SOCIETY FOR NEUROSCIENCE

ABSTRACTS

VOLUME 19, PART 2

23RD ANNUAL MEETING

WASHINGTON, D.C.

NOVEMBER 7–12, 1993

Made in the United States of America.
International Standard Book Numbers:
Part 1 ISBN 0–916110–40–0
Part 2 ISBN 0–916110–41–9
Part 3 ISBN 0–916110–42–7
All parts ISSN 0190–5295
Library of Congress Catalog Card Number 75-7761

Proper citation form for this volume:
Soc. Neurosci. Abstr., Vol. 19, Part 2, p. xxx, 1993.

Published by:
Society for Neuroscience
11 Dupont Circle, N.W.
Suite 500
Washington, D.C. 20036

CONTENTS—PART 2

* 10,975 volunteer abstracts, 18 symposia abstracts, 17 history of neuroscience abstracts, and 56 teaching of neuroscience abstracts.

Please note that due to the increase in abstract submissions this year, the *Abstracts* Volume 19 is printed in **three** parts. The three parts will be mailed, one part at a time, starting in mid-August. The *Abstracts* Volume 19 Part 3, the last part to be mailed, includes the Key Word Index and the Author Index.

1993 Program Committee

Dennis W. Choi, M.D., Ph.D.
Chairperson
Washington University School of Medicine

Irwin B. Levitan, Ph.D.
Incoming Chairperson
Brandeis University

David G. Amaral, Ph.D.
The Salk Institute

Andrew G. Bulloch, Ph.D.
University of Calgary Health Science Center

Marc G. Caron, Ph.D.
Duke University Medical Center

Michael E. Goldberg, M.D.
National Eye Institute, NIH

Mark W. Hamblin, M.D., Ph.D.
University of Washington
Seattle VA Medical Center

Harvey J. Karten, M.D.
University of California, San Diego

Darcy B. Kelley, Ph.D.
Columbia University

James E. Krause, Ph.D.
Washington University School of Medicine

Jean M. Lauder, Ph.D.
University of North Carolina School of Medicine

James O. McNamara, M.D.
Duke University Medical Center
and VA Medical Center

Charles B. Nemeroff, M.D., Ph.D.
Emory University School of Medicine

Mary L. Oster-Granite, Ph.D.
University of California, Riverside

John L.R. Rubenstein, M.D., Ph.D.
University of California, San Francisco

Clifford B. Saper, M.D., Ph.D.
Beth Israel Hospital

Gary Charles Schoenwolf, Ph.D.
University of Utah School of Medicine

S. Murray Sherman, Ph.D.
SUNY, Stony Brook

Roger P. Simon, M.D.
University of California, San Francisco

W. Thomas Thach, Jr., M.D.
Washington University School of Medicine

Leslie G. Ungerleider, Ph.D.
National Institutes of Health

Charles J. Vierck, Ph.D.
University of Florida Health Science Center

Monte Westerfield, Ph.D.
University of Oregon

Richard E. Zigmond, Ph.D.
Case Western Reserve University
School of Medicine

Ira B. Black, M.D., *ex officio*
UMDNJ/RWJ Medical School

Lynn T. Landmesser, Ph.D., *ex officio*
University of Connecticut

Larry R. Squire, Ph.D., *ex officio*
VA Medical Center, San Diego

CHRONOLOGICAL LIST OF SESSIONS

(See page xv for Thematic List of Sessions)

TUESDAY, NOVEMBER 9

WEDNESDAY, NOVEMBER 10

THURSDAY, NOVEMBER 11

Session Number & Title — Page

FRIDAY, NOVEMBER 12

Symposia — 8:30 a.m.

Special Lecture — 10:00 a.m.

Slide Sessions — 8:30 a.m.

Poster Sessions — 8:30 a.m.

THEMATIC LIST OF SESSIONS

(Includes slide and poster sessions, and symposia only)

Session Number	Session Title	Type	Day and Time Mon.	Tue.	Wed.	Thu.	Fri.
THEME A: DEVELOPMENT AND REGENERATION							
192.	Aging processes I	Poster		tAM			
371.	Aging processes II	Poster			wAM		
717.	Aging processes III	Poster					fAM
25.	Axon guidance mechanisms and pathways I	Poster	mAM				
26.	Axon guidance mechanisms and pathways II	Poster	mAM				
103.	Axon guidance mechanisms and pathways III	Slide	mPM				
450.	Axon guidance mechanisms and pathways IV	Poster			wPM		
451.	Axon guidance mechanisms and pathways V	Poster			wPM		
536.	Axon guidance mechanisms and pathways VI	Poster				thAM	
608.	Axon guidance mechanisms and pathways VII	Slide				thPM	
707.	Axon guidance mechanisms and pathways VIII	Poster					fAM
356.	Cell lineage and determination: cellular and molecular specification	Slide			wAM		
21.	Cell lineage and determination: gene expression and growth factors	Poster	mAM				
256.	Cell lineage and determination: immortalization, transplants, cortex	Slide		tPM			
269.	Cell lineage and determination: neural crest	Poster		tPM			
268.	Cell lineage and determination: telencephalon	Poster		tPM			
534.	Cell lineage and determination: visual system	Poster				thAM	
22.	Cell migration and motility: cell-surface molecules	Poster	mAM				
362.	Cell migration and motility: neural plate and crest derivative	Poster			wAM		
361.	Cell migration and motility: olfactory neurons	Poster			wAM		
23.	Cell shape and differentiation: activity	Poster	mAM				
107.	Cell shape and differentiation: immortalized cells and cell lines	Poster	mPM				
705.	Cell shape and differentiation: morphogenesis	Poster					fAM
281.	Cerebral cortex and limbic system	Poster		tPM			
30.	Cerebral cortex and limbic system: molecular determinants and molecular markers	Poster	mAM				
526.	Formation and specificity of synapses	Slide				thAM	
270.	Formation and specificity of synapses: motor neuron muscle interaction	Poster		tPM			
272.	Formation and specificity of synapses: motor neuron to muscle	Poster		tPM			
271.	Formation and specificity of synapses: neuronal target interaction	Poster		tPM			
537.	Formation and specificity of synapses: receptor localization	Poster				thAM	
273.	Formation and specificity of synapses: telencephalon	Poster		tPM			
27.	Formation and specificity of synapses: visual system	Poster	mAM				
516.	**GABA as a Developmental Signal**	**SYMP**				**thAM**	
20.	Genesis of neurons and glia I	Poster	mAM				
360.	Genesis of neurons and glia II	Poster			wAM		
533.	Genesis of neurons and glia III	Poster				thAM	
704.	Genesis of neurons and glia IV	Poster					fAM
28.	Glia and other non-neuronal cells: CNS development	Poster	mAM				
113.	Glia and other non-neuronal cells: PNS development	Poster	mPM				
190.	Glia and other non-neuronal cells: microglia/macrophage	Poster		tAM			
280.	Glia and other non-neuronal cells: neurotransmitters and ion channels	Poster		tPM			
189.	Glia and other non-neuronal cells: response to injury	Poster		tAM			
367.	Hormones and development: androgens	Poster			wAM		
365.	Hormones and development: estrogens	Poster			wAM		
366.	Hormones and development: glucocorticoids	Poster			wAM		
542.	Hormones and development: neuroanatomical finding	Poster				thAM	
712.	Hormones and development: other	Poster					fAM

Session Number	Session Title	Type	Day and Time: Mon.	Tue.	Wed.	Thu.	Fri.
112.	Molecular and pharmacological correlates of development I	Poster	mPM				
262.	Molecular and pharmacological correlates of development II	Slide		tPM			
457.	Molecular and pharmacological correlates of development III	Poster			wPM		
714.	Molecular and pharmacological correlates of development IV	Poster					fAM
621.	Motor systems	Poster				thPM	
187.	Neuronal death I	Poster		tAM			
266.	Neuronal death II	Slide		tPM			
279.	Neuronal death III	Poster		tPM			
368.	Neuronal death IV	Poster			wAM		
620.	Neuronal death V	Poster				thPM	
452.	Neurotransmitters and channels: acetylcholine, amines, peptides, G-proteins	Poster			wPM		
708.	Neurotransmitters and channels: channels	Poster					fAM
274.	Neurotransmitters and channels: glutamate and GABA	Poster		tPM			
175.	Neurotrophic factors: biological effects I	Slide		tAM			
275.	Neurotrophic factors: biological effects II	Poster		tPM			
276.	Neurotrophic factors: biological effects III	Poster		tPM			
277.	Neurotrophic factors: biological effects IV	Poster		tPM			
278.	Neurotrophic factors: biological effects V	Poster		tPM			
453.	Neurotrophic factors: biological effects VI	Poster			wPM		
454.	Neurotrophic factors: biological effects VII	Poster			wPM		
455.	Neurotrophic factors: biological effects VIII	Poster			wPM		
690.	Neurotrophic factors: biological effects IX	Slide					fAM
8.	Neurotrophic factors: expression and regulation I	Slide	mAM				
108.	Neurotrophic factors: expression and regulation II	Poster	mPM				
109.	Neurotrophic factors: expression and regulation III	Poster	mPM				
110.	Neurotrophic factors: expression and regulation IV	Poster	mPM				
111.	Neurotrophic factors: expression and regulation V	Poster	mPM				
538.	Neurotrophic factors: receptors and cellular mechanisms I	Poster				thAM	
539.	Neurotrophic factors: receptors and cellular mechanisms II	Poster				thAM	
540.	Neurotrophic factors: receptors and cellular mechanisms III	Poster				thAM	
541.	Neurotrophic factors: receptors and cellular mechanisms IV	Poster				thAM	
606.	Neurotrophic factors: receptors and cellular mechanisms V	Slide				thPM	
713.	Nutritional and prenatal factors	Poster					fAM
709.	Other factors and trophic agents I	Poster					fAM
710.	Other factors and trophic agents II	Poster					fAM
711.	Other factors and trophic agents III	Poster					fAM
94.	Pattern formation, compartments, and boundaries I	Slide	mPM				
188.	Pattern formation, compartments, and boundaries II	Poster		tAM			
456.	Pattern formation, compartments, and boundaries III	Poster			wPM		
24.	Process outgrowth, growth cones, and sprouting I	Poster	mAM				
185.	Process outgrowth, growth cones, and sprouting II	Slide		tAM			
259.	Process outgrowth, growth cones, and sprouting III	Slide		tPM			
363.	Process outgrowth, growth cones, and sprouting IV	Poster			wAM		
364.	Process outgrowth, growth cones, and sprouting V	Poster			wAM		
448.	Process outgrowth, growth cones, and sprouting VI	Poster			wPM		
449.	Process outgrowth, growth cones, and sprouting VII	Poster			wPM		
535.	Process outgrowth, growth cones, and sprouting VIII	Poster				thAM	
706.	Process outgrowth, growth cones, and sprouting IX	Poster					fAM
178.	Regeneration I	Slide		tAM			
282.	Regeneration II	Poster		tPM			
283.	Regeneration III	Poster		tPM			
543.	Regeneration IV	Poster				thAM	
622.	Regeneration V	Poster				thPM	
715.	Regeneration VI	Poster					fAM
344.	**Regulation of Oligodendrocyte Development**	**SYMP**			**wAM**		

Session Number	Session Title	Type	Day and Time Mon.	Tue.	Wed.	Thu.	Fri.
257.	Sensory and motor systems	Slide		tPM			
29.	Sensory systems I	Poster	mAM				
369.	Sensory systems II	Poster			wAM		
32.	Transplantation I	Poster	mAM				
284.	Transplantation II	Poster		tPM			
357.	Transplantation III	Slide			wAM		
544.	Transplantation IV	Poster				thAM	
623.	Transplantation V	Poster				thPM	
716.	Transplantation VI	Poster					fAM
105.	Visual system	Slide	mPM				
370.	Visual system: LGN and cortex	Poster			wAM		
191.	Visual system: optic tectum/superior colliculus	Poster		tAM			
31.	Visual system: retina	Poster	mAM				
THEME B: CELL BIOLOGY							
288.	Blood-brain barrier: permeability/transport	Poster		tPM			
287.	Blood-brain barrier: structure/function	Poster		tPM			
34.	Cytoskeleton transport and membrane targeting I	Poster	mAM				
436.	Cytoskeleton transport and membrane targeting II	Slide			wPM		
35.	Gene structure and function I	Poster	mAM				
97.	Gene structure and function II	Slide	mPM				
289.	Gene structure and function III	Poster		tPM			
461.	Gene structure and function IV	Poster			wPM		
520.	Gene structure and function V	Slide				thAM	
699.	Gene structure and function VI	Slide					fAM
718.	Gene structure and function VII	Poster					fAM
115.	Membrane composition and cell-surface macromolecules I	Poster	mPM				
193.	Membrane composition and cell-surface macromolecules II	Poster		tAM			
286.	Membrane composition and cell-surface macromolecules III	Poster		tPM			
372.	Membrane composition and cell-surface macromolecules IV	Poster			wAM		
33.	Neuroglia and myelin I	Poster	mAM				
114.	Neuroglia and myelin II	Poster	mPM				
176.	Neuroglia and myelin III	Slide		tAM			
285.	Neuroglia and myelin IV	Poster		tPM			
460.	Neuroglia and myelin V	Poster			wPM		
517.	**Neuronal Functions of Calmodulin-dependent Protein Kinase II**	**SYMP**				**thAM**	
459.	Staining, tracing, and imaging techniques I	Poster			wPM		
545.	Staining, tracing, and imaging techniques II	Poster				thAM	
458.	Staining, tracing, and imaging techniques: confocal microscopy	Poster			wPM		
THEME C: EXCITABLE MEMBRANES AND SYNAPTIC TRANSMISSION							
291.	Calcium channel pharmacology and modulation I	Poster		tPM			
607.	Calcium channel pharmacology and modulation II	Slide				thPM	
720.	Calcium channel pharmacology and modulation III	Poster					fAM
721.	Calcium channel pharmacology and modulation IV	Poster					fAM
549.	Calcium channel physiology	Poster				thAM	
11.	Calcium channel structure, function, and expression I	Slide	mAM				
548.	Calcium channel structure, function, and expression II	Poster				thAM	
463.	Calcium channels: effects of transmitters and hormones	Poster			wPM		
464.	Chloride and other ion channels	Poster			wPM		
295.	Ion channel modulation and regulation I	Poster		tPM			
521.	Ion channel modulation and regulation II	Slide				thAM	
629.	Ion channel modulation and regulation III	Poster				thPM	
722.	Ion channel modulation and regulation IV	Poster					fAM

Session Number	Session Title	Type	Day and Time Mon.	Tue.	Wed.	Thu.	Fri.
723.	Ion channels: cell function	Poster					fAM
119.	Ligand-gated ion channels: glutamatergic	Poster	mPM				
120.	Ligand-gated ion channels: non-glutamatergic	Poster	mPM				
106.	Long-term potentiation I	Slide	mPM				
183.	Long-term potentiation II	Slide		tAM			
374.	Long-term potentiation III	Poster			wAM		
375.	Long-term potentiation IV	Poster			wAM		
376.	Long-term potentiation V	Poster			wAM		
546.	Long-term potentiation VI	Poster				thAM	
547.	Long-term potentiation VII	Poster				thAM	
703.	Long-term potentiation VIII	Slide					fAM
118.	Pharmacology of synaptic transmission I	Poster	mPM				
627.	Pharmacology of synaptic transmission II	Poster				thPM	
117.	Postsynaptic mechanisms I	Poster	mPM				
626.	Postsynaptic mechanisms II	Poster				thPM	
294.	Potassium channel modulation	Poster		tPM			
293.	Potassium channel pharmacology	Poster		tPM			
550.	Potassium channel physiology	Poster				thAM	
292.	Potassium channel structure, function, and expression I	Poster		tPM			
438.	Potassium channel structure, function, and expression II	Slide			wPM		
255.	Presynaptic mechanisms I	Slide		tPM			
373.	Presynaptic mechanisms II	Poster			wAM		
462.	Presynaptic mechanisms III	Poster			wPM		
625.	Presynaptic mechanisms IV	Poster				thPM	
719.	Presynpatic mechanisms V	Poster					fAM
170.	**Role of Calcium in Stimulus-secretion Coupling**	**SYMP**		**tAM**			
121.	Sodium channels I	Poster	mPM				
628.	Sodium channels II	Poster				thPM	
116.	Synaptic structure and function I	Poster	mPM				
290.	Synaptic structure and function II	Poster		tPM			
445.	Synaptic structure and function III	Slide			wPM		
624.	Synaptic structure and function IV	Poster				thPM	
	THEME D: NEUROTRANSMITTERS, MODULATORS, TRANSPORTERS, AND RECEPTORS						
377.	Acetylcholine	Poster			wAM		
10.	Acetylcholine receptors	Slide	mAM				
551.	Acetylcholine receptors: expression of muscarinic receptors	Poster				thAM	
725.	Acetylcholine receptors: muscarinic	Poster					fAM
194.	Acetylcholine receptors: muscarinic antagonists and agonists	Poster		tAM			
465.	Acetylcholine receptors: muscarinic subtypes	Poster			wPM		
552.	Acetylcholine receptors: muscle	Poster				thAM	
726.	Acetylcholine receptors: mutagenesis of muscarinic and nicotinic receptors	Poster					fAM
195.	Acetylcholine receptors: neuronal and α-bungarotoxin-sensitive	Poster		tAM			
123.	Acetylcholine receptors: neuronal nicotinic I	Poster	mPM				
630.	Acetylcholine receptors: neuronal nicotinic II	Poster				thPM	
631.	Acetylcholine receptors: nicotinic antagonists and agonists	Poster				thPM	
466.	Acetylcholine receptors: nicotinic receptor expression	Poster			wPM		
378.	Acetylcholine: ChAT and AChE	Poster			wAM		
122.	Acetylcholine: neuroanatomy	Poster	mPM				
724.	Acetylcholine: release	Poster					fAM
310.	Behavioral pharmacology I	Poster		tPM			
311.	Behavioral pharmacology II	Poster		tPM			
486.	Behavioral pharmacology III	Poster			wPM		

Session Number	Session Title	Type	Day and Time Mon.	Tue.	Wed.	Thu.	Fri.
569.	Behavioral pharmacology IV	Poster				thAM	
101.	Catecholamine receptors	Slide	mPM				
733.	Catecholamine receptors: α- and ß-adrenergic	Poster					fAM
39.	Catecholamine receptors: dopamine I	Poster	mAM				
439.	Catecholamine receptors: dopamine II	Slide			wPM		
562.	Catecholamine receptors: dopamine receptor localization and regulation	Poster				thAM	
302.	Catecholamine receptors: dopamine receptors—molecular biology	Poster		tPM			
38.	Catecholamine receptors: dopaminergic agonists and antagonists	Poster	mAM				
40.	Catecholamines I	Poster	mAM				
203.	Catecholamines II	Poster		tAM			
695.	Catecholamines III	Slide					fAM
382.	Catecholamines: anatomical and developmental aspects	Poster			wAM		
303.	Catecholamines: biosynthesis and degradation	Poster		tPM			
304.	Catecholamines: dopamine release	Poster		tPM			
563.	Catecholamines: electrophysiology	Poster				thAM	
479.	Catecholamines: release	Poster			wPM		
196.	Excitatory amino acids: anatomy and physiology I	Poster		tAM			
379.	Excitatory amino acids: anatomy and physiology II	Poster			wAM		
18.	Excitatory amino acids: excitotoxicity I	Slide	mAM				
553.	Excitatory amino acids: excitotoxicity II	Poster				thAM	
554.	Excitatory amino acids: excitotoxicity III	Poster				thAM	
555.	Excitatory amino acids: excitotoxicity IV	Poster				thAM	
619.	Excitatory amino acids: excitotoxicity V	Slide				thPM	
698.	Excitatory amino acids: excitotoxicity VI	Slide					fAM
727.	Excitatory amino acids: excitotoxicity VII	Poster					fAM
728.	Excitatory amino acids: excitotoxicity VIII	Poster					fAM
729.	Excitatory amino acids: excitotoxicity IX	Poster					fAM
124.	Excitatory amino acids: pharmacology I	Poster	mPM				
197.	Excitatory amino acids: pharmacology II	Poster		tAM			
296.	Excitatory amino acids: pharmacology III	Poster		tPM			
380.	Excitatory amino acids: pharmacology IV	Poster			wAM		
556.	Excitatory amino acids: pharmacology V	Poster				thAM	
730.	Excitatory amino acids: pharmacology VI	Poster					fAM
36.	Excitatory amino acids: receptors I	Poster	mAM				
125.	Excitatory amino acids: receptors II	Poster	mPM				
198.	Excitatory amino acids: receptors III	Poster		tAM			
261.	Excitatory amino acids: receptors IV	Slide		tPM			
297.	Excitatory amino acids: receptors V	Poster		tPM			
381.	Excitatory amino acids: receptors VI	Poster			wAM		
467.	Excitatory amino acids: receptors VII	Poster			wPM		
557.	Excitatory amino acids: receptors VIII	Poster				thAM	
558.	Excitatory amino acids: receptors IX	Poster				thAM	
731.	Excitatory amino acids: receptors X	Poster					fAM
472.	GABA receptors: development	Poster			wPM		
523.	GABA receptors: function—*in vivo* studies	Slide				thAM	
468.	GABA receptors: function—$GABA_A$	Poster			wPM		
471.	GABA receptors: function—$GABA_B$, $GABA_C$	Poster			wPM		
633.	GABA receptors: function—anesthetics	Poster				thPM	
470.	GABA receptors: function—benzodiazepines	Poster			wPM		
351.	GABA receptors: function—molecular modeling I	Slide			wAM		
469.	GABA receptors: function—molecular modeling II	Poster			wPM		
632.	GABA receptors: function—neurosteroids	Poster				thPM	
199.	GABA receptors: structure	Poster		tAM			
3.	**Gender and the Brain**	**SYMP**	**mAM**				
128.	Interactions between neurotransmitters I	Poster	mPM				

Session Number	Session Title	Type	Day and Time Mon.	Tue.	Wed.	Thu.	Fri.
205.	Interactions between neurotransmitters II	Poster		tAM			
484.	Interactions between neurotransmitters III	Poster			wPM		
566.	Interactions between neurotransmitters IV	Poster				thAM	
343.	**Molecular Biology of Neuropeptide Receptors: How Different Are They?**	SYMP			**wAM**		
171.	**Molecular Plasticity to Psychotropic Drugs**	SYMP		**tAM**			
602.	**Neurotransmitter Transporters**	SYMP				**thPM**	
202.	Opioids: anatomy and physiology I	Poster		tAM			
477.	Opioids: anatomy and physiology II	Poster			wPM		
478.	Opioids: anatomy and physiology III	Poster			wPM		
732.	Opioids: behavior	Poster					fAM
348.	Opioids: receptor molecular biology	Slide			wAM		
177.	Opioids: receptor physiology and sigma sites	Slide		tAM			
37.	Opioids: receptors I	Poster	mAM				
475.	Opioids: receptors II	Poster			wPM		
476.	Opioids: receptors III	Poster			wPM		
638.	Opioids: receptors IV	Poster				thPM	
41.	Other biogenic amines and purines: histamine and melatonin	Poster	mAM				
42.	Other biogenic amines and purines: purines	Poster	mAM				
301.	Peptides: anatomical localization I	Poster		tPM			
473.	Peptides: anatomical localization II	Poster			wPM		
474.	Peptides: anatomical localization III	Poster			wPM		
559.	Peptides: biosynthesis, metabolism, and biochemical characterization I	Poster				thAM	
560.	Peptides: biosynthesis, metabolism, and biochemical characterization II	Poster				thAM	
561.	Peptides: biosynthesis, metabolism, and biochemical characterization III	Poster				thAM	
200.	Peptides: physiological effects I	Poster		tAM			
201.	Peptides: physiological effects II	Poster		tAM			
636.	Peptides: physiological effects III	Poster				thPM	
637.	Peptides: physiological effects IV	Poster				thPM	
441.	Peptides: posttranslational processing	Slide			wPM		
104.	Peptides: receptor molecular biology	Slide	mPM				
525.	Peptides: receptor physiology	Slide				thAM	
298.	Peptides: receptors I	Poster		tPM			
299.	Peptides: receptors II	Poster		tPM			
300.	Peptides: receptors III	Poster		tPM			
634.	Peptides: receptors IV	Poster				thPM	
635.	Peptides: receptors V	Poster				thPM	
181.	Receptor modulation, up- and down-regulation I	Slide		tAM			
207.	Receptor modulation, up- and down-regulation II	Poster		tAM			
487.	Receptor modulation, up- and down-regulation III	Poster			wPM		
43.	Regional localization of receptors and transmitters I	Poster	mAM				
129.	Regional localization of receptors and transmitters II	Poster	mPM				
308.	Regional localization of receptors and transmitters III	Poster		tPM			
17.	Second messengers I	Slide	mAM				
130.	Second messengers II	Poster	mPM				
386.	Second messengers III	Poster			wAM		
568.	Second messengers IV	Poster				thAM	
309.	Second messengers: PKC, calcium, and IP3	Poster		tPM			
485.	Second messengers: nitric oxide and calcium	Poster			wPM		
639.	Serotonin receptors: cell biology and effector mechanisms	Poster				thPM	
265.	Serotonin receptors: molecular biology I	Slide		tPM			
480.	Serotonin receptors: molecular biology II	Poster			wPM		
564.	Serotonin receptors: ontogeny and regulation	Poster				thAM	
481.	Serotonin receptors: pharmacology and localization	Poster			wPM		

Session Number	Session Title	Type	Day and Time Mon.	Tue.	Wed.	Thu.	Fri.
93.	Serotonin receptors: pharmacology, localization, regulation	Slide	mPM				
565.	Serotonin receptors: physiology and behavior	Poster				thAM	
437.	Serotonin: anatomy, regulation, and clinical studies	Slide			wPM		
305.	Serotonin: electrophysiology	Poster		tPM			
126.	Serotonin: neurochemistry	Poster	mPM				
482.	Serotonin: neurotoxins, behavior and physiology	Poster			wPM		
640.	Serotonin: other	Poster				thPM	
384.	Storage, secretion, and metabolism I	Poster			wAM		
567.	Storage, secretion, and metabolism II	Poster				thAM	
527.	Transmitters in invertebrates	Slide				thAM	
306.	Transmitters in invertebrates: acetylcholine	Poster		tPM			
204.	Transmitters in invertebrates: amino acids	Poster		tAM			
127.	Transmitters in invertebrates: biogenic amines	Poster	mPM				
483.	Transmitters in invertebrates: nitric oxide	Poster			wPM		
383.	Transmitters in invertebrates: peptides	Poster			wAM		
4.	**Unraveling the Serotonergic System: Insights from Molecular Biology**	**SYMP**	**mAM**				
7.	Uptake and transporters I	Slide	mAM				
95.	Uptake and transporters II	Slide	mPM				
206.	Uptake and transporters III	Poster		tAM			
307.	Uptake and transporters IV	Poster		tPM			
385.	Uptake and transporters V	Poster			wAM		
	THEME E: ENDOCRINE AND AUTONOMIC REGULATION						
393.	Autonomic regulation: central gastrointestinal control	Poster			wAM		
212.	Autonomic regulation: genital innervation	Poster		tAM			
394.	Autonomic regulation: peripheral gastrointestinal control	Poster			wAM		
135.	Autonomic regulation: spinal and peripheral mechanisms	Poster	mPM				
134.	Autonomic regulation: supraspinal control	Poster	mPM				
213.	Autonomic regulation: urinary system innervation	Poster		tAM			
446.	Cardiovascular regulation: brainstem integration	Slide			wPM		
391.	Cardiovascular regulation: descending control	Poster			wAM		
392.	Cardiovascular regulation: hypothalamic control	Poster			wAM		
610.	Cardiovascular regulation: spinal and peripheral control	Slide				thPM	
184.	Cardiovascular regulation: supramedullary control	Slide		tAM			
132.	Cardiovascular regulation: sympathetic system	Poster	mPM				
133.	Cardiovascular regulation: vagal system	Poster	mPM				
390.	Cardiovascular regulation: ventrolateral medulla	Poster			wAM		
312.	Hypothalamic-pituitary-adrenal axis regulation: CRF	Poster		tPM			
488.	Hypothalamic-pituitary-adrenal axis regulation: POMC and steroid receptor studies	Poster			wPM		
387.	Hypothalamic-pituitary-adrenal axis regulation: basic and clinical studies	Poster			wAM		
174.	Hypothalamic-pituitary-adrenal axis regulation: focus on CRF and glucocorticoid receptors	Slide		tAM			
349.	Hypothalamic-pituitary-gonadal regulation: cellular and molecular aspects	Slide			wAM		
258.	Hypothalamic-pituitary-gonadal regulation: control of GnRH secretion	Slide		tPM			
131.	Hypothalamic-pituitary-gonadal regulation: control of LH secretion	Poster	mPM				
572.	Hypothalamic-pituitary-gonadal regulation: gonadotropins, neuropeptides, steroids	Poster				thAM	
570.	Hypothalamic-pituitary-gonadal regulation: neuropeptides and transmitters	Poster				thAM	
734.	Hypothalamic-pituitary-gonadal regulation: regulatory aspects	Poster					fAM
571.	Hypothalamic-pituitary-gonadal regulation: releasing hormones	Poster				thAM	

Session Number	Session Title	Type	Day and Time: Mon.	Tue.	Wed.	Thu.	Fri.
641.	Hypothalamic-pituitary-gonadal regulation: steroid receptors	Poster				thPM	
696.	Hypothalamus and autonomic regulation	Slide					fAM
250.	**Integration in Central Somato-visceral Processing**	**SYMP**		**tPM**			
210.	Neural-immune interactions: CNS effects on immune response	Poster		tAM			
98.	Neural-immune interactions: cytokine effects on the nervous system	Slide	mPM				
211.	Neural-immune interactions: endocrine effects on immune response	Poster		tAM			
45.	Neural-immune interactions: immune mediators in normal CNS	Poster	mAM				
46.	Neural-immune interactions: nervous system pathology	Poster	mAM				
208.	Neural-immune interactions: neurochemical effects of immune stimulation	Poster		tAM			
691.	Neural-immune interactions: neuroendocrine control of immune response	Slide					fAM
209.	Neural-immune interactions: neurophysiological response to immune stimulation	Poster		tAM			
389.	Neural-immune interactions: other neurotransmitters in immune tissues	Poster			wAM		
388.	Neural-immune interactions: sympathetic regulation of immune response	Poster			wAM		
489.	Neuroendocrine regulation: CRF, gonadal and adrenal steroids	Poster			wPM		
573.	Neuroendocrine regulation: catecholamine and GABA	Poster				thAM	
442.	Neuroendocrine regulation: gene expression and co-localization	Slide			wPM		
642.	Neuroendocrine regulation: miscellaneous	Poster				thPM	
735.	Neuroendocrine regulation: oxytocin, vasopressin, fluid balance, and the pineal	Poster					fAM
44.	Osmotic regulation	Poster	mAM				
530.	Osmotic regulation/chemical senses: central pathways	Slide				thAM	
613.	Respiratory regulation	Slide				thPM	
574.	Respiratory regulation: carotid body, pons, hypothalamus, miscellaneous	Poster				thAM	
490.	Respiratory regulation: medullary and spinal cord mechanisms	Poster			wPM		
47.	Thermoregulation and fever	Poster	mAM				
	THEME F: SENSORY SYSTEMS						
739.	Auditory behavior	Poster					fAM
581.	Auditory cortex I	Poster				thAM	
582.	Auditory cortex II	Poster				thAM	
693.	Auditory system	Slide					fAM
494.	Auditory system: central anatomy—brainstem	Poster			wPM		
583.	Auditory system: central anatomy—midbrain, thalamus, and cortex	Poster				thAM	
580.	Auditory system: cochlea	Poster				thAM	
649.	Auditory, vestibular, and lateral line: hair cells	Poster				thPM	
585.	Chemical senses: central gustatory mechanisms	Poster				thAM	
55.	Chemical senses: central olfactory mechanisms	Poster	mAM				
54.	Chemical senses: olfactory bulb	Poster	mAM				
584.	Chemical senses: peripheral gustatory mechanisms	Poster				thAM	
12.	Chemical senses: peripheral mechanisms	Slide	mAM				
53.	Chemical senses: peripheral olfactory mechanisms	Poster	mAM				
431.	**Cortical Oscillatory Responses and Feature Binding**	**SYMP**			**wPM**		
90.	**Functional Organization of Human Visual Cortex**	**SYMP**	**mPM**				
141.	Invertebrate sensory systems I	Poster	mPM				
142.	Invertebrate sensory systems II	Poster	mPM				
219.	Lateral geniculate nucleus: biophysics and pharmacology	Poster		tAM			
218.	Lateral geniculate nucleus: structure and function	Poster		tAM			
530.	Osmotic regulation/chemical senses: central pathways	Slide				thAM	
577.	Pain modulation: pharmacology IV	Poster				thAM	
736.	Pain modulation: pharmacology V	Poster					fAM

Session Number	Session Title	Type	Day and Time Mon.	Tue.	Wed.	Thu.	Fri.
50.	Pain modulation: anatomy and physiology I	Poster	mAM				
102.	Pain modulation: anatomy and physiology II	Slide	mPM				
216.	Pain modulation: anatomy and physiology III	Poster		tAM			
395.	Pain modulation: anatomy and physiology IV	Poster			wAM		
576.	Pain modulation: anatomy and physiology V	Poster				thAM	
51.	Pain modulation: pharmacology I	Poster	mAM				
217.	Pain modulation: pharmacology II	Poster		tAM			
396.	Pain modulation: pharmacology III	Poster			wAM		
444.	Pain: pathways I	Slide			wPM		
575.	Pain: pathways II	Poster				thAM	
646.	Pain: pathways III	Poster				thPM	
493.	Retina: choroid, pigment epithelium, and photoreceptors	Poster			wPM		
578.	Retina: functional organization	Poster				thAM	
519.	Retina: ganglion cells I	Slide				thAM	
579.	Retina: ganglion cells II	Poster				thAM	
52.	Retina: immunocytochemistry	Poster	mAM				
492.	Retina: invertebrate	Poster			wPM		
100.	Retina: photoreceptors and interneurons	Slide	mPM				
48.	Somatic and visceral afferents I	Poster	mAM				
136.	Somatic and visceral afferents II	Poster	mPM				
214.	Somatic and visceral afferents III	Poster		tAM			
49.	Somatosensory cortex and thalamocortical relationships I	Poster	mAM				
313.	Somatosensory cortex and thalamocortical relationships II	Poster		tPM			
644.	Somatosensory cortex and thalamocortical relationships III	Poster				thPM	
645.	Somatosensory cortex and thalamocortical relationships IV	Poster				thPM	
702.	Somatosensory cortex and thalamocortical relationships V	Slide					fAM
491.	Spinal cord I	Poster			wPM		
643.	Spinal cord II	Poster				thPM	
737.	Striate cortex: development and plasticity	Poster					fAM
139.	Striate cortex: functional organization I	Poster	mPM				
140.	Striate cortex: functional organization II	Poster	mPM				
179.	Striate cortex: functional organization III	Slide		tAM			
359.	Striate cortex: plasticity	Slide			wAM		
263.	Striate cortex: response properties I	Slide		tPM			
647.	Striate cortex: response properties II	Poster				thPM	
648.	Striate cortex: response properties III	Poster				thPM	
221.	Subcortical auditory pathways I	Poster		tAM			
222.	Subcortical auditory pathways II	Poster		tAM			
223.	Subcortical auditory pathways III	Poster		tAM			
137.	Subcortical somatosensory pathways: spinal cord and brainstem	Poster	mPM				
215.	Subcortical somatosensory pathways: thalamus	Poster		tAM			
220.	Subcortical visual pathways: retinofugal and retinopetal systems	Poster		tAM			
314.	Subcortical visual pathways: superior colliculus	Poster		tPM			
13.	Subcortical visual systems	Slide	mAM				
138.	Subcortical visual systems: pretectum and pulvinar	Poster	mPM				
397.	Visual cortex: extrastriate—anatomy	Poster			wAM		
19.	Visual cortex: extrastriate—cognitive mechanisms I	Slide	mAM				
398.	Visual cortex: extrastriate—cognitive mechanisms II	Poster			wAM		
618.	Visual cortex: extrastriate—functional architecture	Slide				thPM	
531.	Visual cortex: extrastriate—motion processing	Slide				thAM	
315.	Visual cortex: extrastriate—unit properties	Poster		tPM			
316.	Visual psychophysics and behavior I	Poster		tPM			
738.	Visual psychophysics and behavior II	Poster					fAM

THEME G: MOTOR SYSTEMS AND SENSORIMOTOR INTEGRATION

Session Number	Session Title	Type	Day and Time: Mon.	Tue.	Wed.	Thu.	Fri.
56.	Basal ganglia and thalamus I	Poster	mAM				
57.	Basal ganglia and thalamus II	Poster	mAM				
58.	Basal ganglia and thalamus III	Poster	mAM				
320.	Basal ganglia and thalamus IV	Poster		tPM			
399.	Basal ganglia and thalamus V	Poster			wAM		
586.	Basal ganglia and thalamus VI	Poster				thAM	
587.	Basal ganglia and thalamus VII	Poster				thAM	
650.	Basal ganglia and thalamus VIII	Poster				thPM	
651.	Basal ganglia and thalamus IX	Poster				thPM	
400.	Cerebellum I	Poster			wAM		
401.	Cerebellum II	Poster			wAM		
499.	Cerebellum III	Poster			wPM		
529.	Cerebellum IV	Slide				thAM	
652.	Cerebellum V	Poster				thPM	
146.	Circuitry and pattern generation I	Poster	mPM				
231.	Circuitry and pattern generation II	Poster		tAM			
406.	Circuitry and pattern generation III	Poster			wAM		
656.	Circuitry and pattern generation IV	Poster				thPM	
700.	Circuitry and pattern generation V	Slide					fAM
63.	Control of posture and movement I	Poster	mAM				
64.	Control of posture and movement II	Poster	mAM				
226.	Control of posture and movement III	Poster		tAM			
227.	Control of posture and movement IV	Poster		tAM			
228.	Control of posture and movement V	Poster		tAM			
229.	Control of posture and movement VI	Poster		tAM			
230.	Control of posture and movement VII	Poster		tAM			
404.	Control of posture and movement VIII	Poster			wAM		
405.	Control of posture and movement IX	Poster			wAM		
655.	Control of posture and movement X	Poster				thPM	
692.	Control of posture and movement XI	Slide					fAM
657.	Invertebrate motor function	Poster				thPM	
616.	Motor cortex	Slide				thPM	
498.	Motor cortex: neuropharmacology	Poster			wPM		
65.	Muscle: fiber types	Poster	mAM				
66.	Muscle: gene transfer, contractile properties, fatigue	Poster	mAM				
322.	Oculomotor system: eye-head control	Poster		tPM			
653.	Oculomotor system: neuroanatomy	Poster				thPM	
180.	Oculomotor system: physiology and psychophysics of saccades	Slide		tAM			
144.	Oculomotor system: pursuit and optokinetic nystagmus	Poster	mPM				
321.	Oculomotor system: saccades and superior colliculus	Poster		tPM			
354.	Oculomotor system: superior colliculus and brainstem	Slide			wAM		
145.	Oculomotor system: vergence and accommodation	Poster	mPM				
654.	Oculomotor system: vertical movements, integration, torsion	Poster				thPM	
61.	Reflex function I	Poster	mAM				
62.	Reflex function II	Poster	mAM				
318.	Sensorimotor cortex: behavioral correlates of neuronal discharge	Poster		tPM			
496.	Sensorimotor cortex: functional imaging	Poster			wPM		
495.	Sensorimotor cortex: functional stimulation, models, and behavior	Poster			wPM		
497.	Sensorimotor cortex: neuroanatomy	Poster			wPM		
319.	Sensorimotor cortex: neuronal interactions	Poster		tPM			
317.	Sensorimotor cortex: plasticity	Poster		tPM			
224.	Spinal cord and brainstem I	Poster		tAM			
225.	Spinal cord and brainstem II	Poster		tAM			

Session Number	Session Title	Type	Day and Time Mon.	Tue.	Wed.	Thu.	Fri.
402.	Spinal cord and brainstem III	Poster			wAM		
403.	Spinal cord and brainstem IV	Poster			wAM		
588.	Spinal cord and brainstem V	Poster				thAM	
614.	Vestibular system	Slide				thPM	
59.	Vestibular system: anatomy and pharmacology	Poster	mAM				
60.	Vestibular system: neurophysiology	Poster	mAM				
143.	Vestibular system: psychophysics	Poster	mPM				
	THEME H: OTHER SYSTEMS OF THE CNS						
590.	Association cortex and thalamocortical relations I	Poster				thAM	
591.	Association cortex and thalamocortical relations II	Poster				thAM	
524.	Brain metabolism and blood flow I	Slide				thAM	
615.	Brain metabolism and blood flow II	Slide				thPM	
502.	Brain metabolism and blood flow: PET	Poster			wPM		
501.	Brain metabolism and blood flow: blood flow	Poster			wPM		
500.	Brain metabolism and blood flow: miscellaneous	Poster			wPM		
503.	Brain metabolism and blood flow: nitric oxide	Poster			wPM		
68.	Comparative neuroanatomy I	Poster	mAM				
407.	Comparative neuroanatomy II	Poster			wAM		
67.	Hypothalamus	Poster	mAM				
147.	Limbic system I	Poster	mPM				
148.	Limbic system II	Poster	mPM				
149.	Limbic system III	Poster	mPM				
352.	Limbic system IV	Slide			wAM		
589.	Limbic system V	Poster				thAM	
	THEME I: NEURAL BASIS OF BEHAVIOR						
159.	Aging	Poster	mPM				
75.	Aging: functional anatomy	Poster	mAM				
247.	Aging: memory and cognition	Poster		tAM			
235.	Biological rhythms and sleep I	Poster		tAM			
236.	Biological rhythms and sleep II	Poster		tAM			
434.	Biological rhythms and sleep III	Slide			wPM		
612.	Biological rhythms and sleep IV	Slide				thPM	
662.	Biological rhythms and sleep V	Poster				thPM	
701.	Biological rhythms and sleep VI	Slide					fAM
742.	Biological rhythms and sleep VII	Poster					fAM
254.	Drugs of abuse: alcohol, barbiturates, benzodiazepines	Slide		tPM			
333.	Drugs of abuse: amphetamine and other stimulants—amphetamine	Poster		tPM			
335.	Drugs of abuse: amphetamine and other stimulants—amphetamine derivatives	Poster		tPM			
334.	Drugs of abuse: amphetamine and other stimulants—amphetamine: behavior	Poster		tPM			
336.	Drugs of abuse: amphetamine and other stimulants—nicotine	Poster		tPM			
337.	Drugs of abuse: amphetamine and other stimulants—phencyclidines and other	Poster		tPM			
617.	Drugs of abuse: cocaine	Slide				thPM	
751.	Drugs of abuse: cocaine—behavior	Poster					fAM
752.	Drugs of abuse: cocaine—cell membrane	Poster					fAM
753.	Drugs of abuse: cocaine—glutamate	Poster					fAM
754.	Drugs of abuse: cocaine—locomotor	Poster					fAM
755.	Drugs of abuse: cocaine—microdialysis	Poster					fAM
762.	Drugs of abuse: cocaine—miscellaneous	Poster					fAM
756.	Drugs of abuse: cocaine—monoamines	Poster					fAM
757.	Drugs of abuse: cocaine—neonatal	Poster					fAM

Session Number	Session Title	Type	Day and Time Mon.	Tue.	Wed.	Thu.	Fri.
758.	Drugs of abuse: cocaine—neurophysiology	Poster					fAM
759.	Drugs of abuse: cocaine—nucleus accumbens	Poster					fAM
760.	Drugs of abuse: cocaine—other drugs	Poster					fAM
761.	Drugs of abuse: cocaine—self-administration	Poster					fAM
595.	Drugs of abuse: ethanol and benzodiazepines—tolerance, dependence, withdrawal	Poster				thAM	
74.	Drugs of abuse: ethanol, benzodiazepines, barbiturates I	Poster	mAM				
157.	Drugs of abuse: ethanol, benzodiazepines, barbiturates II	Poster	mPM				
245.	Drugs of abuse: ethanol, benzodiazepines, barbiturates—GABA	Poster		tAM			
749.	Drugs of abuse: ethanol—development	Poster					fAM
750.	Drugs of abuse: ethanol—monoamines	Poster					fAM
512.	Drugs of abuse: opioids and others—developmental effects	Poster			wPM		
596.	Drugs of abuse: opioids and others—miscellaneous	Poster				thAM	
418.	Drugs of abuse: opioids and others—opioids: behavior	Poster			wAM		
417.	Drugs of abuse: opioids and others—opioids: neurochemistry	Poster			wAM		
511.	Drugs of abuse: opioids and others—opioids: withdrawal	Poster			wPM		
72.	Hormonal control of reproductive behavior: hormones and metabolites	Poster	mAM				
416.	Hormonal control of reproductive behavior: immediate early gene expression	Poster			wAM		
241.	Hormonal control of reproductive behavior: male/female/parental	Poster		tAM			
744.	Hormonal control of reproductive behavior: neuroanatomy	Poster					fAM
665.	Hormonal control of reproductive behavior: neuropeptides and transmitters	Poster				thPM	
332.	Hormonal control of reproductive behavior: receptors	Poster		tPM			
233.	Human cognition: attention	Poster		tAM			
532.	Human cognition: attention and memory	Slide				thAM	
347.	Human cognition: audition and language I	Slide			wAM		
740.	Human cognition: audition and language II	Poster					fAM
659.	Human cognition: electrophysiology	Poster				thPM	
232.	Human cognition: hemispheric laterality, gender differences	Poster		tAM			
323.	Human cognition: memory, other	Poster		tPM			
658.	Human cognition: vision, methods	Poster				thPM	
239.	Ingestive behaviors I	Poster		tAM			
240.	Ingestive behaviors II	Poster		tAM			
331.	Ingestive behaviors III	Poster		tPM			
508.	Ingestive behaviors IV	Poster			wPM		
522.	Ingestive behaviors V	Slide				thAM	
697.	Ingestive behaviors VI	Slide					fAM
743.	Ingestive behaviors VII	Poster					fAM
14.	Invertebrate learning and behavior I	Slide	mAM				
238.	Invertebrate learning and behavior II	Poster		tAM			
330.	Invertebrate learning and behavior III	Poster		tPM			
440.	Invertebrate learning and behavior IV	Slide			wPM		
660.	Learning and memory: models	Poster				thPM	
741.	Learning and memory: pharmacology—acetylcholine	Poster					fAM
412.	Learning and memory: pharmacology—benzodiazepines	Poster			wAM		
413.	Learning and memory: pharmacology—excitatory amino acids	Poster			wAM		
153.	Learning and memory: pharmacology—monoamines	Poster	mPM				
154.	Learning and memory: pharmacology—opioids	Poster	mPM				
234.	Learning and memory: pharmacology—other I	Poster		tAM			
507.	Learning and memory: pharmacology—other II	Poster			wPM		
173.	Learning and memory: physiology I	Slide		tAM			
324.	Learning and memory: physiology II	Poster		tPM			
325.	Learning and memory: physiology III	Poster		tPM			
326.	Learning and memory: physiology IV	Poster		tPM			
411.	Learning and memory: physiology V	Poster			wAM		

Session Number	Session Title	Type	Day and Time: Mon.	Tue.	Wed.	Thu.	Fri.
150.	Learning and memory: systems and functions I	Poster	mPM				
151.	Learning and memory: systems and functions II	Poster	mPM				
152.	Learning and memory: systems and functions III	Poster	mPM				
186.	Learning and memory: systems and functions IV	Slide		tAM			
408.	Learning and memory: systems and functions V	Poster			wAM		
409.	Learning and memory: systems and functions VI	Poster			wAM		
410.	Learning and memory: systems and functions VII	Poster			wAM		
447.	Learning and memory: systems and functions VIII	Slide			wPM		
504.	Learning and memory: systems and functions IX	Poster			wPM		
505.	Learning and memory: systems and functions X	Poster			wPM		
506.	Learning and memory: systems and functions XI	Poster			wPM		
592.	Learning and memory: systems and functions XII	Poster				thAM	
747.	Monoamines and behavior: dopamine and movement	Poster					fAM
748.	Monoamines and behavior: electrophysiology	Poster					fAM
243.	Monoamines and behavior: gene expression	Poster		tAM			
745.	Monoamines and behavior: nucleus accumbens	Poster					fAM
244.	Monoamines and behavior: serotonin	Poster		tAM			
242.	Monoamines and behavior: sexual behavior	Poster		tAM			
509.	Monoamines and behavior: stimulants	Poster			wPM		
510.	Monoamines and behavior: stress and depression	Poster			wPM		
746.	Monoamines and behavior: transmitter release	Poster					fAM
155.	Motivation and emotion I	Poster	mPM				
329.	Motivation and emotion II	Poster		tPM			
661.	Motivation and emotion III	Poster				thPM	
328.	Neural plasticity I	Poster		tPM			
414.	Neural plasticity II	Poster			wAM		
69.	Neural plasticity: cerebral cortex	Poster	mAM				
327.	Neural plasticity: hippocampus	Poster		tPM			
237.	Neuroethology: audition	Poster		tAM			
663.	Neuroethology: behavioral strategies	Poster				thPM	
415.	Neuroethology: bird vocalization	Poster			wAM		
156.	Neuroethology: electroreception	Poster	mPM				
70.	Neuroethology: invertebrate	Poster	mAM				
73.	Neuropeptides and behavior: CCK, CRF, vasopressin, and somatostatin	Poster	mAM				
6.	Neuropeptides and behavior: CRF, oxytocin, and other peptides	Slide	mAM				
594.	Neuropeptides and behavior: vasopressin, NPY, neurotensin, and others	Poster				thAM	
603.	**Prefrontal Mechanisms of Disordered Cognition: Relevance to Schizophrenia**	**SYMP**				**thPM**	
246.	Psychotherapeutic drugs: antipsychotics	Poster		tAM			
666.	Psychotherapeutic drugs: antipsychotics and other agents	Poster				thPM	
763.	Psychotherapeutic drugs: anxiolytics and antidepressants	Poster					fAM
158.	Psychotherapeutic drugs: clozapine	Poster	mPM				
353.	Psychotherapeutic drugs: effects on neurotransmitter systems	Slide			wAM		
664.	Stress: behavioral studies	Poster				thPM	
358.	Stress: from molecular biology to behavior	Slide			wAM		
593.	Stress: neurochemistry	Poster				thAM	
71.	Stress: neuroendocrine mechanisms	Poster	mAM				
688.	**The Contribution of Identified Neurons to Neuroscience: A 25-Year Retrospective**	**SYMP**					**fAM**
687.	**View of a Neural System in the Blink of an Eye: The Eyeblink Reflex—Control, Learning, and Cellular Mechanisms**	**SYMP**					**fAM**
	THEME J: DISORDERS OF THE NERVOUS SYSTEM						
15.	Degenerative disease: Alzheimer's—ß-amyloid I	Slide	mAM				

Session Number	Session Title	Type	Day and Time				
			Mon.	Tue.	Wed.	Thu.	Fri.
77.	Degenerative disease: Alzheimer's—ß-amyloid II	Poster	mAM				
162.	Degenerative disease: Alzheimer's—ß-amyloid III	Poster	mPM				
182.	Degenerative disease: Alzheimer's—ß-amyloid IV	Slide		tAM			
338.	Degenerative disease: Alzheimer's—ß-amyloid V	Poster		tPM			
355.	Degenerative disease: Alzheimer's—ß-amyloid VI	Slide			wAM		
421.	Degenerative disease: Alzheimer's—ß-amyloid VII	Poster			wAM		
422.	Degenerative disease: Alzheimer's—ß-amyloid VIII	Poster			wAM		
513.	Degenerative disease: Alzheimer's—ß-amyloid IX	Poster			wPM		
528.	Degenerative disease: Alzheimer's—ß-amyloid X	Slide				thAM	
600.	Degenerative disease: Alzheimer's—ß-amyloid XI	Poster				thAM	
669.	Degenerative disease: Alzheimer's—ß-amyloid XII	Poster				thPM	
79.	Degenerative disease: Alzheimer's—cognitive function: imaging and neuropathology	Poster	mAM				
78.	Degenerative disease: Alzheimer's—cognitive function: neuropsychology	Poster	mAM				
96.	Degenerative disease: Alzheimer's—neuropathology and neurotransmitters	Slide	mPM				
80.	Degenerative disease: Alzheimer's—neuropharmacology and neurotransmitters I	Poster	mAM				
163.	Degenerative disease: Alzheimer's—neuropharmacology and neurotransmitters II	Poster	mPM				
423.	Degenerative disease: Alzheimer's—neuropharmacology and neurotransmitters III	Poster			wAM		
514.	Degenerative disease: Alzheimer's—neuropharmacology and neurotransmitters IV	Poster			wPM		
81.	Degenerative disease: Alzheimer's—other I	Poster	mAM				
99.	Degenerative disease: Alzheimer's—other II	Slide	mPM				
260.	Degenerative disease: Alzheimer's—other III	Slide		tPM			
424.	Degenerative disease: Alzheimer's—other IV	Poster			wAM		
425.	Degenerative disease: Alzheimer's—other V	Poster			wAM		
515.	Degenerative disease: Alzheimer's—other VI	Poster			wPM		
601.	Degenerative disease: Alzheimer's—other VII	Poster				thAM	
670.	Degenerative disease: Alzheimer's—other VIII	Poster				thPM	
264.	Degenerative disease: Parkinson's	Slide		tPM			
164.	Degenerative disease: Parkinson's—free radicals	Poster	mPM				
427.	Degenerative disease: Parkinson's—functional morphology	Poster			wAM		
426.	Degenerative disease: Parkinson's—human neuropharmacology and pathology	Poster			wAM		
428.	Degenerative disease: Parkinson's—human performance and primate models	Poster			wAM		
165.	Degenerative disease: Parkinson's—neurotoxicity I	Poster	mPM				
166.	Degenerative disease: Parkinson's—neurotoxicity II	Poster	mPM				
429.	Degenerative disease: Parkinson's—transplantation and glia	Poster			wAM		
82.	Degenerative disease: other I	Poster	mAM				
167.	Degenerative disease: other II	Poster	mPM				
339.	Degenerative disease: other III	Poster		tPM			
346.	Degenerative disease: other IV	Slide			wAM		
76.	Developmental disorders of the nervous system I	Poster	mAM				
597.	Developmental disorders of the nervous system II	Poster				thAM	
668.	Epilepsy: anticonvulsant drugs	Poster				thPM	
16.	Epilepsy: basic mechanisms I	Slide	mAM				
249.	Epilepsy: basic mechanisms II	Poster		tAM			
420.	Epilepsy: basic mechanisms III	Poster			wAM		
599.	Epilepsy: basic mechanisms IV	Poster				thAM	
764.	Epilepsy: basic mechanisms V	Poster					fAM
161.	Epilepsy: human studies and animal models I	Poster	mPM				

Session Number	Session Title	Type	Day and Time Mon.	Tue.	Wed.	Thu.	Fri.
248.	Epilepsy: human studies and animal models II	Poster		tAM			
419.	Epilepsy: human studies and animal models III	Poster			wAM		
598.	Epilepsy: human studies and animal models IV	Poster				thAM	
160.	Genetic models of nervous system disorders I	Poster	mPM				
667.	Genetic models of nervous system disorders II	Poster				thPM	
694.	Genetic models of nervous system disorders III	Slide					fAM
684.	Infectious diseases	Poster				thPM	
267.	Ischemia I	Slide		tPM			
350.	Ischemia II	Slide			wAM		
435.	Ischemia III	Slide			wPM		
671.	Ischemia: acidosis	Poster				thPM	
672.	Ischemia: calcium	Poster				thPM	
673.	Ischemia: drug treatment I	Poster				thPM	
674.	Ischemia: drug treatment II	Poster				thPM	
675.	Ischemia: glia	Poster				thPM	
676.	Ischemia: heat shock protein	Poster				thPM	
677.	Ischemia: models	Poster				thPM	
678.	Ischemia: molecular biology/immunocytochemistry	Poster				thPM	
679.	Ischemia: neonatal	Poster				thPM	
680.	Ischemia: neurochemistry I	Poster				thPM	
681.	Ischemia: neurochemistry II	Poster				thPM	
682.	Ischemia: neurophysiology	Poster				thPM	
683.	Ischemia: temperature	Poster				thPM	
9.	Mental illness I	Slide	mAM				
84.	Mental illness II	Poster	mAM				
340.	Mental illness III	Poster		tPM			
769.	Mental illness IV	Poster					fAM
251.	**Microglia and Neuronal Injury**	**SYMP**		**tPM**			
443.	Neuro-oncology I	Slide			wPM		
773.	Neuro-oncology II	Poster					fAM
83.	Neuromuscular disease	Poster	mAM				
609.	Neurotoxicity I	Slide				thPM	
686.	Neurotoxicity II	Poster				thPM	
770.	Neurotoxicity III	Poster					fAM
771.	Neurotoxicity IV	Poster					fAM
772.	Neurotoxicity V	Poster					fAM
685.	Neurotoxicity: metals	Poster				thPM	
89.	**Phosphorylation Cascades, Neurofibrillary Tangles, and Alzheimer's Disease**	**SYMP**	**mPM**				
430.	**Thalamocortical Mechanisms Underlying Generalized Absence Seizures**	**SYMP**			**wPM**		
611.	Trauma	Slide				thPM	
765.	Trauma: cord	Poster					fAM
767.	Trauma: miscellaneous I	Poster					fAM
768.	Trauma: miscellaneous II	Poster					fAM
766.	Trauma: treatment	Poster					fAM
OTHER							
85.	History of neuroscience	Poster	mAM, PM	tAM, PM	wAM, PM	thAM, PM	fAM
86.	Teaching of neuroscience I	Poster	mAM, PM	tAM, PM	wAM, PM	thAM, PM	fAM
87.	Teaching of neuroscience II	Poster	mAM, PM	tAM, PM	wAM, PM	thAM, PM	fAM
88.	Teaching of neuroscience III	Poster	mAM, PM	tAM, PM	wAM, PM	thAM, PM	fAM

372.3

A PRIMITIVE NA,K-ATPASE IN TENTACLES OF THE SEA ANEMONE *STICHODACTYLA HELIANTHUS*. S.C. Specht* R. Lopez-Rosado and C. Santos-Berrios. Dept. Pharmacol. Inst. Neurobiol. Univ. PR Sch. Med., San Juan, PR 00901.

Although the Na, K-ATPase is an highly conserved integral membrane enzyme, it has never been described in sub-Arthropod species. We have found that the innervated tentacles of *S. helianthus* contain a ouabain-sensitive Na,K-ATPase. Like the mammalian enzyme, it is phosphorylated by ^{32}P-ATP in the presence of 100 mM NaCl and is 70% dephosphorylated by 20 mM KCl. The M_R of the catalytic subunit is 105 kD, similar to the rat α3 isoform. The ouabain sensitivity is very low, $K_i = 10^{-4}$ M. The affinity for Na^+ is lower than the rat enzyme, although similar to that described in several species of crab, $K_{0.5}$ =24 mM. The affinities for K^+ and Rb^+ are similarly low, $K_{0.5}$ = 3 mM. compared to the rat. In addition, the selectivity for K^+ and Rb^+ vs Cs^+ and NH_4^+ is low, with only a 2-fold difference in affinity compared to 8-fold in the rat. Li^+ is unable to substitute for either Na^+ or K^+. The reduced cation selectivity may represent a true primitive characteristic. [Supported in part by NIGMS grant SS RR 08224].

372.4

LOCALIZATION OF ALPHA 1 ISOFORM OF (NA, K)-ATPase IN SPINAL CORD MICROVASCULATURE BY *IN SITU* HYBRIDIZATION. S.T. Sayers*, T. Khan, G.J. Siegel, N.B. Chauhan, and M.F. Dauzvardis, Rehabilitation R&D Center and Neurology Service, Hines VA Hospital, Hines, IL 60141 and Loyola University Medical School, Maywood, IL 60153.

Expression of (Na, K)-ATPase alpha (catalytic) subunit isoform mRNAs has been established in various cell types in different regions of the central nervous system (CNS). Alpha 1 isoform is found in certain neuronal as well as non-neuronal cells, including choriod plexus ependyma, however its mRNA has not been resolved in CNS capillaries. Immunocytochemical data have supported the presence of alpha 1 isoform in cerebral microvessels. In the present study, we report the expression of alpha 1 isoform in the microvasculature of the spinal cord.

Wistar rats were anesthetized with Ketamine and Xylazine, perfused trancardially with 200 ml saline followed by 500 ml 4% paraformaldehyde in 0.1M phosphate buffer and then with 100 ml 10% sucrose. The spinal cords were removed, cut into small segments and placed in 30% sucrose at 4°C overnight. Ten micron frozen sections were cut on a cryostat. *In situ* hybridization was performed using ^{35}S-labeled complimentary riboprobe transcribed from the cDNA clone which codes for the alpha 1 isoform of (Na, K)-ATPase. Our preliminary results show that most of the capillaries in the spinal cord gray and white matter were labeled with this probe. Due to the limitation of resolution of the microscope, it is very difficult to distinguish whether labeling is in the endothelium or in pericytes around the microvessels. Studies with additional probes, and at the electron microscopic level, are required. Since the blood-brain-barrier is imposed by the specialized structure of the CNS microvessels, regulation of (Na, K)-ATPase alpha 1 isoform in capillaries, or their investing cell processes, may be critical to CNS volume and solute homeostasis.

This research was supported by funds from Veterans Administration, Rehabilitation R&D Service.

372.5

HUMAN NA,K-ATPASE CATALYTIC ALPHA SUBUNIT HAS SOLUBLE DERIVATIVES FOUND IN BODY FLUIDS OF DISEASED PATIENTS. J. H. F. Peng* Univ. of Missouri-Kansas City Sch. of Med., Kansas City, MO 64108.

Na,K-ATPase plays a pivotal role in many important biological functions, and has been a subject of intense investigation. In order to study the possible role of Na,K-ATPase in neurological diseases, such as epilepsy and Alzheimer's disease, polyclonal antisera to the purified human brain holoenzyme, alpha subunit, and 40- & 60-kDa alpha fragments have been prepared. In this study, Western blotting, immunoaffinity purification, and electroelution were used to study the presence of soluble alpha subunit derivatives in the human plasma and cerebrospinal fluid (CSF) employing antiserum to 40 kDa protein. Western blotting reveals that soluble derivatives with molecular weight (mol. wt.) of 59-, 54-, 52-, 50-, and 46- kDa are present in the CSF of eight *Pseudotumor cerebri* and six control patients. Soluble derivatives with mol. wt. of 68-, 66-, 64-, 57-, and 56-kDa are also found in the plasma of thirty-six patients suffering from various illnesses. Antibodies to 40 kDa protein were purified from antiserum by ammonium sulfate precipitation and DEAE-Sepharose chromatography, and used to prepare immunoabsorbant by coupling to CNBr-activated sepharose. Soluble alpha protein derivatives in the plasma were bound tightly to the immunoabsorbants in the presence of 0.5% Triton X-100. Bound proteins were purified by electroelution. Major proteins eluted had mol. wt. of 68-, 64-, 57-, and 56 kDa. Antibodies to 40 kDa could be useful for isolation and quantitation of alpha subunit derivatives. These observations offer the opportunity of using antibodies to alpha protein fragments to probe the role of Na,k-ATPase in various human diseases.

372.6

DIFFERENTIAL AXONAL TRANSPORT OF INDIVIDUAL (Na,K)-ATPase CATALYTIC (α) SUBUNIT ISOFORMS IN RAT SCIATIC NERVE. M. Mata*, C-F. Jin, S. Datta, and D.J. Fink. Dept. of Neurology and GRECC, VAMC, University of Michigan, Ann Arbor, MI 48105.

Three isoforms of the catalytic (α) subunit of the ubiquitous membrane enzyme (Na,K)-ATPase, products of 3 different members of a multigene family, have been identified in the nervous system. We have found that all neurons appear to contain the α2 and α3 isoform mRNAs, and some neurons also contain α1 mRNA. Glial cells contain only α1 and α2 isoform mRNAs. In order to define the dynamics of isoform distribution within the axon, we exploited the phenomenon of axonal transport to study the movement of individual isoforms within the axon.

Two ligatures were placed 1 cm apart along the sciatic nerve of male Sprague Dawley rats, and at 24hrs the amount of isoform-specific peptide in sequential 3 mm segments determined by quantitative Western blot using commercially available polyclonal antibodies directed against the α1, α2 or α3 peptides (UBI).

There was substantial accumulation of immunoreactive α1 peptide immediately proximal to the first ligature, consistent with the rapid axonal transport of that isoform. Neither α2 nor α3 peptides accumulated proximal to the ligature. α3 therefore appears to be relatively stable in the membrane, with only small amounts added incrementally over the 24 hour period. Because α2 is also found in Schwann cells, the relative contribution of glial and axolemmal peptide cannot be determined. There was no evidence for significant retrograde axonal transport of any of the isoform peptides.

Supported by grants from the NIH, VA, and the Zyma foundation.

372.7

SUBCELLULAR DISTRIBUTION OF RAT CEREBELLAR CALRETININ: DISSOCIATION OF CALRETININ BOUND TO SYNAPTIC MEMBRANES BY EGTA. L. Winsky* and J. Kuznicki. Lab. of Clinical Sciences, NIMH Bethesda, MD 20892

Calretinin was quantitated by radioimmunoassay (RIA) in subcellular fractions obtained in the presence of 0.1 mM calcium or 1 mM EGTA from rat cerebellum. Although the majority of the total calretinin was associated with the cytosolic fraction, there was a significant association with several membrane fractions including synaptic membranes, synaptic vesicles and microsomes with least amounts in mitochondria. In addition, cytosol prepared in the presence of EGTA contained more calretinin than when calcium was added to the extraction buffers and there was an apparent increase in calretinin associated with synaptic membranes prepared in the presence of calcium. These results were confirmed by immunoblot which revealed greater intensity of calretinin label in synaptic membrane and synaptic vesicle fractions prepared in the presence of calcium. Overnight incubation of synaptic membranes with EGTA caused a greater release of calretinin from synaptic membranes than when incubated with additional calcium. These results suggest that calcium binding to calretinin could result in membrane association and suggest a direction of future research into the function of this brain enriched calcium binding protein.

372.8

RECOMBINANT CALRETININ BINDS TO HYDROPHOBIC RESINS AND W-7 (CALMODULIN ANTAGONIST) IN A CALCIUM INDEPENDENT MANNER J. Kuznicki, L. Winsky, D.M. Jacobowitz* Laboratory of Clinical Sciences, NIMH, Bethesda, MD 20892.

Calretinin is a neuronal calcium binding protein similar to calbindin D28K. Recombinant calretinin (rCR) binds calcium ions and exhibits calcium-dependent conformational changes. This was shown by differences in binding of rCR to DEAE-cellulose, in proteolytic cleavage, and in ability to aggregate in the presence and absence of calcium. The series of aminoalkyl-agarose resins ($C_{5\text{-}12}$), phenyl-agarose and W7-agarose were used to analyze if hydrophobic region(s) of rCR are exposed by Ca^{2+}-binding. In the presence of calcium and 0.15 M NaCl rCR bound to octyl-, dodecyl- and W7-agarose, but not to phenyl-, pentyl- and hexyl-agarose. The binding of rCR to these resins appeared to be calcium-independent since the majority of the protein was not eluted when the calcium concentration was decreased by EGTA, and the elution could be achieved only using strong agents such as SDS. Moreover, rCR also bound to decyl- and W7-agarose in the absence of calcium. These observations seem to indicate that rCR does not exhibit major changes of hydrophobicity upon calcium binding. It is therefore suggested that any such changes exhibited by native calretinin would be due to possible posttranslation modifications.

373.1

SYNAPTOTAGMIN-DEFICIENT MUTANTS OF *C. elegans*. Michael L. Nonet, Kiely Grundahl, John R. McManus, Barbara J. Meyer and James B. Rand*. Program in Molecular and Cell Biology, Oklahoma Medical Research Foundation, Oklahoma City, OK 73104, and Dept. of Molecular and Cell Biology, University of California, Berkeley, CA 94720.

Synaptotagmin is an abundant synaptic vesicle-associated protein, proposed to be involved in release of neurotransmitters. We have identified the *C. elegans* synaptotagmin structural gene and named it *snt-1* (formerly called *ric-2*). *snt-1* mutants are slow-growing, uncoordinated, small, and impaired in pharyngeal pumping and defecation. Nevertheless, they are active and capable of propagating limited body waves. The mutants are also very resistant to cholinesterase inhibitors and their acetylcholine levels are elevated approximately three-fold. 17 alleles of *snt-1* have been isolated so far; genetic and molecular analyses suggest that many of them are null.

In *C. elegans* neurons, synaptotagmin is localized to regions known to be rich in synapses, and appears to be associated with synaptic vesicles. Preliminary immunohistochemical and hybridization experiments suggest the absence of other synaptotagmin genes in *C. elegans*. We therefore conclude that synaptotagmin is not absolutely required for release of neurotransmitters, although it is clearly necessary for normal synaptic function.

Supported by grants from MDA and OCAST.

373.2

FUNCTIONAL ARCHITECTURE OF SYNAPTOTAGMIN C2 DOMAINS REVEALED BY PEPTIDE BLOCK OF TRANSMITTER RELEASE. W.M. DeBello*, K. Bommert, M.P. Charlton, G.J. Chin, H. Betz, and G.J. Augustine. Dept. Neurobiology, Duke Medical Center, Durham, NC 27710 and MBL, Woods Hole, MA.

Synaptotagmin, an integral membrane protein of synaptic vesicles, possesses 2 copies of a motif known as a *C2 domain*. C2 domains are found in a number of other proteins (including kinase C, phospholipase C, phospholipase A2 and others) and are believed to mediate binding of these proteins to Ca, lipids and to other proteins. The goal of this work was to identify parts of the C2 domains responsible for these functions. To probe for protein-protein interactions, synthetic peptides mimicking different parts of the C2 domains of squid synaptotagmin were microinjected into squid giant presynaptic terminals. Previous work has shown that such peptides can inhibit transmitter release, presumably by competing with the binding of vesicle-associated synaptotagmin to an acceptor protein involved in release (*Nature* 363, in press). C2 domains contain several regions that are conserved in all C2 domain proteins (*Cell* 65, 1043) and an additional region of basic amino acids whose sequence is not conserved among members of the C2 domain protein family. A peptide corresponding to one of the conserved amino acid sequences was ineffective in blocking transmitter release. However, peptides corresponding to the variable basic regions of synaptotagmin were potent blockers of release. These results suggest that the basic regions of C2 domains are responsible for protein-protein interactions, with their variable nature conferring binding specificity upon individual C2 domain proteins. In contrast, the conserved regions of C2 domains may mediate Ca and lipid binding. Supported by NIH grant NS-21624 to GJA, MRC grant to MPC, and DFG grant to HB.

373.3

ON THE STRUCTURE OF THE NEUROSECRETOSOME: EVIDENCE FOR A SYNTAXIN/α-LATROTOXIN RECEPTOR/SYNAPTOTAGMIN/Ca^{2+} CHANNEL COMPLEX. V.M. O'Connor, O. Shamotienko, U. Ernsberger*, M.J. Duggan, A. Siebert, K. Bommert and H. Betz. Max-Planck-Institut für Hirnforschung, 6000 Frankfurt a.M. 71, Germany.

Small synaptic vesicles (ssvs) are clustered at specialized regions of the presynaptic membrane called active zones. The ssv protein synaptotagmin is associated with three distinct presynaptic membrane proteins, the α–latrotoxin (α–Ltx) receptor (Petrenko et al., 1991, Nature 353, 65), syntaxin (Bennet et al., 1992, Science 257, 255) and Ca^{2+} channels (Leveque et al., 1992 Proc. Nat. Acad. Sci. 89, 3625). These interactions are thought to participate in the docking of ssvs at the active zone.

We have been able to co-purify the α–Ltx receptor, synaptotagmin and syntaxin on an α-Ltx affinity column, indicating that these proteins exist within a complex. This was confirmed by the immunoprecipitation of ^{125}I-α-Ltx binding from solubilized brain extracts by syntaxin antibodies. The same antibodies immunoprecipitate ^{125}I-ω-conotoxin binding (Yoshida et al.,1992, J. Biol. Chem. 267, 24925) indicating that Ca^{2+} channels exist within this complex. In favour of this idea α-Ltx receptor antibodies immunoprecipitate ^{125}I-ω-conotoxin binding. Taken together these results indicate an association between syntaxin, α-Ltx receptor, synaptotagmin, and Ca^{2+} channels in a complex we propose to call the neurosecretosome. Syntaxin has been reported to be a plasmamembrane receptor for fusion-competent vesicles that are primed through a mechanism common to all stages of the secretory pathway (Söllner et al., 1992, Nature 362, 318). Thus, the neurosecretosome may represent the specialization of an ubiquitous fusion mechanism to suit the requirements of fast synaptic transmission.

373.4

SYNAPTOPHYSIN EXISTS IN DIFFERENT OLIGOMERIC FORMS. M.J. Duggan, V.M. O'Connor, J. Bormann*, and H. Betz. Max-Planck Institute for Brain Research, 6000 Frankfurt a. M. 71, Germany.

Synaptophysin is the major integral membrane protein found in small synaptic vesicles. It is known to be an oligomeric protein, but the degree of oligomerization is controversial, and has been reported as tetrameric [Rehm, H. et al. (1986) *EMBO J. 5*, 535-541], or hexameric [Thomas, L. et al. (1988) *Science 242*, 1050-1053].

We have attempted to resolve this dispute using a combination of crosslinking and hydrodynamic measurements on a number of different preparations. Freshly solublized synaptophysin from synaptic vesicles has hydrodynamic properties consistent with a trimeric structure. Purified synaptophysin, however, forms a stable structure with the sedimentation velocity of a hexameric structure. Crosslinking experiments indicate that while freshly-solublized synaptophysin appears to be trimeric, synaptophysin in synaptic vesicles can exist in larger complexes at least pentameric in size. All crosslinking of crude preparations shows that a 14 kDa protein is associated with synaptophysin, even after sucrose density sedimentation. These results suggest that synaptophysin is a novel type of integral membrane protein that can exist in multiple oligomeric states.

373.5

CELLUBREVIN: A SUBSTRATE FOR TETANUS TOXIN FOUND ON A CONSTITUTIVE VESICLE RECYCLING PATHWAY IS A HOMOLOGUE OF SYNAPTOBREVIN. H.T. McMahon[1], H. Niemann, R. Jahn*[2], T.C. Südhof[1] [1]Howard Hughes Medical Institute, UT Southwestern Medical Center, Dallas TX 75235; [2]Howard Hughes Medical Institute, Yale University, New Haven CT 06510

We have identified a ubiquitous 16kDa protein found on vesicles undergoing constitutive recycling. This protein has 59% amino acid sequence identity to the mammalian small synaptic vesicle-specific synaptobrevin proteins (also called VAMPs). We have named this protein cellubrevin, because it is presence in all cell. We show that the protein is enriched more than 100-fold in coated vesicles, and by immunofluorescence and organelle immunoprecipitations from cultured CHO cells it co-distributes with the transferrin receptor. Like synaptobrevin II, cellubrevin is proteolysed by tetanus toxin, which inhibits synaptic vesicles exocytosis. By analogy, cellubrevin may function in a fusion complex for constitutive vesicular pathways and so the potential function is now being studied. Our results demonstrate that constitutive and regulated vesicular pathways use homologous proteins for membrane trafficking.

373.6

Staurosporine blocks destaining of frog motor nerve terminals labelled with FM1-43, but does not block transmitter release. A. Henkel and W.J. Betz*. Dept. of Physiology, Univ. of Colorado Med. School, Denver, CO 80262.

The fluorescent dye FM1-43 stains motor nerve terminals in an activity-dependent fashion, apparently by staining membranes of recycled synaptic vesicles. *Destaining* of prestained terminals is also activity-dependent. FM1-43 is thus a useful tool for studying synaptic vesicle trafficking in living nerve terminals. We have investigated the effects of a variety of cytoskeletal drugs on dye movements in frog *cutaneus pectoris* nerve terminals. Drugs that affect the level of protein phosphorylation had the most significant effects. Here we report the effects of **staurosporine**, a kinase inhibitor.

Preparations were first stained with FM1-43 (2 μM in normal frog Ringer; nerve stimulation at 10 Hz for 5 min; wash for 30 min). Next, control intracellular recordings and images were taken, and then staurosporine (2 μM) was added. Further images and recordings were made for 1 hour, after which the nerve was stimulated at 10 Hz for 10 min. This last procedure caused complete destaining of control (no staurosporine) preparations. However, most preparations treated with staurosporine failed to destain, as if stained synaptic vesicles had been immobilized. To our surprise, however, nerve-evoked and spontaneous transmitter release were only modestly reduced. These results suggest that either FM1-43 does not stain synaptic vesicles, or that stained vesicles do not mediate a significant portion of transmitter release.

373.7

ACTIN ASSEMBLY AND EXOCYTOSIS IN CULTURED NEURONS. B.W.Bernstein*, M.DeWit, and J.R.Bamburg. Department of Biochemistry and Molecular Biology and The Program in Neuronal Growth and Development, Colorado State University, Ft. Collins, CO 80523

We have studied the role of actin assembly in neurosecretion from cultured cells and have compared these results to a model based on our earlier synaptosomal studies. Two day cultures of neurons, dissociated from lumbo-sacral sympathetic ganglia of chick embryos and grown in 50µl, paraffin-sealed glass coverslip chambers, show extensive networks of interconnecting processes. Recycled synaptic vesicles in terminals of these processes were stained with a styryl dye (FM1-43; Molecular Probes, Eugene, OR), thus allowing monitoring of exocytosis in real time. The vesicle membranes were labelled through endocytosis that follows exocytosis stimulated by a depolarizing K^+ buffer. Depletion of dye fluorescence, indicating exocytosis, was stimulated electrically. Fixed cells were stained with rhodamine phalloidin, which binds specifically to the filamentous form of actin. Confocal fluorescence microscopy of rhodamine phalloidin stain in exocytotically active areas indicated that actin filaments undergo reversible disassembly during 2 min depolarization. Moreover, if filaments are stabilized by loading cells with phalloidin before stimulating secretion, the rate and extent of exocytosis is reduced. **These data are consistent with our model based on synaptosomal studies and have been confirmed ultrastructurally:** actin filament disassembly is necessary for maximal transmitter release, and reassembly is necessary for modulating release during sustained depolarization. GM35126, NS28338, and NS28323 from the NIH.

373.8

EXOCYTOSIS MONITORED BY CAPACITANCE AND AMPEROMETRY. Ch. Heinemann[1], R.H. Chow[1], R.S. Zucker*[2], and E. Neher[1]. [1]Membrane Biophysics Div., MPI for Biophysical Chemistry, Am Faßberg, D-3400 Göttingen, Germany. [2]Molec. & Cell Biol. Dept., Univ. of Calif., Berkeley, CA. 94720.

Catecholamine release from single vesicles of adrenal chromaffin cells was detected by carbon fiber electrodes at the same time that membrane capacitance (C_m) was monitored. When calcium (Ca^{2+}) was elevated by flashing "caged" Ca^{2+} to give >50 µM free Ca^{2+}, C_m rose immediately with multiple exponential components, the fastest with a time constant of 20-40 ms and a rate of about 40,000 fF/s. The rate increased monotonically with increasing Ca^{2+}, with a power dependency greater than one, and it had reached a plateau for concentrations > 50 µM. For cases in which C_m rose with a time constant faster than 30 ms, the carbon-fiber signals occurred with delays that were statistically longer than expected based on C_m; however, analysis was complicated by a flash-induced carbon-fiber artifact and by the occurrence of multiple, overlapping events. When step depolarizations were used to activate secretion, many carbon-fiber signals occurred even after the rise in C_m had ceased. One possible explanation for discrepancy in the time courses of C_m and amperometry is that the early fusion pore connecting the vesicle interior and the cell exterior is sufficiently large to allow immediate detection of a vesicle fusion as a step in C_m, but too small for the release of catecholamine until the pore dilates to a certain threshold radius -- that is, the time of fusion must be distinguished from the time of release. Supported by NIH and Deutsche Forschungsgemeinschaft.

373.9

SURFACE TOPOGRAPHY AND LOCALIZATION OF CALCIUM CHANNELS AT A CHOLINERGIC PRESYNAPTIC NERVE TERMINAL RELEASE FACE BY ATOMIC FORCE SCANNING MICROSCOPY. P.G. Haydon,+ E.R. Henderson+ R.T. Doyle* and E.F. Stanley*. +Signal Transduction Training Group ISU; *Section on Synaptic Mechanisms NINDS NIH.

There is considerable evidence that transmitter release sites are composed of specific structures that include calcium channels but this membrane surface can not be visualized directly. We have applied Atomic Force Scanning Microscopy to the release face of the chick ciliary ganglion calyx-type nerve terminal to view the surface topography of the release area and to search for membrane-associated particles.

Ciliary ganglia were acutely dissociated and calyces were identified by specific dye staining (J. Neurosci.11:985; Neuron 7:585) and were fixed for scanning. The presynaptic areas generally had very smooth surfaces but with small mounds that, by comparison with transmission EM, we tentatively identified as release sites. Calcium channels in this nerve terminal are blocked by ω-conotoxin GIVA (ω-CTX). We treated the terminals with biotin-ω-CTX followed by streptavidin-30 nm gold. At high resolution scanning, clusters of particles were apparent that presumably reflect the location of the presynaptic calcium channels. Our results suggest that this technique allows a detailed examination of the nerve terminal transmitter release face topology.

373.10

STRATEGIC LOCATION OF Ca^{2+}-GATED K^+ CHANNELS AT RELEASE SITES OF THE FROG NEUROMUSCULAR JUNCTION.

R Robitaille*, ML Garcia, GJ Kaczorowski and MP Charlton. Dept. Physiol., Univ. of Toronto, Canada and Dept of Mem. Biochem. and Biophys., Merck Inst. for Therapeutic Res., New Jersey, USA.

We examined the distribution of Ca^{2+}-gated K^+ channels (gKca) relative to the location of Ca^{2+} channels and transmitter release sites at the frog neuromuscular junction. Charybdotoxin (ChTX), a blocker of gKca channels, increased transmitter release by itself (J Neurosci 12, 297-305, 1992) and when applied after nerve terminals were loaded with a slow Ca^{2+} buffer (EGTA-AM) but not after loading with fast buffers (DMBAPTA-AM, BAPTA-AM). The differential effect of the buffers indicates that the gKca channels must be located close to the Ca^{2+} channels.

Similar to labeling of Ca^{2+} channels with ω-CgTX GVIA (Neuron 5, 773-779, 1990), labeling of gKca channels with ChTX-biotin revealed a series of bands at an interval of 1 µm matching those formed by the cholinergic receptors labeled with fluorescent α-BuTX. ChTX labeling is not blocked by ω-CgTX but is blocked by several treatments known to reduce ChTX affinity and is absent after denervation. We conclude that gKca and Ca^{2+} channels are colocalized at transmitter release sites and that the 10 nm particles at the active zone may include both Ca^{2+} channels and gKca channels. This organization allows a quick and functional narrowing of the AP which helps terminate transmitter release by arresting Ca^{2+} entry.

373.11

A CATION CHANNEL ACTIVATED BY EXTRACELLULAR APPLICATION OF ADENOSINE 5' TRIPHOSPHATE (ATP) ON A PRESYNAPTIC NERVE TERMINAL.

X.P. Sun and E.F. Stanley*. *Section on Synaptic Mechanisms, NINDS, NIH, Bethesda MD 20892.*

Several studies have suggested that ATP may act as a neurotransmitter substance via a purinergic receptor. We have used the patch clamp technique to examine the possibility that such receptors are present on a cholinergic presynaptic nerve terminal (*See J. Neurosci 11:985*).

Application of ATP (0.1-1 mM) to the presynaptic calyx in the whole-cell patch clamp configuration induced a fast-activating and slow-inactivating inward current (6/18) with a reversal potential close to 0 mV. A single channel with a conductance of 50-100 pS was recorded in the cell-attached mode in the presence of 0.1 mM ATP.

Thus, the presynaptic nerve terminal in this cholinergic synapse contains an ATP-sensitive cation channel. Since ATP and ACh are both stored in secretory vesicles and are presumably co-released, ATP may be involved in the feed-back modulation of nerve terminal function.

373.12

RAPID, TRIGGERED ENDOCYTOSIS IN A NEUROENDOCRINE CELL. W. Almers*, J.G. Wong & P. Thomas. Max-Planck-Inst. f. Medizin. Forschung, 6900 Heidelberg, Germany

Single rat melanotrophs were loaded through a patch pipette with the photolabile chelator Ca-DM-nitrophen (Ca-DMn), plus fura-2 or furaptra. We transiently raised cytosolic [Ca], Ca(i), by flash photolysis of Ca-DMn, and tracked the ensuing exo- /endocytic changes in cell surface area via the membrane capacitance, C. Rapidly repeating flashes caused exhaustive exocyt-osis and raised C by 1-2 pF. Thereafter C slowly declined with a time constant (τ > 150 s) that was independent of Ca(i) (0.2-80 µM) and typical of the slow, constitutive membrane retrieval seen with other methods. But after single flashes, C increased and then declined so rapidly that up to 10% of the cell surface were retrieved in 1 s. This was most obvious after cytosolic acidification (pH=6.2) had abolished all but the "exocytic burst", the fastest component of exocytosis in melanotrophs. Endocytosis then lowered C well below the preflash level (excess endoctyosis, EE). The effect remained in cells depleted of Mg and ATP. EE was seen only after the first flash; later, the C changes caused by exo- and exocytosis were equal. Probably, EE retrieved membrane that had reached the cell surface during a previous exocytic episode. EE shows that membrane marked for rapid endoccytosis can accumulate and wait until a new flash triggers its retrieval. The rate of endocytosis grows steeply with the amplitude of the exocytic burst but is independent of Ca(i). Hence the trigger is not Ca but a substance appearing in the course of exocytosis. The resistance to pH 6.2 and independence of MgATP indicates that the rapid endocytosis seen here is not due to clathrin-coated pits. Its speed suggests a novel mechanism of membrane retrieval.

373.13

DEPHOSPHIN: A POTENTIAL REGULATOR OF VESICLE DYNAMICS. E.M. Fykse[1], J.M. Sontag[1], J.P. Liu[2], P.J. Robinson[2], and T.C. Südhof*[1]. [1]Department of Molecular Genetics and Howard Hughes Medical Institute, University of Texas, Southwestern Medical Center, Dallas, Tx, 75235. [2]Endocrine Unit, John Hunter Hospital, Locked Bag 1, Hunter Region Mail Centre, Newcastle, NSW, 2310, Australia.

We are studying the potential role of phosphorylation in synaptic vesicle (SV) exocytosis and recycling. Synaptotagmin and P96 (Dephosphin) are both phosphoprotein with potential key roles in these processes. Dephosphin is located in the nerve terminal. It is rapidly and quantitatively dephosphorylated during depolarization induced calcium influx. Dephosphin is a major substrate for protein kinase C (PKC), and phosphorylation of purified dephosphin with purified PKC showed that one or two sites were phosphorylated. Dephosphin consists of a protein doublet of 94 and 96 kDa. Molecular cDNA cloning and immunoprecipitation showed that this heterogeneity is caused by alternative splicing. A peptide antibody raised against the C-terminal of the short form recognized only the 94 kDa band in brain.

373.14

TRANSPORT OF SYNAPSIN I IN AXONS OF THE LAMPREY SPINAL CORD. T.H. Müller*, V.A. Pieribone, A.J. Czernik, L. Brodin[1], S. Grillner[1], P. Greengard, Laboratory of Molecular and Cellular Neuroscience, Rockefeller University, New York, NY 10021. [1]Nobel Institute of Neurophysiology, Karolinska Inst., Stockholm, Sweden.

The neuronal phosphoprotein synapsin I appears to modulate synaptic efficiency by controlling the fraction of synaptic vesicles available for neurotransmitter release (Greengard et al., Science 259:780, 1993). We have studied the mobility of fluorescently labeled synapsin I in axons of the lamprey spinal cord.

The spinal cord was removed and placed in a recording chamber cooled to 7°C. Dephosphorylated bovine synapsin I was labeled with Texas Red and injected into axons by ionophoresis or pressure. Dye epifluorescence was monitored with a low-light camera system. During continuous injection, synapsin I spread more rapidly in the anterograde than in the retrograde direction. In contrast, both rhodamine-labeled HRP and Texas Red-labeled avidin diffused similarly in both directions. The average anterograde transport velocity for synapsin I, determined from bolus injections, was 0.16 μm/s (14 mm/24h). Adjusting for temperature, using a Q_{10} of 2-3 reported for neuronal transport, yields values of ~100-400 mm/24h at 37°C. These rates correspond to those observed for the fast component of axonal transport in mammalian neurons, and suggests that at least part of the injected synapsin I is transported by this component.

373.15

SYNAPSIN IN MÜLLER AXONS IN THE LAMPREY SPINAL CORD. V. A. Pieribone*, T. H. Müller, L. Brodin#, A. J. Czernik, O. Shupliakov#, E. Schaeffer, S. Grillner#, P. Greengard, The Rockefeller University, New York, NY 10021 and #Karolinska Institutet, Stockholm, Sweden.

Terminal injection of dephosphorylated but not phosphorylated synapsin I into the squid giant synapse reduces the size of the PSP evoked by presynaptic stimulation (Llinás et. al., *J. Physiol. 436:257-82 , 1991*). We have now used immunocytochemical, biochemical and physiological methods to examine the role of synapsin-like proteins in lamprey synapses. The Müller axon/spinal neuron synapse in the lamprey spinal cord is a well characterized synapse with physiological and pharmacological properties similar to those found in mammals and as such is suitable for paired-recording studies.

In tissue sections, antibodies directed against mammalian synapsins strongly cross-reacted with terminal-like structures specifically in the lamprey CNS and not in peripheral organs. Injections of fluorescent-labeled mammalian synapsins were found to be anterogradely transported in axons (see Müller et. al., this volume). Initial results indicate that injection of mammalian dephosphosynapsin I into presynaptic axons results in a specific suppression of the chemical but not the electrical component of the evoked EPSP. These results support the hypothesis that synapsin-like protein(s) are present in the CNS of a cyclostome, and may function in synaptic transmission.

373.16

NICOTINE-INDUCED SYNAPSIN I PHOSPHORYLATION AND ENDOGENOUS ACETYLCHOLINE RELEASE IN CHOLINERGIC NERVE ENDINGS. Sean O'Shea and Enrique L.M. Ochoa * Dept. of Pediatrics, University of California at Davis, Davis CA 95616.

Synapsin II phosphorylation is correlated with the nicotine-induced release of catecholamines from chromaffin cells (Haycock et al., J. Neurosci. **8**:3233, 1988). The present experiments were conducted to test whether nicotine effects both synapsin I phosphorylation and acetylcholine (ACh) release in CNS cholinergic nerve endings. Rat frontal cortex synaptosomes were pre-incubated with $^{32}PO^4$ and SDS-PAGE was performed. Endogenous phosphorylation of two protein bands of 80 and 85 kDa, identified as synapsin I, were demonstrated by autoradiography and quantitated either by densitometry or phosphorimaging. Nicotine-induced endogenous, Ca^{2+}-dependent ACh release was monitored by a continuous fluorometric technique. Synapsin I was rapidly and transiently phosphorylated by (-) nicotine with a maximum at 1 minute. The effect was blocked by pre-incubation with 10 μM mecamylamine but not 10 μM atropine. The increase in phosphorylation was not evident at concentrations lower than 10 μM (-) nicotine, but the EC_{50}-nicotine for ACh release was in the μM range. Pre-incubation of the synaptosomes with 35 μM veratridine and in the presence of EGTA, abolished Ach release or synapsin I phosphorylation. In contrast, the (-) nicotine-induced phosphorylation also occurred in the presence of EGTA. Alpha-bungarotoxin (0.1 μM) blocked neither (-) nicotine-induced ACh release nor phosphorylation, whereas 0.1 μM κ-bungarotoxin blocked ACh release but had inconsistent blocking effects on phosphorylation. We conclude that (-) nicotine requires external Ca^{2+} to induce ACh release, but Ca^{2+} is not essential for synapsin I-phosphorylation. (-) Nicotine may induce synapsin I phosphorylation through a nicotinic neuronal receptor present in rat frontal cortex nerve terminals. *Supported by the Cigarette and Tobacco Surtax Fund of the State of California through the Tobacco-Related Disease Research Program of the University of California, Grant 3RT-0098.*

373.17

IDENTIFICATION AND CHARACTERIZATION OF THE SMALL GTP-BINDING PROTEIN RAB3 IN HELISOMA. R.T. Doyle*, Y. Fang, S. Durgerian, D. Larson, and P.G. Haydon. Department of Zoology and Genetics, Iowa State University, Ames, IA 50011

The small GTP-binding protein rab3A is implicated in regulating vesicular transport in the synapse. To experimentally test for a role for this protein in the synapse we have previously microinjected into Helisoma synapses peptides with sequences corresponding to the rat effector domain of rab3A (Richmond and Haydon, 1992, Soc. Neurosci. Abst). This demonstrated that rab peptide injection does selectively perturb the secretory pathway. As a next step in this study we have now identified the presence of an endogenous rab3 protein in Helisoma neurons using immunocytochemistry and molecular cloning strategies.

An antibody (Cl 42.2 ; provided by R.Jahn) raised against rat rab3A was found to cross-react with Helisoma cultured neurons. Rab3A immunoreactivity was localized within neurites with intense staining in varicosities, the site of synaptic connections.

Using degenerate oligonucleotides corresponding to conserved regions of rab3 we cloned a 300 base pair fragment of rab3 by PCR. Helisoma rab3-specific primers from this sequence were used for PCR sequencing and cloning the remainder of the rab3 coding region from a Helisoma central ganglia cDNA library. The Helisoma rab3 open reading frame is 660 base pairs, coding for a protein of 220 amino acids. Helisoma rab3 is 85% identical to Drosophila rab3 , 77% to rat rab3 and 76% human rab3A, and contains characteristic rab domains such as the GTP binding motifs and C terminal prenylation site.

373.18

STIMULATION OF PROTEIN KINASE C INCREASES INHIBITORY SYNAPTIC TRANSMISSION IN THE RAT HIPPOCAMPUS. M. Capogna, M. Scanziani*, B. H. Gähwiler and S. M. Thompson

Brain Research Institute, Univ. of Zurich, 8029 Zurich Switzerland

Activation of protein kinase C (PKC) facilitates excitatory neurotransmitter release, affects LTP, and modulates ionic channels. The influence of PKC on inhibitory synaptic transmission is less well known. We have addressed this question on evoked and spontaneous $GABA_A$ receptor-mediated synaptic transmission in area CA3 of hippocampal slice cultures. Stimulation of PKC with phorbol ester PDBu (0.5μM) enhanced the amplitude and duration of evoked monosynaptic IPSPs (in CNQX and AP5) and the frequency of spontaneous miniature IPSCs (in CNQX, AP5 and TTX). PDBu did not change the distribution of miniature IPSC amplitudes, suggesting a purely presynaptic mechanism of action. The increase of miniature IPSC frequency by PDBu was antagonized with staurosporin (1μM), a protein kinase inhibitor. Preliminary data indicate that PDBu did not enhance GABA release from presynaptic endings by recruiting axon terminal Ca^{2+} channels: PDBu caused an equivalent increase in mIPSC frequency in the presence of the Ca^{2+} channel blocker Cd^{2+}.

373.19

DEPHOSPHORYLATION OF THE SYNAPTIC PHOSPHOPROTEIN DEPHOSPHIN IS MEDIATED BY CALCINEURIN. R.A. Nichols*, J.M. Brown and G.R. Suplick Department of Pharmacology, Medical College of Pennsylvania, Philadelphia, PA 19129.

Changes in the state of phosphorylation of a number of presynaptic proteins occur during stimulation of brain nerve terminals, and these changes may serve to modulate nerve terminal function. Dephosphin (P96) is one prominent phosphoprotein present in brain nerve terminals, which undergoes stimulation-induced, Ca^{2+}-dependent dephosphorylation. As yet, the protein phophatase mediating dephosphin phosphorylation has not been clearly demonstrated. We have used a pharmacological approach to help identify the dephosphin phosphatase. Synaptosomes were isolated from rat cerebral cortices, labeled with [^{32}P]-inorganic phosphate, and then treated with various selective phosphatase inhibitors. The effect of the presence of inhibitors on subsequent stimulation-induced dephosphorylation of dephosphin was determined by resolving ^{32}P-labeled synaptosomal proteins in SDS gel electrophoresis followed by autoradiography. Treatment with either cyclosporin A (CsA) or L-683,590 (FK-520, a derivative of FK-560), both highly selective inhibitors of the Ca^{2+}/calmodulin-dependent protein phosphatase calcineurin, prior to elevated K^+-depolarization of ^{32}P-labeled synaptosomes attenuated the Ca^{2+}-dependent decrease in ^{32}P-labeled dephosphin. CsA (1 μM) inhibited the K^+-induced decrease in ^{32}P-labeled dephosphin by an average of 76% of control, whereas FK-520 appeared to be less effective. The attenuating effects of CsA and FK-520 were dose- and time-dependent. Similar results were obtained when the calcium ionophore ionomycin was used in place of K^+-depolarization to induce Ca^{2+} entry. Moreover, CsA and FK-520 were without effect on elevated K^+-induced increases in intrasynaptosomal Ca^{2+}, as measured using fura-2. These results implicate calcineurin as the mediator of dephosphin dephosphorylation during stimulated Ca^{2+} entry into brain nerve terminals. (Supported by NS30577)

373.20

RYANODINE DECREASED THE AMPLITUDE AND INCREASED THE RATE OF DECAY OF NERVE-EVOKED CA^{2+} TRANSIENTS AT THE PRESYNAPTIC TERMINALS OF BULLFROG SYMPATHETIC GANGLIA. Y.-y. Peng* Dept. of Pharmacol. & Physiol. Sci., Univ. of Chicago, Chicago, IL. 60637.

In bullfrog sympathetic ganglia, morphological studies have shown that the peptide containing large dense-cored vesicles (LDCV) tend to be away from the plasma membrane (Taxi, 1967; Belhumeur & Tremblay, 1986). This localization of the LDCV's might make peptide release from these vesicles sensitive to the Ca^{2+}-induced Ca^{2+} release (CICR) and Ca^{2+} uptake processes of the intracellular organelles. This hypothesis was tested in intact isolated bullfrog sympathetic ganglia using fura-2 fluorimetry. Presynaptic terminals were selectively filled with fura-2 in its membrane impermeant form (Peng & Zucker, Neuron, 10: 465-473). Ca^{2+} elevation at the presynaptic terminals was evoked by electrical shocks to the preganglionic fibers at 20 Hz, a frequency found to be optimal for evoking peptide release in these terminals (Peng & Horn, J. Neurosci. 11: 85-95). Ryanodine (1 μM) caused up to 50% decrease in the peak amplitude of Ca^{2+} elevation. It increased the rate of the decay of this elevation as well. These findings are consistent with the hypothesis that ryanodine sensitive CICR occurs and is involved in regulating peptide release at these intact presynaptic terminals. Supported by The Louis Block Fund for Basic Research and Advanced Study.

LONG-TERM POTENTIATION III

374.1

THE ROLE OF N- AND P-TYPE CALCIUM CHANNELS IN MOSSY FIBER (MF) SYNAPTIC TRANSMISSION IN THE HIPPOCAMPUS. P.E. Castillo, M.G. Weisskopf and R.A. Nicoll* Depts. of Pharmacol. and Physiol., UCSF, San Francisco, CA 94143-0450.

The MF synapses in the hippocampus exhibit an NMDA-receptor independent form of long-term potentiation. We have presented evidence that both the induction and expression of this form of LTP may be entirely presynaptic (Zalutsky and Nicoll, Science 248:1619, 1990). We carried out two sets of experiments using extracellular field potential recordings in hippocampal slices from guinea pigs to characterize further this form of LTP. First we compared the effect of completely blocking (even during the tetanus) synaptic transmission, either presynaptically (0 mM Ca^{2+}/6 mM Mg^{2+}) or postsynaptically (10 mM kynurenate) on LTP induction. Only the former manipulation blocked LTP indicating that Ca^{2+} entry into the presynaptic terminal is essential for MF LTP. In the second set of experiments we examined the effects of Ca^{2+} channel blockers on MF synaptic transmission and LTP. Nifedipine (30 μM) (L-type) had little effect on baseline MF responses or on LTP. ω-CgTx (1 μM) (N-type) reduced baseline MF responses to ~30% and LTP could still be evoked. ω-AgaTx (1 μM) (P-type) caused a nearly complete blockade of baseline MF responses but LTP-inducing tetani could still enhance transmission in some preparations. Subsequent ω-CgTx application blocked all remaining response.

These results suggest that MF LTP induction requires presynaptic Ca^{2+} entry. The induction and expression of LTP can occur, at least to some extent, after blockade of either N-type or P-type Ca^{2+} channels. Transmitter release involves both N- and P-type Ca^{2+} channels, although the P-type appears to play the primary role in glutamate release at MF synapses.

374.2

A POSTSYNAPTIC- AND NMDA RECEPTOR-RELATED SHORT-TERM POTENTIATION IN RABBIT DENTATE GYRUS X. Xie*[a], T.W. Berger[a] and G. Barrionuevo[b]. [a]*Dept. of Biomedical Engineering and Program in Neuroscience, Univ. of Southern California, Los Angeles, CA 90089, and* [b]*Depts. of Behavioral Neuroscience & Psychiatry, Univ. of Pittsburgh, Pittsburgh, PA 15260.*

In the hippocampus, tetanic stimulation of presynaptic axons could induce four forms of STP[1] which correspond in decay time constants to the STPs earlier found in neuromuscular junction[2] including facilitation I and II, augmentation and potentiation. These forms of STP have been proved to be presynaptic in origin. More recently, a new type of STP was found in the hippocampal CA1 and dentate gyrus[3,4]. In our experiments, rabbit hippocampal slice was perfused with medium including 0.1 mM Mg^{2+} plus 3μM alphaxalone, a $GABA_A$ receptor allosteric agonist. Immediately after high frequency stimulation (50 Hz, HFS) delivered through the same stimulating electrode placed on perforant path axons, the EPSP amplitudes intracellularly recorded from granule cell increased by 73 ± 6% (SEM) and decayed to a stable level with time constant at about 3.66 min (n=4). While one HFS would result in LTP to its saturated level; the same HFS could induce the STP repetitively. The STP could be blocked by D-APV and hyperpolarization of post-synaptic neuron indicating that both forms of use-dependent synaptic plasticity share the same induction mechanism. Preliminary data showed that the paired-pulse facilitation which has been considered as pre-synaptic in origin was reduced during the STP indicating that changes in presynaptic release is probably involved in its expression. Supported by AFOSR, NS01196, NS24288, , MH45156, NH00343 AND MRC GRANT (AFOSR-91-044).

1. Racine,R.J. and MilGram,N.W. Brain Res. 260:201-216; 2. Magleby,K.L. in *Synaptic function* (eds Edelman,G.M. et al) 21-56 (John Wiley & Sons Inc, NY 1987); 3. Malenka,R.C. et al. Neuron 9:121-128,1992; 4. Gustuffson,B. et al. Eur.J.Neurosci. 1:382-389,1989

374.3

A NECESSARY ROLE FOR POSTSYNAPTIC FACTORS IN EXPRESSION OF LONG-TERM POTENTIATION. **K. Shahi* and M. Baudry, Neuroscience Program, USC, Los Angeles, CA 90089-2520.**

One of the arguments supporting a postsynaptic mechanism for the maintenance phase of long-term potentiation (LTP) has been the absence of changes in paired-pulse facilitation (ppf) at potentiated synapses. This argument has been confounded due to the lack of knowledge regarding the exact mechanism underlying ppf. To examine the relationship between LTP and ppf independently of the mechanism responsible for ppf we evaluated the effects of the competitive antagonist of the AMPA receptor, DNQX, on control, facilitated and potentiated synaptic responses in field CA1 of hippocampal slices. DNQX differentially affected the responses to the first and second pulse delivered 50 msec apart to a single pathway. The slope and amplitude of the population excitatory postsynaptic potential (epsp) from the first pulse were decreased more than those from the second pulse in the presence of a submaximal concentration of DNQX. However, when the second pulse was delivered to an independent pathway that had previously been potentiated, the two evoked epsps were equally sensitive to DNQX. These results indicate that ppf is due, at least partly, to an increase in the concentration of glutamate in the synaptic cleft. As this effect is not observed in potentiated synapses, the results indicate that postsynaptic modifications are more likely to underly LTP maintenance. (Supported by grant BNS 9110377 from NSF).

374.4

HIPPOCAMPAL SYNAPTIC PLASTICITY AND MODULATION OF AMPA RECEPTORS IN MICE RESISTANT (C57BL/6J) AND SENSITIVE (DBA/2J) TO AUDIOGENIC SEIZURE. M.F. Tourigny, R. Ward and G. Massicotte*. Département de Chimie-Biologie, Université du Québec à Trois-Rivières, C.P.500, Québec, Canada, G9A 5H7.

Histological and biochemical abnormalities of the hippocampus have been described in several species with epileptic disorders. In this study, we compared formation of hippocampal long-term potentiation (LTP) and modulation of AMPA receptors by phospholipase A_2 (PLA_2) in two inbred strains of mice that differ in their ability to exhibit audiogenic seizure on first exposure to sound. The magnitude of both short term potentiation (STP) and LTP induced by a theta burst stimulation (TBS) paradigm was substantielly enhanced in area CA1 of hippocampal slices from mice sensitive DBA/2J to audiogenic seizure when compared to the resistant C57BL/6J strain. However the initial stage (20 sec) of the STP that follow TBS was not affected in DBA/2J mice suggesting that similar degree of NMDA receptor activation is present in both inbred strains. PLA_2 treatment of tissue sections has been reported to modify properties of AMPA receptors by increasing ^{3}H-AMPA binding and we proposed that PLA_2-induced changes of AMPA receptors is a necessary step in LTP formation (Massicotte and Baudry, Neurosci. Biobehav., Rev. 15: 415, 1991). Exogenous PLA_2 (0,5 μg/ml) and calcium (4.0 mM) were applied at physiological temperature to thin (15 μm), thaw-mounted sections of frozen mice brains and binding properties of AMPA receptors was determined in 10 brain structures by quantitative autoradiography of ^{3}H-AMPA. The quantitative analysis reveals that PLA_2-induced increase in ^{3}H-AMPA binding was significantly enhanced in areas CA3 and CA1 of hippocampus and molecular layer of cerebellum in the sensitive DBA/2J strain. The present results support the idea that PLA_2-induced modification of AMPA receptors is an important component of synaptic plasticity. This work was supported by NSERC Canada.

374.5

SUSTAINED INCREASE IN BASAL AND STIMULUS-DEPENDENT GLUTAMATE EFFLUX DURING LTP IN DENTATE GYRUS: REAL-TIME *IN VIVO* MEASUREMENTS USING A DIALYSIS ELECTRODE. P.T. Galleyt M.L. Errington and T.V.P. Bliss*, †Department of Metabolic Medicine, St Mary's Hospital Medical School, London W2 1PG, UK, and Division of Neurophysiology and Neuropharmacology, National Institute for Medical Research, Mill Hill, London NW7 1AA, UK.

We have used a novel glutamate-sensitive electrode (Albery, Boutelle and Galley, J.Chem. Soc. Chem.Comm., 12, 900-901,1992) to study the release of glutamate during LTP in the dentate gyrus of the anaesthetized rat. The dialysis electrode combines microdialysis with *in vivo* voltammetry and centres around a platinum electrode positioned inside a hollow semi-permeable fibre, allowing the micro-environment around the electrode to be controlled. The removal of interference from electroactive species such as ascorbate and dopamine can be achieved by electropolymerising a coating of polyphenylene diamine onto the platinum and perfusing the enzyme ascorbate oxidase into the probe. Addition of the enzyme glutamate oxidase allows measurement of endogenous extracellular glutamate. In the presence of glutamate the enzyme generates hydrogen peroxide, which is oxidised on the electrode at +650mV, and the resulting current can be measured with a custom designed potentiostat.

To study glutamate release during LTP in the dentate gyrus, stainless steel recording electrodes were attached to the dialysis electrode, and the electrode assembly positioned in the molecular layer of the dorsal dentate gyrus. An increase in glutamate-generated current was routinely obtained when the rate of stimulation of the perforant path was increased to 2Hz from the test frequency of 0.033Hz. Following the induction of LTP by tetanic stimulation there was a sustained increase in basal signal and an approximately two-fold increase in the peak of the stimulus-dependent 2Hz signal measured 60 min after induction. Both LTP and the increase in basal and stimulus-dependent glutamate release were blocked by the intraventricular injection of D-AP5.

374.6

INTERACTION BETWEEN ANIRACETAM AND LTP IN "DISINHIBITED" SLICES. A. Kolta', J. Ambros-Ingerson, J. Larson and G. Lynch. Center for neurobiology of learning and memory, Univ. of California Irvine, Irvine, CA, 92717.

The drug aniracetam, which slows the desensitization of AMPA receptors is reported to have different effects on the waveform of synaptic responses following induction of LTP (Staubli et al., Hippocampus, 1992), a result which suggests that LTP itself modifies the kinetics of the receptor channel. If so, then the interaction between aniracetam and LTP should be still more evident under conditions in which AMPA receptors mediated currents are isolated from other currents normally present in hippocampal responses to afferent stimulation. The present experiments tested this point in mini-CA1 slices where inhibition was blocked with 50 μM picrotoxin and 100 μM of 2-OH-saclofen. Tetrodotoxin (10 μM) was locally injected near the cell body layer to prevent recurrent spiking. Aniracetam (1.5 mM) was perfused before and after the induction of LTP with theta burst stimulation. In several experiments, aniracetam was perfused twice before the induction of LTP. Aniracetam typically produced 3 clearly defined effects on control waveforms recorded under these conditions; namely, an increase in amplitude, rise time and decay time constant (DTC). However, aniracetam effects on waveform's amplitude and rise time were significantly smaller after LTP induction, whereas the increase in DTCs were significantly larger. The effects of aniracetam on control responses were similar after a second perfusion arguing against the possibility that several exposures may alter the receptor responsiveness to the drug. These results support the hypothesis that LTP alters the kinetics of the AMPA receptor.

This work was supported by a NSERC fellowship for A. K. and an AFOSR grant.

374.7

NMDA RECEPTOR-MEDIATED PRESYNAPTIC INHIBITION IN THE CA1 REGION OF THE HIPPOCAMPUS. O. Manzoni*, T. Manabe and R. A. Nicoll Depts. Pharmacol. and Physiol., UCSF, San Francisco, CA 94143-0450.

Previous studies have found that application of NMDA transiently depressed synaptic transmission in the CA1 region of the hippocampus (Collingridge et al. J. Physiol. 334:33-46, 1983; Kauer et al. Nature 334:250-252, 1988; Manabe et al. Nature 355:50-55, 1992). We have used both whole-cell and field recordings to investigate in more detail the mechanism of this depression.

Application of NMDA (10-50 μM) depressed EPSP(C)s with a time course that can outlast the direct postsynaptic actions. This depression was due, at least in part, to a presynaptic inhibition, since paired-pulse facilitation (PPF) was increased during the depression.

Local high intensity tetanic stimulation caused heterosynaptic depression lasting from a few to 15 min (cf. Bashir and Collingridge. Eur. J. Neurosci. 4:485-490, 1992). This depression was blocked or significantly reduced by 50 μM D-APV, was also associated with an increase in PPF and was not blocked by the $GABA_B$ antagonist CGP 35348 (500μM).

These findings indicate that activation of NMDA receptors in the slice can result in a presynaptic inhibition of EPSP(C)s. Presumably, either glutamate can directly activate presynaptic NMDA receptors on nearby excitatory synaptic terminals and decrease transmitter release, or some diffusible factor and/or change in the extracellular ionic environment can mediate this depression. The precise mechanism is currently under investigation.

374.8

THE ROLE OF NMDA AND NON-NMDA RECEPTORS IN SYNAPTIC TRANSMISSION AND LONG-TERM POTENTIATION IN RAT MOTOR CORTEX. **L.Yi, W.T. Greenough and R. Swain*. Neurosci. Prog., Beckman Institute, Univ. of Illinois, Urbana, IL 61801.**

To determine whether N-Methyl-D-aspartate (NMDA) and non-NMDA receptors mediate synaptic responses in rat motor cortex, we tested the effects of D-2-amino-5-phosphonovalerate (APV) and 6,7-dinitro-quinoxaline-2,3-dione (DNQX) on evoked field potentials and induction of long-term potentiation (LTP). In extracellular recording, the field potential elicited in layer II/III of motor cortex in response to stimulation of somatosensory cortex consisted of an EPSP, an apparent population spike (PS) and a second EPSP. Iontophoretic application of APV sightly decreased the amplitude of the first EPSP and PS, whereas the second EPSP was completely depressed. In the presence of DNQX, the amplitude of the first EPSP and PS were greatly reduced, suggesting that the first EPSP and PS include both NMDA receptor-mediated and non-NMDA receptor-mediated components. Tetanic stimulation of sensory cortex in control medium (n=12) induced both LTP of the first EPSP and PS (8 cases), and LTD (3 cases). Tetanic stimulation in the presence of APV (n=12) also induced both LTP of EPSP and PS (4 cases), and LTD (6 cases). It appears that LTD is more likely to occur in the presence of NMDA receptor blockage. LTP of the first EPSP and PS (2 cases), LTD (2 cases), and no significant change (7 cases) were observed in the presence of DNQX (n=11). These data indicate that non-NMDA receptor-mediated component also plays an important role in the occurrence of LTP in motor cortex. In addition, these findings provide evidence for the induction of both LTP and LTD of NMDA receptor-mediated synaptic transmission in motor cortex and demonstrate that the blockade of NMDA receptors favors the occurrence of LTD. Supported by MH35321 and NSF BNS 88 21219.

374.9

CHANGES IN POSTSYNAPTIC AMPA SENSITIVITY DURING LONG-TERM POTENTIATION

Nikolai B. Fedorov, Klaus G. Reymann*

P.O.B. 1810, Federal Institute for Neurobiology, Magdeburg, Germany

The maintenance of LTP in the CA1 region of the hippocampus might involve a slow increase of the postsynaptic sensitivity of AMPA/glutamate receptors. The question is: are these changes mediated by a population of extrasynaptic or synaptic receptors? To answer this question we employed the recently developed one-electrode method for simultaneous local pressure ejection and field potential recording, which allows a more localized site of AMPA ejection and recording. Responses to iontophoretic AMPA ejection were clearly potentiated with a time delay after tetanization of about 10 min, confirming the findings of Davies et al. (1987). AMPA responses to local pressure ejection (fPRs) recorded with the same pipette exhibited more variable changes. Thus, in only 5 of 32 cases which clearly exhibited LTP of the fEPSP there were also increases in the amplitude of fPRs after tetanization. However, in 12 cases a short or long lasting depression of the fPRs (up to 30%) could be seen after LTP induction. In picrotoxin containing solution (20 μM) no long- or short-lasting depressions were observed (n=9), while the long-lasting potentiation of the fPRs was seen in 2 out of 7 cases. These results allow us to suggest that the direction of the changes probably depends on the locus of recording and AMPA ejection and that both the increases as well as the decreases in the sensitivity of neurons to AMPA might be related to LTP. It could be speculated that the increase in the AMPA sensitivity could be mediated by synaptic receptors, while the decrease in sensitivity might be related to changes in receptors on extrasynaptic membrane, where excitatory and inhibitory processes are interacting.

374.10

ASYNCHRONOUS PRE- AND POSTSYNAPTIC ACTIVITY INDUCES LONG-TERM DEPRESSION IN HIPPOCAMPAL CA1 NEURONS IN VITRO.

Dominique DEBANNE, Beat H. GÄHWILER and Scott M. THOMPSON*

Brain Research Institute, University of Zurich, 8029 ZÜRICH Switzerland.

A novel form of associative long term depression (LTD) has been induced in CA1 neurons in hippocampal slice cultures by repeatedly (100 times) stimulating the Schaffer collateral pathway at low frequency (0.3 Hz) after brief periods of postsynaptic firing imposed by injection of depolarizing current pulses. The decrease in excitatory postsynaptic potential amplitude 1) lasted more than 30 minutes, 2) could be reversed by induction of potentiation in the depressed input, 3) could be induced at previously potentiated inputs, 4) was input specific (LTP of another pathway could be induced at the same time) and 5) did not require potentiation of other inputs. The associative character of this LTD was demonstrated by the dependence of the magnitude of the depression upon the interval between postsynaptic depolarization and presynaptic stimulation (maximal LTD =31% with 800 ms interval). No depression was obtained for intervals <500 or >2000 ms. LTD could not be induced in neurons recorded with BAPTA (25 mM) filled micropipettes or in the presence of the NMDA-receptor antagonist AP5 (40 μM) during the conditioning procedure, suggesting that voltage-dependent calcium influx alone is not sufficient for LTD induction. We conclude that associative LTD is induced as a result of NMDA-receptor activation and Ca^{2+} influx, when postsynaptic depolarization is followed by synaptic activity within a limited time window.

374.11

LONG-TERM POTENTIATION OF DUAL COMPONENT EPSCS IN THE RAT HIPPOCAMPAL SLICE. K.A. Clark and G.L. Collingridge*. Dept. of Pharmacology, The Medical School, Birmingham University, Birmingham, U.K.

Long-term potentiation (LTP) is a long lasting increase in synaptic efficiency typically induced by high frequency (tetanic) stimulation. While there is general agreement that the site of LTP induction is post-synaptic, the relative increases in AMPA and NMDA receptor-mediated synaptic components and the site of LTP expression remains controversial. We have used whole-cell patch-clamp recording from CA1 pyramidal neurones to investigate LTP of AMPA and NMDA receptor-mediated conductances simultaneously. To optimise measurement of EPSCs, synaptic inhibition was blocked using picrotoxin (50 μM) and CGP 35348 (200 μM) in the perfusing medium and QX-314 and caesium in the patch solution. Current-voltage plots (n=12), CNQX or NBQX (1-10 μM, n=17) and D-AP5 (50 μM, n=9) were used to define the initial slope of the EPSC as primarily AMPA receptor-mediated and the amplitude measured at 100 ms post-response onset as primarily NMDA receptor-mediated. Neurons, voltage clamped at -60 mV, were dialysed for a minimum of 45 mins, tetanised (100 Hz/1 s) and followed for a further 30 mins. Neurons were sub-divided into two populations on the basis of whether potentiation of the AMPA receptor-mediated component was sustained or not (n=9 and n=6, respectively). In both cases, potentiation of the NMDA receptor-mediated component was similar in amplitude to and paralleled that of the AMPA receptor-mediated component. If following dialysis for 30 mins at -60 mV, neurons were voltage clamped at -50 mV for the rest of the experiment we were unable to obtain LTP of either component (n=8), presumably due to over activation of the NMDA receptor-complex. This study shows LTP is manifest as a parallel increase of the AMPA and NMDA receptor-mediated components of the EPSC. The ability of a neuron to express LTP seems to be independent of factors that would be easily dialysed out of a neuron. K.A.Clark is a Wellcome prize student.

374.12

METABOTROPIC GLUTAMATE RECEPTOR-MEDIATED LTP IN THE LAMPREY RETICULOSPINAL SYSTEM. Simon Alford* and Réjean Dubuc Dép. Kinanthropologie, U. du Québec à Montréal H3C 3P8 and CRSN U. de Montréal H3C 3J7 Québec Canada and *Dep. Physiology Northwestern Univ. Med. School Chicago IL 60611 USA.

This study was conducted to investigate plasticity of synaptic transmission from vestibulospinal (VS) to the reticulospinal (RS) neurones of the lamprey. RS neurones of the posterior rhombencephalic reticular nucleus were recorded with microelectrodes or with whole-cell patch electrodes. Stimuli were applied to the basal plate of the 4th ventricle to stimulate VS axons of the ipsilateral octavomotorius intermediate (OMI) or contralateral octavomotorius posterior (OMP) nucleus. Low frequency stimulation of either pathway evoked a monosynaptic PSP comprising a glutaminergic and electrical component and an inhibitory component. In all cases, using microelectrode recording, tetanic stimulation of the pathway (20 or 50 shocks for 1 s, test intensity) led to a sustained enhancement in amplitude of the PSP (mean increase 42 ± 12 %: n=11). This enhancement was not sustained if the neurone was recorded using a patch pipette (n=6), implying that the potentiation required a diffusible mechanism in the recorded neurone. The tetanus induced enhancement, in synaptic transmission, was pathway specific. Additionally, the sustained enhancement was insensitive to blockade by application of the NMDA receptor antagonist (R,S)AP5 (200 μM; mean increase of 47 ± 15%; n=9) but was abolished by the application of the metabotropic glutamate receptor (mGluR) antagonist 4C3H-PG (100 μM; n=4). Taken together, the latter results indicate that mGluR but not NMDA receptor activation is responsible for evoking this LTP. The response was, however, insensitive to pretreatment with pertussis toxin (mean increase 50 ± 30%; n=3) indicating that the mGluR involved is not coupled to a pertussis toxin sensitive G protein (eg G_i, G_O). In conclusion, inputs to brainstem reticulospinal neurones are capable of use-dependent plastic changes mediated by activation of an mGluR. These changes are likely to play an important role in adapting the activity of brainstem motor systems which are responsible for the initiation and control of locomotion. (Supported by MRC Canada, FRSQ Québec and Fondation de l'UQAM)

374.13

SYNAPTIC PLASTICITY IN THE NUCLEUS ACCUMBENS *IN VITRO*: DIFFERENTIAL REGULATION OF AMPA AND NMDA RECEPTOR-MEDIATED SYNAPTIC TRANSMISSION. S. B. Kombian* and R. C. Malenka. Depts. of Psychiatry and Physiol., University of California, San Francisco, CA 94143.

The nucleus accumbens (NA) is an important limbic-motor interface that is thought to be involved in many complex behaviors. Principal projection cells of NA are quiescent and depend on strong glutamatergic excitation from cortical and subcortical limbic regions. We have examined the mechanisms of activity-dependent changes in NMDA and non-NMDA (AMPA) receptor-mediated synaptic responses, recorded from the "core" region in an *in vitro* parasagital slice preparation. Field EPSPs and whole cell EPSPs/EPSCs were evoked by stimulation of prelimbic cortical afferents in the presence of picrotoxin (25 uM).

Long-term potentiation (LTP) was consistently induced in both field (14/17 slices; 62 ± 20%) and whole cell EPSPs (17/21 cells; 68 ± 9%) by high frequency stimulation (100 Hz-1 sec, given 1 or 2 times separated by 10 sec) at twice the baseline and spike threshold stimulus strengths, respectively. Pairing postsynaptic depolarization with low frequency synaptic stimulation (2 Hz for 2 min) also elicited LTP (7/11 cells; 60 ± 6%). Application of D-APV (25 μM) reversibly blocked LTP (n=6). Loading cells with BAPTA (10 mM; n=4) also prevented the induction of LTP by tetanic stimulation in slices in which LTP was elicited in simultaneously recorded field EPSPs. The same tetanic stimulation and pairing protocols consistently produced a long-term depression (LTD) of the NMDA receptor-mediated EPSP recorded in the presence of CNQX (10 μM; 11/14 slices; 48 ± 12%). The induction of LTD was blocked by D-APV (n=3) and by loading cells with BAPTA (10 mM; n=7). Recording EPSCs at resting potential (-80 mV) and at 30 mV in a single cell permitted simultaneous monitoring of the AMPA-(-80 mV; peak current) and NMDA-(30 mV, late component at 75 msec) mediated EPSCs. Low frequency stimulation at 30 mV produced LTD of the NMDA component and LTP of the AMPA component (35 ± 5% and 55 ± 10% respectively, n=6). These results suggest that at these synapses, an NMDA receptor-mediated rise in Ca^{2+} can cause markedly different effects on AMPA and NMDA receptor-mediated synaptic transmission. SBK is supported by HFSPO.

374.14

LONG-TERM POTENTIATION (LTP) AND NMDA RECEPTORS IN CHICK FOREBRAIN IN VITRO. X. Wang* and H. Scheich. Institute for Neurobiology, Brenneckestr. 6, O-3090 Magdeburg, Germany.

A series of in vitro LTP experiments was carried out in the auditory imprinting-relevant area MNH (mediorostral neostriatum and hyperstriatum ventrale) of the domestic chick. Sagittal slices of 250-300 μm thickness were prepared from forebrain of 0- to 10-day-old chicks. Extra- and intracellular recordings were made in the MNH region. The stimulation was delivered in lobus parolfactorius underneath MNH to the main afferent MNH pathway from the thalamus. A population spike potentiation was recorded extracellularly in about 25% of the tested MNH neurons after application of the afferent tetanic stimulation. There are different neuron types in MNH, only one of which can be potentiated as shown by final intracellular dye-filling. Using intracellular recordings, EPSP potentiation and EPSP-spike (E-S) potentiation was identified. A suitable afferent tetanus could lead to a large depolarization of the postsynaptic membrane. Following this depolarization, the neuronal activity was often increased. The mechanism of these phenomena was investigated with excitatory amino acids and their antagonists. The application of N-methyl-D-aspartate (NMDA, 1 μl, 1 mM) dissolved in the medium near the slice induced many spikes, bursts and a large depolarization of the membrane potential with temporal and amplitude characteristics similar to the tetanus induced depolarization. These effects were blocked by 50 μM APV (DL-2-amino-5-phosphonovaleric acid). These results demonstrate the existence of a large population of NMDA-sensitive cells in the MNH. An activation of NMDA receptors may play a crucial role for induction of LTP in MNH.

374.15

MULTIPLE ISOFORMS OF NEURONAL NITRIC OXIDE SYNTHASE. T.M. Dawson,* J. P. Steiner, J. A. Mong, and S.H. Snyder. Departments of Neuroscience & Neurology., Johns Hopkins University School of Medicine., Baltimore, MD 21205

Nitric oxide (NO) is a recently identified messenger molecule in the central nervous system. NO is produced upon activation of the calcium/calmodulin dependent enzyme, NO synthase (NOS), by a variety of stimuli, including glutamate receptor activation. The major isoform of NOS has been purified to homogeneity, moleculary cloned and its anatomical distribution mapped in detail. Since NO has been implicated in diverse neuronal processes such as long term potentiation (LTP) in the hippocampal formation, but NOS immunoreactivity is absent from CA1 pyramidal neurons, we attempted to identify alternative isoforms of neuronal NOS, which could account for these discrepancies. We purified NOS protein, from rat forebrain and have identified two additional isoforms of molecular mass of 130 Kd and 160 Kd, as well as the major 150 Kd isoform. Peptide antibodies generated against the N-terminal region of the molecularly cloned 150 Kd NOS recognize the purified alternative isoforms and reveal a distinct regional heterogeneity as determined by Western blot analysis and immunohistochemistry. These additional isoforms may be regulated in distinct ways and have unique functions and DNA sequences.

374.16

Nitric Oxide Inhibitors Facilitate the Induction of Hippocampal Long-term Potentiation by Modulating NMDA Responses. *K. Kato, D.B. Clifford and C.F. Zorumski* *, Departments of Psychiatry and Anatomy & Neurobiology, Washington Univ., School of Med., St. Louis, Mo 63110

The effects of the competitive nitric oxide (NO) synthase inhibitor, L-nitroarginine (L-NOArg), on synaptically activated N-methyl-D-aspartate (NMDA) currents and the induction of long-term potentiation (LTP) were studied in the CA1 region of 25-30 days old rat hippocampal slices at 30°C. Whole cell recording techniques were used to measure NMDA currents in the presence of 50 μM picrotoxin and 10 μM CNQX. Application of 10 μM L-NOArg increased the amplitude of NMDA currents by 48.8 ± 4.6% (N=7) in the presence of 2 mM extracellular Mg^{2+} at -70 mV. This augmentation occurred within minutes of L-NOArg administration and was readily reversible on removal of the drug. L-arginine (100 μM), which did not change baseline NMDA responses (N=3), overcame the enhancement produced by L-NOArg (N=5). At 5-100 μM, a 10-25 min application of L-NOArg also facilitated the induction of LTP produced by a single 100 Hz, 300 ms tetanus (+32.0 ± 5.5% change in field EPSP at 10 μM, N=5; +36.7 ± 5.1% change at 100 μM, N=7). In control slices, the 100 Hz, 300 ms tetanus was insufficient to induce LTP (+8.4 ± 2.5%, N=8). The development of LTP in L-NOArg-treated slices was inhibited by 50 μM D-2-amino-5-phosphonovalerate (D-APV), and the effects of 100 μM L-NOArg were overcome by ten-fold higher concentrations of L-arginine (+5.3 ± 1.8, N=9) but not by D-arginine (+36.0% ± 4.3, N=5). Hemoglobin, an agent that binds NO extracellularly, also facilitated the development of LTP when administered for 10 min at 10 μM (+37.1 ± 6.3%, N=3). 10 μM L-NOArg administered for 20 min without a tetanus also induced a slowly developing potentiation (+23.7 ± 2.3%, N=5/7). These results suggest that tonically released NO modulates the threshold for synaptic plasticity in the CA1 hippocampal region by diminishing NMDA receptor-mediated responses.

374.17

NITRIC OXIDE SYNTHASE INHIBITORS BLOCK LTP INDUCED BY WEAK BUT NOT STRONG TETANIC STIMULATION. J.E. Haley*, P.L. Malen and P.F. Chapman. Department of Psychology and Graduate Program in Neuroscience, University of Minnesota, Minneapolis, MN 55455.

Nitric oxide (NO) production during high frequency tetanic stimulation has been implicated in the induction of long-term synaptic potentiation (LTP) in the hippocampus. The dependence of LTP on NO is controversial, however, due to the difficulty in blocking the induction of LTP with NO synthase inhibitors under some experimental conditions. We have attempted to describe the key factors determining the efficacy of NOS inhibitors by testing the potentiating effects of relatively weak and strong tetanic stimulation in the presence of L-nitro arginine (NArg).

Rat hippocampal slices were prepared and field potentials elicited by Schaffer collateral/commissural stimulation were recorded in stratum radiatum. All experiments were performed at 31°C. Pretreatment for 30 minutes with NArg (0.01-1mM) blocked the induction of LTP resulting from subsequent weak tetanic stimulation (2 trains of 100Hz for 250ms, 5 sec intertrain interval) but did not prevent the enhanced synaptic response elicited by a stronger tetanus, where either the duration of the train or the stimulation strength was doubled during tetanus. All three protocols produced LTP of similar magnitude in controls and the size of the response during tetanus was unaltered by NArg. LTP elicited by weak tetanic stimulation was blocked by 50μM AP5, confirming that the enhancement seen in controls was NMDA dependent.

That NO synthase inhibition produces a differential effect on synaptic enhancement produced by weak and strong tetani, may help to explain the controversy surrounding NO and LTP, and raises the possibility that qualitative differences exist between cellular processes activated by different tetanic stimuli.

374.18

PATHWAY SPECIFIC E-S POTENTIATION IS ENHANCED BY BACLOFEN. D.D. Mott*, W.A. Wilson, H.S. Swartzwelder and D.V. Lewis, Depts. of Pharmacology, Medicine (Neurology), Psychology, Pediatrics (Neurology) and Neurobiology, Duke Univ. and V.A. Med. Centers, Durham, N.C. 27710.

As originally described by Bliss and Lomo (1973), long term potentiation (LTP) consists of both a lasting increase in EPSP slope as well as a long term increase in the ratio between the slope of the EPSP and the amplitude of the associated population spike (E-S Potentiation). We and others have previously demonstrated that $GABA_B$ receptor activation can regulate the potentiation of EPSP slope. We now examine the effect of $GABA_B$ receptor activation on the induction of E-S potentiation.

E-S potentiation was studied in the dentate gyrus of the rat hippocampal slice. In control slices a long stimulus train (0.5 s, 100 hz), delivered selectively to the medial perforant path (MPP), produced long term slope and E-S potentiation in that pathway. As previously described, following the MPP train, the non-tetanized lateral perforant path (LPP) exhibited E-S potentiation and a heterosynaptic depression of EPSP slope. In contrast, a brief stimulus train to the MPP (0.1 s, 100 hz) produced no change in either pathway, most likely because of the short train duration. However, application of baclofen (10 μM) during the brief MPP train, enabled the train to produce a lasting E-S potentiation, but no synaptic potentiation, in the MPP. The non-tetanized LPP was not affected. These results indicate that baclofen can selectively facilitate the induction of pathway specific E-S potentiation and suggest that $GABA_B$ receptors may play a role in the mechanism underlying this form of neural plasticity.

Supported by NIH grants NS 27488, NS 17771 and a V.A. Merit Review.

374.19

PROTEIN SYNTHESIS INHIBITORS AND LONG-TERM POTENTIATION (LTP) IN THE INTACT MOUSE: IMPORTANCE OF CONSTITUTIVE RATHER THAN SYNTHETIC PROCESSES. U. Namgung and A. Routtenberg*. Cresap Neuroscience Laboratory, Northwestern University. Evanston, IL 60208.

Does the maintenance of LTP require new synthesis or the presence of existing proteins? We attempted to resolve this controversy (e.g. Neurosci., 28:519, 1989 vs Synapse, 1:90, 1987) by studying LTP in the anesthesized subcutaneously 30 min or 4 hr prior to LTP.

Population responses of dentate gyrus cell layer were obtained by perforant path stimulation from entorhinal cortex. Population spike amplitudes, before and after high frequency stimulation (HFS), were compared among saline, CXM and ANI injected-mice. Initial potentiated responses were similar for all 3 groups, i.e., 200% of baseline spike amplitude 20 min after HFS. This implies that CXM or ANI does not affect the induction stage of LTP in contrast to prior reports (J. Neurosci., 4:3080, 1984 & Synapse, 1:90, 1987). Injection of CXM 4 hr before HFS resulted in a decay of LTP that appeared at 90 min. The decreased responses remained at a lower level (60%) for 4 hr after HFS ($p<0.01$). ANI when injected 4 hr before HFS did not change the potentiated responses. CXM or ANI injected 30 min before HFS had no effect on the response at 4 hr after HFS though the response was temporarily lowered after HFS in the 40-90 min period for both inhibitors.

These results demonstrate that the inhibition of protein synthesis by CXM but not by ANI blocks the maintenance of LTP. In contrast, another study using rats (Neurosci., 28:519, 1989) found ANI to impair LTP maintenance. This inconsistency may reflect different methods or species used. Since CXM injected 4 hr before HFS but not 30 min before blocked LTP persistence, this implies that protein(s) constitutively present at the time of HFS with a half-life of more than 30 min and less than 4 hr is necessary for LTP maintenance. Moreover, the absence of an effect at 30 min suggests that new protein synthesis is not required. [supported by MH25281-18 and AFOSR90-0240]

LONG-TERM POTENTIATION IV

375.1

LONG-TERM POTENTIATION IN ENTORHINAL CORTEX INDUCED BY THETA-PATTERN STIMULATION OF CA1. T.Otto* and H.Eichenbaum. Dept. of Psychology, University of North Carolina, Chapel Hill, NC 27599

The present study sought to determine whether stimulation of the hippocampus, in a pattern consistent with both the learning-related firing patterns of hippocampal neurons (Otto et al., 1992) and the stimulation parameters that optimally induce LTP within the hippocampus itself (Larson et al., 1986), would result in potentiation of the EPSP elicited in a primary cortical target of hippocampal output cells.

Six urethane-anesthetized rats were implanted with a bipolar stimulating electrode in the CA1-subiculum border of the mid-septotemporal hippocampus and a recording electrode positioned in the deep layers of ipsilateral lateral entorhinal cortex (LEC). Single-pulse stimulation of CA1 produced a reliable, monosynaptic EPSP in the LEC that peaked between 5-7ms. Stimuli of an intensity sufficient to evoke an EPSP roughly one-half its maximum amplitude (mean EPSP amplitude = 0.29mv; mean stimulation intensity = 141uA) were delivered to the hippocampus every 15 sec, and the slope of the initial descending phase of the resultant cortical EPSP was measured. Following establishment of a stable baseline, "theta-burst" stimulation of CA1 (2 bouts of 14 bursts [4 pulses at 100Hz], 140ms between bursts [7Hz], 10 sec between bouts) resulted in a brief post-tetanic potentiation followed by stable LTP in entorhinal cortex as indicated by a reliable increase in the slope of the EPSP. The average potentiation at 30min was 161.3% of baseline responses. Responses could be enhanced further by subsequent bouts of theta-burst stimulation. These data are consistent with the hypothesis that learning-related firing patterns of hippocampal output cells support plastic changes in their cortical targets, and could thereby subserve the extrahippocampal consolidation of hippocampal-dependent memory.

Supported by ONR grant N00014-91-5-1881 to HE and TO.

375.2

STRESS IMPAIRS LTP OF THE EPSP IN THE DENTATE GYRUS 2 HOURS AND NOT 24 HOURS AFTER STRESS, BUT ALTERS THE THETA BURST RESPONSE AT BOTH TIME POINTS. **T.J. Shors* and E. Dryver. Department of Psychology and Program in Neuroscience, Princeton University, Princeton, NJ 08544**

Since the effect of stress on hippocampal plasticity and learning bears resemblance to the effect of LTP on similar measures, we tested whether the effect of stress on LTP was long-lasting, as is LTP. In addition, we tested whether stress alters the theta burst response (Larson, Wong, Lynch, 1986) in a manner similar to a previous induction of LTP. Sprague-Dawley rats (n=28) were exposed to 1 hour of restraint and 60, 1 s, 1 mA tail shocks and returned to their home cage. Stimulating the dentate gyrus via the perforant path, rats were tetanized 2 and 24 hours after exposure to the stressor. Unstressed controls (n=23) were tetanized once and then again 2 hours later. Stress impaired EPSP LTP 2 hours ($P<0.05$), but not 24 hours after stress.

In contrast, exposure to the stressor at both time points altered the response to subsequent theta burst stimulation (10, 40 ms bursts at 100 Hz, each separated by 200 ms). While unstressed controls exhibited a decline in the amplitude and area (excluding burst 2) of each successive burst, both stressed groups exhibited no such decline in amplitude, a pattern remarkably similar to that observed in unstressed rats exposed to a second tetanus ($P<0.01$). Assuming dentate LTP lasts longer than 24 hours using these stimulation parameters, these results dissociate stress from dentate LTP with regards to time, but associate stress and LTP with regards to neuronal responsiveness to theta burst stimulation.

[supported by the Office of Naval Research, McDonnell-Pew Program in Cognitive Neuroscience and the Whitehall Foundation].

375.3

CURRENT SOURCE DENSITY ANALYSIS OF ANGULAR BUNDLE-EVOKED RESPONSES IN HIPPOCAMPUS. J.L. Stringer* and C.M. Colbert. Dept. of Pharmacology, Baylor College of Medicine, Houston, TX 77030

Both anatomical and in vitro electrophysiological data suggest that monosynaptic excitation of CA1 pyramids by entorhinal cortical afferents exists. However, available in vivo field potential evidence of monosynaptically driven CA1 cell firing is equivocal because interpreting laminar maps of extracellular voltages is difficult and can be misleading. Current source density (CSD) analysis allows the position of current sinks to be localized more precisely. Here we report, using CSD analysis, that multi-phasic potentials recorded in the CA1 cell layer evoked by stimulating the angular bundle (AB) correspond to sinks in the dentate gyrus (DG). Adult male Sprague-Dawley rats (n=6) were anesthetized with urethane. Stimulating electrodes placed in ipsilateral or contralateral CA3 and the AB were used to evoke extracellular responses recorded by a glass micropipette. Twelve laminar maps were recorded through CA1 and the DG at intervals of 25μm or 30μm. Four responses were averaged to produce the voltage trace at each depth. CSD analysis was performed offline by computer. Stimulating CA3 resulted in current sinks within CA1. Stimulating the AB, however, consistently evoked sinks that localized to the DG. Although there is good evidence that an entorhinal cortex to CA1 pathway exists, these experiments suggest that the multiphasic potentials recorded in CA1 in vivo do not represent activation of this pathway. This work was supported in part by NIH grant NS 28871.

375.4

ORIGINS OF THE VARIATIONS IN LONG-TERM POTENTIATION BETWEEN SYNAPSES IN THE BASAL VS APICAL DENDRITES OF HIPPOCAMPAL NEURONS. A. Arai*, J. Black and G. Lynch. Center for the Neurobiology of Learning and Memory, University of California, Irvine, CA 92717.

Factors which control the magnitude of LTP are of interest as they may be linked to particular features of memory. In apical dendrites of field CA1, the maximum potentiation produced by theta patterned stimulation is in the range of 50%; neither additional stimulation nor employing a smaller initial response can transcend this 'ceiling' value. The present study examined whether the rules which govern LTP in basal dendrites differ from those established for apical dendrites.

Stimulation by ten consecutive bursts produced more than twice as much LTP in basal dendrite field EPSPs as in their apical counterparts. Similar results were obtained with whole-cell clamp recording which indicates that the synapses on the two dendritic arbors of the same neuron differ in their LTP ceiling. In addition, basal synapses required a smaller number of theta bursts to reach their ceiling. Intracellular recording revealed significant differences between basal vs apical responses to trains of bursts: the within burst depolarization was greater and the between burst hyperpolarization was smaller for the basal dendritic responses. These two variables have previously been proposed to influence the magnitude of LTP and the observed differences between basal vs apical synapses are in accord with this hypothesis. Together with recently described immunocytochemical results, the findings reported here suggest that variations in LTP across dendritic subfields of hippocampus reflect a differential distribution of a subclass of GABAergic interneurons. (Supported by ONR grant N00014-89-J-1255).

375.5

DELAYED SIGNAL CONDUCTION FROM CA3 TO CA1 VIA THE CA2 REGION IN RAT HIPPOCAMPAL SLICES REVEALED BY OPTICAL RECORDING.

Y.Sekino*[1,2], M.Tanifuji[1,3] and K.Obata[1]. [1]Lab. Neurochem., National Inst. for Physiol. Science, Okazaki 444, [2]Dept. of Mol. & Cell. Neurobiol. Tokyo Metropolitan Inst. for Neurosci., Fuchu-shi 183, [3]Dept. Information Sci., Fukui Univ., Bunkyo,Fukui 910, Japan.

Many electrophysiological studies using hippocampal slices are based on the anatomical concept of a direct pathway from CA3 to CA1 via the Schaffer collaterals. Anatomical studies, however, show that there could be other CA3 - CA1 pathways, which have not yet been verified by electrophysiological studies. We addressed this problem by optical imaging of activity; using voltage-sensitive dyes. Hippocampal slices of 500 μm thickness were prepared from the temporal part of the rat hippocampus with Schaffer collaterals and mossy fibers in the same preparation. After staining with an absorptive voltage-sensitive dye, RH482, electrical stimulation was applied to the mossy fibers and the neuronal activity was monitored using a SD1001 optical recording system (Fujifilm Microdevices ,Ltd.). Two signal pathways from CA3 to CA1 were observed, which were temporally and spatially different. One was rapid conduction in the distal part of the stratum radiatum, probably through the Schaffer collaterals, starting at 6 ms after the stimulation of the mossy fibers. The other was excitation of the CA2 area with subsequent spread of the excitation to the stratum oriens and the proximal stratum radiatum in CA1. The delay in the excitation of CA2 was about 3 ms. The CA1 region can thus be activated by two different pathways, the direct pathway via the Schaffer collaterals and the delayed pathway via the CA2 region.

375.6

BASAL AND APICAL DENDRITIC SYNAPSES OF HIPPOCAMPAL CA1 PYRAMIDAL CELLS EXHIBIT DIFFERENT LTP PROPERTIES. **L.R.Roth* and L.Stan Leung. Dept. Physiology and Clin. Nuerol. Sci., Univ. Western Ontario, London, N6A 5A5, Canada.**

Long-term potentiation (LTP) at the basal and the apical dendritic excitatory synapses of CA1 pyramidal cells was examined in urethane-anaesthetized rats by stimulation at CA1 str. oriens and str. radiatum, which selectively activated afferents to the basal and apical dendrites respectively. Averaged evoked potentials (AEP) were recorded extracellularly at 50μm depth intervals in *ipsilateral* CA1, and a one-dimensional current source density (CSD) was estimated by a second order spatial differencing. Following baseline recording, the afferent pathway was tetanized with theta-frequency primed bursts(8 bursts of one pulse followed 190ms later by 10 pulses at 100Hz) of either high intensity(400μA), or low intensity(40-70μA). Area under the excitatory sinks 2ms after onset was calculated before and 30 min after the tetanus. Stimulation of str. oriens resulted in significant potentiation of the basal dendritic excitatory sink for both the high intensity(35 $\pm$ 10%, mean $\pm$ SEM, n=7, $p<0.05$) and the low intensity tetanus(78 $\pm$ 19%, n=7, $p<0.05$). Stimulation of str. radiatum resulted in significant potentiation of the apical dendritic sink for the high intensity(73 $\pm$ 46%, n=6, $p<0.05$), but not the low intensity tetanus(-22 $\pm$ 22%, n=9, $p>0.1$). These results suggest that the apical and basal dendritic excitatory synapses of CA1 pyramidal cells may have distinct LTP characteristics; the basal synapses show robust potentiation at low tetanus strengths, however the apical synapses exhibit weak potentiation only following a high intensity tetanus. (supported by NSERC).

375.7

HIPPOCAMPAL EPs HAVE STRONGEST RELATION WITH MOLECULAR RSA OSCILLATION AT MEDIUM STIMULATION INTENSITY. **E.L. Hargreaves,* F. Boon, and D.P. Cain. Dept. Psychology, Univ. Western Ontario, London, CANADA, N6A 5C2.**

That hippocampal EPs recorded during Rhythmical Slow-wave Activity (RSA) are different from those recorded during Large-amplitude Irregular Activity (LIA) has been well documented. Similarly, others have shown that EPs vary systematically within RSA oscillations. We have shown that the RSA/LIA related EP differences change across the I/O curve (Hargreaves and Cain, 1991). Here, we demonstrate a similar change across the I/O curve in EPs recorded during RSA oscillations. Male rats (n=8) were implanted with stimulating and recording electrodes in the perforant-path and dentate-gyrus, respectively. The recording electrodes were bipolar and staggered such that the tips straddled the granule cell layer, enabling the continuous monopolar recording of molecular RSA, in addition to the recording of granular or hilar EPs. Abbreviated I/O curves were recorded during Type I exploratory behaviors at Lo ($\bar{x}$=152μa; se=54.6) Med ($\bar{x}$=289μa; se=73.1) Hi ($\bar{x}$=725μa; se=75) stimulation intensities. EPs were triggered manually and identified along with 6-12 Hz filtered RSA on a polygraph tracing, allowing digitized EP measures to be matched with RSA phase. Averaged 2nd order regressions indicated that EP measures recorded at the medium intensity gave the strongest curvilinear relationship to RSA phase. Supported by NSERC to DPC.

375.8

EFFECTS OF CONDITIONING INPUTS ON THE TRANSMISSION OF NEURAL ACTIVITY IN TRISYNAPTIC CIRCUIT OF HIPPOCAMPUS DETECTED WITH OPTICAL MEASUREMENTS. Tetsurou Kondo, Michinori Ichikawa, Syuji Akiyama, Gen Matsumoto* and Toshio Iijima
Electrotechnical Lab., Molecular and Cellular Neurosci. Sect., Tsukuba, Ibaraki 305, Japan

The main excitatory pathway in the hippocampus is composed of three different groups of neurons (dentate granule cells, CA3 and CA1 pyramidal cells) connected in series with plastic synapses (trisynaptic circuit). We applied conditioning inputs to the beginning of this circuit (perforant path) and studied their effects on the transmission of test input applied also to the perforant path of a rat hippocampal slice. The responses of neurons in the circuit to a test input was monitored simultaneously with high spatio-temporal resolution by applying optical recording which covered almost all surface of a hippocampal slice. A conditioning input of 100 Hz for 1 sec caused LTP in both CA3 and CA1 region. The enhancement of synaptic strength not only had an effect on the amplitude of response of neurons but also on the speed of signal transmission in the trisynaptic circuit. These potentiated synaptic connections were most effectively reduced by the conditioning input of 1Hz for 1 min. However the conditioning input of 0.5 Hz for 2 min had little effect on the synaptic connections in both CA3 and CA1 regions.

375.9

LIKELIHOOD SURFACES ILLUMINATE UNCERTAINTY IN QUANTAL ANALYSIS. A.C. Greenwood*[1], Z.Xiang[2], E.M. Landaw[3], and T.H. Brown[1,2]. Depts. of Physiol.[1] and Psych.[2], Yale Univ., New Haven, CT 06520; Dept. of Biomath.[3], U.C., Los Angeles, CA 90024

We developed a maximum likelihood (ML) approach to quantify uncertainty within several testable models of synaptic transmission. ML theory provides standard errors and likelihood ratio tests, as well as parameter estimates. Monte Carlo simulations amplify the method's power and justify each application of ML theory.

Populations of synaptic responses were analyzed by plotting the dependence of model likelihood on any two quantal parameters in iso-likelihood contour plots. We used the plots to examine the uncertainty in estimates of mean quantal size (q) and content (m) for simulated data. A similar analysis was applied to whole-cell recordings of paired pulse facilitation (PPF) in the rat hippocampal mossy fiber (mf) synapse.

The analysis of simulated data showed that increasing set size and decreasing noise had very different effects. The analysis of the mf PPF data was able to reject the hypothesis that q increased as much as m ($P < 0.05$). It is common practice to test hypothetical LTP mechanisms by extrapolation from pre-induction parameter values, without regard for their uncertainty. This practice leads to false confidence, especially in multi-parameter models. The likelihood contour analysis of mf PPF illustrated these errors (which are not unique to ML analysis) and two satisfactory alternatives (which are). The second derivatives at the ML were found to provide a fast way to screen hypothetical plasticity mechanisms. *Supported by NIMH & ONR.*

375.10

KINETIC ANALYSIS OF THE SPONTANEOUS MINIATURE EPSC OF THE HIPPOCAMPAL CA3 PYRAMIDAL NEURON. S. Smerin*, D. Henze, M. Fleck, T.R. Chay and G. Barrionuevo. *Departments of Behavioral Neuroscience, Psychiatry, and Biological Sciences, University of Pittsburgh, Pittsburgh, PA 15260.*

Toward analyzing long-term potentiation at the four types of glutamatergic synapse -- mossy-fiber, fimbrial, collateral, and perforant-path -- on the hippocampal CA3 pyramidal neuron, we seek to identify the synapse of origin of an EPSC by its kinetics. Since the kinetics of the evoked EPSC are obscured by the asynchronous release of numerous synapses, we are focusing on a quantal EPSC -- the spontaneous miniature EPSC.

The hippocampus of the rat was sliced and maintained *in vitro*. Action potentials were blocked with tetrodotoxin. Spontaneous miniature EPSCs were recorded from the CA3 pyramidal neuron in whole-cell voltage clamp.

The amplitude and 10-85% rise-time for each of at least 500 spontaneous miniature EPSCs were determined for each CA3 pyramidal neuron. Rise-rate was calculated as (0.75 x amplitude)/(rise-time) Amplitude ranged from 4-45pA, with a mean of 18pA. Rise-time ranged from 0.4-5msec. with a mean of 1.4msec. Rise-rate ranged from 2-112pA/msec, with mean at 12 pA/msec.. Cluster analysis is now underway to determine whether the amplitude and rise-rate correlate, and whether spontaneous miniature EPSCs having a given combination of amplitude and rise-rate can be attributed to one of the four types of glutamatergic synapse on the CA3 pyramidal neuron. Supported by NS24288 and NIMH predoctoral fellowships.

375.11

MOSSY FIBER POTENTIATION DECREASES SPONTANEOUS MINIATURE EPSC FREQUENCY IN CA3 PYRAMIDAL NEURONS. D.A. Henze* and G. Barrionuevo. Depts. of Behavioral Neuroscience and Psychiatry. University of Pittsburgh, Pittsburgh, PA 15260.

Previous work has shown that the locus of induction and expression of mossy fiber (MF) potentiation is presynaptic. The underlying mechanisms of presynaptic mossy fiber potentiation could be reflected in a change in the amplitude and/or frequency of spontaneous miniature EPSCs. This study sought to address this issue by recording the frequency and amplitude of spontaneous mossy fiber miniature EPSCs from hippocampal CA3 pyramidal neurons using the whole-cell patch clamp technique.

It has been reported by Randall et al. (1991) that the amplitude range of spontaneous EPSCs in CA1 is 1.3 to 15 pA. In our CA3 recordings, the range of events was 1 pA to 165 pA. Since the mossy fibers are located electrotonically close to the soma, their synaptic currents should be of a larger amplitude than all other events. In an attempt to restrict the analysis to only MF spontaneous EPSCs, events were selected to have amplitudes greater than 15 pA. Events also were selected to have 10-90% rise times greater than 500 μsec and 50% halfwidths of at least 2 ms. It was found that there was no significant change in the average, median, or modal values for three minute epochs both pre- and 15 minutes post-MF tetanus. However, the frequency of the events decreased from 2.13 sec^{-1} to 1.08 sec^{-1} (n=5). All data were collected in the presence of 10 μM bicuculline and 10 μM picrotoxin. The whole-cell electrode solution consisted of 120 mM CsF, 10 mM CsCl, 10 mM EGTA, and 10 mM HEPES. Mossy fiber (MF) tetanus was given in 10 μM MK-801 to block NMDA dependent potentiation of the CA3 collaterals. Supported by NS 24288 and an NIMH predoctoral traineeship.

375.12

FURTHER ELECTROPHYSIOLOGICAL CHARACTERIZATION OF THE LONGITUDINAL ASSOCIATION PATHWAY IN THE RAT DENTATE GYRUS. P.A. Hetherington*, F. Balcomb, K. Austin and M.L. Shapiro. Dept. of Psychology, McGill University, Montréal, PQ, Canada, H3A 1B1.

The hilar ipsilateral longitudinal association pathway (HILAP) arises in the hilus and projects along the longitudinal axis of the dentate gyrus to synapse in the inner one-third of the molecular layer. Here, we better characterize the physiology of this pathway in rats anesthetized with urethane and α-chloralose. Two electrodes were placed to stimulate both perforant path and the HILAP, and a recording electrode was placed in the inner molecular layer of the dentate gyrus, about 1.2 mm temporal to the site of hilar stimulation. Laminar profiles of responses to both perforant path and HILAP stimulation showed that these systems are distinct. The perforant path evoked population negative EPSP was maximal in the middle 1/3 of the molecular layer in contrast to that evoked by hilar stimulation, which was maximal in the inner 1/3 of the molecular layer. The HILAP response was assumed to be excitatory because maximal responses in the molecular layer were negative-going. However, as a further verification that these synapses are excitatory, we performed experiments to observe the effect of GABA antagonists on these responses. Under bicuculline, both perforant path and HILAP responses were affected similarly and potentiation was readily elicited. We also performed experiments where the hilus and the perforant path were stimulated simultaneously such that the population EPSPs coincided. This experiment resulted in a summation of the two responses--not a cancellation as would be predicted if hilar stimulation were invoking an inhibitory projection system. Finally, potentiation of both the HILAP and perforant path responses was blocked by the competitive NMDA antagonist NPC 17742 (see Côté et al., this meeting). These experiments demonstrate that the HILAP makes excitatory synapses on dentate granule cells.

375.13

LONG-TERM POTENTIATION INDUCES HETEROSYNAPTIC EFFECTS ON MEDIAL AND LATERAL PERFORANT PATHWAYS IN SINGLE VOLTAGE-CLAMPED DENTATE GRANULE NEURONS. S. Wang* and J.M. Wojtowicz. MRC Group, Dept. of Physiol., Univ. of Toronto, Toronto, Ont. Canada M5S 1A8.

In this project we asked whether the interactions between medial and lateral perforant pathways reported *in vivo* can be observed in single neurons in an *in vitro* slice preparation. Synaptic transmission in the lateral and medial perforant pathways was monitored with extracellular field recordings or whole-cell voltage-clamp recordings from granule neurons. In all experiments 10 μM bicuculline was included in the bath to block inhibitory inputs. Long-term potentiation (LTP) was induced in one of the two pathways while the synaptic transmission of the other one was monitored simultaneously. Voltage-clamped neurons were depolarized to -20 mV and tetanized with four 0.5 s trains of 100 Hz afferent volleys, delivered at 10 s intervals. With this method the success rate of LTP induction was 55% in medial pathway and 30% in lateral pathway. LTP in medial pathway (253% increment) increases the untetanized transmission in lateral pathway by 24% (n=7), while LTP in lateral pathway (99% increment) depresses the transmission in medial pathway by 19% (n=5). Analogous heterosynaptic interactions but with different time courses were seen during field potential recordings of synaptic transmission. Results suggest that the interactions can occur in single neurons but additional effects may be a property of the cell population. Supported by MRC of Canada.

375.14

QUANTAL BASIS OF FACILITATION AND LONG-TERM POTENTIATION IN RAT HIPPOCAMPAL MOSSY FIBER SYNAPSES. Z. Xiang*[1], A.C. Greenwood[2], & T.H. Brown[1,2] Department of Psychology[1], Department of Cellular and Molecular Physiology[2], Yale University, New Haven, CT 06520

Mossy fiber (mf) synapses are ideal for voltage-clamp recording because of their electrotonic proximity to the soma. The complex circuitry of the CA3 region of the hippocampus, however, makes it difficult to record uncontaminated mossy fiber responses. We devised a set of minimal criteria for eliciting and identifying mf excitatory postsynaptic currents (EPSCs) (Xiang et al, *Neurosci. Abstr., 17*, 1991; Claiborne et al, *Hippocampus, 3(2)*, 1993). Here, we evaluate the quantal basis of paired-pulse facilitation (PPF) and LTP in 5 cells that satisfied our criteria.

Rat hippocampal slices were prepared in the conventional manner and maintained in an interface chamber at 32°C. Whole-cell voltage-clamp recordings were made from CA3 pyramidal cells and mf EPSCs were evoked by minimal stimulation of the dentate gyrus. The quantal basis of the synaptic changes was evaluated using the graphical variance method, which plots the normalized ratio of the mean squared/variance of the EPSCs against the normalized mean of the EPSCs.

In all 5 cells, the data points for PPF and LTP were on or above the diagonal, suggesting that both forms of plasticity reflect an increase in quantal content (m). An alternative interpretation of this result, a plasticity-induced decrease in nonstationarity (Yamamoto et al, *Neurosci. Lett., 138*, 1992), was unsupported by the "runs test" of stationarity. We also examined the interaction between PPF and LTP. The null hypothesis—that LTP and PPF operate on separate parameters that multiply—could be rejected in three of the five cells ($p < 0.05$). In these 3 cells, the slopes of the graphical variance plots for PPF (before and after LTP) and for LTP (of the first and second responses) were qualitatively consistent with a shared mechanism of increased release probability.

The results suggest that both PPF and LTP are due to an increase in m, independently of whether the interaction test implicates a shared quantal parameter. The interaction test alone may not furnish a reliable guide to the locus of LTP expression. *(Supported by ONR and NIMH).*

375.15

A METHOD FOR SIMULTANEOUSLY RECORDING THE CA1 POPULATION SPIKE AND DENDRITIC EPSP IN THE INTACT HIPPOCAMPUS B.J. Branch*, G.M. Rose, K.R. Harris and D.M. Diamond. Dept. of Pharmacology, UCHSC, and Medical Research, VAMC, Denver, CO 80220

Hippocampal connections possess an unusual capacity to show use-dependent changes in strength. This phenomenon, termed long-term potentiation (LTP) or enhancement, has long been considered a physiological model of memory. Electrical stimulation of CA1 pyramidal cell afferents produces a negative EPSP in the dendritic regions and a positive EPSP with a superimposed population spike (PS) in the cell layer. High frequency stimulation of CA1 afferents can produce LTP of both the EPSP and the PS. Experimenters normally record either the EPSP or the PS, thereby limiting an analysis of similarities and differences between these two measures of LTP. This issue is significant because some authors have suggested that the PS is an inadequate measure of LTP. In an accompanying abstract we have addressed this issue directly. Here we present a method that provides for simultaneous recordings of the negative EPSP and the PS in area CA1 in vivo. We have developed a dual electrode system consisting of a pair of 50μ diameter, teflon insulated stainless steel wires, which are glued together with a tip separation of 250-280μ. The electrodes are used in both acute recordings and in a microdrive system for chronic recordings in behaving rats. Simultaneous recordings both EPSP and PS measures may provide significant information concerning potentially different forms of hippocampal plasticity.

375.16

THE POPULATION SPIKE IS A VALID MEASURE OF HIPPOCAMPAL LONG-TERM POTENTIATION. G.M Rose*, B.J. Branch, A.G. Humphreys, C.I. Moore and D.M. Diamond. Dept. of Pharmacology, UCHSC and VAMC, Denver, CO 80220

Long-term potentiation (LTP) has long been considered a physiological model of memory. As described in the original report by Bliss and Lømo (1973), LTP is manifested in several ways, including increases in measures of the field excitatory postsynaptic potential (EPSP), population spike (PS) and unit activity. Subsequent authors have asserted that the term LTP (or LTE) should be restricted to measures of EPSP plasticity, because only this measure reflects the specificity of the effect. However, this view is not supported by published accounts of cellular measures of LTP, (e.g., *J. Neurosci.*, 8:4079, 1988; *Science.*, 232:988, 1986; *Nature*, 266:736 and 266:737, 1977).

We have addressed this issue further by examining the relationship between the negative field EPSP and PS in hippocampal area CA1. Experiments were conducted on the hippocampus *in vitro* and *in vivo* (urethane-anesthetized or unanesthetized rats). In all cases, recording methodology allowed simultaneous examination of both the negative EPSP and PS in response to stimulation of commissural/associational afferents. Long-term plasticity was induced using the primed burst stimulation paradigm, which consists of a single pulse followed 170 msec later by a burst of four pulses at 200 Hz (five pulses total). There was a significant within-subject correlation between the two measures. These results suggest that the EPSP and PS are equivalent indicators of hippocampal LTP.

375.17

PRIMED BURST POTENTIATION IN THE DENTATE GYRUS. A.K. Wiser* and G.M. Rose. Neuroscience Training Program and Department of Pharmacology, UCSHC, and Medical Research, VAMC, Denver, CO 80220

Long lasting hippocampal plasticity, as exemplified by long-term potentiation (LTP), is often used as a model of memory encoding. Because of an emerging awareness of the unphysiological nature of LTP stimulation, interest is increasing in the use of physiologically based patterned stimulation paradigms to generate hippocampal plasticity. One such paradigm, Primed Burst (PB) potentiation, consists of a single pulse followed 170 ms later by 4 pulses at 200 Hz (5 pulses total). PB potentiation has been well characterized in area CA1, but few studies have examined the efficacy of patterned stimulation in the dentate gyrus. The current work compared the effects of PB stimulation in CA1 and the dentate gyrus in pentobarbital anesthetized rats. PB potentiation was generated in the CA1 pyramidal cell layer by stimulating the commissural/associational afferents, and in the dentate granule cell layer by stimulation of the medial perforant path inputs. In experiments to this point, we have observed comparable effects of PB stimulation in both regions. Significant increases in population spike amplitude were seen in 6/8 cases in the dentate, versus 15/26 in CA1. For the sucessful cases, the average amplitude increase was 58% and 74%, respectively. Positive field EPSP slopes changed less frequently: in the dentate, 3/8 cases showed an increase averaging 13%; in CA1, 3/26 cases had an average increase of 11%. These results reinforce the utility of PB potentiation as method to study hippocampal plasticity.

375.18

LONG-TERM POTENTIATION REDUCES AUDITORY GATING IN THE CA3 REGION OF THE HIPPOCAMPUS. C.L. Miller*, G.M. Rose, A.K Wiser, R. Freedman and P. Bickford. Depts. of Pharmacology and Psychiatry and Neuroscience Training Program, U. of Colorado. Health Sciences Center., and Medical Research, VAMC, Denver, CO 80220

A large negative evoked potential (N40) can be recorded in the CA3 region of the hippocampus in response to auditory stimulation. If two stimuli are given at a 0.5 sec interval, the response to the second is reduced compared to the first. This phenomenon is termed auditory gating. Hippocampal connections possess an unusual capacity to show use-dependent alterations in strength, as is best exemplified by long-term potentiation (LTP). The present study was undertaken to assess the potential interaction between LTP and auditory gating in the hippocampus. Experiments were performed in chloral hydrate anesthetized rats. A recording electrode was placed in the CA3 pyramidal cell layer; commissural/associational afferents were activated via a stimulator located in the ventral hippocampal commissure. Paired tone pips were delivered via hollow earbars. Sets of 16 tone pairs were used to establish that auditory gating was present. After this, LTP stimulation (3 trains of 250 Hz for 1 sec) was given. LTP reduced auditory gating by decreasing the response to the first tone and increasing the response to the second. This effect was not observed if LTP failed to develop following high-frequency stimulation. These results indicate that LTP produces functional alterations in hippocampal information processing.

375.19

MUSCARINE BLOCKS LTP OF THE LATERAL PERFORANT PATH IN THE RAT HIPPOCAMPAL SLICE. K. Pang* and J.M. Sarvey, Department of Pharmacology, Uniformed Services University of the Health Sciences, Bethesda, Maryland 20814.

Studies of the cholinergic modulation of long-term potentiation (LTP) may provide insight into how the cholinergic system modulates memory. LTP of the medial perforant path - dentate granule cell synapse (MPP) requires activation of NMDA receptors, whereas LTP of the lateral perforant path - dentate granule cell synapse (LPP) requires activation of opioid receptors. In a previous study, 1 μM muscarine, a cholinergic agonist, enhanced LTP induction in the MPP following subthreshold high frequency stimulation (HFS), but had no effect following suprathreshold HFS. 10 μM muscarine had no effect following either HFS condition. The present study examined the effect of muscarine on LTP of the LPP. In the control condition, suprathreshold HFS induced LTP [population spike (PS): 221%, EPSP: 133%, n=5]. 1 μm muscarine had no effect on LTP of the LPP [PS: 242%, EPSP: 126%, n=4], whereas 10 μM muscarine blocked the induction of LTP [PS: 72%, EPSP: 102%, n=4]. The results provide evidence that the cholinergic system may enhance NMDA-dependent LTP and diminish opioid-dependent LTP. The effect of muscarine following subthreshold HFS of the LPP is currently being examined. Supported by NS 23865.

375.20

A CALCIUM CHANNEL WITH NOVEL PHARMACOLOGY SUPPORTS SYNAPTIC TRANSMISSION AND PLASTICITY IN THE HIPPOCAMPUS. D.B. Wheeler*, R.W. Tsien & A.D. Randall. Department of Molecular & Cellular Physiology, Beckman Center, Stanford University Med. School, Stanford, CA 94305

We have assessed the importance of various Ca^{2+} channels in excitatory synaptic transmission in area CA1. Extracellular recordings were made in s. radiatum of hippocampal slices (3-4 wk rats) during stimulation of the Schaffer collateral-commissural pathway (2/min). EPSP slopes were irreversibly depressed (-46$\pm$1%, n=30) by the N-type channel blocker ω-CTx-GVIA at saturating doses (1 μM). Neither the P-type channel blocker, ω-Aga-IVA (3 or 30 nM, n=4 or 10), nor the L-type channel blocker, nimodipine (5 μM, n=6), altered either basal transmission or that recorded after application of ω-CTx-GVIA. In contrast, the response remaining after ω-CTx-GVIA was abolished by ω-CTx-MVIIC, a potent blocker of Q-type Ca^{2+} channels in cerebellar granule cells (Randall *et al.*, Soc. Neurosci. Abs. 1993) and α_{1A} Ca^{2+} channels expressed in *Xenopus* oocytes (Sather *et al.*, Soc. Neurosci. Abs. 1992). ω-CTx-MVIIC block of transmission was potent (complete at $\geq$150 nM, n=20) and developed slowly (20 min to half-block at 500 nM, n=2), like block of oocyte-expressed α_{1A} channels. Interestingly, 150 nM ω-CTx-MVIIC applied alone strongly reduced basal synaptic transmission (-76$\pm$2%, n=4). The small remaining response was eliminated by ω-CTx-GVIA. Thus, excitatory transmission at the CA3 $\rightarrow$CA1 synapse is dominated by Ca^{2+} channels with pharmacology like Q-type channels in granule cells and α_{1A} channels in oocytes.

Transmission mediated solely by α_{1A}/Q-type Ca^{2+} channels could be readily modulated. This transmission supported enhanced paired-pulse facilitation (+63$\pm$7% vs. +39$\pm$5% in control; 40 ms interpulse interval; n=8, $p<.01$, paired *t*-test), LTP (+38$\pm$1%, 30 min post-tetanus, n=5), and inhibitory modulation by 1S,3R-ACPD (-51$\pm$4%, 200 μM, n=4), carbachol (-53$\pm$5%, 10 μM, n=4), 2-chloroadenosine (-84$\pm$5%, 5 μM, n=4) and (-)-baclofen (-76$\pm$3%, 5 μM, n=8), whilst (+)-baclofen (5 μM, n=4) was ineffective. After application of ω-CTx-GVIA, addition of PDBu (1 μM), but not 4α-PDBu, potentiated transmission (+222$\pm$31%, n=4) and prevented inhibition by the aforementioned modulators.

376.1

STRUCTURAL CORRELATES OF CHEMICALLY INDUCED LONG-TERM POTENTIATION IN HIPPOCAMPAL CA1 AND DENTATE GYRUS.

A. Y. Klintsova*, J. Wenzel, S. Otani, W. B Levy and N. L Desmond.
Dept. of Neurosurgery, Univ. of Virginia, Charlottesville, VA 22908.

Morphological correlates of chemically induced long-term potentiation (LTP) were studied in the CA1 and dentate gyrus (DG) of the rat hippocampus in vitro. Bath application of 25 mM tetra-ethylammonium (TEA) for 5 min increased the Schaffer collateral-CA1 pEPSP slope by 80% and the perforant path-DG pEPSP slope by 40%. 60 min after TEA application, slices were fixed using a mixed aldehyde phosphate-buffered solution and processed conventionally for EM. We looked for changes in the number and size of axospinous synapses in CA1 s. radiatum and the middle third of the DG molecular layer (ca. 800 μm^2 was sampled in each region of each slice). The percentage of concave axospinous synapses decreased greatly (ca. 50%) with TEA-induced LTP in CA1 s. radiatum and slightly increased (16%) in the DG molecular layer. The size of nonconcave synapses decreased both in CA1 and the DG. The size of the concave axospinous synapses, however, changed in opposite directions in the two regions: decreasing in CA1 and increasing in the DG. These results are similar to results obtained in previous experiments correlating morphological changes with stimulation-induced LTP in the DG and in CA1 by various labs. These results suggest the existence of different mechanisms underlying LTP in these two hippocampal regions.

Supported by IBRO Fellowship to AYK, NIMH MH00622 and NIH NS15488 to WBL, and NS26645 to NLD.

376.2

THE HIPPOCAMPAL-PREFRONTAL CORTEX PATHWAY IN THE RAT: LONG-TERM POTENTIATION AND IMPLICATION IN ASSOCIATIVE LEARNING.

F. Burette, T.M. Jay* and S. Laroche. Chaire de Neuropharmacologie, INSERM U114, Collège de France, 75231 Paris, France; NAM, CNRS URA1491, Université de Paris-Sud, 91405 Orsay, France.

We have previously shown that the glutamatergic pathway from the hippocampus to the prefrontal cortex (PFC) supports long-term potentiation (LTP) in the rat. The present experiments were aimed at investigating (1) the involvement of the N-methyl-D-aspartate (NMDA) receptor in the induction of LTP in the anesthetized rat, (2) the duration of LTP in the freely moving rat, and (3) variations in the evoked potentials in the PFC during associative learning. In experiment 1, a push-pull cannula was used to perfuse artificial cerebrospinal fluid into the prelimbic area of the PFC while recording field potentials evoked by stimulation (0.033Hz) of the CA1-subicular region of the hippocampus. High-frequency stimulation (2 series, 6 min apart, of ten 250Hz-200ms trains at 0.1Hz) elicited LTP in the PFC that was blocked by perfusion of D-2-amino-5-phosphonopentanoate (AP5). In experiment 2, field potentials were recorded in the PFC for several days before and after high-frequency stimulation of CA1-subiculum. LTP was induced as in the preceding experiment and found to persist for at least 3 days. Series of 5 short trains (200Hz-50ms) at 7.7Hz, mimicking the theta bursting pattern of CA1 neurons, resulted in LTP lasting for at least 24 hrs. In experiment 3, field potentials were monitored during a tone-footshock associative learning task. Rats were submitted to 4 daily sessions of 8 paired (conditioned group) or unpaired (pseudoconditioned group) stimulus presentations. A decrease in the amplitude of the evoked potential was seen in pseudoconditioned rats, whereas the main effect in the conditioned group was a delayed increase in the evoked potential, occurring on average 30 min after conditioning sessions. The results support the view that LTP-like mechanisms on this pathway may be involved in a process of late consolidation by which the hippocampus can help the stabilization of a cortical representation of the learned association. Supported by grant from the EEC (SC1-CT910685).

376.3

LONG-TERM POTENTIATION AND ASSOCIATIVE MEMORY FUNCTION IN A BIOPHYSICAL SIMULATION OF PIRIFORM CORTEX.

E. Barkai*, G. Horwitz, R.E. Bergman and M.E. Hasselmo, *Dept. of Psych., Harvard Univ., Cambridge, MA 02138.*

Using brain slice experiments and biophysical simulations, we have explored how physiological parameters influence the possible function of the olfactory (piriform) cortex as an associative memory for odor patterns. A simulation of this region with 240 3-compartment pyramidal cells and 58 each of two types of inhibitory interneurons was developed using GENESIS. Hebbian synaptic modification of excitatory intrinsic fiber synapses during learning allows the network to store afferent input patterns and perform completion on degraded versions of these patterns during recall. Afferent input patterns activated 20 neurons each for 500 msec. during learning. Completion was quantified by the dot product between the recall activity in response to the full learned pattern and in response to a degraded pattern, minus the dot product between the response to the full pattern and the response to other stored patterns. Without learning, recall of patterns missing 30% of input gave a performance of 0.685 (5 patterns stored), which improved to 0.815 after learning. Cholinergic suppression of intrinsic synaptic transmission (70%) was required during learning to prevent runaway synaptic modification, which caused a strong homogeneous response to all patterns during recall (performance=0.0). Experimental data on cholinergic modulation of adaptation was modeled by a 40% decrease in M current g(max) and a 30% decrease in AHP current g(max). This change enhanced the learning of new patterns from performance=0.551 to 0.680 (recall patterns missing 70% of input).

Associative memory function in this model depends on synaptic modification of intrinsic synapses. We recorded intracellularly from piriform cortex pyramidal cells during 5Hz stimulation of intrinsic fiber layer coupled synchronously with intracellular current injection. Long-term potentiation was observed in 6 out of 6 neurons tested during perfusion of 20μM carbachol (60% average), but only 1 out of 6 tested without carbachol. These results suggest that acetylcholine is necessary for associative memory function in the piriform cortex.

Support: French Foundation for Alzheimer Research and ONR young investigator award.

376.4

CHRONIC DEVELOPMENTAL LEAD INCREASES LTP THRESHOLD IN RAT DENTATE GYRUS IN VIVO.

M.E. Gilbert* and S.M. Lasley. ManTech Envir. Tech., RTP, NC, 27709 and U. Ill. Coll. of Med., Peoria, IL 61656.

Childhood lead (Pb) exposure has been associated with impaired cognitive function. This study examined the effects of chronic developmental Pb exposure in rats upon the induction of long-term potentiation (LTP), an electrophysiological model of learning and memory. At birth, dams received 0.2% Pb acetate in the drinking water to yield hippocampal Pb levels of 350-400 ng/gm (ca. 35 μ gm/dl whole blood) of offspring maintained on the same solution until adulthood. Field potentials were recorded from the dentate gyrus following stimulation of the perforant path under urethane anesthesia. Threshold for inducing LTP was determined by delivering a set of 15 4-pulse trains at a frequency of 5 Hz at increasing stimulus intensities (100, 200, 400, 600, 1000 μ A). The degree of potentiation of the population spike (PS) was measured 15 m after each train, and 15, 30 and 60 m following the final train. LTP, defined as an increase in PS amplitude >25%, was observed in all animals tested. No difference between Pb and control rats was seen in PS threshold prior to train delivery (311 vs 280 μ A, respectively), or the magnitude of LTP recorded 1 hr after the final train (84 and 117%, respectively). However, a 3.5-fold increase in the intensity of the train required to induce LTP was observed in Pb compared to control animals (489 and 170 μ A, respectively). Only 1 of 10 controls required a train intensity higher than 200 μ A to induce LTP, whereas 8 of 9 Pb-exposed rats required intensities of 400 μ A or greater. These data suggest that the detrimental effects of developmental Pb exposure on cognitive function may be due to its interference with the synaptic processes underlying LTP.

376.5

EXCITATORY SYNAPTIC TRANSMISSION IS MODULATED BY EXTRACELLULAR H^+ BUFFERING IN RAT HIPPOCAMPAL SLICES

T. Taira*, S. Smirnov, J. Voipio and K. Kaila. Department of Physiology and Department of Zoology, 00014 University of Helsinki, Finland.

There is evidence that activity-induced alkaline transients within the interstitial space of nervous tissue are largely due to net fluxes of acid-base equivalents across postsynaptic receptor-gated ion channels. In view of the marked pH sensitivity of certain receptor channels, it has been frequently postulated that synaptically-evoked H^+ shifts might play a neuromodulatory role. We provide here the first evidence to support the above hypothesis in showing that extracellularly and intracellularly recorded glutamatergic responses in area CA1 of rat hippocampal slices are potentiated upon inhibition of fast extracellular H^+ buffering by a poorly-permeant carbonic anhydrase inhibitor, benzolamide (10 μM). Experiments with glutamate receptor antagonists and Mg^{2+}-free solutions suggest that the action of benzolamide is largely mediated by the H^+ sensitivity of *N*-methyl-D-aspartate (NMDA) receptor channels. In agreement with the idea that NMDA receptors provide a target for the intrinsic neuromodulatory action of H^+ ions also under control conditions, addition of the H^+ buffer HEPES (20 mM) produces a selective attenuation of NMDA receptor-mediated responses. An additional interesting possibility to be studied in future work is that the post-synaptically generated alkaline transient acts as a retrograde signal to enhance the influx of Ca^{2+} across presynaptic voltage-gated calcium channels, thereby promoting an increase in transmitter release.

376.6

LOWERING EXTRACELLULAR pH OR INCREASING pCO_2 SUPPRESSES SYNAPTIC TRANSMISSION, BLOCKS LONG-TERM POTENTIATION (LTP) AND ATTENUATES EPILEPTIFORM ACTIVITY INDUCED BY LOW EXTRACELLULAR MAGNESIUM.

L. Velíšek, J.P. Dreier, S.L. Moshé, P.K. Stanton*, Albert Einstein College of Medicine, Bronx, NY 10461, U.S.A.

Recent studies have demonstrated that acidifying extracellular pH reduces NMDA channel conductance. Therefore, lowering extracellular pH, or, raising tissue pCO_2, should influence the magnitude of LTP elicited by submaximal stimulation, as well as suppress epileptiform events elicited by removing extracellular $[Mg^{2+}]$. To test this hypothesis, Schaffer collateral-evoked population spikes and epsp slopes were recorded in CA1 of hippocampal slices (Ringer solution bubbled with 5% CO_2/95% O_2, pH 7.3) and high frequency stimulation (1x100 Hz/1 s) was applied to elicit LTP. We tested two levels of increased pCO_2 (20.6% CO_2 = pH 6.7 or 10.6% CO_2 = pH 7.1). To examine NMDA-mediated epileptiform discharges, $[Mg^{2+}]$-free Ringer was perfused, while recording in entorhinal cortex. To distinguish between actions of high pCO_2 not related to lowered pH, in some experiments Ringer was acidified by lowering $[HCO_3^-]$. Both high pCO_2 and acidic pH suppressed normal Schaffer collateral synaptic transmission (population spike amplitude to 10-70% and epsp slope to 50-70% of baseline). When evoked potentials had stabilized, tetanic stimulation elicited only transient post-tetanic potentiation of both population spikes and epsps, while LTP assessed 30 min post-stimulus was completely blocked. When pH or pCO_2 were returned to baseline values, population spikes and epsp slopes recovered to their original values, and robust LTP now could be elicited. Lowering pH and raising extracellular pCO_2 also increased the interval and decreased the amplitude of low $[Mg^{2+}]$-induced seizure-like events (SLE) in entorhinal cortex. Very low pH (6.2) blocked SLEs completely, as well as status-like recurrent discharges. All these effects were fully reversible. The data suggest that processes leading to local intracerebral pH/pCO_2 changes may have a serious impact on low-frequency synaptic transmission, synaptic plasticity and also seizure generation. *(Supported by the Epilepsy Foundation of America)*

376.7

GLUCOSE METABOLISM AND HIPPOCAMPAL LONG-TERM POTENTIATION Y. Izumi, D.B. Clifford & C.F. Zorumski, Dept. of Psychiatry, Washington Univ. Medical Sch., St. Louis MO 63110

Previously we observed that NMDA blocks the induction of long-term potentiation (LTP) by promoting untimely release of nitric oxide (NO) (Science 257: 1273, 1992). Although the mechanisms underlying this LTP inhibition are uncertain, recent biochemical studies indicate that NO may alter glucose metabolism via ADP ribosylation of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (PNAS 89: 9382, 1992). This prompted us to investigate the effects of glucose and its metabolites on synaptic transmission and LTP induction in the CA1 region of rat hippocampal slices.

When extracellular glucose was decreased from 10mM to 2mM, baseline synaptic responses showed little change. However, in 2mM glucose a single 100Hz x 1s tetanus failed to induce LTP in 5 slices (+6.8 ± 9.2% change in EPSP slope). Similarly, 5μM iodoacetate (IA), an inhibitor of GAPDH, did not alter baseline responses but blocked LTP (-2.2 ± 3.7% change, N=5). The inhibitory effect of IA was overcome by coadministration of 10mM pyruvate (+34.9 ± 16.1% change, N=4) but not by 10mM lactate (-6.4 ± 4.4% change, N=4). Furthermore, substitution of 10mM pyruvate for glucose in normal recording solutions permitted robust LTP (+63.1 ± 31.1% change, N=5) whereas 10mM lactate failed to permit LTP (+6% change, N=2).

These results suggest that glucose metabolism plays an important role in synaptic function beyond maintaining baseline transmission.

376.8

TEMPERATURE-INDUCED ALTERATIONS IN LONG-TERM POTENTIATION. T.L. Ivanco*, K.-A. Moore, and R.J. Racine. Department of Psychology, McMaster University, Hamilton, ON, Canada, L8S-4K1.

Moser et al. (Science, 259 (1993) 1324-1326), reported that changes in extracellular potentials may be correlated with changes in brain temperature. In order to investigate the effects of temperature on response amplitude, as well as on plasticity, we manipulated temperature while monitoring responses evoked in the dentate gyrus by perforant path stimulation. Male, hooded rats were urethane-anesthetized and implanted with electrodes in the perforant-path and dentate gyrus. Following recording of baseline dentate responses at normal temperature, body temperature was elevated, lowered, or maintained in all animals. A second baseline was then taken. Half of the animals in each temperature condition then received a basic 'theta' pattern stimulation (i.e. 5 bursts/s), to induce LTP. The remaining animals were used as controls and remained unstimulated. Responses were retested, after which the body temperature was returned to normal and baseline responses retested for the final time. Results indicated that evoked responses could be potentiated by temperature elevation alone, and this potentiation remained after temperature was returned to normal levels. The addition of stimulation trains did not appear to induce any additional LTP.

376.9

AT TEMPERATURES DOWN TO 17°C LTP CAN OFTEN BE ESTABLISHED IN A HIGH CALCIUM MEDIA (4.5 mM Ca^{++}) IN THE HAMSTER HIPPOCAMPAL SLICE. S.N. Yang and J.M. Horowitz*. Section of Neurobiology, Physiology and Behavior, Univ. of Calif., Davis CA 95616.

Previously we found that LTP can not be established with tetanus at 20°C in the hamster, a hibernator, in 2.0 mM Ca^{++}, but the effect of raised extracellular Ca^{++} was not fully explored (Brain Res. 520:115-122, 1990). Here we determined if calcium-induced LTP could be developed at these low temperatures. Field postsynatpic potential (PSP) slopes were recorded in 2.0 and 4.5 mM Ca^{++} in CA1 hippocampal pyramidal cells using single-shock Schaffer collateral/commissural stimulation. Bath temperature was fixed at levels between 17°C and 25°C. PSPs were recorded (1) in 2.0 mM Ca^{++} (controls), (2) when the bath calcium was then raised to 4.5 mM Ca^{++} for 15 minutes, and (3) throughout a final 60 minute period at 2.0 mM Ca^{++}. At 20°C PSP slope at the end of this final period varied between 135 and 165% of control and averaged 160.5 ± 4.2% [$F(9,80) = 3.8$, $P<0.05$]. At lower temperatures the enhanced PSPs often showed a biphasic response over the 60 min period -- about 15 minutes into the 60 min period the PSP slope decreased markedly, and then, after about 10 min, again increased to peak values. We conclude that, at 20°C and down to 17°C, calcium-induced LTP can often be established as shown by enhanced PSPs at the end of the 60 minute period. [NASA grant NAG-2-788].

376.10

A PRESYNAPTIC ALTERATION SUPPORTING LTP IN HIPPOCAMPAL NEURONS: CONTRAST WITH EFFECT OF α-LATROTOXIN. A. Malgaroli*, D.B. Wheeler, E. Naldi, A. Ciardo, A. Bergamaschi & R. W. Tsien, Ist. Scientifico Raffaele, Dibit, Univ. Milano, Italy & Dept. Mol. Cell. Physiol., Stanford CA.

In CA3-CA1 hippocampal cell cultures, robust LTP can be triggered by a brief application of glutamate (0 Mg^{2+}). Like the amplitude of evoked responses, the frequency of spontaneous minis was strongly increased; such potentiation is induced postsynaptically and expressed presynaptically (Malgaroli & Tsien, *Nature* 1992). We have compared LTP of mini frequency to elevated mini frequency triggered by α-latrotoxin (αLTX). Presynaptic events leading up to exocytosis were probed by rapid changes in external tonicity or by manipulation of presynaptic Ca^{2+} entry. In unpotentiated synapses, *hyper*tonic solutions (500-600 mOsm) increased mini frequency 50-100-fold above basal (normotonic) levels. While the basal release was augmented with mini LTP or α-latrotoxin, the degree of response to hypertonicity was comparably reduced. Evidently, both LTP and αLTX increase the basal frequency without elevating a release ceiling attained by strong, sustained stimulation. *Hypo*tonic challenges (250 mOsm) reveal interesting differences: after αLTX (as in the unpotentiated case), *hypo*tonicity was largely ineffective in suppressing basal mini frequency; with LTP, hypotonicity decreased mini frequency several-fold. The αLTX results are expected if some release sites lose an inhibitory constraint and support a presumptive $[Ca^{2+}]_i$-independent exocytosis, thus generating a pedestal of mini frequency, insensitive to tonicity. In contrast, mini LTP appears associated with a leftward displacement of the stimulus-response curve, toward lower values of tonicity. These data are consistent with enhanced $[Ca^{2+}]_i$-sensitivity of the secretory machinery. Such a mechanism is supported by experiments studying evoked LTP in area CA1 of hippocampal slices. With LTP, the degree of increase in EPSP slope upon raising $[Ca^{2+}]_o$ from 2 to 4 or 5 mM was significantly reduced relative to control. Thus, the EPSP slope vs. $[Ca^{2+}]_o$ curve was displaced toward lower $[Ca^{2+}]_o$, as if the $[Ca^{2+}]_i$ sensitivity of the secretory machinery were enhanced.

376.11

ENHANCED NEUROTROPHIN RECEPTOR EXPRESSION FOLLOWING LONG-TERM POTENTIATION. I.Cavus*, L. Grover & T.Teyler, Neurobiology,NE Ohio Coll.Med.,Rootstown, OH 44272

Tetanic stimulation leading to induction of a form of long-term potentiation (LTP) resulted in increased expression of the *trk*B neurotrophin receptor in area CA1 of rat hippocampal slices. *Trk*B expression was assayed by immunohistochemistry using a polyclonal antibody ($gp145^{trkB}$) raised against the cytoplasmic tyrosine kinase domain of the mouse *trk*B receptor. We compared *trk*B expression 5 hr following induction of two forms of LTP in CA1 dendritic layer: (1) NMDA receptor independent LTP (200 Hz tetanic stimulation in 50 μM APV) or (2) NMDA receptor dependent LTP (25 Hz tetanic stimulation). Induction of NMDA receptor independent LTP lead to greatly enhanced immunoreactivity in CA1 pyramidal cell apical dendrites and somata. *Trk*B immunoreactivity following induction of NMDA receptor dependent LTP was not distinguishable from low frequency stimulated and non-stimulated control slices.

These results provide additional evidence for neurotrophin involvement in LTP, and suggest differential regulation of *trk*B receptor/tyrosine kinase during different forms of LTP.

Supported by NINDS #28698

376.12

THE EFFECTS OF PRIOR ACTIVITY ON LONG-TERM POTENTIATION (LTP) IN RAT DENTATE GYRUS *IN VIVO*. E. Sklar*, M.J. Bianchetta, D.D. Stellwagen, & M.F. Bear. Dept. of Neuroscience, Brown University, Box 1953, Providence, RI 02912.

One theory of synaptic plasticity (Bienenstock *et al., J. Neurosci.* 2:32) postulates that plasticity is regulated by a threshold level of postsynaptic activity that must be surpassed for LTP to occur in response to presynaptic activation, and that this threshold is modulated as a function of recent cell activity. Since LTP is triggered by calcium influx postsynaptically, one possible mechanism for this "sliding threshold" involves the activity-dependent regulation of postsynaptic calcium buffers. Recent evidence suggests a physiological basis for such a sliding threshold. Lowenstein *et al.* (1991, *Neuron* 6:627) found that sustained tetanic stimulation ("2/20 stimulation") of the perforant path caused an increase in the postsynaptic expression of the mRNA to calbindin-D28k, a high-affinity calcium-binding protein. It is known that intracellular injection of calcium buffers such as EGTA interferes with LTP. We hypothesized that an activity-dependent increase in calbindin would have the same effect.

In urethane-anesthetized rats, field potentials were recorded in dentate gyrus as the perforant path was stimulated. After establishment of a 30 min. stable baseline, 1h of 2/20 stimulation was applied, followed by another hour of baseline, and then theta-burst stimulation (TBS) designed to elicit LTP was given. 2/20 stimulation had no lasting effect on synaptic efficacy of baseline responses as measured by EPSP initial slope. However, subsequent TBS produced only a 9% potentiation of EPSP initial slope (25 min. post-tetanus, n=8), as compared to 26% LTP under control conditions (n=6). This reduction in the magnitude of TBS-induced LTP is significant ($p<.05$).

2/20 stimulation showed differential effects on population spike amplitude (PSA), however. PSA was significantly depressed (34% depression, $p<.05$) 50 min. after 2/20 stimulation, and subsequent TBS produced a potentiation that was significantly higher than in unconditioned controls (121% v. 35%, $p<.01$). Work in progress is aimed at determining the extent to which the differential effects of conditioning stimulation on EPSP slope and PSA are due to network properties or to cellular adaptive processes such as calcium buffering.

376.13

CHARACTERIZATION OF EVOKED RESPONSES IN PERFORANT PATH AFFERENTS TO HIPPOCAMPAL AREA CA1 IN VIVO.

R.V. Hernandez, B.E. Derrick, E.J. Barea-Rodriguez & J.L. Martinez, Jr.* Department of Psychology, University of California, Berkeley CA 94720.

Area CA1 of the hippocampus receives monosynaptic input from the entorhinal cortex via the perforant/temporoammonic pathway arising primarily from layer III cells. Field EPSPs evoked in vivo in the rat by stimulation of the medial aspect of the angular bundle were maximal approximately 300 μm below the CA1 layer, followed high-frequency trains, phase reversed in the pyramidal layer, and displayed depth profiles that differed greatly from commissural CA3-CA1 responses; thus these responses are likely monosynaptic perforant path-CA1 responses. Local application of lidocaine (1 μl of 5% solution) in the stratum radiatum of area CA1 eliminated CA1 responses without altering perforant path responses simultaneously recorded in area CA3 or the dentate gyrus. Thus perforant path-CA1 responses were not volume conducted from these sites. Perforant path-CA1 responses were preceded by antidromic spikes, which may reflect direct CA1 projections to entorhinal cortex. Previous reports (Brain Res. 333: 305-310, 1985) indicate that LTP can be induced in this pathway in vitro. We are investigating parameters necessary for LTP induction in vivo.

Supported by DA 04195 (JLM, Jr.), MH 18882 (RVH), and the Rennie Fund of the University of California.

376.14

MAINTENANCE BUT NOT INDUCTION OF MOSSY FIBER LTP DEPENDS ON NEW PROTEIN SYNTHESIS.

E.J. Barea-Rodriguez, B.E. Derrick and J. L. Martinez, Jr.* Department of Psychology, The University of California, Berkeley, CA 94720.

Others reported that the maintenance but not the induction of NMDA-receptor LTP is dependent on protein synthesis (Neuro. Letters 106:175-180,1989). In the present study, we investigated the mechanisms involved in the maintenance of mossy fiber LTP (MF-LTP). Mossy fiber responses were obtained from anesthetized rats as previously described (JPET 263:725-733,1992). Once a stable baseline was obtained, 5μg/μl of the protein synthesis inhibitor anisomycin was administered 30 min prior to the administration of a tetanizing stimulation (100 Hz, 2x). The time course of LTP was affected and LTP began to decay about 30 min after the conditioning train, and returned to baseline at 60-100 min after induction. Anisomycin did not to affect field potentials evoked by low frequency stimulation. These findings indicate that the maintenance of MF-LTP depends on new protein synthesis and agrees with previous findings suggesting that a long-lasting potentiation exists at this synapse. Supported by DA #04195 and Chancellor's Postdoctoral Fellowship (EJBR).

376.15

THE ACTION OF THE RNA- SYNTHESIS INHIBITOR ACTINOMYCIN D ON A LATE STAGE OF LTP IN HIPPOCAMPAL NEURONS IN VIVO AND IN VITRO.

Uwe Frey*, Thomas Seidenbecher and Manfred Krug. Inst. Neurobiol., Gene Regul. & Plasticity, Brennecke- Str. 6, P.O. Box 1860, 3010 Magdeburg; Inst. Pharmacol. & Toxicol., Leipziger Str. 44, 3090 Magdeburg, Germany.

Hippocampal long-term potentiation (LTP) is thought to serve as an elementary mechanism for the establishment of certain forms of explicit memory in vertebrates. As it is the case with behavioral memory, LTP in the CA1 and in the dentate gyrus has stages: a short-term early potentiation (E-LTP) lasting 1-3 hours and is independent of protein synthesis, precedes a later, longer lasting stage (L-LTP), which requires protein synthesis and which can be simulated by analogues of cAMP and dopamine.

Here we present further physiological properties of L-LTP in the CA1 region in vitro and in the dentate gyrus in vivo. So, L-LTP was prevented, when actinomycin D, a RNA-synthesis inhibitor, was applied during induction of LTP at a concentration, which blocked about 90% of total RNA-synthesis.

Moreover, application of a further tetanization to potentiated neurons on top of an established L-LTP caused the induction of a newly generated potentiation. Conventional E-LTP which lasts 1-3 hours normally precludes a further potentiation. This result demonstrates that L-LTP is really maintained by different mechanisms compared to E-LTP.

In conclusion, the maintenance of L-LTP depends not only on protein- but also RNA-synthesis similar to stages observed during the consolidation of behavioral memory.

This work was supported by the German BMFT "Nachwuchsgruppen Biotechnologie", FKZ: 0310258A.

376.16

LTP-DEPENDENT REGULATION OF GENE EXPRESSION: SOME BIOCHEMICAL CONSIDERATIONS.

A.A. Hicks, T. Smirnova, S. Laroche*, M.L. Errington, T.V.P. Bliss and J. Mallet. LGN, CNRS UMRC9923 91198 Gif-sur-Yvette, France; NAM, CNRS URA1491, Univ. Paris Sud, 91405 Orsay, France; Division of Neurophysiology and Neuropharmacology, MRC NIMR, London NW71AA, U.K.

Long-Term Potentiation (LTP), the activity-dependent enhancement of synaptic efficacity often evoked as a model of a process involved in the mechanism of learning and memory, can be established following Ca^{2+} entry through the NMDA subtype of glutamate receptor. The biochemical changes elicited by this Ca^{2+} and which support the induction and maintenance of the potentiation remain obscure. We have identified a number of genes whose expression is regulated in a LTP-dependent manner. One of these encodes a protein of 78 amino acids which, due to the conservation of the calmodulin-binding domain and protein kinase C phosphorylation sequence found in neuromodulin, we refer to as neuromodulin-2 (NM2). We have seen an up-regulation of NM2 gene expression at both 5 and 24 hours after LTP induction in the dentate gyrus *in vivo*. Using anaesthetized rats, we have shown that at 5 hours the change in gene expression is accompanied by changes in the amount of NM2 protein and that this increase in both mRNA and protein can be prevented by the presence of D(-)aminophosphonovalerate (AP5) at the time of attempted LTP induction. The involvement of NM2 with Ca^{2+}-dependent processes and its pattern of temporal regulation highlight its probable role in the maintenance of LTP. Another of these up-regulated genes encodes a novel membrane-associated protein which may be involved in the biochemistry of transmitter release. The importance of modifications in synaptic transmission produced by alterations in the expression of these proteins after induction of LTP will lead to a greater understanding of the biochemical mechanisms underlying synaptic plasticity. Supported by grants from HFSP and EEC (SC1-CT910685).

376.17

LONG-TERM POTENTIATION (LTP) CAN BE INDUCED, BUT IS NOT MAINTAINED, IN HIPPOCAMPAL CA1 AFTER CHRONIC BENZODIAZEPINE (BZ) TREATMENT.

M.S. Mohammed*, X. Zeng and E.I. Tietz, Dept. of Pharmacology, Med. Col. of Ohio, Toledo, OH 43699

Chronic flurazepam (FZP) treatment reduces GABA inhibition in CA1 region of *in vitro* hippocampal slices of BZ tolerant rats. Since GABA antagonists facilitate LTP induction and BZs interfere with induction and maintenance of LTP elicited by theta burst stimulation (TBS) we tested the hypothesis that LTP is altered in CA1 region of hippocampal slices (400 μm) from FZP-treated (100 mg/kg X 3 days; 150 mg/kg X 4 days, p.o. in .02% saccharin) rats. TBS (10 bursts of 4 pulses (100 HZ); 200 ms inter-burst interval) was produced in slices 2 days after treatment, when residual BZs are no longer detectable in hippocampus. The experimenter was blind to rats' treatment histories. Baseline half-maximal EPSPs (CON:0.81$\pm$.04; FZP:0.81$\pm$.05 mV; p=.95) and population spikes elicited by Schaffer collateral stimulation were recorded extracellularly (glass micropipettes, 2M NaCl, 2-5 mΩ). EPSP initial slope (CON:.57$\pm$.12; FZP:.48$\pm$.08 mV/ms, p=.56) was measured as percent change from baseline, 30 sec-60 min after TBS. The percent increase in maximal EPSP slope induced by TBS (CON:151.8$\pm$9.5%; FZP:144.5$\pm$7.1%) was not different (p=.88) between groups. In treated slices, EPSP slope declined to 50% of baseline (122.01$\pm$6.6%) by 30 min and to baseline by 45 min (104.6$\pm$7.9%). EPSP slope was still elevated in control slices after 60 min (144.1$\pm$13.2%). Baseline population spike amplitude in treated slices was greater prior to (CON:4.5$\pm$.05; FZP:7.6$\pm$0.7 mV, p<.01), but not after, LTP induction (p$\geq$.13). Reduced inhibition in CA1 after chronic BZ treatment may be related to the failure to maintain LTP. Supported by NIDA grant RO1-DA04075 and RSDA K02-DA00180 to EIT.

377.1

DIAGONAL BAND STIMULATION INDUCED FOS & SEIZURES IN PIRIFORM CORTEX ARE BLOCKED BY SCOPOLAMINE

L.A. Zimmer, M. Ennis, M. El Etri* and M.T. Shipley

Dept. Anatomy & Cell Biology, Univ. Cincinnati. Col. of Med., Cincinnati, OH 45267

Considerable evidence suggests that seizures induced by organophosphate nerve agents are initiated in piriform cortex (PC) by sustained release of unhydrolyzed acetylcholine (ACh). We have previously shown that systemic, convulsive doses of the irreversible acetylcholinesterase inhibitor, soman, causes the rapid induction of the immediate early gene product, Fos, in neurons in layers II and III of PC. PC is heavily innervated by ACh inputs originating in the basal forebrain, specifically from the nucleus of the diagonal band (NDB). Both muscarinic and nicotinic receptors are present in PC. ACh, acting at muscarinic receptors has been shown to increase the excitability of cortical neurons, an effect that makes them hyper-responsive to other excitatory inputs. PC cells receive and send excitatory amino acid (EAA) synapses. We hypothesize that sustained hypercholinergic stimulation of PC neurons following soman triggers Fos expression and seizures in PC; seizure activity could then spread to hippocampus and cortex. This hypothesis predicts that sustained release of ACh from NDB terminals in PC should cause seizures and selective Fos expression in layer II-III PC neurons as does soman. To test this prediction, microwire electrodes were chronically implanted in NDB to stimulate cholinergic cell bodies, thus causing release of ACh in PC. Shortly after stimulation of NDB (12 Hz; 5 sec on/3 sec off; 20-40 min; 400-500 uA), rats displayed: (a) large amplitude spike & wave EEG activity and (b) intense gnawing, salivation, sniffing, unilateral eye-blinking, rearing, and forepaw reaching. Forty-five minutes following initial NDB stimulation, tissue was processed for immunocytochemical localization of Fos. There was selective, robust induction of Fos in neurons of layers II and III of PC and the hippocampus. Non-stimulated controls had no Fos. Systemic administration of the muscarinic antagonist, scopolamine (2 mg/kg, i.p.), blocked the behavioral signs of NDB stimulation and, in addition, completely blocked or substantially attenuated the expression of Fos. These results demonstrate that activation of NDB, the source of ACh input to PC, produces cortical seizure activity and patterns of Fos expression comparable to soman. The ability of scopolamine to block NDB evoked behaviors and Fos expression indicates that these effects are medited by muscarinic receptor stimulation of PC. *Supported by U.S. Army DAMD17-91-C-1071*

377.2

CHOLINERGIC INTERNEURONS INHIBIT TACHYKININ- AND ENKEPHALIN-mRNA EXPRESSION IN THE RAT STRIATUM

L.R. Lucas* and R. E. Harlan. Neuroscience Training Program and Dept. of Anatomy, Tulane Univ. Sch of Med., New Orleans, LA 70112.

The influence of cholinergic interneurons on neuropeptidergic projection neurons in the striatum is poorly understood. Previously, we have described the effects of the indirect agonist, physostigmine, and the muscarinic antagonist, scopolamine, on SP/NKA-mRNA (substance P/neurokinin A) expression in the striatum (Soc. Neurosci. Abstr. 18:1205, 1992). Chronic treatment with scopolamine resulted in a 46% increase in tachykinin-mRNA expression. To test further the role of cholinergic interneurons, adult male Sprague-Dawley rats were lesioned by an intracerebroventricular injection of AF64A (an aziridinium ion cholinotoxin) prepared stoichiometrically (Moos et al., Drug Dev. Res.. 23:253-260, 1991). The injection site was in the caudate-putamen (CPu) at the level of the lateral septum running medially along the CPu. After 14 days recovery, rats were perfused and coronal cryostat sections were cut for *in situ* hybridization for neuropeptide mRNA- and immunocytochemistry for choline acetyltransferase- (ChAT) detection. The AF64A lesioned CPu resulted in a 63% increase in SP/NKA-mRNA and a 26% increase in preproenkephalin-mRNA (PPE) expression as compared to the vehicle treated hemisphere. Levels of mRNA after AF64A treatment were greatest along the track left by the injection, but were not higher than the highest levels found in the ventrolateral CPu on the vehicle treated side. The difference between the lesioned and vehicle treated sides decreased in magnitude with increasing distance from the injection site. The increase in neuropeptide expression in the lesioned side was complemented by a decrease in ChAT-immunoreactive neurons along the track of the injection. These results suggest that acetylcholine in the striatum may inhibit levels of SP/NKA- and PPE-mRNA expression. Supported by NS24148 and DA06194.

377.3

Effects of Subchronic Nicotine on Rat EEG.

R.J. Radek*, C. Briggs, J. Sullivan, C.H. Kang, S. P. Arneric. Neuroscience, Pharmaceutical Products Division, Abbott Laboratories, Abbott Park, IL. 60064-3500

Acute administration of nicotine activates cortical EEG which is evident by a reduction of slow wave activity. Chronic nicotine exposure associated with cigarette smoking is implicated in the disruption of sleep, an effect consistent with nicotine-induced cortical activation. This study sought to determine the effects of a continuous two week subcutaneous administration of nicotine on EEG in rats.

Rats were implanted with recording electrodes over the frontal cortex. Osmotic minipumps containing saline or 24mg/ml nicotine (2ml/pump) were implanted into the rats. Nicotine plasma levels, determined by HPLC, and 4 hour EEG recordings and were obtained on days 1, 8, and 15 following minipump implantation. A within subject treatment design was used in this experiment such that each rat was recorded for a 15 day period with saline minipumps (control) and another 15 day period with nicotine minipumps. EEG recordings also were obtained 2 and 8 days following termination of nicotine treatment. FFT frequency analysis was used to determine total power for the 1-4 Hz band during each 4 hour recording session.

Average nicotine plasma levels were 67.7±6.2, 67.8±5.8, and 74.6±8.0, on days 1, 8, and 15, respectively. Nicotine treatment significantly lowered (vs. control, $p<0.05$, n=8, within subject ANOVA) 1-4 Hz power by 23.5±8.1%, 16.1±9.3%, and 19.4±7.0% on days 1, 8, and 15, respectively. There were no detectable levels of plasma nicotine and 1-4 Hz activity returned to control levels after nicotine treatment was withdrawn.

These results show that subchronic nicotine produces an attenuation of 1-4 Hz EEG activity, an effect similar to that of acute nicotine administration. Subchronic nicotine exposure may result in physiological consequences of prolonged reduction of cortical slow wave activity.

377.4

CHRONIC NICOTINE PREVENTS NEOCORTICAL NEURONAL LOSS RESULTING FROM NUCLEUS BASALIS LESIONS.

D.J. Socci *and G.W. Arendash. Dept. of Biology & Institute for Biomolecular Science, University of South Florida, Tampa, FL 33620.

We have previously shown that cholinergic denervation of the neocortex through nucleus basalis (NB) excitotoxic lesioning results in a significant 19-28% loss of neurons in neocortical Layer II long-term ($\geq$ 5 months) postlesioning (*Science* 258:952, 1987). The purpose of the present study was to determine whether chronic activation of nicotinic receptors on or near neocortical neurons could rescue them from NB lesion-induced degeneration. Young (2 month old) rats received unilateral infusions of ibotenic acid (5 ug/1 ul PBS) at two sites within the NB. Beginning immediately after lesions, rats received daily i.p. injections of either nicotine (0.2mg/kg) or vehicle. Treatment continued for 5 months, at which time animals were sacrificed and their brains processed histologically. Image analysis of neocortical Layer II at the mid-parietal level from lesioned, vehicle-treated rats revealed a significant 22% neuronal loss ipsilaterally. By contrast, those NB lesioned animals that received chronic nicotine treatment had only a non-significant 9% neuronal loss in Layer II ipsilaterally. These results indicate that: 1) chronic nicotine treatment can provide a cytopreservative action on neocortical neurons following loss of their endogenous cholinergic innervation, and 2) chronic lack of nicotinic receptor activation in neocortex may play an important role in NB lesion-induced loss of neocortical neurons.

377.5

SCOPOLAMINE ATTENUATES MIDDLE LATENCY, VERTEX AUDITORY EVOKED POTENTIALS IN THE RAT.

C.E. Kalmbacher, C.D. Specht, T.R. Gregg, and K.A. Campbell*. Dept. of Psychology, Univ. of Delaware, Newark, DE 19716.

In the freely moving rat, vertex auditory evoked potentials (AEPs), recorded over posterior cingulate cortex, yielded the prominent components P20 and N40 from the averaged waveform. Under pentobarbital anesthesia, rats (n=8) were implanted with skull screw electrodes at vertex (bregma: -4.5 mm A-P, 1 mm M-L) and a far frontal reference (+7 mm). Following a two week recovery, rats were placed in a Plexiglas chamber, and AEPs were recorded in response to a 1 second, 10 kHz tone at 75 dB (ISI = 20-50 seconds): 100-trial averages were obtained before and after s.c. injections of scopolamine and, on the following day, saline vehicle. Each of four doses of scopolamine (0.1, 0.2, 0.5, 1.0 mg/kg) was administered twice, in ascending order, one week apart. Similar procedures were used for administration of methyl-scopolamine (0.5, 1.0 mg/kg) to test for peripheral effects.

Scopolamine produced a significant decrease in amplitude for both the P20 and N40 components in overall tests ($p<.05$). N40 amplitude also showed a significant decrease at each dose of scopolamine ($p<.0125$), and the magnitude of the amplitude decrement was dose dependent. At 23 hours after scopolamine, only N40 amplitude remained significantly depressed overall ($p<.05$). There were no effects of saline injection. These results confirm that middle latency vertex AEPs in the rat are attenuated by muscarinic receptor blockade, consistent with previous findings in cats (Dickerson & Buchwald, 1991).

377.6

IONIC FLUXES UNDERLYING NEOCORTICAL SLOW WAVES AND NUCLEUS BASALIS - MEDIATED ACTIVATION: WHOLE-CELL RECORDINGS *IN VIVO*.

Raju Metherate* and John H. Ashe, Departments of Neuroscience and Psychology, University of California, Riverside, CA 92521.

Slow, rhythmic membrane potential (V_m) fluctuations occur spontaneously in cortical neurons *in vivo*, and underlie EEG activity in the same low-frequency (1-4 Hz) range. Nucleus basalis (NB) stimulation modifies V_m and EEG fluctuations by way of cortical muscarinic acetylcholine receptors, and these changes likely contribute to neocortical activation (Metherate et al., J. Neurosci. 12:4701, 1992).

To investigate the nature of spontaneous V_m fluctuations and their modification by NB stimulation, we have obtained intracellular recordings from the auditory cortex of urethane-anesthetized rats using the whole-cell recording technique. Based on similarities between spontaneous and thalamic-evoked potentials, we hypothesized that spontaneous V_m fluctuations consist of intermixed rapid depolarizations, rapid Cl^--mediated hyperpolarizations, and long-lasting, K^+-mediated hyperpolarizations. NB-mediated cortical activation might then result from muscarinic suppression of K^+ conductance, allowing rapid depolarizations and Cl^--mediated hyperpolarizations to continue uninterrupted. Tests indicated that: 1) Blockade of K^+ channels by Cs^+ from the recording pipette suppressed spontaneous, long-lasting hyperpolarizations. 2) Effects of NB stimulation summated with partial Cs^+ blockade of long-lasting hyperpolarizations, whereas complete Cs^+ blockade occluded NB actions. 3) When Cl^- fluxes were blocked by intracellular picrotoxin, NB stimulation elicited large amplitude depolarizations. These data support the proposed hypothesis, and suggest ionic bases for cortical slow waves and activation.

Supported by the NSF (IBN 9008818, IBN 9118872).

377.7

Cholinergic Agonists Modify Neuronal Excitability in Intralaminar Thalamic Nuclei of the Rat. C.L. Cox*, J.R. Huguenard and D.A. Prince, Dept. Neurology & Neurological Sciences, Stanford University School of Medicine, Stanford, CA

Intralaminar thalamic nuclei (IL) send a widespread projection to the neocortex, and functionally have a role in synchronizing regional neocortical activities. Stimulation of mesopontine nuclei *in vivo* produces cholinergically-mediated changes in IL activity, however the mechanisms underlying these effects are unknown. We investigated the actions of cholinergic agonists and effects of local extracellular stimulation upon IL neurons *in vitro*.

Whole cell recordings were obtained in slices from parafascicular and caudal central lateral nuclei. Brief pressure application (10-100 ms) of carbachol (10-20mM) or methacholine (10-20mM) produced a membrane hyperpolarization followed by a depolarization. The hyperpolarization lasted 10-20s, was associated with a decrease in input resistance, and was attenuated by the specific M_2 subtype muscarinic antagonist methoctramine. The 20-60s depolarization could lead to spike discharge, was associated with little or no change in input resistance, and was attenuated by the selective M_1 subtype antagonist pirenzepine. These data suggest that ACh, by way of different muscarinic receptor subtypes, can produce long-lasting changes in membrane excitability of IL neurons.

Local electrical stimulation evoked a multiphasic synaptic potential consisting of an early EPSP, early IPSP and a late EPSP. The early EPSP was attenuated by the non-NMDA antagonist, CNQX (10μM), whereas the late EPSP was decreased by the NMDA antagonist, D-APV (20μM). The IPSP was blocked by the $GABA_A$ antagonist bicuculline (5-10μM). The cholinergic actions described may modulate subsequent synaptic activity of IL neurons and potentially influence subcortical control of neocortical function.

Supported by NIH grants NS06477 and NS027280.

377.8

INHIBITORY ACTION OF MUSCARINIC AGONISTS ON NEURONS IN THE RAT LATERODORSAL TEGMENTAL NUCLEUS *IN VITRO* R.W. Greene*, J.I. Luebke, and R.W. McCarley, Neuroscience Lab., Harvard Med. School and VAMC, Brockton, MA 02401

The effects of the mixed cholinergic agonist carbachol and the muscarinic agonist methacholine (MCh) on neurons of the laterodorsal tegmental nucleus (LDT) were studied using intracellular and whole cell patch clamp recordings in a rat brainstem slice preparation. Neurons were classified into one of two categories: those that displayed a prominent low threshold calcium burst (LTB, 60%) and those that did not (non-LTB, 40%). Neurons were filled with biocytin, visualized with texas-red avidin and identified as cholinergic or non-cholinergic with NADPH-diaphorase histochemistry. Eighty percent of the LTB neurons processed in this manner were cholinergic and sixty percent of the non-LTB neurons were cholinergic.

Carbachol elicited an outward current that reversed near the equilibrium potential for potassium and displayed marked inward rectification in 95% of the cells tested. The conductance/voltage-relationship was fit to the Boltzmann equation with a $V_{1/2}$=-73+/-4mV and a k value of 10+/-4. The carbachol evoked current was fully blocked by extracellular barium, mimicked by MCh, and blocked by high concentrations of the muscarinic receptor antagonist pirenzepine (IC_{50}=580nM). These data indicate that the muscarinic agonist evoked inhibition of LDT neurons is due to the activation of an inwardly rectifying potassium current mediated by a non-M1 muscarinic receptor.

377.9

NATRIURESIS INDUCED BY ATROPINE MICROINJECTION INTO THE III CEREBRAL VENTRICLE OF THE RAT. Vergara Aragón, P.* Díaz-Pérez, R., Gutiérrez-Mejía, R. and Barrera-Mera, B. Dept. of Physiology, Fac. de Med., UNAM. A.P. 70-250, 04510 México, D.F. MEXICO.

Acetylcholine (ach) intervenously applied to atropinized female dogs has been used to provide additional evidence on the existence of a reflex secretion of vasopressin. Those experiments showed that water diuresis was inhibited during its increasing phase by injecting 5-40 mg i.v. ach, recovering entirely 60 min. later. The data led us to study the effect of atropine on the mechanisms for hydrosaline regulation in the rat. Our purpose was to block the cholinergic effect on water and sodium elimination. Wistar rats, chronically implanted cannula in the bladder and another into the III cerebral ventricle (III CV), were used. They had free access to water and food until the beginning of the test. They remained quietly conscious. Urine samples were collected in 15 min. periods. After the two initial periods the rats received distilled water by stomach tube (3% b.w.), and 15 min. after hydration, 1 μg. atropine sulfate was injected into the III CV during 15 min. The sodium excretion increased .02 to .10 μEq/min. Instead of achieving sodium retention, we obtained an abundant elimination of this ion. This suggests a cholinergic nature in the antinatriuretic mechanisms in the rat. We think this intense natriuresis was induced by the blocking of the sodium sensing mechanisms.

377.10

SEROTONIN DEPLETION DECREASES THE PERFORMANCE IMPROVING EFFECT OF CHOLINERGIC DRUGS IN MECAMYLAMINE TREATED OR BASAL FOREBRAIN-LESIONED RATS. P. Riekkinen Jr*, M.Riekkinen, and J. Sirviö. University of Kuopio, Dept. of Neurology, P.O.B. 1627, SF-70211 Kuopio, Finland.

The present study was designed to investigate the pharmacological consequences of combined cholinergic and serotonergic dysfunctions. Water maze (escape distance, spatial bias) and passive avoidance (entry latency) tests were used to assess behavior. The extent of basal forebrain lesioning (electrolytic medial septal and quisqualic acid nucleus basalis lesions) was investigated from cresyl violet stained coronal sections. The degree of cholinergic cell loss was analysed by the use of hippocampal and cortical choline acetyltransferase activity. P-chlorophenylalanine (3 x 400 mg, i.p.) induced cortical and hippocampal serotonin depletion was measured by HPLC. In intact rats nicotine and tetrahydroaminoacridine did not facilitate water maze or passive avoidance acquisition. Mecamylamine (nicotinic cholinergic antagonist 7.5 mg/kg, i.p.) and basal forebrain lesioning induced passive avoidance and water maze defects were dose-dependently alleviated by nicotine (0.1, 0.3 mg/kg, i.p.) and tetrahydroaminoacridine (1, 3 mg/kg, i.p.). Serotonin lesioning blocked the therapeutic effects of nicotine in mecamylamine and basal forebrain-lesioned rats. However, THA facilitated performance in rats subjected to combined blockade of cholinergic (mecamylamine, basal forebrain lesion) and serotonergic systems.

377.11

IBOTENIC ACID LESIONS LOCALIZED TO NUCLEUS BASALIS CELLS PROJECTING TO TEMPORAL CORTEX DISRUPT CONDITIONAL PAIRING OF TONE AND SHOCK. Nancy J. Woolf* Laboratory of Chemical Neuroanatomy, Dept. of Psychology, UCLA, Los Angeles, CA 90024-1563, U.S.A.

The cholinergic system projects topographically to modules of isocortex and allocortex (Woolf, *Prog. Neurobiol.*, 37: 475, 1991). Cholinoceptive cells in modules of temporal cortex are altered by Pavlovian conditioning to tone (Woolf et al., *Soc. Neurosci. Abstr.*, 17: 480, 1991). To test if cholinergic afferents are critical for the conditioning correlated with cholinoceptive cell changes, cholinergic neurons innervating temporal cortex were ablated. Female Sprague-Dawley rats were used for this study. Ibotenic acid (5 μl/1μl) was bilaterally infused into the inferolateral quadrant of the posterior part of the nucleus basalis at 1.8 mm posterior and 3.5 mm lateral to bregma and 6.3 mm ventral to the pial surface of the brain (n = 5). Other rats (n = 5) received infusions of saline into the same site. Following complete recovery from the surgery, rats were trained with an 80 dB 2KHz tone presented for 30 sec and a 1 sec 1 mA shock. The intertrial interval was 10 min. Rats were trained in 4 trials on day 1. On day 2, rats extinguished conditional responding (CR) to the context after receiving context without shock. CR to tone and to context was scored for each animal on test day 3. Saline infused animals showed a significant increase in CR to tone above that to context ($p < .05$), whereas lesioned animals did not. Thus, cholinergic innervation of temporal cortex was critical to this conditioning to tone. [Support: Sigma Kappa Foundation]

377.12

SELECTIVE CHOLINERGIC DEAFFERENTATION FOLLOWING INTRACORTICAL INFUSIONS OF THE IMMUNOTOXIN 192 IgG-SAPORIN. M. Sarter*[1], L.A. Holley[1], R.G. Wiley[2], and D.A. Lappi[3]. [1]Dept. Psychology, Ohio State Univ., Columbus, OH 43210, [2]DVAMC & Depts. Neurol. & Pharmacol., Vanderbilt Univ., Nashville, TN 37212, and [3]Whittier Inst., La Jolla, CA 92037.

192 IgG-saporin consists of a monoclonal antibody to the rat NGF receptor coupled to saporin, a ribosome-inactivating protein (Wiley 1992). Thus, intracortical infusions of 192 IgG-saporin were expected to result in the internalization of this toxin by cholinergic terminals and the selective destruction of cholinergic afferents. Rats received multiple unilateral intracortical infusions (1 or 0.5 μl/infusion) of 0.01 or 0.05 μg 192 IgG-saporin/1 μl Dulbecco's saline (vehicle was infused contralaterally). Parallel brain sections were stained for cells (cresyl violet), AChE (Kutscher 1990), and normal fibers using Vogt's (1974) reduced silver stain. Nissl-stains did not reveal any effect of the toxin in the cortex. AChE-stained sections demonstrated the concentration and volume-dependent loss of cortical cholinergic inputs. Loss of cholinergic fibers and cells was also observed in the basal forebrain. The extent of the lesion-induced reduction of cortical cholinergic fiber density appeared to account for the decreases in silver-stained normal axons, suggesting a selective loss of cholinergic inputs. 192 IgG-saporin appears to be a significant tool for the examination of the effects of selective cholinergic deafferentation.

377.13

THE PHOSPHATASE INHIBITORS CALYCULIN A AND OKADAIC ACID ALTER PRESYNAPTIC CHOLINERGIC FUNCTION IN RAT BRAIN

A.M. Issa* S. Gauthier and B.Collier. Depts. Neurology & Pharmacology, McGill University, Montreal, Canada H3G 1Y6

It is generally accepted that a tight coupling exists between acetylcholine (ACh) synthesis, storage and release. The underlying mechanisms involved in the process are not well understood, although protein phosphorylation and dephosphorylation have been implicated. We examined the effects of the serine/threonine phophatase inhibitors okadaic acid (OKA) and calyculin A (CA) on parameters of central cholinergic function in brain slice and synaptosome preparations from adult male Sprague Dawley rats. Both OKA and CA produced significant depletion of endogenous ACh content in brain slices in a concentration-dependent manner. OKA (10^{-8} M) depleted the endogenous ACh content of hippocampal slices by 49% and CA (50 nM) produced a 53% depletion. This finding suggested that inhibition of phosphatases 1 and 2A (PP 1 & 2A) by OKA or CA could produce ACh content depletion by either increasing ACh release or inhibiting ACh synthesis. Spontaneous and K^+-evoked ACh release were not directly affected by either OKA or CA ($p>0.05$). Studies were undertaken to determine whether inhibition of PP 1 & 2A would alter choline acetyltransferase (ChAT) activity or high affinity ^{3}H-choline uptake, two parameters involved in the modulation of ACh synthesis. Both of these parameters were significantly decreased ($p<0.05$ and $p<0.001$ respectively) in a concentration-dependent manner. In slices pre-loaded with ^{3}H-choline (1 uM), CA (50 nM) produced an 69 % depletion of ^{3}H-ACh content. This decrease was comparable in magnitude to the inhibition of high affinity ^{3}H-choline uptake produced by the same concentration of CA. Taken together these results suggest that inhibition of PP 1 & 2A may deplete tissue ACh content by inhibiting high affinity choline uptake and subsequent ACh synthesis.

377.14

VITAMIN E ATTENUATES THE EFFECTS OF BOTH REVERSIBLE AND IRREVERSIBLE INHIBITORS OF HIGH-AFFINITY CHOLINE TRANSPORT *IN VIVO*.

T.J. Walsh*, R.W. Stackman & A.C. Bartolomeo. Department of Psychology, Rutgers University, New Brunswick, NJ 08903.

High-affinity choline transport (HAChT), the rate limiting step in the synthesis of acetylcholine (ACh), is modulated by a variety of endogenous factors, including free fatty acids such as arachidonic acid and the enzyme phospholipase A2 (PLA2) (Boksa et al., *J. Neurochemistry* 1988; Saltarelli et al., *J. Neuroscience* 1990). Several compounds have been shown to inhibit HAChT in either a reversible or irreversible manner. AF64A produces an irreversible inhibition of HAChT, a long-lasting cholinergic hypofunction and related cognitive deficits after icv injection. Vitamin E (VE) pretreatment attenuates the behavioral and neurochemical toxicity of AF64A (Walsh et al., SFN Abs. 1992). VE has a variety of biological effects that might alter the dynamics of HAChT and the drugs that bind to this site. For example, VE inhibits (i) oxidative stress and lipid peroxidation, (ii) arachidonic acid synthesis, and (iii) PLA2 activity. The following studies examined the effects of VE on the decrease in HAChT induced by either AF64A, a irreversible inhibitor of HAChT, or hemicholinium-3 (HC-3) a reversible inhibitor. Adult male rats were pretreated with VE (50 mg/kg, i.m.) 24 hrs and 15 min before icv AF64A (0.75, 1.5, 3.0 nmoles) or CSF. AF64A (1.5 & 3.0 nmoles) produced significant 25-40% decreases in hippocampal HAChT that were attenuated by the VE pre-treatment. In a second experiment, rats were pretreated with VE as above, and infused icv with HC-3 (20 μg) or CSF. HAChT in HPC was assessed 30 mins, 4, 12, or 24 hrs after infusion. HC-3 produced a time-dependent decrease in HAChT that recovered within 24 hrs and was significantly attenuated by VE. VE attenuated the effects of both a irreversible and a reversible inhibitor of HAChT without affecting uptake in CSF-injected controls. The mechanisms underlying this effect of VE are currently being explored.

Supported by a Busch Bequest Grant to TJW.

377.15

IMMORTALIZATION OF A CHOLINERGIC CELL LINE DERIVED FROM MURINE CORPUS STRIATUM

M.S. Wainwright*, W-Y Wang, L. A. Won and A. Heller Dept. of Pharmacol. and Physiol. Sci., The University of Chicago, IL 60637.

A cholinergic clonal neuronal cell line was generated from embryonic mouse corpus striatum (CS) by somatic cell fusion techniques. Embryonic day 18 CS cells were fused with the murine neuroblastoma N18TG2 and 23 fusion products were generated. One cell population expressing choline acetyltransferase (ChAT) activity was subcloned. Among the 27 subcloned cell lines, one cell line (X52) expressed substantial ChAT activity and was further characterized. This cell line expressed markedly higher levels of ChAT activity (921.3 ± 97.4 pmol/min/mg protein) than the parent N18TG2 cells (4.3 ± 0.4). The hybrid cells, in contrast to the N18TG2 cells, contain acetylcholine (ACh) (49.6 ± 4.2 ng/mg protein). The hybrid cells spontaneously release ACh which is increased by exposure (20 min) to 70 mM K^+ While both the hybrid cell line and the N18TG2 cells exhibit sodium-dependent choline uptake, this is partially inhibited in the CS cell line, but not the N18TG2 cells, in the presence of a specific choline uptake inhibitor (hemicholinium-3, 100 µM). Following treatment for 2 days with 10 µM forskolin or 5 days with 1 mM n-butyrate, the hybrid cells express neurite-like processes which are immunoreactive to the neuronal marker, neurofilament protein (NFP) but not to glial fibrillary acidic protein (GFAP). These results suggest that this immortalized cell line exhibits some of the properties of fetal CS cholinergic interneurons and may facilitate study of cholinergic function in the developing CS.

Supported by MH 28942 and MH 10262.

ACETYLCHOLINE: ChAT AND AChE

378.1

EXPRESSION OF CHOLINE ACETYLTRANSFERASE WITHIN SPINAL MOTORNEURON FOLLOWING BRACHIAL PLEXUS AVULSION.

Y. Wu, J.K. Terzis, W. Wu, K. Han, W. Bass*. MRC, EVMS, Norfolk, VA.

Total avulsion of brachial plexus is a devastating lesion with major neuronal loss. Previous research in our laboratory revealed that immediate reimplantation of PNS tissue into the rat spinal cord following total plexus avulsion allowed the return of motor function (Han and Terzis,1991). However, the ability of avulsed motor neurons to synthesize neurotransmitter has not been addressed. Immunohistochemistry was used to examine the expression of the choline acetyltransferase (ChAT) within spinal motorneurons over time following brachial plexus avulsion in 22 Sprague-Dawley rats. The results demonstrated that the intensity of the immunoreactivity of ChAT in neuropil in the lesion side increased during the first three days after avulsion, then gradually decreased. The mean number of positive neurons per section is decreased over time post-avulsion; positive neurons on the avulsion side versus the normal side is 84.4% within the first three days following avulsion, 49.7% in the second week, 44.1% in the third week and 24.8% in the sixth week. The reduction of the number of the ChAT-positive neurons was permanent.

378.2

CHOLINE ACETYLTRANSFERASE EXPRESSION IN *DROSOPHILA* cDNA TRANSFORMANTS.

K.Yasuyama, T.Kitamoto* and P.M. Salvaterra. Division of Neurosciences, Beckman Research Institute of the City of Hope, 1450E. Duarte Rd. Duarte, CA 91010

Using a monoclonal antibody specific for *Drosophila* choline acetyl-transferase (ChAT), we have examined the distribution of ChAT-like immunoreactivity (ChAT-IR) in the cephalic and the suboesophageal ganglion of *Drosophila* transformed with wild type ChAT cDNA. Expression of the transgenic ChAT cDNA was controlled by different lengths (7.4, 1.2 and 0.8 kb) of 5' flanking ChAT genomic DNA. Transgenes were carried in animals with a presumptive null genetic background for endogenous ChAT alleles in order to assure that all staining could be attributed to transgenic ChAT. Wild-type flies show a widespread and predominantly neuropile distribution of ChAT-IR. Only a few cell somata, such as bilaterally paired cells in the posterior portion of the protocerebrum, are consistently stained. Some of the same structures stained in wild type flies (e.g., antennal lobe, the noduli and the bilaterally paired somata) also show ChAT-IR in cDNA transformants. In other parts of the ganglia, for example in the central complex, we see distinctive differences in staining of transformants when compared to wild type flies. Although the overall pattern of optic lobe ChAT-IR is preserved in most transformants, different 5' flanking DNA appears to result in altered amounts of ChAT-IR in various layers of medula, lobula and lobular plate. Our observations support the hypothesis that different parts of the 5' flanking region of the ChAT gene contain seperable regulatory elements for various subsets of cholinergic neurons. (Supported by NIH-NIDS)

378.3

HYDROPHILIC AND AMPHIPHILIC FORMS OF CHOLINE ACETYLTRANSFERASE EXIST IN *DROSOPHILA* AND ARE ENCODED BY A SINGLE mRNA. N. Salem, J. Medilanski, N. Pellegrinelli, M Schorderet* and L. Eder-Colli. Department of Pharmacology, CMU, 1211 Geneva 4, Switzerland.

We showed that in the nervous system of the fly *Drosophila melanogaster* choline acetyltransferase (ChAT) exists as hydrophilic and amphiphilic activity. This was based on the demonstration of different interactions with non denaturing detergents. Sequential extraction of *Drosophila* heads produced low salt soluble (87%) and detergent soluble (6%) activities. Sedimentation analysis of detergent soluble ChAT was found to be influenced by the type of non-ionic detergent used (Triton X-100 and Brij 96), whereas this was not the case for soluble ChAT. Using Triton X-114 fractionation of *Drosophila* heads, we found that 76% of the total ChAT activity is hydrophilic and 12% is amphiphilic. We cloned a full length ChAT cDNA from *Drosophila* heads (4.2 kb) and expressed in *Xenopus laevis* oocytes and different type of mammalian cell lines (rat fibroblasts 3T3 and human neuroblastoma SK-NBE). Triton X-114 fractionation of injected oocytes and of transfected cells revealed that 10% and 4% of the total ChAT activity expressed partitioned as amphiphilic enzyme, respectively. Other enzyme activities were assayed among which lactate dehydrogenase (LDH), a soluble marker. Less than 1% of LDH partitioned in the detergent phase suggesting that the presence of amphiphilic ChAT was not due to contamination of the detergent phase by the aqueous phase. Some properties of the hydrophilic and the amphiphilic native and recombinant ChAT were analyzed. The two forms differ in their sensitivity to inhibition by increasing concentrations of acetylcholine and to heat inactivation. In conclusion, we have shown that an amphiphilic form of ChAT exists in *Drosophila* and that hydrophilic and amphiphilic ChAT activities appear to be encoded by a single cDNA.

378.4

THE HUMAN CHOLINE ACETYLTRANSFERASE GENE ENCODES TWO PROTEINS. D.D. Grosman,* M.V. Lorenzi, and W.L. Strauss. Dept. of Pharmacology, Univ. of Miami, Miami, FL 33101.

Two mRNAs are generated by alternative splicing of the primary transcript from the human gene for choline acetyltransferase (ChAT), the enzyme that synthesizes acetylcholine. Based on the sequences of the corresponding cDNA clones, the 2300 nt transcript was predicted to encode the 68 kD human ChAT enzyme. The sequence of the cDNA derived from the 6000 nt mRNA is identical to that corresponding to the 2300 nt mRNA, except for the absence of an 178 bp protein coding exon. This causes a shift in the frame of translation which predicts the synthesis of an ~27 kDa protein consisting of the 203 NH_2 terminal residues of the ChAT enzyme followed by a unique 9 amino acid carboxyl terminus. Based on differences in the amino acid sequences predicted from each transcript, peptides were synthesized for use in the production of polyclonal antibodies specific for each protein. As expected, an antibody raised to the product of the 2300 nt mRNA recognized the 68 kDa human ChAT enzyme. An antibody raised to the product of the 6000 nt mRNA detected a 27 kDa protein in homogenates prepared from human nucleus basalis, but not from a variety of peripheral tissues. The 27 kDa protein can be separated from the ChAT enzyme by either immunoprecipitation or gel filtration and lacks the ability to synthesize Ach. The abundance of the 27 kDa protein was inversely related to ChAT activity in regions of the human CNS known to contain cholinergic elements. These data demonstrate that human brain contains a protein that is immunologically related to ChAT. Possible roles for this protein in Ach synthesis are being examined.

378.5

CHOLINE ACETYLTRANSFERASE IS ASSOCIATED WITH SYNAPTIC VESICLES IN RAT HIPPOCAMPAL TISSUE. P.T. Carroll*, Department of Pharmacology, Texas Tech University Health Sciences Center, Lubbock, TX 79430.

The goal of the present study was to determine whether the membrane-bound choline-O-acetyltransferase (EC 2.3.1.6.; ChAT) in rat hippocampal nerve terminals might be associated with synaptic vesicles. Initially, the subcellular distribution of ChAT was compared with that of occluded acetylcholine (ACh) in rat hippocampal nerve terminals subjected to sucrose density gradient centrifugation. Both non-ionically bound ChAT and occluded ACh peaked in the 0.4 M sucrose fraction of the gradient. This fraction contained almost all of the synaptic vesicle specific SV_2 protein. Immunobeads coated with an antibody directed against the SV_2 protein immunoprecipitated ChAT and occluded ACh from the 0.4 M sucrose fraction, but no other. Immunobeads coated with an anti-ChAT antiserum only immunoprecipitated an anti-SV_2 immunoreactive protein from the 0.4 M sucrose fraction. Pretreatment of the anti-ChAT Immunobeads with purified ChAT blocked this response. Identical results were obtained when the subcellular fractions were incubated with anti-ChAT Immunobeads and the immunoprecipitates probed for synaptophysin. These results suggest that some ChAT is non-ionically bound to synaptic vesicles in rat hippocampal nerve terminals. (2R01NS21289-09)

378.6

KN-62, A Ca2+/CALMODULIN KINASE INHIBITOR, REGULATES CHOLINE ACETYLTRANSFERASE ACTIVITY IN PC12 CELLS. P. W. Scates, C. R. Baitinger and H. L. White*. Div. of Pharmacology, Burroughs Wellcome Co., Research Triangle Park, NC 27709.

In order to determine whether Ca2+/calmodulin kinase II (CaM kinase II) may play a role in the regulation of choline acetyltransferase (ChAT), the effects of the specific CaM kinase II inhibitor, KN-62, 1-[N,O-Bis(5-isoquinoline sulfonyl)-N-methyl-L-tyrosyl]-4-phenylpiperazine, were studied in PC12 cells. Decreases in ChAT activity to approximately half of control values were observed following 48-hour incubations with KN-62 at 0.1-1 μM, a concentration range that corresponds with its inhibition of CaM kinase II. KN-62 at 10 μM did not inhibit ChAT directly when added to cell lysates or to partially purified enzyme. Increases in ChAT activity induced by nerve growth factor (NGF) were blocked by KN-62, while NGF-induced neurite outgrowth appeared to be enhanced. Incubation of PC12 cells with the serine/threonine phosphatase inhibitor, okadaic acid, also produced an increase in ChAT activity, which was completely blocked by 1 μM KN-62. At higher concentrations, KN-62 potentiated the toxic effects of okadaic acid so that cell viability was decreased. Protein phosphorylation patterns were obtained by SDS-PAGE to investigate whether phosphorylation states of ChAT, calmodulin kinase II, and other proteins might be correlated with changes in ChAT activity. We conclude that regulation of ChAT activity in PC12 cells, and perhaps in other systems, is at least partly mediated by calmodulin kinase II.

378.7

THE ALL-*TRANS* AND 9-*CIS* ISOMERS OF RETINOIC ACID (RA) STIMULATE ACETYLCHOLINE (ACh) SYNTHESIS BY INCREASING THE ABUNDANCE OF CHOLINE ACETYLTRANSFERASE mRNA IN A MURINE SEPTAL CELL LINE. W.A. Pedersen[1], B. Berse[2], U. Schüler[1], B.H. Wainer[3] and J.K. Blusztajn[1]*. [1]Boston Univ. Sch. Med., Boston, MA 02118, [2]Harvard Med. Sch. Boston, MA 02215, and [3]Albert Einstein Col. Med., Bronx, NY 10467.

We developed a cell line (SN56.B5.G4) derived from fusion of mouse septal neurons with murine neuroblastoma cells, N18TG2 [*Dev. Brain Res.* 52:219 (1990)], which exhibits several features of cholinergic neurons, including choline acetyltransferase (CAT) activity, sodium-dependent-high-affinity uptake of choline, and depolarization-evoked ACh release [*J. Neurosci.* 12:793 (1992)]. Treatment of the cells with 1 μM of either all-*trans* RA (which binds to the RA receptor) or 9-*cis* RA (which binds to the retinoid X receptor) for 48 hours resulted in an increase in the expression of CAT mRNA by 3-fold that of controls. Both isomers (at 1 μM) increased the cellular levels of ACh and CAT activity by 3-fold. These effects were time- and dose-dependent (EC_{50} values were 20-30 nM for both isomers). A combined treatment with both isomers at concentrations evoking maximal stimulation of cellular ACh content had no additional effect. These results suggest that the cholinergic phenotype is enhanced similarly by activation of the retinoic acid and the retinoid X receptor response pathways.

Supported by: NSF BNS8808942, MH46095, NS25787 & Center for Alternatives to Animal Testing grants.

378.8

EFFECTS OF DITHIOBIURET (DTB) ON REGULATION OF TRANSMITTER SYNTHESIS AND RELEASE IN PC12 CELLS. LM Ireland* and WD Atchison. Dept. Pharmacol. Toxicol., and Neurosci. Program, Michigan State Univ., E.Lansing, MI 48824.

In rats, intraperitoneal administration of DTB appears to affect cholinergic motor neurons selectively, resulting in neuromuscular weakness. Sensory function seems to be largely unaffected by DTB. The apparent selectivity of DTB may result from a specific disruption of acetylcholine (ACh) release. PC12 cells were used to compare the effects of DTB on the content and release of endogenous stores of ACh and dopamine (DA), both of which are released by these cells. Levels of these neurotransmitters were measured using HPLC coupled to electrochemical detection. Incubation of PC12 cells with 50, 100 or 500μM DTB for 24 hr decreased K^+-evoked release of ACh to 60, 40, or 55% of control, respectively. The inhibition of K^+-evoked ACh release by 500μM DTB was potentiated by supplementing the culture media with choline chloride. Neither cellular nor vesicular ACh content was significantly altered by the same 24 hr incubation with DTB. Conversely, cellular DA levels were significantly decreased by 24 hr incubation with DTB. Both 20 and 50 μM DTB decreased total cellular DA content to 25% of control. Vesicular DA content was unaffected by these concentrations of DTB. DOPAC is the major metabolite of DA degradation by monoamine oxidase. Changes in DOPAC content reflect alterations in DA metabolism. Since DTB application tends to decrease both DOPAC and DA levels in the cell, this suggests that DA synthesis is diminished. In spite of decreased DA synthesis, K^+-evoked release of DA is unaltered by DTB. Thus, both ACh and DA stores are affected by DTB but only ACh release is disrupted. This work is supported by NIH grant NS20683.

378.9

SYNTHESIS AND RELEASE OF MUSCLE ACETYLCHOLINE FROM CLONAL CULTURES OF HUMAN SATELLITE CELLS.
J.P. Ternaux*[1], M. Hamann[2], M.C. Chamoin[1], A. Baroffio[2], P. Portalier[1], H. Widmer[2] and C.R. Bader[2] [1]: Unité de Neurocybernétique Cellulaire, UPR 418 CNRS, 13009 Marseille, France. [2]: Département de Physiologie, Centre Médical Universitaire, 1211 Genève 4, Suisse.

Purified satellite cells (SC) were obtained from various human skeletal muscles biopsies following cytometric cell sorting. Acetylcholine (ACh) was extracted with TCA within 12 hours after dissociation, before the start of proliferation. The chemiluminescent assay for ACh revealed the presence of 133 pmoles/10.000 SC (n=2). SC were manually cloned and cultured for proliferation during 4 to 8 weeks. Under such conditions, endogenous level of ACh was 63 ± 76 pmoles/well of 100.000 SC (n=9). Following fusion of the SC into myotubes, ACh content was 33 ± 22 pmoles/well (n=9). When cells were cultured in presence of an esterase inhibitor (10 μm phospholine, 24 hours), the ACh amount was enhanced respectively 2- and 3-fold in proliferative and differentiated state. Conversely, cultivating the cells in presence of bromoACh, a potent inhibitor of choline acetyl transferase (ChAT), gave a 54 ± 19 % (n=5) decrease of ACh content. Presence of ChAT was demonstrated in both proliferative and differentiated states using immunohistofluorescence method. Finally, release of ACh was detected in the supernatant of SC or myotube cultures, following a 15 min incubation. Measured levels of ACh were respectively 3 and 7 pmoles/well/min. These results demonstrate that SC and myotubes synthesize and release ACh. In addition, presence of ACh in freshly dissociated SC is in favour of the "in situ" existence of a muscular pool of ACh.

378.10

TRANSLATION OF THE 6000 NT TRANSCRIPT OF THE HUMAN CHOLINE ACETYLTRANSFERASE GENE MAY BE REGULATED.
A.C. Trinidad* and W. L. Strauss. Dept. of Pharmacology, Univ. of Miami, Miami, FL 33101.

A 2300 nt mRNA encodes human choline acetyltransferase (ChAT), the 68 kDa enzyme that synthesizes acetylcholine. Transcription of the human ChAT gene produces a second, 6000 nt, mRNA that encodes a 27 kDa protein of unknown function, but with high identity to the NH_2 terminus of the ChAT enzyme (D. Grosman *et al.*, this vol.). We have found that a ChAT enzyme positive human neuroblastoma cell line, CHP134, contains both the 2300 nt and 6000 nt ChAT gene transcripts, but does not express the 27 kDa protein. In contrast, the 27 kDa protein is readily detected on immunoblots of proteins from nucleus basalis, a brain region containing amounts of both ChAT mRNAs comparable to those measured in the CHP134 cells. These data suggest that the translation of the 6000 nt mRNA may be regulated. This hypothesis is supported by our inability to detect the 27 kDa protein in homogenates of human ventral spinal cord, which expressed ChAT enzyme (100 pMol Ach synthesized/min/mg protein) and contained both ChAT gene transcripts as measured by RT-PCR. We speculate that the apparent difference in translational efficiency observed between the 2300 nt and 6000 nt mRNAs may derive from the length of their respective 3'-untranslated regions (~200 nt for the 2300 nt mRNA vs. >5000 nt for the 6000 nt mRNA). To test this hypothesis we currently are evaluating the translation of the 27 kDa protein from cRNAs with truncated 3'-untranslated domains.

378.11

REGIONAL VARIATION OF ACETYLCHOLINESTERASE ACTIVITY AND SPECIFIC MRNA IN ADULT RAT BRAIN.
R. Rao, C. Koenigsberger, and S. Brimijoin*, Dept. of Pharmacology, Mayo Clinic, Rochester MN 55905.

Expression of acetylcholinesterase (AChE) varies by region across the nervous system. To investigate this phenomenon, we compared AChE activity with steady state levels of AChE mRNA in different parts of the adult rat brain. RNA was extracted with guanidine isothiocyanate from freshly dissected material, and PolyA+-RNA was isolated by chromatography on oligo-dT cellulose. Following denaturation and electrophoresis on 1.2% agarose, mRNA was blotted onto nylon membranes. Blots were exposed to Kodak XAR-5 film after incubation for 36-40 hr at 42°C with a random prime ^{32}P-labeled cDNA probe for murine AChE (bases 542-1140 of the full length cDNA). The AChE probe hybridized to a 2.4 kb mRNA transcript in all regions (a weaker 3.2 kb band was also seen). AChE mRNA bands were normalized to the density of cyclophilin mRNA bands in the same lanes. Densitometry of the 2.4 kb AChE mRNA showed the following regional abundance: cortex 1.0, striatum 2.4, cerebellum 10, medulla 10.6, thalamus 18, pons 32. Relative abundance of AChE activity in detergent-extracts of the same brain samples also varied greatly: cortex 1.0, striatum 8.0, cerebellum 1.1, medulla 2.7, thalamus 3.0, pons 2.8. Modest AChE activity in the pons contrasted with a high level of message. Even more striking was the low mRNA level of striatum despite the high AChE activity. Possibly, the pons is a net exporter of AChE, while much striatal AChE may be imported by axonal transport from elsewhere in the brain. Regional differences in AChE turnover could also explain these findings. (Supported by Grant NS29646).

378.12

EVOLUTIONARY ORIGINS OF CHOLINESTERASE DUALITY IN THE VERTEBRATES.
Leo Pezzementi*, David Sutherland, Michael Sanders, Weily Soong, and Harry Giles. Birmingham-Southern College, Birmingham, AL

Gnathostome vertebrates possess two evolutionarily related cholinesterases (ChE), acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE). AChE hydrolyzes acetylcholine at cholinergic synapses; the function of BuChE is unknown. The two ChE can be distinguished kinetically and pharmacologically. Differences between the two ChE appear to correlate with position on the phylogenetic scale; in lower gnathostomes, they resemble one another more closely. Also, in the agnathan vertebrates, the lamprey and the hagfish, as well as in the cephalochordate, amphioxus, only one ChE is found. These data have led to the suggestion that the two ChE have evolved as a result of a gene duplication event early in vertebrate evolution, with subsequent structural and functional divergence. To study this question, as well as the molecular basis of the distinction between the AChE and BuChE, we have undertaken a biochemical, pharmacological, and molecular biological investigation of the ChE activities of the hagfish *Myxine glutinosa*, and of the invertebrate chordate, amphioxus (*Branchiostoma* spp.). We have determined the substrate specificity of the enzymes and their inhibition by diagnostic AChE and BuChE inhibitors. We have also used the polymerase chain reaction with degenerate primers to clone 1kb fragments of the ChE genes from the hagfish and amphioxus, comparing these sequences with those of ChE from other vertebrates and invertebrates, and constructing a phylogenetic tree. Additionally, we have conducted a fine-structure analysis of the sequences in terms of crucial aromatic amino acids found in the catalytic site of ChE. The data have provided insights into the evolution of the enzymes and the molecular basis of AChE and BuChE activity. Supported by NSF-RUI grant BNS-9010710 and an NIH-French CNRS Fellowship to L.P.

378.13

HISTOCHEMICAL, BIOCHEMICAL, AND PHARMACOLOGICAL STUDIES OF ACETYLCHOLINESTERASE IN THE NEMATODE *CAENORHABDITIS ELEGANS*.
Y. KAMIYA*, S. HARADA, S. OKOYAMA, H. YAMAMOTO & R. HOSONO. Dept. of Biochemistry and Dept. of Anatomy, School of Medicine, Kanazawa University, Kanazawa, Ishikawa 920, JAPAN.

In *Caenorhabditis elegans* [*C. elegans*], there are three kinetically distinct acetylcholinesterase [AchE] classes (A, B and C). To investigate the role of each class, developmental changes in AchE activity, acetylcholine [Ach] levels, and the sensitivity to AchE inhibitor trichlorfon were examined in mutants which possessed only one of the three AchE classes. Both class A and class B AchE activity were about one half of the total and sufficient for normal locomotion and maintaining normal Ach levels. Class C AchE occupied less than 2% of the total activity and was insufficient for normal locomotion and Ach levels but insensitive to trichlorfon. Although class A AchE localized at the head region, class B enzyme was distributed throughout the head and the body region as seen in wild type. Class C AchE localized at the body region. These results suggest that class B AchE plays a major role, and class A and class C are supplementary.

378.14

EFFECTS OF DIMETHOXY BENZYLIDENE ANABASEINE (DMXB) AND NICOTINE ON AChE EXPRESSING SEPTAL NEURONS IN FIMBRIAL/FORNIX TRANSECTED ANIMALS.
K.S.PANICKAR*, M.A.KING, E.M.MEYER[1] and B.E.HUNTER. Department of Neuroscience., and [1]Department of Pharmacology and Therapeutics, University of Florida College of Medicine, Gainesville, FL 32610-0244.

Nicotine has been shown to have cytoprotective actions in the brain. We therefore conducted a preliminary study using DMXB, a nicotinic agonist, to investigate if DMXB could protect cholinergic neurons in the septum following fimbria-fornix lesions. In the present study 12 male Long-Evan rats were surgically operated for unilateral fimbria-fornix lesions. Following the lesions the animals were injected daily intraperitoneally with 1 mg/ml DMXB (n=4) or 0.2 mg/ml Nicotine (n=4) or saline (n=4). Two weeks following the lesions the animals were sacrificed and the brains removed. Brain sections approximately 40 microns thick were cut and stained using AChE histochemistry. Neurons in the medial septum were counted using a computer-assisted program. Results show that there was a decrease in the number of cells in the ipsilateral side of the lesioned animals when compared to the contralateral side in all three groups. The results from this preliminary study suggest that DMXB does not appear to have a cytoprotective action at this dose. However, there was an increase in the number of stained cells in the unlesioned side in the DMXB and Nicotine groups when compared to the control group suggesting a possible upregulation of this enzyme. Further studies using a wide range of doses of DMXB are suggested. Supported by NIA grant PO1AG10485 and Taiho Pharmaceuticals.

378.15

AB-21: A NOVEL BISQUATERNARY DIOXIME FOR THE REACTIVATION OF ORGANOPHOSPHORYL-INHIBITED AChE. G. Amitai*, L. Raveh, I. Rabinovitz, G. Cohen, B. Manistersky, G. Zomber, R. Adani, Y. Harari and H. Leader, IIBR, Ness Ziona, Israel.

Combined treatment with oximes and atropine confers antidotal protection against highly toxic organophosphorus (OP) poisoning. We have developed a series of quinuclidine containing bisquaternary pyridine mono-oximes designated AB-oximes. The protection ratio (PR) obtained against soman poisnoning for AB-8 was higher than AB-13 in guinea pigs and dogs. AB-13 is a more potent reactivator of tabunyl-AChE and more efficacious against tabun poisoning. A new dioxime compound: 1-(2,4-dialdoxime pyridine) 1'-(3-quinuclidinone) dimethylether dichloride (AB-21) was prepared. AB-21 was designed as a molecular combination of AB-8 and AB-13 so that it will be equi-efficacious against both soman and tabun. AB-21 reactivates tabunyl-FBS-AChE at a higher rate than AB-13 and HLo-7 with the following reactivation bimolecular rate constants (k_r) 48 ,12 and 18 $M^{-1}min^{-1}$, respectively. Diethylphosphoryl-FBS-AChE was reactivated rapidly by AB-21 (k_r=111 $M^{-1}min^{-1}$). Antidotal studies in mice conducted with AB-21 (40 mg/kg, im) together with atropine and benactyzine as post exposure treatment and pyridostigmine as pretreatment demonstrate protection against soman (PR=5.1) and tabun (PR=2.8). Thus, the introduction of two oxime groups into the pyridine ring in AB-21 enhances reactivation potency for inhibited AChE and may increase its antidotal efficacy against OP poisoning.

EXCITATORY AMINO ACIDS: ANATOMY AND PHYSIOLOGY II

379.1

NON-NMDA GLUTAMATE RECEPTOR (GluR) SUBTYPES IN MONKEY HYPOTHALAMUS ARE SELECTIVELY DISTRIBUTED. S.D. Ginsberg*, L.J. Martin, C.D. Blackstone, R.L. Huganir and D.L. Price. The Johns Hopkins Univ. Sch. of Med., Balto., MD 21205.

Glutamate is a physiologically dominant neurotransmitter within discrete hypothalamic circuits. However, the cellular targets for this excitatory synaptic input have not been evaluated systematically. In this study, antipeptide antibodies were used to assess the relative distributions of GluR subtypes throughout the rostrocaudal extent of the macaque hypothalamus (n=7). Antibodies were directed against AMPA (GluR1-GluR4), kainate (GluR6-GluR7), and metabotropic (mGluR1α) receptors. The results indicate that the monkey hypothalamus contains several discrete, differentially distributed populations of AMPA GluR-immunoreactive neurons. Specifically, GluR1-immunoreactive neurons are observed in the posterior hypothalamus and mammillary complex. GluR2/3-immunoreactive neurons have a broader distribution, including the medial preoptic area, arcuate nucleus, lateral hypothalamus, and mammillary complex. GluR4, GluR6/7, and mGluR1α are expressed minimally within monkey hypothalamus. In contrast, GluR3 immunoreactivity is found exclusively in a population of magnocellular neurons within the paraventricular, supraoptic, and accessory magnocellular nuclei and in axons of the hypothalamo-neurohypophysial tract. Double-labeling experiments suggest that GluR3 immunoreactivity is colocalized to a greater degree with oxytocin-containing neurons than vasopressin-containing neurons. The highly selective localization of GluR subtypes within hypothalamic circuits is likely to be related to physiological functions of excitatory synaptic interactions on neuronal targets that are believed to mediate diverse behaviors, including stress, reproduction, osmoregulation, and appetitive responses.

379.2

EXPRESSION OF THE GluR1 GLUTAMATE RECEPTOR IN DEVELOPING RAT BRAIN. C.D. Blackstone*, R.L. Huganir, D.L. Price, and L.J. Martin, The Johns Hopkins Univ. Sch. of Med., Balto., MD 21205.

Ionotropic glutamate receptors (GluR) are thought to have important functions in the developing brain and to harbor potent neurotoxic potential for neuronal damage in the immature central nervous system. This study evaluates, using immunoblotting and immunocytochemistry (ICC), the cellular localization and temporal expression of the GluR1 subunit of the AMPA receptor in developing rat brain. Immunoblots of brain homogenates with anti-GluR1 antibodies showed a single band of protein at 106 kD; there were no cross-reacting proteins with other molecular weights at any developmental stage. GluR1 was first detected at E15.5, and expression increased progressively during late embryonic and early postnatal periods. ICC showed that GluR1 expression within different brain regions is regulated developmentally and that distinct populations of cells show widely different temporal profiles of expression during maturation. In general, mature patterns of GluR1 localization are not reached until approximately postnatal day (P) 21. Cerebral cortex and hippocampus show low levels of GluR1 at P1 and a progressive growth-related enrichment, whereas striatum shows high levels of GluR1 at birth and a gradual reduction during maturation. In cerebellum, radial glial fibers show intense GluR1 immunoreactivity. GluR1 is highly expressed transiently by granule cells (P0-P11) and Purkinje cells (P13-P19), but, by P21, these neurons show scant GluR1 immunoreactivity. These results show that brain development in the rat is accompanied by both rapid and gradual changes in the regional and cellular expression of GluR1. These changes in AMPA GluR expression in the developing brain may be the result of cellular maturation or aspects of complex mechanisms that participate directly in cytodifferentiation.

379.3

NON-NMDA GLUTAMATE RECEPTOR (GluR) PROTEINS ARE EXPRESSED DIFFERENTIALLY IN SUBREGIONS OF THE HIPPOCAMPAL FORMATION IN RAT AND PRIMATE. L.J. Martin*, C.D. Blackstone, S.D. Ginsberg, R.L. Huganir and D.L. Price. The Johns Hopkins Univ. Sch. of Med., Balto., MD 21205.

GluR are essential for the physiological functions of hippocampal circuitry and are believed to mediate synaptic mechanisms of learning and memory. To evaluate the cellular and subcellular localizations of non-NMDA GluR in the hippocampal formations of adult rat and rhesus monkey, immunoelectron microscopy was employed using antibodies that detect AMPA receptor subunits (GluR1; a common epitope on GluR2 and GluR3; GluR3; and GluR4), high-affinity kainate receptor subunits (a common epitope on GluR6 and GluR7), and a metabotropic receptor (mGluR1α). In hippocampus, GluR1 and GluR2/3 were most abundant, GluR6/7 and mGluR1α were expressed moderately, and GluR3 and GluR4 expressions were low or not detectable. GluR1 and GluR2/3 were enriched within dendritic shafts and spines in CA1. CA2, CA3, CA4, dentate gyrus, and subiculum also showed intense immunoreactivity. Most pyramidal neurons expressed both GluR1 and GluR2/3. GluR6/7 was enriched selectively within proximal dendrites in the stratum lucidum of CA3 and in astrocytic processes enveloping synaptic complexes. mGluR1α was localized to nonpyramidal neurons and dendrites within the stratum oriens of CA1 and the polymorph layer of the dentate gyrus. GluR4 was detected within hippocampal astrocytes; hippocampal pyramidal neurons were not GluR4 immunoreactive. These results demonstrate that GluR subtypes are localized differentially to neurons (pyramidal and nonpyramidal) and glia within the hippocampal formation. We conclude that cellular targets within hippocampal circuits can utilize different molecular strategies to transduce glutamatergic synaptic influences.

379.4

ENTERIC NEURONS EXPRESS mRNA FOR THE NMDAR1 RECEPTOR. G.A. Burns* and K.E. Stephens. Department of VCAPP, Washington State University, Pullman, WA 99164-6520.

The distribution of glutamatergic neurons has been described in the central nervous system (Greenamyre, *et al., J. Neurosci.*, 1984 48:2133-44). More recent electrophysiological results indicate that glutamate may be an important excitatory neurotransmitter in the enteric system as well. Exogenous L-glutamate induces contraction of ileal longitudinal smooth muscle/mesenteric plexus preparations, principally via NMDA receptors (Wiley, *et al., Am. J. Physiol.*, 1991 261:G693-G700). However, an actual glutamatergic enteric neuronal subpopulation remains to be identified. In this study, samples from the jejunum, ileum, cecum, and descending colon of perfused, adult Sprague-Dawley rats were processed for *in situ* hybridization histochemistry. Serial, longitudinal (10μ) sections from these samples were hybridized with a 1.4 kb riboprobe, cleaved from a recently characterized NMDAR1 receptor clone (Moriyoshi, *et al., Nature*, 1991 54:31). The riboprobe was transcribed in the presence of digoxigenin-UTP. An enzyme-catalyzed nitroblue tetrazolium color reaction was employed to detect digoxigenin-labeled hybridization products. Enteric neurons expressing mRNA for the NMDAR1 receptor were present within myenteric and submucosal ganglia at all the sites sampled. Discrete, labeled nerve cell bodies were typically nestled among many non-reactive perikarya in any given ganglion. These data localize a population of neurons capable of manufacturing NMDA receptors to the enteric nervous system.

379.5

NMDA R1 RECEPTOR mRNA EXPRESSION IN THE HYPOTHALAMUS OF INTACT, CASTRATE AND DHT-TREATED MALE RATS. L.Kus*[1], A.J. Beitz[1], J.E. Kerr[2], R.J. Handa[2]. Dept. Vet. Pathobiology[1], Univ. of Minnesota, St. Paul, MN 55108 and Dept. Cell Biology, Neurobiology and Anatomy[2], Loyola Univ. Chgo., Maywood, IL 60153.

Glutamate is believed to be the main excitatory neurotransmitter within the hypothalamus. Administration of glutamate to hypothalamic fragments *in vitro* has been shown to stimulate the release of several hormones including GnRH, αMSH, AVP and somatostatin. This study examines the distribution of mRNA for the glutamate receptor subtype NMDA R1. Using a ^{33}P-dATP labeled antisense oligonucleotide probe, we examined the distribution and regulation of NMDA R1 mRNA in the hypothalami of intact, castrate, castrate-DHT (2.5 cm Silastic implant) and intact-DHT-treated adult male Sprague-Dawley rats. Rats were sacrificed 21 days after castration and brains were processed for *in situ* hybridization. In intact rats, the highest levels of NMDA R1 mRNA were observed in the supraoptic, suprachiasmatic and paraventricular nuclei; intermediate levels were observed in the arcuate nucleus, ventromedial nucleus and medial preoptic area; and low levels were observed in the bed nucleus, lateral preoptic area, lateral hypothalamic area and lateral septum. This distribution correlates with the known effects of glutamate on hormone secretion. Neither castration nor DHT treatment had an affect on NMDA R1 mRNA expression in any of the areas examined. This suggests that expression of NMDA R1 mRNA in the hypothalamus is not under the control of testosterone. (NSF BNS 9109226, NIH DA06687, DE06682, DC01086)

379.6

DIFFERENTIAL LOCALIZATION OF A METABOTROPIC GLUTAMATE (mGluR1α) AND THE NMDAR-1 RECEPTOR IN THE CEREBELLAR CORTEX: AN IMMUNOCYTOCHEMICAL STUDY. Z. Vidnyánszky[1], J. Hámori[1], D. D. Schoepp*[2], J. Mody[3], and T. Görcs[1]. [1]Neurobiology Laboratory, United Res. Org. Hung. Acad. Sci. & Semmelweis Univ., Budapest, Hungary, [2]CNS Research, Eli Lilly & Co., Indianapolis, IN, and [3]Depts. of Anesthesiology & Neurology, UT Southwestern Med. Ctr., Dallas, TX.

Polyclonal antibodies raised against the C-terminus of mGluR1α and NMDAR-1 receptors were used for the immunocytochemical localization of these receptors within the cerebellar cortex. mGluR1α was mostly found in Purkinje cells, though some immunolabeling was also observed in basket and Golgi neurons. Most conspicuous was the labeling of dendritic spines, and particularly of postsynaptic specializations in contact with the glutamatergic presynaptic terminals of parallel fiber axons. Golgi cell dendrites in the granular layer were also strongly stained, while granule cells, their processes, as well as glial cells remained unstained. Conversely, the NMDAR-1 antibody labeled the dendrites of granule cells, the presynaptic parallel fibers, and a moderate number of glial processes in the molecular layer.

The postsynaptic localization of the mGluR1α receptor at parallel fiber-Purkinje cell synapses may constitute the morphological basis for the long-term depression of synaptic transmission seen in the cerebellar cortex. The NMDAR-1 receptors are predominantly localized to another excitatory synapse: the synapse between the glutamatergic mossy terminals and granule cells.

Supported by the OTKA S-6066 (Z.V.), 26172 (J.H.), 1107 (T.G), NINDS grants NS-27528, and the Pimley Research Fund (I.M.).

379.7

CELLULAR LOCALIZATION OF NMDAR-1 IMMUNOREACTIVITY IN THE CNS. T. Görcs[1], Z. Vidnyánszky[1], Z. Katarova[2], J. Hámori,[1] and I. Mody*[3]. [1]Neurobiology Laboratory, United Res. Org. Hung. Acad. Sci. & Semmelweis Univ., Budapest, Hungary, [2]Biol. Res. Ctr. Hung. Acad. Sci., Szeged, Hungary, and [3]Depts. of Anesthesiology & Neurology, UT Southwestern Med. Ctr., Dallas, TX.

The physiology, pharmacology, distribution and molecular biology of NMDA receptors is relatively well established. However, no information is available about the ultrastructural localization of these receptors in the mammalian CNS. To determine the cellular localization of NMDA receptors, polyclonal antibodies were raised in rabbits against two unique extracellular domains of the NMDAR-1. The two antibodies immunolabeled the same molecular mass (≈100 kDa) protein band in Western blots of rat brain synaptosomal and microsomal membrane preparations. Light microscopic immunohistochemistry revealed a localization of NMDAR-1 immunoreactivity (NMDAR-1-IR) consistent with previous ligand autoradiography and *in situ* hybridization studies. For example, in the hippocampal formation, the CA1 and dentate regions were diffusely but heavily labeled whereas the CA3 region showed only light immunoreactivity.

At the electron microscopic level, the NMDAR-1-IR was confined to postsynaptic structures and subsynaptic apparatus of asymmetrical synapses. In the hippocampal formation, the immunoreactivity could be observed on dendritic spine heads. NMDAR-1-IR was never found to be associated with symmetrical synapses.

The availability of antibodies to NMDAR-1 and their use for simultaneous immunohistochemical studies with anti-mGluR antibodies will constitute a valuable tool in elucidating the ultrastructural correlates of long-term alterations in neuronal excitability such as LTP and LTD.

Supported by the OTKA 1107 (T.G), and NINDS grants NS-27528, NS-30549, and the Pimley Research Fund (I.M.).

379.8

CELLULAR LOCALIZATION AND LAMINAR DISTRIBUTION OF NMDAR1 mRNA IN THE RAT CEREBRAL CORTEX. F. Conti¶*, A. Minelli¶, M. Molnar°, and N.C. Brecha°. ¶Institute of Human Physiology, University of Ancona, Via Ranieri, I-60131 Ancona (Italy), and °Depts. of Anatomy and Cell Biology and Medicine, UCLA School of Medicine, Los Ageles, CA 90073 (USA)

The cellular localization and laminar distribution of NMDAR1 mRNA have been studied in the neocortex of adult rats by *in situ* hybridization using cRNA probes.

Sections incubated with ^{35}S-labeled sense RNAs were used for specificity controls and for assessing the background level using a semiquantitative analysis. The vast majority of neurons from these series were associated with 5 or less grains. This value was considered as the background level of labeling. In sections incubated with ^{35}S-labeled antisense RNAs, few cells were negative, some were associated with few grains, while a large number of cells were associated with numerous silver grains. The vast majority of cells not associated with silver grains were immunoreactive to a antibody to GFAP, thus indicating that astrocytes do not contain or have low levels of NMDAR1 mRNA. A semiquantitative evaluation of 2,789 neurons revealed that 1.4% were not associated with silver grains, and that the remaining 2,749 neurons were associated with a variable number of grains. The number of grains was counted and neurons were grouped into 5 classes: A (≤ 5 grains), B (6-15), C (16-25), D (26-35), and E (≥ 36). The number of neurons belonging to each of these classes were then evaluated as a function of cortical layer. In layer I most neurons were in classes A and B, whereas in layers II-III and V-VI the majority of neurons were in classes B-E. In contrast, most layer IV neruons were in classes A-C, while only a few were in class E.

These results indicate that virtually all cells containing NMDAR1 mRNA in the cerebral cortex of adult rats are neurons, that about 90% of cortical neurons express NMDAR1 mRNA and that they can be divided into several groups on the basis of the levels of mRNA. The existence of several classes of neurons expressing NMDAR1 indicates that the amount of mRNA, and thus the number of NMDA receptors, may be a critical factor for determining the effect of NMDA receptor activation, while the differential laminar distribution of the several classes suggests that NMDA receptors affect differentially the various stages of cortical processing.

379.9

THE DISTRIBUTION OF GLUTAMATE RECEPTOR SUBTYPES IN THE NORMAL HUMAN ENTORHINAL CORTEX.
J. T. Noga*, T.M. Hyde, R.C. Saunders, D.R. Weinberger, J.E.Kleinman. Neuropathology Laboratory, Clinical Brain Disorders Branch, NIMH, Washington, D.C. 20032

Glutamate receptors are involved in numerous fundamental brain processes. They may play a role in disease processes in Alzheimer's disease, hypoxic brain injury, amyotrophic lateral sclerosis, schizophrenia, and Huntington's disease. The entorhinal cortex (EC) is greatly affected in Alzheimer's disease and likely in schizophrenia; it receives and sends fibers linking associative sensory regions, limbic structures, and cortical regions. We examined the distribution of several glutamate binding sites (tritiated MK-801, CNQX, and kainic acid to label NMDA, AMPA, and kainate receptor sites, respectively; and tritiated D-Aspartate as a label for glutamate terminals) using autoradiography. Twenty micron thick coronal sections of normal human brain were taken from five post-mortem specimens throughout the rostral-caudal extent of the EC and hippocampus. Sections were incubated and apposed to film to make autoradiograms. Review of the autoradiograms, comparing specific to nonspecific binding, revealed the following: MK-801 specific binding was uniformly greater throughout EC without a selective laminar pattern; CNQX bound markedly in a consistently laminar pattern in superficial layer 3 and in layers 5/6 throughout the rostral-caudal extent of EC; kainate strongly labelled layers 5/6 of EC consistently, with less strong, nonlaminar binding in other layers; D-Asp labelled EC layers 1, superficial 3, and 5/6 in a consistently laminar pattern. We conclude that there is a differential mapping in human EC of the glutamate receptor subtypes studied.

379.10

IDENTIFICATION OF GLUTAMINE SYNTHETASE mRNA FROM RAT SPINAL CORD. B. Srinivasan* and K.E. Miller. Dept. Anatomical Sciences, Oklahoma Center for NeuroSciences, Univ. Oklahoma Health Sciences Center, Oklahoma City, OK 73190.

Glutamine synthetase (EC 6.3.1.2) s the enzyme that catalyzes the amidation of glutamate to glutamine and is a key component of the glutamate/glutamine cycle. Immunohistochemical studies have demonstrated that glutamine synthetase is localized to glia in the CNS (A. Martinez-Hernandez, K.P. Bell, M.D. Norenberg, Science 195:1356, '77). As part of a coordinated approach to the study of the genetic regulation of the glutamate enzyme system in the spinal cord, the present study examined the mRNA for glutamine synthetase using slot blot and Northern blot analysis. Spinal cords, brains, and kidneys from rats were rapidly removed, polyA+ mRNA was extracted, and a slot blot and Northern blot were prepared. ^{32}P-labelled glutamine synthetase cDNA (source: ATCC; 1.6 KB insert, plasmid pBR322, PstI site, D.M Burns,...,R.E. Miller, Bioch. Biophys. Res. Comm. 134:146, '86) was used to probe RNA blots. Glutamine synthetase mRNA was found in high abundance in spinal cord, brain, and kidney. The abundance in spinal cord was higher than our previous report for glutaminase mRNA. Northern blot analysis showed 3 bands: one corresponding to 6Kb and dimers of 2Kb and 1.8Kb. In situ hybridization studies are in progress to localize glutamine synthetase mRNA in spinal cord sections. Supported by NS27213 (KEM).

379.11

COLOCALIZATION OF TYROSINE HYDROXYLASE AND GLUTAMATE IN LOCUS COERULEUS: A FLUORESCENCE IMMUNOHISTOCHEMICAL STUDY IN THE RAT, MOUSE, AND KITTEN. V.K. Reddy, S.J. Fung*, R.-H.Liu, Z. Wang and C.D. Barnes. Dept. of VCAPP, Washington State University, Pullman, WA 99164-6520. The central noradrenergic nucleus locus coeruleus (LC) with its diverse axonal trajectories is well-known for its chemically heterogeneous nature (Reddy et al., *Prog. Brain Res.* 88: 103, 1991). In addition to the peptide cotransmitters, the putative excitatory transmitter glutamate (GLU) has also been demonstrated in the LC neurons. In this study we attempted to compare the extent of colocalization of tyrosine hydroxylase (TH) and GLU in LC neurons of the mouse, rat and kitten. Subsequent to perfusion, frozen (coronal) sections of the brainstem were processed, under proper immunostaining control conditions, by the method of Wessendorf and Elde (*J. Histochem. Cytochem.* 33: 984, 1985). Further experiments were performed, in rats, to demonstrate the colocalization of TH and GLU in coeruleo-cortical neurons by using the combined retrograde tracing (with rhodamine-labeled latex microspheres) and dual immunofluorescence labeling techniques (Zhuo et al., *J. Chem. Neuroanat.* 5: 1, 1992).

TH neurons that contain GLU were present in the LC of all three species. In addition, in the kitten such colocalization of TH and GLU was also evident in several nuclear groups of the dorsolateral pontine tegmentum. Preliminary data from these studies indicate that a majority (90%, kitten; 75%, mouse; 56%, rat) of TH immunoreactive neurons in the LC also cocontain GLU. Our data in the rat also demonstrated a substantial population of coeruleo-cortical neurons to cocontain TH as well as GLU. These data provide an anatomical substrate for LC regulation of its target neurons by the release of GLU and NE at axon terminals.

379.12

N-ACETYLASPARTYLGLUTAMATE (NAAG) STUDIED IN MEDIAL PREFRONTAL CORTEX IN RATS USING IN VIVO MICRODIALYSIS. S. Fuhrman* and J. H. Neale. Department of Biology, Georgetown University, Washington, DC 20057.

N-acetylaspartylglutamate (NAAG), a highly prevalent peptide in the vertebrate nervous system, may function in neurotransmission. It is hydrolyzed to N-acetylaspartate (NAA) and glutamate by a membrane-bound peptidase, which has been shown to be extracellular in brain cell cultures. This peptidase may inactivate NAAG after synaptic release of the peptide. Conversely, NAAG may act as an alternate source of glutamate.

The level of NAAG in the extracellular space was studied using the technique of in vivo microdialysis to sample the extracellular fluid. Using anesthetized rats, microdialysis probes were stereotactically inserted into the medial prefrontal cortex, while the probes were perfused with artificial cerebrospinal fluid. Microdialysate fractions were collected and assayed for NAAG using a radioimmunoassay. The concentration of NAAG in the dialysate was approximately 0.5 μM at a perfusion flow rate of 1 μl/min. The basal NAAG level in the extracellular space was calculated to be approximately 10 μM, based on the relative recovery of NAAG from a standard concentration of NAAG in vitro at room temperature. The extracellular NAAG concentration in cortex may be compared with the overall concentration in microdissected samples of cortex of approximately 330 μM (4.7 nmol/mg soluble protein).

Using microdialysis, the synaptic release of NAAG, glutamate, and NAA, as well as the role of peptidase activity against NAAG, will be investigated in the cerebral cortex of the rat.

379.13

DISTRIBUTION OF N-ACETYL-ASPARTYL-GLUTAMATE (NAAG)- AND NAALADASE-IMMUNOREACTIVITIES IN PERIPHERAL NERVOUS SYSTEM. U. V. Berger*, R. E. Carter and J.T. Coyle. Laboratory of Molecular and Developmental Neuroscience, Massachusetts General Hospital, Boston, MA 02129.

The acidic dipeptide NAAG is localized to neurons, released upon electrical stimulation and catabolized to free glutamate by the peptidase, NAALADase. Though the function of NAAG in the CNS is unknown, it may serve as a neurotransmitter, neuromodulator and/or precursor for synaptic glutamate. Several studies have described the distribution of NAAG and NAALADase in the CNS, yet little is known about their localization and function in the peripheral nervous system. Thus, the anatomical distribution of NAAG and NAALADase was investigated in rat retina, dorsal root ganglion (DRG), sciatic nerve, and phrenic nerve at the level of diaphragmatic innervation, using an affinity-purified polyclonal antiserum against NAAG and a specific polyclonal antiserum against NAALADase. Consistent with previous findings in retina, NAAG-like immunoreactivity (LI) was seen in retinal ganglion cells, cells of the inner nuclear layer and in inner and outer plexiform layers. NAALADase-LI was also localized to inner and outer plexiform layers, supporting previous proposals that NAAG may have physiologic actions in the visual system. Consistent with previous findings in the DRG, NAAG-LI was present in ganglion cell bodies and in dorsal and ventral root fibers. NAALADase-LI was localized in DRG to a subset of Schwann cells and to satellite cells supporting the ganglion cells. In sciatic and phrenic nerves, NAAG-LI was observed in virtually all axons, while NAALADase-LI was localized to Schwann cells. In the diaphragm, both NAAG- and NAALADase-LI appeared to be present at the neuromuscular junction. These results suggest that in peripheral nerves NAAG is broadly distributed in axons, while NAALADase appears to be localized primarily to Schwann cells.

379.14

PTERIN-DEPENDENT MONOOXYGENASE AND NITRIC OXIDE SYNTHASE COFACTOR EXPRESSION: LOCALIZATION OF GTP CYCLOHYDROLASE I (GTPCH) mRNA IN THE RAT BRAIN. S.I. Lentz*, K. Hirayama and G. Kapatos. Cellular and Clinical Neurobiology Program, Dept. of Psychiatry, Wayne State Univ. Sch. of Med., Detroit, MI 48201

GTP cyclohydrolase I (GTPCH) is the first and rate-limiting enzyme in the tetrahydrobiopterin (BH4) biosynthetic pathway. BH4 is the essential cofactor for the pterin-dependent monooxygenases which include tyrosine and tryptophan hydroxylase, enzymes that are rate limiting in the biosynthesis of catecholamines and indolamines. BH4 is also required as a cofactor for the family of nitric oxide synthases. The *in situ* hybridization technique was used to study the cellular localization and the relative level of expression of GTPCH mRNA in the rat brain. Coronal sections of 10 μm were taken every 0.4 mm between the diencephalon and the myencephalon. A 302 bp coding portion of GTPCH cDNA was cloned and used to generate ^{35}S-CTP-labelled antisense cRNA probe for the hybridization reaction. For autoradiography, slides were dipped in nuclear track emulsion, exposed at 4°C for 4 to 6 weeks, developed, counterstained, and visualized under darkfield illumination. The nomenclature first described by Dahlström and Fuxe in 1964 was used to identify catecholamine and 5-hydroxytryptamine containing cell groups. Neurons containing GTPCH mRNA in the rat brain corresponded to the known monoaminergic cell groups. The level of GTPCH mRNA expression varied across cell groups with high levels seen in serotonergic neurons (B1-B9) and noradrenergic neurons (A6), moderate levels in dopaminergic neurons (A5, A7, A8, and A10), and low levels seen in dopaminergic neurons (A9, A12-A15). Under the hybridization and autoradiographic conditions used in this study, GTPCH mRNA could not be unequivocally localized to any cell-type within the olfactory bulb, cerebellum or hippocampus, brain regions known to contain large numbers of nitric oxide synthase-positive neurons. (Supported by NIH NS26081)

379.15

ESTRADIOL REGULATES HIPPOCAMPAL DENDRITIC SPINE DENSITY VIA AN NMDA RECEPTOR DEPENDENT MECHANISM. C.S. Woolley* and B.S. McEwen. Lab. of Neuroendocrinology, Rockefeller Univ., New York, NY 10021.

We have previously shown that the density of dendritic spines and synapses associated with dendritic spines on hippocampal CA1 pyramidal cells in the adult female rat are sensitive to ovarian steroids: Ovariectomy results in a decrease in spine and synapse density which can be prevented by administration of estradiol. Furthermore, there is a naturally-occurring fluctuation in the density of hippocampal spines and synapses as ovarian steroid levels rise and fall during the estrous cycle. These morphologic changes correlate well with estradiol effects on hippocampal excitability and seizure susceptibility.

The apparent lack of receptors for estradiol in CA1 pyramidal cells suggests that the effects of this hormone on spine and synapse density may be mediated indirectly, perhaps by an afferent population. In order to investigate the possibility that either excitatory amino acid or cholinergic neurotransmission is involved in the effects of estradiol on dendritic spine density, we have analyzed the density of dendritic spines on Golgi-impregnated CA1 hippocampal pyramidal cells in ovariectomized animals treated concurrently with estradiol and specific excitatory amino acid or cholinergic receptor antagonists. Treatment with the non-competitive NMDA receptor antagonist, MK 801 (0.2 mg/kg) inhibits the effect of estradiol on dendritic spine density. This result was confirmed using a competitive NMDA receptor antagonist. In contrast to treatment with NMDA receptor antagonists, treatment with either the AMPA receptor antagonist, NBQX (30 mg/kg) or the muscarinic receptor antagonist, scopolamine (2 mg/kg) fails to inhibit the effect of estradiol. These results demonstrate that the effects of estradiol in regulation of dendritic spine density require activation of NMDA, but not AMPA or muscarinic, receptors.

379.16

GLUTAMATE RELEASE IN RAT STRIATUM PROMOTED BY STIMULATION OF THE CORTICOSTRIATAL PATHWAY IN A COMPLEX SLICE PREPARATION. S. Bernath*, T.W. Berger[1] and M.J. Zigmond. Univ. Pittsburgh, Pittsburgh, PA 15260 & [1]Univ. Southern California, Los Angeles, CA 90089

Traditionally, *in vitro* studies of transmitter release use electrical and chemical stimuli which depolarize the entire preparation. We have developed an experimental preparation to study the release of glutamate by selectively stimulating the corticostriatal pathway in a complex slice. Horizontal slices (400 µm) containing the striatum and the adjacent anterior cortex of rat brain were cut with a Vibratome. Slices were transferred to a submerged plexiglass recording chamber, and maintained at a temperature of 35°C. Bipolar stimulating electrodes made from teflon-coated silver wire (50 µm in diameter) were placed in the subcortical white matter. Neuronal fibers were stimulated, and extracellular responses of single action potentials were recorded to test the integrity of stimulated pathway within the slice. Next, a glass capillary was positioned above the activated striatal area and 20 sec samples were collected. Corticostriatal fibers were stimulated twice for 30 sec by unipolar square wave pulses (0.6 mA, 100 µsec pulse duration, 10 Hz) with 25 min between the two stimulation periods. Samples were analyzed for their amino acid content by HPLC with fluorometric detection. Stimulation of afferent nerve fibers elevated the extracellular concentration of endogenous glutamate about 6-fold (5.7±1.4; n=4). A similar response usually was observed to the second stimulation. These results demonstrate that glutamate can be released via stimulation of glutamatergic nerve fibers. (Supported by MH45156, NS19608, MH00343, MH29670).

379.17

REGULATION OF 3H-D-ASPARTIC ACID (3H-D-ASP) UPTAKE IN TEMPORAL CORTEX AND HIPPOCAMPAL SLICES. M.J. MELDRUM *, N. NWANNA, P.M. PETTY, Dept. of Pharmacodynamics, Univ. of Florida, Gainesville, Fl. 32610

Endogenous excitatory amino acid induced excitotoxicity has been implicated in several neurodegenerative diseases. In previous studies on endogenous glutamate release using KCl as the stimulus, the release increased in a linear fashion up to KCl concentrations of 100 mM. This data suggested that depolarization with KCl may have an effect on the uptake system which was reflected as increased release. We have therefor tried to characterize the uptake system using 3H-D-Aspartic acid (3H-D-ASP)(an unmetabolized marker for glutamate uptake) in the same slice system used to measure release. Slices were prepared and preincubated for 30 min. in 1 ml Krebs-Ringer-Bicarbonate buffer at 37° C and bubbled with 95%O2/5%CO2. Total uptake was measured after a 5 min exposure to 3H-D-ASP (650,000 DPM). Non-specific uptake was measured in similar slices incubated at 4°C. At the end of the 5 min incubation period the slice were filtered rapidly over GF/C glass fiber filters and washed twice with 5 mls of ice-cold buffer. The slices were then placed in scintillation vials with 10 mls cocktail and vigorously vortexed several times. The vials then allowed to sit overnight were vortexed again and counted. Specific uptake was the total minus that at 4 ° C. Specific uptake was time and temperature dependent and dose dependently inhibited by glutamate and D-Threo-B-hydroxyaspartic acid. Uptake was inhibited in a dose dependent fashion by KCl (56-100mM), uptake was inhibited to the same extent with CH3COOK suggesting the role of potassium. Veratridine (20 uM) also inhibited uptake. The uptake was also very sensitive to O2 as either the bubbling of the buffer with nitrogen or the removal of the O2 tube from the solution decreased uptake to the same extant. These data suggest that the regulation of 3H-D-ASP uptake is very sensitive and can be modified easily and may need to be taken into account when glutamate activity in slices is measured. (Supported by Alzheimer's Association)

379.18

ATP RELEASES EXCITATORY AMINO ACIDS FROM CULTURED GLIA IN A CALCIUM-DEPENDENT MANNER. F. Liu*, K. Jeftinija, and S. Jeftinija, Department of Veterinary Anatomy and Neuroscience Program, Iowa State University, Ames, IA 50011, USA.

Although it has been shown that adenosine 5'-triphosphate (ATP) induces changes in intracellular calcium in glia, relatively little is known about the processes that are influenced by increases in intracellular calcium in glial cells. The specific objective of this study was to study the mechanism by which ATP releases excitatory amino acids (EAA) from cultured peripheral and central glia. Disassociated rat sciatic nerve schwann glia and cerebral cortical glia cultures were grown on polylysine coated coverslips. Cultures were mounted in a perfusion chamber and perfused at a rate of 200µl/min, with gassed Ringer solution at 36±1°C. Quantification of amino acids was performed by high performance liquid chromatography utilizing fluorescence detection and pre-column OPA-derivatization. Following a period of culture equilibration, 1min, 200µl samples were collected. Baseline concentrations of Asp and Glu in schwann glia cultures were 6.7±0.6nM (mean±SEM) and 26.6±2.1nM, respectively. Perfusion application of 100µM ATP for 1min to schwann glia resulted in an 179±4% increase of Asp and 212±10% increase of Glu release. A second applications of ATP 10 min after first application resulted in a increase in the release of both EAA s that was at the level of 42% of that from the first application. Five min application of ATP to glial resulted in a peak increase in the release followed by a decline to a plateau significantly higher than baseline release. Bath application of adenosine (100µM) was without effect on release of EAA suggesting involvement of P2 receptors. Similar results were obtained with cortical glia (>90% GFAP positive). The release of EAA evoked by ATP was not abolished in low Ca -EGTA solution. Pretreatment of the glia cultures with 50µM BAPTA-AM abolished the effect of ATP. Our results show that ATP selectively evokes the release of EAAs from cultured glia by activating intracellular calcium stores. Work was supported by NIH Grant NS27751.

379.19

SYNAPTOSOMAL PRESENCE OF THE GLYCINE-SITE AGONIST OF THE NMDA RECEPTOR, D-SERINE, VERSUS REGIONAL WHOLE-TISSUE LEVELS. M.L. Chouinard*, D. Gaitan and P.L. Wood.
Research Dept. Mayo Clinic, Jacksonville, FL 32224

Glycine, which has several metabolic fates and which modulates diverse neuronal signals is thought to serve as the most probable coagonist of the NMDA receptor. However, the recent discovery of endogenous D-serine (D-S), at levels rivaling free glycine in cortex of adult rat brain (Hashimoto et al. 1992, 1993), indicates that D-S needs to be assessed as an alternative NMDA receptor glycine-site agonist.

The observation of endogenous D-S in several rat brain regions has been extensively reconfirmed in our laboratory with highest levels observed in neocortex where D-S was 35% of the free L-S and levels. In an effort to further evaluate the tentative role of D-S as an excitatory transmitter, D-S was measured relative to L-S and glycine in two astrocytic cell lines (Cambier & Pessac, 1987) and in P2 synaptosomal preparations in an effort to localize specific cellular and subcellular pools of D-S. We observed extremely low levels of D-S in cerebellar type III (granular) and type II (molecular) layer-derived astrocytes, similar to levels in cerebellum, which coincides with the observation of high levels of D-amino acid oxidase in normal type II and type III astrocytes. Recently D-S has been proposed as the major phyisological substrate for D-amino oxidase in mouse cerebellum (Y. Nagata, 1992). The distribution of synaptosomal levels of D-S was similar to that observed in whole tissue with highest levels in cortex followed by hippocampus, striatum, septum and cerebellum. However, D-S levels in cortical synpatosomes, when expressed relative to L-S, were 15-19% higher than in whole tissue. Thus, considering the diverse metabolic fates of glycine and the synaptosomal levels of D-S relative to L-S (a direct glycine precursor), we conclude that D-S is a potential alternative to glycine as a NMDA receptor coagonist in several brain regions.

379.20

ASPARTATE (ASP) AND GLUTAMATE (GLU) MODULATION OF GH SECRETION IN THE PIG: POSSIBLE SITE OF ACTION. C.R. Barb*, R.M. Campbell, J.D. Armstrong, N.M. Cox. USDA-ARS, Athens, GA 30613, Hoffmann-LaRoche, Nutley, NJ 07110, North Carolina State Univ., Raleigh 27695 and Mississippi State Univ., Starkville 39762

In Exp I four female pigs each (70.6 ± 1.3 kg), received iv 50, 100 or 150 mg/kg BW of ASP or GLU. In Exp II OVX female pigs (163 ± 10 kg) immunized against GRF (1-29; n=4) or vehicle (V; n=5) received 150 mg/kg BW ASP or GLU iv in a 2 x 2 factorial design then repeated in a crossover design. In Exp III pig pituitary cells in culture were treated with 10^{-8}, 10^{-6}, or 10^{-4} M ASP or GLU. In Exp I GH response was greater ($P<0.01$) after ASP than GLU. Prior to treatment, GH was 1.8 ± 0.1 ng/ml but increased ($P<0.01$) to 3.8, 4.4, and 9.2 ng/ml (SEM = 0.4) and 2.4, 2.8 and 5.2 ng/ml (SEM = 0.4) after 50, 100 and 150 mg doses of ASP and GLU, respectively. In Exp. II GH increased ($P<0.01$) only in V pigs and avg. 1.2 ± 0.2 ng/ml before and 5.0 ± 0.4 ng/ml and 4.0 ± 0.3 ng/ml first h after ASP or GLU, respectively. In GRF pigs, GH avg. 1.2 ± 0.1 ng/ml and was unchanged. In Exp. III relative to controls (40 ± 6 ng/ml), GH increased ($P<0.01$) 1.9, 2.1 and 2.4 fold and 2.2, 2.2 and 2.5 fold after 10^{-8}, 10^{-6} and 10^{-4} M ASP or GLU, respectively. Therefore, ASP is a more potent secretagogue of GH secretion than GLU, while modulation of GH secretion may occur at both pituitary and brain.

EXCITATORY AMINO ACIDS: PHARMACOLOGY IV

380.1

***IN VIVO* MONITORING OF GLUTAMATE BY MICRODIALYSIS, CAPILLARY ELECTROPHORESIS AND LASER INDUCED FLUORESCENCE DETECTION.** L. Hernández,* S. Tucci, E. Murzi, X. Paez and T. Baptista, Laboratory of Behavioral Physiology, Medical School, Los Andes University, Mérida 5101, Venezuela

Low mass sensitivity of the analytical techniques hinders *in vivo* monitoring glutamate by microdialysis. HPLC and Electrochemical Detection has picomole (10^{-12} moles) detection limit. For this reason, long collection times (one or more minutes) and large sample volumes (5 microliters or more) are required, resulting in poor time resolution. In addition, high flow rates of perfusion (1 microliter/minute or more) cause neurotransmitter depletion around the probe [1]. Capillary electrophoresis with confocal laser induced fluorescence detection (CE-LIFD) has zeptomole (10^{-21} moles) detection limit [2]. Sample volumes for CE-LIFD are nanoliters or less. We successfully measured, with microdialysis and CE-LIFD, release of glutamate every five seconds in the striatum during prefrontal cortex electrical stimulation, release of glutamate in the nucleus accumbens every minute during amygdaloid kindling and release of glutamate every 20 minutes during acute administration of neuroleptics. After electrical stimulation of the prefrontal cortex or the amygdala, glutamate increased in striatal and accumbens dialysates. Acute haloperidol administration, decreased glutamate in striatal dialysates. CE-LIFD improves time resolution of brain microdialysis and lowers the perfusion flow rate to 100 nanoliters/minute or less, to prevent neurotransmitter emptiness around the probe.

1) Gonzalez-Mora, J.L et al. *Monitoring molecules in Neuroscience*,1991
2) Hernández, L. et al. *J. Chromatog.* 1991

380.2

IN VIVO RELEASE OF ARGININE FROM RAT VENTROBASAL THALAMUS EVOKED BY SENSORY AFFERENT STIMULATION. [1]K.Q.Do*, [2]S.A.Eaton, [2]K.E. Binns and [2]T.E.Salt, [1]Brain Res. Inst., Univ. of Zürich, CH;[2]Dept. Visual Science, Inst.Ophthalmology, London EC1V 9EL, UK

We have investigated the possibility that various amino acids may be transmitters of sensory transmission in thalamus. Dual cannulae were introduced in the ventrobasal thalamus of urethane-anaesthetised rats. This allowed extracellular recording of neurone activity in response to either electrical or natural stimulation of vibrissa afferents together with push-pull perfusion (15- 20 µl/min) of the surrounding brain tissue. Successive 1 minute perfusion fractions were assayed with precolumn derivatization HPLC which allowed detection of amino acids at the femtomole level. The extracellular level of arginine (Arg) was raised above levels already present in the perfusate (4-12 pmol/min) during or after a 4 minute period of either electrical or air jet stimulation within the receptive field of neurones which could be recorded near the perfusion site. In some experiments, it was also possible to detect increased levels of glutamate or aspartate during or after stimulation. In separate experiments, iontophoretic application of L-Arg onto thalamic neurones was found to inhibit responses to sensory stimulation, and iontophoretically applied NMDA or AMPA. However, on some neurones, it was possible to observe a facilitatory action of L-Arg at lower iontophoretic application currents. This stimulation-induced Arg release could reflect either an intercellular messenger role of Arg or an intercellular transfer of Arg, possibly as a precursor for nitric oxide, due to metabolic demand.

380.3

RELEASE OF EXCITATORY AMINO ACID NEUROTRANSMITTERS AND GLUTAMINE WITH AGE IN THE NEOSTRIATUM OF THE CONSCIOUS RAT. A. Porras, C.V. Gisolfi, and F. Mora.* Dept. Physiology, Faculty of Medicine, Univ. Complutense of Madrid, 28040 Madrid, SPAIN and Dept. Physiology, Univ. of Iowa, Iowa City, IA 52242, USA.

The effects of age on the extracellular concentrations of glutamate [GLU], aspartate [ASP], and glutamine [GLN] were studied in the neostriatum of the conscious rat. For that a modified continuous push-pull perfusion system was used. After baseline for levels of amino acids was established (50 min), 10 min samples were collected for a total time of 30 min. Groups of rats of 3-4, 12-13, 21-24, and 32-34 months of age were used. Amino acids were analyzed as OPA-derivatives with an HPLC-fluorometric detection technique. The extracellular [GLU] showed an age-related decrease ($r = -0.17$, $p < 0.05$, $n = 191$) while [GLN]= showed an age-related increase ($r = 0.19$, $p < 0.01$, $n = 198$). Extracellular [ASP] did not change during aging ($r = 0.08$, N.S., $n = 191$). Since astrocytes are the main source of GLN in the brain and also these cells are the main compartment for GLU reuptake, the increase in [GLN] and the decrease of [GLU] with age could indicate an increase of astrocyte activity and/or astrocyte proliferation in the neostriatum of the rat during aging. Also the possibility exists for the decrease of [GLU] be a consequence of the degeneration of the cortico-striatal glutamatergic pathway during aging.

Supported by DGICYT PB90-0252.

380.4

DIFFERENTIAL REGULATION AND ION DEPENDENCE OF GLUTAMATE AND ASPARTATE RELEASE FROM CA1 SYNAPTOSOMES. C.P. Duncan*, M. Zhou and J.V. Nadler. Depts. Pharmacology and Neurobiology, Duke Univ. Med. Ctr., Durham, NC 27710.

Slices and synaptosomes prepared from hippocampal area CA1 release both glutamate and aspartate in a Ca^{2+}-dependent manner. Previous studies of CA1 slices indicated that release processes for the two amino acids can be differentially altered. Because amino acid release studies in slices can be confounded by uptake processes and indirect effects, we continued this work with a CA1 synaptosome preparation. Synaptosomes were exposed twice for 1 min to 25 mM K^+; test solutions were substituted for control medium beginning 4 min before the second exposure to elevated K^+.

ISR,3RS-ACPD (100 μM) selectively depressed glutamate release. 1S,3R-ACPD and 1R,3S-ACPD (50 μM) were about equieffective, and the depression was reversed by 100 μM L-AP3. Aspartate release was significantly depressed by 1SR,3RS-ACPD and L-AP3 together, but not by either ligand alone. NMDA increased and D-AP5 depressed aspartate release, but neither ligand affected the release of glutamate. A 4-min exposure to Na^+-free medium (Na^+ replaced by choline and tris; 1 μM atropine) increased K^+-evoked glutamate overflow from CA1 slices by 12-fold, presumably due to prevention of uptake, but had no effect in the synaptosome preparation. In contrast, Na^+-free medium enhanced K^+-evoked overflow of aspartate from CA1 slices by only 4-fold and it reduced synaptosomal aspartate release by 56%. These results suggest that glutamate and aspartate release processes are regulated by different autoreceptor mechanisms and that aspartate release depends on Na^+ as well as Ca^{2+}. (Supported by NIH grant NS 16064.)

380.5

SLOW RHYTHMIC BURSTS PRODUCED BY CA3 NEURONAL CIRCUIT IN THE HIPPOCAMPUS. R. Bianchi* and R.K.S. Wong. Dept. of Pharmacology, SUNY-HSCB, Brooklyn, NY 11203.

In septo-hippocampal slices acutely isolated from adult guinea-pigs, we have recently shown that the muscarinic receptor activation by carbachol application or by electrical tetanic stimulation of the septal area induced slow rhythmic, synchronized depolarizations with spike discharge in CA3 hippocampal pyramidal cells (CA3 HPCs). Each bursting event (called 'phasic burst'; PB) lasted 8-50 s and repeated every 0.3-8.6 min. We observed a similar activity in CA3 HPCs of transverse hippocampal slices (18 out of 19) following addition of picrotoxin (50 μM) to a solution containing excitatory amino acid receptor blockers (CNQX and CPP, 10-20 μM each) and 4-aminopyridine (70 μM). Such activity consisted of action potential discharge elicited by depolarizations (PBs) that lasted 6-28 s and repeated rhythmically every 2.9-9.7 min for up to 3 hours. Simultaneous intracellular recordings from two CA3 HPCs and paired intra- and extracellular recordings showed that the PBs were synchronized in the CA3 field. Paired CA3/CA1 recordings showed PBs in both fields; each PB in CA1 followed the one recorded in CA3 by 1-6 s. If the Schaffer fibers were cut, PBs were recorded only in CA3 and not in CA1. Phasic burst activity was also observed in 'minislices' containing only the CA3 field. These data indicate that the CA3 hippocampal neural network is able to produce a rhythmic, synchronized, excitatory activity in the presence of ionotropic excitatory and inhibitory amino acid receptors blockers.

380.6

GLUTAMATE: POSSIBLE TRANSMITTER ROLE IN *APLYSIA* CNS JacSue Kehoe[1] and Ian Cooke*[2] [1]Laboratoire de Neurobiologie, Ecole Normale Supérieure, 75235 Paris Cedex 05, France; [2]Békésy Laboratory of Neurobiology, University of Hawaii, Honolulu, HI 96822.

It has been known for decades that L-glutamate (Glu) can activate at least three distinct responses (K^+, Cl^-, and cationic) on central molluscan neurons. Kehoe (1978) showed quisqualate (Quis) selectively activates the K^+- and ibotenate the Cl^--dependent response, whereas the cationic response was shown to be selectively transformed by conconavalin A (ConA). Recent work confirmed these findings and showed that the Quis-sensitive K^+ response could be blocked by argiopine (Bolshakov et al. 1991) and induced by an agonist (Katz & Levitan 1993) of the metabotropic response in mammalian neurons. Using classical methods of Glu application, the cationic response could be observed on only a few cells, and often only on axonal membrane. After exposure to ConA it was present on most if not all neuronal somata. We now find, using fast (<20 ms) perfusion-stream switching, that a transient cationic response is detected on almost all cells. Recording during application of ConA, we observe the transformation of the tran-sient to a sustained response; desensitization disappears. This receptor has resisted pharmacological characterization, being insensitive to agonists known to activate the five major categories of Glu receptors in mammalian neurons. However, CNQX, possibly a partial agonist on this receptor, selectively blocks this response. The revelation by the fast-perfusion technique of the prevalence of the cationic Glu response and use of new pharmacological tools has contributed to the identification in the buccal ganglion of *Aplysia* of presumed glutamatergic synapses. Kehoe JS (1978) Nature **274**:866; Bolshakov VY, Gapon SA, Magazanik LG (1991) J Physiol **439**:15; Katz P & Levitan IB (1993) J Neurophysiol **69**:143. IC was supported by a NIH-CNRS Fellowship.

380.7

ANESTHETIC ACTIONS ON GLUTAMATE AND $GABA_A$ PATHWAYS: COMPOUNDS WHICH VIOLATE THE MEYER-OVERTON RULE J.J. Kendig, E.I. Eger II, P. Ionescu, M.J. Laster, and R.G. Giffard*. Depts. of Anes., Stanford Univ. Sch. of Med., Stanford, CA 94305/UCSF, San Francisco, CA 94143

The Meyer-Overton rule relates general anesthetic potency to lipid solubility, but some compounds with large oil/gas partition coefficients are not anesthetics. To probe essential anesthetic actions, we compared two halogenated cyclobutanes, one an anesthetic and one not, on glutamate and $GABA_A$-mediated synaptic transmission in isolated neonatal rat spinal cord. 1-chloro-1,2,2-trifluorocyclobutane (Tri FCB) (1.7%) is anesthetic in rats (predicted minimal anesthetic concentration [MAC] 0.8%); 1,2-dichlorohexafluorocyclobutane (Hex FCB) (10%) is not anesthetic (predicted MAC 4.6%) and increases desflurane MAC by 30%. Spinal cords from 1 - 6 day old rats were arranged to record the monosynaptic reflex (MSR), AMPA-kainate; slow ventral root potential (sVRP), predominantly NMDA; and dorsal root potential (DRP), $GABA_A$. Tri FCB (0.8%) depressed sVRP (70%) and increased MSR latency with no effect on amplitude. Hex FCB (4.6%) slightly depressed sVRP, decreased MSR latency with no effect on amplitude and had no effect on DRP. Anesthetics previously tested markedly depress sVRP; some also enhance DRP. Depression of glutamate transmission may be essential to anesthesia, and increase in $GABA_A$ contributory. MSR latency decrease by Hex FCB may be an anti-anesthetic effect.

380.8

URETHANE DEPRESSES GLUTAMATE BUT DOES NOT ALTER $GABA_A$ TRANSMISSION IN NEONATAL RAT SPINAL CORD L. M. Gibbs*, A. P. Lozier, and J. J. Kendig, Dept. of Anes., Stanford Univ. Sch. of Med., Stanford, CA 94305

Urethane is an anesthetic commonly used in physiology but itself little studied. We compared urethane to other anesthetics in isolated spinal cord of neonatal rats. Cords were arranged to record the monosynaptic reflex (MSR), AMPA-kainate; slow ventral root potential (sVRP), largely NMDA; and dorsal root potential (DRP) evoked directly by muscimol ($GABA_A$) or through an interneuronal pathway from an adjacent dorsal root (glutamate and $GABA_A$). Urethane 20-40 mM reduced MSR amplitude and increased latency; 2-40 mM reduced sVRP amplitude and area under the curve. Muscimol evoked DRP was unaffected; dorsal root-evoked DRP was slightly reduced (20-40 mM). Effects were concentration-dependent and reversible. Isoflurane 0.2-1.2% reduced sVRP; 0.6-1.2% reduced MSR amplitude and increased latency. Isoflurane 1% increased muscimol-evoked but steeply reduced dorsal root-evoked DRP. Isoflurane is anesthetic at 1.4%; reported urethane anesthetic levels are 10-15 mM. Urethane is thus less effective than isoflurane in depressing AMPA-kainate pathways but acts similarly on sVRP. Unlike isoflurane, urethane does not affect $GABA_A$ receptors. Caution should be used in employing urethane as an anesthetic in studies in which glutamate, particularly NMDA, synaptic transmission is a factor.

380.9

TRIPLE WAVELENGTH AND DIFFERENCE SPECTROPHOTOMETRIC DETERMINATION OF NITRIC OXIDE: AN IN VITRO AND IN VIVO MICRODIALYSIS STUDY. A. Balcioglu* and T. J. Maher Mass. College of Pharm., Dept. of Pharmacology, Boston, MA., 02115.

We have previously developed and demonstrated the utility of an improved triple wavelength spectrophotometric technique for the quantification of nitric oxide (NO). The present study determined the applicability of this technique to microdialysis. Using a concentric microdialysis probe perfused with 1μM oxyhemoglobin, the exposure to the thiol-independent NO-generating agent sodium nitroprusside (1mM) enhanced the production of NO as determined by methemoglobin production. Additionally, we implanted such microdialysis probes into the CA3 region of hippocampus in urethane-anesthetized rats where the cordinates were AP - 5.3, RL 4.8, V 6.5 according to Paxinos and Watson. Following treatment with the known excitatory aminoacid receptor agonist, kainic acid (13mg/kg, i.p.) increased the methemoglobin concentration from 2.2 $\pm$ 0.1nM to 13 $\pm$ 1.2nM ($p<0.01$). Co-administration of the NO-synthase inhibitor, L-NMMA (50mg/kg, i.p.) attenuated the kainate-induced increase in NO concentration (3.8 $\pm$ 0.5nM) ($p<0.05$), compared to kainic acid.

These studies demonstrate the utility of hemoglobin trapping of NO in microdialysis applications, both in vivo and in vitro.

Supported in part by a grant from The Center for Brain Sciences.

380.10

ANTAGONISM OF KETAMINE-ANESTHESIA BY INHIBITORS OF NITRIC OXIDE SYNTHETASE. R.A. Mueller, M.D., Ph.D.,* R. Hunt, B.S., Dept. of Anesthesiology and Pharmacology, UNC at Chapel Hill, Chapel Hill, NC 27599.

Ketamine is a non-competitive antagonist of NMDA type glutamate receptors, and some NMDA receptor dependent responses utilize ntiric oxide as a second messenger system. It has been shown that acute administration of an inhibitor of nitric oxide synthetase (NOS), such as nitroarginine methylester (NOA), reduces the apparent MAC of rats to halothane (Anesthesiol. 77:779,1992). The present experiments were designed to see if inhibition of NOS by the administration of NOA would increase the sensitivity of rats to the anesthetic effects of ketamine.

Sprague-Dawley rats of either sex received NOA or saline 30 minutes before administration of i.m. or s.q. (25-75 mg/kg) ketamine. A behavioral scoring system was used at 15 minute intervals to assess the speed of onset and depth of ketamine anesthesia and return to normal behavior. Brain NOS was measured in various areas of brain by the method of Bredt and Synder (Proc. Nat. Acad. Sci. 87:682,1990). Ketamine blood levels were measured by HPLC with UV detection by a slight modification of previously reported methods (Anesthestist 41:619,1992).

Single or repeated (1/day x 3 days) administration of NOA (20 mg/kg i.v.) did not affect the time course of onset, depth or duration of ketamine anesthesia (25-75 mg/kg). Daily 50 mk/kg NOA i.p. for 2 days, and again 30 minutes before ketamine prevented the animals from losing their righting reflex to a ketamine dose of 75 mg/kg s.q. (all saline pretreated rats lost their righting reflex). NOS in vitro activity in cerebellum, cerebral cortex and caudate nuclei was inhibited only 40-50% ($p<.01$) after the repeated intravenous dosing schedule but over 85% in all areas ($p<.01$) given the i.p. repeated dosing schedule. Blood levels of ketamine were significantly different in the control and NOA pretreated rats given 75 mg/kg ketamine.

It is possible that NOA may alter blood levels of ketamine and explain the antagonistic effect of NOA on ketamine induced anesthesia.

380.11

DIRECT AND INDIRECT MEASUREMENT OF NITRIC OXIDE. A.K. Stout* and J.J. Woodward. Dept. of Pharmacology, Medical College of Virginia, Richmond, VA 23298.

Previous studies in our lab have utilized nitric oxide (NO) generators to determine the effects of NO on N-methyl-D-aspartate-stimulated release of preloaded tritiated norepinephrine from rat hippocampal slices. The relative NO-generating ability of these compounds was indirectly determined by measuring their ability to stimulate cyclic guanosine monophosphate (cGMP) formation in cultured cortical neurons. We have now directly measured NO production by these compounds using a commercially available NO microelectrode. In oxygenated Krebs buffer, S-nitroso-N-acetyl-D,L-penicillamine (SNAP) produced approximately ten times more NO than an equimolar solution of sodium nitroprusside (NP). Nitroglycerin solutions did not produce detectable amounts of NO, even in the presence of tissue. These results were in agreement with the cGMP data. NMDA-stimulation of brain tissue did not produce detectable amounts of NO as measured by the microelectrode. However, NMDA did stimulate significant increases in cGMP levels in neuronal tissue. These results suggest that the NO meters which are currently available may not be sensitive enough to detect NO generated by physiologically relevant mechanisms. Supported by NIAAA AA08089, DA 07027, and a grant from the Alcoholic Beverage Medical Research Foundation.

380.12

EMBRYONIC DEVELOPMENT AND POSTNATAL CHANGES IN FREE D-FORMS OF ASPARTATE AND SERINE IN THE HUMAN PREFRONTAL CORTEX. A. Hashimoto*, T. Nishikawa, S. Kumashiro, T. Oka and K. Takahashi. Precursory Res. for Embryonic Sci. and Tech. (PRESTO), Research Development Corporation of Japan, Tokyo, 100 Japan and Dpt. of Mental Disorder Res., Natl. Inst. of Neurosci., NCNP, Tokyo, 187 Japan.

We have measured free D-amino acids in the human frontal cortex of developing and aged brain (from 14 weeks gestation to 101 years of age) by HPLC with fluorometric detection. Extremely high contents of free D-aspartate (0.36 µmol/g, n=2) and D-serine (0.26 µmol/g, n=2) were demonstrated in the fetal cortex at gestational week 14. The content of D-aspartate dramatically decreased to a trace level by gestational week 41 and then remained very low during postnatal stages. In contrast, a persistently high level of D-serine was observed in the frontal tip throughout the embryonic and postnatal life. Because D-aspartate and D-serine are known to interact selectively with the N-methyl-D-aspartate (NMDA) type excitatory amino acid receptor, the present findings suggest that these D-amino acids might play a pivotal role in cerebral development and functions which are related to the NMDA receptor.

380.13

FURTHER EVIDENCE THAT THE PRIMING EFFECT OF QUISQUALATE IS MEDIATED VIA UPTAKE AND RELEASE OF QUISQUALATE. E.W. Harris,* CNS Biology Dept., Fisons Pharmaceuticals, Rochester, NY 14603

Quisqualate (QQ) has the curious ability to sensitize in vitro CNS tissue to certain glutamate analog, such as 2-amino-4-phosphonobutyrate (AP4): AP4 depolarizes quisqualate-treated tissue at ~50μM, but does not depolarize naive tissue at up to 1000μM. The AMPA/KA receptor blocker CNQX reduces depolarization by AP4 in QQ-treated slices, but does not block the induction by QQ of sensitivity to AP4. This evidence points to the involvement of a QQ-sensitive site other than AMPA /KA-preferring receptors, and other than the metabotropic QQ receptor, since only a small sensitization is induced by the metabotropic receptor agonist tACPD. The hypothesis that this puzzling phenomenon is mediated by an known uptake site for which AP4 and QQ are ligands has been investigated further.

In addition to being sensitive to AP4, hippocampal slices exposed briefly to QQ showed also exhibited "primed" sensitivity to the following known uptake-site ligands: D-α-aminoadipate, α-aminosuberate, cystathionine, L-cystine, and 2-amino-6-phosphonohexanoate (AP6). Furthermore, CNQX and pentobarbital, at concentrations that abolish responses to AMPA, only partially blocked the effects of QQ, and of AP4 in QQ-treated slices.

Inclusion of an uptake site ligand, such as AP6, greatly potentiated the effects of QQ (but not of AMPA), and reduced the induction of sensitivity to AP4. In addition, the effects of AP4 or AP6 faded with prolonged applications, but could be restored by re-"priming" with QQ. Finally, the sensitivity to AP4 persists longer if tested in a chamber in which buffer is recirculated than in a "flow-through" chamber continuously superfused with fresh buffer.

These data indicate that uptake and release of underlie "priming" effect of QQ.

380.14

UTILIZATION OF L-AP6 AS A SELECTIVE AGONIST FOR A UNIQUE QUISQUALATE-SENSITIZED RECEPTOR. M.K. Schulte[1], R.J. Roon[1]*, D.C. Sunter[2], and J.F. Koerner[1]. [1]Dept. of Biochemistry, Univ. of Minnesota, Minneapolis, MN 55455, and [2]Dept. of Pharmacology, Univ. of Bristol, BS8 1TD, UK.

Brief exposure of rat hippocampal slices to quisqualic acid (QUIS) sensitizes neurons to depolarization by the α-amino-ω-phosphonate EAA analogues AP4, AP5, and AP6. These phosphonates interact with a novel QUIS-sensitized receptor [Brain Res. 605, 85-92, (1993)]. While L-AP4 and D-AP5 cross-react with other EAA receptors, DL-AP6 is relatively selective for the QUIS-sensitized receptor. This specificity of DL-AP6, in conjunction with the apparent preference of the sensitized receptor for L-isomers, suggested that the hitherto unavailable L-isomer of AP6 would be a highly potent and specific agonist for this receptor. We have measured the pharmacological responses of KAIN/AMPA, NMDA, lateral perforant path L-AP4 receptors, and CA1 QUIS-sensitized receptors to the D- and L-isomers of AP4, AP5, and AP6. D-AP6 and L-AP6 were prepared by fractional crystallization of the L-lysine salt of DL-AP6. The D-isomers of AP4, AP5, and AP6 were 5, 3, and 15-fold less potent for the QUIS-sensitized receptor than their respective L-isomers. While L-AP4 and L-AP5 cross-react with NMDA and L-AP4 receptors, L-AP6 is highly potent and specific for the QUIS-sensitized receptor (IC_{50} = 40 µM). Its IC_{50} values for KAIN/AMPA, NMDA, and L-AP4 receptors were >10, 3, and 0.8 mM, respectively. As with AP4 and AP5, sensitization to L-AP6 was reversed by α-aminoadipate. However, reversal of sensitization to L-AP6 occured at a slower rate, suggesting greater efficacy of L-AP6 for the QUIS-sensitized receptor. (NIH NS 17944)

380.15

L-HOMOQUISQUALATE SENSITIZES HIPPOCAMPAL NEURONS TO DEPOLARIZATION BY L-AP4 AND L-AP6. J.F. Koerner[1]*, S. Venkatraman[2], R.J. Roon[1],and R.L. Johnson[2]. Depts. of [1]Biochemistry and [2]Medicinal Chemistry, Univ. of Minnesota, Minneapolis, MN 55455.

The ability of L-quisqualic acid (L-QUIS) to sensitize neurons to depolarization by α-amino-ω-phosphonate analogues of excitatory amino acids is a highly specific phenomenon. In previous studies of more than 50 analogues, only L-QUIS produced a strong and long-lasting sensitization. For this study we synthesized L-homoquisqualic acid (an analogue of L-QUIS with one additional methylene group in the side chain) from N-Boc-L-aspartic acid α-benzyl ester by the following sequence of reactions: (1) reduction to the alcohol, (2) oxidation to the aldehyde, (3) reductive amination with $BnONH_2$, (4) acylation with $EtO_2CN{=}C{=}O$, (5) cyclization, and (6) deprotection. When hippocampal slices were exposed to 20 μM L-homoquisqualic acid for 20 min and extensively washed with fresh medium, the CA1 pyramidal neurons exhibited a 5-fold increase in sensitivity to depolarization by L-AP4 and 25-fold to L-AP6. The sensitization persisted more than one hour and was reversed by exposure to α-aminoadipic acid. These observations suggest that L-homoquisqualic acid is taken up by the neurons and induces sensitization in a manner analogous to L-QUIS [Brain Res. 605, 85-92, (1993)] but with 10-fold lower potency. (Supported by NIH grant NS 17944.)

380.16

DUAL EFFECTS OF L-NAME ON RESPONSES OF DORSAL HORN NOCICEPTIVE NEURONS TO EXCITATORY AMINO ACID AGONISTS. D. Budai*, K.F.Kitto and A. A. Larson. Department of Veterinary Pathobiology, University of Minnesota, Saint Paul, MN 55108.

Nitric oxide (NO) appears to mediate many actions evoked by excitatory amino acids (EAAs) and play a role in pain. We examined the effect of iontophoretically administered N-ω-nitro-L-arginine methyl ester (L-NAME), an inhibitor of nitric oxide synthesis, on N-methyl-D-aspartate (NMDA)-, (R,S)-α-amino-3-hydroxy-5-methylisoxazole-4-propionate (AMPA)- and kainic acid (KA)-evoked single-cell firing using extracellular recording from dorsal horn nociceptive neurons in the rat. Ejection currents for NMDA, AMPA and KA were selected to produce similar intensities of spike discharges in neurons responding to both noxious and innocuous peripheral stimuli. High doses of iontophoretically applied L-NAME was able to completely inhibit of the firing evoked by NMDA, AMPA or KA. In contrast, low doses of L-NAME increased cell firing evoked under similar conditions. The enhancement of EAA-evoked responses by L-NAME usually lasted for 20-30 min. Enhancement of non-NMDA- (AMPA- and KA-) responses by L-NAME was greater, in terms of both magnitude and duration, than that of NMDA. Similar effects of L-NAME were observed on KA-induced behavioral responses in mice. Pretreatment with L-NAME doubled the number of behaviors in response to a single injection of KA but inhibited KA sensitization at high doses of L-NAME. We hypothesize that L-NAME may exert two opposing effects on EAA activity that may be important in nociceptive transmission. *(Supported by USPHS grants DA04090 and 00124).*

380.17

HIPPOCAMPAL SYNAPTIC FUNCTION AFTER CHELATION OF VESICULAR ZINC. J.D. Easley, D.W. Moncrieff, D.L. Cassel, S.H. Juo, and C.J. Frederickson *. UT-Dallas, Richardson TX, 75080.

Neurons with zinc (and glutamate) in their presynaptic boutons innervate much of the cerebral cortex. In hippocampal field CA1, staining for vesicular zinc suggests that pyramidal apical dendrites receive both zinc-rich inputs (proximally, in s. radiatum) and zinc-free inputs (distally, in s. lacunosum-molecular).

We are testing the effects of zinc chelation upon synaptically-driven field potentials in CA1. For input to the zinc-rich dendritic zone in radiatum, we stimulated Schaffer collaterals, for the zinc-free input in l-m, the perforant path. Stimulation pathway isolation was routinely confirmed by laminar source-sink analysis.

Schaffer collateral responses evoked by slow, repetitive (0.2 Hz) stimulation were unaffected by chelation of vesicular zinc with either TPEN (up to 20 μM) or DEDTC (up to 1.0 mM). Input/output curves spanning threshold to saturation for the EPSP showed no consistent change in either EPSP slope or population spike amplitude after up to 1 hr of perfusion with chelators. However, subsequent histofluorescent tests with zinc probes (TSQ and TFLZ; Texas Fluorescence Labs, Austin. TX) showed that both TPEN and DEDTC had effectively chelated the vesicular zinc of CA1.

These findings fit prior evidence that vesicular zinc is not directly involved in low-frequency classical neurotransmission at EAA synapses. Whether zinc is involved in high-frequency and use-dependent synaptic phenomena (kindling, STP, LTP, LTD, etc.) remains to be explored.

380.18

SPECIFICITY OF CYSTEINE SULFINATE DECARBOXYLASE (CSD) FOR SULFUR CONTAINING AMINO ACIDS (SAA). [1]M.L.Tappaz, [1]K.Almarghini, [2]M.Cuénod* and [2]K.Q.Do. [1]INSERM 171, CHU LYON-Sud, Pierre-Bénite, France; [2]Brain Res. Inst., Univ. of Zürich, Switzerland

CSD which decarboxylates cysteine sulfinic acid (CSA) to form hypotaurine, is thought to be involved in the biosynthesis of taurine. It was recently localized in astrocytes in the cerebellum by immunocytochemistry. This location closely resembled that of another SAA, homocysteic acid (HCA). We therefore investigated the specificity of CSD versus CSA, HCA, as well as related analogs, homocysteine sulfinic acid (HCSA) and cysteic acid (CA). CSD was immunotrapped from brain and liver tissue extracts, using a specific anti-CSD antiserum and incubated with the various SAA. Reaction products were identified and quantified by precolumn o-phtaaldehyde derivatization HPLC. CA and HCA (10 mM) inhibited the formation of hypotaurine from CSA (0.25 mM) by about 70%, while HCSA (10 mM) elicited no inhibition. Incubation with 25 mM of CSA or CA led to the formation of hypotaurine and taurine. The amount of taurine was about 10-fold lower than that of hypotaurine. In contrast, the amount of 3-amino-1-propanesulfonic acid, the decarboxylated reaction product of HCA (25 mM) was less than 1000-fold that of hypotaurine formed from CSA in the same conditions. These results show that CSD has a high specificity for CSA and CA which are the SAA involved in the biosynthesis of taurine. HCA is an inhibitor of CSD but does not appear to be a substrate for CSD in vitro. Accordingly, CSD is unlikely to play a role in the metabolism of HCA in vivo.

380.19

ORALLY ADMINISTERED SEMISYNTHETIC GLYCOSPHINGOLIPID LIGA20 PROVIDES MORPHOLOGICAL AND BEHAVIORAL PROTECTION AGAINST FOCAL ISCHEMIC BRAIN DAMAGE IN RATS. A. Kharlamov, I. Zivkovic, A. Polo, D. M. Armstrong, A. Guidotti and E. Costa*. Fidia-Georgetown Institute for the Neurosciences, Georgetown Univ Med Sch, Washington, DC 20007.

LIGA20 (GM1 with N-dichloroacetyl-sphingosine) added to neuronal cultures of newborn rats prevents glutamate neurotoxicity with a potency 10 fold higher than that of the natural ganglioside GM1 (J. Pharmacol. Exp. Ther. 252:419, 1990). In the present study the antiexcitotoxic effect of LIGA20 was examined *in vivo* using the model of photochemically-induced thrombotic cortical lesion. The size of the infarcted area was analyzed 7 days after the thrombotic insult using the SAMBA4000 computerized imaging system and SigmaScan software in brain slices stained with 2,3,5, triphenyl-tetrazolium chloride. LIGA20 administered via several different treatment paradigms resulted in a considerable reduction of the infarcted area. Oral pretreatment with LIGA20 (70 to 200 μmol/kg/day) for 3 days before the lesion caused up to 30% reduction of the infarcted area. A single intravenous (i.v.) administration of LIGA20 (34 μmol/kg) given 6 hrs after the injury was also neuroprotective resulting in a 20 to 25% reduction of the infarcted area. When this delayed single i.v. injection of LIGA20 was combined with an oral administration of LIGA20 (50 to 200 μmol/kg) a further reduction up to 40% of the infarcted area was observed. The protective action of LIGA20 given orally was related to the brain concentration of this drug. Behavioral studies in rats receiving bilateral cortical lesions demonstrated a clear correlation between the size of the brain lesion and the decreased performance on a water maze. Moreover, a LIGA20 treatment that reduced the size of the infarcted area also improved the rat performance on the water maze.

Supported in part by NINDS grant 2PO1 NS 28130-04.

381.1

NEONATAL MICE LACKING FUNCTIONAL NMDA RECEPTORS GENERATE RESPIRATORY OSCILLATIONS *IN VIVO and IN VITRO*. JL Feldman*§, Y Li¶, GD Funk§, JC Smith§, X-W Dong§, S M Johnson§, J Lai§, S Hsu¶ & S Tonegawa¶. ¶Center for Cancer Research, MIT, Cambridge, MA 02139 & §Dept. Physiol. Sci., UCLA, LA, CA, 90024-1527.

NMDA receptors are important in many CNS functions. We investigated the role of NMDA receptors in the neural control of respiration using mice (129/sv X C57BL/6) with deletions in the NMDAR1 gene of the NMDA receptor (NMDAR1 knockout). The knockout mice generate rhythmic breathing movements from birth. Brainstem-spinal cords (and medullary slices) isolated from control and knockout neonatal (Day 0) mice generated rhythmic motor output in cranial (IX, X, XII) and spinal (C1, C4, C5, T2) nerves. *In preparations from control mice:* (i) Local application of AMPA (200 μM) and NMDA (2 mM) over the C4 and C1 motoneuron pools produced a tonic sustained discharge on the corresponding ventral root. (ii) Application of AMPA and NMDA to the ventrolateral surface of the medulla (at the level of the pre-Bötzinger Complex) produced a rapid, dose-dependent increase in respiratory frequency. *In preparations from knockout mice:* (i) Application of AMPA to the spinal cord and medulla produced similar effects to that in control, whereas NMDA produced no effects. (ii) NMDA did not induce inward currents in hypoglossal motoneurons; AMPA did. We conclude that: i) Functional NMDA receptors are not present in the knockout mice and the NMDAR1 subunit is essential for NMDA receptor activity; ii) NMDA receptors are not essential for development of the neuronal circuitry for respiratory rhythm generation; and, iii) NMDA receptors are not necessary for respiratory rhythm generation or drive transmission to cranial and spinal motoneurons. Supported by NIH Grants HL37941, NS24742, HL40959, and HHMI.

381.2

ANTISENSE OLIGODEOXYNUCLEOTIDE TO THE NMDA-R1 RECEPTOR CHANNEL REDUCES FOCAL CEREBRAL ISCHEMIA INFARCTIONS IN RAT. D.J. Reis, E.V. Golanov, S. Yamamoto, H. Ericson, C.E. Inturrisi[1] and C. Wahlestedt*. Div. Neurobiol., Dept. Neurol. & Neurosci and [1]Dept. Pharmacol., Cornell Univ. Med. Coll., New York, NY 10021.

We have recently observed that antisense oligodeoxynucleotides (ODN) directed against the amino terminus of the primary structure of the NMDA receptor R1-subunit are neuroprotective in rat cortical cell culture (Wahlestedt et al., *Soc. Neurosci. Abstr.* 22:391.7, 1992). We investigated here whether an NMDA-R1 receptor antisense ODN would offer protection *in vivo*.

ODNs were administered intracerebroventricularly in spontaneously hypertensive rats twice daily for 2-3 days. Animals were then subjected to middle cerebral artery (MCA) occlusion and 24h later they were killed and the distribution and volume of the infarction computed. Groups consisted of rats receiving: (a) Antisense ODN (15 nmol, n=6); (b) Matching sense ODN (15 nmol, n=6); or (c) Vehicle (control, n=5). (d) In a fourth group, MK-801 (1 mg/kg) was injected intravenously 30 min after the MCA occlusion (MK-801, n=5) and animals were killed 24h later. Both antisense ODN and MK-801 treatments significantly reduced lesion volume, the former by 43.5% and the latter by 31.4% over controls. Sense ODN was without effect. In separate groups of animals (n=8) treated with ODNs in an identical fashion, it was found that antisense ODN caused a significant (≥35% *vs.* controls) reduction of the B_{max} of cortical NMDA binding sites, labeled by [^{3}H]-CGS19755. Cortical NMDAR1 mRNA concentrations were unaffected by antisense ODN treatment.

The results are consistent with the view that NMDA receptors may participate in the neurotoxicity elicited by focal cerebral ischemia. Antisense ODNs for specific neuronal molecules may be useful for analyses of roles of specific molecules in ischemic lesions and conversely neuroprotection.

381.3

INDUCTION OF A NOVEL NMDA RECEPTOR BY KINDLING IN CA3 IS NOT RELATED TO ALTERATIONS IN NMDAR TRANSCRIPT LEVELS. J.E.Kraus, G-C.Yeh*, Y.Watanabe, D. Bonhaus, J.V.Nadler, and J.O.McNamara . Duke and VA Medical Centers, Durham, NC.

Kindling is an animal model of epilepsy whereby periodic administration of an initially subconvulsant stimulus results in long lasting neuronal hyperexcitability. Enhanced function of excitatory synapases using the NMDA subtype of glutamate receptor (NMDAR) may contribute to the persistence of this hyperexcitability. We have previously shown that kindling induces a novel NMDAR with an increased number of binding sites (B_{max}) for the NMDA receptor antagonist CPP, as measured in whole hippocampal membranes prepared 1 month after the last kindled seizure. Our goals were to determine: (1) the hippocampal region of increased CPP binding; (2) the time course of increased CPP binding; and (3) whether alterations in the expression of distinct genetic isoforms of the NMDAR underlie the properties of the novel NMDAR induced by kindling. To address these goals, we generated equilibrium binding isotherms using [^{3}H]CPP on microdissected hippocampal membranes and used quantitative in-situ hybridization techniques to examine transcript levels of NMDAR subunits. We report that: (1) increases in B_{max} for CPP were specific to region CA3; (2) binding over control was increased 36% at 24 hours and 300% at 28 days after the last kindled seizure; and (3) transcript levels of NMDAR1, NR2A, NR2B, and NR2C were the same in kindled and control animals at both 24 hours and 28 days after the last kindled seizure.

The direction, time course, and location of the kindling-induced increase in CPP binding suggest that this novel receptor may underlie the increased sensitivity of CA3 neurons to NMDA observed in kindled animals (Martin, et. al., J Neuroscience 12:1928, 1992). We have not yet elucidated the molecular mechanisms underlying these changes.

381.4

DOWN REGULATION OF THE GLUR2 SUBUNIT PROTEIN IN AMYGDALOID KINDLING. H.K. Prince, P.J. Conn, C. Blackstone, R. Huganir and A.I. Levey.* Departments of Neurology and Pharmacology, Emory University, Atlanta, GA 30322, and Department of Neuroscience, The Johns Hopkins University, Baltimore, MD 21205.

Previous studies using autoradiography have suggested changes in glutamate receptor density in kindling. More recently, it has been shown that changes in the subunit composition of AMPA receptors (subunits GluR1-4) can result in striking electrophysiological differences in the cell. Based on these data we investigated possible changes in the abundance and distribution of the AMPA receptor subunits in amygdaloid kindling. The GluR1-4 subunits were examined in four brain regions (hippocampus, entorhinal cortex, pyriform cortex/amygdala and limbic forebrain) by quantitative immunoblotting using subtype-specific antibodies. Immunoblots revealed a marked decrease in GluR2/3 immunoreactivity in all four brain regions using an antibody directed against a common epitope, which further analysis with subunit specific antibodies revealed was due to a decrease in GluR2. In contrast, GluR1 and 4 remained constant. The largest change was seen in a region including pyriform cortex and amygdala (32% of control). Immunocytochemistry did not demonstrate any qualitative changes in the cellular or subcellular distribution of the subunits. Because the presence of GluR2 in hetero-oligomeric complexes prevents calcium permeability, our results suggest that decreased GluR2 in kindling may possibly increase neuronal excitability and may contribute to seizure activity.

381.5

THE NMDA RECEPTOR, NMDAR1, IS LOCALIZED TO THE POSTSYNAPTIC DENSITY (PSD) OF RAT BRAIN. P.C. Suen*[1,2], K. Wu[1,2], T.W. Kim[1,2], S.Y. Lin[1,2], Y. Huang[3], R.J. Wenthold[4], J.L. Xu[1] and I.B. Black[1,2]. [1]Dept. Neurosci. and Cell Biol., Robert Wood Johnson Med. Sch.; [2]Program in Physiol. and Neurobiol., Rutgers-The State Univ. of N.J., Piscataway, NJ 08854; [3]Div. of Neurosci., NYSPI, New York, N. Y. 10032; [4]Lab of Neurochem., NIDCD, NIH, Bethesda, M.D. 20892.

Receptors for NMDA (N-methyl-D-Aspartate) are acknowledged to be involved in many physiological and pathological processes in the brain. However, the molecular characteristics of the receptor protein and its exact synaptic function at the postsynaptic site are unknown. We examined NMDAR1, which serves as a fundamental subunit necessary for the NMDA receptor-channel complex, in the PSD, a critical synaptic organelle. Binding of (^{3}H)MK-801 was performed initially to determine whether the PSD contains the receptor. Adult rat cerebral cortical (CTX) PSD contained a single high affinity (^{3}H)MK-801 binding site with a B_{max} = 21 pmoles/mg protein and a K_D= 0.135 μM. To examine regional distribution, Western blot analysis, using specific polyclonal anti-C terminal NMDAR1 antiserum, was performed on total tissue homogenate, synaptic membrane and PSD fractions isolated from cerebral cortex, cerebellum, olfactory bulb and hippocampus. There was no NMDAR1 expression in the cerebellum. In the other three brain regions, NMDAR1 protein was expressed in a region-specific manner and was highest in the PSD. Moreover, the NMDAR1 increased dramatically from postnatal day 4 to day 10, raising the possibility that the receptor may be involved in synaptic maturation. Finally, Western blot analysis revealed that the NMDAR1 in CTX-PSDs of mouse and rat are structurally similar, if not identical. Our findings suggest that the NMDAR1 may exert its physiological role through postsynaptic mechanism involving the PSD.

381.6

EXPRESSION PATTERN OF FIVE METABOTROPIC GLUTAMATE RECEPTOR SUBTYPES IN THE RAT BRAIN UNDER NORMAL CONDITIONS AND FOLLOWING GLOBAL ISCHEMIA. L. Iversen(§), E. Mulvihill(#), B. Haldeman(#), F. Kaiser(§), M. Sheardown(§), L. Frank (&), N. Diemer(&) and P. Kristensen(§)(*). (#) Zymogenetics Inc., Seattle; (§) Institute of Neuropathology, University of Copenhagen and (§) Pharmaceuticals Division, Novo Nordisk A/S, Måløv, DK-2760 Denmark.

The distribution of the mRNA for each of the metabotropic glutamate receptor subtypes was analysed by in situ hybridisation. The glial subtype (mGluR3) showed the highest level of expression in white matter tracts, while the other four subtypes each had a distinct pattern of expression. For example in hippocampus different subsets of metabotropic receptor types were expressed in different areas: CA1 mGluR1(weak) and mGluR5; CA2: mGluR1 (weak), mGluR4 and mGluR5; CA3: mGluR1 and mGluR5. In the dentate gyrus mGluR1, mGluR2 and mGluR5 mRNA could be detected. 24 hours after global ischemia in the neck-cuff and four-vessel occlusion models the expression pattern of mGluR1-mGluR5 mRNA was investigated. In both models the mRNA levels for mGluR1, mGluR2, mGluR3 and mGluR5 were either unchanged or lower following ischemia. In contrast, the mRNA level for the mGluR4 metabotropic receptor was found to be increased in all parts of the hippocampus (except CA2 where it was unchanged) and in the parietal cerebral cortex. These findings together with our recent demonstration that L-AP4 is a strong agonist at the mGluR4 further indicate that mGluR4 is a presynaptic receptor regulating glutamate release and is upregulated following the increase in extracellular glutamate caused by ischemia.

381.7

IMMUNOCHEMICAL STUDIES OF A METABOTROPIC GLUTAMATE RECEPTOR (mGLUR5) IN RAT BRAIN. C. Romano*[1], M.A. Sesma[3], C.T. McDonald[2], M.T. Price[2], and J.W. Olney[2] Depts. of Ophthalmology[1] and Psychiatry[2], Washington University School of Medicine and School of Optometry[3] UM-St. Louis; St. Louis, MO .

The mGluRs are a family of G-protein coupled receptors that interact with a variety of effector systems. Two of these receptors, mGluR1 and mGluR5, stimulate phosphatidyl inositol hydrolysis when expressed in model systems. An antibody selective for mGluR5 was developed using the anti-peptide antibody approach. Western blots of rat cortical synaptic membranes showed a single polypeptide of 148 kD, consistent with the molecular weight predicted from the sequence. The mGluR5 polypeptide was most abundant in cortex, hippocampus, striatum and olfactory bulb, with less in midbrain and little detected in cerebellum and brainstem. This distribution closely matches that found for mGluR5 mRNA (Abe *et al.*, 1992). The dearth of mGluR5 detected in the cerebellum verifies the specificity of the antibody, as mGluR1α is most abundant in cerebellum.

Immunocytochemical studies using either fluorescent or ABC-peroxidase labeling reveal abundant neuropil staining, especially in striatum, lateral septum, and olfactory bulb. Throughout cerebral cortex, labeled puncta and varicose fibers are distributed in layer 2/3. The puncta and fibers may originate from large mGluR5 positive bipolar and multipolar cells in superficial cortical layers. Similar cells and puncta are seen in hippocampus where the puncta encircle unlabeled profiles in the pyramidal layer. mGluR5-positive neuronal somata in the cortex and hippocampus are rare but all resemble local circuit neurons with sparsely branching long dendrites. Distinct puncta and fibers in cortex and hippocampus suggest that mGluR5 is present on presynaptic axon terminals. In the basal forebrain, a small population of mGluR5-positive neurons is found in cholinergic cell groups. Supported in part by Research to Prevent Blindness (to DOVS-WUMS); RSA MH 38894; U.M. Weldon Spring Fund (to MAS).

381.8

IMMUNOCYTOCHEMICAL DISTRIBUTION OF NMDA AND NON-NMDA IONOTROPIC EXCITATORY AMINO ACID (EEA) RECEPTORS IN MONKEY HIPPOCAMPUS S.J. Siegel *[1], W.G. Janssen [1], N. Brose [2], S.W. Rogers [3], T. Moran [1], G.P. Gasic [2], R. Jahn [4], S.F. Heinemann [2] and J.H. Morrison [1], 1 Fishberg Res. Ctr. for Neurobio., Mt. Sinai Sch. of Med., New York, NY 10029, 2 Mol. Neuro. Lab., Salk Inst. for Bio. Studs., La Jolla, CA 92037, 3 Dep. of Pharm. Univ. of Colorado, Denver, CO, 80262, 4 H.H.M.I Yale Univ. New Haven, CT, 06510

The regional, cellular and subcellular distributions of several EAA receptor subunits were investigated in monkey hippocampus using monoclonal antibodies specific for GluR2, GluR5-7 and NMDAR1, members of the pharmacologically defined AMPA/kainate, kainate and NMDA ionotropic receptor categories, respectively. Virtually all pyramidal cells in Ammon's horn, as well as granule cells and polymorphic cells in the dentate gyrus, were labeled with all three antibodies. However, the intensity of immunoreactivity was not constant across regions and strata with each of the antibodies, suggesting a possible variation in the amount and/or subcellular localization of each receptor subunit present in the various cell classes. Dentate granule cell somata and dendrites were intensely labeled with all three antibodies. Additionally, the mossy fibers were labeled with the NMDAR1 antibody, which may correspond to presynaptic receptors. Pyramidal cells in both CA1 and the subiculum were intensely labeled for all three receptor subtypes in virtually every somatic and dendritic segment examined. CA3 pyramidal cell bodies and dendrites were immunoreactive for GluR5-7, and faintly labeled with antibodies directed against GluR2 and NMDAR1. Ultrastucturally, NMDAR1 labeling in the stratum lucidum was localized to mossy fiber axons and terminals, but was not seen in postsynaptic densities, which may explain the NMDA independent nature of long term potentiation in CA3 secondary to mossy fiber stimulation. However, within the molecular layer of CA3, the strata radiatum and moleculare of CA1, and the dentate molecular layer, NMDAR1 labeling was localized to postsynaptic densities, primarily if not exclusively on spines. The data suggest regional and subcellular specificty for each of the three receptor subtypes which may correspond to discrete inputs. These patterns may underlie the individual response profiles of various hippocampal cell types to a variety of physiological, pharmacological and toxicological conditions.

381.9

GLUTAMATE RECEPTOR TRANSCRIPTION IN EMBRYONIC SPINAL MOTONEURONS. R. Temkin*, D. Lowe, P. Jensen, and D.O. Smith. Neuroscience Training Program and Dept. of Physiology, Univ. of Wisconsin, Madison, WI 53706.

Embryonic chick spinal motoneurons respond to AMPA, kainate and NMDA, indicating that receptors for each of these glutamate agonists is present. To determine which known glutamate receptor gene products (GluR) might underlie these responses, we screened cDNA obtained from highly enriched (>92%) motoneuron populations using PCR-based gene amplification. Primers derived from rodent cDNA sequences were used to test for GluRs 1-7 and NMDAR1. Each of these receptor subtypes was detected. Primers designed to distinguish between the *flip* and *flop* splice variants of GluR1 and restriction digests of GluR1 fragments yielded ambiguous results that may indicate slight sequence differences in chick. Sequence analyses of 14 clones encoding GluR6 detected only arginine (and not glutamine) at the site (621) known to regulate Ca^{2+} permeability, thus demonstrating extensive mRNA editing. Since all genes encoding glutamate receptor subtypes appear to be transcribed, synaptic diversity may involve highly regulated translation or intracellular distribution. Supported by NIH grant NS13600.

381.10

EFFECTS OF EXCITATORY AMINO ACIDS ON INTRACELLULAR CALCIUM IN SH-SY5Y HUMAN NEUROBLASTOMA CELLS.

K. Savolainen, P. Nykvist, M. Tuomala, M.-R. Hirvonen* and J. Naarala. Natl. Publ. Hlth. Inst., Dept. Toxicol., Kuopio, Finland.

Free intracellular calcium ($[Ca^{2+}]_i$) regulates many signal transduction processes in cells, including the activation of protein kinase C and calcium/calmodulin dependent protein kinase. It also activates phospholipase C, which generates two second messengers, diacylglyserol and inositol-1,4,5-trisphosphate, from the phosphatidylinositol-4,5-bisphosphate. $[Ca^{2+}]_i$ also plays a critical role both in the toxic cell killing and programmed cell death. We have studied the effects of excitatory amino acids (EAAs; L-glutamate, NMDA, kainate, AMPA and 1S,3R-ACPD) on $[Ca^{2+}]_i$ in SH-SY5Y neuroblastoma cells. The cells were exposed to graded doses of EAAs (100 μM - 3 mM). Changes in $[Ca^{2+}]_i$ by L-glutamate, NMDA and kainate were biphasic, i.e. an increase in $[Ca^{2+}]_i$ was followed by a decrease. L-glutamate, NMDA and kainate all increased $[Ca^{2+}]_i$ in a concentration-dependent manner. The time for reaching a peak in $[Ca^{2+}]_i$ was dose-dependent for both L-glutamate and kainate; at low concentrations (300 - 500 μM) the peaks were at 29 and 6 sec whereas at high concentrations (3 mM) the time points for the corresponding peaks were at 19 and 3 sec for L-glutamate and kainate, respectively. Subsequent to the increase in $[Ca^{2+}]_i$, L-glutamate, NMDA and kainate all caused a decrease in $[Ca^{2+}]_i$, but this effect was not concentration dependent. The decrease took place 2-3 minutes after the cells were exposed to EAAs. 1S,3R-ACPD and AMPA also caused a slight increase, followed by a decrease, in $[Ca^{2+}]_i$, but the changes in $[Ca^{2+}]_i$ were small as compared to those induced by the other EAAs. The present results suggest that $[Ca^{2+}]_i$ is modulated in SH-SY5Y neuroblastoma cells in response to EAAs. These results also suggest that both the ionotropic and the metabotropic EAA receptors may be responsible for EAA-induced changes in $[Ca^{2+}]_i$ in SH-SY5Y neuroblastoma cells. Supported by The Academy of Finland.

381.11

RNA EDITING OF AMPA RECEPTOR SUBUNIT (GluR-B) mRNA. C.M. Burns, P.S. Obermiller and R.B. Emeson.*, Department of Pharmacology, Vanderbilt University, Nashville, Tennessee 37232-6600.

L-glutamate, the major excitatory neurotransmitter in the vertebrate central nervous system, opens cation channels that mediate fast excitatory responses. Recent studies have indicated that the calcium permeability of the AMPA glutamate receptor is dependent upon channel subunit composition. AMPA GluR-B subunit mRNA encodes a critical arginine residue in the second transmembrane spanning domain (TM2) which determines ion flow, yet the GluR-B gene contains a glutamine (CAG) rather than an arginine (CGG) codon at this position. The conversion of the genomic adenosine moiety to the guanosine found in the cDNA has been attributed to RNA editing.

To detect the single nucleotide change and quantitatively assess the extent of GluR-B editing, we have developed a sensitive assay based upon the polymerase chain reaction (PCR) which results in the formation of an artificial restriction fragment length polymorphism (A-RFLP) by introducing Alu I and Hae III restriction endonuclease sites into nonedited and edited templates, respectively. First strand cDNA synthesis was generated from whole mouse brain total RNA using a GluR-B-specific intron primer and the resulting cDNA was PCR amplified using intron-specific primers that spanned the exon encoding TM1 and TM2. DNA sequence and A-RFLP analyses revealed that the GluR-B pre-mRNA transcript was edited, indicating that RNA editing is a nuclear event occurring prior to, or coincident with, RNA splicing. Using this assay, we have also identified a rat glioma cell line which endogenously expresses GluR-B mRNA and edits >75% of the mature transcripts. We are currently using this tissue culture system to identify the *cis*-active regulatory elements controlling RNA editing by transfection of mutant GluR-B transcription units.

381.12

DEVELOPMENTAL CHANGES IN mRNA EDITING OF GLUTAMATE RECEPTORS. D. Lowe* and D.O. Smith. Neuroscience Training Program and Dept. of Physiology, Univ. of Wisconsin, Madison, WI 53706.

The extent of mRNA editing that occurs in the TMII region of glutamate receptors GluRs 2, 5, and 6 was measured in various regions of the mammalian CNS and at multiple ages. Functional analysis of recombinant channels assembled from edited (R) and unedited (Q) forms of GluRs 2 and 6 indicate that this site is a determinant of divalent cation permeability. GluR6-specific cDNA fragments were subcloned into M13mp18RF-DNA, transformed, and plated. Multiple recombinant plaques were sequenced to determine the ratio of edited to unedited subunits present. In hippocampus, a significantly (0.05 level) greater number of plaques contained the unedited form at post-natal day 8 than at 2- or 24-months of age. 33% of the plaques sequenced from 8-day animals were unedited, while 5 and 0% of the plaques were edited in animals aged 2 and 24 months, respectively. Regional and developmental regulation of the editing process may play an important role in neuronal maturation and degeneration. Supported by NIH NS13600.

382.1

NEUROCHEMICAL IDENTIFICATION OF EFFERENT PROJECTIONS OF A_{13} INCERTOHYPOTHALAMIC DOPAMINERGIC NEURONS IN THE RAT. M.J. Eaton*, C.K. Wagner, K. E. Moore and K.J. Lookingland. Dept. Pharmacology/Toxicology, Michigan State University, East Lansing, MI 48824

Anterograde tract tracing from the medial zona incerta (MZI) reveals projections to many limbic structures including the horizontal limb of the diagonal band of Broca (HDB) and the central nucleus of the amygdala (cAMY). The purpose of the present study was to determine if these projections are incertohypothalamic dopaminergic (DA) originating from the A_{13} cell group in MZI by examining changes in the concentrations of dopamine or 3,4-dihydroxyphenylacetic acid (DOPAC) in the HDB and cAMY after bilateral stimulation or lesion of the MZI, or administration of γ-butyrolactone (GBL) or its active metabolite γ-hydroxybutyric acid (GHBA). For comparative purposes, the nucleus accumbens (N. Acc.), which contains terminals of A_{10} mesolimbic DA neurons but no projections from A_{13}, was also examined. Bilateral electrolytic or knife ablation lesions of the MZI reduced dopamine concentrations in the HDB and cAMY, but not in the N. Acc. whereas, electrical stimulation of the MZI increased DOPAC concentrations in HDB and cAMY, but not in N. Acc. Systemic administration of GBL increased the concentrations of dopamine in the MZI, HDB, cAMY and N. Acc., and this action was reversed by apomorphine. Intracerebral injection of GHBA into A_{13} increased dopamine concentrations in the HDB and MZI, whereas its injection into A_{10} increased dopamine concentrations in the HDB and N. Acc. These results suggest that A_{13} DA neurons project to the HDB and cAMY and that the HDB receives a dual DA innervation from both A_{13} and A_{10} cell groups. (Supported by NIH grants NS 15911 and NS 07279).

382.2

EFFERENT PROJECTIONS OF NEURONS IN THE MEDIAL ZONA INCERTA: A *PHASEOLUS VULGARIS* LEUCOAGGLUTININ ANTEROGRADE TRACT-TRACING STUDY IN THE RAT. C.K. Wagner*, M.J. Eaton, K.E. Moore and K.J. Lookingland. Dept. Pharmacology/Toxicology, Michigan State University, East Lansing, MI 48824.

The purpose of the present study was to determine efferent projections of A_{13} incertohypothalamic dopaminergic neurons by examining the distribution of labelled fibers following unilateral injections of the anterogradely transported lectin *Phaseolus vulgaris* leucoagglutinin (PHA-L) into the medial zona incerta. PHA-L-labelled fibers were differentially distributed throughout the brain with the heaviest labelling occuring in the lateral septum, vertical and horizontal diagonal bands, lateral preoptic and hypothalamic areas, midbrain central gray, and ipsilateral parvocellular region of the paraventricular nucleus. Moderate labelling was observed in central amygdala, lateral bed nucleus of the stria terminalis, and in the contralateral parvocellular region of the paraventricular nucleus and the medial zona incerta. Light labelling was detected in the medial preoptic nucleus, supraoptic nucleus, and the dorsomedial aspect of the ventromedial nucleus; whereas little or no labelled fibers were present in the dorsomedial nucleus or magnocellular region of the paraventricular nucleus. These results suggest that neurons in the medial zona incerta project via the lateral hypothalamus to regions in the rostral diencephalon, and to the central amygdala. Additional studies are ongoing to determine if these represent projections of A_{13} incertohypothalamic dopaminergic neurons. (Supported by NIH grants NS 15911 and NS 07279).

382.3

CATECHOL-O-METHYLTRANSFERASE IN THE HUMAN NIGRAL COMPLEX AND STRIATUM: AN IMMUNOHISTOCHEMICAL STUDY. A. Kastner, P. Anglade, E.C. Hirsch*, C. Bounaix, P. Damier, F. Javoy-Agid, N. Bromet, Y. Agid. INSERM U289, Hôpital de la Salpétrière, Paris, France; Biotec Centre, Orleans, France.

The role of pre- and post- synaptic neurons in the degradation of synaptic released dopamine is not well understood, since the location of catechol-O-methyltransferase (COMT), a major enzyme of dopamine metabolism, is controversial. In the present study, we analyzed the cellular distribution of COMT in the nigral complex and the striatum of human brain *post mortem* by means of immunohistochemistry, at the light and electron microscope level.

In the nigral complex, COMT immunostaining was detected in some dopaminergic melanized neurons in the ventral tegmental area and the substantia nigra pars lateralis, but not in the substantia nigra pars compacta. In the striatum, using light microscopy, COMT immunostaining was detected in numerous cells, which at electron microscope level, were identified as glial cells and neurons. Immunonegative putative dopaminergic nerve terminals were observed in synaptic contact with these striatal immunopositive neuronal cell bodies and dendritic spines.

The data suggest that: 1) In the striatum, COMT is not present in the dopaminergic terminals, whereas it is expressed in striatal neurons. Thus, the elimination of striatal released dopamine by its *O*-methylation may involve GABAergic post synaptic neurons but not the dopaminergic presynaptic neurons. 2) In the nigral complex, COMT is expressed by some dopaminergic neurons with an heterogeneous distribution from one mesencephalic region to another. In this context, it is interesting to note that the neurons containing COMT are located in the dopaminergic regions, which are relatively well preserved in Parkinson's disease, suggesting that the absence of COMT in the dopaminergic nigrostriatal neurons might contribute to their susceptibility to the disease.

382.4

THE POSTNATAL DEVELOPMENT OF THE DOPAMINERGIC INNERVATION OF MONKEY PREFRONTAL CORTEX IS PROTRACTED AND REGION-SPECIFIC. D.R. Rosenberg* and D.A. Lewis. Depts. of Psychiatry and Behav. Neurosci., Univ. of Pittsburgh, Pittsburgh, PA 15213.

Developmental abnormalities in the dopaminergic (DA) innervation of the prefrontal cortex (PFC) have been implicated in the pathophysiology of certain neuropsychiatric disorders. In this study, we used immunocytochemical methods to characterize quantitatively the density and laminar distribution of tyrosine hydroxylase (TH)-labeled axons and varicosities in the PFC of 17 monkeys (*Macaca mulatta*) ranging in age from newborn to adult. The anti-TH antibody used in this study has been previously shown to selectively label DA axons in monkey neocortex. In the dorsomedial PFC (area 9), labeled axons were present predominately in layers I-II and V-VI in animals less than 1 month of age, and the density of these axons did not change substantially during early development. In contrast, during the second and third postnatal months, the density of labeled axons increased nearly 4-fold in the middle cortical layers. Axon density in these layers continued to increase with age, reaching a peak (over 6 times newborn values) in animals 2-3 years of age. Fiber density in these layers then declined about 30% in adult animals. The density of labeled varicosities showed similar developmental changes. In contrast, in the adjacent area 46, developmental changes in the distribution of TH-positive axons were much less striking. These findings demonstrate that the development of the DA innervation of monkey PFC is regionally- and laminarly-specific, continues through adolescence into adulthood, and exhibits the greatest degree of change during infancy and around the time of puberty (2-3 years of age).

382.5

SYNAPTIC ASSOCIATIONS BETWEEN DOPAMINE TERMINALS AND GABA INTERNEURONS IN RAT AND MONKEY CORTEX

S.R. Sesack*, C.L. Snyder, and D.A. Lewis, Departments of Behavioral Neuroscience and Psychiatry, University of Pittsburgh, Pittsburgh, PA 15260

Dopamine (DA) terminals regulate the excitability of cortical pyramidal cells via direct synaptic input to dendritic spines. However, it is not known if DA also modulates pyramidal cell activity indirectly through synapses on GABA interneurons, and whether such inputs differ across species. We sought to determine the ultrastructural basis for modulatory interactions between DA and GABA in the prefrontal and motor cortices of rats (Sprague-Dawley) and monkeys (*Macaca fascicularis*). Fixed brain sections were processed according to a dual, peroxidase/gold pre-embedding immunostaining technique. In regions of the neuropil where both markers were detected, terminals containing peroxidase immunoreactivity for DA or tyrosine hydroxylase (TH) synapsed primarily on spines and small dendrites lacking immunogold labeling for GABA. However, DA or TH-labeled (DA/TH) terminals were frequently in direct apposition to GABA-immunoreactive dendrites and in some cases, synaptic specializations were detected at these junctions. Convergence of DA/TH and GABA terminals on common dendrites, some of which were immunoreactive for GABA, was also observed. Axo-axonal appositions, but not synapses, were occasionally detected between DA/TH and GABA terminals. These results demonstrate that DA terminals directly innervate GABA interneurons, as well as pyramidal neurons, in the cortex of both rats and monkeys. These observations also suggest that DA and GABA interact through multiple cellular substrates in the regulation of cortical activity. This work was supported by USPHS grants MH50314, MH00519, and MH43784.

382.6

DOPAMINE SYNAPTIC ORGANIZATION IN THE MONKEY CAUDATE: MEASUREMENT OF SYNAPSE SIZE AND CHARACTERIZATION OF POSTSYNAPTIC PROCESSES. L.F. Smiley* and P.S. Goldman-Rakic. Section of Neurobiology, Yale Univ. Sch. of Med., New Haven, CT 06510.

Despite strong clinical interest in the local circuitry of dopamine neurotransmission in the basal ganglia, a detailed ultrastructural description of dopamine axons in primate caudate is lacking. We therefore measured several morphological parameters of dopamine-immunoreactive axons in the macaque anterior caudate, visualized with a silver precipitation technique which allowed unobscured visualization of the labeled axons. The results from multiple tissue samples were remarkably consistent. As in the rat, dopamine axons were characteristically filled with approximately round clear synaptic vesicles, were never seen to contain dense core vesicles, and formed symmetric synapses. Serial section analysis demonstrated that dopamine synapses were quite small, typically present in only 1 or 2 serial sections, and rarely seen in more than 3 serial sections. Processes receiving dopamine synapses were also characterized by serial section analysis. Fifty percent of postsynaptic profiles were dendritic spine heads, which also received unlabeled asymmetric synapses, similar to the rat (Freund et al., Neurosci. 13:1189, 1984). The remaining dopamine synapses were on dendritic shafts, which were usually seen to be densely spinous when followed in serial sections. The consistent synaptic organization of dopamine axons in the caudate of the nonhuman primate provides a foundation for future ultrastructural analysis of this dopamine system in human pathological states. Supported by MH 44866.

382.7

ORIGINS OF STRIATAL CATECHOLAMINERGIC AFFERENTS IN TWO AMPHIBIANS, THE ANURAN *RANA RIDIBUNDA* AND THE URODELE *PLEURODELES WALTLII*. A.González, M.Muñoz, A.Muñoz, A.Terrés, O.Marín, B.Navarro, R.M.Paz* and W.I.A.J.Smeets•. Dept. Cell Biology, Univ. Complutense of Madrid, Spain and •Dept. Anatomy Fac. Medicine, Vrije Univ. of Amsterdam, The Netherlands.

The distribution of striatal catecholaminergic (CA) immunoreactivity is studied by means of antibodies against dopamine, noradrenaline and tyrosine hydroxylase (TH). In addition, striatal afferents are revealed by retrogradely transported horseradish peroxydase, Fluorogold or fluorescent dextranamines. Double labeling techniques demonstrate the origins of CA projections to the striatum. The CA innervation in *Rana* is strong in the nucleus accumbens but only moderate in the striatum where it is primarily confined to the periventricular cell plate. Differently, in *Pleurodeles* the striatum contains the densest CA innervation arranged in patches whereas the nucleus accumbens is poorly immunoreactive. The striatal afferents in both amphibians originate from the dorsal thalamus, amygdala, hypothalamus, reticular nuclei in the brainstem and the raphe. Remarkably, in the urodele additional afferents from cells in the midbrain tegmentum and in the proximity of the fasciculus solitarius are also present. The present study reveals that in the urodele the mesostriatal CA system is particularly well developed, with additional components from the hypothalamus and the nucleus of the fasciculus solitarius while in the anuran such system seems to be absent and the CA innervation is found to be derived exclusively from the hypothalamic CA cell groups. *(Supported by the DGICYT, PB90-0628)*

382.8

RETROGRADE TRACING THE PROJECTIONS OF PRE-ADOLESCENT TYROSINE HYDROXYLASE NEURONS IN EXTENDED AMYGDALA. C.A. Beltramino[1], G.F. Alheid[2] and L. Heimer* Depts of [1]Otolaryngology and [2]Psychiatric Medicine, Univ. Virginia Health Sciences Center, Charlottesville, Va 22908

In the preadolescent rat, tyrosine hydroxylase (TH) containing cells are found in the extended amygdala, particularly in the intermediate division of the bed nucleus of the stria terminalis (BSTI) and in the central nucleus of the amygdala (CeA). The expression of this transmitter is down-regulated in the adult, so that in rats (over 60 days) few or no catecholamine neurons can be found. Little is known about the functional significance of these neurons, but one starting point is to identify the brain structures that are innervated by these cells. Accordingly, we have initiated a retrograde tracing study using fluorescent tracers (FITC or RITC beads and Fluorogold), combined with TH immunofluorescence (with Texas red or AMCA). Our current results indicate that after retrograde tracer injections in the central and medial amygdala, no double labeling with TH occurred in the BSTI despite dense retrograde cell labeling, nor did these forebrain TH cells appear to project to the parabrachial region, a target common to the BSTI and the CeA. Currently, we are extending these experiments to include especially the medial and lateral hypothalamus, as well as several additional targets of the extended amygdala. Supported by USPHS grant NS17743.

382.9

QUANTITATIVE SURVIVAL OF DOPAMINERGIC NEURONS IN REAGGREGATE TISSUE CULTURE. A. Heller*, S. Price and L. Won. Dept. of Pharmacological and Physiological Sciences, University of Chicago, Chicago, IL 60637.

Three-dimensional reaggregate tissue culture provides a means for the examination of a variety of trophic interactions between dopaminergic (DA) neurons and their target cells. In the present study, an attempt was made to determine the extent of survival of the population of DA neurons cultured in the presence of corpus striatum cells. For this purpose, flasks of reaggregates were prepared from mesencephalon and corpus striatum of individual C57Bl/6J mouse embryos at 14 days of gestation. After 3 weeks in culture, reaggregates were collected for neurochemical analysis and determination of the total number of tyrosine hydroxylase (TH) positive cells present in the cultures. The reaggregates contained DA at a level of 13.8 ± 1.2 ng/mg protein and released the neurotransmitter as evidenced by the presence of 25.5 ± 2.1 ng of DOPAC per ml in the media. In order to determine the level of survival of DA neurons in the reaggregates, the numbers of such neurons in the cultures were compared to that in the mesencephalon of 2 month old C57Bl/6J mice. The total number of TH positive cells in the reaggregates (7868 ± 1030, n=5) was not significantly different from that observed in intact brain. The reaggregation of cells from individual brains therefore provides an approach to the estimation of DA cell numbers in the mouse brain as well as a means for maintaining essentially all of the mesencephalic DA neurons over a fairly extended period of culture during which they continue their morphologic and neurochemical development. Supported by MH28942.

382.10

AMPHETAMINE AND MAZINDOL EFFECTS ON NIGROSTRIATAL DOPAMINE NEURONS DURING POSTNATAL DEVELOPMENT: HEMI-TRANSECTION OF FOREBRAIN/ MIDBRAIN CONNECTIONS.

D. K. Pitts* and G. Zhang. Dept. of Pharmaceutical Sciences, College of Pharmacy & A.H.P., Wayne State University, Detroit, MI 48202.

Amphetamine (AMP) inhibits mesencephalic dopamine (DA) neuronal impulse flow by enhancing both the stimulation of somatodendritic autoreceptors by dendritically released DA and the activity of inhibitory forebrain feedback pathways which are activated by DA. During postnatal development synaptogenesis is still occuring in the rat forebrain. The physiological status of the forebrain feedback pathways during development is not well understood. Extracellular electrophysiological techniques were used to examine the effects of AMP on nigrostriatal dopamine (NSDA) neurons during postnatal development in the urethane anesthetized rat. When given i.v., AMP (3.2 mg/kg) elicited a similar inhibition of spontaneous NSDA neuron activity in adults and 2-week(wk)-old rats. Hemitransections of the midbrain/forebrain (MF) connections, however, significantly accelerated the recovery of adult NSDA neurons from the rapid inhibitory effects of i.v. amphetamine without affecting the recovery of NSDA neurons from 2-wk-old rats. M/F hemitransections significantly enhanced the slowly developing inhibitory effects of i.p. AMP (6.4 mg/kg) on 2-wk-old NSDA neurons with a small more complex effect on adult NSDA neurons. M/F hemitransections significantly attenuated the slowly developing inhibitory effects of i.p. mazindol (6.4 mg/kg; see Zhang and Pitts, Soc. Neuros. Abs., 1993), in adult NSDA neurons with a much smaller marginally significant attenuation in 2-wk-old NSDA neurons. These results suggest that forebrain feedback pathways are still undergoing development in 2-wk-olds and that responses to indirect agonists are dependent on M/F connections, route of administration and the ability to cause catecholamine release. [Supported by MH47857 to DKP]

382.11

EFFECTS OF MAZINDOL AND NOMIFENSINE ON NIGRO-STRIATAL DOPAMINE NEURONS DURING POSTNATAL DEVELOPMENT.

G. Zhang* and D. K. Pitts. Dept. of Pharmaceutical Sciences, College of Pharmacy & A.H.P., Wayne State University, Detroit, MI 48202.

Amphetamine can inhibit mesencephalic dopamine (DA) neuronal impulse flow by enhancing both the stimulation of somatodendritic autoreceptors by dendritically released DA and the activity of inhibitory forebrain feedback pathways which are activated by DA. These effects are the result of the ability of amphetamine to both cause catecholamine release and inhibit its reuptake. Previous extracellular electrophysiological studies examined the effects of amphetamine on spontaneously active nigrostriatal dopamine (NSDA) neurons during postnatal development in the rat. When given by the *i.p.* route, amphetamine had inhibitory effects on NSDA neuron discharge rate which were of equal magnitude across ages 2-days-old through adulthood. However, the time course for the inhibitory effects differed significantly among the various age groups. In contrast to amphetamine which can cause catecholamine release and inhibit reuptake, mazindol and nomifensine are relatively selective DA reuptake inhibitors which do not displace transmitter stores. Therefore these agents are useful for examining the physiological status of the dopamine transporter during postnatal development. In the present extracellular electrophysiological studies using urethane anesthetized rats both *i.p.* mazindol (3.2-12.8 mg/kg) and *i.p.* nomifensine (6.4-12.8 mg/kg) caused a dose-dependent inhibition of spontaneous NSDA neuron activity. NSDA neurons from 2-week-olds were found to be significantly more sensitive to the inhibitory effects of mazindol and nomifensine than NSDA neurons from adults. These results are discussed in the context of previous amphetamine findings. [Supported by MH47857 to DKP]

382.12

POSTNATAL DEVELOPMENT OF MESOACCUMBENS DOPAMINE NEURON SOMATODENDRITIC AUTORECEPTORS.

L. Wang* and D.K. Pitts. Dept. of Pharmaceutical Sciences, College of Pharmacy & A.H.P., Wayne State University, Detroit, MI 48202.

Previous studies of nigrostriatal dopamine-containing (NSDA) neurons indicated that in 2- and 4-week(wk)-old rats these neurons were less sensitive to the inhibitory effects of *i.v.* apomorphine (APO,D2/D1 agonist) on discharge rate than neurons from adults. The sensitivity of NSDA neurons to *i.v.* quinpirole (QUIN,D2/D3 agonist), however, was not found to be age dependent. *Iontophoretic* studies indicated that NSDA neurons from early postnatal periods and those from adults were equally sensitive to the ihhibitory effects of APO (1-wk-old to adult) or QUIN (2-wk-old to adult). This data suggests that the sensitivity of somatodendritic autoreceptors on NSDA neurons during early postnatal development is similar to that of adults. In the present studies extracellular recordings of single antidromically identified mesoaccumbens dopamine-containing (MADA) neurons were made from 2-wk-old and adult rats anesthetized with chloral hydrate. MADA neurons from 2-wk-olds were found to be less sensitive to *i.v.* APO than the neurons from adults. Preliminary evidence suggests a similar age-dependent difference in sensitivity for *i.v.* QUIN. However, *iontophoretic* studies using 7OH-DPAT (D3/D2 agonist) found no significant differences in sensitivity when comparing either MADA or NSDA neurons from 2-wk-olds to similarly identified neurons from adults. The above findings suggest that the differences in sensitivity seen when agonists are given either by the *i.v.* route or by *iontophoresis* is the result of the latter technique reaching a more restricted DA receptor population (e.g., that on the soma & proximal dendrites of DA neurons & neighboring afferents). Further *iontophoretic* and *i.v.* studies with other agonists are currently underway. [Supported by MH47857 to DKP]

383.1

THE NEUROPEPTIDE FMRFAMIDE MODULATES NEURONAL EXCITABILITY IN THE POND SNAIL **HELISOMA.** W.L.Smith*, F. Bahls, and P.G.Haydon. Dept of Zoology and Genetics, Iowa State University, Ames, IA 50011.

FMRFamide, through multiple presynaptic pathways, reduces the amount of transmitter released at the synaptic terminal. These pathways result in a decrease in the activity of high-voltage-activated calcium channels, a decrease in the responsiveness of secretory machinery to calcium, and an increase in an outward potassium current which is mediated by metabolites of arachidonic acid. Current studies are focused on FMRFamide's affects on the excitability of neuron B5 from the buccal ganglia of the pond snail *Helisoma trivolvis*. Two possible effects of the increased potassium current are i) a change in excitability and ii) a change in the duration of action potentials. We have determined that FMRFamide application does result in a decrease in excitability with no apparent change in action potential duration.

Bath application of 10^{-6}M FMRFamide to cultured B5 neurons results in a large decrease in excitability as assayed by the number of action potentials evoked in response to a 5 second depolarizing stimulus. The observed effects are reversible upon washout of FMRFamide. We are currently studying the role of the arachidonic acid pathway in mediating the modulation of neuronal excitability. While FMRFamide decreases excitability, the half-amplitude duration of single action potentials remains unaffected.

383.2

ALTERATIONS IN THE POST-EMBRYONIC EXPRESSION OF FMRFAMIDE IN DROSOPHILA. J.Σ. Silber and P.H. Taghert.* Anat. & Neurobiology, Washington Univ. Med. Sch., St. Louis, MO 63110.

Drosophila melanogaster expresses *FMRFamide* in a discrete, stereotyped pattern in the central nervous system. We examined FMRFamide expression by staging 1st, 2nd, 3rd instar larvae and pupae in 12 hour increments. The expression pattern of the proFMRFamide protein was examined and compared to FMRFamide-lacZ expression in a P-element line known as WF3-Y (Chin et al., 1990, DNA Cell Biol. 9:263; Schneider et al., 1993, Neuron 10:279). Our studies indicate that changes in FMRFamide and FMRFamide-lacZ expression occur on a cell by cell basis. With the exception of one cell type, both patterns are constant from the beginning of the 1st instar through the first half of the 3rd instar. While some cell types continue to express FMRFamide consistently, others exhibit dramatic changes in immunoreactivity. Alterations in the expression pattern occur in at least two different time periods of the 3rd instar. These events take place shortly before, and after, onset of the wandering 3rd instar stage. Changes in the pattern continue through the pupal stages; some cells express FMRFamide and FMRFamide-lacZ consistently, others decrease their expression, and at least one cell type begins to exhibit immunoreactivity late in pupal development. These changes in the FMRFamide pattern should help provide insights into mechanisms regulating post-embryonic transmitter phenotypes.

383.3

A SENSORY NEURON IS SENSITIVE TO ITS ENDOGENOUS PEPTIDE. A. Wenning*[1] and R.L.Calabrese[2]. [1]Fak. f. Biologie, Univ.Konstanz, 78434 Konstanz, Germany; [2] Dept. of Biology, Emory Univ., Atlanta, GA 30322.

Sensory and neurosecretory innervation of each leech nephridium is accomplished by a single neuron, the nephridial nerve cell (NNC). These peripheral neurons contain FMRFamide which they probably release on three targets: the urine-forming cells of the nephridium, the muscles of the urinary bladder sphincter and central neurons (Wenning et al., J Exp Biol, in press). The role of FMRFamide, for example whether it modulates ion transport in the nephridium, is yet unknown. Resting potential and activity of the NNC depend on the extracellular chloride concentration. The NNC is spontaneously active in normal, low blood chloride. It hyperpolarizes and its spike rate decreases in high blood chloride, for example after a blood meal (Wenning & Calabrese, J Comp Physiol, 168:53, 1991).

As shown by extracellular recordings from the NNC in isolated preparations (including nephridium and bladder) bathed in low chloride saline, FMRFamide (bath applied at 10^{-7}M) completely suppresses spike activity. Intracellular recordings from superfused preparations show that the NNC hyperpolarizes when FMRFamide (10^{-5}M) is focally applied to its soma or dendrites. Through its sensitivity to the endogenous peptide, the NNC may modulate its peptide release.

Supported by the DFG (We745/4 to A.W.) and NIH (NS24072 to R.L.C.).

383.4

STRUCTURE - ACTIVITY RELATIONSHIP OF FMRFamide RECEPTOR BINDING IN SQUID (*LOLIGO*) OPTIC LOBE.
G. J. Chin*[1], K. Payza[2], D. A. Price[3], K. E. Doble[3], and M. J. Greenberg[3]. [1]LDN, NICHD, Bethesda, MD, 20892; [2]LBG, NIMH Neuroscience Center at St. Elizabeths, Washington, DC 20032; [3]Whitney Lab, University of Florida, St. Augustine, FL, 32086.

FMRFamide (Phe-Met-Arg-Phe-amide) is a neuropeptide transmitter in many invertebrates. Previously we presented evidence for high-affinity, G protein-coupled FMRFa receptors in membranes of squid optic lobe. In this study we characterized the ligand binding site of the receptors by using ^{125}I-daYFnLRFa as radioligand and performing competition experiments with a large series of FMRFa analogs substituted in each of the four amino acid positions. Of the tetrapeptide analogs tested, FMRFa was the most potent ligand (IC_{50} = 0.4 nM). The first position was very selective for Phe, since all substitutions were >500-fold less potent except for Tyr (IC_{50} = 5 nM). The Met^2 position showed a range of tolerance to substitution, but the C-terminal Arg, Phe, and amide were highly critical for binding activity. Intact and CHAPS-solubilized receptors shared the same binding specificity and G-protein coupling. The CHAPS-solubilized ^{125}I-daYFnLRFa binding sites thus appear to be genuine FMRFa receptors.

383.5

THE STRUCTURE-ACTIVITY RELATIONS OF FMRFamide DEPEND ON THE ASSAY USED TO MEASURE THEM. M.J. Greenberg*, K.E. Doble, W. Lesser, B.M. Dunn and D.A. Price. Whitney Lab, Univ. of Florida, St. Augustine, FL 32086-8623.

We have synthesized 76 of a series of 80 FMRFamide analogs in which each residue in the tetrapeptide is substituted one at a time by the other 19 amino acids. These peptides were bioassayed on the isolated radula protractor muscle (RPM) of a whelk, *Busycon contrarium*, and the isolated, perfused heart of a pulmonate snail, *Helix aspersa*. Radioimmunoassay (RIA) was with two antisera: S253, raised in rabbits to YGGFMRFamide; and Q2, raised to pQDPFLRFamide and DDPFLRFamide. The RPM bioassay and the S253 RIA were most similar, with strict requirements for an Arg^3 and a C-terminal Phe, but with significant tolerance for substitutions at Met^2. The snail heart assay differed in its substantial tolerance for variation at Phe^4, as well as at Phe^1; the data suggest that the C-terminal tetrapeptide of the SCPs - FPRMamide - would be about 8 times more active than FMRFamide, and that a few of the peptides were, in fact, acting at the SCP receptor of the *Helix* heart. Finally, the Q2 RIA is distinct in being very tolerant of changes at Phe^1 and even at Arg^3; but the C-terminal Phe is strictly required. The variability in structure accepted by receptors may be related to the narrowness of the limits within which the tissue functions. Supported by NIH HL28440.

383.6

FMRF-NH_2 EVOKES AN INWARD CURRENT IN HEART INTERNEURONS OF THE LEECH. Joachim Schmidt, Ted W. Simon* & Ronald L. Calabrese Dept. of Biology, Emory University, Atlanta, GA 30322

Pairs of interneurons (HN cells) in the 3rd and 4th ganglion in the CNS of the leech constitute the timing oscillator for heartbeat. These HN cells form reciprocal inhibitory synapses across the ganglion, that lead to a rhythmic bursting in HN cells (Peterson 1983, J. Neurophysiol. 49:256). Bath application of FMRF-NH_2 (10^{-8} to 10^{-6} M) accelerates the rhythm of HN cells and eventually disrupts the bursting pattern (Simon et al. 1992, J. Neurosci. 12:525). FMRF-NH_2 acts in part by hyperpolarizing shifts of steady state activation and inactivation of a K^+ current, I_K.

Focal application of FMRF-NH_2 (10^{-6} M) for 5 to 10 s onto the somata of HN cells causes we a depolarizing effect of FMRF-NH_2. This response was subject to strong desensitization. Similar to bath appliction of FMRF-NH_2, we also observed acceleration of the rhythm after focal application.

Membrane properties underlying the depolarization were characterized using the single electrode voltage clamp technique. Focal application of FMRF-NH_2 increased membrane conductance, causing an inward current. This inward current appeared to be voltage dependent. Focal application of FMRF-NH_2 did not depolarize HN cells that were bathed in Na-free saline, but did depolarize HN cells when Li^+ was substituted for Na^+. These experiments indicate, that the inward current is carried by Na^+.

Reduction of the normal 1.8 mM Ca^{2+} concentration to 0.1 mM Ca^{2+} in the bathing saline did not effect the responsiveness of HN cells to focal FMRF-NH_2 application. This result indicates that the response does not depend on external Ca^{2+}. When 1.8 mM Co^{2+} substituted for Ca^{2+} in the bathing saline the depolarizing response of the HN cells was blocked .

383.7

ISOLATION OF pQFYRFamide FROM SNAIL GANGLIA. D.A. Price[*1], K.E. Doble[1], W. Lesser[1], M.J. Greenberg[1], G.A. Cottrell[1,3], K.M. Swiderek[2], T.D. Lee[2], E.M. Lutz[2], J. Sommerville[2]. [1]Whitney Lab., St.Augustine, FL 32086, [2]City of Hope, Beckman Res. Inst, Duarte, CA and [3]Dept. of Biol., University of St. Andrews, Scotland, UK.

We have isolated and sequenced two different cDNAs that encode FMRFamide-related peptides from a snail (Helix aspersa) ganglia library. Both begin with the same stretch of 250 identical bases which includes the 5'non-coding region and the first 25 amino acids, but the sequences diverge thereafter, so we assume that they are products of alternative splicing as was previously found for the homologous mRNAs in Lymnaea (Saunders et al., J.Neurosci. 12:1033, 1992). One precursor encodes the two tetrapeptides FMRFamide and FLRFamide as well as the pentapeptide pQFYRFamide. The other precursor encodes a number of heptapeptides, most conform to the consensus sequence XDP(F/Y)LR(F/I)amide. Since the tetrapeptide precursor is expressed by neurons that innervate the heart, we tested synthetic pQFYRFamide on the isolated heart and found it to be a more potent cardioexcitor than FMRFamide. We have isolated a peptide from ganglion extracts by HPLC which has the expected elution position, immunoreactivity and UV absorbance for pQFYRFamide. Electrospray mass spectrometric analysis of this peak showed a prominent, protonated molecular ion at 742.6 daltons in agreement with the calculated value of 742.9 for pQFYRFamide confirming the predicted structure.

383.8

EFFECTS OF NEUROPEPTIDES ON CRAYFISH HEART. M. Srivastava and A. J. Mercier*, Dept. of Biol. Sci., Brock University, St. Catharines, Ont., L2S 3A1.

Effects of four neuropeptides on isolated crayfish hearts were examined. The FMRFamide-related peptide DRNFLRFamide (DF_2) and proctolin increased the frequency and amplitude of cardiac contractions, while met-enkephalin and leu-enkephalin did not alter cardiac activity. Chronotropic effects presumably arise from peptide action on the cardiac ganglion; inotropic effects may potentially result from actions on the cardiac ganglion (via changes in number or frequency of impulses within bursts), on motor nerve terminals or on myocardial cells.

Intracellular recording from myocardial cells was used to characterize the effects of DF_2 in more detail. Muscle potentials exhibited a wave of depolarization, due to summated EPSP's, and an action potential. DF_2 increased the amplitude and duration of such waves and increased the width (but not amplitude) of the action potential. Input resistance did not change. Attempts are being made to examine myocardial effects by obliterating cardiac ganglion activity and stimulating myocardial cells with extracellular electrodes. Procaine (at 10^{-9} to 10^{-4} M) is ineffective at blocking cardiac ganglion activity. Contractions can be elicited by stimulating preparations in which the dorsal myocardium (containing the cardiac ganglion) is excised. These experiments may provide some evidence for direct effects on cardiac muscle cells.

Supported by NSERC Canada.

383.9

MOLECULAR ORGANIZATION AND EXPRESSION OF THE *DROSOPHILA MELANOGASTER* DROSULFAKININ GENE. R. Nichols[1]* and S.A. Schneuwly[2]. [1]Biological Chemistry Department, University of Michigan, Ann Arbor, MI 48109 and [2]Der Universität Würzburg, Germany. The *Drosophila melanogaster* drosulfakinin gene (Dsk) can be predicted to encode three peptides, DSK 0, DSK I, and DSK II. Based on sequence similarity, DSK I and DSK II are cholecystokinin homologues, while DSK 0 is not. The Dsk transcript has been shown to be expressed in all developmental stages by *in situ* tissue localization and Northern blot analysis. Sequence-specific antisera to DSK I and DSK II indicate that the *Drosophila* cholecystokinin homologues are expressed throughout development from late embryo through adult in the central nervous system. The entire nucleotide sequence of the Dsk gene and surrounding DNA has been obtained for *Drosophila melanogaster* and a single P element enhancer trap line insertion near the Dsk gene.

383.10

DROSOPHILA MELANOGASTER DROMYOSUPPRESSIN. J. McCormick* and R. Nichols. University of Michigan, Ann Arbor, MI 48109-1048. We have previously reported the isolation of an abundant peptide TDVDHVFLRFamide from *Drosophila melanogaster*. Based on structure similarity, this peptide has been designated dromyosuppressin (DMS). The myosuppressin peptides isolated to date from other organisms are highly conserved being either identical or differing only by the N-terminal amino acid residue. Studies indicate that myosuppressin peptides inhibit gut and oviduct motility. We have generated and characterized sequence-specific antisera to DMS. Our immunocytochemical study indicates that DMS is expressed throughout all developmental stages in the central nervous system and the gastrointestinal tract, indicating that expression is under developmental and tissue-specific regulation. A cDNA encoding DMS has been amplified from *Drosophila melanogaster* adult head RNA using a sequence-specific primer. This amplified cDNA has been used as a hybridization probe to screen a genomic library to isolate the Dms gene. The cDNA has also been used to cytologically localize the Dms gene to the left arm of the second chromosome.

383.11

BEHAVIORAL ANALYSIS OF RESPIRATION IN *LYMNAEA STAGNALIS*. L.L.Moroz*, S.U.Hasan, A.G.Bulloch, K.Lukowiak and N.I.Syed. University of Calgary, Neuroscience, Reproductive Medicine and Respiratory Research Groups, Calgary, Alberta, CANADA.

Gas exchange in fresh water snail, *Lymnaea stagnalis* primarily occurs via the lung through episodic opening and closing of the lung orifice, the pneumostome. Although the neuronal network of the respiratory central pattern generator of *Lymnaea* has recently been identified *in vivo* and subsequently reconstructed *in vitro* by Syed *et al* (Science; 250, 1990), little is known about the peripheral component of this network or the normal respiratory behavior. Therefore, we investigated the respiratory behavior of the *Lymnaea* under normoxic, hypoxic, hyperoxic, hypercapnic states and following administration of principal neurotransmitters. Under normoxic (control) conditions, the pH, PCO_2 and PO_2 of the hemolymph were 7.97±0.05, 9.49±0.18 torr and 27.0±0.9 torr (Mean±SD) respectively. During control periods, animals (n=8) visited the water surface 5±1.3 times per hour, the duration of each stay was 6±2.3 min., the pneumostome opened 5±1.3 times per h (once per visit) and for 25±15 seconds whereas during hyperoxia (100% O_2; n=12), these variables changed to 8±3.2 visits per h, 4±1.8 min./ stay, 3±1.6 times/h and <1 second ($p<0.001$) respectively. Under anoxic conditions, the animals (n=7) constantly stayed above the water surface, and the opening frequency and duration of the pneumostome were 15±4.0 per hour and 80±40 sec respectively ($p<0.01$). During hypercapnia, the animals (n=6) visited the water surface 11±5.2/h and stayed there for 7±3.2 minutes whereas the frequency and duration of the pneumostome opening were 9±3.6/h and 30±19 sec respectively. FMRF-amide suppressed the opening of pneumostome whereas dopamine initiated the breathing cycles. These studies provide a model system in which the control of breathing can be studied from behavioral to cellular level.

383.12

PARAMETERS WHICH DETERMINE THE RELEASE OF PEPTIDE COTRANSMITTERS (SCPS) VARY BETWEEN *APLYSIA* MOTOR NEURONS. M.D. Whim* and P.E. Lloyd. Dept. Pharm. Physiol. Sci. & Comm. on Neurobiol. Univ. Chicago, Chicago, IL 60637.

Aplysia motor neurons B1, B2 and B15 were placed in primary culture. Newly synthesized peptides were labeled with [35]S-methionine. Using a combination of HPLC and liquid scintillation counting, the release of peptides by intracellular stimulation was monitored. It is known that the SCPs can be released from B1 and B2 in a stimulation- and Ca-dependent manner (Lloyd et al, 1986). We now find that stimulation of B15 at 12 Hz with 4 sec bursts and 3 sec interburst intervals results in the release of authentic SCPA, SCPB and buccalin A, in a stimulation- and Ca-dependent manner. We then examined the effect of spike pattern on the release of the SCPs from B15. Spike number was kept constant and spike pattern was varied. We compared the effects of stimulating B15 at a tonic 5 Hz to 50 Hz for 1 sec with 9 sec interburst intervals. About 6-fold more peptide was released per spike by the latter paradigm. When we compared 5 Hz tonic firing to ~9 Hz with 4 sec bursts and 3 sec interburst intervals (a more physiological pattern; Cropper et al, 1990), the bursting pattern was still more effective at eliciting peptide release. In contrast the release of the SCPs per spike from B1 and B2 neurons in culture is pattern-insensitive over the physiological range. To ensure that this release was from neurites we compared the release of the SCPs from the soma and from intact B1 and B2 neurons. We conclude that peptide release is predominantly from the neurites. Thus the parameters which determine the release of the same peptides differ from neuron to neuron.

383.13

MODULATION OF PYLORIC NETWORK ACTIVITY BY TWO FAMILIES OF TACHYKININ-LIKE PEPTIDES. D.M. Blitz and M.P. Nusbaum*. Neurobiology Research Center and Dept. of Physiology & Biophysics; Univ. of Alabama at Birmingham; Birmingham, AL 35294.

The stomatogastric ganglion (STG) of the crab, *Cancer borealis*, is innervated by modulatory neurons that contain many different neuropeptides. One of these peptides is a Substance P-like peptide (Goldberg et al., Cell & Tiss. Res. 252:515-522, 1988). Many of these peptides elicit distinct motor patterns from the pyloric neural network in the STG. However, bath-application of Substance P (SP) elicits no response from the pyloric network. Recently, two families of tachykinin-like peptides, including eight leucokinins and two locustatachykinins, were identified in insect nervous systems (Holman et al., Comp. Biochem. Physiol. 88C:31-34, 1987; Schoofs et al., FEBS 261:397-401, 1990).

Exogenous application of members from both peptide families (10^{-6} M) excites the pyloric rhythm. Each peptide increased both the pyloric cycle frequency and the impulse activity in the LP neuron. Thus far, the leucokinins assayed include L-I, III, IV, VI and VIII. L-I was the only one of these peptides to also reproducibly elicit rhythmic bursting in the DG neuron, a member of the gastric mill network within the STG. Neither of the locustatachykinins (LOM TK-I & TK-II) elicited bursting activity in the DG neuron. In preliminary experiments aimed at determining whether either peptide family represents the source of the SP-like immunolabeling (SPLI) in the STG, we preincubated the SP antiserum with either L-I (10^{-4} M) or LOM TK-II (10^{-4} M, 10^{-3} M). SPLI was not blocked by preincubation with L-I, but was partially blocked with the lower and completely blocked with the higher concentration of LOM TK-II. We are continuing to characterize the effects of these peptides on the STG networks, and are working to determine which members of each family are the native peptides within the stomatogastric nervous system. Supported by BNS-8909613 & HFSP (MPN).

383.14

LIGHT LEVEL AND ULTRASTRUCTURAL ANALYSIS OF A PUTATIVE NEUROHEMAL ORGAN IN THE COMMISSURAL GANGLION OF THE CRAB, *Cancer borealis*. V.L. Kilman*, A.E. Christie, and E. Marder. Dept. of Biology, Brandeis Univ., Waltham, MA 02254.

Many peptides have neuromodulatory effects on the pattern generating networks of the crustacean stomatogastric nervous system. Substance P-like immunoreactivity is found throughout this system in cell bodies, in neuropil regions, and in a large club shaped structure in each commissural ganglion (CG) (Cell and Tiss Res 252:515, 1988). The club originates from fibers in the commissure anterior to the CG, follows the anterior-medial edge of the CG to the level of the inferior esophageal nerve (ion), and then curves back into the center of the ganglion and ends. Its function is unknown. We are using confocal microscopy of immunolabeled whole mounts along with sectioned toluidine-blue stained tissue to study this structure.

Confocal microscopy of the antibody-labeled club shows fibers entering the CG which contain elongated swellings. Posteriorly these swellings become larger, denser, and less discrete as individual structures. The maximum cross-sectional diameter of these swellings is 6-18 μm, consistently larger than the varicosities stained by this antibody in the neuropil region. Serial optical sections show the club is fenestrated by tubular unstained regions.

Toluidine-blue stained 2 μm sections show a concentration of large darkly staining structures in the club region of the CG. There is a gradation in length of these structures from 4 μm near the commissure to 6-15 μm near the ion. They are distinctly different in size and location from the smaller neuropilar varicosities. The most posterior of these structures cluster around empty lacunae but do not contact cell bodies or enter the fine neuropil region. Ultrastructure of this region will be presented.

At present our findings suggest that this structure may mediate non-directed neurohemal release of a substance P-related peptide in the commissural ganglion. Supported by NS17813 and T32NS07292.

383.15

PHARMACOLOGICAL CHARATERIZATION AND HORMONAL MODULATION OF VOLTAGE-GATED CURRENTS IN *APLYSIA* BAG CELLS. L.A. Fieber*, NIEHS Marine and Freshwater Biomedical Sciences Center, Univ. of Miami, Rosenstiel School of Marine and Atmospheric Science, Miami, FL 33149.

The bag cells of the marine gastropod *Aplysia* are neurosecretory cells that when stimulated undergo extended periods of action potential discharge culminating in the release of bag cell hormones that bring about egg laying behavior. Egg laying is a stereotyped behavior that supersedes foraging and all other activities for several hours, and presumably is tightly regulated. The voltage-gated currents contributing to the depolarizing phase of the action potential in bag cells are found only in cells from sexually mature animals. When maintained in short term tissue culture and studied via whole cell voltage clamp, inward currents for Na^+ and Ca^{2+} were found in 67% and 86%, respectively, of bag cells from mature *A. braziliana* but were not present in cells from immature *Aplysia*. The Na^+ current activated at -20 mV and peaked at approximately +10 to 20 mV. Na^+ currents were absent in Na^+-free medium and reversibly inhibited by tetrodotoxin (10 μM), but were not blocked by Cd^{2+} (100 μM). The Ca^{2+} current, using 11 mM Ba^{2+} as the current carrier, activated at -20 mV, peaked at +20 to 30 mV, and was reversibly inhibited by nifedipine (5 μM). ω-conotoxin (10 μM) produced a slight inhibition. Both Na^+ and Ca^{2+} currents were insensitive to holding voltage in the range of -70 to -40 mV. Both currents were strongly inhibited by bath applied α–bag cell peptide (5 μM), a hormone released from bag cells, while the outward currents were slightly inhibited. Bag cell ionic currents are modulated by intracellular second messengers found elevated in bag cells undergoing action potential afterdischarge (Conn et al, 1889, J. Neurosci., 9: 473-479). A phorbol ester which may mimic the effects of protein kinase C on these cells, phorbol 12-myristate 13-acetate (25 nM), increased both inward currents for Na^+ and Ca^{2+}, and the outward currents.

Supported by USPHS ES05705.

383.16

ANATOMY AND PEPTIDE IMMUNOREACTIVITY OF THE NEUROSECRETORY CELLS OF THE SUBESOPHAGEAL GANGLION OF *MANDUCA SEXTA*. N.T. Davis*, H.K. Lehman, P.E.A. Teal, and J.G. Hildebrand. Div. of Neurobiology, Univ. of AZ, Tucson, AZ 85721.

Four groups of neurosecretory cells residing in the subesophageal ganglion (SEG) and projecting to neurohemal release sites in the corpus cardiacum (CC) were identified. Two anterior median pairs of cells (MdAM) are in the mandibular neuromere, and a pair of ventral median triplets (MxVM) is in the maxillary neuromere. These cells project contralaterally to the CC via the ventral nerve of the CC. A pair of lateral neurosecretory cells (MxL) in the maxillary neuromere and a ventral median pair (LbVM) in the labial neuromere project contralaterally to the brain and thence to the CC via nerve 3 of the CC. Cells MdAM, MxVM, and LbVM were immunoreactive to antisera against Pheromone Biosynthesis Activating Neuropeptide (anti-PBAN) at all stages of postembryonic development of males and females. Anti-PBAN also stained 2 pairs of interneurons closely associated with the MxVM cells; these interneurons descend in the ventral nerve cord, provide branches to the dorsal neuropil of each of the ganglia, and branch extensively in the terminal ganglion. The 3 groups of median neurosecretory cells were also stained by anti-FMRFamide Anti-Proctolin stained only the MdAM and MxVM cells; anti-Pigment Dispersing Hormone stained only the LbVM cells; anti-Crustacean Cardioacceleratory Peptide stained the MxL cells. Staining for PBAN was especially intense in the middle stages of adult development but diminished in late stages. During this period of development, a corresponding change in concentration of PBAN immunoreactivity of a fraction isolated from the SEG was demonstrated by competitive ELISA; early to middle stages contained 0.38-0.42 pmoles/SEG, whereas young adults contained 0.13 pmoles/SEG. Studies are underway to determine if there is neurohemal release during late development of the adult. [Supported by the Whitehall Foundation (to NTD).]

383.17

THE ALLATOSTATINS INFLUENCE THE GASTRIC SYSTEM OF THE CRAB, *Cancer borealis*. P. Skiebe-Corrette*, J. C. Jorge-Rivera and E. Marder, Dept. of Biology, Brandeis University, Waltham, MA 02254,

The allatostatins (AST)1-4 are a family of amidated peptides that share a C-terminal sequence. These peptides were isolated from brains of virgin female cockroaches (Woodhead et al 1989, Proc Natl Acad Sci USA 86, 5997). In cockroaches, the ASTs inhibit the synthesis and release of juvenile hormone. In crabs, AST 1-4 inhibit the pyloric rhythm, and the presence of one or more allatostatin-like peptides in the stomatogastric nervous system was shown by immuncytochemical studies with an antibody raised against AST 1 (Skiebe-Corrette and Schneider, in preparation).

We now show that the ASTs influence both the gastric mill rhythms and the neuromuscular junctions of gastric mill muscles. 10^{-7} to 10^{-6}M AST inhibits ongoing gastric rhythms in *in vitro* preparations of the somatogastric nervous system. DG neuron bursts decrease in frequency, and LG activity is inhibited. Moreover, pyloric/gastric interactions characteristic of "switching" are modified by the ASTs.

AST-3 reduces the gain of gastric motor neuron to muscle interactions. 10^{-8} M AST-3 reduces the tension evoked by stimulation of the motor nerve in the gm 9 and gm8 muscles. AST-3 also reduces the amplitude and modifies the facilitation of nerve-evoked Excitatory Junctional Potentials (EJPs) in gm8.

In summary, the ASTs appear to inhibit motor function at multiple sites within the somatogastric system.

Research supported by DFG SK 38/1-1 (PSC), T32 NS07292 (JCJR), and NS17813 (EM).

383.18

NEUROPIL ARBORIZATION AND TRANSMITTER COMPLEMENT OF A MODULATORY PROJECTION NEURON. A.E. Christie[1*], B.J. Norris[2], M.J. Coleman[2], E. Marder[1], and M.P. Nusbaum[2]. [1]Dept. of Biology, Brandeis Univ., Waltham, MA 02254,[2]Neurobiology Research Center & Dept. of Physiology & Biophysics; Univ. of Alabama at Birmingham; Birmingham, AL 35294.

The peptide proctolin excites the pyloric and gastric mill rhythms when bath-applied to the stomatogastric ganglion (STG) of the crab, *Cancer borealis*. There are three distinct proctolin neuron pairs that innervate the STG. At least two of these pairs have different effects on the STG rhythms, which include separate subsets of the proctolin-application effects. This suggests that, within the STG neuropil, (1) these proctolin neurons may arborize in spatially separate regions, (2) there are restricted spheres of influence of neurally-released proctolin, and/or (3) each neuron has different co-transmitters that modify the proctolin effects. We are studying these issues by analyzing the arborization of a proctolin neuron, Modulatory Commissural Neuron 1 (MCN1), within the STG.

Using confocal microscopic analysis of Lucifer yellow (LY) dye-fills of the MCN1 arbor in the STG neuropil, we have found LY-labeled neurites and varicosities throughout the peripheral neuropil. Sectors densely-packed with MCN1 arbor often alternate with nearly empty regions. In ganglia that were also immunolabeled with proctolin antisera, MCN1 varicosities were double-labeled, and areas near those lacking MCN1 branches were replete with proctolin-immunoreactivity. There is thus no large scale segregation of branches from each proctolin neuron, although there may be distinct microdomains for each one. We have also found that MCN1 exhibits Substance P-like immunoreactivity (SPLI), and that it is the sole source of SPLI in the STG. This SPLI indicates that MCN1 has a co-transmitter unique among the proctolin neurons. Thus, both branching patterns and cotransmitters may contribute to the distinct effects of each proctollin neuron within the STG. Supported by NS29436 (MPN), NS17813 (EM) & HFSP.

383.19

THE DISTRIBUTION OF MYOMODULIN-LIKE IMMUNOREACTIVE MATERIAL IN THE NERVOUS SYSTEM AND PERIPHERAL TISSUES OF THE RAGGED SEA HARE, *BURSATELLA LEACHII*. M.W. Miller*. Institute of Neurobiology, Univ. of Puerto Rico, Blvd. del Valle 201, San Juan, PR 00901.

The neuropeptide myomodulin A was originally identified in the neuromuscular system underlying feeding behavior of *Aplysia californica*, where it was proposed to act as a modulatory cotransmitter (Cropper et al., 1987). Immunocytological studies showed that myomodulin-like immunoreactive material is present in specific neurons throughout the central nervous system of *Aplysia* and in fibers and varicosities on target organs belonging to several additional physiological systems (Miller et al., 1991). In order to begin to assess the degree to which the functions of myomodulin-related peptides have been evolutionarily conserved, the distribution of myomodulin-like immunoreactive material was examined in *Bursatella leachii*, a member of the Aplysidae family (Subfamily: Notarchinae). Cell bodies containing myomodulin-immunoreactive material were located in each of the central ganglia. The visceral ganglion, which in *Bursatella* is fused with the circumesophageal ring, contains a large immunoreactive neuron that may be homologous to the L10 interneuron of *Aplysia*. In the buccal ganglion, immunoreactive material was located in eight to ten large neurons in a region corresponding to the ventral motor neuron cluster of *Aplysia*. In addition, a very large spherical structure (400-600 μm in diameter) was present in the most ventrolateral part of each hemiganglion. This structure may be an extraordinarily large neuron or may be a capsule in which several neurons are encased. Its surface was covered with branching fibers containing immunoreactive material. Myomodulin-like immunoreactivity was also observed in peripheral sensory and motor systems. In the eye, labelling occurred in a population of widely-dispersed monopolar neurons and in several fibers in the optic nerve. Finally, branching immunoreactive nerve fibers were present on a well-developed bilateral longitudinal muscle running the entire length of the animal, from the mouth region to the tail. Experiments in progress are directed toward the functional characterization of myomodulin-immunoreactive neurons in *Bursatella*.

STORAGE, SECRETION, AND METABOLISM I

384.1

STEREOSELECTIVITY OF NICOTINE TO INDUCE DOPAMINE (DA) RELEASE FROM SUPERFUSED RAT STRIATAL SLICES: DEPENDENCE UPON PRIOR DA LOADING. L.H. Teng, S.T. Buxton, P.A. Crooks and L.P. Dwoskin*. Graduate Center for Toxicology and College of Pharmacy, University of Kentucky, Lexington, KY 40536.

In agreement with previous reports, in the current study nicotine (1.0 nM - 100 μM) enhanced [^{3}H]overflow from rat striatal slices preloaded with [^{3}H]dopamine (DA) in a concentration-dependent and stereoselective manner, in both the absence and presence of nomifensine (10 μM) and pargyline (10 μM) in the superfusion buffer. Determination of the effect of S(-) and R(+)nicotine on endogenous DA release revealed a concentration-dependent increase in dihydroxyphenylacetic acid (DOPAC) overflow, in both the absence and presence of nomifensine (10 μM) in the superfusion buffer. In contrast, the effect of nicotine on endogenous DA release was not stereoselective. Furthermore, when pargyline (10 μM) was included in the buffer, nicotine no longer enhanced DA release. Moreover, when striatal slices were loaded with cold DA (0.1 μM) rather than loading with [^{3}H]DA (0.1 μM), stereoselectivity of the effect of nicotine on DA release was observed. The results suggest that nicotine releases DA from two separate pools within the DA terminal. The effect of nicotine on the newly uptaken pool of DA is stereoselective and probably receptor mediated. Whereas, the effect of nicotine on the DA storage pool is not stereoselective and may not be receptor mediated. Generalization of results from experiments using [^{3}H]DA as a tracer to load DA terminals and subsequently monitor DA release should be interpreted with caution. (Supported by a grant from the Tobacco & Health Research Institute, Lexington, KY).

384.2

pH-JUMP UPTAKE OF ACETYLCHOLINE BY SYNAPTIC VESICLES. M. L. Nguyen and S. M. Parsons*. Neuroscience Research Institute, University of California, Santa Barbara, California 93106.

Cholinergic synaptic vesicles purified from the electric organ of *Torpedo californica* can be loaded with buffers of known pH by hyposmotic lysis. [^{3}H]ACh is taken up in a vesamicol-sensitive manner when the vesicle lumen is set at pH 5.5 and the external pH is jumped to 7.8. Uptake of [^{3}H]ACh is dependent on the vesicular pH gradient, as less uptake occurs at higher luminal pH values and uptake is blocked by very low concentrations of nigericin. The maximal uptake at five minutes after the pH-jump saturates at 13 nmol/mg with a K_M of 200 uM, which is very similar to the K_M for ATPase driven uptake of ACh. After five minutes the occluded [^{3}H]ACh leaks out of the vesicles; this leakage is blocked by vesamicol added at the peak of uptake, indicating that the leakage occurs through the ACh transporter. The pH gradient as monitored by the absorbance of acridine orange substantially dissipated over the time course of the experiment, suggesting that the efflux of [^{3}H]ACh is due to reversal of the uptake mechanism. Nonradioactive ACh (50mM) added at the peak of uptake does not stimulate the rate of leakage of the [^{3}H]ACh, which suggests that efflux is rate-limited by reorientation of the loaded ACh transporter from the inside to the outside of the vesicle. The pH-jump approach to inducing active transport of ACh will simplify mechanistic study of the transporter by removing the dependence of transport on the V-type ATPase.

384.3

MYOSIN MAY BE INVOLVED IN THE NUEROTRANSMITTER RELEASE MECHANISM AT THE SYNAPSE FORMED BETWEEN RAT SYMPATHETIC NEURONS IN CULTURE. S. MOCHIDA[a], Y. NONOMURA[b], H. KOBAYASHI[a] and B. LIBET*[c], [a]Dept. of Physiology, Tokyo Med. Coll., Tokyo 160, [b]Dept. of Pharmacology, Univ. of Tokyo, Sch. of Med., Tokyo 113 and [c]Dept. of Physiology, Univ. of California, Sch. of Med. San Francisco, CA 94143.

Some evidence pointed to the possibility that myosin could be involved in the neurotransmitter release, because it was found that myosin light chain kinase (MLCK) seemed to play a role in exocytosis of secretory cells[1,2] Therefore, we looked at the histochemical distribution of myosin in neuronal terminals and tested its possible role in synaptic transmitter release by depression of MLCK activity and by antibody on myosin itself at the synapse formed between cultured sympathetic ganglion cells in which some cells make functional synaptic contacts with other ganglionic cells. Myosin antibody recognized at the same synaptic terminals that showed synaptophysin presence with its antibody. Myosin antibody or SM-1, the pseudosubstrate inhibitor of MLCK, introduced into the presynaptic neuron inhibited the synaptic activation of the post synaptic cell. Wortmannin, a selective inhibitor of MLCK, also inhibited transmitter release when applied into the presynaptic neuron or to the bath solution. These results indicate that phosphorylation of myosin by MLCK may be necessary for transmitter release.

1. Kitani, S. et al., BBRC., 183, 48-54, 1992.
2. Ohara-Imaizumi, M. et al., BBRC., 185, 1016-1021, 1992.

384.4

IMMUNOPHILIN REGULATION OF NEUROTRANSMITTER RELEASE. J.P. Steiner*, T.M. Dawson and S.H. Snyder. Dept. Neuroscience, Johns Hopkins University Sch. of Med., Baltimore, MD 21205.

The immunophilins, FK506-binding protein (FKBP) and cyclophilin, mediate the immunosuppressant actions of drugs such as FK506 and cyclosporin A, respectively. In the brain, the immunophilins occur in substantially higher concentrations than in the immune tissues and are highly localized in discrete neuronal populations together with the calcium/calmodulin-dependent protein phosphatase, calcineurin, whose activity is inhibited by immunosuppressant drug-immunophilin complexes. The immunophilins regulate exocytosis of secretory granules in mast cells and regulation of synaptic functions such as neurotransmitter release may be a function for immunophilins working with calcineurin in the brain. FK506 enhanced the release of dopamine, norepinephrine, serotonin, GABA and glutamate from striatal synaptosomes in a dose dependent manner evoked by potassium depolarization. Concentrations of FK506 as low as 100 pM significantly enhanced the release of the transmitters. Rapamycin, which interacts selectively with FKBP to inhibit effects of FK506, blocked the FK506-induced increases in transmitter release. Cyclosporin A also potentiated the release of these neurotransmitters. Since immunophilins colocalize with and regulate the activity of calcineurin, we measured the levels of phosphorylation in synaptosomes in response to FK506 and depolarization. Phosphorylation of several synaptic vesicle proteins was enhanced by FK506, indicating that they are calcineurin substrates and thus might mediate the enhancement of transmitter release elicited by FK506. Our results suggest that a major function of the immunophilins maybe to regulate neurotransmitter release.

384.5

TACHYKININ RELEASE FROM RAT VENTRAL SPINAL CORD IS HIGHLY FREQUENCY-DEPENDENT. J. FRANCK, E. BRODIN(1) and G. FRIED*. Departments of Physiology and Pharmacology(1), Karolinska Institutet, S-104 01 Stockholm, Sweden.

The release of endogenous 5-hydroxytryptamine (5-HT), substance P (SP) and neurokinin A (NKA) from superfused tissue slices of rat ventral lumbar spinal cord, where SP and NKA coexist with 5-HT in terminals of descending bulbo-spinal neurons, was investigated. Electrical field stimulation was carried out using square-wave pulses with 2 ms duration and 30 mA stimulus intensity. Four different patterns of stimulation were used; 2 Hz continuous, 20 Hz continuous, 20 Hz intermittent and 50 Hz intermittent stimulation. 5-HT was measured in the slice superfusates by high performance liquid chromatography with electrochemical detection (HPLC-ED). SP and NKA were measured by radioimmunoassay (RIA). The release of 5-HT was significantly enhanced using all stimulation paradigms and the evoked release of 5-HT per pulse was independent of the stimulation frequency. The release was found to be calcium-dependent and there was no increase in the efflux of 5-hydroxy-indole acetic acid (5-HIAA) in response to stimulation. At 2 Hz (continuous) no significant increase in the release of SP was observed. Stimulation at higher frequencies yielded a significant increase in the release of SP per pulse. At 20 Hz, the release was increased by 73% (continuous) and 74% (intermittent), and at 50 Hz (intermittent) by 175% of basal efflux. The evoked release of NKA was also frequency dependent. At 2 Hz (continuous), no significant increase in the release of NKA was observed. At 20 Hz (intermittent), the evoked release per pulse was increased by 33% and at 50 Hz (intermittent) by 53% compared to the basal efflux of NKA. The results suggest that coexisting neurotransmitters/neuromodulators in the spinal cord may be released in different proportions depending on the stimulation frequency, and that only 5-HT is released when the nerve terminal is activated by low frequency stimulation.

384.6

EFFECT OF NICOTINE ON NEUROTRANSMITTER RELEASE FROM THE RAT HIPPOCAMPUS. R.Miyoshi*, J.Semba, S.Kito, M.Furutsu, A.Ando and L.Shimada, *Department of Pharmacology, Tokyo Women's Medical College, Tokyo 162, Division of Health Sciences, University of the Air, Chiba 261, Japan.

Nicotine receptors are contained in the hippocampus in a high density. On the other hand, there are several papers published in which nicotine is considered to have beneficial effects on impaired memory function.

In order to directly assess the changes of release of endogenous amino acids, acetylcholine (ACh) and catecholamines from rat hippocampus induced by systemic administration of nicotine, we measured release of these substances using an intracerebral microdialysis technique.

A dialysis probe of Carnegie Medicine was inserted into rat hippocampus of a Wistar strain. ACh was assayed by HPLC with an electrochemical detector and amino acids with a fluorescence detector.

Intraperitoneal injection of nicotine caused decrease of glutamate and increase of GABA release. Aspartate and glycine release remained unchanged. ACh also stayed at the same level after the injection, while norepinephrine decreased.

It is assumed that these effects of nicotine on neurotransmitter release from the hippocampus may be related with its cytoprotective effect on hippocampal neurons.

384.7

ACTIVATION OF PROTEIN KINASE C (PKC) POTENTIATES K^+-STIMULATED 5-HT RELEASE IN RAT REGIONAL BRAIN AREAS. D.J. Jones*, A. Biediger and V. Gandhi. Dept. Anesthesiology, Univ TX Hlth Sci Ctr, San Antonio, TX 78284

Previous studies from our laboratory and others demonstrated that phorbol esters which activate PKC enhance either electrical or K^+-stimulated release of various neurotransmitters. As a prelude to studies evaluating the role of PKC activation in transmitter release in brain ischemia, we evaluated the effects of the phorbol ester, phorbol 12-myristate 13-acetate (PMA), on K^+-stimulated [^{3}H]5-HT release from slices of regional brain areas of the rat.

Slices (300 μm) were prepared from regional brain areas and superfused with control (5 mM K^+) or depolarizing (15 mM K^+) buffer. K^+-stimulated release was lowest in the medulla/pons (0.74 $\pm$ 0.06% total [^{3}H]) and highest in the corpus striatum (2.69 $\pm$ 0.22%). The percent enhancement of K^+-stimulated release was dependent on the concentration of PMA in the incubation medium. Depending on the brain area, 0.1 μM PMA enhanced K^+-stimulated release 25-55%. The highest increase occurred in the midbrain and hippocampus. PMA alone in the absence of K^+-depolarization produced no increase in release. Biologically inactive phorbol produced no enhancement. Receptor selectivity was demonstrated by blockade of the PMA-enhancement with the PKC-inhibitor staurosporine (0.1 μM). The present studies demonstrate that PKC modulates the voltage-dependent release of 5-HT in rat brain. Supported by Miles Laboratories.

384.8

PROPERTIES OF GLYCINE AND ß-ALANINE RELEASE FROM HIPPOCAMPAL SLICES FROM DEVELOPING AND AGEING MICE. S.S. Oja* and P. Saransaari. Tampere Brain Res. Ctr., Dept of Biomed. Sci., Univ. of Tampere, SF-33101 Tampere, Finland.

The release of two structurally related amino acids, glycine and ß-alanine, was studied form hippocampal slices from developing and ageing mice. The spontaneous and potassium-stimulated releases were monitored using a superfusion system. The response of glycine release to potassium ions (50 mM), an about 1.2-fold increase, remained fairly similar throughout the whole life-span of the mice studied (from 7-day- to 22-month-olds). Potassium stimulation evoked more ß-alanine release from slices from adult mice than from slices from immature animals, this response being thereafter of the same order of magnitude until 22 months of age. The release processes of both glycine and ß-alanine were not markedly Ca^{2+}- or Mg^{2+}-dependent, but they were affected by the glutamate receptor agonists. The release of glycine was potentiated by kainate, N-methyl-D-aspartate (NMDA) and quisqualate in developing mice but only kainate was effective in adults. The effect of kainate was abolished by CNQX and that of NMDA by MK-801. The release of ß-alanine was also stimulated by the three agonists only in the immature hippocampus. The actions of kainate and quisqualate were again antagonized by CNQX and that of NMDA by MK-801. The results point out similarities in the releases of glycine and ß-alanine, showing that both processes are similarly affected by the three subtypes of the glutamate receptor in the developing hippocampus.

Supported by the Emil Aaltonen Foundation, Finland.

384.9

QUANTITATIVE AUTORADIOGRAPHY OF (3H) BEFLOXATONE BINDING SITE IN RAT BRAIN. O. CURET* AND C. SAUVAGE Centre de Recherche Delalande Groupe Synthelabo 92500 Rueil Malmaison FRANCE

Befloxatone is a new potent, reversible, selective and competitive MAO-A inhibitor. Previous binding studies in Rat brain membranes have shown that (3H) Befloxatone is a selective and a high affinity radioligand for MAO-A.In the present studies the autoradiographic distribution of (3H) Befloxatone was performed in the presence of Harmaline (10 µm) to determine specific binding. The specific binding of (3H) Befloxatone to brain sections was saturable, reversible and of high affinity. Scatchard analysis revealed a single binding site with a Kd of 1.3nM and a Bmax of 7.5 fmol/mg protein. In competition binding studies with (3H) Befloxatone, the displacing potency of substrates and MAO-A inhibitors correlated highly and positively with their corresponding values from MAO-A inhibition assays. The rank of (3H) Befloxatone binding sites densities was: locus coeruleus>>interpeduncular nucleus> habenula, solitary tract nucleus, paraventricular thalamus> dorsal raphe and median hypothalamus. Low levels of binding were detected in white matter. In conclusion, (3H) Befloxatone is a valuable radioligand for quantitative autoradiographic distribution of MAO-A in Rat brain.

384.10

DOPAMINE AND SEROTONIN METABOLISM IN THE DA-DENERVATED AND 5-HT-HYPERINNERVATED NEOSTRIATUM OF ADULT RAT AFTER NEONATAL 6-OHDA. E. Molina-Holgado*, K.M. Dewar, L. Descarries, L. Grondin and T.A. Reader. Centre de recherche en sciences neurologiques and Départements de physiologie, psychiatrie et pathologie, Université de Montréal, Montréal, Québec, Canada.

Dopamine (DA) and serotonin (5-HT) metabolism were studied in the rostral neostriatum (rNS) of 3-month-old rats, following neonatal DA denervation by intraventricular 6-OHDA (lesioned) or saline injection in littermates (controls). 5-HTP and L-DOPA were measured by HPLC at times 0 and 30 min after inhibition of aromatic amino acid decarboxylase (AAAD) by NSD-1015 (100 mg/kg). DA and 5-HT were similarly measured after 0, 5, 10 and 20 min of monoamine oxidase (MAO) inhibition by pargyline (75 mg/kg). In the lesioned rNS (DA-denervated and 5-HT-hyperinnervated), inhibition of AAAD induced an accumulation of L-DOPA equivalent to 4.5% of that in controls (0.62 compared to 14 nmol/g/h), for an L-DOPA/DA ratio of 0.63, 187% above that in controls. 5-HTP accumulation was 79% greater than in controls (4.44 vs. 2.47 nmol/g/h), for a 5-HTP/5-HT ratio of 0.45, 54% lower than in controls. MAO inhibition induced total DA accumulations over 20 min that were equivalent to 30% of initial, normal values in the controls (35.4 nmol/g/h), and 70% of initial, depleted values in the lesioned rNS (1.38 nmol/g/h). Total 5-HT accumulations represented 66% of initial, normal values in the controls (4.32 nmol/g/h), and 30% of initial, elevated values in the lesioned rNS (6.0 nmol/g/h). Expressed as % of initial values at each time interval after pargyline, these measurements indicated a higher rate of DA and lower rate of 5-HT accumulation in the lesioned than control rNS. It could therefore be concluded that the turnover rate of DA was increased and that of 5-HT decreased in the DA-denervated and 5-HT-hyperinnervated tissue, in keeping with the respective L-DOPA/DA and 5-HTP/5-HT ratios. (Supported by MEC, Spain, and grants MT-3544 and 6967 from MRC, Canada).

384.11

CONTROL OF EVOKED DOPAMINE OVERFLOW IN THE BASOLATERAL AMYGDALOID NUCLEUS AND CAUDATE-PUTAMEN. P.A. Garris* and R.M. Wightman. Department of Chemistry and Curriculum in Neurobiology, University of North Carolina, Chapel Hill, North Carolina, 27599-3290.

The pharmacology of evoked dopamine (DA) overflow was compared in the basolateral amygdaloid nucleus (BAN) and caudate-putamen (CP). Extracellular DA concentration was measured *in vivo* in anesthetized rats by fast-scan cyclic voltammetry at carbon-fiber microelectrodes and elicited by electrical stimulation of ascending DA fibers over the frequency range of 10 to 60 Hz. The DA antagonist, haloperidol (0.5 mg kg^{-1} i.p.), and the reuptake inhibitor, nomifensine (25 mg kg^{-1} i.p.), elicited marked increases in extracellular DA in the CP. The increases were most pronounced at lower frequencies and were approximately 500 % above control at 20 Hz for both drugs. The DA agonist, apomorphine (2 mg kg^{-1} i.p.), reduced the effects of haloperidol by about half. Much smaller effects of haloperidol, apomorphine or nomifensine were observed in the BAN. In addition, the monoamine oxidase inhibitor, pargyline (75 mg kg^{-1} i.p.), had no effect on evoked DA overflow in either region. In conclusion, the regulation of extracellular DA levels in the BAN is distinct from that in the CP.

384.12

MODULATION OF STRIATAL DOPAMINE SYNTHESIS AND RELEASE BY ANTIPSYCHOTIC AGENTS: EVIDENCE FOR SEROTONERGIC INVOLVEMENT. C. Wiley Aretha, R.E. Arthur, Jr., L.D. Alphs and W.A. Wolf. V.A. Medical Center, Allen Park, MI 48101 and Cellular and Clinical Neurobiology Program, Dept. of Psychiatry, Wayne State University, Detroit, MI 48201.

Haloperidol is a typical antipsychotic with a propensity for eliciting movement disorders. Clozapine is an atypical antipsychotic which elicits little or no extrapyramidal side effects. It has been suggested that differential alterations in central serotonergic tone may be relevant to clozapine's clinical profile. A comparative analysis of the ability of haloperidol and clozapine to modify the synthesis and release of dopamine (DA) in specific brain regions was undertaken. In superfused rat striatal slices, haloperidol (30 nM) increased electrically stimulated release of endogenous DA to 200% of control. However, under the same conditions of stimulation, clozapine (300 nM - 3 uM) did not significantly alter DA release. Studies on a possible serotonergic involvement in this differential local control of striatal DA release will be presented. With respect to DA synthesis, it is established that acute haloperidol administration activates striatal DA synthesis and turnover and that tolerance to synthesis activation occurs upon chronic administration. In the present study, pre-treatment with the 5-HT_3 antagonist, zatosetron (100 µg/kg i.p., 30 min prior to haloperidol), antagonized the ability of haloperidol (2 mg/kg i.p., 60 min prior to sacrifice) to activate tyrosine hydroxylase (TH) in rat striatum (Km for the cofactor, tetrahydrobiopterin = 78 ± 7 µM, 55 ± 4 µM, and 74 ± 4 µM for vehicle, haloperidol and haloperidol + zatosetron, respectively). These results are consistent with the hypothesis that 5-HT_3 blockade can inhibit the neuronal activation of midbrain DA neurons. The ability of zatosetron to modify the chronic effects of haloperidol on TH activity (i.e. tolerance to activation by drug challenge) is currently under investigation. The importance of serotonergic mechanisms in modulating the effects of haloperidol and clozapine on DA neurotransmission will be discussed.

384.13

THE EFFECT OF RESERPINE PRE-TREATMENT UPON L-DOPA AND AMPHETAMINE EVOKED DOPAMINE AND DOPAC EFFLUX FROM SUPERFUSED STRIATAL TISSUE. D.E. Dluzen* and B.-J. Liu. Department of Anatomy, Northeastern Ohio Universities College of Medicine, Rootstown, OH 44272-0095.

Male rats were treated with reserpine (5 mg/kg) or its vehicle at 24 hours prior to sacrifice and superfusion of the corpus striatum. Two different modes of L-DOPA (5 µM) and amphetamine (10 µM) stimulation, a brief 10-minute and a continuous 60-minute infusion, were tested for their ability to evoke striatal dopamine and DOPAC efflux. Reserpine significantly reduced the amount of basal dopamine and DOPAC released from superfused striatal tissue fragments of male rats. Although basal release rates were significantly reduced, the amount of dopamine and DOPAC released in response to in vitro L-DOPA infusions (10 or 60 minute infusions) was equivalent between reserpine and vehicle treated animals. In contrast, amphetamine stimulated DA release was significantly reduced in male rats treated with reserpine. For both L-DOPA and amphetamine, significantly greater amounts of dopamine were obtained with the 60- versus 10-minute infusion modes. These results demonstrate that the capacity for L-DOPA, but not amphetamine, to evoke dopamine efflux is unaltered under conditions when monoamine storage ability is diminished.

384.14

REAL TIME DOPAMINE OVERFLOW IN BEHAVING RAT MEASURED BY FAST CYCLIC VOLTAMMETRY. Z.L.Kruk*, J.G.Williams & P.Willner. Dept.Pharmacology, Queen Mary & Westfield College, Mile End Rd, London E1 4NS UK

We report voltammetric measurement of electrically stimulated dopamine (DA) overflow in conscious rats for up to 17 days using the same electrodes. A miniaturised carbon fibre electrode, a Ag/AgCl reference and steel auxiliary electrodes, were permanently implanted, and fast cyclic voltammetry (Millar et al 1985, Eur.J.Pharmac,109, 341) was used to measure electrically stimulated endogenous dopamine (DA) overflow in the nucleus accumbens, in response to electrical stimulation (50Hz; 30 to 100µA; 0.5 to 5s) of the ipsilateral ventral tegmental area in conscious rats. DA overflow was stimulus intensity and duration dependent, and ranged from 50 nMolar to 5 µMolar equivalent DA; the limit of detection in vitro was 10 nMolar DA. All connections to the rat were by a multi way swivel. Voltammetric, electrophysiological, anatomical and pharmacological evidence indicate that DA overflow could be measured for up to 17 days.

384.15

MONOCLONAL ANTIBODIES REACTIVE WITH A MONOAMINE TRANSPORTER PREPARATION PURIFIED FROM BOVINE ADRENAL CHROMAFFIN GRANULE MEMBRANES. J.A. Near* C.E. Odya and X. Li Medical Sciences Program and Department of Pharmacology, Indiana University School of Medicine, Bloomington, IN 47405.

Western blotting and ELISA data obtained using polyclonal serum from a mouse immunized with a highly purified chromaffin granule monoamine transporter preparation (*Mol. Pharmacol.* 40:889) yielded data consistent with the presence of antibodies to the transporter. Hybridomas produced by fusing spleen cells from the mouse with X63/Ag8.653 myeloma cells were screened in an ELISA. Positive wells were sub-cloned by limiting dilution, stocked, grown up in mouse ascites, and antibodies purified on immobilized Protein A. Immunoreactivity with a mixture of the 6 antibodies coincided with dihydrotetrabenazine (TBZOH) binding activity in fractions eluted from all columns employed in the transporter purification. Antibody from one of the clones was capable of removing TBZOH binding activity from a partially purified preparation of transporter. Studies are currently under way to confirm that the monoclonal antibodies are reactive with the transporter and not with a component that co-purifies with the transporter.

Supported by the Indiana University Project Development Program.

384.16

CYTOCHROME b_{561} REDUCTION BY ASCORBATE: CONCERTED PROTON/ELECTRON TRANSFER. Patrick M. Kelley* and David Njus, Dept. of Biological Sciences, Wayne State Univ., Detroit, MI 48202

Ascorbic acid (vitamin C) is regenerated by cytochrome b_{561} in chromaffin vesicles. The ascorbate reacts with the cytochrome via a mechanism that we have termed concerted proton/electron transfer. This mechanism differs from the purely electron-transfer type of reaction by which ascorbate reacts with cytochrome c. The dianion of ascorbate (pK=11.3) reduces cytochrome c by a reaction which is strongly dependent upon pH. The reaction is faster at higher pH but quite slow at physiological pH. Cytochrome b_{561}, by contrast, reacts very rapidly at physiological pH even though its midpoint potential is 120 mV lower than that of cytochrome c. Moreover, the reaction is much less dependent on pH, suggesting that cytochrome b_{561} is reduced by the ascorbate monoanion, the predominant form at physiological pH. These observations argue that cytochrome b_{561} oxidizes the ascorbate monoanion to the semidehydroascorbate radical directly by extracting both the proton and the electron from ascorbate in a single step. In accordance with the concerted transfer of both the proton and the electron together, substitution of a deuteron for the proton should significantly affect the rate of reduction of the cytochrome by ascorbate. Initial results show that the rate of reduction is approximately 2.5 times faster in H_2O than it is in D_2O at pH 6.8. The change in the rate of reduction of cytochrome c is insignificant under these conditions. The possibility of a direct effect of D_2O on the cytochrome rather than on the concerted H^+/e^- transfer itself has not yet been excluded. Cytochrome b_{561} may be a paradigm for reactions between ascorbate and other ascorbate-requiring enzymes, all of which may react via concerted proton/electron transfer.

385.1

NEUROCHEMICAL EFFECTS OF NOVEL COCAINE ANALOGS ON CULTURED NEURONS. *B.A. Bennett* C.E. Hyde, J.R. Pecora, E. Sakali and H.W. Davies*, Dept. Physiol/Pharmacol, Bowman Gray Sch. Med. and Dept. of Chemistry, Wake Forest Univ., Winston-Salem, NC 27157

A unique scheme utilizing vinlycarbenoid precursors has been developed for the synthesis of novel tropane analogs of cocaine. These studies describe the ability of several analogs to inhibit dopamine (DA) and/or serotonin (5HT) transport in cultured neurons. We examined analogs that showed selective affinity for either the DA tranporter, the 5HT transporter, or were equipotent at both. Inhibitory potencies (IC_{50}'s) were determined and are shown in the table. As shown, all of these compounds have a much higher potency for inhibiting DA transport than does cocaine (IC_{50} = 540 nM). We have previously determined that repeated cocaine administration to cultures does not alter dopamine or serotonin transporter function (no change in K_m or V_{max}). We exposed DA and 5HT cultures to these analogs for 5 days and examined amine uptake and cell survival. While DA or 5HT cell survival was unaffected, there were significant reductions in transporter activity. The results indicate that these analogs interact with the transporters and bind with such high affinity that a long washout period (approximately 24 hrs) is necessary in order to resume normal transporter function. Structure activity relationships of these compounds and that of cocaine will be discussed. *Supported by NIDA grants 05073, 06634 (BAB), 07246 (CEH) and 06301 (HWD).*

IC50 Values (nM)

	DA	*5HT*
WF11	18.6	660
WF31	67.0	990
WF23	.011	.021

385.2

SUGARS ENHANCE THE BINDING OF THE COCAINE ANALOG, WIN 35428 IN RAT STRIATUM. A.L. Kirifides*, J.A. Harvey and V.J. Aloyo Dept. of Pharmacology, Medical College of PA, Philadelphia PA, 19129.

WIN 35428 (WIN) has been employed in both phosphate and Tris buffers to characterize the striatal dopamine transporter. When investigating WIN membrane binding, many researchers add sucrose to the incubation mixture. Our study focused on the effects of sucrose on WIN binding under conditions of single-site kinetics (Kirifides et.al. Life Sci. 1992). Binding assays were performed using crude membrane fraction prepared from fresh rat striatum at 0°C in 20 mM buffer (Phosphate, Hepes, Tris or Tris with NaCl), pH 7.4, at 0°C with or without sucrose. Nonspecific binding was defined as the binding remaining in the presence of 30 µM cocaine. In all four buffers 0.32M sucrose increased specific binding without altering nonspecific binding. The effect of sucrose was not due to an increase in osmolality since NaCl and choline chloride inhibited WIN at iso-osmolar concentrations. Furthermore, other sugars (glucose, glycerol, mannitol as well as sucrose) also increased WIN binding in a concentration dependent manner. Scatchard analysis revealed that in all three buffers sucrose significantly increased WIN's affinity without altering the Bmax. Thus we conclude that the presence of sugars enhances WIN binding by altering the affinity state of the dopamine transporter. (Supported by NIDA Grant DA06871-01).

385.3

SEX, ESTROUS CYCLE AND GONADECTOMY INFLUENCE STRIATAL DOPAMINE UPTAKE SITES. M. Morissette* and T. Di Paolo. School of Pharmacy, Laval University and Department of Molecular Endocrinology, CHUL Research Center, Ste-Foy, Quebec, Canada.

Reuptake of dopamine (DA) into nerve terminals is the primary mechanism of inactivation of this neurotransmitter in the synaptic cleft. We investigated the hormonal modulation of rat striatal DA uptake sites. During the estrous cycle, peak density of striatal DA uptake sites labelled with [^{3}H]GBR-12935 occurred in the morning of proestrus in coincidence with peak DA, serotonin, dihydroxyphenyl-acetic acid and 5-hydroxytryptophan levels pointing to a pre-synaptic effect of gonadal hormones. Striatal homovanillic acid and 5-hydroxyindoleacetic acid levels as well as [^{3}H]GBR-12935 binding affinity remained unchanged throughout the estrous cycle. Density of [^{3}H]GBR-12935 striatal binding sites was lower in ovariectomized rats compared to intact female rats during the estrous cycle, whereas it was similar in ovariectomized rats, gonadectomized and intact male rats. Binding affinity was in general similar for all groups of rats examined. The affinity of DA for striatal [^{3}H]GBR-12935 binding sites was similar in males and ovariectomized females, and did not change during the female estrous cycle. In summary, striatal DA uptake sites density was lower in male compared to intact females rats and fluctuated during the female estrous cycle. Gonadectomy left unchanged striatal DA uptake sites in male but decreased density in female rats indicating a specific effect of female sex hormones. These results suggest that gonadal hormones could influence the activity of psychoactive drugs acting on neuronal DA uptake sites. Supported by a MRC of Canada Grant to T. D. P.

385.4

DOPAMINE TRANSPORTER ACTIVITY IN THE RAT MIDBRAIN: RELATIONSHIP TO CALBINDIN-D_{28k}-CONTAINING DOPAMINERGIC NEURONS. M.K. Sanghera*, K.F. Manaye, C.-L. Liang, A.M. Iacopino, M.J. Bannon, and D.C. German. Dept. of Psychiat., UT Southwestern Med. Cntr., Dallas TX; Dept. of Biomedical Sci., Baylor Col. of Dentistry, Dallas, TX; Dept. of Psychiat., Wayne State Univ., Detroit, MI.

The dopamine transporter is the site at which MPP^+, the neurotoxic metabolite of MPTP, gains access to the midbrain dopaminergic (DA) neurons. The midbrain DA neurons that contain the calcium-binding protein, calbindin-D_{28k} (CaBP), are less vulnerable to MPTP toxicity, compared to the DA neurons that lack CaBP (German et al., 1992). The present study sought to determine whether there is comparable dopamine transporter activity among the DA cells with and without CaBP. Using in situ hybridization with a riboprobe from the human dopamine transporter, and immunohistochemical staining techniques, we found markedly less mRNA activity over neurons in the ventral tegmental area (VTA; a region with many CaBP-containing neurons) than over the substantia nigra neurons (a region lacking CaBP-containing neurons). These data suggest that VTA neurons are spared, following MPTP administration, because of a less active dopamine transporter.

385.5

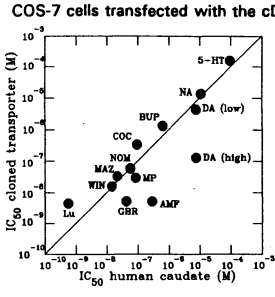
HUMAN DOPAMINE TRANSPORTER cDNA: COMPARISON OF [^{3}H]DOPAMINE UPTAKE AND [^{3}H]WIN 35,428 BINDING. Z.B. Pristupa*, J.M. Wilson, B.J. Hoffman^, S.J. Kish, and H.B. Niznik. *Labs of Mol. Neurobiol. and Human Neurochem. Pathology, Clarke Institute of Psychiatry, Toronto, Ontario, Canada M5T 1R8, and Lab. Cell Biol.^, NIMH MD*

Dopamine (DA) reuptake is thought to be involved in several neuropsychiatric disorders as well as in the reinforcing effects of drugs of abuse such as cocaine and amphetamine. Some evidence in the literature suggests that various inhibitors of DA uptake interact with the DA transporter at pharmacologically distinct sites. To assess this possibility, we compared the ability of various DA uptake blockers and substrates to inhibit [^{3}H]DA uptake and [^{3}H]WIN 35428 binding to COS-7 cells expressing the cloned human DA transporter. [^{3}H]DA uptake was concentration dependent and saturable, with K_m of 1.4 µM. Similarly, [^{3}H]WIN 35,428 binding was saturable with an estimated K_d of ≈ 6 nM. [^{3}H]DA uptake was inhibited by numerous compounds in a concentration dependent and **uniphasic** manner with the following rank-order of potency: (+) diclofensine > amfonelic acid> Lu 19,005 ≈GBR 12,909≈ WIN 35428 > methylphenidate > mazindol ≈ nomifensine > (-) diclofensine > cocaine ≈ bupropion > dopamine > norepinephrine > serotonin. Most compounds inhibited the binding of [^{3}H]WIN 35428 in a uniphasic manner except for Lu-19005, WIN and DA in which two affinity states were clearly evident. For both Lu-19005 and WIN the IC_{50} for the high affinity site [4 and 15 nM, respectively] correlates well with functional values obtained for DA uptake [~13nM] while for dopamine the IC_{50} of the low affinity site [~4 µM] matches well with that of uptake [1.7 µM]. While data on the cloned DA transporter suggests strong pharmacological homology between DA uptake and [^{3}H]WIN binding, comparisons of [^{3}H]WIN binding to the cloned transporter and to membrane preparations from human caudate reveal some differences specifically with dopamine, GBR 12,909, and amfonelic acid, each of which exhibits affinities for the native receptor ~ 10 fold lower than that of the cloned protein. Given possible differences in assay conditions and/or membrane composition to account for these alterations, we conclude that the binding of [^{3}H]WIN to the cloned DA transporter and to human caudate membrane preparations appears to be pharmacologically similar.

385.6

[^{3}H]WIN 35,428 BINDING IN HUMAN CAUDATE: PHARMACOLOGICAL PROFILE. J.M. Wilson*, Z.B. Pristupa, H.B. Niznik and S.J. Kish. Clarke Institute of Psychiatry and University of Toronto, Ontario, Canada.

[^{3}H]WIN 35,428 (WIN) is a cocaine analogue which has been used to label the dopamine (DA) transporter in brain of experimental animals. Since little information is available on the pharmacological profile of [^{3}H]WIN binding in human brain, we assessed the rank orders of various displacers in inhibiting [^{3}H]WIN binding in human caudate (n = 2 to 4). Our preliminary results show that the rank order potency was Lu 19005 > WIN > mazindol > GBR12,909 > nomifensine > methylphenidate > cocaine > amfonelic acid > bupropion > DA > NA > 5-HT. As shown in the figure, this rank order correlated (r = 0.87) with that observed in COS-7 cells transfected with the cDNA for the human DA transporter, with the exception of amfonelic acid, DA (high) and GBR 12,909 which were 10-100 fold more potent in inhibiting [^{3}H]WIN binding to the cloned transporter than native transporter in human caudate. Despite these pharmacological differences, which could be accounted for by minor variations in assay conditions and/or composition of membrane preparations, [^{3}H]WIN appears to recognise the same site on the cloned and native DA transporter. (US NIDA DA07182).



385.7

RAPID INCREASE OF STIMULANT BINDING TO THE DOPAMINE TRANSPORTER AFTER ACUTE COCAINE ADMINISTRATION: PHYSIOLOGICAL BASIS OF DRUG CRAVING? M.M.Schweri*, Div. Basic Med. Sciences, Mercer Univ. Sch. Med., Macon, GA 31207.

Although cocaine is thought to exert its reinforcing effect via stimulant binding sites on the dopamine (DA) transporter, little is known regarding the actual molecular basis of the drug's powerful addicting effects. These studies used the radiolabeled stimulant, [^{3}H]methylphenidate ([^{3}H]MP), to probe the acute response of stimulant binding sites to cocaine in drug-naive rats. Binding of [^{3}H]MP was found to be 17% higher in unwashed striatal tissue homogenates from rats treated 1 hr previously with 15 mg/kg cocaine HCl (*i.p.*), compared to saline-injected controls (38,600 ± 1,050 vs 33,100 ± 900 CPM's/mg protein; $p<0.005$, Student's independ. t-test). This increase persisted when the tissue was washed twice with 50 mM Tris-Cl assay buffer before determination of [^{3}H]MP binding. Scatchard analyses of twice-washed tissue showed that both the affinity and receptor density were significantly affected: the K_D decreased 22% (from 109 ± 15 nM to 85 ± 14 [$p<0.03$]) and the B_{MAX} increased 11% (from 6.4 ± 0.4 to 7.1 ± 0.5 pmols/mg protein [$p<0.02$]) in samples from vehicle- and cocaine-treated rats, respectively (paired t-test, 4 separate experiments). If future work shows that DA transport is affected in a parallel manner, a homeostatic mechanism may be hypothesized to underlie cocaine craving, as follows. The cocaine-induced rise in synaptic DA may trigger a compensatory increase in the efficiency of the transporter in order to reduce DA levels to normal. If the increased efficiency continues after cocaine is no longer present, however, synaptic DA concentrations may be reduced *below* baseline levels, leading to renewed cocaine use to counteract the resulting dysphoria.

385.8

EVALUATION OF DOPAMINE UPTAKE INTO THE SUBSTANTIA NIGRA, VENTRAL TEGMENTAL AREA AND STRIATUM OF THE RAT: EFFECTS OF COCAINE TREATMENT. Ivory Baker, Richard J. Wyatt and Joseph M. Masserano*. Neuropsychiatry Branch, NIMH Neuroscience Center, Washington, D.C. 20032.

This study evaluates and compares dopamine uptake into cell body regions (substantia nigra and ventral tegmental area) with that into a nerve terminal region (striatum). Kinetic analysis of [^{3}H]dopamine uptake was linear for all three brain regions indicating the presence of one form of dopamine uptake in the slices that is apparently of the high affinity type. The apparent Km for dopamine uptake is 0.36 µM for the striatum, 0.49 µM for the substantia nigra, and 0.84 µM for the ventral tegmental area. The apparent Vmax for dopamine uptake in the striatum was 39.0 pmols/min/mg protein and was approximately 8-fold higher than the Vmax in the substantia nigra (5.3 pmols/min/mg protein) and ventral tegmental area (4.1 pmols/min/mg protein). IC50 values for GBR12909 ($5x10^{-7}$), cocaine ($5x10^{-6}$), and WIN, 528 ($5x\ 10^{-7}$) are similar in all three brain areas. The administration of cocaine 10 mg/kg, i.p., twice a day for 7 days produced no differences in the uptake of [^{3}H]dopamine in the three brain areas compared with saline treated rats at 1 or 6 weeks after the last injection. Previous studies have shown a decrease in dopamine uptake in the frontal cortex after cocaine treatment. These data illustrate a selective uptake of dopamine in the dopaminergic cell body regions of the mesencephalon that is not affected by cocaine administration.

385.9

SPECT IMAGING OF DOPAMINE TRANSPORTERS IN NON-HUMAN PRIMATE STRIATUM WITH 123I-MMG 142(E). R.T. Malison*, M.P. Kung, W. McElgin, G. Romaniello, H.I. Kim, M.M. Goodman, H.F. Kung. Depts. of Psychiatry and Radiology, Univ. of Pennsylvania School of Medicine and VA Medical Center, Philadelphia, PA 19104, and Dept. of Radiology, Univ. of Tennessee Medical Center, Knoxville, TN 37920.

The regional distribution, kinetics, and pharmacological specificity of uptake of a new radioiodinated cocaine analog, N-((E)-3-iodoproprenyl-2-yl)-2ß-carbomethoxy-3ß-(4-chlorophenyl) tropane (123I-MMG 142(E)) were examined in brain SPECT studies (n=12) of non-human primates. Synthesis and purification of the iododestannylated trialkyltin precursor yielded the tracer at greater than 90% radiochemical purity and high (>20,000 Ci/mmol) specific activity. Cynomologous monkeys were injected with 7.0 ± 0.4 mCi (mean ± SEM) of the tracer, and serial 10-minute images were acquired on the Picker PRISM 3000 (total scan time=170 ± 10 min). Images were reconstructed as transaxial slices (2 mm) using restorative techniques (Wiener prefiltering). Radioactivity concentrated quickly in striatal regions (time of peak =26±4 min) and cleared gradually thereafter (6.9 ± 1.9%/hr). Striatal to cerebellar ratios of 2.4 ± 0.5 (n=12), 5.4 ± 0.8 (n=12), 8.4 ± 1.1 (n=7), and 16.7 ± 4.4 (n=4) were observed at time of peak and 1 hr, 2 hr, and 3 hr p.i., respectively. In contrast, extrastriatal activity peaked earlier and at lower levels, cleared more rapidly, and resembled time-activity curves seen for the cerebellum. Displacing doses of non-specific antagonists of monoamine transporters (mazindol and ß-CIT) showed that 95% of striatal 123I-MMG 142(E) binding was reversible, while selective antagonists (e.g., paroxetine, nisoxetine, and GBR 12909) suggested that striatal activity was specificially associated with dopamine transporters. These results indicate that 123I-MMG 142(E) may be a useful radioligand for in vivo SPECT imaging of striatal dopamine transporters.

385.10

COMPARISON OF ß-CIT ENANTIOMERS USING HOMOGENATE BINDING AND SPECT IMAGING. B.E. Scanley, R.M. Baldwin, M. Laruelle*, M.S. Al-Tikriti, Y. Zea-Ponce, S. Zoghbi, S.S. Giddings, S. Wang, Y. Gao, J.L. Neumeyer, P.B. Hoffer, R.B. Innis. Yale Univ. & VA Med Ctr, West Haven, CT 06516 and Research Biochemicals Int., Natick, MA.

ß-CIT, (2ß-carbomethoxy-3ß-(4-iodophenyl)tropane; also designated RTI-55) is an analogue of cocaine which has been developed as a SPECT radiotracer that labels dopamine and serotonin transporters. We have prepared the ^{125}I and ^{123}I labeled (1R)- ("active") and (1S)- ("inactive") enantiomers of ß-CIT. Total [^{125}I](S)-ß-CIT homogenate binding to rat striatum (0.24±0.01 fmol/mg tissue, ±S.E.M.) and cortex (0.08±0.004 fmol/mg) was approximately equal to nonspecific binding of the active isomer (0.23±0.02 and 0.06±0.01 fmol/mg in striatum and cortex, respectively) and much lower than total binding of the active isomer (7.9±0.25 and 1.1±0.03 fmol/mg in striatum and cortex, respectively). *In vivo*, the plasma clearance of [^{123}I](S)-ß-CIT (121±15 l/h, n=4) was not significantly different than that of [^{123}I](R)-ß-CIT (138±19 l/h, n=4). SPECT imaging was performed in two baboons. A bolus dose of cold (R)-ß-CIT (0.94µmol/kg) rapidly displaced uptake of [^{123}I](R)-ß-CIT. However, during a prolonged [^{123}I](S)-ß-CIT equilibrium infusion, this same dose of cold (R)-ß-CIT did not appreciably displace activity in any region. Peak uptake of the active isomer in striatum, normalized to injected dose and weight (µCi/cm^3/µCi/g) was double uptake of the inactive isomer, (7.0±0.5, n=4 and 3.5±0.4, n=4, respectively). Peak normalized occipital activity was not significantly different between the isomers (3.7±0.2, n=4 vs 3.1±0.4, n=4; active and inactive isomers, respectively). Percent washout from peak was significantly faster and time to peak was earlier for the inactive isomer in all regions (striatal time to peak: 17±2 vs 168±60 min and striatal washout rate: 26±5 vs 1±0.6 %/h, inactive and active isomers, respectively). These studies demonstrate ß-CIT stereoselectivity using both homogenate binding and *in vivo* imaging and suggest that the inactive enantiomer may be a useful measure of the kinetics of blood brain barrier transport and nonspecific binding.

385.11

SPECT IMAGING AND *EX VIVO* AUTORADIOGRAPHIC EVALUATION OF [^{123}I]ISOPROPYL ß-CIT OF MONOAMINE UPTAKE SITES IN BABOON. M.S. Al-Tikriti*, B.E. Scanley, S.S. Zoghbi, Y. Zea-Ponce, R.M. Baldwin, M. Laruelle, P.B.Hoffer, J.L.Neumeyer, R.B. Innis, Yale University and VA Medical Center, Psychiatry Dept. West Haven, CT 06516.

Several ligands have been synthesized to image the DA transporter site *in vivo*. CIT is an iodinated cocaine analog with an affinity for DA uptake site almost 10-fold>CFT. However, CIT also binds to 5-HT sites. IPCIT (Isopropyl 3 ß-(4-iodophyenyl)tropane-2 ß-carboxylate) is more selective for DA uptake site than to 5-HT and NE sites. The purpose of this study was to evaluate IPCIT as *in vivo* brain imaging ligand in primates. Three SPECT scans were conducted in baboons (10 kg *Papio anubis*), Animals were injected with 11.1±5.7 mCi i.v. of [^{123}I]IPCIT and scanned on the CERASPECT device for 460±34.6 min. Highest activities were seen in striatum, reaching peak level at 280 min p.i.. At 2h p.i. the striatal/occipital ratio was 1.66±0.1(n=3). Parent compound in plasma was 60±20% compared to 30±10%(n=4) for ß-CIT. Plasma clearance was 99±18 L/h vs 138.6±19 L/h for ß-CIT. Afterwards, striatal activity was monitored over 200 min and washout rate was negligible (1%/h and $T_{1/2}$=47 h). *Ex vivo* autoradiography was carried out in a baboon injected with 18 mCi, scanned for 2 h then sacrificed. Autoradiograms showed highest radioactivity in caudate and putamen. The ratio of putamen/occipital cortex was 3.2, and for caudate/occipital was 3, the cortical grey/white matter ratio was 1.9. Autoradiograms showed no increased activity associated with regions enriched in 5-HT transporter, like the colliculi. The *Ex Vivo* studies demonstrate selectivity of IPCIT for DA transporters and suggest that imaging with [^{123}I]IPCIT may be used to quantify DA transporters *In Vivo*.

385.12

APPARENT RECEPTOR-LINKED REGULATION OF SEROTONIN UPTAKE IN THE HUMAN PLATELET.

G.M. Anderson*, L.M. Hall, and J.X. Yang. The Child Study Center & Department of Laboratory Medicine, Yale Univ. School of Medicine, New Haven, CT 06510.

We have previously reported (Anderson & Horne, BBA 1137:331-337, 1992) that activation of protein kinase C (PKC) decreases serotonin (5HT) uptake in the human platelet. This finding, and reports of receptor-linked control of other transporters, has prompted us to study possible receptor-linked regulation of platelet 5HT uptake.

We have observed that adenosine diphosphate (ADP) causes a rapid decrease (to 42.3 ± 11.4 % of controls, N=15) in the V_{max} of platelet 5HT uptake without significantly affecting the K_m value. The IC_{50} for ADP determined from dose-response studies was 24 ± 15 µM (N=5). Time course studies showed the effect to be very rapid, with ADP pre-incubations as short as 2.5 sec bringing about the maximum effect.

Studies on the mechanism have strongly indicated that the effect is not mediated through changes in cytosolic pH or Ca^{+2}, cAMP inhibition, PKC activation, or the cyclooxygenase pathway. The implications of an apparently closely-coupled receptor-linked regulation of 5HT uptake will be discussed.

Supported by NIMH grant MH30929 and Gettner Research Fund.

385.13

EFFECT OF (+) 3,4-METHYLENEDIOXYMETHAMPHETAMINE (MDMA) ON SEROTONIN AND DOPAMINE UPTAKE IN RAT HIPPOCAMPUS AND STRIATUM. R. Lew*, K.E. Sabol, G. Vosmer, J.B. Richards, K. Layton and L.S. Seiden. Dept. Pharmacol. and Physiol., Univ. Chicago, Chicago. Ill 60637.

The present study examines the long term effects of MDMA on serotonin and dopamine uptake in synaptosomal preparations of rat hippocampus and striatum. Age-matched Holtzman Sprague-Dawley rats (275 - 300 g) were injected with either saline (0.9% ; control) or MDMA (20 mg/kg free base; treated) twice daily (12hrs apart) for four days and sacrficed 2, 8, 16, 32 or 52 weeks after the final injection. On the day of sacrifice, striatum and hippocampus were removed from control and treated animals and synaptosomes were prepared in 0.32 M sucrose. For serotonin uptake, ^{3}H-5-HT (10 nM final : spec. act = 26.4 Ci/mmol) was preincubated in buffer with unlabeled 5-HT for 10 min at 30 ^{0}C before addition of hippocampal synaptosomes (170 µg protein). Uptake was terminated after 5 min by addition of 3 ml ice-cold 0.32 M sucrose and then filtered through GF/B filters. Dopamine uptake was performed in a similar manner except uptake was terminated 3 min after addition of striatal synaptosomes (50 µg protein). Preliminary experiments showed uptake in striatal and hippocampal synaptosomes was temperature dependent and had pharmacological profiles typical of dopamine and serotonin uptake. In dopamine uptake studies, no significant difference was observed between control and treated animals sacrificed 2, 8, 16, 32 and 52 weeks after treatment. In contrast, serotonin uptake in treated animals was 33.3 ± 1.4 % of control animals after 2 weeks recovery (control: Bmax = 9.37 ± 0.57 pmol mg prot; MDMA treated: Bmax = 3.09 ±0.12 pmol/mg prot). By 16 weeks, serotonin uptake had recovered to 74.1 ± 3.7 % of that in control animals. However full recovery was not observed by 52 weeks as serotonin uptake in treated animals was 84.6 ±4.5 % of control. Consequently serotonin uptake in hippocampus is reduced after MDMA treatment. However, since dopamine uptake was not affected by MDMA treatment, it appears that there is no relationship between dopamine and serotonin uptake systems. (This work was supported by research grant DA 00085; L.S.S is supported by RSA MH-105 62)

385.14

PHORBOL ESTERS DECREASE DOPAMINE UPTAKE AND COCAINE ANALOG BINDING IN COS CELLS EXPRESSING RAT DOPAMINE TRANSPORTER. S. Kitayama*, G.R. Uhl#, and T. Dohi. Dept. of Pharmacology, Hiroshima Univ. Sch. Dentistry, Hiroshima 734, JAPAN, #Mol. Neurobiol. Lab., ARC/NIDA and Depts. of Neurology and Neuroscience, JHUSM, Baltimore, MD 21224.

Recent elucidation of the amino acid sequence of the dopamine transporter(DAT) reveals several consensus sequences for phosphorylation by protein kinases. To test possible roles of protein kinase C in modulating transporter function, we investigated the effect of phorbol esters on the function of DAT expressed in COS cells. Cell treatment with phorbol 12-myristate 13-acetate(PMA) reduced the affinity of binding of the radiolabeled cocaine analog CFT without affecting Bmax. The velocity of uptake of [^{3}H]dopamine was reduced by the treatment with PMA without affecting affinity. There was also a small reduction of affinity for Na^+. These results demonstrate that activation of protein kinase C alters dopamine transporter function in ligand recognition and substrate translocation, and suggest that the regulation of transporter activity might provide important synaptic fine tuning under physiological conditions.

385.15

MODULATION OF DOPAMINE TRANSPORT BY NITRIC OXIDE (NO). M.J. Kuhar* and S. Pögün. Neuroscience Branch, NIH-NIDA, Balto., MD 21224.

The dopamine transporter (DAT) terminates the action of dopamine (DA) by removing the released transmitter from the synaptic cleft. In this study, we report the modulation of the DAT by NO, a diffusable messenger with possible presynaptic action which is present in brain regions containing dopaminergic terminals. The effect of NO on ^{3}H-DA uptake was examined in striatal synaptosomes from male Sprague Dawley rats using standard procedures. Tissue was preincubated at 37^{0}C for 15 min and then at 30^{0}C for 5 min. Uptake was begun by adding ^{3}H-DA (0.5 nM) and terminated in 3 min by adding cold sucrose. Sodium nitroprusside (SNP) which generates NO independent of the enzyme nitric oxide synthase inhibited ^{3}H-DA uptake in a time, temperature and dose dependent fashion: At a concentration of 300 µM, uptake was inhibited 60%. In the absence of calcium, a condition which inhibits release of DA and activates guanylate cyclase, the observed inhibition by NO was more pronounced: 95% at 300 µM and 84% at 30 µM SNP concentration. Potassium ferricyanide had no effect on DA uptake. The effect of SNP was prevented by reduced Hb and methylene blue, and augmented by superoxide dismutase. Dibutyril cGMP (dbt-cGMP) mimicked the effect of NO on uptake: a 60% inhibition was observed at a concentration of 100 µM. Evaluation of the effect of NO on the kinetics of DA uptake revealed that NO increases K_m and reduces V_{max} in a dose dependent manner; dbt-cGMP also has a similar effect. DAT binding is not affected by either SNP or dbt-cGMP. Our results suggest that NO can inhibit DA transport in vivo and alter dopaminergic neurotransmission. This mechanism could be functionally important and may be involved in neuropsychiatric disease.

385.16

ATP-DEPENDENT MONOAMINE UPTAKE BY DIGITONIN-PERMEABILIZED PC12 WAS INHIBITED BY DIBUTYRYL CYCLIC AMP PRETREATMENT OF THE CELLS. N. Nakanishi*, S. Onozawa, R. Matsumoto, ¶H. Hasegawa and S. Yamada Dept. of Biochem., Meikai Univ. Sch. Dent., Sakado, Saitama 350-02, Japan, and ¶Dept of Bioscience, Nishi-Tokyo Univ., Uenohara, Yamanashi 409-01, Japan.

In pheochromocytoma, dibutyryl cyclic AMP (dBcAMP), forskolin and cholera toxin extremely elevate the extracellular dopamine. We found that inhibition of vesicular catecholamine transport by cAMP was a key to understand this process. In order to elucidate the mechanism of this cAMP effect, an assay system specific to vesicular monoamine uptake was developed applicable to PC12 cells by permeabilizing plasma membranes with digitonin. The permeabilized cells retained the ability to uptake exogenous serotonin (5HT) in an ATP-dependent manner. The ATP-dependent 5HT uptake by the permeabilized cells treated with dBcAMP prior to the permeabilization was decreased to about 50% of the control value. However, an addition of dBcAMP into the incubation medium for 5HT uptake by the permeabilized cells did not inhibit the uptake. The results indicated that the inhibition of vesicular monoamine transport by dBcAMP was not due to a direct effect but was mediated by some process. Protein phosphorylation was suggested to be involved in such process.

385.17

CYCLIC AMP INHIBITION OF CATECHOLAMINE UPTAKE BY PHEOCHROMOCYTOMA CELLS. S. Onozawa, N. Nakanishi, R. Matsumoto, ¶H. Hasegawa* and S. Yamada. Dept. of Biochem., Meikai Univ. Sch. Dent., Sakado, Saitama 350-02, and ¶Dept. of Biosci., Nishi-Tokyo Univ., Uenohara, Yamanashi 409-01, Japan.

Dibutyryl cAMP (dBcAMP) increases dopa content but decreases cellular dopamine (DA) in PC12h cells, a subclone of PC12. This decrease was accompanied by a large increase in DA in the culture medium, suggesting a modulation by cAMP of some transport process of catecholamine. We have examined how dBcAMP affected the intra- and extra-cellular DA. dBcAMP did not induce the exocytotic DA release. dBcAMP was inhibitory to the uptake of exogenous norepinephrine (NE) by the cells while no stimulation occurred in exocytotic or non-exocytotic release of NE pre-loaded. The uptake inhibition by dBcAMP was compared to those by reserpine and nomifensine. The inhibition profile was similar to that of reserpine but to that of nomifensine; inhibition was observable after a certain amount of NE was accumulated inside the cells, suggesting that the inhibition took place at the step of transportation from inside the cytosol to the vesicle. The same results were obtained with other cAMP-elevating agents, forskolin and cholera toxin. This type of inhibition increases DA concentration outside the cell by reducing reuptake capacity and hence by enhancing the leakage out from the cells.

385.18

DIFFERENTIAL REGULATION OF RAT NOREPINEPHRINE TRANSPORTER AND TYROSINE HYDROXYLASE EXPRESSION FOLLOWING RESERPINE. J.F. Cubells*, K.S. Kim, T.C. Wessel, T.A. Houpt, and T.H. Joh. Burke Med. Res. Inst., and Dept. of Psychiatry, Cornell U. Med. Coll., White Plains, NY 10605.

The norepinephrine transporter (NET) terminates the synaptic action of norepinephrine, thereby playing a critical role in noradrenergic transmission. To study the regulation of NET expression in rats, we used PCR to amplify a rat cDNA fragment that hybridized on Northern blots to PC-12 cell mRNA of the same size as the major band recognized by human NET (H-NET) probe, and was almost 90% identical in sequence to bp 988-1512 of H-NET cDNA (Pacholczyk, *et al.*, 1991, *Nature, 350*: 350). *In situ* hybridization of this probe to rat tissue sections labeled both locus ceruleus (LC) and adrenal medulla (AM), but neither substantia nigra nor dorsal raphe. It therefore appears to be a fragment of rat NET (R-NET) cDNA.

Reserpine 10 mg/kg s.c., administered to rats 24 hr prior to perfusion and *in situ* hybridization, caused almost no change in LC and AM levels of R-NET mRNA, but consistent with previous findings (Wessel and Joh, 1992, *Molec. Brain Res.*, *15*, 349), robustly induced tyrosine hydroxylase (TH) mRNA in both of those tissues. Thus, *in vivo* the TH and R-NET genes exhibit differential expression in response to acute catecholamine depletion. Supported by a Reader's Digest Fellowship to JFC and MH49762.

385.19

CHLORPROMAZINE BLOCKS THE UPTAKE OF HISTAMINE INTO PRESYNAPTIC TERMINALS OF BARNACLE PHOTORECEPTORS AND AFFECTS SIGNALS GENERATED IN THE POSTSYNAPTIC CELL.
Ann E. Stuart*, Elizabeth C. Schmid and Harold E. Mekeel, Dept. of Physiology, Univ. of North Carolina, Chapel Hill, NC 27599-7545.

Barnacle photoreceptor (PR) synapses function by disinhibition: postsynaptic cells preferentially signal a decrease in the cleft concentration of the PR's transmitter, histamine (HA). Uptake appears to be the primary mechanism for quickly decreasing the cleft concentration of this inhibitory transmitter. Using autoradiography, we found that chlorpromazine (20uM) but not phenoxybenzamine (7uM), desiprimine (100uM), or cocaine (100uM), consistently blocked uptake of 3H-histamine into PR terminals. In each of 6 preparations incubated in 20uM chlorpromazine, PR terminals did not take up 3H-HA; 2 preparations incubated in 7uM showed only light labeling compared to normal. From dose-response curves, 20uM chlorpromazine was found to reduce specific 3H-HA uptake to one-third its original value of 72.2 +/-5.7 pmol/mg protein and 50% block could be achieved at 0.7 uM. Chlorpromazine did not affect presynaptic voltage or Ca influx into the PR presynaptic terminals, as measured by Ca spikes induced in the presence of tetraethylammonium ion, but it did dramatically affect the postsynaptic response. The PRs' inhibition of the postsynaptic cell was abnormally prolonged so that disinhibitory responses, normally evoked by hyperpolarization of the PRs, could not be generated. These results suggest that the drug inhibits the uptake of HA, causing it to accumulate in the cleft during presynaptic depolarization and to saturate postsynaptic receptors. They argue for a crucial role of the HA transporter in the generation of the postsynaptic response at this synapse. Supported by EY03347.

385.20

ALLOSTERIC EFFECTS OF MONOVALENT IONS ON THE BINDING OF INHIBITORS TO AMINE TRANSPORTERS.
L.Raymon,* M.Eldefrawi
Pharmacol., Univ. of MD Sch. Med., Baltimore, MD 21201.

Amine uptake is Na+ and Cl- dependent and regulated by other ions. This study investigates the ionic regulation of the binding of uptake inhibitors to the transporters.

The effects of NaCl, KCl, NaHCO3 and KHCO3 on binding of BTCP to the dopamine transporter (DAT), desipramine (DMI) to the norepinephrine transporter (NET), citalopram (CTL) to the serotonin transporter (5HTT) and cocaine (COC) to the three transporters in rat whole brain less cerebellum was studied in 1mM NaH2PO4. BTCP specific binding to DAT increased 3-fold with increasing concentrations of NaCl whereas DMI, CTL and COC specific binding decreased. Addition of KCl to the buffer decreased the specific binding of all 4 ligands. When HCO3- was the anion, the specific binding of BTCP to DAT increased at low doses of the salt, then decreased at higher concentrations. A mirror image was observed for DMI. The binding of both CTL and COC decreased with increasing concentrations of NaHCO3 or KHCO3.

Non specific binding of all inhibitors decreased to similar values with increasing NaCl or KCl. However, non specific binding of BTCP and COC increased 8- and 1.5 to 2-fold, respectively, when HCO3- was present.

CSF is buffered by HCO3-. It is therefore important to study binding and uptake in HCO3- buffers, preferably artificial CSF, for physiological relevance.
(This research was funded in part by NIDA grant DA03680)

SECOND MESSENGERS III

386.1

DISTRIBUTION OF G PROTEIN α SUBUNITS AND THEIR COUPLING TO CLONED ADRENERGIC α-1 RECEPTORS IN COMMONLY USED TRANSFECTION HOSTS.
J.A. Salon*, J.A. Bard, C. Forray, R.L. Weinshank, and T.A. Branchek. Synaptic Pharmaceutical Corp., Paramus, NJ 07652.

The G protein-coupled receptor (GPCR) superfamily mediate neurotransmitter activity by coupling highly specific receptor binding events to finite cellular responses via the action of the intracellular G protein complex. The α subunit of the heterotrimeric G protein complex is believed to confer receptor and effector specificity. The importance of this specificity is critical for efficient coupling of heterologously expressed receptors. It would therefore be advantageous to know a priori which α subunits are required for a particular receptor and what G proteins are expressed in commonly used transfection hosts. To this end we have examined the Gα mRNA content of 12 commonly used host cells and their coupling to transiently expressed human α-1 adrenergic receptors (AR).

Using northern analysis and PCR we have found that both Gα11 and Gαq subunits are expressed in varying amounts in all lines tested with some evidence for message heterogeneity of the Gαq subunit. Coupling to cloned AR α-1a, b, and c was also examined and found to be dependent upon both receptor and cell line. Intracellular calcium and PI turnover was demonstrated with α-1b and α-1c in LMtk-, CHO, and NIH3T3 lines while α-1a was functionally responsive in HEK 293 hosts only.

This cataloging of G protein expression in transfectant cell hosts should benefit the characterization of cloned GPCRs. Failure to couple efficiently, as seen with α-1a AR in multiple transfection hosts, may indicate the need for additional cellular components or very strict requirements for receptor/G protein interaction which are selectively expressed in mammalian cells. Supported by NIH grant 1R43AG10653-01.

386.2

SELECTIVE EXPRESSION OF A NOVEL G-PROTEIN γ7 SUBUNIT IN THE RODENT NEOSTRIATUM.
J.B. Watson†*, P.M. Coulter II†, J.E. Margulies†, L. deLeceaǂ, M.G. Erlanderǂ, and J.G. Sutcliffeǂ. †Mental Retardation Research Center, Department of Psychiatry and Biobehavioral Sciences, UCLA School of Medicine, Los Angeles, CA 90024, ǂDepartment of Molecular Biology, The Scripps Research Institute, La Jolla, CA. 92037.

G-protein γ subunits in the form of β-γ dimers modulate phospholipases, ion channels, and adenylate cyclases after their dissociation from α subunits. We used subtractive hybridization to clone a new G-protein γ subunit, named γ7, that is expressed almost exclusively in the rodent striatum. Northern blot analysis detects two rare, brain-specific mRNAs of 3.0 Kb and 0.6 Kb, expressed predominantly in the striatum. Conceptual translation of the nucleotide sequences of full-length cDNA clones of each mRNA reveals a novel 69 amino acid sequence which is highly similar to the sequences of previously identified γ subunits. *In situ* hybridization detects the γ7 subunit mRNA primarily in medium-size neurons of the neostriatum and nucleus accumbens, neurons of the olfactory tubercle and ventral pallidum, and at low levels in the dentate gyrus of hippocampus and laminae II,III,VI of the neocortex. The γ7 subunit's selective pattern of mRNA expression is highly reminiscent of those of the striatum-enriched adenylate cyclase AC_{ST}, the dopamine receptors subtypes D1 and D2, and the α subunit of $G_{OLF\alpha}$. This suggests that, in striatum, γ7 is a subunit of a $G_{OLF\alpha}$,-containing G-protein that couples dopamine receptors selectively to AC_{ST}. This putative selective coupling pathway to cAMP production in medium-size spiny neurons represents a potential site of dysfunction in basal ganglia-selective disorders. Supported by the NIH, HD25831 (J.B.W.), and the Hereditary Disease Foundation (J.B.W.).

386.3

PURIFICATION OF FUNCTIONAL GL1α AND GL2α CO-EXPRESSED WITH $\beta\gamma$ SUBUNITS IN A BACULOVIRUS EXPRESSION SYSTEM.
F. Nakamura, M. Kato, K. Kameyama, T. Nukada, and T. Haga*. Dept. Biochem., Inst. Brain Res., Facl. Med., Univ. Tokyo, Hongo, Tokyo 113 Japan

We have previously cloned cDNAs encoding two kinds of G-protein α subunits, GL1α and GL2α, which correspond to mouse Gα14 and Gα11 of the Gq subfamily of G-proteins (Nakamura et al. J. Biol. Chem. 266, 12676-12681 (1991)). To characterize these proteins, we have expressed GL1α and GL2α in Sf9 cells using a recombinant baculovirus expression system. GL1α and GL2α were expressed as major proteins in the particulate fractions but were not significantly solubilized in buffers containing 1% sodium cholate or 1% Lubrol PX. By contrast, when Sf9 cells were co-transfected with a recombinant baculovirus that expressed bovine G-protein β1 and γ2 subunits, the α and $\beta\gamma$ subunits could be solubilized together in buffers containing 1% sodium cholate. The trimeric forms of G-proteins were partially purified by sequential DEAE-sephacel and heptylamine sepharose column chromatography. Purified trimers containing either GL1α or GL2α activated phospholipase C-β purified from bovine brain in the presence of GTPγS or aluminium fluoride. This procedure, thus, provides a new way to obtain relatively large amounts of functional GL1α and GL2α proteins.

386.4

CHIMERIC G PROTEINS DEFINE G-PROTEIN-TUBULIN INTERACTION SITES IN β-ADRENERGIC ACTIVATION OF ADENYLYL CYCLASE
J.S. Popova[1]*, G.L. Johnson[2] and M.M. Rasenick[1]. U. Illinois College of Medicine[1] Chicago, IL 60612-7342 and Nat'l. Jewish Ctr. for Immunology[2], Denver CO 80206

The cytoskeletal protein, tubulin [Tub], has been shown to bind to Gαs and Gαi1, but not to Gαi2, Gαi3 or Gαo. Tub with hydrolysis-resistant GTP analog bound (Tub-GppNHp) has also been found to activate adenylyl cyclase [AC] in permeable C6 glioma cells, bypassing the β-adrenoceptor [β-AR]. Nucleotide transfer from Tub and thus direct activation of Gαs was hypothesized to explain this. In order to determine the role of Tub as an intracellular signal transducing protein, we studied the effects of GppNHp and Tub-GppNHp on AC activity in permeable COS-1 cells after transient overexpression of wild type and chimeric Gα proteins. Concommittant β-AR stimulation was assesed with (-)-isoproterenol [(-)-iso]. In naive COS-1 cells, Tub-GppNHp was significantly more potent and twice as efficacious as GppNHp in augmenting (-)-iso- stimulated AC activity. COS-1 cells overexpressing the chimeric protein Gαs/i(38) [Gαs 1-356; Gαi2 357-392] demonstrated a 13-fold increase in AC activity upon GppNHp or Tub-GppNHp addition and (-)-iso caused no further increase in activity. The chimera Gαi/s(bam) [Gαi2 1-212; Gαs 213-292] was found to be nearly identical to naive COS-1 cells in activating AC upon GppNHp or Tub-GppNHp stimulation. However, (-)-iso was found to potentiate GppNHp and not Tub-GppNHp responses. A third construct, Gαi(bam)s/i(38) [Gαi2 1-212; Gas 213-356; Gαi2 357-392] was poorly responsive to GppNHp or Tub-GppNHp and unresponsive to (-)-iso. Permeable COS1 cells expressing these constructs as well as chimera Gαi(54)/s [Gαi2 1-54; Gαs 55-396] were exposed to tubulin with the hydrolysis-resistent photoaffinity probe, (^{32}P)-AAGTP (azidoanilido GTP] bound, to study the ability of Tub to bind to G protein and transfer nucleotide. The results obtained support the view that: a) the loss of the C-terminal part of Gαs molecule inhibits β-AR potentiation of Tub-guanine nucleotide stimulation of AC activity and b) the region between the 54th and 212th amino acids of Gαs is important for guanine nucleotide binding and transfer from Tub as well as Tub-GppNHp stimulation of AC.

386.5

SUBSYNAPTIC LOCALIZATION OF GTP-BINDING PROTEINS. R.S. Cohen*, M.M. Rasenick and D.R. Manning. Dept. of Anatomy and Cell Biology and Dept. of Physiology and Biophysics, U. of Illinois at Chicago, Chicago, IL 60612 and Dept. of Pharmacology, U. of Pennsylvania, Philadelphia, PA 19104.

To localize GTP-binding proteins at the subsynaptic level, we used affinity purified antibodies to detect $G\alpha_i$, $G\alpha_o$ and $G\alpha_s$ using the immunoperoxidase method on vibratome sections of rat cerebral cortex and hypothalamus. Following the reaction, the tissue was processed for EM. $G\alpha_i$, $G\alpha_o$ and $G\alpha_s$ were localized at both pre- and postsynaptic sites. Synaptic vesicles (SVs) of some, but not all, presynaptic terminals were labeled in both brain regions. Dense-cored vesicles in the hypothalamus also showed reaction product. In addition to the presynaptic membrane, the presynaptic dense material was labeled. In postsynaptic processes, the postsynaptic membranes (PSMs) and postsynaptic densities (PSDs) were labeled by all the antibodies, although not all PSMs and PSDs showed reaction product. Some unlabeled PSMs and PSDs were adjacent to labeled ones and, in the case of $G\alpha_s$, a single presynaptic terminal was seen in association with both an unlabeled and labeled PSM and PSD. Subjunctional bodies beneath PSDs showed reaction product. The antibodies also labeled the membranes around and cytoskeleton within dendritic spines. Microtubules of dendritic synapses were also reactive. Preliminary observations of cells in the two regions showed differences in the labeling patterns of $G\alpha_i$ and $G\alpha_o$ compared to $G\alpha_s$. The two former proteins appeared in a punctate pattern around cell bodies, while the latter appeared to show some cytoplasmic reactivity. Significant labeling by $G\alpha_s$ was seen in glia. The presynaptic localization of these proteins suggest a possible role in docking of SVs and in other events related to vesicular transport. At the postsynaptic site, these proteins may undergo membrane-cytoskeletal interactions which modify signal transduction. Supported by NIH grants HD24553, MH39595, and GM34781.

386.6

PARTICIPATION OF TUBULIN IN Gs-MEDIATED SIGNAL TRANSDUCTION THROUGH THE ASSOCIATION OF Gs PROTEIN. S. Hatta*, H. Ikeda, T. Saito, and H. Ohshika. Depts. of Pharmacology and Neuropsychiatry, School of Medicine, Sapporo Medical University, Sapporo 060, Japan.

Previously, we have demonstrated that tubulin dimers (Tu) interact with and transfer guanine nucleotide (GN) to Gi, and thereby inhibit adenylyl cyclase (AC) in rat cerebral cortex membranes (CCM). Under conditions used in those experiments (23°C/1 m*M* $MgCl_2$), transfer of GN to Gsα from Tu was not observed. In this study, we examined the association between Tu and Gs, and effects of Tu on Gs-mediated signal transduction in rat CCM. Tubulin was prepared by polymerizing with GppNHp (Tu-GppNHp) or photoaffinity GTP analog, AAGTP (Tu-AAGTP) from rat brains. Incubation of CCM with Tu-[^{32}P]AAGTP at 30°C with 5 m*M* $MgCl_2$ resulted in incorporation of [^{32}P]AAGTP into Gsα and Giα, indicating transfer of AAGTP from Tu to both Gsα and Giα. Tu-GppNHp decreased agonist binding affinity for the β-adrenergic receptor (i.e., right shifted the isoproterenol competition curves of [^{3}H]CGP12177 binding) with potency similar to that of GppNHp. Furthermore, Tu-GppNHp activated AC and stimulated isoproterenol-sensitive AC activity in CCM. These results suggest that in rat cerebral cortex, Tu participates in the regulation of Gs-mediated signal transduction by transferring GN to Gs as well as affecting Gi-mediated pathway.

386.7

EFFECT OF CHRONIC ANTIDEPRESSANT TREATMENT ON TUBULIN FUNCTION IN RAT BRAIN. H. Kamada[1], T. Saito[1]*, H. Ozawa[1], S. Hatta[2], E. Hashimoto[1], T. Ashizawa[1], M. M. Rasenick[3] and N. Takahata[1]. Dep. of Neuropsychiatry[1] and Pharmacology[2], Sapporo Med. Univ., Sapporo, Japan 060. Dep. of Physiology and Biophysics, Univ. of Illinois.[3], Chicago, Ill 60608.

Tubulin, the cytoskeletal element, is a GTP-binding protein (G proteion) with similarities to other G proteins. Tubulin, polymerized with the hydrolysis-resistant GTP analog, 5'-guanylylimidodiphosphate (GppNHp), can promote inhibition of rat cerebral cortex synaptic membrane adenylate cyclase. Although dimetric tubulins have been implicated as modulators of the adenylate cyclase system, the total mechanism of this regulation has not been clear. In this study, the effect of a chronic antidepressant on tubulin function in rat brain was examined. Male SD rats aged 1 to 6 months were treated with amitriptyline (AMT) by intraperitoneal injection (10mg/kg) for 21 days. Tubulin-GppNHp was prepared according to the method of Shelanski. The quantity of tubulin in cerebral cortical membranes was measured by immunoblotting. Adenylyl cyclase (AC) activity was determined by the method of Salmon. GppNHp-stimulated AC activity was increased in the cortical membranes from AMT-treated rats. Tubulin-GppNHp prepared from AMT-treated rats was more potent than that from control rats in stimulation of cortical membran AC from both AMT-treated rats and control rats. The amount of tubulin in cortical membranes of rats was not altered by AMT treatment. These results indicated that altered tubulin function, at least in part, contributed to an increased AC activity in chronic antidepressant-treated rats.

386.8

ENDOTHELINS-INDUCED CALCIUM MOBILIZATION IN CANINE TRACHEAL SMOOTH MUSCLE CELLS. C.M. Yang* and Y.-L. Yo. Department of Pharmacology, Chang Gung Medical College, Taiwan, R.O.C.

Endothelins (ETs)-mediated rises of intracellular Ca^{2+} ($[Ca^{2+}]_i$) were monitored in cultured canine tracheal smooth muscle cells (TSMCs) using a fluorescent Ca^{2+} indicator fura-2. ET-1, ET-2, ET-3 and S6b elicited an initial peak and followed by a sustained elevation of $[Ca^{2+}]_i$, in a dose dependent manner. In the absence of external Ca^{2+}, only an initial peak of $[Ca^{2+}]_i$ was seen, the sustained elevation of $[Ca^{2+}]_i$ can then be evoked by addition of 1.8 m*M* Ca^{2+}. Ca^{2+} influx was required for the changes of $[Ca^{2+}]_i$, since the Ca^{2+}-channel blockers, diltiazem, verapamil, and Ni^{2+}, decreased both the initial and sustained elevation of $[Ca^{2+}]_i$ in response to these peptides. TSMCs pretreated with phorbol 12-myristate 13-acetate (PMA, 1 μM) for 30 min attenuated Ca^{2+} mobilization induced by ETs. While long-term (24 hr) PMA treatment resulted in a recovery of responsiveness. These results suggest that the inhibitory effect of PMA was mediated through the activation of protein kinase C. The changes of $[Ca^{2+}]_i$ induced by ETs were attenuated by cholera toxin pretreatment, but not by pertussis toxin. These results conclude that the initial detectable increase in $[ca^{2+}]_i$ stimulated by these peptides is due to the release of Ca^{2+} from internal stores, whereas the contribution of external Ca^{2+} follows and is involved in a diltiazem- and verapamil-sensitive process. (supported by NSC82-0412-B182-007 and CMRP-340).

386.9

DIACYLGLYCEROL ANTAGONIZES THE INHIBITORY EFFECT OF ARACHIDONIC ACID ON THE GTPase ACTIVATING PROTEIN. P. Homayoun* and DW. Stacey. Dept. of Mol. Biol., Cleveland Clinic Foundation, Cleveland, OH 44106.

The Ha-ras proto-oncogene product p21 participates in the transduction of proliferative signals by a mechanism that is largely unknown. P21 is active when bound to GTP and becomes inactive upon GTP hydrolysis. A cytoplasmic protein, GTPase activating protein (GAP) has been shown to stimulate the rate of hydrolysis of p21-bound GTP. Stimulation of DNA synthesis by ras has been shown to require protein kinase C (PKC) activity in many cell types. In fact, transformation of cells by oncogenic ras results in stimulation of phospholipid metabolism, the release of diacylglycerol (DG), arachidonic acid (AA) and stimulation of PKC. Since in vitro studies have shown that AA, but not DG, inhibits GAP activity, we were interested to determine if DG has a synergistic effect with AA in inhibiting GAP activity. Ras protein was laoded with [α-32p]GTP and incubated in the presence of GAP alone or with lipids substrates. The rate of hydrolysis of p21-GTP was measured by precipitation of ras-bound-nucleotides by anti-ras antibody and the separation of ras-GTP from ras-GDP by thin-layer chromatography and measured by phosphorimager analysis.

Results have shown that: 1) DG (1-stearoyl, 2-arachidanoyl) did not affect GAP activity even at high concentrations (200 μg/ml). 2) AA inhibits GAP activity in a dose dependent manner and reaches a saturation point at 200 μg/ml. 3) DG antagonizes the inhibitory effect of AA on GAP in a dose dependent manner (IC_{50}=75 μg/ml). These results suggest that a rise in DG concentration in cells not only stimulates protein kinase C activity but may also indirectly modulate the activation of GAP.

386.10

DIFFERENTIAL LOCALIZATION OF p42 AND p44 MAP KINASES IN ADULT BRAIN. R.S. Fiore*, J.S. Fosnaugh, and J.M. Baraban. Department of Neuroscience, Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205.

p42 and p44 mitogen-activated protein (MAP) kinases are expressed at high levels in brain and are activated rapidly following neurotransmitter or growth factor receptor stimulation. To help determine their role in neuronal function, we have localized these isoforms immunohistochemically in rat brain sections. In previous studies, we found that p42 MAP kinase is prominently expressed in neuronal cell bodies and dendrites, where it is associated with microtubules (Fiore et al., *Neuroscience*, in press). Preliminary light-microscopic studies with antibodies selective for p44 MAP kinase suggest that this isoform is also found in neuronal cell bodies and dendrites. In addition, intense staining for p44 MAP kinase appears to be localized in oligodendrocytes and axons in several brain areas. Results indicating that p44 MAP kinase is present in axons support recent *in vitro* biochemical studies suggesting that tau, which is associated with axonal microtubules, is a substrate for MAP kinase (Drewes et al., *EMBO J.*, 1992) and suggest that the p44 isoform may be preferentially involved in regulation of tau.

386.11

TYROSINE PHOSPHORYLATION OF PLC-γ_1 INDUCED BY ELECTROCONVULSIVE SHOCKS IN RAT BRAIN.

Y. M. Ahn[1], Y. H. Lee[2], P. G. Shu[2], S. H. Ryu[2], J. B. Park[3], H. L. Kim[4*], and Y. S. Kim[1]. Departments of [1]Psychiatry and [3]Biochemistry, Seoul National University College of Medicine, Seoul, Korea 110-460, [2]Division of Neurobiology, Department of Life Science, Pohang Institute of Technology, Pohang, Korea 790-784. [4]LMB, NINDS, NIH, Bethesda, MD USA 20814.

Electroconvulsive shock(ECS) has long been used in psychosis but it's mechanism of action is still unclear. So we studied the possible involvement of PLC signal transduction system in rat brain by ECS.

The authors report here that ECS increased tyrosine phosphorylation of PLC-γ_1 in rat cerebral cortex and hippocampus. The tyrosine phosphorylation of PLC-γ_1 began to increase at 0.5 min. At 2 min, it showed further increase, reached peak at 10-20 min and returned to the baseline after 60 mins. However, the amount of PLC-γ_1 assessed with immunoblotting showed no difference. And the degrees of PLC-γ_1 tyrosine phosphorylation were not reflected on the PLC activity that was measured with PLC-γ_1 immunoprecipitates. These findings suggest that tyrosine phosphorylation of PLC-γ_1 could be observed in the brain of an intact animal, providing a way to access the mechanism of ECS with tyrosine kinase and PLC-γ_1.

386.12

CYCLIC AMP/VANADATE-SENSITIVE PHOSPHORYLATION OF SYNAPTOSOMAL PROTEINS.

M.A.N. Edgar & L.A. Dokas*, Depts. of Biochemistry & Molecular Biology & Neurology, Medical College of Ohio, Toledo, OH 43699.

The central nervous system exhibits elevated levels of protein phosphorylation relative to other tissues, reflecting higher activities of protein kinases, as well as higher concentrations and larger numbers of substrates. The role of many of these proteins in maintenance and regulation of neuronal function remains unclear. A combination of immunoblotting and alkali digestion of SDS/PAGE-resolved [γ-^{32}P]ATP-labelled phosphoproteins reveals the presence of three prominent proteins with apparent molecular masses of 39, 50, and 58 kDa in the synaptosomal (P2) and synaptic plasma membrane (SPM) fractions of rat cortex. The 58 kD protein has been further separated by SDS/PAGE and one component corresponds to pp60^{src}. Further studies have revealed two cAMP-sensitive phosphoproteins in the P2 fraction with apparent molecular masses of 37 kD (IEP: 6.3) and 32 kD (IEP: 5.1). Phosphorylation of these two proteins is dependent upon both cAMP and vanadate. Phosphorylation of both proteins is maximal by 1 min., followed by dephosphorylation to basal levels by 5 min. Analysis of a number of brain regions demonstrates a widespread distribution of these proteins. Amino acid analysis indicates multiple phosphorylation sites for most of these proteins, ^{32}P-labelling occurring on serine, threonine and tyrosine residues. Supported by NIH grant NS30792.

386.13

SUPERSENSITIVE DOPAMINERGIC BEHAVIORAL RESPONSES FOLLOWING 6 HYDROXYDOPAMINE LESIONS OF THE SUBSTANTIA NIGRA ARE ASSOCIATED WITH ALTERED PHOSPHORYLATION OF CREB IN RAT STRIATUM

D. Cole*, C. Konradi, S Hyman Lab. of Molecular and Developmental Neurobiology, MGH East, Charlestown, MA 02129

Following destruction of the substantia nigra (SN), rats develop a repertoire of supersensitive behavioral and cellular responses to dopaminergic (DAergic) drugs. Levodopa (L-DOPA) induction of c-*fos* expression in the striatum of these rats occurs via D1 receptors but without D1 receptor upregulation, suggesting that altered post-receptor signal transduction plays a role in DA receptor supersensitivity. A cAMP and calcium responsive element that binds CREB mediates c-*fos* expression when CREB is phosphorylated by protein kinase A or CaM kinase II *in vitro*. We have begun to test the hypothesis that SN ablation alters CREB phosphorylation in striatal neurons by L-DOPA *in vivo*.

Rats received unilateral 6-hydroxydopamine lesions of the SN. Rats rotating $\geq$5 times/min contralateral to the lesion after receiving apomorphine were given carbidopa/levodopa and perfused. Tissue was prepared for immunohistochemistry using polyclonal antibodies that recognize either 1)CREB regardless of phosphorylation state or 2)only CREB phosphorylated at serine-133 (PCREB; antibodies courtesy of M Greenberg and D Ginty). Staining on the lesioned side was compared with staining on the unlesioned (control) side.

Preliminary results revealed copious PCREB immunostaining in the nuclei of striatal neurons ipsilateral to the lesioned SN and a paucity of immunostaining contralaterally. CREB immunostaining was robust and symmetric on the two sides.

These preliminary results suggest that SN destruction alters DAergically mediated phosphorylation of critical signal transduction proteins, such as CREB. Modification of post-receptor pathways associated with supersensitive responses *in vivo* may play an important role in determining these responses.

386.14

DOWN-REGULATION OF CREB EXPRESSION IN A "LOCUS COERULEUS(LC)-LIKE" CELL LINE.

K.L. Widnell* and E.J. Nestler. Laboratory of Molecular Psychiatry, Depts. of Psychiatry and Pharmacology, Yale School of Medicine, New Haven, CT 06508.

The cAMP response element binding protein (CREB) and its related proteins belong to a family of leucine zipper transcription factors which bind DNA as dimers. We have found that CREB is expressed in a newly derived "LC-like" cell line. This cell line, developed by Suri et al., exhibits a noradrenergic neural phenotype (*J. Neurosci.*, **13**:1280, 1993). Moreover, Duman et al. have found that the cells, like the LC *in vivo*, possess VIP- and CRF-stimulated-, as well as opiate-, α_2-adrenergic-, and NPY-inhibited-adenylyl cyclase (*Soc. Neurosci. Abs.* **18**:343.5, 1992). In the current study, we addressed the effects of acute perturbation of the cAMP pathway on cell regulation. It is generally believed that the CREB gene is constitutively expressed and not subject to regulation. However, we have shown a 50% down-regulation of CREB mRNA levels after two hours of forskolin treatment, which was still present at six hours. This decrease in CREB mRNA was not observed when the cells were treated with 1,9-dideoxyforskolin, an analogue of forskolin which does not activate adenylyl cyclase. Short-term forskolin exposure causes these cells to differentiate modestly. However, two observations suggest that the forskolin-induced decrease in CREB mRNA is not a consequence of this differentiation per se: CREB mRNA levels were comparable in undifferentiated LC-like cells and in cells that were differentiated by serum starvation; and forskolin down-regulated CREB mRNA to the same extent in undifferentiated and differentiated cells. This study represents the first demonstration that CREB expression is subject to dynamic regulation, in this case mediated by the cAMP pathway.

386.15

INTRACEREBROVENTRICULAR FORSKOLIN INCREASES PHOSPHORYLATED-CREB AND FOS IMMUNOREACTIVITY IN RAT STRIATUM.

J.N. Simpson*, W. T. Bohler, and J. F. McGinty. Department of Anatomy and Cell Biology, East Carolina University School of Medicine, Greenville, NC 27858-4354.

Activation of the adenylate cyclase/cAMP cascade leads to an increase in phosphorylation of cAMP responsive element binding proteins (CREBs) on Ser[133]. Phosphorylated-CREBs (P-CREBs) then bind to cAMP responsive elements (CREs) in the promotor regions of several genes and induce their transcription. Intracerebral infusion of forskolin, an activator of adenylate cyclase, has been shown to induce striatal preproenkephalin (PPE) and preprodynorphin (PPD) mRNA signal (Simpson et al., Soc. Neurosci. Abst. 18: 1006, 1992). Because promotor regions of PPE, PPD, and c-fos genes contain CREs, the effect of forskolin on phosphorylated-CREB- and FOS-immunoreactivity (ir) was examined.

Five male Sprague Dawley rats (220-240 g) received intracerebroventricular (ICV) injections of forskolin (1 mM in 10 µl ACSF) in their right ventricle and ACSF alone (10 µl) in their left ventricle. One hour following ICV injections, rats were transcardially perfused with 4% paraformaldehyde and 50 µm serial sections were collected through the striatum for immunocytochemistry. Incubation with polyclonal antisera against CREB, P-CREB (kindly donated by D. D. Ginty, Dept. Microbiology and Molecular Genetics, Harvard Medical School) and FOS (Oncogene Sci.) was followed by avidin-biotin peroxidase reagents (Vectastain Elite kit).

Although striatal CREB-ir was not altered by forskolin treatment, striatal P-CREB-ir was markedly increased on the forskolin-treated side as compared to the vehicle-treated side. In addition, striatal FOS-ir was induced only on the forskolin-treated side.

These results strengthen the hypothesis that forskolin acts through the adenylate cyclase/cAMP second messenger cascade to induce striatal opioid gene expression in vivo. Supported by DA 05470 (JNS) and DA 03982 (JFM).

386.16

TRACING THE SIGNAL TRANSDUCTION CASCADE BY WHICH S100β INDUCES FOS PROTEIN IN GLIA.

Stefan Strack and Linda J. Van Eldik*
Dept. Pharmacology, Vanderbilt Univ., Nashville, TN 37232-6600.

S100β is a glia-derived protein that stimulates proliferation when added to cultures of rat primary astrocytes and the C6 glioma cell line (1). S100 also induces a transient rise in intracellular calcium (2) and c-fos mRNA (1). We have developed a rapid assay for measurement of fos protein expression in order to study early events in S100-stimulated second messenger cascades. C6 glioma cells are serum-starved and pulse-labeled with ^{35}S-amino acids in the presence of S100, and expression of fos protein is quantitated by immunoprecipitation with a fos antibody. Following stimulation of cells with low nanomolar S100, there is a transient increase in fos labelling that is maximal at 60 minutes and still slightly above baseline at 90 minutes after stimulation. Fos induction by S100 is somewhat delayed when compared to induction by PDGF in the same cells (peak at 45 minutes, baseline levels after 60 minutes), suggesting that these two growth factors induce fos via different pathways. Consistent with our previous results (1,2,3), disulfide-linked dimers of S100 are more potent than monomeric S100. We have begun to investigate the role of calcium transients in the S100-induced fos response. Preincubation with the intracellular calcium chelator BAPTA blunts S100-induced fos expression almost to control levels, suggestings that a rise in intracellular calcium is an obligatory step. Experiments are also in progress to investigate the potential contribution of protein kinase A and C activation and tyrosine phosphorylation to the mechanisms of S100-stimulated signal transduction. [Supported by a Pharmaceutical Manufacturers Association postdoctoral fellowship to SS and NIH grant AG11138 to LVE]

1. Selinfreund et al. (1991) PNAS 88:3554-3558
2. Barger & Van Eldik (1992) J.Biol.Chem. 267:9689-9694
3. Barger et al. (1992) Biochim.Biophys.Acta 1160:105-112

386.17

NEUROANATOMICAL DISTRIBUTION OF *DUNCE* HOMOLOGS IN THE MOUSE. J.A. Cherry* and R.L. Davis. Center for Learning and Memory, Beckman Neuroscience Center, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724.

In *Drosophila*, the *dunce* gene codes for a low Km, cAMP-specific phosphodiesterase. Flies bearing mutations at this locus perform poorly in a variety of associative learning tasks, including negatively-reinforced olfactory conditioning. Recently, 4 separate homologs of the *dunce* gene have been identified in both rats and humans. As a first step in determining whether a conservation exists in the function of *dunce* genes in flies and mammals, we have begun to examine the distribution of *dunce* in mouse brains. Using sequence obtained from rat (kindly provided by G. Bolger and M. Wigler), primers directed to the 3' region of each of the 4 genes were constructed. PCR was then used to amplify mouse brain cDNA; the product of each reaction was cloned into pGEMEX vectors (Promega) and sequenced to verify homology. Fusion proteins for each gene were expressed in E. coli, gel purified, and injected into rabbits. Resultant polyclonal antibodies were purified on affinity columns and are being used for immunohistochemistry (Vector Labs) of mouse brain tissue sections. Preliminary evidence for one of these proteins, the mouse homolog of the rat clone RD1 (Davis et al., PNAS 86:3604, '89), suggests that it is specific for olfactory sensory neurons. Strongest immunoreactivity (IR) is seen in axon bundles that project to the olfactory bulb and in dendritic knobs. Moderate IR occurs in dendrites and the cytoplasm of these neurons; little staining of nuclei occurs. The only staining that we have yet observed in brain is in the olfactory nerve layer that surrounds the olfactory bulbs and terminates in the olfactory glomeruli. Continued characterization of the neural distribution of *dunce* homologs in mouse will help direct future studies aimed at determining the function of these genes in mammals.

386.18

IMMUNOHISTOCHEMICAL LOCALIZATION OF CAM II KINASE IN THE SPINAL CORD OF THE HEN K. F. Jensen*, R. P. Gupta and M. B. Abou-Donia. Neurotoxicology Division, HERL, U. S. EPA, RTP, NC 27711 and Dept. Pharmacology, Duke University, Durham, NC 27710

CaM II kinase has been hypothesized to be involved in the regulation of numerous aspects neural structure and function including the cytoskeleton, ion channels, gene regulation and neurotransmitter release. Previous immunohistochemical studies have emphasized that this enzyme is particularly abundant in the forebrain and less abundant in more caudal structures such as the brainstem. Such studies, however, have usually employed antisera recognizing the alpha subunit. In this study we employed an antiserum that recognizes both the alpha and beta subunits of hen CaM II kinase (Gupta et. al., 1992, Biochem. Pharmacol. 43:1975). With this antiserum substantial CaM II kinase immunoreactivity was detectable in more caudal regions of the hen brain and was particularly abundant in the spinal cord. The difference between our findings and previous studies may be related to differences the ratios of alpha and beta subunits in different brain regions. This finding is particularly important since some neurotoxic organophosphates that produce spinal cord pathology, such as TOCP, also produce dramatic increases in CaM kinase II activity.

HYPOTHALAMIC-PITUITARY-ADRENAL AXIS REGULATION: BASIC AND CLINICAL STUDIES

387.1

PRENATAL STRESS AND ADOPTION HAVE OPPOSITE LONG-TERM EFFECTS ON HYPOTHALAMO-PITUITARY-ADRENAL AXIS ACTIVITY. **S. Maccari*, C. Henry, M. Kabbaj, P.V. Piazza, H. Simon and M. LeMoal. INSERM U259-Rue Camille St Saëns, 33077 Bordeaux, France.**

Stressors during pregnancy result in offspring with morphological and behavioral alterations. Recently, we have also shown that prenatal stress increases the propensity to develop intravenous amphetamine self-administration in adult offspring. The hypothalamo-pituitary-adrenal (HPA) axis may play a critical role in these behavioral alterations. This study addressed two questions (i) Could prenatal restraint stress during the last week of pregnancy produce long-term alterations in stress-induced corticosterone secretion via modification of hippocampal type I and type II corticosteroid receptors? and If so (ii) is this effect due to a direct effect of prenatal stress on the offspring or is it mediated by altered maternal behavior? In order to answer these questions half of the animals were raised by their biological mother while the other half were assigned at birth to a foster mother. Our results show that in adult offspring: i) prenatal stress induces a prolonged corticosterone secretion in response to novelty exposure and a decrease (60%) of type I corticosteroid receptors ii) adoption per se decreases stress-induced corticosterone secretion iii) adoption and prenatal stress interact such that adoption totally reverses the effect of the prenatal stress procedure on corticosterone secretion and on type I corticosteroid receptors. In conclusion we might suggest that long lasting modifications of HPA axis activity may be one of the biological mechanisms by which the early enviroment influences behavior of the adult animal. Furthermore, prenatal and postnatal events seem to exercise opposite effects on HPA axis activity.

387.2

ALTERED HYPOTHALAMIC-PITUITARY-ADRENOCORTICAL AXIS IN ADULT RATS AFTER PRENATAL IMMUNE CHALLENGE. J.M.H.M. Reul*, J. Stec, G.J. Wiegers, M.S. Labeur, A.C.E. Linthorst, E. Arzt and F. Holsboer. Max Planck Institute of Psychiatry, Clinical Institute, Department of Neuroendocrinology, Munich, Germany.

The Hypothalamic-Pituitary-Adrenocortical (HPA) axis plays a pivotal role in the maintenance of homeostasis. Cytokines, released from immune and other cells by immune challenges, not only coordinate the host defense reaction but also stimulate HPA axis activity. Both cytokines and HPA axis hormones play crucial roles during pregnancy. Maternal-fetal immune interactions are important for maintenance of gestation and maternal adrenocortical secretions influence fetal brain development. It remains unknown whether maternal cytokines also affect fetal brain development. We investigated whether prenatal immune stimulation affects fetal brain development and causes an altered HPA axis when adult. Pregnant rats were injected i.p. with lipopolysaccharide or human erythrocytes (HE) to stimulate their immune system by T-cell-independent and T-cell-dependent mechanisms. Adult male progeny of both groups had increased basal plasma corticosterone levels, whereas ACTH concentrations were unchanged. In addition, the HE group showed an increase in plasma ACTH and corticosterone in response to stress. Both immune-challenged groups had decreased levels of mineralocorticoid and glucocorticoid receptors in the hippocampus, a brain structure critical for HPA axis regulation. Infection during pregnancy can thus induce anomalies in fetal brain development and subsequent maladaptive adrenocortical responses to stress in adulthood.

387.3

ADRENOCORTICOTROPIN STRESS RESPONSE IN CONGENITAL LEARNED HELPLESS RATS. S.Y. Nguyen*, R.V. Thompson and E. Edwards. Dept. Pharmacology & Toxicology, University of Maryland at Baltimore, Baltimore, MD 21201.

The learned helpless (LH) rat is an animal model of depression and/or anxiety. Through a genetic breeding program, we have established two strains of rats demonstrating susceptibility (cLH) or resistance (cNLH) to helpless behavior. We have compared the stress response of adult males cLH and cNLH rats to three different stressors: 40 min, 0.8mA fooshock; 60 min restraint and 1 min ether exposure. Stress responsiveness was assessed by monitoring the changes in plasma levels of adrenocorticotropin hormone (ACTH). Shock exposure resulted in a significant 3 to 5 fold increase in plasma ACTH levels for both cLH and cNLH rats. However, cLH rats exhibited a blunted ACTH response as compared to cNLH rats ($p < 0.001$). Similarly, restraint stress produced a significant rise in ACTH levels in both cLH and cNLH rats (17 to 26 fold). Plasma ACTH levels were again lower in cLH rats as compared to cNLH. By contrast to footshock and restraint, 1 min ether exposure only cause a slight change (50-52% ↑) in ACTH plasma levels of both cLH and cNLH rats. In addition, no strain differences were observed. A similar pattern of stress responsivity was observed in cLH and cNLH female rats for all three stressors. We have previously demonstrated a differential corticosterone stress response in cLH rats. This similar stress responsiveness to various stressors at the level of the pituitary is of potential significance suggesting that the pituitary gland may be one potential site for the dysfunctional HPA axis which characterizes the cLH rat.

387.4

EFFECTS OF PRENATAL DEXAMETHASONE ADMINISTRATION ON BRAIN BIOGENIC AMINE AND 3H-PAROXETINE BINDING CAPACITIES IN RAT OFFSPRINGS. K.Muneoka, M.Mikuni, T.Higuchi*, K.Takahashi and K.Matsumoto, Div.of Mental Disorder Res.,Natl.Inst.of Neurosci.,NCNP,Tokyo,187 and Dept.of Neuropsychi.,Faclt.of Med.,Kagoshima Univ.,Kagoshima 890.

In this study, we have investigated wether, or not, glucocorticoid receptor activation in an immature brain can result in permanent changes in monoamine metabolism and receptor function once that brain is mature. Pregnant rats were given 0.05mg/kg(lower dose) or 0.2mg/kg(higher dose) of dexamethasone(DEX) on gestational days 17,18 and 19. Brain biogenic amines including 5-HT,NE and 5-HIAA were measured by HPLC with ECD in hypothalamus of the offsprings at postnatal 21-day(P21D) and 12 weeks of age(12Ws). In addition, 3H-paroxetine binding assay was also performed for hypothalamus of male offsprings at 12Ws. In male rats at P21D, lower dose group showed significantly higher concentration of 5-HT compared to control and higher dose groups while higher dose group showed significantly higher 5-HT levels than control. 5-HIAA levels showed no significant changes among each groups. NE levels increased significantly in lower dose group compared to the other groups. At 12Ws, similar dose-specific effects of prenatal DEX treatment such as P21D on 5-HT levels of hypothalamus were seen in male offsprings. But NE levels showed no significant changes at this period. 3H-paroxetine binding capacities increased significantly in lower dose group rather than control. Higher dose group showed a tendency to be increased in 3H-paroxetine binding sites compared to control, but not significant. These results indicated that prenatal DEX administration altered brain biogenic amine levels and influenced the development of serotonergic neurons.

387.5

FISCHER AND LEWIS RAT STRAINS DIFFER IN CORTICOSTERONE REGULATION OF TYROSINE HYDROXYLASE AND OTHER PHOSPHOPROTEINS IN THE VENTRAL TEGMENTAL AREA. J. Ortiz* and E. J. Nestler. Laboratory of Molecular Psychiatry, Dept. of Psychiatry, Yale Univ. School of Medicine, New Haven, CT 06508.

Fischer and Lewis inbred rat strains show different inherent preference for cocaine, alcohol and opiates. They also differ in their hypothalamic-pituitary-adrenal (HPA) responses to several pharmacological agents, with the Lewis strain being hyporesponsive as compared to the Fischer strain. We have studied HPA activity by measuring plasma concentrations of corticosterone (CORT) at different time points of the circadian cycle. We have also examined CORT regulation of several phosphoproteins that show Fischer-Lewis differences in the ventral tegmental area (VTA), locus coeruleus (LC) and dorsal raphe (DR), by means of back-phosphorylation and 2D gel electrophoresis, and immunoblotting techniques.

Naive male Lewis rats did not show an evening rise of plasma CORT, which indicates a clear impairment of HPA axis function. However, after the implantation of subcutaneus CORT pellets, plasma CORT increased 60% more in Lewis than in Fischer rats. This suggests that slower CORT metabolism might counteract partially a deficiency in CORT release in Lewis rats. In the brain regions studied, CORT administration regulated tyrosine hydroxylase (TH), glial-fibrillary acidic protein (GFAP) and a protein of Mr~70Kd whose identity is unknown. TH levels increased in the VTA of Fischer rats by 40-70%, abolishing pretreatment differences between these strains. GFAP back-phosphorylation increased in the VTA of Fischer rats and decreased in the DR and LC of Lewis rats, in a similar tendency to abolish inherent strain differences. The 70Kd protein showed increased back-phosphorylation in the VTA and DR of Fischer rats. No CORT regulation of neurofilament proteins was observed. Studies are currently underway to correlate CORT regulation of VTA phosphoproteins with behavioral measures of cocaine preference in Lewis and Fischer rats.

387.6

RAT LINES GENETICALLY SELECTED FOR DIFFERENTIAL RESPONSIVENESS OF THE DOPAMINERGIC SYSTEM DISPLAY DISTINCTLY DIFFERENT PITUITARY-ADRENAL CHARACTERISTICS. N.Y. Rots[1,2], M. Oitzl[2*], A. Berod[3], W. Rostene[3], A.R. Cools[1], E.R. de Kloet[2]. [1]Dept. Pharmacol., Nijmegen Univ., 6500 HB Nijmegen; [2]Div. of Medical Pharmacology, Cent. Biopharm. Sci., Leiden Univ., 2300 RA Leiden, The Netherlands; [3]INSERM U339, Hopital Saint-Antoine, 75571 Paris-Cedex 12, France.

Rats of a normal outbred Wistar population were selected after an injection with the dopamine agonist apomorphine which induces a gnawing response. Apomorphine susceptible rats (apo-sus) showed an increased locomotor activity and a fleeing response in a defeat test while the apomorphine unsusceptible (apo-unsus) rats display a freezing response under the same conditions. Our studies were aimed to examine the neuroendocrine reactivity of the hypothalamic-pituitary-adrenal system of the two rat lines in order to obtain more insight into the physiological basis of their different behavioral performance. Apo-sus rats have the following characteristics (i) increased tyrosine hydroxylase mRNA in the dopaminergic A_9 cell group and altered features of striatal dopamine D_2-receptors, (ii) enhanced and prolonged ACTH release in response to a conditioned emotional stimulus, while total plasma corticosterone was similar, (iii) reduced basal and enhanced stress induced free corticosterone; binding of corticosterone to CBG was reduced, furthermore, the in vitro responsiveness of the adrenocortical secretion to ACTH was increased, (iv) MRs in the hippocampus assessed with quantitative in vivo autoradiography showed increased retention of radiolabeled corticosterone. In conclusion: This study with genetically selected rat lines suggests that increased nigro-striatal dopaminergic responsiveness is correlated with enhanced pituitary-adrenal reactivity.

387.7

SOCIOREGULATORY EFFECTS ON SQUIRREL MONKEY PITUITARY-ADRENAL ACTIVITY. D.M. Lyons* and S. Levine. Dept. of Psychiatry and Behavioral Sciences, Stanford Univ. Sch. of Med., Stanford, CA 94305.

Squirrel monkeys show unusually prolonged elevations in plasma cortisol when separated from like-sex social companions. To determine whether this sustained hypersecretion of cortisol reflects a deficiency in neuroendocrine feedback mechanisms that normally inhibit the prolonged activation of the pituitary-adrenal axis, we collected simultaneous measures of plasma cortisol and plasma corticotropin (ACTH) from 30 juvenile monkeys (15 males, 15 females) over a 9-week period while animals were living in previously established groups, in individual cages, and in newly-formed groups. As found in recent studies of adults, mean cortisol titers were consistently higher in individually housed animals than when the same animals were housed in small like-sex social groups. When cortisol was elevated, however, ACTH titers were significantly and chronically reduced. These results suggest that elevated cortisol does indeed inhibit ACTH synthesis or release, and that hypercortisolism in squirrel monkeys living without social companions is not a consequence of chronic elevations in ACTH. Similar peculiarities in pituitary-adrenal activity have been noted in clinical studies of anorexia and endogenous depression.

387.8

RESISTANCE OF PLASMA CORTICOSTERONE LEVELS TO SUPPRESSION BY DEXAMETHASONE IN A TRANSGENIC MOUSE MODEL FOR MAJOR DEPRESSION. N. Barden*, F. Holsboer, J.M.H.M. Reul and I. Stec. Max Planck Institute of Psychiatry, Clinical Institute, Department of Neuroendocrinology, Munich, FRG and Molecular Psychogenetics Laboratory, Department of Physiology, Laval University Hospital Research Centre, Ste Foy, Québec, Canada.

Transgenic mice bearing a transgene expressing antisense RNA complementary to a fragment of the glucocorticoid receptor cDNA have hypothalamic-pituitary-adrenal (HPA) axis changes similar to those seen in depression including a hyperactive HPA axis as demonstrated by elevated early morning plasma corticosterone and ACTH levels, a reduced glucocorticoid binding capacity and, as a result, a deficient glucocorticoid feedback inhibitory mechanism[1]. We have further investigated these changes in HPA axis regulation by use of different neuroendocrine challenge tests including a dexamethasone suppression test (DST) and/or stress. Transgenic mice required a ten fold higher dose (20 µg/100g BW) of dexamethasone to suppress the basal corticosterone levels than did normal mice. Pre-treatment of normal mice with 10 µg dexamethasone/100g BW completely suppressed their corticosterone stress response to a 15 minute exposure to a novel environment. In contrast, transgenic mice pre-treated with 0-20 µg dexamethasone 6h before exposure to the same stress paradigm always responded with an increase of plasma corticosterone levels. These findings are similar to those often observed in depressed patients who show non suppression in the DST and an increase of cortisol in a combined DST-corticotropin-releasing hormone challenge test[2] and support the hypothesis of a defective corticosteroid feedback mechanism in depressive illness.

1. Pepin, M-C., Pothier, F. & Barden, N. *Nature* **355** (1992) 725-728
2. Holsboer, F. *Eur Arch Psychiatr Neurol Sci* **238** (1989) 302-322

387.9

STRESS-SPECIFIC EFFECTS OF CHRONIC INTERMITTENT STRESS ON TUMOR GROWTH IN NEONATALLY HANDLED AND NONHANDLED RATS: CORRELATES WITH HPA ACTIVITY

M. Walker, A. Steverman, S. Bhatnagar, N. Shanks* & M.J. Meaney, Depts. of Psychiatry & Neurology and Neurosurgery, Douglas Hospital Research Centre, McGill U., Montreal, Quebec H4H 1R3 CANADA

It has previously been demonstrated that elevated plasma corticosterone (B) levels support increased growth of Fujinami sarcoma virus (FSV) transfected fibroblasts in adult rats. Neonatal handling is known to permanently alter HPA responding such that nonhandled (NH) animals exhibit greater hypothalamic-pituitary-adrenal (HPA) responding to stress as adults than do neonatally handled (H) animals. We assessed the impact of two chronic intermittent stressor regimens (cold and restraint) on tumor incidence and growth in adult H and NH rats following inoculation with FSV transfected cells. Cold stress increased the incidence of tumors in both H and NH rats, whereas restraint stress did so only in the NH animals. Following 3 weeks of daily cold (4 hours, 4°C), H or NH plasma B and ACTH responding were similar to that seen on the first day of responding. In contrast, following 3 weeks of restraint (20 minutes daily) plasma B and ACTH responding was reduced in H animals relative to responses seen following the first stress exposure. Diurnal HPA activity was not affected by the chronic stressor exposure in either H or NH animals. Glucocorticoid receptor binding was determined for tumors and spleens, and did not vary across treatment groups. However, thymus binding was decreased in restraint stressed H animals, but was not altered in the other stress groups. These data indicate a greater incidence of tumors following a chronic stressor regimen when HPA adaptation was not observed and a lower incidence when HPA stress responding is dampened.

387.10

HYPOTHALAMIC PITUITARY ADRENAL RESPONSES TO ENDOTOXIN IN CHRONIC INTERMITTENTLY COLD STRESSED NEONATALLY HANDLED AND NON HANDLED ANIMALS. S. Sharma*, S. Bhatnagar, N. Shanks, S. Gelber and M.J. Meaney. Developmental Neuroendocrinology, Lab., Douglas Hosp. Res. Ctr., McGill Univ., Montreal, Quebec, Canada H4H 1R3.

As adults, neonatally handled (H) rats secrete less corticosterone (B) and adrenenocorticotropin (ACTH) following exposure to acute stress than non-handled (NH) animals. We have previously shown that when H and NH animals exposed to chronic intermittent cold stress (4°C for 4 h a day for 21 days; H CHR and NHCHR) are presented with a novel, heterotypic stressor (20 min restraint), NH CHR hypersecrete both ACTH and B compared to NH control (CTL) but H CHR are not different from H CTL. In the present study, we attempted to determine if this facilitation of HPA responses to novel stressors in NH animals extends to a stimulus that is thought to activate the HPA axis through the immune system. We administered endotoxin (*Salmonella enteritidis;* 1mg/kg) intravenously and examined the time course of plasma B and ACTH responses. Peak B and ACTH responses were seen at 1 or 2 h after endotoxin injection in all groups. However, neither H CHR nor NH CHR exhibited facilitation of B or ACTH responses to the endotoxin. Indeed, integrated B and ACTH levels suggested that prior exposure to chronic stress decreased responsiveness to endotoxin. These data suggest that facilitation of, and individual differences in, HPA responses to novel stressors in chronically stressed animals are dependant on the nature of the novel stress.

387.11

EFFECTS OF REPEATED ELECTROCONVULSIVE SHOCK (ECS) ON TYROSINE HYDROXYLASE ACTIVITY IN STRESSED RATS. Linda S. Brady* and Sung-Kwan Hong. Section on Functional Neuroanatomy, NIMH, Bethesda, MD 20892.

It is thought that the locus coeruleus (LC)-norepinephrine (NE) system may play a role in the therapeutic efficacy of ECS treatment for human depression, which may be associated with elevated NE activity in the CNS and periphery. We compared the effects of repeated ECS on tyrosine hydroxylase (TH) enzyme activity in stressed and unstressed rats. The treatment groups were: *stress* (immobilized for 2 h), *ECS* (80 mA, 0.5 sec, via earclips), *stress+ECS* (in immediate succession), and *control* (handled daily) for 7 days. All rats were sacrificed 24 h after the last treatment. The *stress* animals showed non-significant increases in adrenal weights and in TH enzyme activity in the adrenal gland and the LC. In both *ECS* and *stress+ECS* groups, the adrenal glands were enlarged (27% increase, $P < 0.05$), adrenal TH activity was increased (4.5-fold, $P < 0.001$), and LC TH activity was increased (4-fold, $P < 0.001$) relative to *control*. TH enzyme activity in the adrenals and LC of *stress+ECS* animals was ~2-fold greater ($P < 0.05$) than in *stress* animals but did not differ from activity in *ECS* animals. Thus, ECS effects on TH activity do not differ in stressed and unstressed rats. Repeated ECS does not restore TH activity to control levels in stressed rats, as might be predicted from its therapeutic effects in depression.

387.12

CHRONIC ANTIDEPRESSANT TREATMENT IMPROVES SPATIAL MEMORY PERFORMANCE IN YOUNG BUT NOT AGED RATS. J.L.W. Yau*, T. Olsson, R.G.M Morris[1], M.J. Meaney[2] and J.R. Seckl. Dept Med, Western Gen Hosp and [1]Dept Neuroscience, Edinburgh Univ and [2]Douglas Hosp Res Centre, Montreal, Canada.

The emergence of cognitive deficits in a subgroup of aged rats is associated with increased hypothalamic-pituitary-adrenal axis activity, decreased hippocampal glucocorticoid receptor (GR) expression and neuronal loss. Neonatal manipulations that permanently increase hippocampal GR expression prevent these age-related deficits. We have previously shown that chronic antidepressant treatment increases GR gene expression in the hippocampus and have now investigated whether antidepressants improve spatial memory in young and aged rats. Lister-hooded rats aged 8 and 22±1 months were ranked according to performance in a watermaze (4 trials/day for 4 days followed by a free swim probe trial). In each group matched pairs of rats were treated with amitriptyline (10 mg/kg, i.p.) or saline daily for 9 wks. Thereafter the rats were reassessed in the water maze. Amitriptyline treatment significantly improved spatial memory in the young rats (30% increase in probe transfer time, $p<0.05$ vs previous test and saline-treated controls). By contrast, in aged rats amitriptyline had no effect, either in rats showing normal or impaired spatial memory (compared to young controls). Our data indicate that antidepressant drugs improve spatial memory in young but not aged rats, presumably reflecting a loss of plasticity of cognitive function that preceeds overt deficits and neuron loss. The relationship of this to hippocampal GR expression is currently being assessed by *in situ* hybridization.

387.13

NEUROTENSIN/NEUROMEDIN N mRNA LEVELS ARE SELECTIVELY REGULATED BY CORTICOSTERONE IN FOREBRAIN CELL GROUPS. A.G. Watts* & G. Sanchez-Watts. NIBS Program & Dept. of Biol. Sci., USC, Los Angeles, CA 90089.

Recent results from our laboratory have identified changes in neurotensin/neuromedin N (NT/NMN) gene expression in a number of forebrain cell groups after disturbance to fluid homeostasis in the rat. Increased plasma osmolality leads to decreases in NT/NMN mRNA levels in the central nucleus of the amygdala (CEA), increases in neurons in parts of the lateral hypothalamic area (LHA), but no changes in the hypothalamic paraventricular nucleus (PVH). However, hypovolemia induced by subcutaneous (sc) injection of polyethylene glycol (PEG) leads to increases in NT/NMN mRNA levels in CRH neurons in the medial parvicellular PVH, in neurons of the periventricular nucleus (PV), but little change in neurons in the LHA. A feature common to both types of dehydration is increased plasma concentrations of corticosterone (CORT). To investigate the potential role of CORT in mediating these changes in NT/NMN mRNA levels we have used *in situ* hybridization to determine the regulation of NT/NMN mRNA levels by CORT. Male SD rats were bilaterally adrenalectomized under halothane anesthesia and 4 days later implanted sc with either a placebo pellet or a pellet containing 50mg, 100mg or 200mg of CORT (Innovative Research of America). Seven days later animals were anesthetized, and perfusion-fixed. Frozen 15μm coronal sections were cut through the forebrain and hybridized using a cRNA probe for NT/NMN. Analysis of the dipped slides (8 day exposures) and X-ray autoradiographs showed that CORT did not alter the levels of NT/NMN mRNA in the CEA or the LHA. In the PVH no NT/NMN mRNA was detected after any of the treatments. However in the PV immediately caudal to the PVH, substantial dose-dependent increases were observed. The location of these neurons raises the possibility of colocalization of NT in the tyrosine hydroxylase-containing neurons found in this same region. These results suggest that alterations in NT/NMN gene expression seen in the LHA and CEA after osmotic stimulation are not dependent on increased plasma CORT. The increases seen in the PVH after hypovolemia are also CORT-independent. However, CORT may mediate the increases seen in PV neurons after osmotic stimulation or hypovolemia. (Supported by NS 29728)

387.14

REGULATION OF VASOPRESSIN AND OXYTOCIN GENE EXPRESSION IN PARVOCELLULAR NEURONS OF THE PVN BY STRESS AND STEROIDS IN THE HYPOOSMOLAR RAT. T.G. Sherman*, J. Dohanics† and J.G. Verbalis†. Departments of Behavioral Neuroscience and †Medicine, University of Pittsburgh, Pittsburgh, PA 15260.

Chronic hypoosmolality induces a down regulation of hypothalamic magnocellular vasopressin (AVP) and oxytocin (OT) synthesis. A first-order decay of AVP and OT mRNA levels results in only 10-15% of the mRNA content of control rats by day 14 of hypoosmolality. Although the ability of the hypothalamo-neurohypophyseal system to down-regulate has important implications for studies of magnocellular physiology, it is also an attractive model for studies of the hypothalamic-pituitary-adrenal axis. We have previously shown that the effects of parvocellular-specific stressors on ACTH secretion are not impaired in hypoosmolar rats. Hypoosmolality, therefore, may prove to be an effective means to study parvocellular AVP and OT gene expression in the absence of the dominating influence of magnocellular AVP and OT. Measures of hypothalamic AVP and OT transcriptional activity, as measured by intron-directed *in situ* hybridization, were undetectable in 14 day hypoosmolar rats, consistent with results from nuclear run-on studies. In hypoosmolar rats, a dramatic increase in AVP primary transcript levels occurred 60 min after a single *sc* injection of metyrapone or after a 30 min restraint stress. Neither of these stimuli resulted in detectable changes in AVP or OT mRNA levels when measured by exon-directed *in situ*. The metyrapone-induced increases in nuclear AVP transcripts were mostly confined to parvocellular neurons, whereas the restraint-stress increases, while also largely parvocellular, resulted in magnocellular expression as well. These results demonstrate that using intron-directed *in situ* hybridization in hypoosmolar rats enables detection of rapid up-regulation of AVP and OT transcription in parvocellular neurons of the PVN.

387.15

REVERSAL OF SEX DIFFERENCES IN HPA RESPONSE TO ENDOTOXIN CHALLENGE IN THE NEONATE BY GONADECTOMY. C.M. McCormick*[1], N. Shanks[2], & M.J. Meaney[2]. [1]Dept. of Psychology, Bates College, Lewiston ME 04240, & [2]Dept. of Psychiatry, Douglas Hospital Research Centre, McGill University, Montreal PQ H4H 1R3.

The interaction between gonadal and adrenal steroids in their roles as immunoregulators is unclear. We recently reported a sex difference in HPA responsivity in rat pups to immune challenge with endotoxin. In the present study, we assessed the effects of neonatal gonadectomy on the sex difference in HPA responsivity. One-day-old pups were gonadectomized or subjected to sham surgery. At three days of age, pups were injected i.p. with .05mg.kg *salmonella enteriditis* endotoxin. Four hours later, we collected trunk blood and tissues. Sham females showed higher levels of plasma ACTH and corticosterone compared to sham males. The reverse was found in gonadectomized animals: Males showed elevated, whereas females showed decreased, corticosterone levels relative sham same-sex controls. Gonadectomy did not change ACTH response to endotoxin in males, and elevated ACTH response in females. The reduction in CRH levels of gonadectomized animals following endotoxin did not differ between the sexes. These data suggest that sex differences in HPA response to immune challenge may be mediated at the level of the CNS in females, but may be due to altered adrenal sensitivity in males. Currently we are investigating the role of circulating sex steroids on immune/HPA function via gonadectomy and sex steroid replacement, and via administration of sex steroid antagonists.

387.16

ORIGIN OF NEUROHYPOPHYSEAL NEUROPEPTIDE FF. E.A. Majane*, J. Zhu, A.A. Aarnisalo†, P. Panula#† and H.-Y.T. Yang. Lab. Biochem. Genetics, NIMH, Washington, DC 20032, Univ. of Helsinki# and Abo Akademi Univ., Finland.†

Neuropeptide FF (FLFQPQRF-NH_2) is an FMRF-NH_2-like peptide with morphine modulating activity. Neuropeptide FF (NPFF) is unevenly distributed in the rat CNS with the highest concentrations found in posterior pituitary and spinal cord. In rat pituitary, NPFF is found exclusively in the neural lobe where it is localized in nerve terminals and fibers suggesting that hypothalmus is the source of neural lobe NPFF. In this study we investigated the origin of neurohypophyseal NPFF using electrolytic lesion studies and an anterograde tracing experiment. Lesions of the supraoptic nucleus (SON) caused a 50% reduction in pituitary NPFF while the lesion of a prominent NPFF cell body group found in the medial hypothalamus failed to alter NPFF content in neural lobe. HPLC analysis of punches taken from the SON confirm the existence of authentic NPFF in this area. An anterograde tracing experiment, showing that Phaseolus vulgaris-leucoagglutinin injected to SON is found in NPFF positive fibers in neurohypophysis, also supports the hypothesis that at least part of neural lobe NPFF originates from SON.

387.17

HYPOTHALAMIC/PITUITARY/ADRENAL AXIS RESPONSES TO AN EXERCISE CHALLENGE IN WOMEN WITH PERIMENSTRUAL MOOD ALTERATIONS. C.A.Cahill*, J.Mallory, C.Thomas and K. Zuninga. University of Kansas Medical Center, School of Nursing, Kansas City, KS 66160.

The search for a neuroendocrine cause of altered mood states in some women during the late luteal phase of the menstrual cycle has traditionally focused on alterations in gonadal axis hormones. Yet, there is no evidence that women with perimenstrual symptomatology experience greater any greater incidence of infertility or other gynecologic disorders. This suggests that altered gonadal axis function is not involved in mood changes. Psychosocial studies of causes of perimenstrual symptoms suggest that daily life stressors are predictive of perimenstrual symptom occurrence and severity. Recent evidence of stress axis and gonadal axis interaction suggests that the interface between these axes may be involved in perimenstrual symptom etiologies. Using the Stress Diathesis Model of Depression as an analogous paradigmatic for the investigation of stress axis function in perimenstrual symptomatology, we propose to describe the effects of a physical challenge (physical exercise) on plasma ACTH, prolactin and cortisol levels during the follicular phase of the menstrual cycle and the late luteal phase of the menstrual cycle. Five women who report few symptoms during both phases of the cycle will serve as the control group. Five women with low symptoms in the follicular phase and severe symptoms during the late luteal phase and 5 women with moderate symptoms during the follicular phase and severe symptoms during the late luteal phase will form the 2 experimental groups. Comparison of the hormone responses between cycle phases and among groups will be done to determine the tone and responsivity of the stress axis in women with perimenstrual symptoms.

387.18

Uncertainty in Hormone Concentration Estimation: Advantages of Complete Error Propagation Through Standard Curves by Coupling to a Discrete Variance Function Martin Straume[1] Michael L. Johnson[1,2] Johannes D. Veldhuis[1] and David J. Hudson,*[3] Center for Biological Timing and [1]Dept. of Med./Int. Med., [2]Dept. of Pharm., [3]Dept. of Biol., Univ. of Virginia, Charlottesville, VA 22901

Uncertainty in experimental determination of hormone concentration is quantified by coupling (i) discrete estimates of standard deviation in observed response from standard curves (*model-independent empirical variance "function"*) with (ii) nonlinear asymmetric error propagation through standard curve model parameters (*complete variance space mapping*). Advantages include (a) statistically accurate uncertainty estimation even from *singlet* data (precision improves as replicate number increases), (b) reliable estimation of concentration and uncertainty for hormone levels approaching zero, and (c) rigorous quantitation of *all* factors contributing to uncertainty. Many commonly encountered biasing and error-causing limitations are overcome by explicitly accounting for the contribution of (ii), not requiring replicate measurements, not basing uncertainty solely on variance from replicate measurements, and not requiring any particular analytical function for the variance model.

Supported by NSF Center for Biological Timing

387.19

IMPLICATIONS OF MODEL SIMULATIONS DESIGNED TO REPLICATE OBSERVED PLASMA ACTH PULSATILE PHENOMENA IN HUMANS. B.M.Goodman, M. Carnes, S.J. Lent, and B.R. Brooks*. GRECC Service, Middleton VA Hospital and Dept. of Medicine, Univ. of Wisconsin, Madison, Wisconsin 57606.

ACTH is secreted in a pulsatile fashion forming a comlex signal in the plasma compartment. Using high intensity venous sampling (1–2 min intervals) we have observed rapid short term (less than 10 min) elevations and suppressions in plasma ACTH concentrations in rats and human subjects which can not readily be explained by accepted ACTH clearance kinetics. We performed model simulations of pulsatile ACTH secretion using bi-compartmental clearance with time invariant parameters and were unable to replicate observed plasma ACTH phenomena. We were, however, able to generate the observed rapid fluctuations in plasma concentration by allowing the fraction of the ACTH signal shunted between the two clearance mechanisms (decay times of 3 and 30 min) to be modulated according to the rate of pulsatile bursts, independent of sign. This modeling work will be presented and its physiological implications discussed.

Supported by the Department of Veterans Affairs and NIH R29 DK40759

387.20

RESPONSIVENESS OF THE HYPOTHALAMIC-PITUITARY-ADRENAL AXIS TO A GLUCOSE CHALLENGE IN ALZHEIMER'S DISEASE. [1]R.A. Mulnard,*[2] S. Moore, [2]C.A. Sandman, [1]C.W. Cotman. Irvine Research Unit in Brain Aging[1], and Department of Psychiatry and Human Behavior[2], University of California Irvine.

As previously reported by Gold and associates, glucose has been shown to enhance memory function in well young and elderly subjects as long as glucose metabolism is normal. But, abnormal glucose metabolism, characterized by severe elevations in serum glucose, causes memory impairment. In elderly subjects with Alzheimer's disease (AD), enhancement of memory function under glucose conditions is demonstrated by improved performance on the Weschler Paragraph test for narrative memory. DeLeon has also reported that cortisol response to glucose as a biological challenge in AD subjects was characterized by a sustained rise in the hormone up to two hours after ingestion of the glucose solution. Our work aims to combine both of these projects by stimulating memory capabilities and the HPA axis with a biological challenge of 75 grams of glucose solution consumed over a ten minute period of time. 16 patients (10 AD and 6 non-AD) were tested with a modified Gold protocol. A wide range of neuropsychological tests were utilized to discriminate the effect on memory in the demented population. HPA axis response (ACTH, cortisol and B-endorphin) was measured in 10 patients at baseline and at the peak peripheral glucose level. The results, while preliminary in nature, have facilitated a revision in the original protocol and have shown a diversity of responses among the subjects, perhaps a phenomenon related to proposed physiological subtypes of AD.

NEURAL-IMMUNE INTERACTIONS: SYMPATHETIC REGULATION OF IMMUNE RESPONSE

388.1

THE EFFECT OF CHEMICAL SYMPATHECTOMY ON PRIMARY ANTIBODY RESPONSE IN YOUNG AND OLD F344 RATS. A. L. Langsam , C. L. Low, K. S. Madden, S.Y. Felten, D.L. Felten*& D. L. Bellinger. Dept of Neurobio & Anat. Univ.of Rochester Sch. Med., Rochester NY 14642.

Studies from our laboratories, as well as others, have demonstrated noradrenergic (NA) sympathetic innervation of primary and secondary lymphoid organs. Further, we have shown a striking progressive decline in the density of NA nerves that distribute to spleens from old F344 rats, and a greater than 50% decline in splenic NE content. The time course of this age related loss of NA innervation of the spleen parallels an age-related decline in cell-mediated immunity. In the present study, we have examined the role of NA innervation of the spleen in primary antibody response in young and old F344 rats following chemical sympathectomy (SympX) with 6-hydroxydopamine (6-OHDA). KLH-induced antibody proliferation of splenocytes and serum antibody titers for keyhole limpet hemocyanin (KLH) were assessed at 2, 4, 5, 7, and 14 days after SympX (or vehicle injection) and subsequent immunization with KLH (150 µg i.p.). The number of anti-KLH secreting splenocytes was reduced, but serum antibody titers (both IgM and IgG) were elevated in vehicle-treated 17-mo-old rats compared with their young adult controls. SympX in both young and aged F344 rats altered both KLH-induced proliferative response and serum KLH antibody levels. The finding of altered primary antibody response in aged SympX animals demonstrates that NA sympathetic innervation is still capable of altering immune response with age, despite the significant age-associated decline in innervation. Supported by Sandoz Foundation for Gerontological Research,NIMH R29 MH47783-02.

388.2

IMMUNOCYTOCHEMICAL AND NEUROCHEMICAL ANALYSES OF SYMPATHETIC NERVES IN RAT BONE MARROW. K.L. Gibson-Berry, C. Richardson, S.Y. Felten,*D.L. Felten, Dept. Neurobiol. & Anat., Univ. Rochester Sch. Med., Rochester, N.Y. 14642

The innervation of femoral and tibial bone marrow was investigated utilizing immunocytochemical staining of fixed and decalcified tissue sections from normal Fisher 344 rats. Staining was achieved with antibodies recognizing PGP 9.5, a general neural marker, tyrosine hydroxylase (TH), the rate limiting enzyme in the synthesis of catecholamines, and neuropeptide Y (NPY), a neuropeptide often co-localized with norepinephrine in sympathetic fibers. Many PGP 9.5-, TH- and NPY-immunoreactive (ir) fibers were observed in association with the vasculature with a smaller number of fibers coursing in the parenchyma adjacent to hematopoietic elements. PGP 9.5-ir fibers appeared to be more abundant than either TH- or NPY-ir fibers suggesting that the TH- and NPY-ir fibers are only one component of the total innervation. To further investigate the neural origin of TH- and NPY-ir fibers, rats were chemically sympathectomized with the 6-hydroxydopamine (6-OHDA), with similar immunocytochemical analyses as described above. The vast majority of TH- and NPY-ir fibers were depleted by 6-OHDA treatment. Relatively fewer PGP 9.5-ir fibers were destroyed and these are believed to represent fibers contianing additional neuropeptides such as substance P and CGRP . Additionally, fresh frozen samples of tibial and femoral marrow are being analyzed for norepinephrine content by HPLC, and for NPY content by radioimmunoassay. Extensive reduction in norepinephrine and NPY levels is anticipated in sympathectomized rats compared with control rats. Bone marrow from sympathectomized mice demonstrate enhanced proliferative responses (Madden et al., submitted for publication). Thus, we hypothesize that the presence of noradrenergic fibers and other peptidergic fibers in the parenchyma of the marrow and along the vasculature provides the anatomical substrate for neurotransmitter signaling of the hematopoietic marrow, and for potential central nervous system modulation of marrow proliferation, differentiation, or cell trafficking. (Supported by NIMH R37M442076)

388.3

INTERLEUKIN-2-INDUCED POTENTIATION OF IMMUNE FUNCTION IS MEDIATED BY THE SYMPATHETIC NERVOUS SYSTEM. S. Zalcman*, J. Green-Johnson, L. Murray, W. Wan^, D.M. Nance^ & A. H. Greenberg. Manitoba Institute of Cell Biology, and ^Department of Pathology, University of Manitoba, 100 Olivia St., Winnipeg MB. Canada. R3E 0V9.

Interleukin (IL)-2, a lymphokine produced by activated T cells, stimulates T and B cell responses, including antigen-specific immunoglobulin production. We have shown that peripherally administered recombinant IL-2 also induced marked increases in hypothalamic norepinephrine (NE) utilization but did not affect plasma corticosterone levels in mice. The hypothalamus mediates sympathetic outflow to lymphoid organs, and catecholaminergic stimulation during the early phases of the in vitro IgM plaque forming cell (PFC) response enhances the peak splenic PFC response (see Sanders and Munson, 1985). Hence, we assessed whether the effects of IL-2 on the IgM PFC response are mediated through the sympathetic nervous system. IL-2 (100 or 200 ng) administered ip to mice and rats either 1 day before or immediately prior to sheep red blood cell (SRBC) immunization markedly enhanced the subsequent peak splenic IgM PFC response, compared with controls receiving vehicle. IL-2 administered at a later time frame after immunization (i.e., 2 days) did not affect the number of antibody-forming cells. Intact sympathetic innervation of the spleen was required for the IL-2-induced immunoenhancement to occur in that cutting the splenic nerve (which reduced splenic NE content by approx. 98%) blocked the enhancing effects of IL-2 on the IgM PFC response in rats. The IL-2-induced immunopotentiation was likewise blocked in mice administered the ß-adrenergic antagonist propranolol (5 mg/kg) immediately and 1 day after IL-2 administration. The α-adrenergic antagonist phentolamine (5 mg/kg) had no effect. Taken together, these data suggest that IL-2-induced potentiation of the IgM PFC response requires intact sympathetic innervation of the spleen and is blocked by ß-adrenergic antagonists. It is suggested that lymphokines released during the early phases of the IgM PFC response may activate the sympathetic nervous system which in turn potentiates the ongoing immune response. Supported by NIMH and MRC of Canada.

388.4

ENHANCED CATECHOLAMINERGIC INNERVATION OF LYMPHOID TISSUES IN NGF TRANSGENIC MICE. D. Beiting, K.M. Albers†, B.M. Davis and S.L. Carlson*. Depts. of Anatomy & Neurobiology and †Pathology, University of Kentucky Medical Center, Lexington, KY 40536-0084

Lymphoid tissues are innervated by the sympathetic nervous system with a distinctly compartmentalized distribution of fibers. Nerve growth factor (NGF) is essential for the maintenance of peripheral sympathetic neurons, however the mechanism by which the pattern of innervation of lymphoid tissues is determined remains unknown. We have examined the lymphoid tissues of NGF transgenic mice to determine if the presence of increased NGF modifies the pattern of innervation. These transgenic mice express high levels of NGF in the skin starting late in prenatal development and continuing into adulthood. To study the sympathetic innervation of the spleen and lymph nodes (peripheral and mesenteric), 30µM sections were stained with anti-tyrosine hydroxylase. The immunoreactivity in the spleen and peripheral lymph nodes of the transgenic mice is tremendously enhanced compared to controls, but maintains the compartmentation of the innervation patterns. In control spleens, the central artery innervation is more prominent than that in the marginal zone, and fibers are rarely found in the red pulp. In the spleens of transgenic mice, the fibers are very dense in the marginal zone, with somewhat less than normal innervation surrounding the central artery. In some spleens, many fibers are found in the red pulp as well. The peripheral lymph nodes of control mice have sympathetic innervation in the medulla in association with vessels. In contrast, the peripheral lymph nodes of the transgenic mice have extremely dense innervation in the medulla and capsule, with the fibers sometimes ringing B-cell follicles but not extending into the cortex or paracortex. Interestingly, innervation of the mesenteric lymph nodes of the transgenic mice is not much different than in controls. In summary, these transgenic mice may provide a good model system to study the factors that direct the pattern of innervation of these tissues and the consequence of hyperinnervation on immune function. {Supported by MH 48644 to S.L.C. and NS 31826 to B.M.D.}

388.5

CUTTING THE SPLENIC NERVE DIFFERENTIALLY AFFECTS CATECHOLAMINE AND NEUROPEPTIDE LEVELS IN THE SPLEEN. C.Y. Vriend*, W. Wan, A.H. Greenberg and D.M. Nance. Depts. of Psych. and Path. and Inst. of Cell. Biol., Univ. of Manitoba, Winnipeg, MB, R3E 0W9

Sympathetic innervation of the spleen has been demonstrated, and norepinephrine (NE) and the neuropeptide Y (NPY) have been shown to be colocalized in splenic nerve fibers. In addition, vasoactive intestinal peptide (VIP) as well as substance P (SP) fibers have been identified in the spleen. Further, splenic lymphocytes have been shown to bear receptors for these neurotransmitters. We have previously shown that cutting the splenic nerve abrogates the immunosuppressive effects of footshock, and central injection of IL-1β. In addition, splenic nerve cuts attenuate the immunoenhancement resulting from the peripheral injection of IL-2. To determine the effects of surgical denervation on splenic neurotransmitters, splenic nerve cuts or sham operations were performed on male S/D rats. One week later, spleens were removed and NE was assayed by HPLC. NYP and VIP were assayed by RIA. Previous attempts to assay SP by RIA failed to detect measurable quantities of this peptide so SP was not assayed in nerve cut rats. As expected, the NE content in spleens of nerve cut animals was reduced 90-100%. However, there was no change in NPY or VIP content. Additional nerve cut and sham operated animals were perfused and spleens were processed for immunocyto-chemistry of dopamine-beta-hydroxylase (DBH) and NPY in order to localize NE/NPY fibers. Staining for DBH/NPY fibers was eliminated in nerve cut animals. The discrepancy between the RIA data and the immunocytochemical data for NPY could be the result of endogenous production or increased uptake from the circulation in response to deinnervation. It could also be a result of differences between the antibodies used in the two methods. The possibility of an alternate source of splenic NPY should be further examined as well as the disposition of VIP and SP before and after nerve cut. Supported by MRC and NIMH.

388.6

NORADRENERGIC INNERVATION OF MURINE SPLEEN: NE CONTENT AND CONCENTRATION SHOW MINOR STRAIN DIFFERENCES AND MINIMAL OR NO DIURNAL VARIATION. S.P. Kelley, L.J. Grota , S.Y. Felten, K.S. Madden,* D.L. Felten. Dept. Neurobiol. & Anat. Univ. Rochester Sch. Med., Rochester, NY 14642.

Strain differences have been invoked to explain differing results when studying neural-immune interactions in laboratory animals. Recently, Lyte et al. (J. Neuroimmunol. 1991. 31:1-8) reported 6-fold strain differences in norepinephrine (NE) concentration (conc.) in C57BL/6 compared with DBA/2 mice. We investigated the splenic NE content and conc. in 3 strains of male mice (BALB/C, C57BL/6, & DBA/2), as well as possible diurnal variability in this innervation. Mice were housed on a 12 hr on/12 hr off light/dark cycle for 3 weeks, then sacrificed at one of 6 times during the 24 hr. cycle, and spleen NE total content and concentrations were determined using high performance liquid chromatography with electrochemical detection. We found a small but significant difference between strains in total resting spleen NE content (BALB/C > C57BL/6 > DBA/2) and in resting NE conc. (C57BL/6 > BALB/C > DBA/2). This may reflect differences in spleen weight (BALB/C > DBA/2 > C57BL/6). We were unable to find more than subtle differences in NE content or concentration based on the time of day sampled; it is unlikely that NE innervation of spleen shows conspicuous diurnal variation for these parameters.

Strain	Total NE (pMoles)	NE (pMoles/mg)	Spleen Wt. (mg)
BALB/c	340.7 ± 58.4	3.26 ± 0.73	108 ± 27
C57Bl/6	216.4 ± 43.8	4.49 ± 1.99	52 ± 12
DBA/2	174.9 ± 59.8	2.37 ± 0.76	73 ± 11

n=48

Supported by R37 MH42076 and a Lucille P. Markey Charitable Trust Award.

388.7

CHEMICAL SYMPATHECTOMY AND THYMECTOMY EFFECTS ON SPLENIC ANATOMY IN THE FROG *XENOPUS LAEVIS*. K.S. Kinney*, S.Y. Felten, J.D. Horton, and N. Cohen. Univ. of Rochester Sch. of Med. and Dent., Rochester, NY 14642 and Univ. of Durham, Durham DH1 3L3, U.K.

The spleen of the adult frog *Xenopus laevis* is separated into clearly defined compartments of red pulp and lymphopoietic white pulp, much as is seen in mammals. Also similar to mammals, sympathetic innervation of the spleen is noradrenergic (NA) and largely confined to the white pulp. To further characterize the resemblances to and differences from the mammalian system, selective depletion of portions of the nervous or lymphoid components of the spleen were performed.

6-Hydroxydopamine (6-OHDA) selectively destroys noradrenergic nerve fibers. Treatment of adult frogs with 6-OHDA results in a striking loss of NA fibers in the spleen, as assessed by SPG histofluorescence. This observation has been confirmed using immunocytochemistry directed against tyrosine hydroxylase and PGP 9.5, a pan-neuronal marker. Regeneration of the sympathetic nerve fibers occurs during the next two months.

To examine the possible influences of T-cells on splenic innervation, *Xenopus* larvae were thymectomized at 7 days post-fertilization. This results in a loss of T-lymphocytes and T-cell dependent immunity which persists through metamorphosis into adult life. In such frogs overt sympathetic innervation develops as in intact controls (i.e., at the end of metamorphosis) despite the lack of T-cell compartments.

388.8

SPLENIC REDUCTION IN NOREPINEPHRINE IN MRL-*LPR/LPR* MICE IS PREVENTED WITH CYCLOPHOSPHAMIDE. S.M. Breneman*, J.A. Moynihan, L.J. Grota, S.Y. Felten. Depts Neurobiology & Anatomy, Psychiatry and Immunology, Univ. Rochester Sch. Med., Rochester, NY 14642.

The MRL-*lpr/lpr* mouse has been used extensively as a murine model of systemic lupus erythematosis. These mice develop an autoimmune disease characterized by increased anti-DNA titers, hypergammaglobulinemia, glomerulonephritis, marked lymphadenopathy and splenomegaly. The increased size in the lymphoid organs is due to a massive expansion of a subset of T-cells which are CD3+, CD4-, and CD8- (double negative, DN) which has been shown previously to be reduced with treatment by the DNA alkylating agent, cyclophosphamide (Cy).

Sympathetic involvement in neural communication with the immune system has been examined by our lab in this autoimmune model as well as others (arthritis). We have shown previously that the NE sympathetic innervation of the MRL-lpr/lpr spleen was reduced as the disease developed. Eight week old female MRL-lpr/lpr and their congenic control strain, MRL-+/+ were treated weekly with 100mg/kg i.p. of Cy . Treament continued through 17 wks and the animals were sacrificed at 18 wks. Cy treated lpr/lpr animals had disease measures equal to untreated +/+ and total splenic NE (as measured by HPLC) equal to untreated +/+ indicating that the innervation reduction is not a direct genetic effect or an epiphenomenon but rather a direct result of disease state. This work supported by grants from the Whitehall Foundation and the NIMH, MH10347.

388.9

BETA-ADRENERGIC RECEPTOR REGULATION OF MACROPHAGE (MO)-DERIVED TUMOR NECROSIS FACTOR (TNF) PRODUCTION IN EXPERIMENTAL ARTHRITIS. R.C.Chou, M.W.Stinson, C.M.Smith* and R.N.Spengler. Depts. of Pathology, Microbiology, and Pharmacology and Therapeutics, SUNY-Buffalo, Buffalo, NY 14214.

The nervous system may play a role in the pathogenesis of rheumatoid arthritis (RA). It is also known that TNF is found in the synovial fluid of RA patients. In the present study, we have determined that β-adrenergic agonists inhibit lipopolysaccharide (LPS)-stimulated TNF production and gene expression in complete Freund's adjuvant (CFA)-elicited female Lewis rat peritoneal MOs. Therefore, we examined β-adrenergic regulation of LPS-induced TNF production in female Lewis rats with streptococcal cell wall (SCW)-induced arthritis. Peritoneal MOs were harvested from rats with chronic arthritis and from non-arthritic CFA rats. Elicited MOs were stimulated with LPS (100ng/ml) and the β-adrenergic agonist regulation of TNF production was assessed. MOs from both groups showed similar TNF production after LPS stimulation alone. Upon addition of isoproterenol (ISO), a β-adrenergic agonist, MOs from the CFA group showed a concentration-dependent inhibition by ISO of LPS-stimulated TNF production. A change in β-adrenergic sensitivity was produced after pretreatment of the CFA group MOs with appropriate agonists/antagonists for one hour before LPS challenge. However, MOs from the SCW group did not show similiar inhibition of TNF production upon addition of ISO. Furthermore, changes in sensitivity could not be induced in MOs from SCW group as compared to CFA group. Results of our studies show that MOs from the SCW group are unable to transduce the β-adrenergic signals into effector function, which may help explain the elevated TNF levels in rheumatoid synovium. (Supported by Arth Fdn and in part by AHA)

388.10

TRYPSIN ENHANCES 6-HYDROXYDOPAMINE–INDUCED NEUROGENIC PLASMA EXTRAVASATION IN THE RAT KNEE JOINT: EVIDENCE FOR A PEPTIDE INHIBITOR OF EXTRAVASATION FROM THE SYMPATHETIC NEURON. Paul G. Green*, Jian Luo, Elena R. Hammond and Jon D. Levine
Depts. Oral and Maxillofacial Surgery, Anatomy and Medicine, UCSF, San Francisco, CA 94143

Perfusion of 6-hydroxydopamine through the rat knee joint causes an increase in plasma extravasation by activation of sympathetic postganglionic neuron (SPGN) terminals. Similarly, the increase in plasma extravasation in the rat knee joint produced by the inflammatory mediator bradykinin is dependent on the SPGN. There is evidence that both 6-hydroxydopamine and bradykinin release a number of mediators, some of which appear to enhance plasma extravasation and some which inhibit it. We attempted to determine the nature of inhibitory factor(s) by co-infusing trypsin (which rapidly cleaves peptides) with 6-hydroxydopamine. We observed a marked enhancement of 6-hydroxydopamine–induced plasma extravasation by trypsin. This effect appeared to be specific to neurogenic plasma extravasation since trypsin alone had little effect on plasma extravasation and trypsin did not affect non-neurogenic plasma extravasation (that produced by platelet activating factor). Taken together, the data suggests that 6-hydroxydopamine not only releases mediators from the SPGN that enhance plasma extravasation, but also an inhibitor(s) of plasma extravasation that is peptide in nature.

388.11

CATECHOLAMINES INHIBIT LYMPHOCYTE BINDING TO ENDOTHELIAL CELLS. C. Kioni, K. Abell, J.P. McGillis†* and S.L. Carlson. Dept. of Anatomy & Neurobiology and †Microbiology and Immunology, University of Kentucky Medical Center, Lexington, KY 40536-0084

The autonomic nervous system, through the catecholamines norepinephrine and epinephrine, can modulate a variety of immune responses. Little is known, however, of the exact mechanisms by which this occurs. One hypothesis is that catecholamines may alter the patterns of lymphocyte migration or homing to lymphoid tissues or inflammatory sites. Lymphocyte homing is accomplished through the interaction of adhesion molecules expressed by lymphocytes and specialized endothelial cells (high endothelial cells, HEC) lining postcapillary venules in lymphoid tissues or at inflammatory sites. To examine the possibility that catecholamines can alter these interactions, we have studied lymphocyte binding to ECs in vitro. ECs were isolated from human umbilical vein (HUVEC), and cultured in 24 well plates until confluent. On the day of the experiment, half of the HUVECs were preincubated with 1U/ml interleukin-1ß (IL-1ß) for 4 hours to enhance their expression of adhesion molecules. Human peripheral blood T-cells were isolated, loaded with a fluorescent dye, then added to the HUVEC cultures, with or without the addition of various concentrations of epinephrine (Epi) or the ß-adrenergic agonist isoproterenol (Iso). After 30 or 60 minutes incubation, the non-adherent lymphocytes were washed away, and the adherent cells counted using a fluorescence microscope. IL-1 increased the number of T-cells bound by two fold compared to adhesion to unstimulated HUVECs. The presence of Epi or Iso inhibited lymphocyte binding to the IL-1 stimulated HUVECs by up to 50%, with a dose response over the range of 1-100nM. Preincubation of only the lymphocytes or HUVECs with Epi or Iso prior to the adhesion assay did not change the number of lymphocytes bound. It is known that some of the adhesion molecules change to a high affinity state during the process of lymphocyte-EC binding. Thus catecholamines may modulate the dynamics of the interactions between adhesion molecules expressed on the lymphocytes and ECs. (Supported by R29 MH48644)

NEURAL-IMMUNE INTERACTIONS: OTHER NEUROTRANSMITTERS IN IMMUNE TISSUES

389.1

COMPARISON OF CHOLINE-O-ACETYL TRANSFERASE (ChAT) IN ADULT MOUSE BRAIN, THYMUS, AND SPLEEN. M. Badamchian*, T. Damavandy, and K. Bulloch. Department of Biochemistry and Molecular Biology, The George Washington University School of Medicine and Health Sciences, Washington, DC 20037. Department of Psychiatry School of Medicine, University of California San Diego, La Jolla, CA 92161.

Recently, we characterized the ChAT in BALB/C mouse thymus anatomically and biochemically (Badamchian et al., Progress in NeuroEndocrinology, 5(4), 1992). In this present study: 1) we continued to analyze the possibility of cholinergic immunomodulation of immune tissues by determining if ChAT is also present in the BALB/C mouse spleen, and 2) we compared the biochemically characterized ChAT in the adult BALB/C mouse brain, thymus and spleen. ChAT activity in the spleen extract was found to be 0.05 nmoles/min/mg protein compared to the 0.035 and 0.1 nmoles/min/mg protein found in the thymus and whole brain extract controls, respectively. Western Blotting and immunochemical visualization of ChAT using the anti ChAT monoclonal antibody, MB16, demonstrated two bands with molecular weights of 28 and 59 kDa in the brain membrane-bound ChAT. In contrast, the spleen and thymus demonstrated only one identical band with a molecular weight of 28 kDa. Immunoprecipitation of the enzyme from the brain, thymus and spleen resulted in a recovery of 59%, 60% and 60% of the activity, respectively.

389.2

CHOLINERGIC- AND ß-ADRENERGIC RECEPTOR INVOLVEMENT IN NICOTINE'S IMMUNO-SUPPRESSIVE EFFECTS C.G. McAllister, A.R. Caggiula, S. Knopf, L.H. Epstein, A.L. Miller, S.M. Antelman, K.A. Perkins, and D.J. Edwards*. *Univ. of Pittsburgh and Pittsburgh Cancer Institute, Pittsburgh, PA 15213*

We have reported that acute nicotine (NIC) produces a dose-dependent decrease in the response of rat peripheral blood leukocytes (PBL) to mitogens (Caggiula et al, *Drug Dev Res* 26 1992), and a similar, but less consistent effect on splenic leukocytes (SL; Knopf et al, *Soc Neurosci* Abst. #288.8, 1992). In the present work, male rats were given 1 mg/kg of mecamylamine (MEC), an antagonist of central and peripheral nicotinic cholinergic receptors 10 min before 1.32 mg/kg (free base) of NIC. MEC antagonized NIC's effects on PBL responses to both concanavalin A and phytohemagglutinin (PHA), and SL responses to PHA. Currently we are using MEC and chlorisondamine, a peripheral blocker, to determine the involvement of central vs peripheral cholinergic receptors. In a second study, the ß-adrenergic antagonist propranolol (2 mg/kg, 30 min before NIC) attenuated the suppressive effects of NIC on the SL response to PHA but did not antagonize NIC's effects on the PBL mitogenic response. These initial results suggest that while both SL and PBL effects of NIC are mediated by cholinergic receptors, activation of ß-adrenergic mechanisms is more important for the SL than for the PBL action. Supported by DA07546.

389.3

CELLULAR LOCALIZATION OF QUINOLINIC ACID IN THE RAT
MG Espey, JR Moffett, SJ Gaudet*, and MAA Namboodiri. Dept. of Biology, Georgetown University, Washington, DC 20057.

Quinolinic Acid (QUIN) is an endogenous neurotoxin of unknown origin and is thought to be involved in a variety of neuropathological conditions. QUIN is a metabolite in the kynurenine pathway of tryptophan degradation and is produced in large amounts in response to immune stimulation via an interferon gamma mechanism, resulting in increased levels in the brain and peripheral tissues. In the present study, we have determined the cellular localization of QUIN using a highly specific polyclonal antibody preparation recently produced in our laboratory.

Polyclonal antibodies against QUIN were made in a New Zealand white rabbit using protein coupled and gold absorbed QUIN. The antiserum was purified by preabsorbtion with carbodiimide (CDI) coupled protein conjugates of glutamic, nicotinic, and picolinic acids and with CDI treated brain protein homogenate. Anesthetized Sprague-Dawley rats were perfused transcardially with an aqueous solution of CDI (6%), DMSO (5%), and N-hydroxysucciniimide (1mM) to covalently couple QUIN to tissue proteins. Immunohistochemistry was performed on frozen cryostat tissue sections using the ABC-peroxidase method. No significant QUIN immunoreactivity was detected in the brain. In contrast, strong QUIN immunoreactivity was detected in the spleen, thymus, lymph node, bronchial and gut associated lymphoid tissue, skin, and intestinal villi. QUIN immunoreactive cells have the morphology of macrophage and dendritic cells. These findings indicate a specific involvement of QUIN in the immune system and support a role for this NMDA receptor agonist as a unique cytokine involved in antigen processing.

389.4

SPONTANEOUS LOSS OF T CELL SELF TOLERANCE TO GLUTAMATE DECARBOXYLASE IS A KEY EVENT IN THE PATHOGENESIS OF MURINE INSULIN-DEPENDENT DIABETES.
J. Tian, P.V. Lehmann, T. Forsthuber, G. S.P. Ting, D. Newman, M.A. Atkinson, E.E. Sercarz, A.J. Tobin, M. Clare-Salzler and D.L. Kaufman* University of California, Los Angeles, CA.90024

Insulin-dependent diabetes mellitus (IDDM) in the nonobese diabetic (NOD) mouse results from a T lymphocyte mediated destruction of the insulin producing β cells of the pancreas and serves as a model for human type 1 diabetes. While a number of autoantibodies have been associated with IDDM, it is unclear whether, when and to what β cell antigens (βCAs) pathogenic T cells actually become activated *in vivo* during the spontaneous disease process. We report that a T helper 1 (Th1) response to glutamate decarboxylase (GAD) develops in NOD mice concurrent with the onset of lymphocytic infiltration into the islets (insulitis). This T cell response is initially confined to the carboxy-terminal region of GAD, but later spreads intramolecularly to additional GAD determinants. Subsequently, T cell reactivity arises to other βCAs, consistent with the intermolecular diversification of the autoimmune response. Experimentally induced tolerance to GAD blocked the development of T cell responses to GAD, the spread of autoimmunity to secondary antigens, insulitis and IDDM. Thus, tolerization to key early targets of autoimmunity can prevent expansion of the autoimmune repertoire and the ensuing β cell destruction. As a similar autoimmune progression is also likely to occur during the development of human IDDM, these findings will be useful for the design of immunotherapies.

389.5

DECREASES IN PERIPHERAL-TYPE BENZODIAZEPINE BINDING SITES IN SPLEEN OF BULBECTOMIZED RATS.
T. Dennis*, V. Beauchemin and N. Lavoie. Department of Psychiatry, McGill University, Montréal, Canada.

Numerous parallels can be drawn between the behavioral and biochemical changes observed in rodents following bilateral olfactory bulbectomy (OBX), and in patients presenting with major depression. Several studies have shown that these patients present an immunitary dysfunction. The *in vitro* and *in vivo* immunomodulatory effects of peripheral-type benzodiazepine binding site (PBBS) ligands are consistent with the high densities of these sites on cells of the T- and monocytic lineages in the rat immune system organs (Benavides *et al. J. Pharmacol. Exp. Ther. 249: 333, 1989*). In the present study, we investigated the effects of OBX on PBBS densities and autoradiographic distribution in rat thymus and spleen.

Four weeks after OBX or sham surgery, male Sprague-Dawley rats were sacrificed by decapitation, organs were quickly removed, weighed and frozen on dry ice. Tissue sections were processed for autoradiography, PBBS were labelled with 1 nM [^{3}H]PK-11195. Thymus glands from OBX rats showed a significant involution. PBBS were heterogeneously distributed throughout the thymus tissue with higher densities of [^{3}H]PK-11195 binding sites in medulla than in cortex. There was no alteration in PBBS binding densities in the thymus following OBX. Similar to thymus, spleen weights from OBX rats were significantly decreased. In control animals, higher densities of [^{3}H]PK-11195-labelled PBBS were found in the white pulp compared to the red pulp. Following OBX, PBBS densities were decreased in both regions of the spleen.

The present results confirm that cell lines of the rat immune system possess high densities of PBBS and are consistent with the notion that OBX in rats may induce an immunitary dysfunction similar to that found in major depression in humans.

389.6

MODULATION OF THE IN VIVO ANTIBODY RESPONSE BY THE BENZODIAZIPINE INVERSE AGONIST (DMCM) ADMINISTERED CENTRALLY OR PERIPHERALLY.
M. Fleshner*, M. M. Brohm, M. L. Laudenslager[1], L. R. Watkins, S. F. Maier. Dept. of Psychology, Univ.of Colorado, Boulder, CO 80309, Univ. Colorado Health Sci. Cntr., Denver, CO[1].

Stressors can result in changes in immune function. Although there is increasing information concerning the peripheral hormonal and neural mediators of stress-induced changes in immune function, there is little information concerning the central nervous system mechanisms which lead to the peripheral changes. The following experiments examined the possible involvement of the benzodiazipine-$GABA_A$-chloride complex in modulation of the *in vivo* antibody response. Rats were given either peripheral or intracerebral ventricular injections of methyl-6,7-dimethoxy-4-ethyl-beta-carboline-3-carboxylate (DMCM), a drug which has been shown to act at the benzodiazipine-$GABA_A$ complex and produces a behavioral state similar to "anxiety". Rats were then immunized with keyhole limpet hemocyanin (KLH) and serum levels of KLH-specific antibody were measured for two weeks after immunization. Both peripheral and central administration of DMCM modulated the *in vivo* antibody response. The dose-response relationship of DMCM and changes in antibody levels was non-monotonic, with high doses resulting in an increase in serum antibody levels and moderate doses resulting in a decease in serum antibody levels. A possible role of the benzodiazipine-$GABA_A$ system in stress-induced immunomodulation is currently being investigated. NIH-MH45045

389.7

SUBSTANCE P (SP)-POSITIVE NERVE FIBERS IN INTERSTITIAL CYSTITIS (IC) -
J. Marchand[2], X. Pang[1], G.R. Sant[3], R.M. Kream[2] and T.C. Theoharides[1]*. Departments of Pharmacology and Experimental Therapeutics[1], Anesthesiology[2] and Urology[3] Tufts University School of Medicine and New England Medical Center, Boston, MA 02111, USA

IC is a painful bladder disorder occurring mostly in women and is characterized by frequency, nocturia, suprapubic pain and sterile inflammation. Most prevalent pathologic findings are those of mucosal tears, and increased numbers, as well as activation of bladder mast cells over controls. Experimental studies using perfusion of human biopsies showed bladder mast cell activation with SP.

Immunohistochemistry was performed blindly on 10 biopsies which were immersed immediately in 4% paraformaldehyde/0.1 M phosphate buffer for 24 hr at 4°C, and transferred to 20% sucrose/0.1 M phosphate buffer for 24 hr at 4°C. Cryostat sections were cut at 7 μm, thaw-mounted, and incubated for 48 hours with rabbit anti-SP primary antibody and visualized using the ABC technique (Vector). A dense plexus of SP-positive fibers was evident in the submucosa, immediately deep to the epithelia and often perivascularly. Numerous SP-positive fibers were also found surrounding bundles of smooth muscle in the muscularis lamina. SP may, therefore, be involved in mast cell activation, as well as in the painful and inflammatory aspects of IC.

389.8

FLOW CYTOMETRIC FLUORIMETRY REVEALS THAT SUBSTANCE P CAUSES CHANGES IN MEMBRANE POTENTIAL AND CYTOPLASMIC CALCIUM CONCENTRATION IN THYMIC LYMPHOCYTES.
C. B. Lacey*[1], D. Maric[2], I. Maric[2], G. D. Lange[3], R. Elde[1] and J. L. Barker[2]. [1]Dept. of Cell Biol. and Neuroanat., Univ. of Minn., Mpls, MN 55455 and [2]Lab. of Neurophys. and [3]Instrumentation and Computer Sect., NINDS, NIH, Bethesda, MD 20892.

Substance P has been implicated as a neuromodulator in the communication between the nervous and immune systems. In the present study we have begun to examine possible mechanisms by which SP may function in cellular signal transduction in thymocytes. To study the effects of this neuropeptide on membrane potential and Ca^{2+} regulation, we utilized the voltage-sensitive oxonol dye, DiBAC4(3) and the Ca^{2+} indicator dye, fluo-3. Single cell suspensions were prepared from embryonic (E20-22) and maternal adult rat thymus by mechanical dissociation and separated according to their specific buoyant densities by selective centrifugation on a four-step discontinuous Percoll gradient. The fluorescence (FL) distributions of the indicator dyes were monitored by flow cytometric analysis. The resting membrane potential (RMP) was estimated by adding 1μM gramicidin to oxonol-stained cells while varying the $[Na^+]_o$ and correlating to the Nernst equation. The RMP's were -82 ± 3 mV and -70 ± 7 mV (mean ± S.E.M.) for embryonic and adult thymocytes, respectively, and were at least partially dependent on $[K^+]_o$. SP produced a hyperpolarization of the RMP in both cell populations in a dose-dependent relationship. This effect was blocked by the SP-antagonist [D-Arg[1], D-Phe, D-Trp[7,9], Leu[11]]-SP. Doses of SP that hyperpolarized cells also produced an elevation of the resting cytoplasmic levels of Ca^{2+} ($[Ca^{2+}]_c$) in a subpopulation of embryonic thymocytes. This was seen as an increase in the fluo-3 FL signal and converted into quantitative estimates of $[Ca^{2+}]_c$. These results indicate the possibility that SP released from peripheral nerve fibers may interact with receptors on thymocytes to change Ca^{2+} homeostasis and this may be related to changes in membrane potential. Supported by DA06209.

389.9

IN VITRO AND IN VIVO SUPPRESSION OF CON A INDUCED PROLIFERATION OF CD4 BUT NOT CD8 BY CALCITONIN GENE RELATED PEPTIDE. K. Bulloch,* S. Graeber, A. Diwa and S. Baird . Dept. of Psych. and *Path. UCSD, San Diego, CA 92093.

Calcitonin Gene Related Peptide (CGRP) nerve fibers and their corresponding receptors have been characterized in the mouse thymus. Our in vitro studies show that CGRP significantly suppressed (55%) a Con A induced proliferation of murine thymocytes at physiological doses. Furthermore, CGRP was found to inhibit proliferation of murine lymphoid cells by 30% in a mixed lymphocyte reaction. These doses are within the physiological range of the Kd for the receptor in the thymus. In the present study we have extended these findings by examining the effect of CGRP on subsets of thymocytes. The results of these experiments show CGRP has no effect on unstimulated thymocytes either at 24 hrs or 72 hrs. When thymocytes are stimulated with Con A in the presence of CGRP the CD 4 cell response is inhibited by 50% compared to thymocytes stimulated with Con A alone. There was no detectable change in CD 8 cell proliferation. Injections of 24 ul of 10^{-8}M and 10^{-7}M CGRP directly into the thymus produced similar inhibitory effects when thymocytes harvested from these mice were stimulated with Con A. Since CGRP is reported to inhibit Il 2, TNF, and IFN-γ in murine T cells, it is possible that in the thymus CGRP selects against TH1 cells.

389.10

INVOLVEMENT OF CHOLECYSTOKININ IN THE DEVELOPMENT OF TOLERANCE TO MORPHINE-INDUCED IMMUNOSUPPRESSION. X-Z. Ding[1], D-M. Chuang*[2] and B. M. Bayer[1], [1]Dept. of Pharmacology, Georgetown Univ. Med.Center, Washington DC 20007. [2]Biological Psychology Br., NIMH, MD 20892

The possible role of neuropeptide cholecystokinin (CCK) in the development of tolerance to immunosuppressive effect of morphine was investigated. Tail-flick latency was used as a monitor to examine the development of tolerance to the morphine analgesia. Rats were injected with increasing doses of morphine (10-40mg/kg,s.c.) twice a day for 6 days. The analgesic effect induced by a challenge dose of morphine (6 mg/kg) was significantly decreased from 3 to 6 days after the morphine injection. Two hours following acute exposure to morphine (6-10mg/kg), concanavalin A stimulated whole blood lymphocyte proliferation (WBLP) was inhibited by 80 percent. However, following 4 to 6 days of morphine injections, no suppression in WBLP was apparent. The measurement of CCK biosynthesis showed that levels of both CCK mRNA and peptide were significantly increased by 2-3 fold selectively in the hypothalamus, brain stem and in the spinal cord in morphine tolerant animals. In order to further determine whether the increase of CCK biosynthesis was directly related to the development of tolerance to morphine-induced immunosuppression, the effect of selective antagonists L-365260 (CCK-B) and MK-329 (CCK-A) were examined. Chronic pretreatment of L-365260 (0.5mg/kg,s.c.) 10 min before the daily morphine injection was found to be accompanied by a significantly suppressed WBLP compared to the response of morphine tolerant animals. In contrast to L-365260, chronic pretreatment of MK 329 (1mg/kg) had no significant effect on WBLP in morphine tolerant animals. Neither L-365260 nor MK 329 alone, acutely or following chronic treatment, had a significant effect on WBLP. These results suggest that changes in central CCK biosynthesis may be involved in the development of tolerance to the immunosuppressive effect produced by morphine through the CCK-B receptor.

389.11

THE EFFECT OF EXOGENOUSLY ADMINISTERED METHIONINE ENKEPHALIN ON T CELL DEVELOPMENT IN SPECIFIC STRAINS OF MICE. Ching-Ping Ho, Joseph Acosta, Frank Andriani, Conrad Cean, Leslie David and (Marie Metlay) *. SUNY/College at Old Westbury, Old Westbury, N.Y 11568.

Previously we had shown that the level(s) of endogenous (met)-enkephalin bound to receptors on both developing and mature T lymphocytes depended upon the age, genetic background, and disease state of the animal. We had demonstrated that as mice of the RF/J strain aged and or developed spontaneous thymomas, the level of endogenous (met)-enkephalin bound to receptors on T cells significantly increased. In contrast, mice of Balb/C strain exhibited an undetectable level of (met)-enkephalin binding at any stage of their life. In this study we are reporting on the effect of exogenously administered (met)-enkephalin (via a subcutaneous osmotic pump) on the T lymphocytes in the mice of both the RF/J and Balb/C strains. The drug was administered at physiological levels over a 7 day period, and developing T cells (thymocytes) and T lymphocytes of the peripheral lymph nodes were analyzed by flow cytometry. The results indicated that exogenously administered drug alters the ratio of $CD4^+$ to $CD8^+$ T cells in both an age-dependent and strain-dependent manner. The effect, an increase of $CD4^+$ cell, was observed in aged RF/J mice, but not in age-matched mice of the Balb/C strain. An interesting observation was that an increase in $CD4^+$ cells can be induced in Balb/C mice if the animals are heat stressed. This research was supported by NIMH MARC-17138 and NIGMS-MBRS-08180.

389.12

POTENTIAL ROLE OF THE AUTONOMIC NERVOUS SYSTEM IN THE IMMUNOSUPPRESSIVE EFFECTS OF ACUTE MORPHINE ADMINISTRATION. L.R. Flores, B.B. Wolfe* and B.M. Bayer. Dept. of Pharmacology, Georgetown Univ. Med. School, Washington D.C., 20007.

These studies investigated the mechanism by which acute morphine administration inhibits peripheral blood lymphocyte proliferation in rats. We have previously reported that morphine administration inhibits Concanavalin A stimulated lymphocyte proliferation by a centrally mediated mechanism which appears to be independent of the hypothalamic-pituitary-adrenal axis. In the current studies we investigated the role of the autonomic nervous system (ANS) in mediating the immunosuppressive effect of morphine. To determine the contribution of the ANS, rats were pretreated with the ganglionic blocker chlorisondamine (CHL, 5 mg/kg) prior to morphine (7 mg/kg) administration. Ganglionic blockade with CHL completely antagonized the inhibitory actions of morphine, suggesting that intact ganglionic transmission is required for the inhibition to occur. In an attempt to identify the postganglionic mechanisms involved, a pharmacological approach was utilized. Blockade of postganglionic parasympathetic neurotransmission with atropine methylbromide (1 mg/kg) did not attenuate the suppressive effects of morphine. Similarly, blockade of sympathetic neurotransmission with the α-adrenergic antagonist phentolamine (1 mg/kg) failed to antagonize the suppression of lymphocyte activity observed following morphine treatment. Blockade of ß-adrenergic receptors with propranolol (2.5 mg/kg) resulted in partial antagonism, but this action was not shared by the peripherally acting ß antagonists nadolol (6 mg/kg) or atenolol (15 mg/kg). These results suggest that the inhibitory effect of morphine on blood lymphocyte proliferation may be mediated through activation of the ANS, however, individual blockade of either the parasympathetic or sympathetic division of the ANS was not sufficient to attenuate this immunosuppressive effect.

389.13

DIFFERENTIAL ROLE OF MET-ENKEPHALIN ON ANTIBODY PRODUCTION WHEN B LYMPHOCYTES ARE STIMULATED WITH DIFFERENT MITOGENS. K.P. Das, J.S. Hong* and V.M. Sanders Neuropharmacol. Sect., Lab. Integr. Biol., Natl. Inst. Env. Hlth. Sci., Res. Tri. Pk., NC 27709.

The amount and isotype of antibody secreted by high density mouse splenic B lymphocytes stimulated *in vitro* with lipopolysaccharide 0(LPS) or LPS/dextran sulfate (LPS/DxS) in the presence of met-enkephalin (ME) was studied. These mitogenic stimuli were chosen since LPS and LPS/DxS, respectively, stimulate either one-third or the majority of B cells to differentiate. Increasing concentrations of ME (10^{-16}-10^{-8} M) were added to cultures at 0, 3, 6 and 24 hr after mitogen. At all time points of ME addition, LPS/DxS stimulation shifted the dose response curve for an inhibition of IgM secretion to lower concentrations of ME (10^{-16}-10^{-10} M) as compared to cells stimulated with LPS alone (10^{-12}-10^{-10} M). Both mitogens induced a biphasic inhibition in that IgM production returned to control levels at 10^{-8} M. In LPS/DxS-stimulated cells only, a concomitant biphasic decrease in IgG3 production occurred after all times of addition, while IgG2a production decreased only when ME (10^{-14}-10^{-8} M) was added at 24 hr. No significant changes occurred in either IgG1 production or B cell proliferation under any of the culture conditions. These data show a differential sensitivity of B cells to ME depending on the type of B cell stimulus used. We suggest that LPS/DxS, in contrast to LPS, activates a subpopulation of B cells that is either more responsive to ME receptor stimulation or secretes ME to elevate the *in vitro* exposure concentration of ME above that added exogenously.

389.14

MORPHINE ATTENUATES THE SURGERY-INDUCED INCREASE IN TUMOR CELL RETENTION: EVIDENCE FOR NATURAL KILLER (NK) CELL MEDIATION G.G. Page*, S. Ben-Eliyahu, V. Shabanzadeh, & J.C. Liebeskind UCLA Dept. of Psychology, Los Angeles, CA 90024.

Painful stressors such as surgery have been shown to suppress immune function and to enhance tumor development although the role immunity plays in tumor development remains unclear. Further, the role of pain per se is not known. To explore these issues, we used the MADB106 tumor cell line, syngeneic to the Fischer 344 rat and known to be sensitive to natural killer (NK) cell control. Rats were either subjected to abdominal surgery with anesthesia or anesthesia alone, and were either treated or not with morphine. Morphine was administered pre- and postoperatively in saline and in a slow release suspension, respectively. Normal and LGL/NK-depleted (using the mAb 3.2.3) rats were assigned to the above 4 groups with the rationale that if LGL/NK cells were necessary to mediate an event, then in their absence, that event would not occur. Four h after surgery, all animals were injected i.v. with 10^5 radiolabeled MADB106 tumor cells. Lungs were removed 20 h later and radioactive content assessed in a gamma counter. In normal animals, there was a significant interaction of morphine and surgery such that morphine attenuated the surgery-induced increase in tumor cell retention without affecting tumor cell retention in the anesthesia groups. In the LGL/NK-depleted animals however, although there was a main effect of surgery, morphine had no effect on the surgery-induced increase in tumor cell retention. These results imply that: (a)factors in addition to LGL/NK cells play a role in the surgery-induced increase in tumor cell retention; and (b)LGL/NK cells mediate morphine's attenuating effects on the observed surgery-induced increase in tumor cell retention. Supported by the UCLA Psychoneuroimmunology Program and NIH grant NS 07628.

389.15

EQUIANALGESIC DOSES OF SYSTEMIC BUT NOT INTRATHECAL MORPHINE ALTER RAT LYMPHOCYTE PROLIFERATION. I.G. Hamra*, and T.L. Yaksh. Dept. of Anesthesiology, UCSD, LaJolla, CA 92093.

Surgical trauma and opiates are linked with suppression of immune function. Evidence suggests a probable supraspinal action of opiates which mediates effects upon the immune system. However, the role of spinal systems in morphine-induced immunosuppression have not been evaluated. In this study, we compared the effects of equianalgesic doses of systemic and intrathecal morphine on lymphocyte proliferative responses and phenotypic expression of lymphocyte cell surface markers in rats. Equianalgesic doses of subcutaneous (10 mg/kg) or intrathecal (30 µg, by a chronic intrathecal catheter) morphine were given twice (at time 0 and 2.5 hours) to give a 5 hour period of analgesia. Immediately following the five hour period, spleens were harvested and lymphocytes isolated by density gradient centrifugation. The lymphocytes were then incubated with the mitogens Concanavalin A (ConA), phytohemagglutinin (PHA), pokeweed mitogen (PWM) and lipopolysaccharide (E. Coli, LPS) or labeled with monoclonal antibodies directed at cell surface markers (T cell, B cell, CD4+, CD8+, CD25+). Systemic morphine dramatically suppressed lymphocyte proliferation to the mitogens PHA, PWM, and ConA, but not LPS., The number of lymphocytes expressing the CD4+ receptor were modestly increased. Morphine did not alter the ability of the lymphocytes to express the IL-2 receptor (CD25+ cells) following stimulation with either PHA or ConA, suggesting that morphine inhibition of proliferation does not occur through decreased IL-2 receptor expression. Intrathecal morphine did not alter lymphocyte proliferative responses to any of the mitogens, nor did it change the expression of cell surface markers. These results suggest that spinal opiates may have theoretical benefits for the analgesic management of both normal and immunocompromised patients.

389.16

AGE- AND SEX-DEPENDENT EFFECTS OF (+)FENFLURAMINE (d-FEN) ON SPLENIC MONOAMINE METABOLISM, RECEPTORS AND IMMUNE FUNCTION. **S.A. Lorens,[1]* M. George,[1] C. Dersch[3], J. Clancy[2] and R. Zaczek.[3] Depts. Pharmacology[1] and Anatomy,[2] Loyola University Chicago Medical Center, Maywood IL 60153, and NIDA-ARC,[3] P.O. Box 5180, Baltimore MD 21224.**

Subchronic administration of the serotonin (5-HT) releaser and reuptake inhibitor, d-fenfluramine (d-FEN), enhances some splenic immune functions, especially natural killer cell (NK) activity, only in young males and old females. The objective of the present study was to determine whether these effects of d-FEN were associated with alterations in splenic monoamine turnover and receptor binding. F344 rats (5 and 21 mo old at sacrifice) received d-FEN (old rats, 0.6 mg/kg/day, p.o.; young rats, 1.2 mg/kg/day, p.o.) for 30-38 days. d-FEN treatment did not affect body weight or water intake. Although the concentration of 5-HT in the spleen was 3.5 fold greater than the level of NE, splenic NE turnover (MHPG/NE ratio = 0.88) was higher than 5-HT turnover (5 HIAA/5-HT ratio = 0.25). d-FEN treatment increased splenic NE turnover (41-61%) and decreased 5-HT synthesis in both young and old rats. In contrast, subchronic d-FEN affected splenic [^{3}H]5-HT, [^{3}H]paroxetine and [^{3}H]dihydroalprenolol binding in an age and sex dependent manner: increasing (14-40%) binding in the young male rats, and reducing (24-46%) binding in the old female rats. These effects were not due to differences in splenic drug and metabolite concentrations. 5HT immunoreactive elements are densely distributed throughout the splenic red pulp (RP). This is in marked contrast to the reported localization of NE fibers predominantly in the white pulp (WP). d-FEN thus may augment splenic NK activity by modifying splenic 5-HT availability and/or receptor density in the RP, and may enhance splenic T and B cell function by altering NE metabolism and/or β-adrenergic receptor number in the WP. The age and sex related nature of these effects suggest, moreover, that d-FEN will enhance splenic immune functions only if plasma testosterone levels are high (young males) or estrogen levels are low (old females).

389.17

Development of Cellular Immunity Following Fetal Alcohol Exposure A.N. Taylor*, M. Pilati, R. Yirmiya, D. Tio & F Chiappelli. Dept. Anatomy & Cell Biology, UCLA-1763, & West Los Angeles VAMC, Brentwood Research, Los Angeles, CA 90024.

Age-associated differences exist in proliferative responses to concanavalin A (ConA) of thymocytes (Wong et al, 1990; Chiappelli et al, 1992) and splenocytes obtained from fetal alcohol exposed (FAE) male Sprague Dawley rats compared to control animals (Norman et al, 1989,1991). The proliferation of thymocytes from prepubertal (day 44) FAE rats is significantly greater than control, but this difference disappears by day 72. ConA responses of FAE splenocytes are significantly suppressed compared to control in prebubertal and young adult rats. We have tentatively attributed these differences to the maturation stage of thymocytes and splenocytes; but we find no age-associated outcomes of FAE on ConA proliferation by blood mononuclear cells. These important differences in T lymphocyte populations among immune compartments are now being characterized functionally and phenotypically. (VA Medical Research Service; UCLA Psychoneuroimmunology).

389.18

CHANGES IN BRAIN MAST CELLS DURING DEVELOPMENT IN DOVES **X. Zhuang[1]*, H. Machuca[2], and R. Silver[1,2], [1]Columbia University and [2]Barnard College, New York, N.Y. 10027.**

Mast cells originate in the bone marrow and migrate to tissues where they differentiate. We previously reported that few mast cells expressing GnRH-like ir are present in the medial habenula (MH) of isolated ring doves, but they are detected after courtship. To understand their origin, we studied mast cells in doves aged 1 day to 10 mo. Alternate brain sections were stained with toluidine blue (a classical mast cell marker), antiserum to GnRH (LR-1), alcian blue (specific for low sulfated glycosaminoglycans characteristic of mucosal and immature mast cells) and safranin (specific for highly sulfated heparin characteristic of serosal mast cells). In 2-3 mo. birds Indian ink/gelatin infusions were made to demonstrate blood vessels. Mast cells stained by alcian blue and toluidine blue are seen in the pia and choroid plexus at 2 days, rapidly increase in number within the first week, and decrease thereafter. A few mast cells expressing GnRH-ir can be seen in the pia mater after 6 days. Mast cells expressing GnRH-ir are detectable in the dorsal most aspect of MH at 21 days and gradually increase in number. They occur outside Indian ink delineated blood vessels in MH. By 4 months, mast cells (visualized with antiserum to GnRH-ir, alcian blue or toluidine blue) are distributed throughout MH, and a few remain in the surrounding pia. Safranin positive mast cells are only seen in MH, and only in adults (10 mo). The sequential appearance of mast cells first in the pia and the choroid plexus, then at the tip of MH, and ultimately throughout MH, suggest that they migrate into MH. Granule contents show a biochemical alteration during ontogeny. The physiological function of parenchymal mast cells remains to be explored.
Supported by NIMH grant 29380 (to RS).

389.19

FLOWCYTOMETRIC CROSSMATCH TEST AS A METHOD FOR COMPARISON OF PERIPHERAL ANTIBODY PRODUCTION TO MULTIPLE INJECTION SITES IN GUINEA PIGS. P.M. KRUEGER*, F.A. KAPLAN, D.F. EMERICH. CytoTherapeutics, Inc., Providence, RI 02906.

The purpose of this study is to compare peripheral guinea pig anti-rat IgG and IgM antibody production in guinea pigs using multiple injection sites. Previous studies in our lab using immunocytochemical evaluation have demonstrated that rat adrenal pheochromocytoma (PC12) cells injected into the brains of guinea pigs are rejected within 1 to 3 weeks. PC12 cells at a concentration of 10^6/4ul resuspended in phosphate buffered saline (PBS) were injected into either the brain (striatum), intraperitoneally, or subcutaneously (nape of the neck). Serum samples were collected from all animals prior to, and at 1, 7, 23 and 51 days following implantation and then frozen at -70^{o}C until testing. Flowcytometric crossmatches (FCXM) were done using target Lewis rat spleen cells at a concentration of $3x10^5$/100ul which were incubated with 10ul of sequential guinea pig sera in dilutions from neat to 1:32 for 1 hour at room temperature. Cells were washed twice with PBS + 0.25% bovine serum albumin (BSA). Cells were then incubated with 100ul of FITC-labeled goat anti-rat IgG (Fab')2 and anti-rat IgM (mu chain) for 1 hour in the dark at room temperature. After incubation, cells were washed twice with PBS + 0.25% BSA, resuspended in 500ul of wash solution and analyzed by fluorescence intensity over 256 channels with LYSIS I software. Comparing the shift in the mean channel fluorescence of tested samples over the negative control was used for data analysis. Anti-rat IgG antibody production was demonstrated to occur in animals who received implants either in the striatum or intraperitoneally. No significant shift was observed in the subcutaneous group. The FCXM has proven to be both a very sensitive and reproducible method for demonstration of antibody production.

390.1

ELECTRICAL ACTIVITY AND SYNAPTIC RESPONSES OF RAT ROSTRAL VENTROLATERAL MEDULLA NEURONS IN VITRO. S. Y. Wu* and N. J. Dun. Dept. Anatomy, Medical College of Ohio, Toledo, OH 43614

Whole-cell patch recordings were obtained from rat rostral ventrolateral medulla (RVLM) neurons of 500 μm coronal slices. RVLM neurons had a resting membrane of -55 to -60 mV and input resistance of 300 to 700 mΩ. Some of the neurons exhibited a time-dependent anomalous rectification that was sensitive to cesium. When labeled intracellularly with lucifer yellow, RVLM neurons appeared oval and had few cell processes. In addition, some of the labeled neurons seemed to be dye-coupled. The majority of RVLM neurons exhibited spontaneous discharges, some of which were blocked by Ca-free solution, indicating that they were synaptic in origin. The discharges recorded in about 30% of RVLM neurons were not blocked by Ca-free solution nor by the excitatory amino acid antagonists CNQX and AP5, indicating that they were intrinsic in origin. Na-free and tetrodotoxin blocked the spontaneous activities. Electrical stimulation of the nucleus of the solitary tract (NTS) elicited, after a synaptic delay of several to over 10 ms in most cases, an excitatory synaptic current (EPSC), an inhibitory synaptic current (IPSC) or a mixed EPSC and IPSC in spontaneously active and quiescent RVLM neurons. EPSCs had a reversal potential close to 0 mV and were largely suppressed by the non-NMDA receptor antagonist CNQX. IPSCs had a reversal potential of -70mV and were blocked by bicuculline in some cells and by strychnine in others. The results indicate that a population of rat RVLM are intrinsically active and may subserve a role of pacemaker neurons. This type of neurons also receives excitatory and inhibitory inputs following stimulation of the NTS. On the basis of synaptic delays, neurons in the NTS appear to contact RVLM neurons via a polysynaptic pathway. Glutamate, GABA and glycine appear to be the excitatory and inhibitory transmitters to the RVLM neurons.

390.2

ROLE OF A5 NORADRENERGIC NEURONS IN CAROTID-SYMPATHETIC CHEMOREFLEX. N. Koshiya*, D. Huangfu and P.G. Guyenet. Department of Pharmacology, University of Virginia, Charlottesville, VA 22908.

Splanchnic sympathetic nerve discharge (SND), phrenic nerve discharge (PND) and the activity of A5 reticulospinal neurons (A5 units) were recorded in urethane-anesthetized vagotomized rats without aortic baroreceptor afferents. Carotid chemoreceptor stimulation (CCS) with 8 - 12 sec N_2 inhalation increased SND by 117±14% (sympathetic chemoreflex, SCR) and PND (amplitude and rate), raised MAP by 27±5 mmHg and increased discharge rate of A5 units from 1.9±0.2 to 6.0±0.7 spikes/sec. During chemoreceptor activation, SND and most A5 units displayed a pronounced post-inspiratory peak with inspiratory depression. Bilateral microinjection of the $GABA_A$ agonist, muscimol (Mus, 175 pmol in 100 nl) into the A5 area attenuated the SCR by 65±4% while reflex activation of PND by CCS was unchanged. SCR was also attenuated by 61±7% in 2 hrs after bilateral microinjection of the noradrenergic neurotoxin 6-hydroxy-dopamine (6-OHDA, 4μg in 200 nl) into upper thoracic cord, while resting SND level was unchanged. Intraspinal 6-OHDA also blocked antidromic activation (ADA) of A5 units from spinal segments caudal to the injection while ADA of sympathetic premotor neurons in the rostral ventrolateral medulla (RVLM) was preserved. Rats chronically treated with intracisternal 6-OHDA (250 μg, 2 wks) had reduced SCR (71±21% increase in SND) which was not altered by acute injection of Mus (175 pmol) into the A5 area.

These results suggest that A5 neurons are activated by the bulbar respiratory center and that their spinal projections contribute to SCR.

390.3

ROLE OF SEROTONIN AND CATECHOLAMINES IN SYMPATHETIC RESPONSES EVOKED BY STIMULATION OF ROSTRAL MEDULLA. D.Huangfu*, T.A.Riley and P.G.Guyenet. Dept. of Pharmacology, Univ. of Virginia, Charlottesville, VA 22908.

In halothane-anesthetized and paralyzed rats, single-pulse stimulation of the rostral end of nuc. raphe pallidus/obscurus (NR) produced an excitatory response in i) the discharge of the lumbar sympathetic nerve (lSND; peak latency: 210±14 ms) and splanchnic nerve (sSND; peak latency: 161±5 ms), and ii) the activity of lumbar sympathetic vasoconstrictor neurons (LSVNs). Stimulation of the rostral ventrolateral medulla (RVL) produced two excitatory responses in lSND, sSND, and LSVNs activity. These consisted of an early peak (RVL peak I; latency: 98±8 ms for lSND and 62±2 ms for sSND) followed by a second smaller one (RVL peak II; latency: 210±15 ms for lSND and 160±6 ms for sSND). NR peak (sSND) was attenuated by i) intracisternal (i.c.) 5,7-dihydroxytryptamine (200 μg, 2 weeks, 76% decrease), or ii) acute intrathecal (i.t.) methiothepine (10 μg, 69% decrease) or methysergide (40 μg, 72% decrease). These treatments had no effect on RVL peak I but modestly attenuated RVL peak II. 6-Hydroxydopamine (200 μg, i.c., 2 weeks) attenuated neither NR peak nor RVL peak II. The α-adrenergic blockers phentolamine (20 μg, i.t.) or prazosin (20 μg, i.t.) massively reduced RVL peak II (95% or 94% respectively) and also attenuated NR peak (67% or 46% respectively). The excitatory amino acid (EAA) receptor antagonist kynurenic acid (i.t.) produced proportionately larger reduction of RVL peak I than raphe peak or RVL peak II. Interpretations: i) LSVNs receive convergent excitatory inputs from NR and RVL; ii) bulbospinal serotonergic neurons mediate most of the sympathoexcitation evoked from NR and a portion of RVL peak II; iii) slow conductive adrenergic cells may mediate most of RVL peak II and a portion of the NR response; iv) the short-latency RVL peak I is predominantly mediated by an EAA.

390.4

EFFECT OF OPIOIDS ON CARDIORESPIRATORY FUNCTION IN THE RAT: AN ELECTROPHYSIOLOGICAL STUDY. S.C. Baraban* and P.G. Guyenet. Dept. of Pharmacology, University of Virginia, Charlottesville, VA 22908

In chronic studies, we measured naloxone-precipitated changes in phrenic nerve discharge (PND), mean arterial pressure (MAP) and splanchnic sympathetic nerve discharge (sSND) in urethan-anesthetized, vagotomized, paralyzed and artificially ventilated rats. Sprague-Dawley rats (310-380g) were implanted with morphine pellets (75 mg; NIDA) or placebo pellets over a 2 day regimen. In morphine-dependent rats (n=6) naloxone (1 mg/kg i.v.): i) raised resting MAP (+13 mmHg), ii) increased resting PND (+301%), iii) increased T_e (+122%), iv) decreased T_i (-51%), and v) increased the respiratory modulation of sSND. A significant naloxone-precipitated increase in resting sSND (+43%) was revealed only when MAP was artificially lowered to the pre-naloxone level. Naloxone exerted no effect in placebo-treated rats (n=5). Clonidine (5 μg/kg i.v.) lowered MAP and sSND in both morphine-dependent and placebo-treated rats. Clonidine pretreatment did not alter the naloxone-precipitated increase in MAP nor the respiratory activation. However, clonidine was effective in reducing the sympathoactivation associated with withdrawal. The naloxone-precipitated rise in MAP could be reversed by subsequent injection of an arginine-vasopressin antagonist, O-Et-AVP (50 μg/kg i.v.). Thus, clonidine pretreatment reduces SND and its activation by withdrawal but appears to exert only minor effects on vasopressin release and respiratory changes.

In acute studies, we examined the effect of morphine on the activity of clonidine-sensitive, sympathetic premotorneurons of the rostral ventrolateral medulla (RVLM) using extracellular recording techniques *in vivo*. These cells had a low discharge rate (2-9 spikes/sec) and a slow conduction velocity (2.9-5.1 m/sec). Morphine (1-7 mg/kg i.v.) dose-dependently inhibited (50-100%) the firing rate of most of these neurons (8/10). Naloxone (1 mg/kg i.v.) reversed this effect. These findings support the hypothesis that the sympathoactivation produced during morphine withdrawal may be mediated, in part, by activation of clonidine-sensitive neurons of the RVLM.

390.5

IRREVERSIBLE HYPOTENSION ASSOCIATED WITH KAINATE LESIONING OF A ROSTRAL VENTROLATERAL MEDULLARY VASOMOTOR SITE IN THE CAT. L.M. Sexcius*, D.G. Bernard, R.M. Douglas, R. Millis, C.O. Trouth. Department of Physiology & Biophysics, Howard University, College of Medicine, Washington, D.C. 20059

Electrical stimulation (40 Hz, 1 msec, 1-5 V) of the brainstem of cats 3 mm below the rostral ventrolateral medullary surface (rVMS) consistently resulted in a marked increase in systemic arterial blood pressure (BP) and heart rate (HR). Respiratory effects were inconsistent and not predictable. Occlusion of either the carotid or vertebral artery resulted in an increase in blood pressure as well as increases in the activity of neurons recorded extracellullarly at this site and from neurons at the caudal respiratory chemosensitive area. Electrolytic (10-20 μA for 10-15 sec) or chemical (2.7 mM kainate) lesioning of this site bilaterally resulted in an irreversible fall in blood pressure despite carotid or vertebral arterial occlusion. This site appears to be located in the nucleus reticularis parvocellularis of the cat.

SUPPORT: NIH-NIGMS GRANT #SO6GMO8016

390.6

RVLM IS HYPERSENSITIVE TO NMDA IN THE SPONTANEOUSLY HYPERTENSIVE RATS. Y. Wang*, J.C.Lin, W.L.Tsao. Department of Pharmacology, National Defense Medical Center, Taiwan.

The purpose of this study is to investigate the cardiovascular effects of NMDA (N-methyl-D-aspartate) in rostral ventral lateral medulla (RVLM) of spontaneously hypertensive (SH) rats. Adult SH rats and their normotensive controls (Wistar Kyoto, WKY) were anesthetized with urethane (1.25g/kg, i.p.), cervical vagotomized and placed on a sterotaxic frame. We found local application of electrical stimuli or NMDA produced hypertension in both animals. SH rats had a greater pressor response to NMDA and electrical stimuli than their normotensive controls. Local injection (4 nmole) of AP5 (2-amino-5- phosphono-valerate), a specific NMDA antagonist, to the RVLM, did not affect resting blood pressure, however, did antagonize the electrical (5-20 Hz) evoked hypertension in WKY and SH rats. The high blood pressure evoked by the high frequency stimulation (60 Hz), on the other hand, were not affected by AP5. Interestingly, AP5 also abolished the difference of evoked hypertension between WKY and SHR during low frequency (5-10 Hz) electrical stimulation. The difference during high frequency (60Hz) was not affected by AP5. We previously found that NMDA is involved in carotid clamping induced hypertension and neuronal excitation in the RVLM. In the present study, we found that local applied AP5 in RVLM antagonized carotid clamping evoked hypertension in SHR and WKY at the same extend. These suggested that the hypersensitivity of RVLM to the low frequency stimulation in SH rats involved NMDA. High frequency stimulation or carotid clamping may involve NMDA and other mechanisms.

390.7

ACTIVATION OF THE ROSTRAL VENTROLATERAL MEDULLA (RVLM) IN DOGS ELICITS AN INCREASE IN ARTERIAL PRESSURE (BP) WITHOUT AN INCREASE IN HEART RATE (HR). L.W. Dickerson,*F.E. Kuhn, W.H. Panico, W.P. Norman, R.A. Gillis. Departments of Pharmacology and Medicine, Georgetown University, Washington, D.C. 20007.

Studies in the cat indicate that stimulation of the RVLM, specifically, neurons in the subretrofacial nucleus (SRFN), increases BP and HR, and enhances sympathetic tone in specific vascular beds. To determine whether the SRFN exerts a similar influence in the dog, these neurons were activated by microinjection of L-glutamic acid (L-glu) into a site approximately 8.0 mm caudal to the foramen cecum, 5.0 mm lateral to the midline, and 1.5 mm below the ventral surface while monitoring BP, HR, renal and femoral arterial flows in vagotomized, pentobarbital-anesthetized animals. Microinjection of L-glu produced increases in BP (+40±5 mm Hg, $p<0.05$, N=8) (from a baseline of 123±7 mm Hg), femoral resistance (+60±1%, $p<0.01$), and renal resistance (+41±11%, $p<0.05$). There was no increase in HR (+3±2 beats/min, $p>0.05$) from a baseline of 162±10 beats/min. These data suggest that neurons in the SRFN in the dog, as in the cat, influence vascular tone, but unlike the cat, have no effect on sinus rate. [NIH Grant R01-DA-05-333].

390.8

Alpha$_2$-adrenoceptor-mediated inhibition of bulbospinal barosensitive cells of rat rostral medulla. Patrice G. Guyenet* and Andrew M. Allen. Dpt. of Pharmacology, University of Virginia, Charlottesville, VA, 22908.

Bulbospinal barosensitive neurons of the rostral ventrolateral medulla (RVLMbb cells, presumed sympathetic vasomotor premotor neurons) were recorded with iontophoretic electrodes in urethane-anesthetized rats. The majority of RVLMbb cells were insensitive to i.v. clonidine (clo, up to 20 μg/kg) and insensitive to iontophoretically applied clo or α-methyl norepinephrine (αMNE). These cells (n=47/76) had a spinal conduction velocity of 4.1 ± 0.2 m/s and a mean firing rate of 20 ± 1 spikes/s. A second population (n=29) was powerfully inhibited by i.v. clo (5-10 μg/kg, activity decreased by 83 ± 11 %), iontophoretically applied clo (decreased by 51 ± 7 %) and iontophoresis of αMNE (decreased by 69 ± 3 %). These cells had a slower conduction velocity (2.0 ± 0.3 m/s) and a much slower discharge rate (6 ± 1 spikes/s). Both populations were pulse-synchronous at resting AP. The inhibitory effects produced by iontophoresis of αMNE or clo were reduced to the same degree (86 - 98 %) by iontophoresis of idazoxan (an α_2-adrenergic antagonist with imidazoline structure) and by iontophoresis of piperoxan (65-77%, a non-imidazoline α_2-antagonist). The inhibition of RVLM cells by i.v. clo was reversed by iontophoresis of idazoxan and by i.v. injection of yohimbine (non-imidazoline α_2-antagonists). This data suggests that: i) i.v. clo only inhibits a subpopulation of RVLM sympathetic premotor neurons, possibly the C1 adrenergic cells, ii) this effect of clo is due to activation of α_2-adrenergic receptors rather than "non-adrenergic imidazoline" binding sites, iii) these α_2 receptors are located on or close to the clo-sensitive cells and these cells may be tonically inhibited by endogenously released catecholamines.

390.9

α2A-adrenergic receptors are present in noradrenergic, adrenergic and serotonergic spinally projecting cells of rat pons and medulla. R.L. Stornetta*, P.G. Guyenet, T. Riley, F.N. Norton, D.R. Rosin, K.R. Lynch. Dept. of Pharmacology, Univ. of Virginia Sch. of Med., Charlottesville, VA 22908.

The brainstem distribution of the α2A-adrenergic receptor (α2AR) has been previously characterized using a subtype specific antibody. We sought to determine whether some of these receptors are located in the catecholaminergic or serotonergic spinally projecting neurons known to contribute to the control of sympathetic tone. 12 μm frozen sections were cut on a cryostat and reacted sequentially with the α2AR antibody and with commercially available antibodies to either tyrosine hydroxylase (TH) or serotonin (5HT). In some cases α2AR-like immunoreactivity (LIR) was visualized with a nickel enhanced DAB reaction (black), TH- or 5HT-LIR was visualized with DAB (brown). In other cases, α2AR-, TH- and 5HT-LIR was detected by immunofluorescence. Cells immunopositive for α2AR, TH and 5HT were plotted using the Neurolucida software in conjunction with Ludl motor stage controllers and maps of the exact location of these cells in medulla were constructed. All 5HT immunopositive cells in raphe pallidus and obscurus contained α2AR-LIR and virtually all TH positive cells showed α2AR-LIR. Only 22% ± 2.4% of cells with α2AR-LIR in RVL were also TH positive. Bulbospinal neurons were tagged in some rats by retrograde labeling with FITC-conjugated latex microspheres injected at T3 (7-15 days prior). The brainstem was processed for simultaneous fluorescent detection of α2AR-LIR (Texas Red), TH (AMCA)(n=3) or 5HT (AMCA)(n=3) in combination with the FITC microspheres. Virtually all (>90%) spinally projecting C1 cells, and all A5 and lower brainstem serotonergic cells contain α2AR-LIR. Thus α2ARs are present in a majority of the bulbospinal neurons which are presumed to provide an excitatory drive to the sympathetic outflow and this may explain the profound effects of clonidine on sympathetic nerve activity.

390.10

ABDOMINAL VAGAL STIMULATION INDUCES FOS IMMUNOREACTIVITY IN RABBIT MEDULLARY A1 AND C1 CATECHOLAMINE-SYNTHESIZING NEURONS. Z.J. Gieroba* and W.W. Blessing. Centre for Neuroscience, Flinders University, Bedford Park, SA 5042, Australia.

Stimulation of the abdominal vagus nerve (AVN) in the rabbit increases plasma vasopressin and arterial pressure. The A1 and C1 catecholamine-synthesizing medullary neurons could be involved in these reflexes. We tested, in unanesthetized rabbits, whether electrical stimulation of the AVN induces fos immunoreactivity in A1 and C1 neurons. Rabbits were anesthetized with thiopentone (30 mg/kg i.v.), then with halothane (1.5 %). The anterior trunk of the AVN was cut and placed inside a cuff electrode. The rabbit recovered from anesthesia. After 24 hours the AVN was electrically stimulated (0.5 ms, 20 Hz, 500 μA, 4.5 min on, 0.5 min off, 2 hours). One hour later rabbits were perfused with 4% paraformaldehyde. Brains were cut (40 μm) and processed for demonstration of fos (Cambridge Research Biochemicals, 1:1000) and tyrosine hydroxylase (TH) immunoreactivity (Incstar Corp., 1:25000) by a two-color immunoperoxidase procedure. Fos-positive nuclei were observed within the nucleus tractus solitarius, (78 % on left side). TH was present in 9 ± 1% (n = 5) of fos-positive cells (A2 neurons). The ipsilateral caudal and intermediate ventrolateral medulla contained 16 ± 3 fos-positive nuclei per section (90% left side). TH was present in 70 ± 6% (n = 10) of fos-positive cells (A1 neurons). The rostral ventrolateral medulla contained 34 ± 4 (n = 4) fos-positive neurons per section, (82 % left side) and 54 ± 5% neurons were double labeled for TH (C1 cells). Our results indicate that stimulation of the abdominal vagus in unanesthetized rabbits activates A1, A2 and C1 medullary cell groups.

390.11

BULBOSPINAL BAROSENSITIVE NEURONS IN ROSTRAL VENTROLATERAL MEDULLA ARE EXCITED BY ABDOMINAL VAGAL STIMULATION. I.P. Messenger, Z.J. Gieroba and W.W. Blessing*. Centre for Neuroscience, Flinders University, Bedford Park, SA 5042, Australia.

Electrical stimulation of the abdominal vagus increases arterial pressure. We have tested, in rabbits anesthetized with urethan (1.5 g/kg, i.v.), whether the discharge rate of bulbospinal neurons in the rostral ventrolateral medulla (RVLM) is affected by electrical stimulation of the abdominal vagus (cuff electrode at the level of the diaphragm, 0.5 ms, 200 Hz, 1-3 cathodal pulses). RVLM neurons were identified by antidromic activation from the ipsilateral dorsolateral funiculus of the thoracic spinal cord, and by a collision test. Peristimulus time histograms relating vagal stimulation to RVLM neuronal discharge were constructed using an ITC16 interface and a Macintosh IIfx computer. Recordings were made from 31 RVLM neurons. Conduction velocity of the descending axons was 7.5 ± 0.6 m/s. Discharge rate of the RVLM neurons was 3.5 ± 0.6 spikes/s. Twenty three neurons (74%) were excited by stimulation of the vagus, 6 were unaffected and 2 were inhibited. The latency to maximum excitation was 255 ± 7 ms (vagal conduction velocity 0.6 m/s) and was followed by inhibition lasting approximately 240 ms. Sixteen neurons excited by the vagus were tested for baroreceptor sensitivity; 14 were inhibited by activation of baroreceptors using intravenous phenylephrine (discharge rate reduced to 0.8 ± 0.2 spikes/s) and those tested showed cardiac-related rhythmicity. These results indicate that the majority of bulbospinal neurons in the RVLM, with properties of sympathoexcitatory barosensitive neurons, are excited by electrical stimulation of the abdominal vagus. Our study shows a direct involvement of RVLM neurons in the reflex pathway in which abdominal vagal stimulation increases arterial pressure.

390.12

PHENYLEPHRINE DECREASES OPTICAL REFLECTANCE IN THE KITTEN VENTRAL MEDULLARY SURFACE. R.K. Harper*, D. Gozal, X-W. Dong, D.M. Rector and R.M. Harper. Brain Res. Inst. & Dept. Anat. & Cell Biol., UCLA Sch. of Med.; Div. Neonatol. & Ped. Pulmonol., Childrens Hosp., USC Sch. of Med., Los Angeles, CA.

Regional neuronal activation on the ventral medullary surface (VMS) of 10, 20, and 30 day old kittens during phenylephrine-induced blood pressure elevation by optical recording procedures. The VMS was exposed through a ventral surgical approach under pentobarbital anesthesia. Arterial pressure, end-tidal CO_2, costal diaphragmatic EMG, and ECG were monitored. A coherent image conduit, coupled to a charge-coupled device camera, was positioned over the VMS. Reflected 660nm light was digitized continuously at 2-3 sec intervals triggered by the peak of ECG during a baseline period, and after 10, 20, and 40 μg/kg i.v. phenylephrine injection. Forty images within each epoch were averaged, and were subtracted from baseline. Regional differences within the image were determined by ANOVA procedures (α=.05). Phenylephrine induced a significant, transient elevation of blood pressure concomitant with diminished respiratory EMG activity. A pronounced dose-dependent decrease in optical reflectance (increased neural activity) was found over widespread regions of the VM surface during the early pressor response with a subsequent return to baseline values. We conclude that pharmacologically-induced rapid blood pressure elevation results in a generalized increase in neural activity within the VMS, in contrast to the decreased activity found in adult animals.

Supported by HD-22506 and NIDR DE07212

390.13

THE A1 CATECHOLAMINE CELL GROUP: FINE STRUCTURE AND SYNAPTIC INPUT FROM THE NUCLEUS OF THE SOLITARY TRACT. C.A. Peto, R.K.W. Chan and P.E. Sawchenko*. The Salk Institute, La Jolla, CA 92037.

Preembedding immunostaining methods were used to characterize tyrosine hydroxylase-immunoreactive (TH-ir) elements in the caudal ventrolateral medulla, and to determine whether neurons of the A1 cell group are innervated by projections of the nucleus of the solitary tract (NTS). Immunoperoxidase-stained TH-ir neurons in the A1 region were medium-sized and multipolar, giving rise to 1-3 primary dendrites. They possessed rounded nuclei with infrequent invaginations, well-developed Golgi apparati, high cytoplasmic densities of mitochondria and lysozomes, and a low-moderate tendency for rough endoplasmic reticulum (RER) to align in parallel stacks. Reaction product was distributed diffusely through the cytoplasm, but avoided regions occupied by Golgi and RER. A1 cell bodies were commonly juxtaposed to TH-positive and TH-negative neurons, myelinated profiles, glia and/or vascular elements, but close membrane appositions with any of these were rare. Synaptic input to A1 neurons was predominantly asymmetric in type, provided virtually exclusively by non-TH-ir terminals, and directed principally to dendritic shafts; TH-ir somata were sparsely innervated. In a second experiment, silver-intensified immunogold localization of TH-ir was combined with immunoperoxidase labeling for anterogradely transported PHA-L, following tracer injections in the caudal aspect of the medial division of the NTS. These experiments revealed a small proportion of PHA-L- labeled axon terminals that contacted postsynaptic TH-ir elements. Dually labeled synapses most commonly comprised asymmetric contacts between anterogradely labeled axon terminals and dendritic shafts of TH-ir neurons, mimicking the overall pattern of synaptic input to A1 neurons. These results suggest that the fine structure and synaptic input of A1 neurons is somewhat distinct from that of rostrally-situated the C1 catecholamine neurons (cf, Milner et al., *Brain Res.* 411:28, 1987). In addition, they document a direct NTS-A1 projection that may participate in the interoceptive control of vasopressin secretion.

390.14

EFFECTS OF CLONIDINE AND RILMENIDINE ON CNS NEURONS ACTIVATED BY BARORECEPTOR UNLOADING IN CONSCIOUS RABBITS. Y.-W. Li* and R.A.L. Dampney, Dept. of Physiology, University of Sydney, NSW 2006, AUSTRALIA.

Intravenous injection of the centrally-acting anti-hypertensive drugs clonidine and rilmenidine has been shown to inhibit pressor neurons in the rostral ventrolateral medulla (RVLM) that are normally excited by baroreceptor unloading. We have found that baroreceptor unloading also causes neuronal activation (as indicated by the expression of Fos) in several other brain regions, including the nucleus tractus solitarius (NTS), A1 and A5 areas, locus coeruleus and subcoeruleus, lateral parabrachial nucleus, and various forebrain nuclei (Dampney and Li, Society for Neuroscience Abstracts, this meeting). The purpose of this study was to determine whether neuronal Fos expression that is normally induced by baroreceptor unloading within these brain regions could be affected by clonidine or rilmenidine. In conscious NZW rabbits, baroreceptor unloading was produced by i.v. injection of clonidine (7-10 μg/kg) or rilmenidine (140-200 μg/kg) followed by continuous i.v. infusion of sodium nitroprusside sufficient to lower arterial pressure by 20-25 mmHg for 1 hr. Compared with experiments in which the same degree of baroreceptor unloading was produced by sodium nitroprusside alone, pre-treatment with clonidine or rilmenidine greatly reduced the number of Fos-labelled cells (by at least 70%) in the RVLM, A1 and A5 areas, locus coeruleus and subcoeruleus, but not in other brain regions. The findings suggest that both drugs selectively suppress Fos expression in pontomedullary catecholamine cell groups.

390.15

CAUDAL VENTROLATERAL MEDULLA MEDIATES CARDIOVASCULAR RESPONSES EVOKED FROM THE CAUDAL PRESSOR AREA. R.R. Campos Jr., O.S. Pôssas, S.L. Cravo*, O.U. Lopes and P.G. Guertzenstein. Dept. of Physiology, Escola Paulista de Medicina, 04023-900 São Paulo, Brazil.

Recent evidences show that the caudal pressor area (CPA) contains tonically active neurons involved in arterial blood pressure (AP) regulation. CPA stimulation produces hypertension while its inhibition evokes falls in AP. The present study was designed to determine whether neurons in caudal ventrolateral medulla (CVL) are involved in these responses. Adult rats (Wistar, 250-350 gr) were anesthetized (urethane 1.2 gr/kg, IP) and AP was recorded. Dorsal medullary surface was exposed and microinjections (200 nl) were made through microcanulae. Results showed that: a) CPA excitation with glutamate (50 nmol) produced pressor responses (44 ± 5.7 mmHg, n = 6); b) previous microinjections of bicuculline (200 pmol) in the CVL reduced these responses significantly (7 ± 2.5 mmHg, $P < 0.01$); c) inhibition of the CPA by application of glycine (100 nmol) evoked hypotension (-40 ± 5.2 mmHg, n = 6); d) these falls in AP were virtually abolished by microinjections of kyurenic acid (4 nmol) in the CVL (-3 ± 1.7 mmHg, $P < 0.01$). We conclude that CVL mediates cardiovascular responses evoked from CPA responses and that both GABA and glutamate synapses are involved.

(supported by FAPESP grant 91/0506-0, Brazil).

390.16

EFFECT OF LESIONS IN THE CAUDAL VENTROLATERAL MEDULLA ON FREQUENCY COMPONENTS OF SYMPATHETIC NERVE ACTIVITIES. H.M. Wilfehrt, D.R. McCrimmon* and S.F. Morrison. Dept. of Physiology, Northwestern Univ. Med. Sch., Chicago, IL 60611.

Interruption of neuronal activity in the periambigual region of the caudal ventrolateral medulla (CVLM) produces a large increase in splanchnic sympathetic nerve activity (SNA) resulting from elimination of both a tonic sympathoinhibition and the vasomotor component of the baroreceptor reflex. The present study was performed to determine whether a shift in the frequency components of SNA contributes to this increase and whether CVLM lesions produce differential changes in the SNAs to functionally distinct targets. The power spectra of SNA were compared before and after microinjection of kainic acid into the CVLM of chloralose/urethane-anesthetized, artificially ventilated rats. Lesions of CVLM neurons consistently produced both an increase in the total power and an upward shift in the mean frequency components of splanchnic SNA that was particularly evident when CVLM lesions were made in baroreceptor-denervated animals. In comparison to splanchnic SNA, CVLM lesions resulted in greater increases in cardiac SNA and smaller increases in cervical SNA. We conclude that elimination of the sympathoinhibitory influences of neurons in the CVLM increases both the number and the discharge frequency of active elements within sympathetic generating networks and that there is a differential influence of this inhibitory system on the sympathetic outflow to different organs. Supported by NIH HL47196.

390.17

NTS EFFERENT TERMINALS SYNAPSE ON NEURONS IN THE CAUDAL VENTROLATERAL MEDULLA THAT PROJECT TO THE ROSTRAL VENTROLATERAL MEDULLA. S.A. Aicher*, O.S. Kurucz, D.J. Reis and T.A. Milner. Dept. Neurol. & Neurosci., Cornell Univ. Med. Coll., NY, NY 10021.

The caudal ventrolateral medulla (CVL) contains neurons that are vasodepressor and are a critical component of the baroreceptor reflex arc. Electrophysiological studies suggest that CVL neurons are intercalated in the baroreceptor pathway between the nuclei of the solitary tract (NTS) and the rostral ventrolateral medulla (RVL) (Jeske, et.al., AJP, 264, '93). However, synaptic contacts between NTS efferents and CVL neurons that project to the RVL have yet to be demonstrated. In the present study, the retrograde tracer wheat germ agglutinin-apo-horse radish peroxidase conjugated to gold (WAHG) was pressure injected into the RVL (10-30nl) and the anterograde tracer biocytin (1.5%) was iontophoresed into the NTS (5μA, 7s on/off, 15 min) of anesthetized rats. After 4-6h rats were perfused transcardially with 3.75% acrolein in 2% paraformaldehyde and sections through the CVL were processed for both markers. By light microscopy, WAHG-labelled cells were seen in the CVL, LTF, NTS and the area postrema. Numerous biocytin-labelled varicose processes overlapped neurons containing WAHG in the CVL. By electron microscopy, biocytin was found in myelinated and unmyelinated axons and in terminals (0.2-0.4μm) that contained primarily small clear vesicles. These terminals formed both asymmetric and symmetric synapses primarily on large dendrites within the CVL. Many of these post-synaptic dendrites contained WAHG associated with lysosomes and multivesicular bodies. Some dendrites received contacts from 2 or more biocytin-labelled terminals. These data indicate that (1) NTS efferents terminate in the CVL; and (2) some of the target neurons project to the RVL. These data support the hypothesis that CVL neurons are intercalated between the NTS and the RVL in the baroreceptor reflex pathway. (Support: NIH HL08251 & HL18974).

390.18

A METABOTROPIC GLUTAMATE RECEPTOR IS RESPONSIBLE FOR MEDIATING EXCITATORY AMINO ACID NEUROTRANSMISSION IN THE CAUDAL VENTROLATERAL MEDULLA (CVLM) OF THE CAT. K. Chen, K.L. Dretchen* and R.A. Gillis. Dept. of Pharmacology, Georgetown Univ. Washington, DC 20007

Neurons in the CVLM exert an important role in controlling cardiovascular function. Excitation of these neurons results in hypotension and bradycardia due primarily to a decrease in sympathetic outflow. In our previous study we demonstrated that microinjection of a glutamate uptake inhibitor L-trans-2,4-pyrrolidine dicarboxylic acid (L-trans-2,4-PDC) into the CVLM (0.2 nmol/side) decreased mean arterial blood pressure (MAP) and heart rate (HR) of chloralose-anesthetized cats (FASAB. 7:A552, 1993). The responses were not prevented by blockade of NMDA and non-NMDA ionotropic glutamate receptors. Furthermore, microinjection of 1S,3R-ACPD (0.08 nmol/side), a specific agonist of the metabotropic receptor, into the CVLM mimicked the effect of L-trans-2,4-PDC on MAP and HR. These data suggested the presence of a metabotropic receptor at the CVLM. To obtain supportive evidence, we determined whether inhibitors of guanine nucleotide binding protein (which links the metabotropic receptor to its second messengers) would counteract the BP and HR effects of L-trans-2,4-PDC and 1S,3R-ACPD. The two inhibitors tested were pertussis toxin (PTX, 50 ng/side) and N-ethylmaleimide (NEM, 5 nmol/side). PTX pretreatment reduced the MAP and HR effects of L-trans-2,4-PDC from -65 ± 8 mmHg to -35 ± 2 mmHg ($P < 0.05$, N = 5) and -27 ± 3 beats/min to -14 ± 3 beats/min ($P < 0.05$), respectively. NEM pretreatment exerted a similar antagonistic effect on L-trans-2,4-PDC (N = 8) and also counteracted the hypotensive and bradycardiac effects of 1S,3R-ACPD (N = 4). These results indicate that a metabotropic receptor exerts a major role in mediating cardiovascular response in the CVLM.

391.1

AUTONOMIC RESPONSES TO STIMULATION AND INHIBITION OF THE INSULAR CORTEX IN SPONTANEOUSLY HYPERTENSIVE AND WISTAR RATS. K.S. Butcher* and D.F. Cechetto. Robarts Research Institute, Univ. of Western Ontario, London, ON, Canada N6A 5K8.

Lesion of the insular cortex (IC) results in elevated renal sympathetic nerve activity (RNA) and arterial pressure (AP) in the Wistar rat, while the opposite effect is observed in the spontaneously hypertensive rat (SHR). In order to further test the hypothesis that the IC differs between the two strains, acute changes in AP, heart rate (HR) and RNA were measured in propofol-anesthetized and chronically instrumented Wistar (n = 15) and SHR (n = 17) rats during pressure injection of D,L homocysteic acid (DLH; 100 mM) and lidocaine (LID; 20 mg/ml) into the IC. DLH injections (200 nl) into the rostral posterior IC of anesthetized Wistar rats resulted in a significant increase in AP (mean change = +27 ± 7 mmHg; $p<0.05$), and a significant decrease in HR (-22 ± 9 BPM) and RNA (-11 ± 4 μv.s). DLH injections into the IC of anesthetized SHR did not significantly affect AP or RNA, but HR did decrease significantly (-6.5 ± 3 BPM). LID injections (200 nl) throughout the IC of anesthetized Wistar and SHR rats did not result in any significant autonomic changes. DLH injections (500 nl) into the IC of conscious and freely moving Wistar and SHR rats did not result in significant autonomic changes, although AP and HR were elevated in 3 of the 4 Wistar rats. LID injections (500 nl) into the IC of conscious Wistar rats resulted in a significant increase in HR (64 ± 42 BPM), but had no effect in SHR. These results confirm a difference in the excitability of the IC in Wistar and SHR rats which may be related to the different responses to lesion of the IC. (Supported by the Heart and Stroke Foundations of Canada and Ontario)

391.2

POWER SPECTRUM ANALYSIS OF HEART RATE VARIABILITY IN PATIENTS WITH INSULAR CORTEX LESIONS

WM Martin and SM Oppenheimer* Cerebrovascular Program, Dept of Neurology, Johns Hopkins Univ. Sch of Med. Baltimore MD 21287

The insular cortex likely is important in control of cardiac rhythm. Predominant lateralisation of cardiovascular sympathetic sites is demonstrable in the right insula and parasympathetic sites in the left.In an ongoing investigation, we devised a new portable platform to record ECG, arterial pressure and respiration non-invasively in 3 patients with destructive insular lesions.Heart rate variability was determined by spectral and RR distribution analysis.Results were compared with matched control groups.Sequential spectra were elicited over 5 minute time bins for 1 hour in supine relaxed patients breathing spontaneously. The two left insular patients had increased stability of low frequency spectral peaks over the hour compared with the right insula patient or controls.High frequency peaks were reduced in left insular lesions compared with right insular lesions or controls.Distribution analysis indicated decreased heart rate variability with left insular lesions.Pulse rate was higher in the left insular patients but blood pressure was not noticeably lower than that of the control groups.These preliminary data suggest that destructive left insular lesions decrease parasympathetic cardiac tone which may predispose towards cardiac arrhythmogenesis.

391.3

PREFRONTAL STIMULUS-PRODUCED HYPOTENSION: MEDIATION BY HYPOTHALAMO-MEDULLARY RELAYS? S.G.Patrick Hardy*[1,2] and Paul J. May[2]. Depts. of Physical Therapy[1] and Anatomy[2], Univ. of Miss. Med. Ctr., Jackson, MS 39216.

Stimulation of the rat prefrontal cortex (PFC) results in a pronounced degree of hypotension (Hardy and Holmes, 1988; Sun, 1992). This prefrontal stimulus-produced hypotension (SPH) occurs secondary to the inhibition of cardiovascular, reticulospinal neurons within the *rostroventrolateral medulla* (RVL) (Sun, 1992). In addition, prefrontal SPH is dependent upon neurons residing within the *posterolateral hypothalamus* (Hardy and Mack, 1990). It is therefore conceivable that prefrontal SPH is mediated over hypothalamic relays to the RVL. To test this hypothesis, a series of anatomical experiments were performed, using a variety of tracers. Within the posterolateral hypothalamus, orthogradely labelled PFC axon terminals and retrogradely labelled hypothalamic neurons, projecting to the RVL, were observed to co-exist with an overlapping distribution. This finding supports the hypothesis that prefrontal SPH is mediated by hypothalamic relays to cardiovascular centers of the RVL.

To better understand this pathway, studies incorporating electromicroscopy have recently been initiated. It was observed that PFC terminals, within the posterolateral hypothalamus, typically were small and contained clear, round synaptic vesicles. These axon terminals made asymmetric synapses, primarily upon small caliber dendrites. Hypothalamo-medullary neurons typically contained a prominent Golgi apparatus and an indented nucleus. (Funded by the SHRP Faculty Development Fund, University of Mississippi Medical Center)

391.4

PHARMACOLOGICAL STUDY OF RECEPTORS MEDIATING THE DEPRESSOR RESPONSES TO INTRA-HIPPOCAMPAL INJECTION OF DYNORPHIN-A(1-8) AND L-GLUTAMATE IN CONSCIOUS SPONTANEOUSLY HYPERTENSIVE AND WISTAR-KYOTO RATS. Q. Wang, A.J. Ingenito and J.P. DaVanzo*. Department of Pharmacology, East Carolina Univ. Sch. of Med., Greenville, NC 27858.

In conscious and unrestrained spontaneously hypertensive rats (SHR) and normotensive Wistar-kyoto (WKY) rats, receptors which mediate the depressor responses to microinjection of dynorphin-A(1-8) (Dyn) and l-glutamate (Glu) into hippocampal formation (HF) were studied by using receptor antagonists. Dyn (10 nmol) and Glu (10 nmol) unilaterally injected into the dorsal HF decreased mean blood pressure by -25.3±2.4 (Dyn) and -27.1±3.4 (Glu) mmHg in SHRs and -15.8±1.7 (Dyn) and -17.8±2.6 (Glu) mmHg in WKY rats, but without significant alteration of heart rate in both strains. In both strains of rats, while pretreatment of the HF with kappa opioid receptor antagonist nor-binaltorphimine (nor-BNI, 2 nmol) considerably antagonized the depressor responses to intra-HF Dyn but not Glu, pretreatment of the HF with glutamate subclass N-methyl-D-aspartate (NMDA) receptor antagonist 2-amino-5-phosphonopentanoic acid (AP-5, 5 nmol) markedly blocked the depressor responses to intra-HF Glu but not Dyn. The depressor responses to intra-HF injection of either Dyn or Glu were found to be insensitive to antagonism by non-NMDA subclass receptor antagonist 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX, 4 nmol) in SHRs as well as WKY rats. All three antagonists injected alone into the HF showed no significant effect on basal blood pressure and heart rate in SHRs and WKY rats. The results indicate that kappa opioid and NMDA receptors selectively process the depressor responses to intra-HF Dyn and Glu in SHRs and WKY rats.

391.5

LONGITUDINAL COLUMNAR ORGANIZATION OF FOS -REACTIVE NEURONS IN THE PERIAQUEDUCTAL GRAY FOLLOWING CHANGES IN BLOOD PRESSURE.

A.Z. Murphy*, M. Ennis, M.T. Shipley and M.M. Behbehani,

Depts. Physiol. and Anat. & Cell Biol., Univ. of Cincinnati Col. of Med., Cincinnati., OH 45267.

The midbrain periaqueductal gray (PAG) plays an important role in autonomic function, including cardiovascular regulation. Recent studies suggest that PAG is comprised of anatomically and functionally defined longitudinally (rostrocaudally) oriented neuronal columns. Activation of neurons in the lateral column of PAG elicits pressor responses; activation of neurons in the ventrolateral column evokes depressor responses. However, it is not known if there are PAG neurons that respond to changes in blood pressure (BP). If BP responsive cells exist it is of further interest to know if they are selectively located in functionally appropriate columns, i.e. are pressor-depressor responsive neurons restricted to columns that cause pressor-depressor responses, respectively? We have addressed these two issues by determining the distribution of PAG neurons expressing the immediate early gene product, Fos, following pharmacologically selective induction of high or low blood pressure.

In 15 anesthetized rats, a femoral artery and vein were cannulated for BP measurement and iv infusion (flow rate 1 ml/hr) of either the *vasoconstrictor*, phenylephrine (5 µg/ml), the *vasodilator*, sodium nitroprusside (5 µg/ml), or physiological saline. Animals were sacrificed 60 min later and the brain was processed for immunocytochemical detection of Fos-like-IR (FLI). Pressor and depressor drugs produced robust, selective FLI in neurons confined to discrete, longitudinally-organized columns within PAG. Specifically, vasoconstriction resulted in extensive FLI cells within the lateral column in PAG; in contrast, vasodilation resulted in extensive FLI located almost exclusively within the ventrolateral column in PAG. Importantly, far fewer FLI cells were found in PAG after saline infusion indicating that volume receptor activation does not account for the pressor/depressor evoked FLI in PAG. These results demonstrate that PAG neurons activated by pressor/depressor stimulation are restricted to as separate, discrete longitudinally organized columns, similar to those functionally defined by Bandler and colleagues. Specifically, pressor responses preferentially activate neurons in the lateral column while depressor responses activate neurons in the ventrolateral column. *(Supported by TGHL07571, NS20643 and NS29635.)*

391.6

THE MIDBRAIN PERIAQUEDUCTAL GRAY (PAG) DENSELY INNERVATES MEDULLARY REGIONS CONTAINING CHOLINERGIC VAGO-CARDIAC NEURONS

M. Ennis*, S.-j. Xu, T.A. Rizvi, M.M. Behbehani & M.T. Shipley.

Dept. Anatomy & Cell Biology, Univ. Cincinnati. Coll. Med., Cincinnati, OH 45267.

Activation of PAG elicits selective pressor and depressor responses. PAG projections to the rostral ventrolateral medulla sympathoexcitatory zone is a likely substrate for PAG-evoked pressor responses. Circuits mediating PAG-evoked depressor responses are less clear. Here, we report PAG projections to the rat nucleus ambiguus (NA) and periambigual area (pNA), a potent depressor region in the ventral medulla.

WGA-HRP and PHA-L injections into lateral and ventrolateral PAG labeled a rostrocaudally oriented, longitudinal fiber plexus that densely innervates the entire rostrocaudal axis of NA and pNA. Labeling begins at the level of the facial nucleus, corresponding to the compact formation of NA; labeled fibers at this level are primarily located in the region immediately surrounding, and to a lesser extent, within NA. The density of labeled fibers increases along the rostral-caudal axis of pNA, terminating most heavily in the loose and caudal, external formation of pNA. Both NA and pNA contain cholinergic vagal neurons that project to the heart. To directly assess the relationship between PAG projections and these cholinergic neurons, anterograde tracing was combined with immunohistochemical staining for choline acetyltransferase (ChAT), the synthetic enzyme for acetylcholine. Anterogradely labeled fibers terminated among ChAT-positive neurons in NA and the loose and caudal, external divisions of pNA. Additional double labeling studies assessed PAG projections in relation to neurons labeled after Fluorogold injections into 1-2 cardiac ganglia. PAG terminal projections were intermingled among labeled neurons in caudal, external formation of pNA.

These results indicate that PAG has direct, robust connections with NA and pNA. This projection terminates most heavily in the caudal pNA region that contains preganglionic parasympathetic cholinergic neurons that project to the heart. This PAG→NA projection may comprise a direct anatomical substrate that mediates the potent depressor responses produced by PAG stimulation. In addition, the projection from PAG to the rostral, compact division of NA may mediate PAG-evoked respiratory and vocalization responses.

(Support: NS29635 & NS20643).

391.7

VIGILANCE REACTION ELICITED BY ELECTRICAL STIMULATION OF THE MIDBRAIN PERIAQUEDUCTAL GRAY AND THE HYPOTHALAMUS MAY INVOLVE SEPARATE NEURAL PATHWAYS IN RABBITS. Y-F. Duan*, P. M. McCabe, R.W. Winters, E.J. Green, Y. Huang, and N. Schneiderman. Department of Psychology, University of Miami, Coral Gables, FL, 33124.

The vigilance reaction (VR), characterized by behavioral freezing, increased alertness, an increase in blood pressure, bradycardia, and respiratory apnea or shallow tachypnoea, can be elicited by electrical stimulation of the hypothalamus. It has been suggested that the midbrain periaqueductal gray (PAG) , instead of the hypothalamus, may be the integrative site for the VR. The present study was conducted to assess the functional relationship between the PAG and the hypothalamus in the integration of the VR.

Adult New Zealand albino rabbits were anesthetized with pentobarbital. Electrical stimulation of the PAG and hypothalamus was accomplished via separate bipolar stainless electrodes (50-400 μA, 100 Hz, 0.5 ms duration, 10 s train). In selected animals surgical transection of the caudal PAG was performed stereotaxically. Arterial blood pressure, EKG, and respiratory signals were recorded.

The hypothalamic and PAG vigilance areas were identified by cardiorespiratory responses to electrical stimulation. Concurrent stimulation of the PAG and hypothalamic VR areas yielded a larger VR. Ipsilateral and bilateral lesions of the PAG VR areas did not seem to affect the hypothalamic VR response. Transection of caudal PAG attenuated VR of the rostral PAG without affecting the hypothalamic VR. These findings suggest that the VR elicited by hypothalamic stimulation is mediated by a neuronal pathway that is different from the one that mediates the VR elicited by stimulation of the PAG. (Supported by NIH HL 36588 and HL 07426).

391.8

SYMPATHOEXCITATORY RESPONSES ELICITED FROM THE AREA OF THE CUNEIFORM NUCLEUS IN THE RAT. A.J.M. Verberne*. University of Melbourne, Clinical Pharmacology and Therapeutics Unit, Austin Hospital, Heidelberg 3084, Australia.

Electrical stimulation of the area of the cuneiform nucleus (CNF) in the rat produces elevations in arterial blood pressure. In this study, the effect of electrical and chemical stimulation of the CNF on lumbar sympathetic nerve discharge (LSND) and on putative sympatho-excitatory, medullospinal neurones of the rostral ventrolateral medulla (RVLM) in halothane-anaesthetized, paralysed rats has been examined. Twin pulse stimulation (0.5 Hz, 0.5 ms duration, 3 ms pulse interval, 150-300 μA) of the CNF produced sympathoexcitation with an onset latency of 78 ± 4 ms (n=6). Electrical (50 Hz, 0.5 ms, 25-100 μA, 10 s) and chemical (DL-homocysteic acid 0.16M, pH 7.4, 25-50 nl) stimulation of the CNF produced pressor responses accompanied by sympathoexcitation. Twin pulse stimulation of the CNF also activated 8/10 RVLM barosensitive neurones (resting firing rate = 24 ± 4 spikes/s, range = 6-35 spikes/s, n=10) with an onset latency of 15 ± 3 ms. Train stimulation of the CNF produced an intensity-dependent excitation of RVLM neurones. Non-barosensitive neurones within the RVLM were not excited by CNF area stimulation (n=5). These studies suggest that CNF stimulation produces a sympathoexcitatory response which is mediated by putative premotor sympathoexcitatory neurones of the RVLM. (Supported by the NH&MRC of Australia).

391.9

NEUROCHEMICAL ORGANIZATION OF THE PARABRACHIAL NUCLEUS IN CARDIOMYOPATHIC INBRED AND NORMAL HAMSTERS. Gary V. Allen* and David A. Hopkins, Department of Anatomy and Neurobiology, Dalhousie University, Halifax, N.S., Canada, B3H 4H7.

In animals born with a genetic predisposition for cardiomyopathy, sensory signals from diseased heart tissue would likely differ from that of normal heart tissue. It is hypothesized that this difference would affect the rate of synthesis and release of specific neurochemicals in autonomic structures. In order to test our hypothesis, the brains of normal golden hamsters, albino hamsters (strain CF-148, Canadian Hybrid Farms) and cardiomyopathic inbred hamsters (strain CHF-146, Canadian Hybrid Farms) were prepared for the immunohistochemical localization of antibodies to the catecholamine-synthesizing enzyme, tyrosine hydroxylase (TH), and serotonin (5-HT). Brain sections from each group were processed identically and simultaneously. Central autonomic regions were surveyed for differences in immunohistochemical staining. The results show that there were striking differences among strains with respect to the two neurochemicals examined. In golden hamsters and albino control hamsters, a small cluster of TH-positive cell bodies and a few TH-positive fibers were found in the lateral portion of the parabrachial nucleus (Pbl). In contrast, TH-positive fibers and cell bodies were dense in the Pbl of the cardiomyopathic hamsters (CF-146). 5-HT labeled fibers were dense in the Pbl of the CF-146 and CHF-148 strains but there was no detectable staining of 5-HT in the PB of the golden hamster. Because the parabrachial nucleus is known to receive visceral afferent input from the nucleus of the solitary tract (Mantyh and Hunt,'84; Hubert et al.,'90), it is possible that the differential distribution of TH and 5-HT in the PB of the different groups of animals may reflect varying afferent input from the heart, other autonomic changes or genetic differences. Supported by Dalhousie Medical Research Foundation to G.V.A. and MRC to D.A.H..

391.10

NEUROPEPTIDE CHANGES IN THE PARABRACHIAL NUCLEUS FOLLOWING CERVICAL VAGAL STIMULATION. TAREK M. SALEH* and DAVID F. CECHETTO. Robarts Research Institute and the University of Western Ontario, London, Ontario, Canada N6A 5K8

Previous studies in our laboratory have shown that the peptides; neurotensin (NT), cholecystokinin (CCK), substance P (SP) and calcitonin gene-related peptide (CGRP), have a role in modulating ascending visceral sensory information ascending relaying in the parabrachial nucleus (PB). In this investigation, we examined the changes in the levels of these peptides detected by immunohistochemistry in response to cervical vagal stimulation in the inactin-anesthetized male Wistar rat. Paired control and experimental animals were instrumented to monitor blood pressure and heart rate. The vagus nerve was stimulated for 0.5, 2 or 4 hours after which time the animals were perfused and the brains were processed immunohistochemically for the peptides NT, CCK, SP and CGRP. Vagal stimulation produced a depletion in the immunolabeling of NT and CCK in the external lateral (el) and external medial (em) subnuclei. This depletion increased with the length of vagal stimulation. In contrast, the immunolabel of the peptides SP and CGRP increased after one half hour reaching a maximum after two hours of vagal stimulation in the el and em subnuclei. After four hours of vagal stimulation, the immunolabel for SP and CGRP was depleted in the two PB subnuclei.

Thus, the neuropeptides NT, CCK, SP and CGRP, which modulate the visceral sensory information in the PB, are also influenced by the level of activity in the vagus nerve. (Supported by the Heart & Stroke Foundation of Ontario).

391.11

THE EFFECTS OF CHEMICAL BLOCKADE OF THE LATERAL PARABRACHIAL NUCLEUS ON THE BAROREFLEX. R.B. Felder*, A.L. Pence and L.F. Hayward. Cardiovascular Center and Dept. of Internal Med. University of Iowa College of Medicine, Iowa City, IA 52242

Previous studies have shown that electrical or chemical activation of the lateral parabrachial nucleus (LPBN) produces an increase in arterial pressure and sympathetic nerve activity and an attenuation of baroreflex control of blood pressure and heart rate. Electrolytic lesion of the LPBN produces an augmentation of baroreflex control of heart rate and increases in plasma norepinephrine and renin activity. Since electrolytic lesions destroy fibers of passage in addition to cell bodies, the present study was designed clarify the role of the LPBN in the control of the sympathetic nervous system. In 5 urethane anesthetized Sprague Dawley rats, mean arterial pressure (MAP) and renal sympathetic nerve activity (RSNA) responses to electrical stimulation of the left aortic depressor nerve (L-ADN) were recorded before and following chemical blockade of the ipsilateral LPBN (200 nl of 2 mM kainic acid [KA]) microinjected into the left LPBN). Immediately following chemical blockade of the left LPBN there was a significant increase in resting MAP (77+5 vs 97±9 mmHg; pre vs post KA block; $p<0.05$; mean±SE) and an increase in RSNA. (100 vs 113±5% $p<0.2$). Before chemical blockade, L-ADN stimulation at 2, 5 and 10 Hz (10 V, 0.2 ms) resulted in a 12, 18 and 19 mmHg drop in MAP and a 26, 44 and 37% decline in RSNA. Two minutes following chemical blockade 2, 5 and 10 Hz L-ADN stimulation resulted in a 14, 24*, 25mmHg drop in MAP (* significantly different from control; $p<0.05$) and a 12, 61 and 76*% decline in RSNA. The results suggest that the LPBN may produce some tonic inhibition of baroreflex control of arterial pressure and RSNA in the anesthetized animal. (Support: HL44546 & HL14388)

391.12

RESPIRATORY MODULATION OF SPLANCHNIC SYMPATHETIC NERVE ACTIVITY IN RAT AFTER PONTINE LESIONS THAT PROLONG INSPIRATION. S.F. Morrison* and S.L. Cravo. Dept. of Physiology, Northwestern Univ. Med. Sch., Chicago, IL, 60611.

Respiratory modulation of the amplitude of sympathetic nerve activity provides an indication of the neural circuitry by which the central respiratory generating networks can influence those controlling sympathetic nerve activity (SNA). To determine which class of neuron involved in respiratory control might be responsible for the respiratory modulation of splanchnic SNA, the temporal characteristics of this modulation were examined in vagotomized, decerebrate, unanesthetized rats (n=7) before and after pontine lesions which increased the mean phrenic burst duration from 380 ±12 ms to 1371 ± 240 ms. Relative to the onset of the phrenic burst, averages of splanchnic SNA prior to pontine lesions contained an excitatory potential with an onset of 49 ± 7 ms and a mean latency of 111 ± 10 ms. Neither the duration of the sympathoexcitation nor the temporal relationship between the onset of inspiration and enhanced SNA was significantly different during the prolonged phrenic bursts produced by pontine lesions. We conclude that this respiratory-linked sympathoexcitation is mediated by neurons in the respiratory generating network that exhibit a change in discharge pattern synchronized to the onset of inspiration. Supported by NIH HL47196.

391.13

REGIONAL HAEMODYNAMIC EFFECTS OF URAPIDIL IN NORMOTENSIVE ANAESTHETIZED RATS: COMPARISON WITH FLESINOXAN, PRAZOSIN AND CLONIDINE. H. Dabiré, K. Chaouche-Teyara, P. Lacolley* and M. Safar. INSERM U337, 15, Rue de l'Ecole de Médecine; 75270 Paris 06, France.

Using pulsed Doppler technique, we have compared i.v. and i.c.v. systemic and regional haemodynamic effects of urapidil (U, α_1-antagonist/5-HT_{1A} agonist), flesinoxan (F, 5-HT_{1A} agonist), clonidine (C) and prazosin (P). The results observed [Table: decrease (Dec.), increase (Inc.) or no change (-)] suggest that 1) I.v. U, F and P decreased mean arterial pressure (MAP) by a reduction in total peripheral resistance

	MAP	HR	CO	TPR	HQVR	MVR	RVR
U (0.1-3 mg/kg i.v.)	Dec.	-	-	Dec.	Dec.	Dec.	-
F (3-300 μg/kg i.v.)	Dec.	Dec.	-	Dec.	Dec.	-	-
P (1-100 μg/kg i.v.)	Dec.	-	-	Dec.	Dec.	Dec.	Dec.
C (1-100 μg/kg i.v.)	Dec.	Dec.	Dec.	-	Inc.	-	Inc.
U (10-300 μg/kg i.c.v.)	Dec.	-	-	Dec.	Dec.	-	-
F (3-30 μg/kg i.c.v.)	Dec.	-	-	Dec.	Dec.	-	-
P (0.1-3 μg/kg i.c.v.)	-	-	-	-	-	-	-
C (1-10 μg/kg i.c.v.)	Dec.	Dec.	-	Dec.	Dec.	-	-

(TPR) and C cardiac output (CO). The hindquarters vascular bed is the main contributor to the reduction in TPR. 2) U, F, P and C have differential effects on hindquarters (HQVR), mesenteric (MVR) and renal (RVR) vascular resistances. 3) Central administration smooths these differences . 4) U ressembles more closely F than P and C. The combined peripheral and central properties of U may explain its effectiveness to decrease blood pressure.

391.14

CHARACTERIZATION OF RECEPTORS INVOLVED IN THE CARDIOVASCULAR ACTIONS OF CENTRALLY-ADMINISTERED NICOTINE (N). R. Chen and S.E. Robinson*. Department of Pharmacology & Toxicology, Medical College of Virginia, VCU, Richmond, VA 23298-0613.

Intracerebroventricular injection (i.c.v.) of N exerts multiphasic actions on the cardiovascular system at doses too small to be acting peripherally. The ability of nicotinic receptor antagonists to block the cardiovascular effects of i.c.v. N was studied in male Sprague-Dawley rats (300-350 g), which had lateral ventricular cannulae implanted under Equithesin anesthesia 3 days prior to the experiment. Blood pressure (BP) and heart rate (HR) were measured in urethane-anesthetized rats via a polyethylene cannula (PE 50) inserted in the femoral artery and connected to a pressure transducer coupled to a cardiotachograph and a polygraph recorder. I.c.v. injection of N (160 nmol/2 μl artificial CSF) produced a triphasic effect on BP, consisting of an initial brief increase, followed by a decrease, and then a prolonged increase in BP, and a biphasic effect on HR, consisting of a decrease and then an increase in HR. Mecamylamine (M, 108 nmol/2 μl artificial CSF, i.c.v.) alone had no effect on BP and HR, and neuronal bungarotoxin (n-btx, 4.5 μg/2 μl artificial CSF, i.c.v.) alone produced slight, but significant increases in BP and HR. Both M and n-btx blocked the hypertensive responses when injected i.c.v 10 or 30 min, respectively, prior to N. M also reduced the hypotensive response. M blocked the bradycardia, but not the tachycardia, response to N. n-Btx did not block HR responses to N. Thus, multiple receptor subtypes are involved in the cardiovascular actions of N. (Supported in part by a Staton Heart Fund Fellowship).

391.15

RENIN-ANGIOTENSIN SYSTEM INVOLVEMENT IN STRESS-INDUCED HYPERTENSION. D.C. Hatton*, S.C. Coste, Y.Qi, and D.A. McCarron. Oregon Health Sciences University, Portland, OR 97201

Daily exposure to air jet stress (AJS) has been shown to cause sustained elevations of blood pressure in borderline hypertensive rats (BHR). Both AV3V lesions and renal denervation have been shown to prevent the development of stress-induced hypertension in this strain suggesting that altered fluid balance regulation may be responsible. This study assesses the involvement of the renin-angiotensin system in stress-induced hypertension. Eight and nine week old rats were randomized to 4 groups receiving either captopril or vehicle with and without AJS. Captopril was administered in the drinking water (100mg/l) throughout the study. Water intake data indicated that the average daily dose was 6.2 mg/day. After 10 days of stress, catheters were placed in the femoral artery and the animals were allowed 48 hrs for recovery before measuring BP. Mean arterial pressure in conscious, restrained animals were as follows: vehicle/AJS = 148±5; captopril/AJS = 130±3; vehicle/no stress = 138±2; captopril/no stress = 134±2 mmHg. ANOVA indicated that AJS caused a significant elevation of blood pressure in the vehicle/AJS group but not in the captopril/AJS group. Circulating norepinephrine was significantly lower in captopril/AJS rats than vehicle/AJS rats during exposure to stress (850±91 vs 551±66 pg/ml) but not during baseline (443±65 vs 432±181 pg/ml). A similar trend was seen for epinephrine. The outcomes suggest that the formation of angiotensin II may be critical in the development of stress-induced hypertension and that the influence may be exerted through sympathetic nervous system activity. Supported by the Medical Research Foundation of Oregon and NHLBI grant 5 T32 HLO7332.

391.16

CARDIOVASCULAR RESPONSES TO CIGARETTE SMOKE EXPOSURE IN RESTRAINED CONSCIOUS RATS. A.A. Houdi*, R.T. Dowell, M. Welch and J.N. Diana. Coll. of Pharmacy and Dept. of Physiology, Coll. of Med. and THRI, University of Kentucky, Lexington, KY 40546.

The mechanism(s) responsible for cigarette smoke- or nicotine-induced changes in cardiovascular functions have not been fully characterized. In this study, we investigated the effect of cigarette smoke exposure with different nicotine content on cardiovascular functions using a nose exposure system in conscious restrained rats. Male Sprague-Dawley rats (300 ± 25 gm) were exposed to 3,6,9 puffs during the "break in" period and 10 puffs the day of experiment (day # 4). Blood pressure (BP), heart rate (HR) and cardiac output (CO) were recorded continuously throughout the experimental period. On the day of the experiment, rats exposed previously to cigarette smoke generated from high nicotine cigarette (2.19 mg/cig.) showed a marked decrease in HR (-35%) and CO (-23%) and an increase in TPR (+100%) and BP (+9%) in response to restraint stress. Whereas rats exposed previously to air puffs showed a lesser decrease in HR (-14%) and CO (-17%) and an increase in BP (+20%) and total peripheral resistance (TPR, +44%). In addition, cigarette smoke exposure produced a further fall in HR (-67%) and in CO (-55%) with marked increase in BP (+60%) and TPR (+290%). Air puffs exposure had no effect on these parameters. This effect of cigarette smoke was dose dependent on the nicotine content of the cigarette and antagonised by pretreatment with mecamylamine or hexamethonium. Pretreatment with atropine methyl bromide blocked the bradycardia in response to both restraint stress and cigarette smoke exposure and produced a mild increase in HR, indicative of sympathetic activation. These data further support the involvement of specific nicotinic receptors in mediating the effects of cigarette smoke on cardiovascular functions by activating both sympathetic and parasympathetic systems. Supported by KTRB.

391.17

COMPARATIVE EFFECTS OF INTERMITTENT AND STEADY AIR JET ON BLOOD PRESSURE IN SPRAGUE-DAWLEY RATS. E. S. Halas* and L. M. Klevay. USDA, ARS, Grand Forks Human Nutrition Research Center, Grand Forks, ND 59202.

Prior research has shown that steady or intermittent air jet (Drolet, G., Laforest, S. and Bachelard, H. *Soc. Neurosci. Abstracts* 1992; 18: 1183) effectively raises blood pressures of borderline hypertensive rats. These rats were restrained; our research (Klevay, L.M. and Halas, E.S. *Physiol. Behav.* 1991; 49: 309-314) has shown that chronic restraint is capable of elevating blood pressure in normotensive rats. Experiment 1 utilized three groups of adult, male rats. Group 1 was restrained and subjected to a steady air jet for 30 minutes a day, five days per week for eight weeks. A second group was restrained similarly while the third group (control) was left in their cages. No significant difference in blood pressure measured by tail-cuff was found. Experiment 2 was similar except that exposure to air jet was randomized by computer both in intertrial interval (10 to 60 sec.) and duration (0.5 to 10 sec.). All groups had a mean blood pressure of 110 mmHg at the start of the experiment. After two weeks, mean (SE) blood pressures were 119 ± 3.0, 111 ± 2.2 and 103 ± 4.2 for the respective groups. Most of the significance by ANOVA ($p<0.008$) was attributed to a difference between the extreme values as adjacent pairs were not different ($p>0.05$) by Tukey's standardized range test. Intermittent air jet is far more effective in raising blood pressure than steady air jet, which has been used in most prior studies. Most experiments have used borderline or Spontaneous Hypertensive Rats; these results show that normotensive rats respond to this stress with increased blood pressure. This method should be useful in nutritional experiments related to human hypertension.

391.18

AUTONOMIC AND METABOLIC CHANGES WITH EXERCISE IN CHRONIC FATIGUE SYNDROME. **S Sisto, D Cordero ,MT Bergen, S Drastal, WN Tapp, BH Natelson*. Neurobehavioral Unit, EOVAMC 127-A, East Orange, NJ 07018.**

Chronic Fatigue Syndrome (CFS) is a disabling illness of unknown etiology that often strikes middle aged women. Earlier work noted an abnormal heart rate response to exercise. To study this further, CFS patients and sedentary controls walked on our treadmill protocol consisting of alternating stages of exertion and rest (1 mph , 2, rest, 1.5, 2.5, rest, 3, 3.5). Subjects continued exercising until they reached maximal workloads. Electrocardiogram, respiration, and expired gases were collected for autonomic and metabolic analysis.

We analyzed the area under the heart rate spectrum at frequencies greater than .15 Hz to estimate the parasympathetic component in heart rate variability. As expected, vagal power dropped when subjects, control or CFS, exercised ($p<.05$). Initial resting vagal power was not different between groups. However, during the rest periods following submaximal exercise, controls recovered parasympathetic power more than CFS subjects ($p<.01$).

VCO_2 in CFS subjects was elevated above controls at 2.5, 3 and 3.5 mph 0% grade ($p<.01$). O_2 pulse (VO_2 / heart rate) was also elevated compared to normals during exercise ($p<.01$). No other metabolic parameters showed significant differences across groups. These differences in metabolic and autonomic responses to exercise suggest an organic etiology for CFS which requires further investigation. Supported by NIH (U01-AI32247) and VA medical research funds.

391.19

IMPAIRMENT OF CARDIOVASCULAR AUTONOMIC REGULATION IN AMYOTROPHIC LATERAL SCLEROSIS. T. Kiauta*, B. Žvan and S. Šega. Dept. of Neurology, University Medical Centre, SLO-61105 Ljubljana, Slovenia.

Few studies of autonomic nervous system function in amyotrophic lateral sclerosis (ALS), performed in a limited number of patients and yielding conflicting results, can be found in the literature. The aim of this study was to apply a standardised battery of cardiovascular tests to a sizable group of ALS patients and healthy controls.

The Valsalva manoeuvre, deep breathing test, sustained handgrip test, orthostatic test and spectral analysis of heart rate variability were tried in a group of 15 ALS patients, 7 of them female, aged 36 to 69 years (mean ± S.D.: 54.7 ± 9.0 years). All of them had both upper and lower motor neuron signs, while brainstem involvement was found in 7 patients. In all cases the diagnosis of ALS was confirmed electrophysiologically and gross symptoms of autonomic involvement were absent. Several patients were not up to performing the complete testing battery because of muscular weakness.

No abnormalities were found in 7 patients, while in the remaining 8 patients combinations of test results suggesting both sympathetic and parasympathetic hypofunction were established.

CARDIOVASCULAR REGULATION: HYPOTHALAMIC CONTROL

392.1

CHEMICAL TOPOGRAPHY OF EFFERENT PROJECTIONS FROM THE MEDIAN PREOPTIC NUCLEUS TO PONTINE MONOAMINERGIC CELL GROUPS IN THE RAT. A.M. Zardetto-Smith*, T.G. Beltz and A.K. Johnson, Depts. of Psychology and Pharmacology, Univ of Iowa, Iowa City, IA 52242.

The median preoptic nucleus (MnPO), a major cell group in the anteroventral third ventricular (AV3V) region, is hypothesized to be a critical integrative area for hydrational information derived hormonally from blood-borne angiotensin II (ANG) acting on the subfornical organ, and neural volume/pressure information relayed from visceral receptor input to the brainstem. Previous anatomical studies in our laboratory demonstrated the MnPO is densely innervated by dopamine-beta-hydroxylase (DBH) immunoreactive fibers, and behavioral studies have indicated that norepinephrine must be present in this area to elicit normal drinking and pressor responses to exogenously administered ANG . In addition to this major noradrenergic input, the MnPO also receives efferents from brainstem serotonergic groups. Using anterograde tracing combined with glucose oxidase immunocytochemistry, this study focused on the reciprocal innervation of noradrenergic/serotonergic cell groups in the upper brainstem by efferents from the MnPO. Small deposits of Phaseolus vulgaris leucoagglutinin (PHA-L) were iontophoretically placed within the MnPO of male, Long-Evans rats. PHA-L fibers and terminals were visualized using the avidin-biotin immunoperoxidase technique. Monoaminergic cell bodies were localized using antibodies to dopamine-B-hydroxylase or serotonin (5-HT) and visualized with a glucose-oxidase nitro blue tetrazolium reaction. The results indicate that the A6 group was the most densely innervated of the upper brainstem catecholamine groups, with sparser innervation of the A5 and A7 groups. The B6 and B7 serotonergic groups received moderate innervation, with light innervation of the B8 group. Efferents from the MnPO to these groups may provide feedback important in modulating the forebrain projections originating from these areas. (Supported by NRSA F32HL-08349 and the Iowa Affiliate of the American Heart Association).

392.2

ACTIVITY OF ANTERIOR HYPOTHALAMIC NEURONS IS INCREASED BY HIGH DIETARY NaCl IN SPONTANEOUSLY HYPERTENSIVE RATS. I. Kadisha, K. King, T. van Groen, S. Oparil* and J. M. Wyss. Departments of Cell Biology and Medicine, University of Alabama at Birmingham, Birmingham, AL 35294.

Previous data from our laboratory indicate that the anterior hypothalamic area plays an important role in NaCl-sensitive hypertension in the spontaneously hypertensive rat (SHR) on a high NaCl diet. In the NaCl-sensitive SHR (compared to NaCl-resistant rats) neurons in the anterior hypothalamic nucleus appear to be less responsive to a high NaCl diet, and this results in a lack of restraint on the sympathetic nervous system and a subsequent rise in arterial pressure. To more precisely test this hypothesis, 6 week old SHR were placed on a diet containing either 8% or 1% NaCl for 4 weeks. After the 4 weeks on the diet, arterial pressure was significantly elevated in the SHR on the 8% (192 ± 5 mm Hg) NaCl diet compared to the SHR on the 1% (150 ± 4 mm Hg) NaCl diet. Subsequently, the rats were perfused, the brains were removed and sectioned, and the sections were stained for cytochrome oxidase, an indicator of neuronal activity. In the SHR on the 1% NaCl diet, cytochrome oxidase labeling was present in the anterior hypothalamic area, but the labeling was light. In contrast, in the SHR on the 8% NaCl diet, cytochrome oxidase labeling in the anterior hypothalamic nucleus was more distinct and moderately elevated, suggesting that neuronal activity in the anterior hypothalamic nucleus was increased in these rats.

392.3

PARAVENTRICULAR NUCLEUS NEURONS PROJECTING TO THE SPINAL CORD IN THE RAT ARE INFLUENCED BY SUBFORNICAL ORGAN STIMULATION. J.S. Bains* and A.V. Ferguson. *Department of Physiology, Queen's University, Kingston, Ontario, Canada, K7L 3N6.*

The subfornical organ (SFO) is an important site in mediating the central nervous system actions of angiotensin II (ANG). Peripheral administration of this peptide results in: increased blood pressure, increased circulating levels of neurohypophyseal hormones, as well as increased drinking behaviour. Anatomical studies have shown the presence of efferent projections from SFO to the paraventricular nucleus (PVN). Electrophysiological as well as cardiovascular studies suggest that SFO neurons projecting to the paraventricular nucleus (PVN) are influenced by ANG. The actions of this peptide at SFO can be mimicked by electrically activating this structure. It has been suggested, based on anatomical evidence, that a component of the pressor response may be mediated by PVN projections to the intermediolateral cell column (IML) in the spinal cord.

The present study carried out in urethane anaesthetized (1.4 g/kg), male, Sprague-Dawley rats, examined the effects of systemic angiotensin and SFO stimulation on PVN neurons antidromically identified as projecting to IML. Recordings were made from a total of 44 cells which were antidromically activated from the spinal cord. The antidromically evoked action potentials showed a mean latency of 102.3± 7.6 ms, while the mean threshold for activation was 1.61 ± 0.14 mA. Systemic ANG administration had no effect on the excitability of these neurons (n=18). A total of 13 neurons were also tested for responses to stimulation of SFO. Stimulation of SFO resulted in excitation (n= 8), or had no effect (n= 5). These findings suggest SFO efferents provide excitatory input to PVN neurons projecting to IML, although ANG apparently does not activate this pathway in this preparation.

392.4

CHARACTERIZATION OF CARDIOVASCULAR RESPONSES TO PARAVENTRICULAR NUCLEUS (PVN) INJECTIONS OF A μ OPIOID AGONIST IN CONSCIOUS RATS. Hélène Bachelard* and Guy Drolet. Unité de Recherche sur l'Hypertension, Centre de Recherche du CHUL, Université Laval, Québec, G1V 4G2

The present study was designed to investigate the mechanisms of the cardiovascular responses produced by a μ opioid agonist, D-Ala^2, MePhe^4-Gly^5-ol-enkephalin (DAGO), bilaterally injected into the PVN of conscious Wistar Kyoto rats. The rats were chronically instrumented with intracerebral cannulae, intra-vascular catheters and pulsed Doppler flow probes in a three step surgery. PVN microinjection of artificial CSF had no consistent effects whereas DAGO produced dose-related cardiovascular effects. DAGO (1.0 nmol) produced significant increases in mean arterial blood pressure (MAP) (+24 ± 4 mm Hg) and heart rate (+97 ± 15 bpm), falls in renal (-28 ± 5%) and mesenteric (-38 ± 4%) vascular conductances and an increase in hindquarter (+91 ± 15%) vascular conductance. In the presence of phentolamine injected i.v. DAGO (1 nmol) produced a decrease in MAP (-24 ± 5 mm Hg), while the renal and mesenteric vasoconstrictor responses to DAGO were inhibited. The heart rate response and hindquarters vasodilatation to DAGO were unchanged in the presence of phentolamine. In the presence of propranolol injected i.v. only the tachycardic and hindquarters vasodilatator responses to DAGO (1 nmol) were inhibited, while the others responses were unchanged. However following intravenous pretreatment of rats with a mixture of phentolamine and propranolol the cardiovascular responses to DAGO (1 nmol) were completely inhibited, while intravenous pretreatment of rats with captopril or a V1 vasopressin receptor antagonist had no effect on the cardiovascular responses to DAGO (1 nmol). Together these results suggest the participation of the sympathetic nervous system to the cardiovascular responses elicited by PVN injection of DAGO in conscious rats. The work was supported by the FRSQ , FQMC and the MRC.

392.5

NITRIC OXIDE AFFECTS BLOOD PRESSURE AND AMINO ACID RELEASE WITHIN THE PARAVENTRICULAR NUCLEUS. T. Horn*, P.M. Smith, B.McLaughlin, L .Bauce, G .Marks, Q. J. Pittman and A. V. Ferguson.; Univ. of Leipzig, Germany; Queen's University, Kingston, Canada & Neuroscience Research Group, Univ. of Calgary, Canada.

Nitric oxide (NO) synthase immunoreactivity in neurons of the hypothalmic paraventricular nucleus (PVN) implicates a possible role of NO as an important messenger in central cardiovascular regulation within this area. Thus, we have tested whether sodium nitroprusside (SNP), a NO donor, affects blood pressure when microinjected into the PVN of urethane anaesthetized rats. A dose of 50 pmol SNP resulted in a significant decrease in mean arterial blood pressure (MAP, mean response -3312 ± 1189 mmHgsec over 330 sec response time, $p < 0.05$), but no significant change in heart rate. Furthermore, we delivered artificial cerebrospinal fluid (aCSF), which was pregassed with a mixture of 5% NO in nitrogen (NO-aCSF), via microdialysis bilaterally into the PVN in order to distinguish between effects due to the action of SNP or free NO. Perfusions of the PVN with NO-aCSF over a period of 30 min resulted in a statistically significant decrease of MAP (-5121 ± 817 mmHgsec , $p < 0.005$) when compared with the MAP during a control perfusion (aCSF). The perfusates of the PVN microdialysis were collected and subsequently analyzed for amino acids by HPLC. The levels of aspartate (432%) , glutamate (1158%), taurine (344%) and GABA (2536%) in samples, obtained from perfusions with NO-aCSF, were found to be significantly elevated when compared with controls (aCSF) whereas alanine, glutamine, and serine concentrations of the perfusates did not change. Our study provids the first direct evidence that NO may play a significant role in the regulation of cardiovascular function within the PVN.

Supported by the ICCS , Heart and Stroke Foundation and NATO.

392.6

Selective Loss of Hypothalamic Neurons in Spontaneously hypertensive rats (SHR). R. Eilam , R. Malach*, Brain Research, The Weizmann Institute of Science, Rehovot, Israel 76100

The likelihood that the brain undergoes severe morphological changes during the development of hypertension is indicated by a marked loss in brain weight and volume in SHR. We have found a specific cell loss in the PVN of 3-6 months old SHR; immunohistochemical studies revealed a gradual reduction in vasopressin (VP+) parvocellular (P) cells but not in tyrosine hydroxylase P cells in this region when compared to normotensive (WKY) rats. Alternate sections stained for Nissl or vasopressin revealed a loss in the number of cells and appearance of pycnotic cells in regions of the PVN normally reach in VI+ P cells. The cross-sectional area profile of VP+ P neurons was markedly reduced. This phenomenon was already significant in 2 months old SHR. Our findings strongly indicate that VP+ P cells undergo a degenerative process (possibly programmed cell death) characterized by cell contraction in their initial stage of dying.

392.7

NEUROTRANSMITTER MEDIATING HYPOTENSION INDUCED C-FOS EXPRESSION IN THE SUPRAOPTIC NUCLEI OF RATS. E.Shen*,X.Sun and Z.H.Jiang. Shanghai Brain Research Institute, Shanghai 200031, China.

It has been reported that hemorrhage or hypotension induces Fos-immunoreactivity (Fos-IR) in the supraoptic (SO) and paraventricular nuclei of the hypothalamus in rats, mostly in vasopressin-containing cells (Shen et al., Soc.Neurosc.Abstr.18:1176,1992). The aim of the present study is to explore the neurotransmitter mediating this effect. Adult male Sprague Dawley rats anesthetized with nembutal were used. The hypotension induced Fos-IR in the SO was suppressed by microinjection of the alpha-adrenergic blocker phentolamine (1-5ug) into the SO 10min before intravenous infusion of nitroprusside (2mg/ml), but not by the beta-adrenergic blocker propranolol (2-10ug) microinjected into the SO. The hypotension induced Fos-IR in the SO was also diminished by microinjection of the alpha-2 blocker yohimbine (0.1-1ug) into the SO. Microinjection of the norepinephrine (NE) reuptake blocker desipramine (2ug) into the SO to accumulate the spontaneously released NE in the SO could induce Fos-IR in the SO. It is concluded that the hypotension induced Fos-IR in the SO is mainly mediated by NE via alpha receptors.

(Supported by the Chinese Academy of Sciences Grant ks852024 and the National Natural Science Foundation of China Grant 39270241)

392.8

FOREBRAIN *FOS* STAINING PRODUCED BY CAROTID SINUS NERVE (CSN) STIMULATION IN NEMBUTAL (NEM) AND URETHANE (URE) ANESTHETIZED RATS. J.T. Cunningham*, A.M. Zardetto-Smith, M.Z. Cicha, A.K. Johnson & S.J. Lewis. Depts. of Pharmacol., Psych. and the Cardiovascular Center, Univ. of Iowa, Iowa City, IA 52242.

This study examined the central expression of the protein product of the intermediate-early gene *c-fos* as an indicator of cellular activation (*fos*-IR) following electrical stimulation of the CSN in anesthetized rats. Adult male rats were anesthetized with either NEM (50 mg/kg i.p.) or URE (1.5 g/kg i.p.) and a catheter was placed in their left femoral artery for blood pressure measurement. Their left CSN was isolated and a bipolar stimulating electrode was placed on it for 20 min. In control rats (SHAM) no current was passed through the electrode while stimulated rats received CSN stimulation for 20 min (5 V, 2 ms duration, 2 pps). All rats were perfused one hour after the 20 min test period. The *fos*-IR was localized immunocytochemically (ABC, Vector) and visualized with a nickel-intensified DAB reaction. In SHAM rats anesthetized with NEM, *fos*-IR was observed in the lateral septum, the OVLT, the subfornical organ, the central nucleus of the amygdala (CeA), the medial amygdala, the proximal supraoptic nucleus of the hypothalamus (SON), the paraventricular nucleus of the hypothalamus (PVN), and paraventricular thalamus. In CSN stimulated rats anesthetized with NEM, *fos*-IR was also observed in the lateral hypothalamus and the pattern of staining was altered in the SON, the PVN and the CeA. In general, URE anesthetized rats showed more background *fos*-IR and slightly higher stimulated *fos*-IR levels than NEM anesthetized rats. In addition to the regions listed above, CSN stimulated, URE anesthetized rats also demonstrated *fos*-IR in the diagonal band of Broca. These results indicate that, while surgical stress and anesthesia can affect the levels of *fos*-IR, CSN stimulation influences *fos*-IR differentially in the hypothalamus and the basal forebrain. (Supported by HL 4546 and HL 14388).

392.9

C-FOS IMMUNOREACTIVITY IN THE FOREBRAIN AND THE MEDULLA AFTER INTRAVENOUS HYPERTONIC SALINE INFUSION IN ANESTHETIZED RATS. M.L. Weiss* and M.J. Kenney. Dept of Anatomy and Physiology, Kansas State University, Manhattan, KS 66506-5602.

Intravenous hypertonic saline (HTS) infusion increases arterial blood pressure, but the mechanism of this pressure increase is not well established. We employed c-fos immunocytochemistry as a marker for neurons activated by iv HTS in chloralose-anesthetized rats. Mean arterial pressure (MAP) and heart rate (HR) were recorded continuously during the 30 min intravenous infusion of 2.5M NaCl (10µl/100gBW/min) and the subsequent 20-40 min period. HTS infusion increased MAP (24±4 mm Hg, N=7) but did not affect HR. In the forebrain, many c-fos immunoreactive (IR) neurons were found in areas involved with cardiovascular regulation: the anteroventral wall of the third ventricle (AV3V) region (median preoptic nucleus, organum vasculosum lamina terminalis, periventricular region), the subfornical organ, and the hypothalamic magnocellular neuroendocrine system. In the medulla, c-fos IR cells were found within the area postrema, the medial nucleus of the solitary tract and within the ventral lateral medulla after iv HTS. While there was no difference in the pattern of c-fos IR in the brain between animals injected ip or iv, there was more intense staining after ip injection. We conclude that neurons within sites involved with blood pressure regulation contain c-fos IR after iv HTS. This technique may provide insight into the mechanisms responsible for the blood pressure increases seen during peripheral hyperosmolality.

392.10

INCREASED *c-FOS* STAINING IN MULTIPLE HYPOTHALAMIC REGIONS AFTER SLOW HEMORRHAGE. M.L. BLAIR*[1] AND J.A. OLSCHOWKA[2]. Departments of Physiology[1] and Neurobiology and Anatomy[2], University of Rochester School of Medicine. Rochester, NY 14642.

Immunohistochemical localization of the oncoprotein Fos was employed to identify hypothalamic regions which respond to a slow hemorrhage (HEM). Conscious male Sprague-Dawley rats were subjected to blood withdrawal, 0.8 ml/kg/min, to a cumulative loss of 12.8 ml/kg bwt. HEM caused a transient decrease in mean arterial pressure (93±1 mm Hg to 76±6 mm Hg, $p < .05$) and a sustained 3-fold increase in plasma renin activity ($p < .01$). The hypothalamic Fos distribution of HEM rats (n=5) was compared with that of control rats (n=5) for which arterial pressure was measured but no blood was withdrawn. HEM rats showed a significant increase in the number of Fos immunoreactive cells ($p < .05$), as compared with control rats, in the magnocellular and parvocellular paraventricular nuclei, supraoptic nucleus, ventromedial nucleus, dorsomedial nucleus, arcuate nucleus, posterior hypothalamic nucleus, and supramammillary area, but not the posterior lateral hypothalamus. In conclusion, Fos immunoreactivity indicates that neuronal activity is increased by HEM in multiple hypothalamic regions which include, but are not restricted to, those known to be involved in regulation of extracellular fluid volume and arterial pressure. Supported by PHS NS29400, PHS S7RR05403 and Amer. Heart Assoc. (N.Y. State affiliate) 89-032.

392.11

CARDIOVASCULAR EFFECTS OF MICROINJECTION OF KAINIC ACID OR BICUCULLINE INTO THE DORSOMEDIAL OR PARAVENTRICULAR HYPOTHALAMIC NUCLEUS IN ANESTHETIZED RATS. J. A. DiMicco*, Dept. Pharmacol. & Toxicol., Ind. Univ. Sch. of Med., Indianapolis, IN 46202.

We have previously reported that microinjection of either $GABA_A$ receptor antagonists, such as bicuculline methiodide (BMI), or excitatory amino acid receptor agonists into the dorsomedial hypothalamic nucleus (DMH) elicits marked tachycardia and modest increases in arterial pressure in anesthetized and conscious rats. However, others have reported similar effects upon microinjection of BMI into the neighboring paraventricular nucleus (PVN). To assess whether the cardiac effects of microinjections into the DMH might be a consequence of diffusion to the PVN, BMI 5 pmol/15 nL (5 rats) or kainate (KA) 1-3 pmol/15 nL (5 rats; dose kept constant in a given experiment) was injected at each of three sites in random order at 30-40 min intervals in urethane-anesthetized rats. Target stereotaxic coordinates corresponded to (1) the PVN, (2) the DMH, and (3) an intermediate site equidistant to each, and were confirmed histologically at the end of each experiment. Mean maximal increases in heart rate (ΔHR; beats/min ± SEM) and changes in arterial pressure (ΔBP; mmHg ± SEM) evoked at each site were:

	DMH		Intermediate		PVN	
Agent	ΔHR	ΔBP	ΔHR	ΔBP	ΔHR	ΔBP
BMI	64 ± 7	7 ± 3	30 ± 10	-3 ± 2	14 ± 3	-2 ± 2
KA	59 ± 6	1 ± 3	28 ± 8	-1 ± 3	8 ± 6	2 ± 2

These data suggest that the tachycardia observed after microinjection of these agents into the DMH is not attributable to diffusion or spread to the neighboring PVN. Rather, the tachycardia seen after microinjection of these agents at higher doses and in larger volumes into the PVN may result from diffusion to the DMH, a region recently implicated in the generation of the cardiovascular response to stress. (Supported by USPHS Grant NS 19883)

392.12

THE INFLUENCE OF HYPOTHALAMIC NUCLEAR STIMULATION ON ROSTRAL VENTROLATERAL MEDULLA CARDIOVASCULAR NEURONS OF THE RAT. T.P. Wong, Y.S. Chan* and T.M. Wong. Department of Physiology, Faculty of Medicine, The University of Hong Kong, Sassoon Road, Hong Kong.

The contribution of the hypothalamic nuclei to the arterial blood pressure and the spontaneous activity of cardiovascular neurons in the rostral ventrolateral medulla (RVL) was investigated in pentobarbitol-anesthetized rats. Extracellular activities of RVL neurons and arterial blood pressure were recorded simultaneously during the experiments. Only RVL neurons which exhibited cardiac-locked activity as well as barosensitivity to phenylephrine were classified as cardiovascular neurons. Electrical microstimulation of the paraventricular nucleus of hypothalamus (PVH) increased the discharge rate of 90% of the RVL cardiovascular neurons tested. About 60% of these was accompanied by pressor response while 30% was associated with depressor response. Electrical microstimulation of the dorsomedial nucleus of hypothalamus (DMH) also elicited changes in both the discharge rate of RVL cardiovascular neurons and arterial blood pressure. Most neurons also exhibited an increase in discharge rate in response to the stimulation of DMH. Microinjection of L-glutamate, an amino acid which is known to stimulate the soma without exciting the axons of passage, into PVH or DMH also elicited alterations in both the firing rate of RVL cardiovascular neurons and arterial blood pressure changes. These results indicate that RVL cardiovascular neurons, which mediate the baroreceptor reflex, are under the influence of inputs from the hypothalamic nuclei.
(Supported by grants from the Croucher Foundation and U.P.G.C.)

392.13

WITHDRAWN

392.14

DISINHIBITION OF POSTERIOR HYPOTHALAMIC NEURONS ELICIT SPLANCHNIC MICROVASCULAR CONSTRICTION. R.W. Stremel* and I.G. Joshua. Department of Physiology & Biophysics, School of Medicine, University of Louisville, Louisville, KY 40292.

Inhibition of γ-aminobutyric acid (GABA) receptors within the posterior hypothalamus (PH) elicits an "exercise-like" redistribution of blood flow. This redistribution should be reflected at the microvessel level and thus splanchnic vasoconstriction is predicted. To test this, we observed microvessels of the intestinal serosa during PH activation ($GABA_A$ receptor antagonist, bicuculline methiodide (BMI)). Sprague-Dawley rats, anesthetized with pentobarbital (50 mg/kg, ip), were prepared for video microscopic observation of microvessels within intestinal smooth muscle suspended in an oxygenated, pH balanced tissue bath. Arterioles of 10 to 30 μ were observed before and after microinjection of 15 - 45 nl of BMI (5 ng/nl) into the region of the PH. The expected increases in blood pressure (44% ± 10, average ± sem increase in mean arterial pressure), heart rate and locomotor activity were observed, as was vasoconstriction of intestinal smooth muscle microvessels (-35% ± 2, mean ± sem reduction in diameter). These results support the concept that the PH is responsible for eliciting a "central command" for exercise and a redistribution of blood flow at the microvessel level. Supported by American Heart Association.

392.15

ROLE OF CORTICOTROPIN-RELEASING FACTOR IN MEDIATING CARDIOVASCULAR RESPONSES TO BLOCKADE OF $GABA_A$ RECEPTORS IN THE HYPOTHALAMIC DORSOMEDIAL NUCLEUS. F. Onat, A. Dedeoğlu and L.A. Fisher*. Department of Pharmacology, College of Medicine, University of Arizona Health Sciences Center, Tucson, AZ 85724.

Disruption of GABAergic neurotransmission in the hypothalamic dorsomedial nucleus (DMN) elicits cardiovascular changes that are characteristic of the defense reaction, a patterned response to stressful stimuli. Likewise, corticotropin-releasing factor (CRF) acts within the central nervous system (CNS) to produce stress-like changes in cardiovascular function. CRF-containing neurons are located within the DMN and hence the purpose of the present study was to test the hypothesis that blockade of $GABA_A$ receptors in the DMN produces cardiovascular activation through disinhibition of CRF-containing neurons. All experiments were performed in conscious, unrestrained male Sprague-Dawley rats (220-250 g) instrumented with intracerebroventricular (icv) and/or intraparenchymal (aimed at the DMN) guide cannulas for drug administration and iliac arterial catheters for direct measurement of arterial pressure (AP) and heart rate (HR). Icv administration of bicuculline methiodide (BMI), a $GABA_A$ receptor antagonist (0.01-0.3 nmol), produced dose-related increases in AP (5-17 mm Hg) and HR (10-70 beats/min) that were not altered appreciably by pretreatment with the CRF receptor antagonist, α-helical CRF_{9-41} (9 nmol, icv). When injected into the DMN (250 nl), very small doses of BMI (0.0025-0.01 nmol) were required to elicit robust changes in AP (3-13 mm Hg) and HR (20-110 beats/min) that were attenuated by 50-70% after pretreatment with α-helical CRF_{9-41} (9 nmol, icv). These results suggest that GABAergic mechanisms within the DMN tonically inhibit CRF-containing neurons and that BMI-induced cardiovascular elevations of AP and HR are in part mediated by the release of CRF.

392.16

α_2-ADRENERGIC REGULATION OF HYPOTHALAMIC PROOPIOMELANOCORTIN (POMC) mRNA AND BLOOD PRESSURE (BP) IN THE RAT.
M.N. Scanlon, S-J. Li, E. Lazar-Wesley, K. Varga, N.S. Gantenberg and G. Kunos*. Dept. of Pharmacol. & Toxicol, Med. Coll. of Virginia, Richmond, VA 23298

Several lines of evidence indicate that activation of central α_2-adrenergic receptors (α_2AR) by clonidine or α-methyldopa (αMD) reduces BP in part through the release of ß-endorphin and subsequent stimulation of opiate receptors in the brainstem. Lesion studies suggest that the source of the ß-endorphin released is neurons that originate in the arcuate nucleus of the mediobasal hypothalamus (MBH). To obtain an indicator of the activity of these neurons, we have measured steady state levels of POMC mRNA in the MBH of rats, and correlated drug-induced changes in BP measured by the tail cuff technique with changes in POMC mRNA levels, measured by a DNA excess solution hybridization assay as well as by in situ hybridization histochemistry. αMD, 200 mg/kg/day i.p. for 4 days, reduced BP and significantly increased POMC mRNA levels compared to vehicle-treated controls, in an area of the MBH limited to the arcuate nucleus. In animals treated with yohimbine (2 mg/kg/day) + αMD, there was no change in BP, and POMC mRNA levels were similar to that in controls. In animals treated with naltrexone (2 mg/kg/day) + αMD, BP remained unchanged, but POMC mRNA levels were increased to the same extent as with αMD alone. Hydralazine, 2 mg/kg/day for 4 days, decreased BP but did not modify POMC mRNA levels. We conclude that the opioid-mediated component in the hypotensive action of αMD is related to an α_2AR-mediated activation of endorphinergic neurons in the arcuate nucleus.

392.17

HYPOVOLEMIA INCREASES THE LEVELS AND THE EXTENT OF CO-LOCALIZATION OF NEUROTENSIN AND ENKEPHALIN mRNAS IN HYPOTHALAMIC CRH-CONTAINING NEURONS. G.Sanchez-Watts*, S.M. Tanimura & A. G. Watts. NIBS Program & Dept. of Biol. Sci., USC, Los Angeles CA 90089.

Disturbances of fluid homeostasis lead to widespread alterations in neuropeptide gene expression suggesting that altered peptidergic function makes important contributions to the different components of fluid balance regulation. Iso-osmotic hypovolemia caused by subcutaneous (sc) injections of polyethylene glycol (PEG) leads to a well-documented series of physiological and behavioral responses, including increased vasopressin (VAS) and corticosterone secretion, hematocrit, thirst and sodium appetite. Here, using *in situ* hybridization (ISH), we report the time-course and extent of neuropeptide and immediate-early gene mRNA response in the forebrain to PEG-induced hypovolemia. We have also used combined digoxigenin- and ^{35}S-labeled cRNA probes to investigate the effects of hypovolemia on the colocalization of peptide mRNAs in the hypothalamic paraventricular nucleus (PVH). Groups of male SD rats (280-300g BW) were anesthetized and injected sc with 5mls of either 30% (w/v) PEG in 0.9% saline or vehicle alone. At various times up to 24h after PEG injections animals were anesthetized and perfusion-fixed. Frozen 15µm coronal sections were cut through the forebrain and hybridized with a cRNA probe for CRH, proenkephalin (ENK), c-fos, neurotensin (NT), oxytocin or VAS. Sections from animals perfused 18-20h after PEG were also hybridized with a digoxigenin-UTP labeled (DIG) cRNA probe for CRH cocktailed with a ^{35}S-UTP labeled cRNA probe for either NT, ENK or VAS. Analysis of the dipped slides and X-ray autoradiographs showed significant increases in the levels of c-fos, CRH, NT and ENK mRNAs in the medial parvicellular PVH. No increases were seen in the LHA. Increased c-fos mRNA levels were also seen in the supraoptic nucleus and other limbic forebrain regions. Combined DIG and ^{35}S ISH showed a significant percentage of the increased levels of ENK and NT mRNA occurred in CRH neurons, suggesting that increased peptidergic colocalization in the PVH regulated part of the neuroendocrine response to hypovolemia. These results are also in marked contrast to changes in PVH mRNA levels seen after hyperosmotic dehydration, when decreases in CRH mRNA occur without concomitant increases in NT or ENK gene expression. (Supported by NS 29728)

392.18

VASOPRESSIN-MEDIATED PRESSOR RESPONSE TO THE INTRA-VENTRICULAR NORADRENALINE IN CONSCIOUS RATS. F.M.A. CORRÊA* Dept. Pharmacol., School Medicine Ribeirão Preto, Univ. of São Paulo, Ribeirão Preto, SP, Brazil, 14049-900.

The i.c.v. injection of noradrenaline (NA) causes blood pressure increase in unanesthetized rats that is blocked by i.v. injection of vasopressin antagonists (Corrêa et al., J. Neuropharmacology 24: 831-838, 1985). Presently we observed similar responses when NA was injected into the III or IV ventricles and dose-effect curves were generated. Similarly to the lateral ventricle, responses were blocked by i.v. injection of a vasopressin antagonist suggesting a common mediation via vasopressin release into the systemic circulation. Obstruction of selected ventricular spaces with cream plugs were performed to identify the ventricular area involved in the pressor response to centrally injected NA. NA injections were followed by injections of Evan's blue dye for macroscopic observation of the distribution of drug within ventricles. Obstructions of the III ventricle or the aqueduct blocked the pressor response to NA injected into the lateral ventricle. Obstructions of the IV ventricle, although less conclusive, indicated that the access to the ventral portion of the brainstem is essential for the response.

Technical support: Idalia I.B. Aguiar
Grants: FAPESP, CNPq

392.19

A CENTRAL ACTION OF ADENOSINE IN A HYPOTHALAMICALLY EVOKED CARDIOVASCULAR RESPONSE. K.M. Spyer*, J.H. St. Lambert, M.S. Dawid-Milner, L. Silva-Carvalho., Dept. of Physiology, Royal Free Hospital School of Medicine, London NW3 2PF.

In pentobarbitone anaesthetised cats and rats, stimulation in restrictive sites in the hypothalamus elicits a characteristic cardiovascular response that is an integral component of the defense reaction. The evoked rise in arterial pressure is reduced significantly (47.43%, $p<0.01$ in the cat; 33.53%, $p<0.05$ in the rat) by the intravenous injection of the A_1 adenosine antagonist DPCPX by not by 8-SPT; the former enters the CNS but not the latter. In the cat, DPCPX also reduces the pressor response, but not the respiratory changes, to carotid body stimulation and facilitates the baroreceptor reflex lowering of arterial pressure. These observations are consistent with adenosine release being involved in mediating these cardiovascular responses. The pattern of action of the A_1 antagonist is suggestive of a site of action within the nucleus tractus solitarius, and this possibility is under investigation.

Study supported by Wellcome Trust

AUTONOMIC REGULATION: CENTRAL GASTROINTESTINAL CONTROL

393.1

LATERAL HYPOTHALAMIC LESIONS PRODUCED BY N-METHYL-D-ASPARTATE OR IBOTENIC ACID INDUCE GASTRIC EROSIONS IN RATS. C. V. Grijalva*, J. Rios-Jimenes and J. Landeira-Fernandez. Dept. of Psychol., Univ. of California, Los Angeles, CA 90024

Bilateral electrolytic lesions of the lateral hypothalamus (LH) produce gastric erosions. However, electrolytic lesions destroy both neurons as well as axonal fibers of passage. Because several fiber tracts course through the LH area it is unclear whether the formation of gastric erosions following LH lesions is due to damage to intrinsic neurons or to the interruption of certain fibers systems. The present study was conducted to examine whether damage to intrinsic LH neurons induced by microinfusion of N-methyl-D-aspartate (NMDA) or ibotenic acid (IBO) could also produce gastric erosions. In Experiment 1, LH lesions were produced by IBO (10 µg/µl) or electrolytic current (1.2 mA, 10 sec). Rats receiving electrolytic lesions displayed motor impairments while those receiving IBO lesions did not. Both electrolytic and IBO lesions produced eating deficits and gastric erosions 24 h after surgery. In Experiment 2, bilateral LH lesions were produced by NMDA (20 µg/µl). Again, both electrolytic and NMDA lesions produced gastric erosions and eating deficits but only electrolytic lesions induced motor impairment. In Experiment 3, the extent of LH neuronal damage was varied by infusing different doses of NMDA (20 µg/µl or 10 µg/µl). Both doses reliably produced gastric ulceration and the magnitude of the effect was dose dependent. It is concluded that damage to intrinsic LH cell bodies are responsible for the induction of gastric erosion formation.

393.2

N-METHYL-D-ASPARTATE LESIONS IN THE SUBSTANTIA NIGRA BUT NOT IN THE VENTRAL TEGMENTAL AREA PRODUCES GASTRIC EROSIONS IN THE RAT. J. Landeira-Fernandez* and C. V. Grijalva. Department. of Psychology, University of California, Los Angeles, CA 90024

Bilateral electrolytic lesions in the substantia nigra (SN) or ventral tegmental area (VTA) lead to stomach ulceration (Roland & Grijalva, Brain Res, 1993, 605, 110-120; Ray, Henke & Sullivan, Physiol and Behav, 1988, 42, 359-364). However, electrolytic lesions destroy both neurons as well as axonal fibers of passage. Recently we showed that damage to intrinsic lateral hypothalamic (LH) neurons are involved in the formation of gastric erosions (Grijalva, Rios-Jimenes & Landeira-Fernandez, Soc. Neurosci. Abstr. 1993). Because descending fibers from the LH travel through the region of the SN and the VTA (Berk & Finkelstein, Brain Res Bull, 1982, 8, 511-526) it is unclear whether the formation of gastric erosions following SN and VTA lesions are due to damage to intrinsic neurons or due to the interruption of the descending LH fibers systems. The present study was conducted to examine whether damage to intrinsic SN and VTA neurons by microinfusion of N-methyl-D-aspartate (NMDA, 20 µg/µl) could also produce gastric ulceration. A control group received microinjections of the vehicle either in the SN or VTA. It was found that bilateral NMDA lesions in the SN but not in the VTA reliable produced gastric erosions 24 h after surgery. These results indicate that intrinsic neurons in the SN play an active role in the development of gastric mucosal injury.

393.3

VIRAL TRANSNEURONAL LABELING OF THE VISCERAL NEURAXIS AFTER LONG SURVIVAL TIMES FOLLOWING DIFFERENT ALIMENTARY STRUCTURE INJECTIONS. M. Yang*, X. Zhao and R.R. Miselis, Dept. of Animal biology, School of Veterinary Medicine, University of Pennsylvania, Phila., PA 19104

Brainstem labeling by Bartha pseudorabies virus (PRV) following long survival periods after injections of the stomach, esophagus or cecum retains a high degree of viscerotopic labeling of primary motoneuronal pools. Secondary and /or tertiary labeling within the nucleus of the solitary tract (NTS) progressively increases with longer survival times with the stomach injection producing the most labeling. Differences in the number of neurons labeled and their topography are also retained over long survival periods. Parvocelluar subnuclei of paraventricular n. of the hypothalamus are virally labeled following the above injections with only small differences in topography. Greater differences in the number of neurons labeled occurs in the tuberomammillary n., juxtapeduncular n., dorsal medial and lateral hypothalamus, central n. of the amygdala, substantia innominata, preoptic area, bed n. of the stria terminalis, and insular cortex. In all cases the labeling within any nucleus was greatest with the stomach injections and least with the cecum. Labeling progressed from normal morphological appearance to the gradual occurrence of cytopathology. In most cases cytopathology did not occur before upstream labeling of another order of neurons began to label indicating specific transsynaptic passage of PRV. Supported by GM 27739.

393.4

MIDBRAIN LABELING FOLLOWING BARTHA PSEUDORABIES VIRUS (PRV) INJECTIONS OF ALIMENTARY STRUCTURES. R. R. Miselis*, X. Zhao and M. Yang, Dept. of Animal biology, School of Veterinary Medicine, University of Pennsylvania, Phila., PA 19104

Analysis of midbrain labeling by PRV following injections of the stomach, esophagus and cecum were focused on in this study. Rats were injected with PRV and sacrificed at 48, 60, 72, 84, 90, and 96 hours post injection. Initial labeling of the central gray occurs at 72 hrs p.i. following stomach or esophagus injections and at 84 hrs after cecal injections. Subsequently labeling increases throughout the rostro-caudal extent of central gray and occurs in the dorsal raphe and ventrolateral region of the mesencephalic reticular nucleus and lightly in the ventral tegmental area. Esophageal injections resulted in distinct pattern differences in labeling of the central gray and greater labeling of the caudal linear raphe nucleus, retrorubral field and ventral tegmental area. For all organs injected labeling continues into the lateral aspects of the pontine reticular formation. The earliest time of labeling of neurons of the midbrain suggest that they could be second to third order neurons from the dorsal or ventral vagal complex. These data indicate that midbrain neurocircuitry is more involved in esophageal control than either gastric or cecal control. Supported by GM 27739.

393.5

TRANSNEURONAL LABELING OF NEURONS IN THE BRAIN AFTER INJECTION OF PSEUDORABIES VIRUS (PRV) INTO THE RAT ESOPHAGUS. X. Bao, R. Barrett and S.M. Altschuler*. Children's Hospital of Phila., Univ. of Pa. Sch. of Med., Phila., PA 19104.

Premotor neurons (PMNs), through contact with both afferents and motoneurons, initiate and control swallowing. The location and connectivity of PMNs innervating the esophagus were determined using a Bartha strain of PRV. In 30 rats, PRV injections were made into the esophagus. Following a 48-120 h survival, brain sections were processed immunocytochemically for PRV. Neuronal labeling was limited to the compact formation (c) of the NA for survival times of 48-52 h. At 60-62 h survivals, PRV-labeled 2nd order neurons (PMNs) were localized to the central subnucleus (cen) of NTS. At longer survivals, labeling occurred in adjacent subnuclei of the NTS, PTI, dorsal and ventral medullary reticular formation, spinal trigeminal complex, raphe nucleus and forebrain. Esophageal PMNs are localized to the cenNTS, the site of termination of esophageal vagal afferents, and have direct synaptic contact with cNA motoneurons. The pattern of labeling at longer survivals suggests widespread CNS control over esophageal peristalsis. Supported by NIH grant DK-44487.

393.6

NITRIC OXIDE SYNTHASE INHIBITION REDUCES THE EFFECT OF SUBSTANCE P MICROINJECTED INTO THE NUCLEUS RAPHE OBSCURUS ON INTRAGASTRIC PRESSURE IN THE RAT. Z.K. Krowicki* and P.J. Hornby, Dept. of Pharmacology and Experimental Therapeutics, Louisiana State University Medical Center, New Orleans, LA 70112.

We have demonstrated that the inhibitory effect of substance P (SP) microinjected into the nucleus raphe obscurus (NRO) on intragastric pressure (IgP) can be completely abolished by bilateral vagotomy but only slightly reduced by atropine (Krowicki and Hornby, Soc. Neurosci. Abstr., 1277, 1992). Since nitric oxide (NO) may be an inhibitory vagal transmitter to the stomach (Allescher et al., Am. J. Physiol. 262:G695-G702, 1992) we tested the hypothesis that the gastric inhibitory effect of SP (135 pmol), microinjected into the NRO, could be abolished by N^G-nitro-L-arginine (L-NAME; 10 mg/kg, i.v.) alone, or in combination with systemic cholinergic blockade by atropine (1 mg/kg i.v.). The IgP inhibition was quantified by measuring the area of the response (cm^2) below the baseline using a computer-based imaging system. The decreases in IgP evoked by microinjection of SP into the NRO in two groups of animals were -1.43±0.24 (n=7) and -1.46±0.33 (n=8). The response to SP microinjected into the NRO 15 min after L-NAME or atropine was significantly reduced to -0.74±0.19 (n=7) and -0.61±0.18 (n=8), respectively. Microinjection of SP into the NRO 15 min after atropine with subsequent L-NAME or after L-NAME with subsequent atropine, did not evoke any changes in IgP when compared with vehicle. These findings indicate a role of NO in the decrease in IgP induced by microinjection of SP into the NRO. Supported by PHS grant DK42714.

393.7

EVIDENCE FOR AN INDEPENDENT ACTION OF SEROTONIN AND SUBSTANCE P MICROINJECTED INTO THE NUCLEUS RAPHE OBSCURUS (NRO) ON INTRAGASTRIC PRESSURE IN THE RAT. P.J. Hornby* and Z.K. Krowicki, Dept. of Pharmacology, Louisiana State University Medical Center, New Orleans, LA 70112.

Serotonin (5-HT) microinjected into the NRO increases and substance P (SP) decreases intragastric pressure (IgP) in the rat (Krowicki and Hornby, J. Pharmacol. Exp. Ther., 265:468-476, 1993; Soc. Neurosci. Abstr., 1277, 1992). Since 5-HT is co-localized with SP we attempted to test whether rapid sequential administration of these transmitters into the NRO would alter their effect on IgP. Intragastric pressure was monitored in α-chloralose anesthetized rats before and after sequential (30 sec intervals) microinjections of vehicle, 5-HT (0.6 and 6.0 nmol) and SP (135 pmol) into the NRO in a volume of 60 nl. Changes in IgP were quantified as areas of the response below (-) or above (+) baseline levels. The results (cm^2) are shown in two tables below as avg±SEM (n). The substances injected first are given in left column.

Treat.	5-HT 0.6	SP
Veh.	0.4±0.1(5)	-1.7±0.5(9)*
5-HT0.6	0.3±0.1(3)	-1.6±0.4(4)*
SP	0.4±0.1(3)	-0.6±0.3(7)

Treat.	5-HT 6.0	SP
Veh.	1.0±0.2(7)*	-1.7±0.5(9)*
5-HT6.0	0.7±0.3(2)	-1.6±0.4(6)*
SP	1.2±0.4(6)*	-0.6±0.3(7)

* Significantly different from control

Our results indicate that 5-HT and SP act independently in the NRO to affect IgP. Supported by PHS grant DK41714 and A.P. Sloan Foundation.

393.8

MICROINJECTION OF L-ARGININE AND S-NITROSO-AMINO-PENICILLAMINE (SNAP) INTO THE ROSTRAL DORSAL MOTOR NUCLEUS OF THE VAGUS (DMV) PRODUCES AN INCREASE IN GASTRIC MOTILITY. W.H.Panico, R.A. Travagli, A. Raines*, D.M. Armstrong, C.Nguyen, S.B. Benjamin, and R.A.Gillis, Georgetown Univ. Medical Center, Washington D.C. 20007

Electrophysiological studies performed in our laboratory with the use of the patch clamp technique on thin slices have shown an excitation of DMV neurones upon treatment with drugs that produce NO (L-Arginine and SNAP). The purpose of the present study was to confirm this finding in an in vivo cat preparation. For this purpose L-Arginine (40nl of a 2M solution) and SNAP (60nl of a 0.5M solution) were microinjected unilaterally into the DMV of chloralose-anesthetized cats while monitoring antral and pyloric motility using extraluminal force transducers. Microinjections of L-Arginine caused increases in motility of both the antrum and the pylorus. Minute motility index (MMI) of the antrum increased from 1.3±1.1 to 9.4±1.2 ($p<0.05$, N=8), while MMI of the pylorus increased from 3.0±1.6 to 9.7±2.7 ($p<0.05$, N=8). Similar increases were observed after microinjection of SNAP. Ipsilateral vagotomy was effective in abolishing the increases in MMI elicited by NO producing drugs. These results suggest that NO acting on DMV neurons can evoke vagally-mediated increases in gastric motility.

393.9

FUNCTIONAL HETEROGENEITY OF NEURONS OF THE DORSAL MOTOR NUCLEUS OF THE VAGUS (DMNV) R. Fogel*, W.E. Renehan and X. Zhang. GI Div., Henry Ford Hosp., Detroit MI 48202

Stimulation of gastric and duodenal mechanoreceptors initiates a number of vagally-mediated changes in gastrointestinal function including gastric and intestinal motility and gastric acid secretion. It is currently unknown 1) whether individual DMNV neurons are affected by distention of both the duodenum and stomach and 2) whether all DMNV neurons projecting to the abdomen respond to distention in the same manner. To enhance our understanding of the function of the DMNV, we investigated the response of DMNV neurons to gastric and duodenal distention. Glass micropipettes filled with 2% Neurobiotin (Vector Labs) were used to label individual physiologically-characterized DMNV neurons. Neurons were reconstructed using the Eutectics Neuron Tracing System. Fifty one DMNV neurons were studied: 3 cells were unaffected by distention; 8 neurons responded to only one stimulus; and 40 neurons responded to both stimuli. Twenty eight of the 40 neurons had an inhibition of firing rate, 2 increased firing rate and 10 neurons had a mixed response- excitation by one stimulus, inhibition by the other. Forty nine of 51 neurons sent axons out of the brainstem. The morphology of the neurons that were inhibited by both stimuli was compared to the group with a mixed response. No significant differences in soma-dendritic morphology were detected. These results support a functional heterogeneity of DMNV neurons. The nature of the response to duodenal and gastric distention is not associated with soma-dendritic morphology.

393.10

TUMOR NECROSIS FACTOR (TNF_α) IN THE DORSAL VAGAL COMPLEX (DVC) SUPPRESSES GASTRIC MOTILITY G.E.Hermann* and R.C.Rogers, Dept.of Physiology, College of Medicine, Ohio State University, Columbus, OHIO 43210

TNF_α is one of the proinflammatory cytokines released in response to antigenic challenge. TNF_α has been demonstrated to increase plasma CORT levels, increase body temperature, suppress appetite and cause nausea and vomiting when injected systemically. Thus, it appears that TNF_α may be responsible for provoking many of the autonomic signs associated with critical infectious illness. Receptors for TNF_α have been localized within the brainstem. The DVC are brainstem nuclei which participate in vagal regulation of gastric function and have fenestrated capillaries which may allow entry of large "afferent" peptide signals from the bloodstream. Our present studies demonstrate that microinjection of TNF_α (20 femtomoles) into the DVC significantly suppresses gastric motility for prolonged periods of time (in excess of 30 min) even in TRH-stimulated preparations.

Resolution of infectious challenges requires coordinated patterns of systemic responses by the host involving interactions of immune-neuro-endocrine systems. TNF_α may be one of the cytokines that provide afferent information from the immune system to the CNS which can elicit appropriate physiologic or homeostatic responses. (Supported, in part, by NIH NS 30803 to RCR)

393.11

PANCREATIC POLYPEPTIDE IN THE DORSAL VAGAL COMPLEX STIMULATES GASTRIC ACID SECRETION AND MOTILITY. D.M. McTigue and R.C. Rogers* Dept. of Physiology College of Medicine, Ohio State University, Columbus OH 43210

Pancreatic polypeptide (PP), an endocrine hormone released from the pancreas during all phases of digestion, has specific receptors located within the dorsal vagal complex (DVC) in regions known to contain fenestrated capillaries. Since the DVC participates in vagal regulation of gastric activity, the present studies were designed to examine the effect of PP microinjected directly into this region on gastric function. Rats were equipped for recording of either gastric acid secretion or gastric antral motility. Following establishment of baseline values, 20 nl of 0.9% saline or PP [4.0 pmol, 0.04 pmol, or 0.004 fmol] was microinjected into the DVC and acid secretion or antral motility was monitored for 2 hours. Microinjection of PP into the DVC resulted in a dose-dependent increase in gastric acid secretion and gastric motility that was sensitive to atropine (0.2mg/kg) and vagotomy. Thus, PP placed into the DVC increases gastric acid secretion and motility by stimulating vagal cholinergic pathways. Preliminary data from our laboratory suggest that PP administered intravenously also enhances gastric acid secretion. These results provide an interesting model in which a hormone produced in the periphery interacts directly with brainstem autonomic nuclei leading to altered gastric functions. (NS 30803 to RCR)

393.12

TUMOR NECROSIS FACTOR (TNF_α) IN THE MEDIAL NUCLEUS TRACTUS SOLITARIUS (NTS) DECREASES ARTERIAL PRESSURE, HEART RATE, AND EFFERENT SYMPATHETIC NERVE ACTIVITY. C.M. Heesch*, G.E. Hermann and R.C. Rogers, Dept. of Physiology, College of Medicine, The Ohio State University, Columbus, OHIO 43210.

TNF_α, a cytokine produced by macrophages during septic shock and following myocardial infarction, is a potential endogenous mediator of untoward cardiovascular effects. Intravenous administration of TNF_α results in systemic hypotension in animals and humans and a peripheral vasodilatory action has been described. The current study was performed to determine if a central nervous system (CNS) effect of TNF_α on cardiovascular pathways could contribute to the cardiovascular depressant actions. Unilateral microinjections of TNF_α (20-30 femtomoles in 20-30 nl) into the medial NTS resulted in significant decreases in mean arterial pressure (-17$\pm$ 5.5 mmHg) and heart rate (-48$\pm$ 10.8 bpm) in 6 male Long-Evans rats. In four rats efferent renal sympathetic nerve activity was also recorded and decreased by 22$\pm$ 5.0 % following microinjection of TNF_α into the NTS. Thus, in addition to vasodilatory effects on vascular smooth muscle, circulating TNF_α may also depress autonomic function through an action at central nervous system sites with an incomplete blood brain barrier. (Supported by NIH HL-36245 to CMH and NIH NS-30803 to RCR)

393.13

PREPRO-TRH-(160-169) POTENTIATES TRH IN THE DMN-INDUCED STIMULATION OF GASTRIC ACID SECRETION . H. Yang* and Y. Taché. CURE/VA Wadsworth Medical Center, Dept. of Medicine and Brain Research Institute, UCLA, Los Angeles, CA 90073.

Prepro-TRH-(160-169) (Ps4) is distributed in similar area as TRH and is generated from the processing of TRH prohormone. Ps4 potentiates TRH-induced TSH release in quartered rat anterior pituitaries (Proc. Natl. Acad. Sci. USA, 87:4439, 1990). The possibility that Ps4 modulates TRH action in medullary nuclei was studied by co-injection of Ps4 with TRH into the dorsal motor nucleus of the vagus (DMN) or the nucleus ambiguus (Amb). In urethane anesthetized rats, gastric acid secretion (GAS) was determined by flushing the stomach every 10 min and heart rate (HR) was measured with a polygraph through a PE-90 cannula inserted into the cervical artery. Peptides were microinjected using a glass pipette. TRH (50 ng/50 nl) microinjected into the DMN induced an increase in GAS which was dose-dependently potentiated by co-injection of Ps4. The net GAS (μmol/60 min) were 13.5 $\pm$ 3.1 after TRH alone (n=13) and 15.4 $\pm$ 3.6 (n=7), 23.8 $\pm$ 1.2 (n=8) and 38.2 $\pm$ 8.7 (n=8) in groups in which Ps4 was co-injected with TRH at 100, 150 and 200 ng respectively. Microinjection of Ps4 alone (200 ng/167 pmol) did not influence basal GAS (net GAS was 2.0 $\pm$ 1.8, n=7). By contrast, co-injection of TRH with prepro-TRH-(178-199) in the DMN (438 ng/167 pmol) did not modulate TRH induced GAS (15.4 $\pm$ 3.3, n=8). When Ps4 (200 ng) was co-injected with TRH into the Amb, it did not modulate TRH (50 ng) induced GAS (net μmol/60 min: 19.9 $\pm$ 5.3 TRH alone, n=9, vs 18.5 $\pm$ 3.7, TRH + Ps4, n=10). However, three min after microinjection of TRH alone, the HR (beat/min) was 234 $\pm$ 86 (n=8) whereas it was 326 $\pm$ 10 in TRH + Ps4 group (n=9). These results indicate that Ps4 potentiates the action of TRH in the DMN to stimulate GAS and its effect is site and peptide specific.

393.14

INTRACISTERNAL (IC) INJECTION OF PANCREATIC POLIPEPTIDE (PP) STIMULATES GATRIC SECRETION THROUGH THE VAGUS NERVE IN RATS. T. Okumura, T. N. Pappas* and J. L. Taylor. Department of Surgery and Gastroenterology, Duke University Medical Center, Durham, NC 27710

It has been recently demonstrated that PP can bind to the dorsal vagal complex in the medulla oblongata. There is however little evidence whether PP has a central effect on gastrointestinal functions. The present study was carried out to examine the effect of IC administered PP on gastric secretion. Under isoflulene anesthesia, 24 hr -fasted male Sprague-Dawley rats, weighing 250-300 g, received IC (10 μl) or intraperitoneal (0.5 ml) injection of rat PP or BSA-Saline and the pylorus was ligated. Rats were sacrificed 2 h after pylorus ligation and gastric acid secretion was measured. IC injection of PP dose-dependently increased gastric acid output. In contrast, intraperitoneal injection of PP failed to change gastric acid output.

PP	Gastric acid output (μEq/2 hr) (n)	
Dose (ng)	Intracisternal	Intraperitoneal
0 (BSA-Saline)	146 ± 16 (6)	183 ± 14 (6)
62.5	206 ± 5 (4) *	
250	259 ± 28 (4)*	186 ± 12 (4)
1000	362 ± 67 (4) *	184 ± 17 (7)
2500		179 ± 18 (4)

Mean ± SEM, * $p < 0.05$ vs BSA-Saline

In acute bilateral gastric branch vagotomized rats, IC injection of PP (1000 ng) did not alter gastric acid output. These results suggest that central PP stimulates gastric acid secretion at a dose-dependent manner in conscious rats. It is also demonstrated that increase in gastric secretion by central PP was prevented by vagotomy. These results suggest that PP may have a physiological role in central regulation of vagal-mediated gastric secretion.

394.1

THE NEUROANATOMICAL BASIS FOR VAGAL CONTROL OF GASTRIC EMPTYING. M-C. Holst* and T.L. Powley, Purdue University, West Lafayette, IN 47907.

Physiological studies have highlighted the importance of the vagus in pyloric mechanisms of gastric emptying. In this study, vagal afferents and efferents of the rat's pyloric region were labeled by injections of DiI into the nodose ganglion (left, right or both) or dorsal motor nucleus of the vagus (DMNX; right, left or both) respectively. Other animals had injections of DiA in the DMNX and DiI in the nodose. The DMNX, nodose ganglia and enteric neurons were counterstained by an i.p. injection of Fluorogold. Transverse or longitudinal sections (140 μm) of the pylorus were examined with epifluorescence and confocal microscopy. Vagal preganglionic fibers and terminals are prominent in an extensive complex of ganglia which extends from the myenteric plexus into the enlarged circular muscle layer (the torus) of the pyloric region. Additional vagal efferents are in a second plexus specific to the inner part of the torus. Only the outer plexus is continuous with those in the antrum and duodenum. Vagal preganglionics also occur in ganglia of the sparse submucosal layer, including a plexus over the thickened muscularis mucosa at the base of the pyloric glands. Vagal afferent fibers and endings are found in ganglia of both parts of the torus and the submucosa. In cases with double label, some neurons, particularly in the inner plexus of the torus, are innervated by both vagal afferents and efferents. Vagal afferents are also abundant in the mucosa, especially in the duodenal villi and the distal torus. In the latter, they form a dense network of fibers and terminals intimately associated with the processes of interstitial cells of Cajal in a distribution resembling that of pyloric CCK binding sites. The unique pattern of vagal fibers in the torus provides the anatomical basis for physiologically defined pyloric mechanisms. NIH DK27627.

394.2

VAGAL EFFERENT PROJECTIONS TO THE ENTERIC NERVOUS SYSTEM: COMMAND NEURON HYPOTHESIS RECONSIDERED. D.B. Boyd, J.B. Kelly, D.L. Kim, M-C. Holst and T.L. Powley*. Purdue University, West Lafayette, IN 47907.

The proposal that a limited subset of enteric neurons with widespread integrative functions are targeted selectively by vagal preganglionics, i.e. the mother cell (Langley) or command neuron (Wood) hypothesis, is based largely on the relative numbers of preganglionic and enteric neurons. However, the number of postganglionics or ganglia in the gut contacted by vagal motor fibers has not been directly determined. To assess the extent of divergence in this parasympathetic outflow, male rats received injections of DiI or PHA-L in the dorsal motor nucleus of the vagus. Three weeks after tracer administration, animals were injected (ip) with 2mg of Fluorogold to counterstain the enteric nervous system. Five days later, animals were perfused. Sections of medullas (56μ) and whole mounts of stomachs, small intestines, and ceca were prepared and examined with conventional epifluorescence and confocal microscopy. Individual motor fibers were identified as they entered the target organs and then traced and digitized in their entirety (Eutectics Neuron Tracing System). In the viscera, vagal axons travel long distances, collateralize frequently, ramify extensively, and make putative contacts on large numbers of postganglionic neurons in numerous enteric ganglia. In the stomach, which receives the densest and most extensive vagal motor innervation, virtually all myenteric ganglia receive vagal projections, and most neurons within these ganglia are contacted by varicosities of vagal efferents. The finding that vagal preganglionics project so divergently within the enteric nervous system strongly suggests that the pre- to postganglionic interface might be better considered a station in an extensively interconnected neural network, rather than a relay in a command neuron circuit. NIH DK27627.

394.3

VAGAL AND ENTERIC INNERVATION OF THE RAT ESOPHAGUS. W.L. Neuhuber*1, J. Wörl1, B. Mayer2 and H.R. Berthoud3, 1Dept. Anatomy, Univ. Erlangen-Nuremberg, D-8520 Erlangen, FRG, 2Dept Pharmacol.&Toxicol., Univ. Graz, A-8010 Graz, Austria, and 3Pennington Biomed. Res. Ctr, Baton Rouge, LA 70808

The act of deglutition is thought to be controlled by a bulbar swallowing center in concert with vago-vagal reflexes (Cunningham et al., *Dysphagia* 5:35-51,1990). However, the presence of a myenteric plexus also in striated portions of the esophagus is puzzling. Using anterograde DiI and DiA tracing, NADPH-diaphorase and AChE histochemistry, and immunocytochemistry for NO synthase and CGRP, we attempted to elucidate the distribution of efferent and afferent vagal fibers, as well as the intrinsic wiring of enteric neurons in the wall of the rat esophagus. DiA injections into the dorsal motor nucleus labeled the rare varicose fiber in no more than 16% of all myenteric ganglia, while DiI injections into nodose ganglia led to profuse labeling of afferent terminals in up to 100% of enteric ganglia. Afferent fibers were sparse in mucosa and virtually absent from outer and inner muscle layers proper. DiI injections into the nucleus ambiguus resulted in anterograde labeling of motor endplates. No convincing terminal labeling in myenteric ganglia was seen. Most of enteric neurons were NADPH-diaphorase/NO synthase positive. They represent the most likely source for nitrergic fibers in about two thirds of motor endplates where they could be distinguished from CGRP immunoreactive motor terminals. In conclusion, there is extensive co-innervation of esophageal striated muscle fibers from nucleus ambiguus (cholinergic) and myenteric plexus (nitrergic), while myenteric ganglia appear to be influenced mainly by vagal afferent rather than efferent terminals. If these afferent terminals are considered a mechanosensor-local effector device influencing enteric neurons as suggested previously (Neuhuber, *J Auton. Nerv. Syst.* 20:243-255,1987), a local reflex arc involving nitrergic co-innervation of motor endplates might be proposed for the fine-tuning of peristalsis in the striated esophagus.

394.4

VAGALLY-MEDIATED SEROTONIN (5HT) RELEASE INHIBITS GASTRIC ACID SECRETION VIA RECEPTORS OF THE $5HT_1$ FAMILY. K.J.LePard and R.L.Stephens Jr.*, Dept. of Physiology, Ohio State Univ., Columbus, Ohio 43210.

Vagal stimulation releases 5HT into the portal circulation. Exogenous 5HT inhibits gastric acid secretion, and it is proposed that endogenous 5HT in the gut exerts an inhibitory tone on acid secretion. Therefore, the physiologic effect of 5HT released from gut stores on acid secretion was determined. In urethan-anesthetized rats with acute portal vein cannula, 5HT in the portal circulation was elevated 90% by vagal stimulation with the TRH analogue RX77368 (100 ng, i.c.) [mean ± SEM, μg/30 min, (n=5): basal, 135 ± 19; stimulated, 256 ± 35]. To characterize possible sites of action, the effects of various 5HT antagonists on exogenous 5HT-induced inhibition of acid secretion was determined. Pretreatment with methysergide ($5HT_{2/1}$), methiothepin ($5HT_1$) or spiperone ($5HT_{2/1A}$) but not ritanserin ($5HT_{2/1C}$), renzapride ($5HT_{1P/3}$) or ICS 205-903 ($5HT_{3/4}$) significantly reversed 5HT-induced inhibition of acid. Moreover, methysergide augmented RX77368 (30 ng, i.c.) stimulated acid secretion by 267%. Therefore, vagally-mediated release of 5HT from the gut appears to provide an inhibitory tone on acid secretion via activation of receptors of the $5HT_1$ family. Supported by NIH DK 42880.

394.5

PERIPHERAL CCK AND BOMBESIN INFLUENCES AFFERENT ACTIVITY OF GASTRIC VAGAL TERMINALS RESPONSIVE AND NOT RESPONSIVE TO GASTRIC DISTENSION: SINGLE UNIT ANALYSIS. T.J. O-Lee, E. Yoshida-Yoneda, Y. Taché*, and J.Y. Wei. CURE/DDC VA Wadsworth Medical Center, Dept. of Medicine and Brain Research Institute, UCLA, Los Angeles, CA 90073.

We previously reported that different mechanisms are involved in gastric distension, bombesin (bom), and CCK induced stimulation of gastric vagal afferent discharge (Gastroenterology 102:A537, 1992). It is not clear, however, whether all responsive terminals have the same responsiveness to iv bom and CCK injection and gastric distension. **Purpose:** To assess, with single unit analysis, the sensitivity of gastric distension responsive vagal afferent terminals to iv bom and CCK injection. **Methods**: Male SD rats (280-320 g) were anesthetized with urethane (1.5 g/kg, im). Gastric afferent activity was recorded from a nerve filament dissected from distal cut end of ventral gastric branch of the vagus nerve. A single unit was isolated with a window discriminator and monitored on a digital oscilloscope. Consecutive responses to gastric distension, 100 ng or 1 μg of bom or CCK and saline (0.2 ml) were assessed. **Results**: 25 units were analyzed, 15 units were activated by gastric distension. 12 of these 15 units (80%) showed sensitivity to all doses of iv bom and CCK. 2 units were unaffected by small doses of bom, 1 of the 2 was also unaffected by small doses of CCK. 1 unit showed no response to large the dose of bom. 10 of the 25 units analyzed were not sensitive to gastric distension. 7 of the 10 were sensitive to all doses of bom and CCK (70%). 3 were unaffected by low doses of bom, 2 of the 3 were also unaffected by low doses of CCK. **Conclusions:** 1. All gastric afferent units responding to CCK also responded to bom; 2. bom and CCK actions can be exerted on units responding and not responding to gastric distension (Supported by NIH grants NS28433 & DK 30110).

394.6

ORIGIN OF NITRIC OXIDE SYNTHASE-CONTAINING TERMINALS IN THE COELIAC GANGLION OF THE GUINEA PIG. C.R. Anderson*, J.B. Furness and S.L. Edwards Department of Anatomy and Cell Biology, University of Melbourne, Parkville, Australia, 3052.

The distribution of nitric oxide synthase (NOS)-containing terminals in coeliac ganglia from guinea pigs of either sex was studied using the NADPH diaphorase procedure and NOS immunohistochemistry, both of which showed identical patterns of staining. Some groups of postganglionic nerve cell bodies were surrounded by dense pericellular baskets of NOS-containing terminals. The proportion of nerve cell bodies forming such groups was highest in the medial parts of the ganglion (> 50%) and lowest in the lateral parts (<10%). In addition, all groups of postganglionic neurons were surrounded by a relatively sparse plexus of weakly stained NOS-containing fibres. Injection of Fast Blue into the coeliac ganglia of guinea pigs anaesthetised with sodium pentobarbitone, fentanyl and droperidol resulted in labelling of preganglionic neurons in the thoracolumbar spinal cord. More than 40% of the Fast Blue-labelled preganglionic neurons were positive for both NOS-IR and NADPH diaphorase and were predominantly in the lateral parts of the intermediolateral column. Severing of the nerves connecting the coeliac ganglion with the gut resulted in degeneration of the dense pericellular baskets of NOS-containing terminals, which are hence of enteric origin, but spared the sparse plexus of less intensely stained NOS-containing terminals, which are hence likely of preganglionic origin. Studies using double-labelling histochemistry showed that the NOS-containing fibres of enteric origin terminated around somatostatin-containing postganglionic neurons, which have previously been identified as noradrenergic neurons that control intestinal fluid transport.

394.7

EXTRINSIC DENERVATION INCREASES NADPH DIAPHORASE STAINING BUT NOT CITRULLINE PRODUCTION BY NITRIC OXIDE SYNTHASE IN MYENTERIC PLEXUS OF GUINEA PIG ILEUM. A.M. Yunker*, J.B. Moldovan, M.L. Contreras, and J.J. Galligan. Dept. Pharmacol. and Toxicol. and Neuroscience Program, Michigan State University, E. Lansing, MI 48824.

The functions of the small intestine are controlled by the enteric nervous system and extrinsic nerves. At least one population of enteric neurons contains nitric oxide synthase (NOS) and NADPH diaphorase (NADPH d.), and has been reported to co-localize with vasoactive intestinal polypeptide immunoreactivity (VIP-ir). We tested the hypothesis that extrinsic denervation alters NADPH d. staining in enteric neurons. Seven weeks after extrinsic denervation, the number of vasoactive intestinal peptide immunoreactive (VIP-ir) (6 ± 1.2 cells/ganglia) and NADPH d. stained neurons (0 cells/ganglia) in the submucosal plexus was not significantly different from either normal or control preparations. In control myenteric plexus, 9 ± 1 cells/ganglia contained NADPH d. and 3 ± 0.03 cells/ganglia contained VIP-ir. However, 7 weeks after extrinsic denervation the number of NADPH d. stained neurons increased by 85%, whereas the number of VIP-ir neurons did not change. To determine if the increase in NADPH d. staining correlated with an increase in NOS activity, we examined the formation of L-[^{14}C] citrulline from L-[^{14}C] arginine in normal and denervated small intestine. Following denervation, citrulline production decreased by 47% (7.6 ± 0.67 pmole citrulline/100 µg protein) as compared to control preparations (13.3 ± 1.9 pmole citrulline/100 µg). The NOS antagonist N_w-nitro-L-arginine methyl ester (0.01 M) decreased citrulline production in both control (80%) and denervated (79%) tissues. Although EGTA (0.001 M) decreased citrulline production 96% in control tissues, 34% of citrulline production remained in denervated tissues, implying a denervation-induced Ca^{2+}-independent form of NOS. These data suggest extrinsic denervation alters NOS found in enteric neurons. This alteration may be part of the adaptive process that allows normal digestive functions to continue in the absence of extrinsic nerves. (Supported by NIH DK 40210 and a grant-in-aid from the American Heart Association.)

394.8

EXPRESSION OF FOS RELATED ANTIGENS BY ENTERIC NEURONS: EFFECTS OF INTESTINAL TRANSECTION. T. Karaosmanoglu, E. Blaugrund, P. R. Wade, V.M. Tennyson*, M.D. Gershon Dept. of Anatomy and Cell Biology, College of P & S, Columbia University. New York, NY 10032 USA.

The enteric nervous system (ENS) is capable of mediating intestinal reflexes in the absence of connections to the CNS. The activity of enteric neurons is thought to mediate the inhibition of myogenic muscle contraction and patterned activity (e.g. migrating myoelectric complexes and the peristaltic reflex). Nuclear immunoreactivity for Fos, a 62 kDa protein encoded by the *c-fos* proto-oncogene, has previously been used to visualize activated enteric neurons. We have attempted to use a commercial antibody to Fos (Oncogene Sciences Ab2) to investigate the effects of sectioning the intestine on the activity of neurons in the ENS. Fos immunoreactivity (F-IR) was found in virtually all neuronal nuclei in ganglia of both submucosal and myenteric plexuses in preparations fixed immediately after removal from guinea pigs and rats. Demonstration of all myenteric neurons by staining with quinolinic phthalocyanine (cuprolinic blue) revealed that neurons with F-IR comprised a subset (50-70%) of all neurons. Following transection and reanastomosis of the rat ileum, no nuclear F-IR was observed for up to 2 wks post-surgery in a zone 5 cm above and below the anastomotic site. In order to determine if the antibodies were detecting Fos, immunoblots were prepared and segments of guinea pig ileum small and large intestine were subjected to treatments known to affect expression of *c-fos*. These preparations were exposed either to cold or to tetrodotoxin (TTX) in order to decrease expression of Fos, or they were incubated with veratridine to increase it. None of the treatments altered the pattern of F-IR seen in the tissue. No 62 kDa immunoreactivity was detected in immunoblots, which revealed instead immunoreactive material of lower molecular weight, the amount of which could not be altered by exposure of tissue to TTX or veratridine. We conclude that this antibody does not react with Fos in the ENS, but probably with one or more Fos related antigens (FRA). FRA expression thus characterizes a subset of enteric neurons and is abolished by transecting the bowel. Supported by NIH grant NS12969.

394.9

NEUROTRANSMITTER CONTENT OF NEURONS IN GANGLIA OF THE GUINEA PIG SPHINCTER OF ODDI. D.G. Wells*, E.K. Talmage and G.M. Mawe. Dept. of Anatomy and Neurobiology, Universtiy of Vermont, Burlington, VT 05405.

The sphincter of Oddi (SO) is a smooth muscle sphincter that regulates the flow of bile into the duodenum. To identify potential chemical coding in SO neurons, immunohistohemistry and histochemistry were employed to assay for putative neurotransmitters and related synthetic enzymes in whole mount preparations, with and without colchicine treatment. Immunoreactivity (IR) for Leu-enkephalin (L-ENK), substance P (SP), vasoactive intestinal peptide (VIP), neuropeptide Y (NPY), calcitonin gene-related peptide (CGRP), and tyrosine hydroxylase (TH), was demonstrated within the SO ganglionated plexus. Most SO neurons expressed either SP- or VIP-IR, and most SP-IR neurons were also L-ENK-IR. The VIP-IR neurons also stained for NADPH-DA activity [a form of nitric oxide synthase], but neurons that contained L-ENK- or SP-IR did not stain for NADPH-DA. A small contingent of non-SP-IR SO neurons was immunoreactive for NPY. Although CGRP-IR neurons were not observed in SO ganglia, CGRP-IR fibers that were also SP-IR were observed. A vast network of TH-IR fibers was seen throughout the ganglionated plexus of the SO; however, no TH-IR cell bodies were seen in SO ganglia. These results indicate that most neurons in SO ganglia are either SP/L-ENK-IR or VIP-IR with NADPH-DA activity. We propose that these classes of neurons represent excitatory and inhibitory motor neurons, respectively. Neural and/or hormonal input to these sets of neurons may be an important means of regulating SO tone. Supported by DK-45410.

394.10

INTRACELLULAR CALCIUM-LOWERING EFFECT OF CGRP ON INTESTINAL MUSCLE. Y.D.Sun and C.G.Benishin* Dept. of Physiol., Univ. of Alberta, Edmonton, Canada T6G 2H7.

Calcitonin gene-related peptide (CGRP) is a neuropeptide which shares the same gene with the calcium-regulating hormone, calcitonin. CGRP relaxed the tension induced by different stimulants of longitudinal muscle of guinea pig ileum. In the present study, we investigated the effect of CGRP on intracellular calcium concentration ($[Ca^{2+}]_i$) of the muscle by using the tension-$[Ca^{2+}]_i$ simultaneous recording technique. Histamine (0.5 µM) and KCl (30 mM) increased both tension and $[Ca^{2+}]_i$. CGRP decreased the tension (by 78% and 85% respectively) while it showed minimal reduction of $[Ca^{2+}]_i$ (by 25% and 0% respectively). In histamine pre-contracted tissue, forskolin (0.5 µM), an activator of adenylate cyclase, decreased the tension (by 93%) to a larger extent than $[Ca^{2+}]_i$ (by 58%) while nifedipine (10 nM), a calcium antagonist, lowered both tension and $[Ca^{2+}]_i$ to similar extent (by 100% and 97%). CGRP and forskolin, unlike nifedipine, caused a dissociation between force and $[Ca^{2+}]_i$, indicating that they may interfere with other process(es) involved in the muscle contraction in addition to the $[Ca^{2+}]_i$-lowering effect. CGRP, similar to forskolin, was found to increase cAMP level. These results suggest that CGRP may affect the muscle tension by a mechanism similar to forskolin.

394.11

CAPSAICIN (C) INHIBITS RAT SMALL INTESTINAL ALANINE ABSORPTION. N.E. Saadé*, K. Barada, J. Atallah, S. Itani, L. Abdallah, S. Nayfeh and C.F. Nassar. Departments of Physiology and Internal Medicine, American University of Beirut, Beirut, Lebanon.

Capsaicin sensitive primary afferents (CSPA) are involved in gastric mucosal defense mechanisms and gastro-intestinal-motility through the release of peptides and neurotransmitters. The effects of capsaicin on L-alanine absorption across the rat small intestine were investigated using the single pass perfusion technique and the two compartment incubation system.

Intraluminal perfusion of 160 and 800 µM C produced a sustained and significant decrease in alanine jejunal absorption. Acute block of vagal CSPA increased basal alanine absorption and reduced the C inhibitory effect, while neonatal treatment produced a decrease of both basal alanine absorption and the C inhibitory effect. Incubation of intestinal strips with different concentrations of capsaicin (100-2400 µM) produced a dose dependent inhibitory pattern of intracellular alanine accumulation, which was maximal at 1600 µM. Mucosal scrapings incubated with 1600 µM capsaicin did not show a similar effect. We conclude that capsaicin exerts a CSPA mediated decrease in rat small intestinal alanine absorption.
(Supported by the Lebanese National Research Council and the D.T. Sabbagh Fund).

394.12

SUBSTANCE P DEPOLARIZES GALLBLADDER NEURONS BY OPENING CATION CHANNELS. G.M. MAWE* Dept. of Anatomy and Neurobiology, The Univ. of Vermont, Burlington, VT, USA 05405

In the guinea pig gallbladder, substance P (SP) is coexpressed with CGRP in sensory nerves and it is synthesized by most neurons. SP-immunoreactive nerve fibers are abundant in gallbladder ganglia, where they appear to surround the neurons. Since SP is thought to be a major mediator of slow excitatory postsynaptic potentials (EPSPs) in the gut, and since slow EPSPs occur in gallbladder, experiments were done to determine whether SP could mediate these synaptic events in the extrahepatic biliary system. Intracellular voltage and current recordings were made from neurons in ganglia of the guinea pig gallbladder, and SP was applied by pressure microejection (0.1 mM; 15 PSI, 100-1000 msec) or superfusion (0.1 - 100 nM). SP caused a prolonged depolarization that was accompanied with a decrease in input resistance and an increase in excitability. The magnitude of the SP response increased as the duration of pressure microejection was increased. When applied to gallbladder neurons in the single electrode voltage-clamp recording mode, SP caused an inward current. Using both current- and voltage clamp recordings the responses to SP had a reversal potential between 0 and 10 mV. Also, the inward current elecited by SP was deminished in a low Na^+ solution. The NK1 antagonist CP96345 (Pfizer; 1.0 µM) decreased the responsiveness of cells to SP and decreased the amplitude of slow EPSPs. These results indicate that SP may be a mediator of slow EPSPs in ganglia of the gallbladder. Since CP96345 decreased the responsiveness of SP and the amplitudes of slow EPSPs, it is likely that SP mediates its effect by acting at NK1 receptors. Supported by NS26995 and NS45410.

395.1

CONTRASTING EFFECTS OF INTRACEREBROVENTRICULAR (ICV) AND INTRATHECAL (IT) ADMINISTRATION OF YOHIMBINE ON NALOXONE POTENTIATION OF NOVELTY-INDUCED HYPOALGESIA. J. Rochford*. Dept. Psychiatry, McGill University, Montreal, Quebec.

Exposure to novel stimuli, such as a hot plate apparatus, has been shown to induce hypoalgesia. With repeated exposure, novelty-induced hypoalgesia (NIH) habituates. Naloxone administration has been found to attenuate the rate at which NIH habituates. Thus, by the fourth or fifth exposure to the hot plate, naloxone-treated animals appear hypoalgesic relative to saline-treated controls. Moreover, it has been found that the hypoalgesia observed in naloxone-treated animals is mediated, at least in part, by noradrenergic substrates. The present series of experiments were conducted to determine the relative contribution of supraspinal and spinal noradrenergic substrates in the mediation of this effect.

Male, Wistar rats (275-300g) were implanted either with a ventricular canula (right lateral ventricle) or an IT catheter. Following recovery animals were administered (s.c.) either saline or 10 mg/kg naloxone, 30 min prior to placement on a 48.5° C hot plate once a day for 8 days. The paw lick latencies for saline-treated animals declined over tests, whereas the latencies in naloxone-treated animals did not. These results confirm previous findings demonstrating that naloxone attenuates the habituation of NIH. On days 9-10, animals were administered, in counterbalanced order, either vehicle, or the alpha-2 noradrenergic antagonist yohimbine (7.5 - 30.0 µg) IT or ICV 15 min prior to their allotted s.c. injection. ICV yohimbine administration enhanced the paw lick latencies in naloxone-treated animals, whereas IT administration inhibited them. Yohimbine was without effect in saline-treated animals. These results suggest that the contributions of spinal and supraspinal noradrenergic pathways to naloxone's effect on NIH are qualitatively different (Funded by NSERC).

395.2

INJECTION OF APV INTO RAT HIPPOCAMPAL DENTATE GYRUS PRODUCES ANALGESIA IN THE FORMALIN TEST. J.E.McKenna* and R.Melzack
Department of Psychology, McGill University, 1205 Dr. Penfield Ave., Montreal, Que. Canada, H3A 1B1

Previous research indicates that prolonged noxious stimulation causes NMDA-mediated changes in the CNS; glutamate and aspartate are released in the dorsal horns of the spinal cord after peripheral formalin injection, and the competitive NMDA antagonist dl-2-amino-5-phosphono-valeric acid (APV) produces analgesia when applied intrathecally to rats or mice in the formalin test. Recent studies in our laboratory have indicated that lidocaine block of the dentate gyrus significantly decreases formalin pain scores. In the present experiment Long-Evans rats received 0.75 µl intracranial injections of APV (5.0 µg/µl) or saline via infusion pump, either before or after an injection of 2.5% formalin acetate into one hindpaw. Intradentate injection of APV caused a significant reduction in pain scores for 20-40 min. These results indicate that the NMDA receptor ion complex may be implicated in supraspinal pain mechanisms. Supported by NSERC grant A7896.

395.3

THE EFFECT OF LATERAL HYPOTHALAMIC STIMULATION ON TAIL-FLICK AND BOTH PHASES OF THE FORMALIN PAIN RESPONSE. P.N. Fuchs* and V.C. Cox. Dept. of Psychology, Univ. of Texas, Arlington, TX 76019-0528.

The purpose of the study was to employ a between and within-subjects design to investigate the effect of lateral hypothalamic (LH) stimulation on pain responding during the tail-flick test and during both phases of formalin induced pain responding. Experiment 1 examined the effects of LH stimulation on both phases of formalin induced pain responding. LH stimulation which produced analgesia during the second phase of the formalin pain response did not produce analgesia during the first phase of the formalin pain response. Experiment 2 examined the effects of LH stimulation on tail-immersion thermal pain and the second phase of the formalin pain response. Only posterior LH stimulation sites were effective in both increasing tail-flick withdrawal latencies and attenuating the second phase of the formalin pain response. The results indicate that the first and second phase of pain responding during the formalin test are mediated by functionally distinct neural substrates that are differentially responsive to LH stimulation. In addition, electrode sites that were effective in attenuating spinally mediated phasic pain responses, in the same subject, also attenuated tonic pain response.

395.4

Effect of Midbrain Stimulation on the Magnitude of a Rat Operant Response to Noxious Thermal Stimuli. D.K. Douglass* & E. Carstens. Section of Neurobiology, Physiology & Behavior, Univ. of California, Davis, CA 95616.

We have used our previous method, measuring the magnitude of an operant response elicited by a range of noxious thermal stimulus intensities, to investigate effects of stimulation in midbrain analgesia areas.

Under barbiturate anesthesia, male Sprague-Dawley rats were implanted with bipolar stimulation electrodes in midbrain periaqueductal gray (PAG) and lateral reticular formation (LRF). After recovery, rats were trained to push up on a lever with the nose in response to a brief (5 s) 55°C heat pulse on the tail. The external end of the lever was connected to a force transducer interfaced with a computer to measure push magnitude (peak; area under force trace).

Responses to 55°C stimuli were recorded at 2 min intervals without and during PAG or LRF stimulation (3 100 Hz trains/s, 0-300 µA). Response magnitude tended to increase with stimulus temperature from 51-59°C. PAG and LRF stimulation significantly suppressed response magnitudes at all temperatures. Response magnitude was often "quantally" suppressed with a 50 µA increase in PAG (8/10 rats) or LRF (5/7 rats) stimulation intensity.

The results show that the operant response can be supressed by midbrain stimulation. However, previously reported parametric differences in PAG and LRF-evoked suppression of nocifensive responses and dorsal horn neurons were not seen with our present model, possibly because of the apparent quantal suppression of the operant response.

We are grateful for Dr. L. R. Watkins' help in developing this model.

395.5

SELECTIVE ANTINOCICEPTIVE EFFECTS OF THE SUBSTANCE P ANTAGONIST CP96,345 ON RESPONSES TO LOW SKIN HEATING RATES. D. C. Yeomans* and H. K. Proudfit. Dept. of Pharmacology, U. Illinois at Chicago. Chicago, IL 60612.

We have previously provided evidence that foot withdrawal responses to low rates of radiant skin heating are mediated by the activation of C-fiber mechanoheat nociceptors. In contrast, high rates of skin heating evoke foot withdrawal responses that are mediated by myelinated A-fiber nociceptor activation. This hypothesis is supported by the following evidence: 1) Topical capsaicin, which selectively sensitizes C-fiber nociceptors, selectively decreased foot withdrawal latencies (FWL) for low skin heating rates. 2) Topical DMSO, which selectively sensitizes A-fiber nociceptors, selectively decreased FWL for high skin heating rates. 3) Low doses of systemic morphine (0.01 to 1.0 mg/kg), which selectively attenuate nociception produced by C-fiber activation, increased FWL only for low heating rates.

Substance P is a putative neurotransmitter released from the central terminals of C-fiber nociceptors by noxious stimulation. CP96,345 (Pfizer), a highly selective substance P antagonist with subnanomolar affinity for NK1 receptors, would reduce nociceptive responses mediated by the activation of C-fiber nociceptors. To provide additional evidence that responses to low heating rates are mediated by activation of C-fiber nociceptors, we determined whether intrathecal administration of CP96,345 would selectively increase FWL evoked by high or low skin heating rates. CP96,345 (0.2 to 200µg) produced dose-dependent FWL increases for low skin heating rates, but did not effect responses evoked by high skin heating rates. These results are consistent with our hypothesis that low skin heating rates evoke responses that are mediated by C-fiber nociceptors, and that responses to high heating rates are mediated by the activation of A-fiber nociceptors.

395.6

DELTA AND KAPPA OPIATE RECEPTORS MEDIATE ANTINOCICEPTION IN THE RAT TAIL FLICK TEST PRODUCED BY NOXIOUS THERMAL STIMULATION OF THE HIND PAW G.M. Pitcher, K. Yashpal* and J.L. Henry, Departments of Physiology & Psychiatry, McGill Univ., Montreal, Quebec H3G 1Y6

Noxious thermal stimulation of one hind paw in the lightly anaesthetized rat produces a transient (< 6 min), naloxone-reversible antinociception in the tail flick test. The present study was done to determine the specific type of opiate receptor involved in mediating this antinociceptive response. Sprague-Dawley rats (250-300 g) were lightly anaesthetized with Na-pentobarbital (20mg/kg) and chloral hydrate (120 mg/kg) i.p. After recording baseline readings at 3 min intervals in the tail flick test, the animals were administered 10 µl of one of the following intrathecally to the lumbar spinal cord: artificial CSF (n = 7); the kappa opiate receptor antagonist, nor-binaltorphiminedihydrochloride (nor-BNI; 6.5 nmol in CSF; n = 11); the highly specific delta opiate receptor antagonist, H-Tyr-Tic-Phe-Phe-OH (TIPP; 6.5 nmol in CSF; n = 6; generously supplied by Dr. P. Schiller, IRCM, Montreal; PNAS 89:11871, 1992). Three min later one more reading was taken and then one hind paw was immersed in water at 55°C for 1.5 min; subsequent readings of tail flick latency were taken at 3 min intervals, beginning 30 s after the immersion. In the group of rats given CSF, the full antinociceptive response was observed. However, in the two groups pretreated with the opiate antagonists, readings after immersion did not differ from the baseline readings. The data indicate that at least two types of opiate receptor, delta and kappa receptors, are involved in eliciting the antinociceptive response to extrasegmental aversive sensory inputs to the spinal cord. (supported by the MRC of Canada)

395.7

ANTINOCICEPTION EVOKED BY HIGH INTENSITY, LOW FREQUENCY PERIPHERAL STIMULATION IN INTACT AND SPINAL RATS: MEDIATION BY NK-1 AND OPIATE RECEPTORS V.V. Romita* and J.L. Henry, Departments of Physiology & Psychiatry, McGill Univ., Montreal, Quebec H3G 1Y6

In on-going experiments on an animal model of electroacupuncture, in intact rats lightly anaesthetized with Na-pentobarbital (20mg/kg) and chloral hydrate (120 mg/kg) i.p., high intensity, low frequency electrical stimulation was applied for 20 min at 20 X threshold to evoke muscle contraction. Stimulation of hind limb meridian points *femur-futu* (ST-32) and *fengshi* (GB-31), but not of non-meridian points, increased tail flick latency during stimulation and produced a long lasting antinociceptive effect which lasted greater than one hour. In unanaesthetized, chronic spinal transected rats, this stimulation evoked a similar though smaller effect. Both the substance P (NK-1) receptor antagonist, CP-96,345 (5 mg/kg, s.c., n=6), and the opiate antagonist, naloxone (25 mg/kg, i.p., n=8), attenuated the antinociception during the stimulation and blocked the post-stimulation antinociception in intact rats. In addition, CP-96,345 applied intrathecally (65 nmoles, n=5) at the lower lumbar level also attenuated the evoked antinociception, implicating the action of the antagonist at the spinal level. In chronic spinal rats, CP-96,345 (5 mg/kg, s.c., n=17) attenuated and naloxone (25 mg/kg, i.p., n=11) blocked the evoked antinociceptive response. These results suggest that the antinociception evoked by electroacupuncture-like stimulation is mediated via activation of NK-1 and opiate receptors in the spinal cord, and that the spinal cord is capable of independently maintaining mechanisms of antinociception. (Supported by the Fonds de la recherche en santé du Québec)

395.8

EXPOSURE TO WHITE NOISE RESULTS IN DECREASED SENSITIVITY TO THERMAL STIMULI IN HUMANS.

N.A. Johnson* and F.J. Helmstetter. Dept. of Psychology, University of Wisconsin Milwaukee, Milwaukee, WI 53201

Previous research has demonstrated a decreased sensitivity to nociceptive stimuli in humans following exposure to a noxious environmental stimulus (e.g., shock). The present study was designed to determine whether similar changes could be produced in response to the presentation of a non-noxious stressor. Withdrawal and detection latency were measured to a radiant heat source focused on the index finger of the subject's right hand. Independent groups of subjects were exposed to either a 0dB, 70dB, or 90dB white noise for 20 seconds. Exposure to high intensity noise resulted in a significant increase in withdrawal latency relative to baseline. This increase in latency correlated with stress related galvanic skin response, which was recorded simultaneously. These results are consistent with previous studies from our laboratory on anxiety and thermal pain responsivity in humans, which demonstrated an increase in withdrawal latency in subjects reporting high state anxiety, as well as with recent animal data which indicates that white noise hypoalgesia depends on brain mechanisms critical for fear and anxiety.

395.9

"SECONDARY" HYPERALGESIA (CAPSAICIN) MEDIATED BY C-NOCICEPTORS. J.Serra, M. Campero, J.Ochoa*. Depts. of Neurology and Neurosurgery, Good Samaritan Hospital , Oregon Health Sciences University, Portland, OR 97210.

The "secondary" hyperalgesia (2y-H) that develops surrounding an area of skin injury is generally described to be selective to mechanical stimuli and to disappear with A-fiber block; it is attributed to activation of pain-evoking CNS cells by innocuous input from A-β afferents.

Methods: We gave intradermal capsaicin to 15 healthy volunteers and made the following rigorous measurements 15-70+ mm beyond the injection site: mechanical (von Frey's hairs) and heat (1 cm^2 Peltier thermode) pain detection thresholds; mechanical and heat suprathreshold pain magnitudes; "touch" (von Frey's hairs) detection threshold; and maximal area of the flare (thermography and naked eye).

Results: In the area of 2y-H: 1) The subjective detection thresholds did not change significantly for touch, mechanical pain nor heat pain. 2) Stimuli above threshold for touch perception, but below the threshold for pain found prior to capsaicin, evoked touch but not pain. 3) The magnitude of suprathreshold pain evoked by quantified punctate mechanical or heat stimuli was exaggerated in the same area. 4) The volatile maximal area of flare matched the areas of mechanical hyperalgesia (MH) and heat hyperalgesia (HH).

Conclusions: 1) The tight spatial match of MH, HH and flare suggest C nociceptor mediation for all three phenomena. This is in keeping with our selective nerve fiber blocks and intraneural microstimulation studies (unpublished). 2) The unchanged threshold for pain in the area of "secondary" MH and HH indicates that C nociceptors in that area do not have sensitized receptors. 3) The increased mechanical and heat suprathreshold pain magnitudes in the area of "secondary" hyperalgesias suggests amplification of afferent responses at a site (proximal to receptors) theoretically inclusive of CNS and not exclusive of the nociceptor axon. 4) Capsaicin-induced "secondary" MH in response to punctate mechanical stimuli is probably not mediated by low threshold mechanoreceptors. 5) Several alternatives may explain disappearance of C-mediated hyperalgesia during apparently "selective" A-fiber block .

(J. Serra was the recipient of a "La Caixa" Fellowship Program Grant)

395.10

THERMAL SECONDARY HYPERALGESIA IS INDUCED BY APPLICATION OF MUSTARD OIL TO THE TAIL OF THE SPINALIZED RAT. C.L. Cleland* and G.F. Gebhart. Dept. of Pharmacology, Univ. of Iowa, Iowa City, IA 52242

Noxious stimuli, such as mustard oil (MO), can induce long-lasting hyperalgesia to distant cutaneous stimuli. Alterations in central processing are likely to underlie such secondary hyperalgesia. The aim of these studies is to develop a spatio-temporal description of the hyperalgesia evoked by the application of MO to the tail of the spinalized rat in order to provide insight into the underlying central mechanisms.

Male Sprague-Dawley rats were anesthetized with pentobarbital and spinalized at T8-T10. Following recovery (24-48 hrs), MO (25-100 µl, 50-100%) or vehicle (mineral oil) was applied to the tail. Thermal sensitivity was tested every 6 min by immersing the lower part of the tail (40-80 mm below MO) into warm water (47-51^{o}C) and measuring the latency to withdrawal.

MO produced a long-lasting decrease in the latency to withdrawal from warm water, while vehicle had no effect. Typically, thermal hyperalgesia exhibited distinct early and late components. The early component (0-18 min, 80% baseline) coincided with the expected duration of C-fiber activity evoked by MO, suggesting that a portion of the hyperalgesia was due to peripheral activity. The late component (45-60 min, 85% baseline) was most likely due to central changes because: 1) hyperalgesia persisted after MO evoked activity should have ended, and 2) the long separation of MO application warm water (80 mm) argues against peripheral sensitization.

In contrast to other studies that have shown mechanical but not thermal secondary hyperalgesia in response to chemical C-fiber activation, our results show that centrally-mediated thermal secondary hyperalgesia can be evoked by MO. Further studies will investigate the spatio-temporal features of warm, cold and mechanical hyperalgesia evoked by MO.

395.11

DO THE EFFECTS OF REPETITIVE TRANSCUTANEOUS ELECTRICAL NERVE STIMULATION (TENS) ON EXPERIMENTAL PAIN CUMULATE OVER TIME? J. Liu and C.W.Y. Hui-Chan*. School of physical and Occupational Therapy, McGill University, Montreal, Quebec, Canada H3G 1Y5.

The objective of this study was to determine whether the effects of repeated TENS applications on the human flexion reflex (FR) and subjective pain sensation would cumulate over time.

Ten young healthy subjects were randomly assigned to either a TENS or a placebo group. Ten daily 60 min TENS or placebo stimulation was applied to the lumbro-sacral region over a two week period. The FR was elicited by electrically stimulating the sole of subject's right foot and recorded electromyographically from the biceps femoris (BF) and tibialis anterior (TA). Subjective pain sensation was measured using the visual analogue scale (VAS). ANOVA and Tukey tests were used to analyze the data obtained prior to, during, and up to 60 min after TENS or placebo stimulation, and to compare the data obtained on day_1, day_5 and day_{10} of the treatment period between the two groups.

For the BF FR area on day_1, a significant inhibition of the group mean value ($p < 0.01$) was found *after* TENS in the treatment group (to 71.8% of control value), but not in the placebo group (to 86.1%). Of interest is that the pre-TENS control value itself was decreased significantly from a mean of 100% on day_1 to 52.4% on day_{10} ($p < 0.01$) in the TENS group. This reduction was significantly greater ($p < 0.01$) than that found in the placebo group, who showed a mean of 82.7% on day_{10}. Similar results were obtained for the TA FR area and VAS scores.

The above results indicated that repeated daily TENS applications produced cumulative inhibitory influence on both FR responses and subjective pain sensation over a two week period. Such a gradual development probably implicates plastic changes in the neural pathway.

This study was financed by the Fonds de la Recherche en Sante du Quebec.

395.12

EVIDENCE THAT THE TAIL-FLICK RESPONSE IS SPINALLY MEDIATED IN RATS IRRESPECTIVE OF TEST TEMPERATURE. T.E. KING*, M. PAYNE, & J.W. GRAU.Department of Psychology, Texas A&M University, College Station, TX 77843.

Researchers frequently test pain reactivity in rodents by measuring the latency at which subjects withdraw their tail from a radiant heat source (the "tail-flick test"). It is commonly held that this response is spinally mediated when the heat source is intense, but depends on supraspinal mechanisms when intensity is decreased (Jensen &Yaksh, 1986). Contrary to this claim, we report that the tail-flick response is spinally mediated over a wide range of test conditions.

Rats (N=16) were tested a day after they experienced either a spinal transection (at T2) or a sham operation. Testing was conducted with a radiant heat tail-flick device while subjects were loosely restrained in tubes. Each subject was tested at 4 different intensities, the order of which was counter-balanced using a latin square design. At each intensity, the subjects received 3 tail-flick tests at 2 m intervals. The mean tail-flick latencies (±SE) observed in sham operated rats were: 3.50 (±.35), 4.47 (±.48), 5.71 (±.43), and 9.92 (±.74) (from hottest to coolest). Similar values were obtained from spinalized subjects: 3.77 (±.52), 5.32 (±.87), 6.91 (±.56), and 11.07 (±.98). An ANOVA confirmed that changing the heat intensity had a significant impact ($p < .0001$). Neither the main effect of operation ($p > .20$), nor its interaction with heat intensity ($p > .76$), approached statistical significance. Supported by MH48994 to J.W.G.

395.13

A SUPRA-THRESHOLD TAIL-FLICK TRIAL INDUCES HYPERALGESIA IN PENTOBARBITAL ANESTHETIZED RATS BUT NOT IN AWAKE RATS. C.F. Kallina*, T.E. King, & J.W. Grau, Department of Psychology, Texas A&M University, College Station, TX 77843.

The tail-flick test is frequently employed to test pain reactivity in rats. To reduce variability, and to monitor the time course of effects, researchers often repeatedly test the same subjects. This methodology poses no problem unless the test itself affects pain reactivity. Interestingly, Baldwin et al. (Neurosci. Abstracts, 18, 292) recently showed that a single supra-threshold exposure to radiant heat induces hyperalgesia on subsequent tests in pentobarbital anesthetized rats.

Experiment 1 was designed to replicate this effect and evaluate whether a change in tail temperature was responsible for the hyperalgesia. We placed pentobarbital-anesthetized (40 mg/kg) rats in restraining tubes and administered 5 tail-flick tests at 1 m intervals (mean=4.7). Half of the subjects (n=10) then received a supra-threshold exposure to heat. This manipulation was achieved by manually preventing the rat from flicking prior to 6 s. Ten more tail-flick tests were given at 1 m intervals. Tail temperature was also monitored. We found a supra-threshold exposure induced hyperalgesia and that this effect could not be accounted for by a change in tail temperature.

Experiment 2 assessed whether a similar effect could be observed in spinally transected (T2) rats. No change in tail-flick latencies were observed suggesting the hyperalgesic effect is supraspinally mediated.

Experiment 3 tested whether a supra-threshold exposure to radiant heat affects pain reactivity in intact/awake rats. We found it had no effect on pain reactivity in awake subjects.

395.14

FORMALIN INDUCED NOCIFENSIVE BEHAVIOR IS INCREASED DURING FEVER IN RATS. N.E. Ferguson* and P. Mason. Dept. of Pharmacological & Physiological Sciences and Committee on Neurobiology, University of Chicago, Chicago, IL 60637.

Although febrile humans report somatic aches and pains, nociceptive responsiveness has not been well studied during experimental fever. To determine whether exogenous pyrogen administration increases nociceptive responsiveness in rats, nocifensive behavior evoked by the formalin test was compared in rats that received pretreatments of yeast, lipopolysaccharide (LPS) or vehicle.

Male Sprague-Dawley rats (240-360g) were handled and exposed to the formalin testing chamber for 2 days prior to testing. To induce fever, rats received either an i.p. injection of LPS or a s.c. injection of yeast. When a fever was established, 19-20 hrs after the yeast injection or 2 hrs post-LPS, rats were briefly anesthetized with halothane in order to inject formalin or saline, s.c., into the ventrum of the left hind paw. Upon recovery from the anesthetic, animals were placed into the testing chamber and behavioral scoring, on a 4 point scale, commenced. Core body temperature, measured using either a thermistor probe in the colon or a telemetric transmitter implanted in the peitoneal cavity, was recorded at 5 min intervals. All testing was done between 0900-1630 h at ambient temperatures of 22-23 ^{o}C.

Core temperature in yeast-injected rats was significantly higher than in control rats. Pain scores for rats receiving formalin in the hind paw were higher in those pretreated with yeast or LPS than in rats pretreated with vehicle. Rats injected with saline in the hindpaw did not exhibit significant nocifensive behavior in any group studied (yeast, vehicle).These results are evidence that nociceptive responsiveness increases during fever in the rat.

PAIN MODULATION: PHARMACOLOGY III

396.1

Kainate and AMPA Enhance Capsaicin-Evoked Release of iCGRP from Rat Dorsal Horn. J. Durnett Richardson, M.G. Garry, K.M. Hargreaves,* Univ. of MN, Depts. of Restorative Sciences and Pharmacology, Minneapolis, MN.

A number of studies suggest a role for glutamate receptors in nociception. Most studies have focused on the role of the NMDA receptor subtype in mediating spinal mechanisms of hyperalgesia. In contrast, little is known about the roles of the kainate and AMPA receptor subtypes in nociception. In the present study, we evaluated the effect of kainate and AMPA on evoking release of immunoreactive calcitonin gene-related peptide (iCGRP), a marker for activation of certain primary afferent neurons. Briefly, rat spinal cords were removed using hydraulic extrusion. The dorsal horn of the lumbar region was dissected, chopped into 200μm cubes and placed in chambers for *in vitro* superfusion. Tissue was superfused with oxygenated Krebs buffer (pH 7.4, 37°C). After allowing for baseline recovery, tissue was superfused with 100μM kainate or 10μM AMPA for 6 minutes. Twenty-one minutes later, the tissue received 10μM capsaicin (CAP), a neurotoxin which selectively stimulates certain nociceptive fibers. The levels of iCGRP in the superfusates were measured using radioimmunoassay. Data were analyzed with ANOVA. The results indicate that neither kainate nor AMPA alone affected the spontaneous release of iCGRP. Both kainate and AMPA, however, significantly increased the release of iCGRP in response to CAP when compared to a group which received only CAP. Specifically, the release of iCGRP evoked by CAP was 7953 ± 913 fmol/g/3min while the release in response to CAP pretreated with kainate was 27,936 ± 6179 fmol/g/3min. In addition, release of iCGRP in response to CAP pretreated with AMPA was 18,038 ± 3170 fmol/g/3min ($F_{(3,24)}$=5.47; p<0.01). These results demonstrate that kainate and AMPA enhance CAP-evoked release of iCGRP from the dorsal horn. These data support the hypothesis that kainate and AMPA have a modulatory effect on certain primary afferents containing iCGRP. This research was supported by a Predoctoral Fellowship from the Howard Hughes Medical Institute and DE09860.

396.2

An Evaluation of the Effects of Excitatory Amino Acids in Bovine Dental Pulp. D.L. Jackson*, L.M. Aanonsen†, J.D. Richardson, H.G. Geier, K.M. Hargreaves. Dept. of Rest. Sci., Univ. of Minnesota , and †Dept. of Biol., Macalester College, Minneapolis MN. 55455.

Noxious stimuli are capable of increasing the release of glutamate and other excitatory amino acids within the spinal cord and it is currently thought that these amino acids may contribute to the development of centrally mediated hyperalgesia. While there is considerable information about the release profile and the receptor types involved in the centrally mediated excitatory amino acid effect, relatively little is known about the activity of these compounds in peripheral tissue. The previously descibed method of *in vitro* bovine dental pulp superfusion (Hargreaves et al. Abs. Soc. Neurosci. 17:1371, 1991) was used to investigate the role of excitatory amino acids in the secretion of immunoreactive CGRP (iCGRP) from capsaicin sensitive peripheral tissue. Dental pulp tissue was obtained from the mandibular incisors of freshly slaughtered holstein cows. The tissue was cut into 200μM cubes and placed into 1.0cc superfusion chambers through which physiologic Kreb's buffer (37°C @ pH 7.4) was continuously pumped. Following baseline collections the tissue was stimulated with either 50mM potassium or kainic acid (100μM). The superfusate was collected for subsequent analysis with a validated radioimmunoassay for iCGRP. Additionally, amino acids were measured by pre-column derivatization with NDA followed by HPLC with fluorescent detection. Data was analyzed using Student's-t tests and expressed as mean ± s.e.m. The administration of 50mM potassium resulted in a greater than five-fold increase in the release of glutamate relative to baseline levels (89.52 ± 21.86 nmol/ml vs. 15.9 ± 2.72 nmol/ml). This stimulus did not produce significant increases in the the levels of asparagine, serine, glycine/tyrosine, alanine, or arginine, thus ruling out cell lysis as an explanation of this result. The glutamate analogue kainic acid produced a 231.8 ± 114.7% increase in the secretion of iCGRP from dental pulp relative to controls receiving no stimulant (p<0.05). Collectively, these results suggest that glutamate is released in stimulated peripheral tissue and is capable of eliciting the release of iCGRP from a select population of primary afferent fibers. This research was funded by: K16-DE0027-03, DE10096, and P3ODEO9737.

396.3

ANTAGONISM OF THE HEMODYNAMIC AND MOTOR RESPONSE TO INTRATHECAL (IT) N-METHYL D-ASPARTATE (NMDA) IN RATS. H. El Sayed-Awad; T.L. Yaksh, and M.B. Weinger*, Department of Anesthesiology, University of California, San Diego, La Jolla, CA 92093-0818

NMDA receptors play a role in pain transmission at the spinal level. We studied the effects of spinal NMDA receptor activation in the halothane (1%) anesthetized rat on blood pressure (BP), heart rate (HR) and truncal EMG activity. IT injection of NMDA (3ug/10ul) caused a short-lasting increase in HR, BP and EMG that could be reliably repeated at 30 min intervals. The pharmacology of this effect was investigated by giving escalating doses of a single agent IT, each dose 5min before each of the 4 sequential injections of IT NMDA, each 30 min apart. In the present study, NMDA (MK801 or AP-5), non-NMDA (CNQX) and NMDA-glycine site (7-chlorokynurenate) antagonists produced dose dependent reductions in the elevations in HR/BP and EMG otherwise produced by the IT NMDA. In contrast, ketamine at the highest doses was ineffective. To investigate the role of nitric oxide synthase in this model, IT L-NAME was given and found to suppress the evoked EMG but not the BP/HR response. These data suggest that the spinal action of NMDA receptors may evoke a facilitated state of processing in the anesthetized animal that has been described for dorsal horn windup and in unanesthetized animal models of protracted afferent input, such as on the formalin test. (DA02110)

396.4

NMDA EVOKES SPINAL RELEASE OF AMINO ACIDS AND PROSTANOIDS IN THE ANESTHETIZED RAT. L.S. Sorkin*. Anesthesiology Research Lab., Univ of CA at San Diego, La Jolla, CA. 92093-0818.

Activation of N-methyl-D-aspartate (NMDA) receptors in the spinal cord dorsal horn has been linked to initiation of hyperalgesia. In order to examine the pharmacological sequelae of NMDA receptor occupancy, this study measured the spinal release of glutamate, glycine, citrulline (as an indirect measure of NO production), prostaglandin E2 and thromboxane B2 in response to intrathecal (IT) NMDA. Rats with IT catheters were anesthetized with halothane, implanted with transverse dialysis probes across the dorsal horns of the rostral lumbar enlargement and dialyzed with artificial CSF (5μl/min). End-tidal halothane was maintained at 2-2.5% during surgery and then reduced to 1.1%; incisions were treated with 0.5% bupivacaine. After 3 hrs of washout and 2 basal samples (1 hr), 3 μg of NMDA was given IT and 3 samples taken in the next 1.5 hrs. In some experiments, IT administration of MK-801 (NMDA antagonist), L-NAME (NO syntase inhibitor) or ketorolac (cyclooxygenase inhibitor) preceded the NMDA. Following NMDA, mean peak responses of Glu, Cit, Gly, PGE2 and TxB2 were significantly elevated over baseline. Pretreatment with MK-801 prevented all evoked release. L-NAME, but not its inactive stereoisomer D-NAME, blocked the evoked release of both Glu and Cit and reduced, but did not eliminate the release of PGE2 and TxB2. Ketorolac blocked the NMDA-induced increase in PGE2 and TxB2 and reduced the basal and evoked release of Cit. These data support the hypotheses that spinal release of Glu, NO and prostanoids participates in the generation of hyperalgesia and that Glu release is secondary to NO production. While prostanoid and NO release appear to be interrelated, the relationship is not clear. This work was funded in part by an Arthritis and Prostaglandin Research Challenge Grant from GD Searle & Co.

396.5

MK-801 REDUCES PERSISTENT HINDLIMB FLEXION SUBSEQUENT TO INDUCTION R.D.Moore,D.J.Mokler, and B.J.Winterson*,Depts.Physiol.&Pharm.,UNECOM,Biddeford,ME 04005

NMDA receptors have been implicated in spinal nociceptive transmission and the *induction* of persistent hindlimb flexion (PHF). NMDA receptors may be involved in the *maintenance* of PHF. PHF was induced in Long-Evans rats with constant current square wave pulses (7ms, 2 mA, 100Hz) across the thigh for 60 min. PHF was assessed by adding weight until stimulated and unstimulated leg lengths were equal. After flexion measurements on day 0, rats were assigned to 4 groups (n=9-12) in which a rat either received an i.p. injection of saline (Controls) or MK-801 (0.1 mg/kg, 1 mg/kg or 3 mg/kg). Flexion was measured at 1 hr and rats were allowed to recover. On day 1, rats were anesthetized, flexion measured, then given saline or MK-801 (same dose as day 0) and flexion was measured at 1 hr. On day 2, rats received an injection of saline or MK-801 (same dose as day 0) without anesthesia or measurement. On day 3, following anesthesia, flexion was measured. Rats were spinalized at T7 and flexion was measured. All rats showed flexion after stimulation but Controls showed no change in flexion after saline injection nor any significant change in mean flexion over 3 d. Rats that received 0.1 mg/kg MK-801 did not show a significant effect on day 0. However, mean flexion significantly dropped by day 3 (6.5 to 3.5gm). Rats that received 1 or 3 mg/kg MK-801'showed a drop in mean flexion on day 0 (1:9.3 to 6.3 gms; 3:10.7 to 7.5 gms). On day 3 mean flexion had dropped in rats receiving 1 or 3 mg/kg MK-801 (1: 3.8 gms, 3: 5 gms). These results suggest that the maintenance as well as induction of PHF involves NMDA receptors. (supported by the American Osteopathic Association)

396.6

THE COMPETITIVE NMDA ANTAGONIST CPP PLUS NON-NMDA ANTAGONIST DNQX POTENTIATES ANTINOCICEPTION MORE THAN MK-801 PLUS DNQX IN FORMALIN PAIN MODEL. Y.M. Goettl* and A.A. Larson, Graduate Program in Neuroscience, University of Minnesota, St. Paul, MN 55108, U.S.A.

Activity at *N*-methyl-D-aspartate (NMDA) and non-NMDA excitatory amino acid receptors has been implicated in the acute phase and NMDA receptors in the tonic phase of the formalin pain model at the spinal cord level. How these two receptor systems interact to transmit pain is not known. Using doses at about ED_{50} values, we injected 6 nmoles of DNQX, a competitive non-NMDA antagonist, 80 pmoles of CPP, a competitive NMDA antagonist or 1 nmole of MK-801, a PCP ligand and non-competitive NMDA antagonist, individually or in combination in 23-30 g male Swiss-Webster mice. Drugs were administered intrathecally 5 min before injecting 20 µl of 5% formalin s.c. in the dorsum of the rear foot. All three drugs individually were antinociceptive in the acute phase (0-5 min) inhibiting behaviors to 45-60% that of control mice. While MK-801+DNQX decreased behaviors to 34% of control, CPP+DNQX was profoundly antinociceptive with decreases to 5% of control. CPP+DNQX also produced hind limb paralysis or paresis that appeared to dissipate by the tonic phase. In the tonic phase (20-30 min), CPP and MK-801 each significantly decreased behaviors to 38% and 64% of control, respectively, while DNQX had no effect. CPP+DNQX decreased behaviors to 7% of control whereas MK-801+DNQX was not antinociceptive. DNQX interacts differently with MK-801 than with CPP suggesting that MK-801 has activity not linked to inhibition of NMDA receptors. (Supported by NIDA training grant T32 DA07234, NIDA 04190, 04090 and 00124.)

396.7

MECHANISMS OF ACUTE THERMAL AND MECHANICAL HYPERALGESIA: RECEPTOR SUBTYPES AND CELLULAR EVENTS A. Burnett*, S.T. Meller, C. Dykstra and G.F. Gebhart, Dept. Pharmacol., Univ. Iowa, Iowa City, IA 52242, USA.

This study examined the excitatory amino acid (EAA) receptor subtypes and the cellular events that mediate acute thermal and mechanical hyperalgesia in the rat. Rats were injected with EAA agonists through a chronically implanted intrathecal (i.t.) catheter, and tested 0.5, 1, 2, 5, 10 and 15 min post-drug. Receptor antagonists or blockers of intracellular enzymes were tested against agonists that produced thermal or mechanical hyperalgesia. Only NMDA produced a dose-dependent thermal hyperalgesia; it was blocked by APV but not by DNQX or AP3. In contrast, QA or a 1:1 combination of AMPA + trans-ACPD, but not NMDA, AMPA or trans-ACPD alone, produced a dose-dependent mechanical hyperalgesia; DNQX or AP3, but not APV, attenuated the mechanical hyperalgesia. Thermal hyperalgesia was attenuated in a dose-dependent manner by L-NAME, methylene blue (MB), hemoglobin (Hb), H-7 or chelerythrine chloride (CC) but was unaffected by mepacrine, neomycin, baicalein, NDGA or indomethacin. Mechanical hyperalgesia was unaffected by L-NAME, MB, Hb, H-7, CC, neomycin or baicalein, but was dose-dependently attenuated by mepacrine, NDGA or indomethacin. In conclusion, acute thermal and mechanical hyperalgesia are produced by activation of NMDA and AMPA + metabotropic receptors, respectively. Thermal hyperalgesia is produced by activation of NOS, GC-S and PKC, but not PLA_2 or PLC or cyclooxygenase or lipoxygenase products. Mechanical hyperalgesia is mediated produced by activation of PLA_2 and production of cyclooxygenase products, but not by NO, cGMP, lipoxygenase products or activation of PKC or PLC.

396.8

CHARACTERIZATION OF THE SPINAL MECHANISMS OF THERMAL AND MECHANICAL HYPERALGESIA FOLLOWING INTRAPLANTAR ZYMOSAN S.T. Meller,* C. Dykstra and G.F. Gebhart, Dept. Pharmacol., University of Iowa, Iowa City, IA 52242.

The excitatory amino acid (EAA) receptor subtypes and the intracellular cascade of events produced following intraplantar injection of zymosan A were investigated in awake rats.

Intraplantar zymosan A produced a rapid onset (< 30 min), long-lasting (24-72 h) and dose-dependent (0.0625-5 mg) thermal and mechanical hyperalgesia accompanied by edema. Three hours after zymosan, the effect of EAA receptor antagonists (MK-801, APV, DNQX or AP3) or blockers of intracellular enzymes (L-NAME, hemoglobin, mepacrine, chelerythrine chloride or indomethacin), injected through a chronically implanted intrathecal catheter, were examined. APV, MK-801, L-NAME, hemoglobin and chelerythrine chloride attenuated or blocked the thermal but not the mechanical hyperalgesia produced by zymosan. In contrast, DNQX and AP3 attenauted both the thermal and mechanical hyperalgesia and mepacrine or indomethacin attenuated the mechanical but not the thermal hyperalgesia. None of the antagonists or enzyme blockers produced any change in latencies or thresholds in sham rats.

These data suggest that (1) zymosan produces reliable and reproducible, rapid onset and long-lasting thermal and mechanical hyperalgesia, (2) the thermal hyperalgesia is produced by activation of NMDA, AMPA and metabotropic glutamate receptors, production of NO and activation of PKC, and (3) mechanical hyperalgesia is produced by activation of AMPA and metabotropic glutamate receptors, activation of PLA_2 and production of cyclooxygenase products, but not by NMDA receptors, production of NO or activation of PKC or PLC.

396.9

INHIBITION OF THERMAL HYPERALGESIA BY L-NAME FOLLOWING UNILATERAL HINDPAW INFLAMMATION. R.J. Traub*, S.T. Meller and G.F. Gebhart, Dept. Pharmacology, Univ. of Iowa, Iowa City, IA 52242.

Carrageenan-induced unilateral hindpaw inflammation results in thermal and mechanical hyperalgesia of the inflamed hindpaw. Studies from our laboratory have suggested that nitric oxide (NO) mediates thermal hyperalgesia associated with sciatic nerve injury and pharmacologically-induced facilitation of the thermal withdrawal reflex. The role of NO in hyperalgesia following hindpaw inflammation was examined.

Male Sprague-Dawley rats were injected in the left hindpaw with 100µl 2% carrageenan. L-NAME (2, 20, 200 nmol), D-NAME (200 nmol) or saline were injected intrathecally (2 µl, 10 µl saline flush) 20 min prior to (and again 2 hrs later; pre-treat) or 4 hrs following (post-treat) carrageenan.

Carrageenan produced inflammation and thermal and mechanical hyperalgesia that peaked at 3 hrs and persisted beyond 12 hrs. Post-treatment with L-NAME produced a dose-dependent attenuation of the thermal, but not mechanical, hyperalgesia that lasted 3-5 hrs. There was no effect upon the non-inflamed hindpaw. D-NAME and saline had no effect. Pre-treatment with L-NAME (all doses) delayed the onset of thermal hyperalgesia by 3 hrs.

L-NAME blocks NO production. These data suggest that NO mediates thermal but not mechanical hyperalgesia and is necessary for the development and maintenance of hyperalgesia resulting from hindpaw inflammation.

396.10

POTENTIATION OF (+)-*CIS*-DIOXOLANE ON ANTINOCICEPTION INDUCED BY I.C.V. ß-ENDORPHIN IS MEDIATED BY NO-CGMP IN THE MOUSE. J. Y. Xu and L. F. Tseng*, Department of Pharmacology and Toxicology, Medical College of Wisconsin, Milwaukee, WI 53226.

(+)-*cis*-Dioxolane (CD), a muscarinic receptor agonist, at doses (0.5-2 µg, i.c.v.) produced a dose-dependent inhibition of the tail-flick response (TF) and at subantinociceptive doses (0.25 µg, i.c.v.) potentiated i.c.v. administered ß-endorphin(ß-EP)-induced TF inhibition in male ICR mice. The potentiation of ß-EP-induced antinociception by CD is attenuated by hemoglobin (120 µg), Nω-nitro-L-arginine (1 µg) or methylene blue (5 µg), given i.c.v., indicating that NO-cGMP system is involved in the potentiation. The same treatment of mice with hemoglobin, Nω-nitro-L-arginine or methylene blue also blocked CD-, but not ß-EP-induced TF inhibition. CD selectively potentiated the TF inhibition induced by ß-EP, an ε receptor agonist, but not morphine or DAMGO, µ receptor agonists, DPDPE, a δ receptor agonist, or U50,488H, a κ receptor agonist. It is concluded that ß-EP-induced antinociception is selectively potentiated by the activation of muscarinic receptors by CD which is mediated by NO-cGMP system (supported by NIH grant DA 03811).

396.11

INVOLVEMENT OF GLYCINE IN SPINALLY-MEDIATED NOCICEPTION CHRISTIE MCNICKLE and LIN AANONSEN*. Department of Biology, Macalester College, St. Paul, MN 55105.

Previous studies indicate that glutamate, acting at the NMDA receptor, may be involved in spinally-mediated nociception. Since glycine has been shown to enhance the effects of glutamate at the NMDA receptor complex, we hypothesized that intrathecal (i.t.) glycine may also be involved in spinally-mediated nociception. In addition to glycine's effect at the NMDA receptor complex, glycine also acts at a lower affinity site where it is thought to be one of the major inhibitory neurotransmitters in the spinal cord. To eliminate activation of this site by glycine, we used the selective antagonist strychnine. For activation and antagonism of the glycine site linked to the NMDA receptor complex, we used the agonists glycine and serine and the selective antagonist 7-chlorokynurenic acid (7-CK). We proposed that the agonists, acting at the NMDA receptor complex, would produce hyperalgesia, while the antagonist would be analgesic. To test this we administered the drugs (i.t.) in mice. The pain threshold was determined by placing the mouse on a hot-plate and measuring the latency to lick its hind-paw. At low doses, strychnine alone produced hyperalgesia, which could be blocked by the noncompetitive NMDA antagonist PCP. 7-CK (≥2.72mM) increased hot-plate latencies, but animals also displayed motor impairment. The glycine dose response curve was biphasic; at low doses glycine produced hyperalgesia which could be attenuated by PCP and at high doses, glycine was analgesic. These results support the presence of a high affinity and a low affinity glycine site in the rat spinal cord. The results also suggest that endogenous pools of glycine can be experimentally shifted to activate the NMDA site, and thereby enhance pain responses.

396.12

INTRATHECAL CPP AND DAMGO ACT SYNERGISTICALLY TO DECREASE THERMAL HYPERALGESIA IN INFLAMED RAT HINDPAW. G.M. Williams*, R.M. Caudle, K. Ren, D.A. Thomas, and R. Dubner. Neurobiol. Anesth. Br., NIDR, NIH, Bethesda, MD 20892.

We have demonstrated that both intrathecal NMDA antagonists (EJP 219:235) and intrathecal opioid agonists (EJP 194:135) reverse the hyperalgesia produced by complete Freund's adjuvant (CFA) injection into the rat hindpaw. Here we investigate if co-administration of a competitive NMDA antagonist ((±)-3-(2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid; CPP) and a mu opioid agonist ((D-Ala^2,MePhe^4,Gly-ol^5) enkephalin; DAMGO) produces a supra-additive antinociceptive action. An isobolographic approach was utilized to analyze for synergism.

Rats were cannulated intrathecally and received a unilateral hindpaw injection of CFA to produce thermal hyperalgesia (paw withdraw latency inflamed paw = 4.5 ± 0.2 sec; contralateral = 9.5 ± 0.2 sec). Dose-response curves for DAMGO alone (0.01 - 10 nmol), CPP alone (0.3 - 3000 nmol) and CPP + DAMGO were determined. CPP + DAMGO combinations were administered in a constant dose-ratio of 62 nmol CPP/ 1 nmol DAMGO, with the total drug dose ranging from 0.26 - 26 nmol. The doses estimated to produce paw withdraw latencies of 12.2 sec on the inflamed paw (half-way between inflamed baseline and 20 sec cutoff) were: CPP , 62 nmol; DAMGO, 1.5 nmol; and CPP + DAMGO, 4.4 nmol. This CPP + DAMGO value is 7-fold lower than that predicted for a simple additive interaction (isobolographic prediction using the individual dose-response curves). After transforming the data to account for the potency difference between CPP and DAMGO, general linear modelling of the individual and combination dose-response curves showed that the supra-additive interaction was statistically significant ($p < 0.05$). The finding of a synergistic interaction suggests that NMDA antagonist/opioid agonist combination therapy may provide increased pain relief, decreased toxicity, or both, in a clinical setting.

396.13

MORPHINE AND EXCITATORY AMINO ACID ANTAGONISTS DISTINGUISH ALLODYNIA FROM OTHER PAIN STATES S.E. Sherman* and C.W. Loomis. School of Pharmacy, Memorial Univ., St. John's, NF, Canada A1B 3V6

The effects of intrathecal (i.t.) morphine and excitatory amino acid antagonists on i.t. strychnine (STR)-dependent allodynia were investigated in the lightly anesthetized rat. With i.t. STR (40 μg), normally innocuous hair deflection (HD) evoked a motor withdrawal response, a pressor response and tachycardia; responses that are normally evoked by noxious pinch or heat, but not HD alone. I.t morphine (50 μg) blocked responses to noxious heat and pinch but was ineffective against STR-dependent responses to HD. These HD-evoked, STR-dependent responses were significantly attenuated by the non-selective excitatory amino acid antagonist gamma-D-glutamylglycine (i.t), and the AMPA-selective antagonist NBQX (i.t.). The failure of i.t morphine to inhibit HD-evoked, STR-dependent responses indicates that allodynia is not mediated via a conventional nociceptive pathway. Since non-NMDA receptor antagonists are reported to be ineffective in experimentally-induced hyperalgesia, sensitivity to NBQX distinguishes allodynia from hyperalgesia. (Supported by MRC Canada)

396.14

NITRIC OXIDE AND *N*-METHYL-D-ASPARTATE RECEPTORS: ROLE IN SPINAL REFLEXES AFTER PERIPHERAL INFLAMMATION M.L. Semos and P.M. Headley, SPON: Brain Research Association. Physiology Dept., University of Bristol, BS8 1TD, U.K.

Single motor unit activity was recorded electrophysiologically under *α*-chloralose anaesthesia in both spinalized and non-spinalized rats. The flexion withdrawal reflex was evoked with noxious pinch stimuli applied to the hindpaw receptive field (2.2N/4mm^2 intact: 2.8N/4mm^2 spinalized, every 3 mins). The NMDA receptor contribution to the reflex was first tested with ketamine, a non-competitive NMDA antagonist. Inflammation of the hindpaw was induced by topically applied mustard oil (100%) and a ketamine test repeated. The role of nitric oxide (NO) was then assessed using the NO synthase inhibitor N^G-nitro-L-arginine methyl ester (L-NAME) given in a dose-doubling regime to 40mg/kg I.V.. Drug effects are expressed as the mean % of control pre-drug values (±S.E.M).

All drugs significantly affected the reflex to a greater degree in non-spinalized animals. Ketamine 6.7mg/kg I.V. significantly inhibited the pinch reflex (33±6%, n=8 intact: 61±8%, n=6 spinalized). Mustard oil significantly enhanced responses (219±70%, n=12 intact: 128±10%, n=6 spinalized). There was no difference in either group in the effectiveness of ketamine in the hyperalgesic state. Without inflammation, L-NAME 40mg/kg had no effect on reflexes to noxious pinch in intact rats (Semos & Headley, 1993, J. Physiol. **459**, 503P). However, in the hyperalgesic state L-NAME significantly inhibited this reflex, but only when the cord was intact (70±8%, n=10 intact: 113±6%, n=6 spinalized).

These data indicate that there is no enhancement of the NMDA receptor contribution to reflexes after acute inflammation, whereas NO pathways are activated. There is thus no clear link between NO and NMDA mechanisms in this system. The role of NO in nociception with peripheral inflammation is predominantly supraspinal.

This work is supported by the M.R.C.

396.15

NITRIC OXIDE (NO) MEDIATED MUSCARINIC ANALGESIA: SITES OF NO SYNTHASE AND GUANYLYL CYCLASE mRNA-CONTAINING NEURONS IN RAT ROSTRAL VENTRAL MEDULLA. E.T. Iwamoto*, L.J. Marion, R.M. Booze, and M.C. Tyler§. Dept. of Pharmacology, Univ. of Kentucky Col. of Med., Lexington, KY 40536, and §Du Pont Res. and Devel./NEN Products, Boston, MA 02118.

To investigate the hypothesis that the analgesia produced by microinjections of the muscarinic agonist (+)-*cis*-methyldioxolane (CD) into the rostral ventral medulla (RVM) is mediated by nitric oxide (NO), we studied the effects of the NO synthase inhibitor L-N^5-nitroarginine (NNR). Five min preadministration of 6 nmol NNR antagonized CD-produced antinociception in both the 52°C hot-plate and tail-flick tests. The NO precursor L-arginine (LR), but not DR, reversed the NNR-induced inhibition of CD-produced antinociception. The guanylyl cyclase inhibitor methylene blue also significantly decreased CD antinociception. Dibutyryl cGMP or 8-bromo cGMP produced antinociception when injected alone in the RVM; buffer, NNR, LR, DR, or methylene blue alone were without effect. As a morphologic test of our hypothesis, the sites of NO synthase (NOS) and soluble guanylyl cyclase (sGC) in the RVM were localized using *in situ* hybridization histochemistry with ^{33}P-labeled oligonucleotide probes complementary to NOS or sGC mRNA. Significant levels of NOS and sGC mRNA were localized in a region that includes both the gigantocellular reticular nucleus pars alpha and the lateral paragigantocellular nucleus. The data support the hypothesis that muscarinic antinociception is mediated by a LR/NO/cGMP cascade in the RVM. (Supported by NIH NS 28847)

396.16

NADPH-DIAPHORASE STAINING IN THE SPINAL CORD OF MONONEUROPATHIC RATS IS REDUCED WITH ADRENAL MEDULLARY TRANSPLANTS. A. T. Hama and J. Sagen. Department of Anatomy and Cell Biology, Univ. of Illinois at Chicago, Chicago, IL 60612.

Previous work in our laboratory has shown that adrenal medullary tissue transplanted into the subarachnoid space of rats with unilateral painful peripheral neuropathy reduces thermal allodynia and thermal hyperalgesia. Adrenal medullary transplants may alleviate pain directly by releasing opioid peptides and catecholamines and, possibly indirectly, by inhibiting or reducing the neurochemical changes that lead to the chronic pain state. Increased activity or synthesis of nitric oxide synthase (NOS), leading to increased levels of nitric oxide, could increase and prolong abnormal pain sensations. Thus, levels of NOS, NADPH-diaphorase (NADPH-d) in spinal cord, may serve as a marker for cellular activity. By loosely ligating the right sciatic nerve in rats, various pain symptoms such as thermal hyperalgesia and cold allodynia were induced. Animals were given either adrenal medullary or control striated muscle transplants two weeks after nerve ligation. One week after transplantation, animals that received adrenal medullary tissue showed significantly reduced hyperalgesia and allodynia in contrast to control transplanted animals. After behavioral evaluation, transplanted animals and age-matched animals that received no surgery were perfused, and histochemistry for NADPH-d was performed on spinal segments L_4-L_5. In control transplanted animals with nerve ligation, there was a marked increase in NADPH-d stained cells and processes in medial superficial dorsal horn, in laminae IV-VII and in lamina X ipsilateral to the side of nerve ligation. In contrast, in animals with adrenal medullary transplants, NADPH-d staining was similar to non-ligated control animals. The number and intensity of stained cells and processes were reduced in all three spinal regions of adrenal transplanted animals compared to animals with control transplants. These results suggest that adrenal medullary transplants may reduce chronic neuropathic pain by decreasing spinal levels of NADPH-d/NOS and levels of nitric oxide, which has been implicated in long-term persistence of intractable pain. Aided by a grant from the Paralyzed Veterans of America Spinal Cord Research Foundation and the National Spinal Cord Injury Association, Illinois Chapter.

396.17

SODIUM NITROPRUSSIDE POTENTIATES CAPSAICIN-INDUCED RELEASE OF PEPTIDES FROM CULTURED RAT SENSORY NEURONS. J. Dymshitz*, M.R. Vasko. Dept. of Pharmacology and Toxicology, Indiana U. School of Medicine, Indianapolis., IN 46202.

Administration of substances that spontaneously release nitric oxide (NO) onto the spinal cords of rodents results in an increased sensitivity to noxious stimuli. One possible mechanism for these hyperalgesic actions of NO donors is that NO enhances the release of neurotransmitters from nociceptive sensory neurons. To address this issue, we examined whether sodium nitroprusside (SNP), a donor of NO, could alter the resting or stimulated release of substance P (SP) and of calcitonin gene-related peptide (CGRP) from rat sensory neurons grown in culture.

Dorsal root ganglia were dissected from rat fetuses (E15-17), mechanically dissociated after incubation with collagenase, and the cells were grown for 10-12 days in DMEM supplemented with fetal bovine serum, NGF and mitotic inhibitor. Release experiments were performed by incubating sensory neurons with a HEPES buffer containing 3.5 mM KCl (resting release) or buffer containing various concentrations of SNP (0.1 μM -1000 μM) for 10 min. The cells were then exposed to 50 nM capsaicin in the presence or absence of SNP.

When neurons were treated with SNP, the resting release of CGRP was not significantly altered, whereas 1 mM SNP increased SP release 3 fold. Pretreating cells with 10 μM or 100 μM SNP (concentrations that did not alter resting release) enhanced the release of SP and CGRP evoked by 50 nM capsaicin approximately 2-3 fold. Exposure of the cells to 100 μM potassium ferrocyanide did not alter resting or stimulated release of the peptides. Furthermore, the stimulatory actions of SNP were not mimicked by a 1 hr pretreatment with 500 μM 8'-bromo-cGMP.

These results suggest that NO enhances the capsaicin-evoked release of SP and CGRP from primary sensory neurons. Because 8'-bromo-cGMP does not augment release, the effect of NO may not be mediated through an increase in intracellular cGMP. (Supported by PHS DA07176)

396.18

APPLICATION OF L-NITRO-ARGININE METHYL ESTER ATTENUATES THE SENSITIZATION OF STT NEURONS FOLLOWING ACUTE ARTHRITIS. H. Rees*, J. Palecek and W.D. Willis. Marine Biomedical Institute, UTMB, Galveston, Texas 77555-0843.

The sensitization of STT neurons following acute arthritis was studied in 8 anesthetized adult monkeys (Macaca fascicularis). STT neurons were recorded with single barreled carbon fibre microelectrodes and identified by antidromic activation from the contralateral thalamus. Cells were selected with large receptive fields on the hind limb and which also showed some response to knee stimulation. Control responses to graded cutaneous stimuli (brush, press, pinch & heat) as well as joint flexion were established. Acute arthritis was then induced by injection of kaolin/carrageenan into the knee capsule. Following injection, the knee joint was flexed for 15 min periods every 30 mins for 4 h. The responses to cutaneous stimuli and flexion were then retested. The responses of the same STT cells to flexion of the knee joint were increased markedly for all degrees of flexion tested. The responses to cutaneous mechanical stimuli at sites distant from the inflamed knee were also significantly increased. There was little change in the responses to intense mechanical or thermal cutaneous stimuli. The nitric oxide synthase inhibitor L-nitro-arginine methyl ester (L-NAME) was then administered via a microdialysis fiber previously inserted in the dorsal horn of the spinal cord. L-NAME significantly attenuated the increases in responses to mechanical cutaneous stimuli while application of the inactive isomer D-NAME did not. The results suggest that acute arthritis sensitizes STT cells, increasing the efficacy of the input from low threshold mechanoreceptors. This sensitization is partly reversed by central administration of L-NAME, which indicates that the process is at least partly dependent on the production of nitric oxide in the spinal cord. However, the enhanced responses to flexion were unaltered by L-NAME, suggesting that peripheral sensitization was more important than central effects in increasing these responses. (Supported by NS 09743, NS11255 and Bristol Myers-Squibb)

396.19

POTENTIAL ROLE FOR NITRIC OXIDE IN THE DEVELOPMENT OF HYPERALGESIA PRODUCED BY CAPSAICIN. H.D. Gilchrist[1,2]*, B.A. Allard[1] and D.A. Simone[1,2]. [1]Neuroscience Research in Psychiatry and [2]Graduate Program in Neuroscience, Univ. of Minnesota, Minneapolis, MN 55455.

Intradermal (ID) injection of capsaicin (CAP) in humans produces cutaneous hyperalgesia to heat and mechanical stimuli, and enhances excitability of primate dorsal horn neurons. In order to study underlying pharmacological mechanisms, a similar model of hyperalgesia was developed in rat. Rats received one ID injection of vehicle or CAP (1, 10 or 30 μg in 10 μl Tween saline) into the plantar surface of one hindpaw. Nociceptive responses evoked by heat and mechanical stimuli (von Frey monofilaments) applied to the plantar surface of both hindpaws were assessed before and after injection. Hyperalgesia to heat and mechanical stimuli was defined as facilitation of withdrawal responses (decreased withdrawal latency to heat and increased withdrawal response frequency to von Frey stimuli). CAP produced a dose-dependent decrease in withdrawal latency and increased the frequency of withdrawal responses. Withdrawal latency decreased 50% and withdrawal response frequency increased 60-70% following the 30 μg dose. Facilitation of withdrawal responses typically lasted 1-3 hr.

Additional rats were pretreated 30-60 min before CAP with the nitric oxide (NO) synthesis inhibitor N-nitro-L-arginine methyl ester (L-NAME) either systemically (100 mg/kg, IP) or into the plantar hindpaw at the site of the subsequent CAP injection (200 μg in 30 μl, ID). Systemic L-NAME decreased the magnitude of hyperalgesia to both heat and mechanical stimuli. L-NAME injected directly into the paw did not alter the development of hyperalgesia. We conclude that 1) ID injection of CAP in rats is a useful model of hyperalgesia, and 2) NO in the CNS plays a role in the development of hyperalgesia following CAP. Supported by NIH NS31223 and the Minnesota Medical Foundation.

396.20

NERVE GROWTH FACTOR ALLEVIATES A PAINFUL PERIPHERAL NEUROPATHY IN RATS. D. Thomas*, K. Ren and R. Dubner. Neurobiology and Anesthesiology Branch, NIDR, NIH, Bethesda, MD 20892.

Nerve growth factor (NGF) reverses some effects of axotomy and prevents toxic neuropathy in adult rodents. We tested the possibility that NGF may block behavioral signs of hyperalgesia and allodynia resulting from a chronic constriction injury (CCI) of the sciatic nerve. CCI was induced in rats by ligation of the sciatic nerve under sodium pentobarbital (50mg/kg) anesthesia. This procedure produced behavioral hyperalgesia from post-operative day (POD) 1-3 as demonstrated by a quicker paw withdrawal from a noxious thermal stimulus on the side of injury, when compared to the contralateral hindpaw. The mechanical sensitivity of the ipsilateral hindpaw, assessed with von Frey filaments, was also significantly increased. A cuff was placed around the sciatic nerve at the site of ligation and 2.5S NGF was infused into the cuff via an Alzet osmotic pump at a rate of 0.5 μg/μl/h for 7-10 days. When NGF infusion was started immediately after the nerve ligation, thermal hyperalgesia was significantly reduced or abolished from POD 5 up to the end of the test period (POD 42), when compared with rats receiving vehicle infusion. CCI-induced increase in mechanical sensitivity was also abolished by NGF infusion. Delayed infusion of NGF (POD 4) failed to block thermal hyperalgesia. Infusion of NGF had no significant effect on paw withdrawal latency of the rats that had no CCI. Thermal hyperalgesia developed, but was not enhanced in CCI rats receiving an infusion of anti-NGF antiserum. These results suggest that alterations in neurotrophic factor(s) may contribute to the development of behavioral hyperalgesia in an animal model of neuropathy and that NGF may have therapeutic value in the treatment of neuropathic pain.

VISUAL CORTEX: EXTRASTRIATE—ANATOMY

397.1

Little cells ending on big cells: An Oligosynaptic Retino-tecto-pulvinar system in Pigeon. H.J.Karten, K.Cox, J. Mpodozis, H.J.Bischof, T.Shimizu* Dept. Neurosciences, UCSD, La Jolla, CA 92093-0608; Dept. Psychology, USF, Tampa, FL 33620.

The retino-tecto-pulvinar system appears to be one of the most ancient of visual pathways to the telencephalon of all vertebrates. The projections upon the rotundus/pulvinar (RtPl) from layer 13 of the tectum has been confirmed with injection of Cholera Toxin B (CTb) into the RtPl of pigeons. In addition to dense bilateral retrograde labeling of cells of layer 13 of the tectum, we observed massive retrograde filling of widely arborizing dendrites in layer 5b of the tectum. Layer 5b is the recipient of a dense retino-tectal input of small ganglion cells. We have tentatively identified the specific retinal ganglion cells terminating in layer 5b as extremely small neurons with somal diameters of 4.5-6 um, and dendritic fields of 25 um. The cells of layer 13 are exquisitely motion sensitive and have extremely large receptive fields (>50 degrees), whereas the retinal ganglion cells that appear to terminate upon their distal dendrites are extremely small neurons. The morphology of layer 13 neurons is similar to the tecto-pulvinar neurons of squirrel (May et al., 1982) and of lizards (P.Ramon, 1896). These findings suggest the common presence of a "fast" throughput pathway from retina upon the thalamus.

(Supported by NS-24560 and EY-06890 to H.J.K.)

397.2

ANATOMICAL CONNECTIONS OF SOME VISUAL CORTICAL AREAS IN THE MONGOLIAN GERBIL (*Meriones unguiculatus*). L. I. Cudmore*, C. G. Ellard, and A. I. Long. Department of Psychology, University of Waterloo, Waterloo, Ontario, CANADA N2L 3G1.

Despite a growing corpus of experimental studies of visual behaviour in the gerbil, relatively little is known about the anatomical organization of the visual system in this species. Small quantities of 1% wheat germ agglutinated horseradish peroxidase (0.1-0.2 ml) were micro-injected into various locations in the posterior neocortex of the gerbil. Following a survival period of 48 hours, gerbils were perfused. Brains were sectioned at 40μ and three adjacent series of sections were saved. One series was reacted with a modification of HRP histochemistry, using tetramethylbenzidine as the chromogen. Adjacent sections were stained with gallocyanin for Nissl substance. A third series was stained with a gold chloride stain for axons. Results from the three stains were correlated in order to describe the cytoarchitectonics of visual cortex in the gerbil and its connectional organization. Results suggested that there are at least two distinct visual cortical areas adjacent to primary visual cortex in the gerbil. Each of these areas has distinct patterns of reciprocal connections with the lateral geniculate nucleus and with the lateral posterior nucleus of the thalamus. Of particular note was our finding of a cortical zone lateral and anterior to primary visual cortex with high myelinization. This zone, which may be analogous to area TP in the squirrel as defined by Sereno et al. (1991) and area MT in the primate, is presently being tested for its motion sensing properties.

This research was funded by a grant to CGE from the Natural Sciences and Engineering Research Council of Canada.

397.3

PATTERN AND LAMINAR DISTRIBUTION OF INTRINSIC CONNECTIONS IN CAT LATERAL SUPRASYLVIAN CORTEX. A.J. Cook and G. Einstein*. Dept. of Neurobiology, Duke Univ. Med. Ctr., Durham, NC 27710.

The topographic map and receptive field properties of neurons in the cat's posterior medial lateral suprasylvian cortex (PMLS) are quite different from those of area 17. While different inputs may account for these unique properties, these may also be contributed to by differences in intrinsic circuitry. In order to investigate this possibility, we made small, focal injections of biocytin into layers 2, 3, 5, and 6 of area PMLS and reconstructed injection sites and filled axons through serial sections cut in the coronal plane. We found that in pattern and extent, the intrinsic connections of PMLS resembled those of area 17. Injections restricted to layer 2 revealed axons that traveled horizontally in the superficial layers for long distances (up to 3 mm) terminating in tufts at 500 um intervals. Injections restricted to layer 3 revealed axons that traveled horizontally for long distances (upwards to 3.5 mm) but which also traveled vertically, tufting in layer 5 before entering the white matter. Injections restricted to layer 5 filled axons that traveled horizontally in layer 5 for 2-3 mm and coursed obliquely for 1-2 mm terminating in layer 3. Injections restricted to layer 6 filled axons that formed a cup around the injection site, traveling vertically to terminate in layer 2, obliquely to terminate in layer 3, and horizontally for long distances along the 5/6 border terminating in periodic light tufts. These results suggest that there may be a typical pattern of intrinsic connections throughout visual cortex. However, at most AP levels, 3.5 mm of PMLS represents a larger region of visual space than 3.5 mm of area 17. Thus, horizontal connections in PMLS may serve to connect more disparate regions of visual space. Supported by NEI grant EY07840.

397.4

A CROSS-CORRELATION STUDY OF FUNCTIONAL CONNECTIVETY BETWEEN AREA 17 AND LATERAL SUPRASYLVIAN AREA IN CATS. N. Katsuyama, H. Sato, H. Tamura and T. Tsumoto*. Dept. Neurophysiol., Osaka Univ. Med. Sch., Suita, Osaka, 565 Japan.

The posteromedial lateral suprasylvian area (PMLS) of cat's cortex is one of extrastriate visual areas, and is believed to be involved in visual motion perception. The PMLS receives main cortical inputs from area 17 (V1) and sends feedback outputs to V1. To assess the functional significance of this reciprocal connection between PMLS and V1, we carried out cross-correlation analysis of neuronal spike trains recorded simultaneously from the both areas. Adult cats were anesthetized with a mixture of 70 % N_2O, 30 % O_2 and 0.3 - 0.5 % halothane. Animals were paralysed and maintained under artificial ventilation. Visual stimuli were presented on a computer display. Positions of receptive field center of V1 cells were located at foveal and parafoveal areas of the visual field, and those of cells in the posterior and anterior portions of PMLS tended to locate at foveal and peripheral visual fields, respectively. Among 84 pairs of cells recorded so far, significant correlation of firings was observed in some of the cases when receptive fields of both cells were completely overlapped. Typical significant correlograms obtained without visual stimuli had peaks at center (time zero), indicating common excitatory inputs and/or synchronized activities. Some correlograms showed an asymmetric positive peak, which indicates an excitatory drive from V1 to PMLS cells.
(supported in part by a HFSP grant to T.T.)

397.5

LOCAL PATCH PYRAMIDAL NEURONS ARE DISTINCT FROM CORTICOCORTICAL AND CALLOSAL CELLS IN CAT AREA 18. J.A. Matsubara* [1,2], R. Chase[3], J. Zhang[1] and D.M. Thejomayen [2]. Depts Ophthalmology[1] and Anatomy[2], U.B.C., Vancouver, B.C.; Dept of Biology[3], McGill University, Montreal, P.Q. CANADA.

Intrinsic, callosal and corticocortical pathways are distributed in patches within the upper layers of cat visual cortex. Our earlier studies showed that local patch pyramids and callosal cells form separate cell populations, but not separate patch networks. Here,we compare the corticocortical (area 18 to area 17) cells to local patch pyramids in 18. Using intracellular injections into prelabeled targets in fixed slices, cells were filled with lucifer yellow and imaged en bloc with confocal microscopy.

The vast majority of corticocortical neurons were pyramidal cells (99 of 105 cells), but a small number of nonpyramidal (3/105) and fusiform (3/105) cells were also observed. The most common pyramidal cell type was the standard pyramid (80/99), but 'armed' standards (8/99), modified (7/99), star (2/99) and inverted (2/99) types were present. All three projection populations contained standard pyramids, but of different size ranges. Modified and star pyramids were only found in the corticocortical and local patch populations, while 'armed' standards were only found in the callosal and corticocortical populations. Although cell types often overlapped, double labeled studies show less than 5% of the local patch neurons also project corticocortically, while none of the local patch neurons project callosally. These results suggest that most local patch pyramidal cells are distinct from projection pyramids. Whether it is cell lineage or chemotropic factors that dictate if a cell becomes a local patch or projection pyramid remains unknown. This work was funded by MRC (Canada) and NSERC.

397.6

NOVEL AND EXPANDED VISUAL THALAMIC PROJECTIONS TO LATERAL SUPRASYLVIAN CORTEX FOLLOWING REMOVAL OF AREAS 17 & 18 FROM THE DEVELOPING CAT. S.G. Lomber*, M.A. MacNeil and B.R. Payne. Dept. of Anatomy & Neurobiology, Boston University School of Medicine, Boston, MA 02118.

Both behavioural and physiological studies have implicated lateral suprasylvian (LS) cortex in the sparing of visual functions following the removal of areas 17 & 18 in the developing cat. We tested the hypothesis that thalamic projections to LS cortex expand following removal of immature areas 17 & 18 on P1 or P28 by injecting retrograde tracers into LS cortex when the cats were at least six months of age or older. Patterns of cell labelling were then compared to the patterns in intact cats and cats which underwent comparable lesions in adulthood.

Compared to intact cats, lesions on both P1 and P28 result in an increase in the numbers of labeled neurons and a change in their distribution. In the dorsal lateral geniculate nucleus, lesions induce novel projections from the A-laminae and increase projections from the C-complex. In the extrageniculate visual nuclei of the P1 group, there is a 100% increase in projections from the medial division of the lateral posterior nucleus. The magnitude and pattern of labelling in the adult lesion group is indistinguishable from that in the intact cat.

These results show that visual thalamic inputs to LS increase following lesions of immature visual cortex and it is likely that the magnitude of the visual signals conveyed along these pathways is increased. These signals may contribute to the spared functions attributed to LS cortex following removal of areas 17 & 18.
(Supported by MH44647 and NS07152)

397.7

AMPLIFICATION OF THE PATHWAY FROM AREAS 20 AND PS TO LATERAL SUPRASYLVIAN CORTEX AFTER REMOVAL OF AREAS 17 & 18 IN THE NEWBORN CAT. M.A. MacNeil*, S.G. Lomber, and B.R. Payne. Department of Anatomy & Neurobiology, Boston University School of Medicine, Boston, MA 02118.

Visual areas 17 & 18 provide the major cortical input to lateral suprasylvian (LS) cortex in the cat. If these visual areas are removed in the adult, functional deficits result that are not observed when the same type of damage is incurred shortly after birth. The purpose of our study was to determine whether anatomical rewiring of cortico-cortical projections to LS cortex may help explain the behavioral compensation following neonatal ablations of areas 17 & 18.

Retrograde tracers were injected into LS cortex and the pattern of cortical labelling seen in intact adult cats was compared to that present in adult cats which had incurred lesions of visual areas 17 & 18 either on the day of birth (P1 group) or during adulthood (adult group). When equating animals by injection size, the P1 group had at least 30% more cells labelled throughout cortex compared to either the intact or adult groups. In addition, the projection from areas 20 and PS to LS cortex in the P1 group was significantly increased. Comparisons between intact and adult groups yielded no significant differences.

Our results indicate that removal of areas 17 & 18 on the day of birth leads not only to a numerical compensation in the numbers of cells projecting to LS, but also to an increase in the magnitude of visual projections from ventral extrastriate regions. These shifts may be responsible for behavioral compensation demonstrated in neonatally damaged cats. (Supported by MH44647 and NS07152)

397.8

COMPARISON OF INPUTS FROM AREA V2 TO AREAS V4, MT AND PO IN CEBUS. R. Gattass, S. Nascimento-Silva, M. Fiorani Jr. and A.P.B. Souza*. Dept. Neurobiologia, IBCCF, UFRJ, Rio de Janeiro, RJ, 21941-900, Brazil.

To investigate the differential inputs from area V2 to areas V4, MT and PO in Cebus monkeys we injected multiple retrograde tracers and analysed the distributions of labeled neurons in V2. We sectioned one brain in the parasagittal plane, one in the coronal plane and in the last one we used flattened preparations of the cortex. In V2 we found labeled neurons arising from V4, MT and PO. Both V4- and MT-projecting neurons were located in stripes running orthogonal to the V1/V2 border. As in the macaque MT-projecting neurons were located in the cytochrome oxidase-rich thick stripes, and V4-projecting neurons were in both cytochrome oxidase-rich thin stripes and interstripes regions. Most of the PO-projecting neurons were found in the thick stripes, but they were also found in thin stripes and in the interstripe regions. PO-projecting neurons were occasionaly intermingled with those from MT and V4. The results indicate that three distinct visual pathways arise from different sets of modules in V2. (Supported by CEPG/UFRJ, CNPq and FINEP).

397.9

CORTICAL CONNECTIONS OF INFEROTEMPORAL AREA CITv OF MACAQUE MONKEYS. E. McClendon* and D.J. Felleman. Dept. of Neurobiology and Anatomy, Univ. of Texas Med. School, Houston, TX 77030.

Area CITv was first identified as one of the four projection zones of V4 in inferotemporal cortex (IT). To compare the connections of CITv to previously studied connections of PITv (Felleman and McClendon, '91), and to investigate further the organization of anterior IT, we examined the distribution and laminar patterns of labeled cells and/or terminals following injections of neuroanatomical tracers into CITv and PITv in 5 hemispheres. The distribution of callosal projecting neurons and/or myeloarchitecture served as an independent reference for various areal borders. In 2 monkeys, paired CITv injections of nuclear yellow (NY) and bisbenzamide (BB) produced multiple patches of labeled cells in layer 3 of areas V4 and VOT, and in layers 3 and 5 of areas PITv and PITd. A complementary injection of ^{3}H-proline into CITv yielded robust bilaminar terminations indicative of clear feedback projections in areas V4, VOT, and PITv, and weak terminations in area PITd. Dense reciprocal connections were observed between area CITv and several projection zones within anteroventral IT and temporal polar cortex, while weaker connections were observed to anterior IT within the STS. Paired PITv injections of NY and BB produced multiple clusters of cells in areas V4 and VOT, and in contrast to CITv injections, also labeled cells in areas V3A, V4t, VTF, and TH. PITv connections with anterior IT were less robust and were more restricted to posterior regions of anterior IT than the CITv projections. These data reinforce our views of hierarchical processing within the inferotemporal cortex and suggest the presence of multiple subdivisions within the anteroventral portion of IT. Supported by NEI EY-08372, the Sloan Foundation, the Whitehall Foundation, and the Texas Advanced Research Program 11618025.

397.10

INTRINSIC CONNECTIONS OF ROSTRAL INFERIOR TEMPORAL CORTEX IN SQUIRREL MONKEYS. R. E. Weller* and G. E. Steele. Dept. of Psychology, Univ. of Alabama at Birmingham, Birmingham, AL, 35294.

On the basis of cortical and subcortical connections, rostral inferior temporal cortex (ITR) of squirrel monkeys may be homologous to anterior inferotemporal cortex (IT) or area TE (TEa) of macaques, an area important in visual recognition and memory. Neurons in TE are unusual in possessing large receptive fields and complex stimulus selectivities. The present study examined the intrinsic connections of ITR of squirrel monkeys (genus Saimiri), following pressure injections of wheat germ agglutinin conjugated to horseradish peroxidase (WGA-HRP) or iontophoretic injections of biocytin in ITR.

Injections of WGA-HRP in ITR resulted in an irregular distribution of surrounding label that sometimes formed patches. Anterograde label was more extensive than retrograde, and was most extensive in layer I. Reconstruction of axons individually labeled by the biocytin injections revealed that axons exhibited en passant boutons that included all layers of cortex. Individual axonal terminal fields ranged from 400-600 microns in areal extent, or were not particularly widespread. Patterns of intrinsic connections in ITR of squirrel monkeys thus appear similar, in general, to those reported for other areas of visual cortex in primates.

Supported by EY07147 to REW and MH10070 to GES.

397.11

INTRINSIC CONNECTIONS IN THE MACAQUE INFEROTEMPORAL CORTEX: ANATOMICAL SUBSTRATE FOR FUNCTIONAL COLUMNS. I. Fujita*,[1,2] and T. Fujita[2], [1]PRESTO, Research Development Corporation of Japan, and [2]Laboratory for Neural Information Processing, Frontier Research Program, RIKEN, Wako, Saitama 351-01, Japan

Neurons in area TE of the monkey inferotemporal cortex are arranged into columns according to visual features of objects to which they respond (Fujita et al., *Nature*, 360:343-346,1992). Multiple columns appear to be selective for similar object features, because 2 or 3 separate clusters of cells respond to similar stimuli along a tangential recording penetration. To explore anatomical substrate for this organization, we made extracellular iontophoretic injections of biocytin into area TE of Japanese macaques.

After injections into single or restricted layers (2-3, 3, 3-4, 4, 5 or 6), most retrogradely labeled cells were found above or below the injection site to make a columnar appearance. Labeled cells were distributed through layers 2-6, except that those after layer 6 injection avoided layer 4. In addition to this radial labeling, a smaller number of cells were labeled lateral to the injection site, especially in layers 2 and 3. These cells, mostly pyramidal type, tend to cluster within terminal arbors of axons which radiated horizontally from the injection site.

A single injection produced more than 15 patches of terminal arbors in successful cases. The diameter of the patches was 0.5±0.2 mm (range: 0.3-0.9 mm). The mean center-to-center distance between adjacent patches was 0.7 mm (range: 0.5-1.8 mm). The distribution of the patches around the injection site was elongated in a direction parallel to the superior temporal sulcus.

Strong vertical connections between layers may contribute to the shared stimulus selectivity within a column. The results show that intrinsic horizontal axons in area TE arborize in a clustered manner as in the striate and prestriate cortices. Size and spacing of the terminal patches are, however, larger than those reported for V1 and V2, and roughly correspond to those of TE cell clusters with similar selectivity measured in physiological studies. We thus suggest that horizontal axons may link columns with similar stimulus selectivity.

397.12

NEUROCHEMICAL GRADIENT ALONG THE VENTRAL VISUAL PATHWAY IN THE MACAQUE MONKEY : CALBINDIN POSITIVE PYRAMIDAL NEURONS IN LAYERS II AND III OF AREAS TE, TG, AND 35/36 H. Kondo[1], T. Hashikawa[1], K. Tanaka*[1], and E.G. Jones[1,2], [1]RIKEN, Wako, Japan and [2]Univ. California, Irvine, USA.

V1, V2, V4, TEO, and TE compose a serial chain called the ventral visual pathway. This pathway is thought to be essential for object vision, and the receptive fields become larger and the complexity of stimulus selectivity increases towards the anterior. TE projects to areas 35/36 and TG, which in turn project to the hippocampus via area 28. To obtain clues to the relative roles of the different areas in this serial pathway, we compared neurochemical properties among the areas. Three Japanese monkeys (*M. fuscata*) were used. Sections were cut parallel to the superior temporal sulcus so that all the areas of interest were contained within single sections. They were stained immunocytochemically for calcium-binding proteins; calbindin (CB), parvalbumin (PV), and calretinin, as well as for GABA and with SMI32. Remarkable regional differences were found in CB and PV immunoreactivity. In sections stained for CB, besides densely stained nonpyramidal neurons, there were more lightly but definitely stained pyramidal neurons in the anterior areas. Their number gradually increased from V1 to TE, and was also large in 35/36 and TG. These CB immunoreactive pyramidal neurons were mostly confined to layers II and III. Similar CB immunoreactive pyramidal neurons were also dense in area 28 and the hippocampus. The number of CB immunoreactive nonpyramidal neurons was relatively constant from V1 to TE. PV immunoreactive neurons were all nonpyramidal. Their number was rather constant from V1 to TE, but with fewer in area 35/36. The density of neuropil stained for PV gradually decreased from V1 to TG and 35/36.

The CB immunoreactive pyramidal neurons in layers II and III, which were predominant in the anterior areas, may play a crucial role in integration of information or memory.

397.13

ORGANIZATION OF PROJECTION FROM THE ANTERIOR TE (TEa) TO THE PERIRHINAL (AREAS 35/36) AND FRONTAL CORTICES IN THE MACAQUE MONKEY: PHA-L STUDY. K.S. Saleem*, K. Cheng and K. Tanaka. RIKEN, Wako-shi, Japan.

Area TE of the inferotemporal cortex is located at a latter portion of the ventral visual pathway, which is thought to play a crucial role in visual discrimination and recognition of objects. We previously reported that single site in TEO projects to several focal columnar regions in TE (Soc Neurosci Abstr, 1992; Vol 18, p 294). In the present study, we analyzed how the output of TE to perirhinal and frontal cortices is organized. We made a single iontophoretic injection of PHA-L into the ventral part of the anterior TE (medial bank of amts) in two Japanese monkeys (*M. fuscata*). The injection sites were 0.7 mm in width and involved layers 2-6.

Projection to the lateral part of the perirhinal cortex (area 36) was restricted to several (4-7) focal regions. The clustering of labeled terminals extended through all the cortical layers in these foci, although terminals were denser in layers I-IV. These foci measured 0.3-0.9 mm mediolaterally, and 0.5-2.2 mm anterioposteriorly. The space between neighboring foci was up to 3.3 mm, and the foci were distributed over a large part of area 36. Projection to the medial part of the perirhinal cortex (area 35) was more distributed. The labeled terminals were denser in the upper layers and were distributed continuously through most of the mediolateral extent of the area 35.

Projection to the prefrontal cortex was directed to the basoventral prefrontal area (area 11) in the medial and lateral bank of the mediál orbital sulcus, as others reported previously using HRP. The terminals were restricted to one focal region in one case and 3 foci in the other case. The foci were 0.2-0.4 mm in diameter, and separated by 1.5-1.6 mm in the second case. The terminals were mostly limited to layers I-III.

These different organizations of projections from anterior TE to areas 36, 35, and 11 may suggest that the information from TE is used in different ways in these target regions.

397.14

PHA-L STUDY OF THE SUBCORTICAL PROJECTIONS OF THE MACAQUE INFEROTEMPORAL CORTEX. K. Cheng*, K.S. Saleem and K. Tanaka. RIKEN, Wako, Japan.

Area TE of the inferotemporal cortex is a latter stage of the ventral visual pathway, which is essential for discriminating and recognizing visual images of objects. Previous studies have demonstrated global structures of the connections from TE to the subcortical areas. Especially, some degree of topography has been shown for projections from TE to the striatum and the amygdala. To analyze the detailed organization of these projections, single focal PHA-L injection was made iontophoretically into the ventral part of TEa (medial bank of the anterior middle temporal sulcus; 2 cases), TEp (1 case) and TEO (1 case) in Japanese monkeys (*M. fuscata*). The size of injection sites was 0.7-1.0 mm in diameter. The injections involved layers 2 to 6.

After injections into TEa and TEp, labeled terminals were mainly found in the tail of the caudate and its adjacent ventrolateral part of the putamen, whereas sparse labeling was observed only in the tail of the caudate after TEO injection. Injections into TEa, TEp, and TEO gave rise to labelings at different rostrocaudal levels in the tail of the caudate, which were topographically arranged. In particular, these labelings were more restricted in the anterior-to-posterior plane (TEa: 2.5mm; TEp: 2.8mm; TEO: 0.5mm) compared to labelings in the previous studies based on mass injections in TE. However, in the two TEa cases, the mediolateral (1.5 and 1.6 mm) and dorsoventral (3.1 and 3.5 mm) extents were comparably close to those described previously. Thus, the projection is not organized in a point-to-point, but a considerably distributed fashion. The divergent termination pattern was also revealed by serially reconstructed single axons (n=4). A single axon branched upon arriving in the striatum, and often terminated in a number of arbors (6-10), which was more than that in the corticocortical projections (2-4). The size of arbors typically measured 200-300μm in diameter. In addition to terminating in the striatum, some axons also sent collaterals to the lateral nucleus of the amygdala, and formed arbors there similar to those in the striatum.

397.15

TRANSIENT INPUTS TO INFERIOR TEMPORAL CORTEX IN INFANT MACAQUES. H.R. Rodman*, K.L. Nace, T. M. Woods, C.G. Gross, and N. S. Rebmann. Dept. Psychology, Princeton Univ., Princeton NJ 08544.

Inferior temporal (IT) cortex is a "high-order" visual area involved in pattern perception and recognition in adult primates. We previously reported (Rodman et al., ARVO Abs.,1990) that the pattern of inputs to IT from posterior, visual cortical regions is adult-like at 7-18 weeks, an age range we have also studied physiologically. Here we report apparently transient inputs from more anterior cortical zones at the same ages. Injections of the retrograde tracers WGA-HRP or cholera toxin B-subunit (CTB) were made in *M. fascicularis* at 6-7 weeks (n=3), 13-18 weeks (n=3) or in adulthood (n=2). 1) Projections from lateral and ventral frontal cortex (areas 46 and 12)derived from both hemispheres at 6-7 weeks, but only from ipsilateral areas in older animals. 2) Substantial inputs from the ipsilateral insula were seen in all infants but one of the 18 week olds and not in adults. 3) Label was found in ipsilateral cingulate cortex in WGA-HRP infant cases, but not in the smaller CTB injection cases in infants or adults; cingulate label was also seen contralaterally in the 7 week WGA-HRP case. 4) At 6-7 weeks, a small but significant number of labelled cells was observed in infragranular layers of contralateral IT, as well as the heavy label in supragranular layers also seen in older infants and adults. More widespread inputs to IT from contralateral cortices in infant monkeys, including the deep layer projection from contralateral IT, may reflect the development of connectivity needed for large bilateral receptive fields in IT and behaviorally for perceptual equivalence of objects in the two visual half-fields. More generally, the data suggest that development of mature patterns of connectivity between IT and cortical areas that are *not* primarily visual in function may be important in the emergence of IT's role in pattern recognition.

Supported by by NIH MH-19420, NIH MH-42102 and the NERPRC.

397.16

TRANSIENT SUBCORTICAL CONNECTIONS OF INFERIOR TEMPORAL CORTEX IN INFANT RHESUS MONKEYS. M.J. Webster*, J. Bachevalier[1] and L.G. Ungerleider. Lab. Neuropsychol., NIMH, NIH, Bethesda, MD 20854 & [1]Dept. Neurobiol. & Anat., Univ. Texas Medical School, Houston, TX 77025.

Previously we reported transient projections in infant monkeys from inferior temporal (IT) cortex to several medial temporal-lobe regions, including those from posterior IT cortex (area TEO) to the amygdala and parahippocampal cortex and from anterior IT cortex (area TE) to perirhinal and parahippocampal cortex (Webster et al., J. Neurosci., 1991, 11:1095). Here we report that the projections of IT cortex to subcortical structures are similar in infant and adult monkeys, but some appear to undergo refinement during development. Quantitative analyses showed that: 1) whereas the projection from TE to the superior colliculus is consistent (5/5 cases) and widespread in infants, it is unreliable (2/7 cases) and limited in areal extent in adults; 2) although the projections from TE to n. medialis dorsalis and the tail of the caudate are present in infants and adults, they are reduced in adults; 3) there appears to be a projection from TE to n. paracentralis in infants (2/5 cases), but it is absent in adults (0/7 cases); and 4) TEO receives input from the dLGN in both infants and adults, but the number of cells giving rise to this projection is fewer in adults. No such differences between infants and adults were apparent in a number of other subcortical projections, including those to the pulvinar, reticular nucleus, claustrum, and putamen.

397.17

CORTICOSTRIATAL PROJECTIONS OF THE PRESTRIATE REGIONS IN RHESUS MONKEYS. E.H. Yeterian* and D.N. Pandya. Dept. of Psychology, Colby College, Waterville, ME 04901, E.N.R.M. V.A.M.C., Bedford, MA 01730, and Boston University School of Medicine.

In 18 animals, radioactively labeled amino acids were injected into various subdivisions of the prestriate cortex. The results show that the ventrolateral prestriate and caudal inferotemporal regions project preferentially to the ventral portion of the body, to the genu, and to the lateral portion of the tail of the caudate nucleus, and also to the caudoventral portion of the putamen. The projection from the caudal bank of the superior temporal sulcus (area MT) is directed mainly to the central and lateral sector of the body of the caudate nucleus, to the genu and the tail, as well as to the caudal portion of the putamen. In contrast, the dorsolateral prestriate region is related strongly to the dorsal and lateral portions of the head and the body, and to the genu of the caudate nucleus, as well as to the dorsal sector of the putamen. The medial prestriate region, like the dorsolateral areas, projects predominantly to the dorsolateral sector of the head and the body of the caudate nucleus, and to the dorsal portions of the genu and of the putamen. The ventromedial prestriate cortex is connected to the dorsal portions of the head and the body of the caudate nucleus and of the putamen, as well as to the genu and to the lateral sector of the tail. Thus, it seems that the ventrolateral prestriate and caudal inferotemporal regions subserving central or object vision have a differential distribution of corticostriatal connections compared to dorsolateral, dorsomedial, and ventromedial regions that are involved in peripheral or spatial visual processes. (Supported by the Dept. of Veterans Affairs, NIH grant 16841, and Colby Coll. grant 01 2203.)

397.18

PARVALBUMIN STAINING DELINEATES SUPERIOR TEMPORAL POLYMODAL CORTEX IN MACACA MULATTA. B. Seltzer*, M. Cola, C. Weldon, M. Massee, and C.G. Cusick. Depts. of Psychiatry & Neurology and Anatomy, Tulane Univ. Sch. of Med., V.A. Med. Ctr., New Orleans, LA 70112.

Thalamic areas staining heavily for parvalbumin (PVA) project to layer IV of the cerebral cortex so that PVA fiber plexuses reflect the thalamic input to a given cortical region (DeFelipe and Jones, '91). To examine this aspect of cortical organization within the superior temporal sulcus (STS), and relate it to cortical connections, we performed PVA staining on hemispheres in which injections of 1 or more anterograde tracers had been made into cortical areas that project to the STS.

Cortex in the caudal upper bank of the STS, at the level of area MT, exhibits marked depletion of PVA staining in layer IV compared to adjacent cortexes of the temporal lobe. At the level of primary auditory cortex, the upper bank has a lateral division, with poor layer IV staining, and a medial, where layer IV is better demarcated. All 3 zones are the targets of overlapping and nonoverlapping input from prearcuate cortex, the superior temporal gyrus, inferior parietal lobule, and intraparietal sulcus, thus identifying the caudal sector as "caudal area TPO," the lateral zone as "mid TPO," and the medial zone as "area PGa" (Seltzer and Pandya, '89). The medial pulvinar, which connects with area TPO (Yeterian and Pandya, '91), also stains lightly for PVA.

Supported by the V.A., N.I.H. grant EYO8906, and Tulane University.

397.19

PHYSIOLOGICAL AND CONNECTIONAL EVIDENCE FOR FUNCTIONAL SUBDIVISIONS IN POSTERIOR CINGULATE CORTEX OF THE RHESUS MONKEY. S.Y. Musil*, M. K. Smith, & C.R. Olson. Laboratory of Sensorimotor Research, National Eye Institute, Bethesda, MD 20892, and Department of Anatomy, University of Maryland at Baltimore, Baltimore, MD 21201.

Neurons in posterior cingulate cortex (CGp) of the rhesus monkey carry signals related to recent eye movements and current eye position and they respond to physically salient visual stimuli. In the experiments described here, we have explored the distribution within CGp of neurons carrying oculomotor and visual signals and have correlated their location with patterns of connectional topography.

We have analyzed the efferent connections of CGp by placing deposits of multiple distinguishable retrograde tracers in distant cortical areas (area 7a, area 7b and prefrontal area 9). We have found that neurons projecting to these areas are concentrated in distinct subregions of CGp. Neurons projecting to area 7a are most numerous caudally; those projecting to area 9 are concentrated at ventral levels; and those projecting to area 7b tend to be located. rostrally and dorsally.

We have compared the distribution of physiologically characterized neurons to subdivisions defined by efferent connectivity in three monkeys. We have found that neurons with oculomotor, and especially those with visual, properties are located at caudal levels where connections with area 7a are predominant.

397.20

Occipital Projections to the Cingulate Motor Cortex from the Anterior Calcarine Region in the Rhesus Monkey: Evidence for Direct Prostriate and Parastriate Input to M3 (Area 24c). R.J. Morecraft[1]*, K.S. Rockland[3] and G.W. Van Hoesen[2,3]. Dept. of Anatomy and Structural Biology[1], Univ. of South Dakota, Vermillion, SD 57069 and Depts. of Anatomy[2] and Neurology[3], Univ. of Iowa, Iowa City, IA. 52242

It has long been assumed that the organization of medial visual cortex connectivity is similar to the well-established organization of the lateral visual cortex. However, little information is available to support this supposition.

As part of an investigation on the connectivity of cortex forming the depths and lower bank of the cingulate sulcus, we observed direct projections to the cingulate motor cortex (M3 or area 24c) arising from the anterior part of the calcarine fissure. These findings were based on injections of retrogradely transported fluorescent dyes in 5 rhesus monkeys. Correlation of labeled fields with Nissl, myelin, acetylcholinesterase, and cytochrome oxidase stained sections demonstrated that the labeled cortex in the anterior calcarine fissure corresponded to a portion of V2 that adjoins the anterior part of V1 and to Sanides' area prostriata. Prostriate cortex is anterior to V1 and also adjoins the posterior parasubiculum. In V2, labeling occurred in layer 3 and spanned a distance of 0.1 mm in a rostrocaudal direction and 2 mm in a dorsoventral direction. Within area prostriata, labeled neurons occurred in layers 3 and 5/6, spanning a distance of 3.5 mm in a rostrocaudal direction and 2 mm in a dorsoventral direction. The heaviest distribution of labeling occupied the deep laminae. V2 and prostriate cortex did not project to area 23c, but only to M3.

These results document an unexpected, direct linkage from medial visual cortex to cortex which gives rise to corticospinal axons. This projection may originate from areas subserving the peripheral visual field and be involved in photic detection. (Support by NS 14944 and PO NS 19632, EY 07058).

397.21

PROGRESSIVE CHANGES IN SYNAPTIC BOUTON ORGANIZATION IN THE VISUAL CORTEX: FROM RAT TO HUMAN. G. Kenan-Vaknin*, O. Varlamov, G.E. Ouaknine, N. Razon, Z. Rappaport, R. Malach. Neurobiology Department, Weizmann Institute, Rehovot, 76100, Tel-Aviv and Beilinson Medical Centers, Israel.

What characterizes the progressive elaboration of cortical circuitry in different mammalian species? To begin addressing this question, the density and distribution of synaptic boutons on biocytin-labeled axon collaterals of layers II-III neurons in rat, cat, monkey and human were studied. A total of 2700 boutons in 93 axons were quantitatively analyzed. Two bouton populations were studied: axonal varicosities and bouton-bearing stalks. Moving from rats to humans, the incidence of stalks increased progressively from 9.1% in the rat through 49% (cat), 93% (macaque) and 93.5 % (human). Average inter-bouton interval also showed progressive increase from 4.2um ±2.5 (±S.D) in the rat to 10.8±8.4, 11.7±6.9, 16±12.2 in cat, monkey and human respectively. The size of the most frequent inter-bouton interval appears more stable, ranging from 2-4um in the rat to 4-6um in humans. These results may help define the significant parameters for high level information processing in cortical circuits.

VISUAL CORTEX: EXTRASTRIATE—COGNITIVE MECHANISMS II

398.1

DISSOCIATIONS AND LOCALIZATION OF MOTION PERCEPTION AND PURSUIT AFTER HUMAN CEREBRAL HEMISPHERIC LESIONS. J.J.S.Barton, J.A.Sharpe*, J.Raymond. Division of Neurology, University of Toronto, Toronto, Ontario, Canada M5T 2S8, and Department of Psychology, University of Calgary, Calgary, Alberta, Canada.

We investigated motion perception and pursuit in 20 patients and correlated defects with lesion sites on CT or MRI. We hypothesized that abnormal pursuit could occur with normal motion perception but abnormal motion perception would always impair pursuit. We tested motion perception with random dot cinematograms, using a staircase method that varied directional coherence of motion to determine a coherence perceptual threshold. We obtained foveal thresholds for the four cardinal directions separately and we tested 16 points in the peripheral field while monitoring fixation. Smooth pursuit gain was measured to predictable sinusoidal targets to find directional defects and during pursuit initiation to step-ramp targets to find retinotopic defects. We compared directional and retinotopic symmetry in perceptual and motor tasks to those of normal controls.

Six patients had directional asymmetries of motion perception, all worse for motion towards the side of their lesions: 5 of the 6 had symmetric smooth pursuit. Five had hemianopias which excluded retinotopic testing. The overlap region for the directional motion perception defect was the junction of Brodmann areas 19 and 37. Three other subjects with parietal and internal capsular lesions had ipsidirectional pursuit defects despite symmetric motion perception. We did not find any retinotopic defects. Since lesions in monkey MT/MST cause similar defects, we conclude that the 19/37 area may be the human homologue of MT, MST or their inputs. A double dissociation exists between smooth pursuit and perception of coherent motion.

Supported by MRC grants 9004FEN-1222-21813(JB) MT 5404, ME 5509 (JS).

398.2

CORTICAL REGION RELATED TO VISUAL MOTION PERCEPTION IN HUMAN SUBJECTS. Y. Nakamura, K. Ohtsuka*, H. Maekawa, M. Kiyosawa, T. Kawasaki, and K. Ishii. Dept. of Ophthalmology, Sapporo Medical Univ. and Tokyo Medical and Dental Univ., Dept. of Radiology, Tokyo Metropolitan Medical Research Institute, Sapporo 060, Japan

Previous studies indicated that the MT of the monkey is related to the visual motion perception. In the present experiment, we investigated the cortical region related to the visual motion perception in human subjects. Transcranial magnetic stimulation (TMS) in healthy human subjects can produce transient visual motion sensation or specific reversible defects of visual motion perception. Single TMS pulses were applied systematically at various locations over the occipital, parietal and temporal cortex using a magnetic stimulator with an 8 shaped coil at an intensity of 1.5 Tesla. We identified the location where visual motion sensation is elicited in two subjects. At the same location TMS degraded motion direction detection of random dot stimuli. The effective area was marked on the head by a vitamin-D tablet which produces high signal intensity on T1 images of MRI. We made 3-D MRI of the brain in each subject. In addition, we also used positron emission tomography (PET). Images of regional cerebral blood flow were obtained by using intravenous infusions of $H_2{}^{15}O$. The subjects viewed computer displays of moving or static random dot patterns. We coregistered statistical parametric maps (SPMs) of PET onto the respective MRI. It was located ventrolaterally in the anterior part of the occipital lobe.

398.3

ATTENTIONAL MODULATION OF NEURONAL RESPONSES IN MT AND MST OF A MACAQUE MONKEY PERFORMING A VISUAL DISCRIMINATION TASK. G. H. Recanzone*, R. H. Wurtz, and U. Schwarz. Laboratory of Sensorimotor Research, National Eye Institute, Bethesda, MD, USA 20892.

The primate visual system has been proposed to be comprised of a ventral, shape and color processing pathway and a dorsal location and motion processing pathway. We examined the responses of single neurons in the dorsal pathway (areas MT and MST) in a behaving Rhesus monkey that was trained to discriminate either the location or the shape of two moving visual stimuli.

All stimuli were 2 X 2 ° shapes projected onto a tangent screen by a video projector. The monkey was trained to fixate a lone stimulus, then to visually track a target which was one of two extrafoveal moving stimuli that appeared after 300-800 msec. One stimulus was always inside the receptive field (RF) of the neuron under study and the other was in the opposite visual hemifield. In the location task, before the start of each trial a stimulus was briefly presented in the location at which the target stimulus would appear. In the shape task, the shape of the fixation stimulus matched one of the two extraretinal stimuli (the target), but no location cue was given. Neuronal responses were compared between trials with identical visual stimuli in the RF of the studied neuron when the target was inside vs. outside of the RF.

In area MT, the response of approximately 3/4 of the neurons was modulated for stimuli moving in at least 1 of 8 directions in the location task, as was the response of nearly 9/10 of the neurons in the shape task. Of these, approximately 2/3 had a decreased response when the target was inside the RF. Similar changes were observed in MST for the location task. In both areas, the spontaneous activity of approximately 3/4 of the cells was also modulated in the location task. We conclude that attention to a visual stimulus can modulate the responses of neurons in the dorsal pathway for both location and shape-discrimination tasks.

398.4

Modulation of visual and non-visual neuronal responses in area MT and MST by monkeys behavior in a direction discrimination task

A.Thiele*, A.Wachnowski, K.-P.Hoffmann, Dept. Neurobiol., Ruhr-University Bochum, D-4630 Bochum, Germany

Area MT and MST contain direction selective cells and MT neurons are essential for direction discrimination near threshold (Newsome et al.1990, Komatsu and Wurtz 1988).

To investigate whether the monkeys behavior is influenced by responses of neurons in area MT and MST or vice versa, the monkey had to perform a peripheral detection task while fixating a central target. Unidirectional moving bars (4 directions) were presented in the receptive field near contrast threshold (5 luminances) with randomly varying onset. Detected or guessed movement directions were indicated by arm movements (4 touch bars), whereafter fixation and stimulus presentation were continued for another 500 ms.

In area MT 44/56 neurons responsed to stimulus presentation. This response was reduced in 17/44 neurons following the monkeys decision. The response of 89/165 MST neurons was related to stimulus presentation (visual neurons). 35/89 of these MST neurons changed their activity with the monkeys behavior. 26/165 MST neurons were characterized as non-visual neurons. Their activity was only related to the monkeys behavior. 13 of these neurons started to fire after the monkeys behavior. Another 13 cells were active before (100-0ms) or during the arm movement of the monkey.

The modulation of the visual response in MT and MST seems to be an attentional effect. The activity of non-visual cells in MST could be induced by higher cortical areas related to the decision or arm movements of the monkey.

Supported by DFG Neurovision

398.5

PURSUIT EYE MOVEMENTS, OPTIC FLOW, AND VISUAL CORTEX: A NETWORK MODEL OF HEADING DETECTION. M. Lappe*, Dept. Zoology & Neurobiology, Ruhr University Bochum, D-44780 Bochum, Germany

Humans are able to recover their direction of heading from optic flow during egomotion even in the presence of eye movements. While often the retinal flow pattern alone seems sufficient to perform this task, some situations also require extraretinal information. A recently proposed, purely visual, neural network model (Lappe and Rauschecker, *Neural Computation* 5, 1993) accounts for results of human psychophysics and generates neurons that exhibit selectivities to expanding, rotating and translating random dot patterns, similar to a class of cells found in monkey visual area MSTd. However, MSTd appears to also contain nonvisual information: Visual tracking neurons are active during smooth pursuit even in the absence of visual stimulation. The present work extends the model to include extraretinal eye movement information. The network starts out with a layer of direction selective neurons, modelled after area MT and assumed to contain a population encoding of the optic flow field. In a second layer the direction of heading is recovered by populations of neurons with selectivities for various optic flow patterns, resembling cells in MSTd. In addition, a population of tracking neurons independently provides the second layer cells with the direction of an eye movement. Eye speed, on the other hand, is available only with a variable gain factor. Zero gain amounts to no extraretinal input at all.

While the network is usually able to detect the direction of heading without the need for extraretinal information, it reproduces a characteristic deficiency of humans when being subject to a self-movement towards a vertical plane: Heading direction is correctly recovered only as long as the eye movement input is present. Without extraretinal information the network yields large errors by erroneously choosing the direction towards the fixation point. Reliable eye speed information, however, is not required. Instead, the network performs even better with imperfect gain that is smaller than one. Simulations of single neurons of the second layer reveal a behavior also described for MSTd: Neurons respond directionally selective to translating visual stimuli, but fail to respond to retinal image motions generated by an eye movement, i.e. they discriminate between active, self-induced and passive, real-world motion.

398.6

COLOUR CONSTANCY IN V4 MONKEYS, NORMAL MONKEYS AND HUMAN OBSERVERS. V Walsh*, J J Kulikowski, S R Butler & D Carden Dept Exp Psychol, Oxford Uni, Oxford OX1 3UD, UK & Visual Sciences Lab, UMIST, Manchester, M60 1QD, UK.

Colour constancy is the ability to identify a surface colour despite changes in the spectral content of the illuminant, the location of the surface and the angle from which the surface is viewed. We tested three groups of subjects (humans, normal macaque monkeys and monkeys with lesions of visual area V4) for the ability to identify surface colours when only the spectral content of the illuminant was changed. The human subjects were shown a coloured patch under one illuminant and asked to select the patch under a different illuminant from an array of distractors. The monkeys were required to select a particular coloured patch for a food reward in a WGTA testing apparatus. Human subjects showed good but not perfect colour constancy. They showed a tendency to select patches that belonged to the same perceptual colour category as the target item. Normal monkeys also showed good constancy. Monkeys with V4 lesions, however, did show a deficit in colour constancy, sometimes selecting a colour from a different colour category. The size of the errors was much greater than, and thus cannot be explained by, the limits of the animals' wavelength discrimination abilities. Analysis of the human data suggests that colour constancy, like colour memory, is organized within fundamental perceptual colour categories. It is argued that while monkeys with V4 lesions have access to the categorical information, they cannot correct for changes in the viewing conditions to maintain the stability of these categories.

Supported by the Wellcome Trust, The Nuffield Foundation and The UMIST Academic Resource Fund.

398.7

CUE-DEPENDENT DEFICITS IN CONTOUR ORIENTATION DISCRIMINATION AFTER V4 LESIONS. L.G. Ungerleider*, P. De Weerd, M. Mishkin & R. Desimone. Laboratory of Neuropsychology, NIMH, NIH, Bethesda, MD 20892.

We investigated the effects of V4 lesions involving one quadrant of the visual field on orientation discrimination in two fixating rhesus monkeys using low frequency, phase-randomized gratings. The gratings were defined by luminance, color, texture or occlusion cues. The texture gratings consisted of either a 90-degree orientation difference between the line elements of adjacent texture patches or by a size difference between random dots. The occlusion-defined grating (illusory contour) consisted of opposing line segments of matched orientation but shifted in phase. Relative to orientation thresholds in control quadrants, there was a 104% increase in thresholds in the lesion quadrant for the texture- and occlusion-defined gratings, but only a 35% increase for the color- and luminance-defined gratings. Control experiments showed that the greater deficits with texture gratings and illusory contours were caused neither by reduced acuity nor by differences in difficulty among perceptual tasks. These results demonstrate a role for V4 in the processing of second-order boundaries, and open the possibility that neuronal responses to such boundaries previously described in areas V1 and V2 might be due in part to feedback from area V4.

398.8

EFFECTS OF FOCAL ATTENTION ON RECEPTIVE FIELD PROFILES IN AREA V4. C.E. Connor*, J.L. Gallant and D.C. VanEssen. Dept. of Anatomy and Neurobiology, Washington University School of Medicine, St. Louis, MO 63110.

Moran and Desimone (1985) demonstrated that stimulus-evoked responses of cells in areas V4 and IT of macaque cortex are diminished when attention is focused on a second, ineffective stimulus inside the classical receptive field (CRF). This effect was interpreted as a shrinkage of the receptive field around the attended stimulus. We undertook to measure changes in V4 receptive field profiles as a function of the position of focal attention relative to the CRF. The attentional focus was manipulated by altering the position of a behaviorally relevant ring-shaped stimulus. Concurrently, a behaviorally irrelevant optimal bar stimulus was used to map the receptive field of the cell under study. The ring stimuli were part of a serial size discrimination task. Each ring was flashed briefly, and after a 100 msec delay a bar stimulus was flashed at one of a range of positions inside and outside the CRF.

Shifts of attention produced alterations in receptive field profiles for over half the cells studied. In most cases the receptive field center of gravity translated towards attentional foci in or near the CRF. Changes in width and shape of the receptive field profile were also observed, but responsive regions were not typically limited to the location of the attended ring stimulus. Attention-related effects often included enhanced responses at certain locations as well as diminished responses at other locations. These observations impose significant constraints on models of focal attention.

398.9

RESPONSES OF MONKEY LOCUS COERULEUS (LC) NEURONS TO CS+ STIMULI REFLECT COGNITIVE PROCESSING: DELAYED ACTIVITY DURING ACQUISITION IN A VIGILANCE TASK. P. Kubiak*, J. Rajkowski and G. Aston-Jones, Div. Behavioral Neurobiol., Dept. Mental Health Sci., Hahnemann University, Philadelphia, PA 19102.

LC neurons respond preferentially to CS+ stimuli in a vigilance task (Aston-Jones et al., Prog. Brain Res. 88: 501, 1991). Here we report the effect of reversal training on such LC responses.

Two cynomolgus monkeys were overtrained in a vigilance task with vertical and horizontal bars on a video display as CS+ or CS-. The animal was required to release a pedal shortly after presentation of infrequent CS+ stimuli (10-20% of trials) to receive juice reward, and withhold responding to CS- stimuli. Upon reaching criterion (< 10% errors) stimulus meaning was reversed. Acquisition of this new task was followed by re-reversal, etc. Monkeys more rapidly acquired reversal with continued reversal training. LC responses became selectively driven by the new CS+ stimuli in parallel with behavioral acquisition of the new response contingency. However, LC and behavioral responses (pedal release latency and cue directed gaze) were markedly prolonged (+0.2 s) during reversal acquisition. Improvements in task performance were accompanied by shorter response latencies of LC neurons. However, the delay in LC and pedal responses to target stimuli persisted for many trials after criterion performance was re-established. Prolonged processing of cues during reversal acquisition may be responsible for the delays in behavioral and neural activities. These results indicate that recognition of target stimuli results in phasic activation of LC cells during a vigilance task, and that such LC responses are more cognitively- than sensorially-driven. Supported by AFOSR Grant F49620-93-1-0099.

398.10

CORRELATIONS BETWEEN LOCUS COERULEUS (LC) NEURAL ACTIVITY, PUPIL DIAMETER AND BEHAVIOR IN MONKEY SUPPORT A ROLE OF LC IN ATTENTION. J. Rajkowski*, P. Kubiak and G. Aston-Jones, Div. Behav. Neurobiol., Dept. Mental Health Sci., Hahnemann Univ., Philadelphia, PA 19102.

We reported that during prolonged recording sessions (0.5 to 2 hours) in a vigilance task LC neurons varied tonic discharge rates in close relation to varying levels in task performance, e.g., as indicated by foveations to initiate trials (Abst. Soc. Neurosci. 18:538, 1992). Here we report that other experimental variables indicative of changing levels of attentiveness are also closely related to LC tonic activity.

Several eye-related signals (eye movements, eye blinks, pupil diameter; ISCAN video eye tracking system), along with task codes and discharges of individual LC neurons, were recorded from 2 cynomolgus monkeys performing a vigilance task. This task required that the animal foveate a spot on a video monitor to initiate each trial, and release the lever within 700 ms of an infrequent visual CS+ (10% of trials). Performance epochs were defined by plotting averaged measures of the signal detection parameters ß (decision criterion) and d' (discriminability) in 10 min bins. Periods of good performance (few false alarms and misses = high ß and d') were best correlated with epochs of low tonic LC discharge, whereas periods of worst performance, as indicated by frequent false alarms, occurred during elevated LC activity. Pupil diameter also varied throughout the recording session, was directly correlated with the rate of LC tonic discharge, and was consistently smaller during periods of high ß and d'. Poor performance was characterized by frequent intertrial bar releases, larger amplitude eye movements and pupillary dilation. It is proposed that high tonic LC activity corresponds to a labile or scanning mode of attention, associated with high autonomic arousal as reflected in pupil diameter. Conversly, focused attention is associated with lower autonomic arousal and moderate LC tonic activity. Supported by AFOSR Grant F49620-93-1-0099.

398.11

EFFECT OF STIMULUS TRANSFORMATIONS ON SHORT-TERM MEMORY MECHANISMS IN INFERIOR TEMPORAL CORTEX. A. Lueschow, E.K. Miller and R. Desimone.* Lab Neuropsychology, NIMH, Bethesda, MD 20892.

We are typically able to recognize a previously seen object even if it is transformed in size or location on the retina. Thus, a horse is a horse (of course) even if we can also see that it is large or small or that it is present in central or peripheral vision. We sought evidence for a neural basis of this object invariance in recordings from inferior temporal (IT) cortex in two rhesus monkeys. The task was a delayed matching-to-sample task, in which the monkey had to match a test stimulus to a previously seen sample. Several intervening nonmatch stimuli could occur between the sample and final match. The stimuli were digitized pictures of complex objects, such as faces, several of which were tested on each cell. On some trials, the final match stimulus was identical to the sample and on other trials it was larger or smaller or at a different retinal location. The monkeys immediately recognized the transformed object as the same. As was found in previous studies, responses to the test stimuli were a joint function of the current stimulus and the memory trace. For most of these cells, responses to a stimulus that matched the sample were suppressed. These cells also showed suppressed responses to the transformed matching stimuli, although the amount of suppression was often less than if the stimulus was an identical match. Information about both the object and its transformation may therefore be available in the responses of IT neurons.

398.12

DUAL MECHANISMS OF SHORT-TERM MEMORY: INFERIOR TEMPORAL (IT) CORTEX. E.K. Miller* and R. Desimone. Lab Neuropsychology, NIMH, Bethesda, MD 20892.

Consistent with a role in short-term memory (STM), responses of IT neurons are determined jointly by current stimuli and memory traces. Psychological studies suggest multiple components mediating STM and we sought evidence for such mechanisms in IT recordings in two rhesus monkeys. The task was a modified delayed matching-to-sample (DMS) task, in which stimuli could intervene between the sample and match. On some trials (standard trials), the only stimulus that was repeated was the sample/match stimulus. These trials could be solved using a repetition or recency rule. On other trials (ABBA trials), two of the intervening nonmatch stimuli matched each other. On such trials, the monkey had to ignore these "repeated nonmatch" stimuli and respond only to the match. Both monkeys were initially trained using standard trials only. When ABBA trials were first introduced, the monkeys mistakenly responded to the repeated nonmatch, suggesting that they had learned a repetition rule. After training on the ABBA trials, about half the cells responded differently to a stimulus depending on whether or not it matched the sample. Similar to previous studies, a majority showed a suppression of their responses to the match. However, these "suppression" neurons also showed suppression to the repeated nonmatch, even though the monkey had learned not to respond to it. By contrast, the remaining neurons with memory effects showed enhanced responses to **only** the match. These results suggest that IT cortex contains at least two mechanisms mediating STM: passive suppression that is sensitive to repetition and active enhancement that mediates working memory. The greater incidence of such enhancement effects compared to previous studies suggests that learning the ABBA strategy resulted in a shift from the suppression to the enhancement mechanism.

398.13

DUAL MECHANISMS OF SHORT-TERM MEMORY: VENTRAL PREFRONTAL CORTEX. L. Chelazzi*, E.K. Miller, A. Lueschow, R. Desimone. Lab Neuropsychology, NIMH, Bethesda, MD 20892

In a companion study, we recorded the responses of neurons in inferior temporal (IT) cortex during performance of a short-term memory (STM) task that revealed dual passive and active memory mechanisms. Since prefrontal cortex has also been linked to STM (Wilson et al., 1993; Fuster, 1973) and because its ventral portion (below the principal sulcus) is anatomically connected with IT cortex, we recorded from this ventral region in one rhesus monkey during the same task.

Unlike in IT cortex, the responses of some cells appeared to be related to the monkey's behavioral response. Otherwise, many prefrontal neurons had properties similar to those of IT neurons, including stimulus selective responses and either suppressed or enhanced responses to items that matched the sample. As in IT cortex, the enhanced responses were specific for the stimulus that matched just the sample item, suggesting an active memory mechanism. Further, many prefrontal cells showed either non-specific or sample-selective activity during the delay intervals of the task (delay activity). However, unlike IT cortex, prefrontal delay activity conveyed sample information across intervening stimuli. Thus, delay activity in prefrontal cortex seems to reflect maintenance of the sample in memory. While an intervening stimulus typically interrupted the ongoing delay activity, it quickly recovered in subsequent delay intervals. Some cells showed increasing delay activity with increasing trial length and some did not show any delay activity until after at least one intervening stimulus occurred.

These results suggest that prefrontal neurons participate in actively maintaining a memory trace and that STM is mediated by interactions between prefrontal and IT cortices. Prefrontal neurons may prime certain IT cells to give enhanced responses to stimuli matching the sample memory.

398.14

EFFECTS OF ADULT LEARNING ON THE STIMULUS SELECTIVITY OF CELLS IN THE INFEROTEMPORAL CORTEX. E. Kobatake,* K. Tanaka, G. Wang & Y. Tamori RIKEN, Wako-shi, Japan

The anterior part of the macaque inferotemporal cortex (TE), which is situated at a latter stage of the ventral visual pathway, contains cells which selectively respond to various visual object-features. To determine whether the stimulus selectivity changes with changes in the visual environment, two adult Japanese monkeys (Macaca fuscata) were trained to discriminate new stimuli: one monkey was trained with a set of shape stimuli and the other with color stimuli. The shape set was composed of 28 shapes made by superimposing 2 or 3 primitive shapes (circles, squares, triangles and bars). The color set was composed of 18 stimuli made by combining 9 colors with two primitive shapes (circle or square). One of the stimuli was presented as the sample, and the monkey was required to select it from among 5 (shape) or 15 (color) stimuli presented together after a delay period (~16s). After reaching a saturated level of performance, the monkeys were overtrained for more than two months, and then recording was begun. During recording, monkeys were anesthetized with N2O and isoflurane and immobilized. For each cell, the most effective object-stimulus was selected from dozens of 3D models of animals and plants, and then the response to the object stimulus was compared with responses to the shape or color stimuli used in the training. Control recordings were made in two monkeys which were not trained with either the shape or the color set. Effects of the shape training were evident. A large number of cells recorded in TE of the trained monkey were most strongly activated by some of the 28 shapes (39% of 44 cells), while such cells were rare (9% of 67 cells) in the untrained monkeys. Effects of the color training were smaller if present. The cells which were most strongly activated by some of the 18 color stimuli comprised 11% of 19 TE cells in the trained monkey and 3% of 67 TE cells in the untrained monkeys. We conclude that the stimulus selectivity of TE cells can be changed by training in the adult. The degree of change, however, depends on the type of stimulus used in the training.

398.15

BEHAVIORAL PERFORMANCE IN PATTERN DISCRIMINATION AND PATTERN SELECTIVITY OF INFERIOR TEMPORAL NEURONS OF MONKEY. J. WATANABE* AND A. KITAOKA Dept. of Behav. Physiol. Tokyo Metropol. Inst. Neurosci., Tokyo 183, Japan

We studied a relation between behavioral performance in visual pattern discrimination and neuronal selectivity to patterns in the inferior temporal area, which is thought to be an important center of pattern perception. Monkeys were trained to perform a modified delayed non-matching to sample task, in which a sample pattern was presented on a color CRT for 0.5s with 1s interval repeatedly up to 4 times. On each trial, a stimulus-pair was selected randomly from 8 patterns, which were made up of 4 originals (+, Square, Y, and H) and their rotated figures (x, Diamond, inverted-Y and laid-H). Monkeys responded with a longer reaction time and made more omission errors at square/diamond, +/x, Y/inverted-Y or H/laid-H discrimination, i.e. they had a difficulty in discrimination of rotated patterns. In addition, the performance was poor at +/square, +/diamond, x/square, or x/diamond pairs. A trial with either H or inverted-H was performed with a relatively short reaction time and less omission error.

Single neuronal activity from the inferior temporal area (TEO) of one monkey performing the task was recorded through a chamber attached on a lateral skull with a glass-coated Elgiloy electrode. Of 938 neurons recorded, 242 neurons gave excitatory responses to pattern stimuli: 63 neurons responded to one or two patterns, 131 to three to five patterns, and 84 to six to eight patterns. The neurons preferred H most and laid-H second; this finding might be related to the high behavioral performance at pairs including either H or laid-H. However, our neurophysiological data did not explain the behavioral findings fully.

398.16

RETRIEVAL OF CONCURRENT VISUAL DISCRIMINATIONS WHILE INFEROTEMPORAL CORTEX IS SUPPRESSED WITH COLD. James A.Horel* and Geoffrey M. Stegner. Anatomy & Cell Biology, & Neuroscience Training Program, SUNY HSC Syracuse, NY 13210

Three *Macaca fascicularis* monkeys were trained on three sets of concurrent 8 visual discriminations and tested for their recall while inferotemporal cortex (IT) was suppressed with cold. Cryodes covered IT from the superior temporal sulcus to the anterior middle temporal sulcus, from the temporal pole to the border of TEO. The two-choice visual discriminations were presented on a computer monitor equipped with a touch screen. Forty eight complex colored images were scanned into the computer from magazine and catalogue illustrations. Touching a correct stimulus produced a food pellet. The discriminations were presented in sets of 8, with 8 pairs presented together, one pair at a time, in random order. The three sets of 8 were presented separately. The animals performed these nearly perfectly without IT suppression, but were significantly impaired during cooling. However, performance on many of the pairs was unaffected, while a few were strongly affected, performance on some pairs was consistentlly below chance. The pairs that produced the strong deficits varied from animal to animal. Some of the stimuli that were particularly difficult for an animal were removed and run by themselves in concurrent 2 or 4. The animals continued to be impaired on these stimuli for the most part, although not consistently so. When these stimuli were replaced into the set of 8, performance was improved on these, but the impairment shifted to others that had not previously shown an impairment. Since different animals were impaired on different stimuli, and experience with the stimuli could shift the impairment to others that were not impaired, it suggests the impairment relates more to experience with the stimuli than to their particular physical dimensions. (supported by NIH, RO1 NS1829)

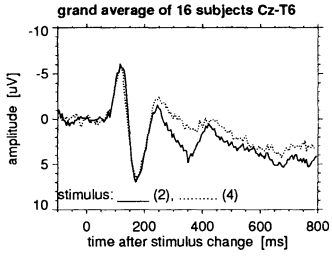
398.17

THE DIRECTION OF GAZE IN FACE STIMULI LEADS TO A MODIFICATION OF „FACE-RESPONSIVE" COMPONENTS (P3) IN VISUALLY EVOKED EEG-POTENTIALS. S. Bork, L. Fuhry, O.-J. Grüsser, Margitta Seeck (SPON: European Brain and Behaviour Society). Dept Physiol., Freie Univ., W-1000 Berlin 33 (FRG).

In former studies components of visually evoked EEG-potentials (EPs) related to the presentation of faces were described. In 16 volunteers (8 male, 8 female; 23-31 years) EPs were recorded through the electrodes F3, F4, T5, T6, Cz and Oz with reference to linked mastoids (intern. 10/20 system). 160 slides (4° × 6°) were projected onto a white screen. Stimulus change lasted less than 6 ms. Duration of stimulus presentation varied randomly between 2.2 and 4.2 sec.

Four stimulus categories were applied: (1) face and line of sight turned sideways; (2) face directed straight ahead, but line of sight sideways; (3) face turned sideways, but line of sight directed to the viewer; (4) face and line of sight directed to the viewer.

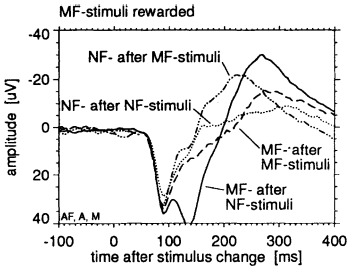
The face-responsive component with a maximum through electrode Cz was confirmed, consisting of a pronounced positive peak (P2) at about 160 ms with a rapidly following negativity. Both of the „dissociated face" categories (line of sight and face are turned in different directions) led to a slow positive potential between 250 and 400 ms (P3). This potential was most prominent with bipolar recordings Cz-T6 ($p<0.01$, fig. 1).



398.18

DEPENDING ON STIMULUS CATEGORY SEQUENCES, FIRST-ORDER MARKOV-CHAIN PROPERTIES APPEAR IN FACE-RELATED COMPONENTS OF JAVA MONKEY VISUAL EVOKED POTENTIALS. L. Fuhry, O.-J. Grüsser, W. Seidler*, Dept Physiol., Freie Univ., Arnimallee 22, W-1000 Berlin 33 (FRG).

EPs were recorded with seven epidural electrodes implanted above the right hemisphere of a Java monkey trained to distinguish monkey faces (MF) from complex non-face stimuli (NF; b/w slides 5.1° diameter). One set consisted of 53 MF- and 59 NF-stimuli which were applied three times per session. Either MF- or NF-stimuli were rewarded. Stimuli were presented within a randomized time slot of 2.9-4.1 s. EPs were averaged according to the category of the current and the preceding stimulus. The fig. illustrates the grand averages of EP-responses over the temporal lobe from eight sessions. MF-stimuli preceded by MF-stimuli evoked responses more similar to those of NF-stimuli preceded by either NF- or MF-stimuli.



398.19

ELECTROPHYSIOLOGICAL STUDIES OF FACE RECOGNITION IN HUMAN EXTRASTRIATE CORTEX. T. Allison*, H. Ginter, G. McCarthy, A.C. Nobre, A. Puce, M. Luby, K. McCarthy and D.D. Spencer. Neuropsychology Laboratory, Veterans Administration Medical Center, West Haven, CT 06516 and Depts. of Neurology, Neurosurgery, Biology and Psychology, Yale University, New Haven, CT 06510.

Recognition and identification of faces is a complex but normally effortless task. However, patients with lesions in the temporal-occipital region may lose the ability to recognize familiar faces (family members, famous faces, or their own mirror image), suggesting that a region of visual cortex is specialized for processing of facial information. Here we report the first study of face recognition with recordings made directly from the cortical surface of patients with indwelling electrodes for localization of an epileptogenic region.

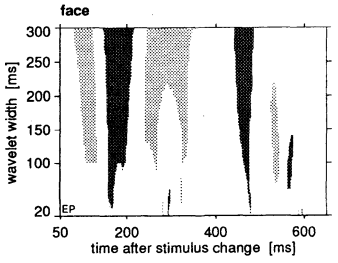
Ten patients with a total of 576 electrodes on the inferior and lateral cortical surface viewed a video monitor which at 2 sec intervals displayed unfamiliar faces, scrambled faces (face pixels rearranged to be unrecognizable), cars, scrambled cars, words, and butterflies. Locally generated evoked potentials were recorded simultaneously from 32 or 64 locations, digitized at 250 Hz, and averaged for each stimulus type.

Major results are: 1) Portions of inferior extrastriate cortex respond only to faces with a large-amplitude (100-200 μV) negativity at about 200 msec. 2) Across subjects, the responsive locations (determined by MRI) include mid to posterior portions of the fusiform and inferior temporal gyri, but in individuals the face-responsive region is small, perhaps 1-3 cm^2. 3) Similar potentials were recorded from the left and right hemispheres. 4) Other discrete regions of inferior cortex respond to cars or words. 5) Cortical stimulation (5 sec trains, 50 Hz, 2-10 mA) via the same electrodes which recorded face-specific potentials sometimes produced a transient prosopagnosia. These results suggest a remarkable degree of "modularity" in the processing of faces and other categories of complex objects.

398.20

SIGNIFICANCE OF CATEGORY-RELATED DIFFERENCES IN EEG-POTENTIALS OF MAN EVOKED BY FACE-, HAND- AND "NON-BODYPART"-STIMULI AND EVALUATED WITH THE WAVELET ANALYSIS. Elke Heusser, L. Fuhry, O.-J. Grüsser, Dept. Physiol. Freie Univ. 1 Berlin 33, FRG (SPON: European Neuroscience Association)

The evoked potentials related to the presentation of b/w-photographs of hand-, face- and "non-bodypart"-stimuli were investigated. 160 diapositives (40 each category) were presented in random order for 2.1 - 4 sec each to 19 subjects (10 males, 9 females). The EPs were analyzed with the wavelet method (Dittberner et al. Vision Res. submitted 1993): To describe the significance of category-related differences of the resulting grand averages, they were compared with the "random-shuffeled-grand average", i.e. the average of 30 × 19 EPs over all 4 categories. The 4 resulting grand averages and the "random-shuffeled-grand average" were convoluted by wavelets of different widths and compared with each other. Areas exceeding the ± 3 SD-range of the random-shuffled EPs were defined as significant. In the 2-dimensional contour-plots these significant ranges are depicted (fig.). For faces significant responses appeared between 150–300 ms after stimulus change and within a temporal frequency range between 3 and 15 Hz.



398.21

FUNCTIONAL BRAIN MRI OF CORTICAL AREAS ACTIVATED BY VISUAL MENTAL IMAGERY. S. Ogawa*[1], D.W. Tank[1], R. Menon[2], J.M. Ellermann[2], H. Merkle[2] and K. Ugurbil[2]. [1]AT&T Bell Laboratories, Murray Hill, NJ 07974 and [2] U.of Minn. Medical School, Minneapolis, MN 55455

We have used Magnetic Resonance Imaging (MRI) of Blood Oxygenation Level Dependent (BOLD) contrast to provide functional brain maps of cortical areas activated by two mental imagery tasks. Imaging experiments were performed on 10 normal subjects using a Siemens/SIS 4T system (FLASH pulse sequence: 5 sec/image; 40 msec echo time; 1.6x3.3mm in-plane resolution). Areas in the occipital pole were examined along two slice orientations: an oblique plane located along the calcarine fissure and a sagittal plane located 1-2 cm off midline. Areas were defined as activated if the average signal intensity in a pixel during the mental imagery period increased above the average in a control period at the .01 t-test confidence level.

Imagination of simple, static objects from auditory cues produced variable results depending upon the subject. In some subjects, small (~2%), but statistically significant, signal changes were observed in areas of primary visual cortex that were also strongly activated by direct visual stimulation (alternating red/green checkerboard; 8 Hz; ~3 fold larger signal change). However, in many subjects, no signal change was observed that was statistically significant given present signal-to-noise ratios.

An imagery task in which the subject was instructed to mentally navigate through a known complex environment produced activation of a previously uncharacterized, highly localized region in the parietal lobe. The region activated is on the parietal side of the occipital/parietal sulcus approximately 2 cm from the midline and 5 cm from occipital pole in a plane parallel to the calcarine fissure. In three subjects signal changes of 2 to 7% were always observed that were easily visible in the activation map. Three others showed occasional statistically significant localized changes in this same area. Activated areas were not observed in the remaining 4 subjects. In subjects that showed a robust parietal lobe response, no activation of this area was produced by imagination of static objects or scenery, or direct visual stimulation.

398.22

BINDING FORMS A CEREBRAL CODE WHICH ERROR CORRECTS: Scattered Feature Detectors Generate a Hexagonal Code via Synchronizing Excitation among Pyramidal Neurons.
WILLIAM H. CALVIN* Univ. of Washington NJ-15, Seattle WA 98195

Pyramidal neurons of the superficial neocortex are excitatory to other pyramids and, since they tend to cluster axon terminals at a standard distance ("0.5mm"), some corticocortical cell pairs will mutually re-excite. Though refractoriness should prevent reverberation, even weak positive coupling can *entrain* oscillators when cells are active for other reasons. Because each pyramid sends horizontal axons in many directions, simultaneous arrivals may recruit a 3rd and 4th pyramid at 0.5mm from the synchronized parental pair. A triangular mosaic of synchronized superficial pyramids could thus form, extending for some mm (Calvin, *Abstr.* '92). Consider N neuron pairs responding to different aspects of an apple, scattered across extrastriate areas, forming N triangular mosaics that interdigitate. There is thus a recycling temporal pattern (since synchrony is not needed across pairs) imposed on a widespread cortical area. It has a spatial repeat, an elementary spatiotemporal pattern of N neurons which, for geometric reasons, is no larger than a 0.5mm hexagon. This compact pattern becomes standardized across the mosaic (a variant must overcome six simultaneous EPSPs); such pattern "crystallization" is a form of error correction. The synchrony could induce longer-term NMDA connectivity changes; later, partial patterns could "pop out" the complete spatiotemporal pattern for *Apple* by resonance with this connectivity pattern, even in cortex lacking the original feature detectors. *A hexagonal matrix could thus house a Hebbian cell assembly and function as a cerebral code.*

399.1

MODULATION OF SPINDLING OSCILLATIONS BY SYNAPTIC INPUTS. D. Contreras* and M. Steriade. Lab. of Neurophysiol., Laval University, Quebec, Canada G1K 7P4.

Sleep spindles are generated in the thalamus and distributed to wide cortical territories. To explore the influence of synaptic inputs on spindles in urethane or ketamine-xylazine anesthetized cats, stimulating electrodes were inserted in the rostral intralaminar (CL) thalamus and the pericruciate motor cortex, and multi-site intracellular and extracellular simultaneous recordings were performed in the anterolateral sector of the reticular thalamic (RE) nucleus and from thalamocortical (TC) neurons in ventroanterior-ventrolateral (VA-VL) thalamic nuclei as well as in the pericruciate cortical areas. Crosscorrelations between action potentials and focal or intracellular waves were done.

Spindle oscillations induced by cortical or thalamic stimulation were similar to those appearing spontaneously. Cortical and RE cells responded with a short-lasting EPSP followed by a hyperpolarization lasting for 60 to 250 ms that led to a series of depolarizing waves at 8 to 12 Hz. TC cells responded with an EPSP followed by a hyperpolarization of similar duration, leading to a rebound spike-burst and a sequence of cyclic (8-12 Hz) IPSPs. Cellular events were correlated to the EEG from the pericruciate cortex: a surface-negativity (depth-positivity) was coincident with the cellular hyperpolarization, followed by surface-positive (depth-negative) waves at 8-12 Hz that were correlated with cortical- and RE-cell firing or TC-IPSPs. Variations in the duration of the initial EEG surface-negativity were strictly followed by the three cell-types. A decrease in the intensity of cortical or thalamic stimulation led to a progressive decrease in the amplitude of the oscillation, but not in its frequency, until a single EPSP was elicited by the testing volley. These results suggest that the magnitude of synaptic inputs to the pacemaker RE thalamic nucleus effectively modulates the strength of the oscillation and that a generalized inhibitory phenomenon is used by the network to correctly time the spindle oscillation.

Supported by MRC of Canada (grant MT-3689).

399.2

SPONTANEOUS SYNAPTIC POTENTIALS INDUCED BY 4-AMINOPYRIDINE IN NEOSTRIATAL NEURONS.

Flores-Hernández J., Galarraga E., Pineda J. C. and Bargas J*. Departamento de Neurociencias, Instituto de Fisiología Celular, UNAM, AP.: 70-253, México DF, 04510.

Of the fast synaptic actions, $GABA_A$, GLU, and ACH-nicotinic, GLU comes from neurons extrinsic to the neostriatum. CNQX/AMPA and NMDA receptors have been characterized using orthodromic responses. However, it is believed that a special dissection is needed to preserve GLU transmission. Intracellular recordings from an *in vitro* slice preparation were used to analyze the spontaneus synaptic potentials (SSPs) that appear after superfusion with 4-AP (1μM-1mM). Normally, the frequency of SSPs recorded in neostriatal neurons is low: less than one event per second. However, 4-AP increases this frequency in a dose-dependent manner (e.g., > 5 per second after 4-AP 100 μM): SSPs appeared in a random manner, with frequency oscillations, and for more than 60 min. Either 0.5 μM TTX or 400 μM Cd^{2+} blocked the SSPs. Bicuculline (BIC 4μM) or CNQX (10 μM)+2APV (100 μM) decreased their frequency. When BIC and CNQX+APV were given together SSPs and basal noise decreased. However, in a few cases some SSPs potentials remained after these blockers. It is concluded that: 1) extrinsic glutamatergic transmission is greatly preserved in slices, for some time, even if most fibers are cut, 2) 4-AP induces both GABA- and GLU-SSPs, 3) Most 4-AP-induced SSPs require action potential firing and $[Ca^{2+}]_o$, and 4) 4-AP could be used to study transmitter release with electrophysiological methods.

Financed by DGAPA-UNAM and CONACyT (México).

399.3

SYNAPTIC INTERACTIONS BETWEEN THE DOPAMINERGIC AFFERENTS AND THE CORTICAL OR THALAMIC INPUT AT THE SINGLE CELL LEVEL IN THE STRIATUM OF MONKEY. Y. Smith*[1], B.D. Bennett[2], J.P. Bolam[2], A. Parent[1] and A.F. Sadikot[1]. [1]Centre de Recherche en Neurobiologie, Hôp. Enfant-Jésus and Univ. Laval, QUÉBEC, CANADA; [2]MRC Unit, Univ. Dept of Pharmacology, OXFORD, UK.

The anterograde transport of *Phaseolus vulgaris*-Leucoagglutinin (PHA-L) or biocytin (Bio) was combined with the immunostaining for tyrosine hydroxylase (TH) to test the possibility that the dopaminergic afferents converge with cortical or thalamic inputs on single neurones in the sensorimotor sector of the primate striatum (St). In a series of squirrel monkeys, PHA-L was delivered into the centromedian nucleus (CM) of the thalamus whereas in other animals, Bio was injected in the primary motor cortex. After the appropriate survival period, sections including the St were processed to reveal the anterograde tracers and TH immunoreactivity in material suitable for light and electron microscopic analysis. Although TH-immunoreactive fibres and terminals invaded the entire extent of the St, the cortical and thalamic inputs formed bands that were confined to the post-commissural region of the putamen. Electron microscopic analysis revealed that more than 80% of the TH-positive terminals formed symmetric synapses with dendritic shafts, whereas the cortical and thalamic terminals formed asymmetric synapses predominantly with the heads of dendritic spines and with dendritic shafts, respectively. Examination of striatal regions where the cortical or thalamic inputs overlapped with the TH-immunoreactive structures revealed that the cortical boutons form synapses onto striatal elements that also receive inputs from TH-positive terminals. In contrast, none of the striatal structures examined received convergent synaptic inputs from thalamic boutons and TH-positive terminals.

These results suggest that the dopaminergic afferents interact differently with the cortical and thalamic input to influence the neuronal activity in the sensorimotor sector of the striatum in primate. Supported by the Canadian and UK MRCs and NATO.

399.4

CONVERGENCE OF THALAMIC AND CORTICAL INPUTS IN THE SENSORIMOTOR SECTOR OF THE STRIATUM IN MONKEY. J.P. Bolam*[1], Y. Smith[2], B.D. Bennett[1], A. Parent[2] and A.F. Sadikot[2]. [1]MRC Unit, Univ. Dept of Pharmacology, OXFORD, UK; [2]Centre de Recherche en Neurobiologie, Hôp. Enfant-Jésus and Univ. Laval, QUÉBEC, CANADA.

The anterograde transport of *Phaseolus vulgaris*-Leucoagglutinin (PHA-L) was combined with that of biocytin (Bio) to determine whether the thalamic input from the centromedian nucleus (CM) and the cortical afferents from the primary motor cortex (MC) converge onto single elements in the striatum of squirrel monkey. After simultaneous injections of PHA-L in CM and Bio in MC, rich plexuses of anterogradely labelled fibres and terminals occured in the post-commissural region of the putamen. Although both sets of anterogradely labelled elements formed patches rostrally, they aggregated in the form of bands that largely overlapped in the caudal half of the putamen. Occasionally, the two sets of labelled fibres overlapped with striatopallidal neurones that had been identified by retrograde transport of the lectin-conjugated horseradish peroxidase from the internal pallidum. At the electron microscopic level, the cortical terminals were found to be heterogeneous in size (max. diam. 0.5-2.0 μm), contained round synaptic vesicles and formed asymmetric synapses with the head of dendritic spines. The thalamic terminals had a small to medium size (max. diam. 0.3-1.0 μm), contained pleomorphic vesicles and formed asymmetric synapses predominantly with dendritic shafts and less frequently with spines. Electron microscopic analysis of striatal regions where the two sets of anterogradely labelled terminals overlapped revealed that the cortical and thalamic afferents form synapses on different neuronal elements. Therefore, despite the fact that the cortical and thalamic afferents largely occupy the same territory in the sensorimotor sector of the primate striatum, our results suggest that they terminate on different post-synaptic targets. The possibility that the cortical and thalamic inputs innervate different regions of single striatal neurones is currently under investigation. Supported by the Canadian and UK MRCs and by NATO.

399.5

BASAL GANGLIA REGULATION OF THALAMOCORTICAL ACTIVITY: VENTRAL PALLIDUM EXERTS OPPOSITE ACTIONS ON MEDIODORSAL AND DORSAL THALAMIC NUCLEI. A. Lavin* and A.A. Grace, Depts. Behavioral Neuroscience and Psychiatry, Center for Neuroscience, Univ. Pittsburgh, Pittsburgh, PA 15260.

The primary output pathway of the accumbens occurs via the ventral pallidal projections to the thalamus. Studies indicate that this pathway innervates the mediodorsal nucleus (MD) and the reticular nucleus (RTN) within the thalamus. Using *in vivo* intracellular recording and staining in rats, we investigated the effects of ventral pallidal (VP) stimulation on the activity of thalamic cells identified by Lucifer yellow injection.

VP stimulation caused a short-latency inhibition of neurons in the MD (ipsp latency = 2.2±1.0 msec; amplitude = 6.9±1.6 mV), with repetitive stimuli causing a tonic hyperpolarization of MD cells. Similarly, VP stimulation also elicited short-latency ipsps (latency = 2.7±1.1 msec, 5.4±1.1 mV amplitude) in approx. 70% of the RTN cells recorded. In contrast, approx. 60% of the neurons recorded in the dorsal thalamic nuclei responded to VP stimulation with longer-latency epsps (latency = 3.5±2.7 msec; amplitude = 5.2±4.7 mV). Furthermore, repetitive stimulation caused a tonic depolarization which often led to spike firing.

In schizophrenics, either hypofrontality or increased dopamine inhibition of the accumbens should result in activation of the VP. In this study, VP activation caused: 1) a direct inhibition of the MD thalamic nucleus which, in the absence of other excitatory inputs, should augment the hypofrontality, and 2) a disinhibition of the dorsal thalamic nuclei secondary to direct RTN inhibition. The latter action could conceivably contribute to the breakdown of thalamic filtering hypothesized to occur in schizophrenia.

399.6

EFFECTS OF DOPAMINE DEPLETION ON DYE- AND TRACER-COUPLING BETWEEN SPINY CELLS AND BETWEEN ASPINY CELLS IN STRIATUM. **S.-P. Onn* and A.A. Grace. Depts. of Behavioral Neuroscience and Psychiatry, University of Pittsburgh, Pittsburgh, PA 15260.**

Previous studies using *in vivo* intracellular recording have shown that injection of the dye Lucifer yellow (LY) into single striatal neurons (18%) results in labelling of pairs of neurons of the medium spiny class. In contrast, when coupling was assessed using intracellular injection and subsequent histochemical localization of Neurobiotin (NB), a significantly higher proportion of neurons (LY=18%; NB=47%) and more cells per injection (2-4 cells) were found to be coupled. However, although LY did not reveal coupling between aspiny cell types (n=5), in each case in which NB was injected into an Aspiny I or Aspiny II cell, the cell was found to be coupled to neighboring cells of the same morphological class (n=4).

In rats which had received 6-hydroxydopamine-induced depletions of striatal DA (95-99% loss of tyrosine hydroxylase immunoreactivity), there was a substantial increase in the incidence (92% coupling) and the extent (14/16 cases of 3 or more cells labelled per injection) of dye coupling revealed by LY injection, including a case of coupling among a cluster of 3 aspiny neurons. In contrast, DA depletion produced little alteration in the incidence (52% coupling) or the extent (2-3 cells coupled/injection) of tracer coupling revealed by NB injection. Therefore, although DA depletion produced an up-regulation in the incidence and the extent of dye coupling revealed by LY injection, it did not have a significant impact on the level of tracer coupling revealed by NB injection. This finding is consistent with the hypothesis that LY is only transferred between neurons when the gap junctions are in a high-conductance state, whereas NB appears to be capable of crossing gap junctions in either the open or the closed configuration. (Support by NS19608, Tourette Syndrome Association Grant and National Alliance for Research on Schizophrenia and Depression (NARSAD) Young Investigator Award).

399.7

MICROINJECTION OF BICUCULLINE TO THE CAT STRIATUM PRODUCES INHIBITION OF SINGLE UNIT DISCHARGES IN THE SUBSTANTIA NIGRA.

H.Yamada, K.Fujimoto* and M.Yoshida.
Dept. of Neurology, Jichi Medical School, Minamikawachi-machi, Tochigi, 329-04, Japan

By the unilateral injection of bicuculline (BIC) into the putamen (Put), dystonia of the neck was produced in accordance with spike discharges of the Put . On the other hand, by the unilateral injection of BIC into the caudate nucleus(Cd), locomotor hyperactivity was produced in accordance with spike discharges of the Cd. The response of the single unit activity of the substantia nigra pars reticulata (SNr) during these BIC injections was studied. Tubes for injections of BIC and recording electrodes were inserted stereotaxically into the Cd and Put. Single unit discharges in the SNr were recorded with an Elgiloy-electrode. Injection as well as recording sites were histologically identified. Shortly after the BIC (3μg/1.5μl) injection into the Put or Cd, SNr neurons showed marked inhibition in correspondence with the spikes in the Put or Cd. The striatum exerts GABAergic inhibition on the SNr and SNr in tern exerts GABAergic inhibition on the ventromedial nucleus of the thalamus, tectum and other structures. Thus the excitation of the striatum is hypothesized to stimulate the structures receiving influences from the basal ganglia output and produces dystonia or locomotor hyperactivities. We confirmed this hypothesis electrophysiologically by recording single unit activities in the SNr.

399.8

CA^{2+}-DEPENDENT MODULATION OF L-TYPE CA^{2+} CURRENTS IN ACUTELY DISSOCIATED RAT NEOSTRIATAL NEURONS. A. R. Howe* and D. J. Surmeier, Dept. of Anatomy and Neurobiology, College of Medicine, University of Tennessee, Memphis, Memphis, TN 38163.

We have recently reported that L-type Ca^{2+} currents are modulated by muscarine receptor activation in acutely-dissociated rat neostriatal neurons based upon the ability of muscarine to modulate the BayK 8644 enhanced tail current. As previously reported for sympathetic ganglion neurons (Mathie et al., 1992), the modulation of L-type current in neostriatal neurons was dependent on the absence of high levels of Ca^{2+} chelator. This dependence suggested that fluctuations in intracellular Ca^{2+} levels were crucial to the modulation. However, photometric studies in sympathetic ganglion neurons have suggested that an elevation in intracellular Ca^{2+} is not a necessary antecedent to L-current modulation. We have followed-up this work in neostriatal neurons by asking 1) how Ca^{2+} currents are affected by elevating intracellular BAPTA concentration and 2) how they are altered by Ca^{2+} mobilizing agents.

Whole-cell voltage-clamp recordings from acutely dissociated neostriatal neurons employed conventional techniques at room temperature. Elevations in intracellular BAPTA from 0.1 to 20 mM had several effects on whole-cell Ca^{2+} currents, including increasing peak currents, slowing de-activation kinetics and reducing the effects of BayK 8644. Ca^{2+} mobilizing agents, such as thapsigargin and caffeine, produced potent reductions in Ca^{2+} currents, including BayK 8644 enhanced tail currents, but in preliminary experiments these modulations were not strongly attenuated by high internal BAPTA concentrations. The impact of dialysable chelators on these modulations and those of other Ca^{2+} mobilizing agents (IP3, ryanodine) is currently being pursued. This work is supported by USPHS grants NS 28889 (DJS) and GR MH-10400-01(ARH).

399.9

DOPAMINE D1 RECEPTOR ACTIVATION REDUCES CA^{2+} CURRENTS IN ACUTELY DISSOCIATED RAT NEOSTRIATAL NEURONS. D. J. Surmeier*, Jose Bargas and A. R. Howe, Dept. of Anatomy and Neurobiology, College of Medicine, University of Tennessee, Memphis, Memphis, TN 38163.

The actions of dopamine in the neostriatum are mediated by a family of G-protein coupled receptors that can pharmacologically be divided into D1- and D2-classes. The D1-class receptors are positively coupled to adenylyl cyclase through G_s-family proteins. This signaling pathway is coupled to ion channel modulation in many excitable cells. Previous electrophysiological studies have reported that D1-class receptor activation produces a reduction in evoked discharge produced either by intracellular current injection or cortical stimulation. Consistent with this modulation, we have shown that Na^+ currents are reduced by D1-class agonists in neostriatal neurons. We set out to determine whether Ca^{2+} currents, which also contribute to spike generation and patterning, were also modulated by D1-class receptors.

Neostriatal neurons from young adult rats (3-8 weeks postnatal) were acutely-dissociated and whole-cell voltage-clamped at room temperature as previously described by our group. Only medium-sized neurons that previous work had shown to be projection neurons were examined. D1 receptor agonists (SKF 38393, SKF 82958) reduced peak Ca^{2+} currents in over 80% (n>100) of neurons studied. This modulation was blocked by the D1 receptor antagonist SCH 23390 but not by the 5-HT_2 antagonist ketanserin. Inclusion of GTP-γ-S in the patch pipette prevented reversal of the D1 modulation, suggesting G-protein mediation. Application of membrane permeant cAMP analogs (CPT-cAMP, Sp-cAMPS) mimicked the modulation produced by receptor stimulation. Co-application of the phosphatase inhibitor calyculin A with either cAMP analog potentiated their effects, implicating protein phosporylation in at least a portion of the modulation. This work is supported by USPHS grants NS 28889 (DJS) and GR MH-10400-01(ARH).

399.10

MECHANISM FOR AGE-RELATED DECREASE IN THE DURATION OF Ca^{2+}-MEDIATED PLATEAU POTENTIALS IN STRIATAL NEURONS. F. A. S. Villar, R. Dunia, T. H. McNeill, and J. P. Walsh*, Andrus Gerontology Center & Dept. of Biological Sciences, University of Southern California, Los Angeles, CA 90089-0191.

Plateau potentials induced in striatal neurons from aged rats (>24 mo.) are significantly shorter in duration than those obtained from young rats (3-6 mo.)(Dunia and Walsh, 1992). We tested the hypothesis that the reduced duration of plateau potential may be due to an age-related increase in the basal intracellular Ca^{2+}. To test this hypothesis slices were bathed in an extracellular medium where Ca^{2+} was replaced by Ba^{2+}. The Ba^{2+}-saline increased the average duration of the plateau potentials in neurons from young rats by 300% (500 to 2000 msec). By contrast, the Ba^{2+}-saline increased the average duration of plateau potentials in neurons from aged rats by only 40% (190 to 270 msec). These data indicate that the age-related reduction of the plateau potential is not due to a Ca^{2+}-mediated inactivation of Ca^{2+} currents.

Plateau potentials were tested separately for their sensitivity to NMDA (2 μM). NMDA reversibly reduced the duration of the plateau potentials, suggesting that NMDA may act to modulate Ca^{2+} currents in striatal neurons.

Neurons tested for plateau potentials were also filled with biocytin and reacted for HRP histochemistry. Measurements were then made of the total dendritic length. The data suggests that the duration of the plateau potential reflects the size of the neuronal dendritic field. Research was supported by a grant from the NIA (5 P01 AG0979).

399.11

AGED-RELATED DECLINE IN PAIRED-PULSE FACILITATION IN THE RAT STRIATUM: ROLE OF GABA MODULATION AND CALCIUM HOMEOSTASIS. X. Qu* and J.P. Walsh, Andrus Gerontology Center & Dept. of Biological Sciences, Univ. of Southern California, Los Angeles, CA 90089-0191

Our previous work has shown that there is an age-related loss of paired-pulse facilitation at the corticostriatal synapse. In the present study we tested the hypothesis that this change may be due to an alternation in GABAergic influence. Coronal slices (450 μm) from young (2-6 mo) and aged (24-26 mo) male Fisher 344 rat neostriatum were used. We gave two cortical stimuli to the corpus callosum at various interstimulus intervals and recorded the EPSPs from striatal neurons. Bicuculline (20 μM) and saclofen (400 μM) were added to the artificial cerebral spinal fluid (ACSF) to block $GABA_A$ and $GABA_B$ receptors respectively. Facilitation was slightly enhanced by the GABA receptor antagonists in both age groups, but the age-related difference in synaptic facilitation still persisted ($p < 0.01$). These data indicate that a change in GABAergic modulation can not account for the age-related difference in paired-pulse facilitation.

We next tested the hypothesis that the age-related loss of paired-pulse facilitation was due to an increase in intracellular Ca^{2+}. Slices from young and aged rats were bathed in a reduced Ca^{2+} ACSF. This solution significantly increased facilitation in both age groups ($p < 0.01$). These data indicate that multiple Ca^{2+} ions cooperate in the release process and that this mechanism may be saturated in aged corticostriatal terminals.
Supported by NIA grant 5 P01 A60979.

399.12

COMPUTER SIMULATION OF SPONTANEOUS MEMBRANE POTENTIAL SHIFTS IN STRIATAL NEURONS.

C. J. Wilson Dept. of Anat. and Neurobiol., Univ. of Tennessee , Memphis, TN.*

Striatal spiny neurons exhibit spontaneous membrane potential transitions from a hyperpolarized (down) state (-70 to-90 mV) to a depolarized but still subthreshold (up) state (-45 to -55mV). State transitions require excitatory synaptic transmission in the corticostriatal and thalamostriatal pathways but have been shown to be shaped by nonlinear properties of the striatal neuron. Biophysical data suggest two classes of potassium channels, one inwardly rectifying and activated at hyperpolarized membrane potentials, and one outwardly rectifying and activated by depolarization, are dominant over the relevant range of membrane potentials. Computer simulations of somatic current injections and synaptic excitation were employed using a morphologically accurate model of the striatal spiny neuron to assess the expected behavior of the cell based solely upon these two classes of channel.

No passive model of the striatal neuron could duplicate the voltage transients evoked in the striatal spiny neurons by small current pulses. Model neurons containing the two potassium channels accurately reproduced the behavior of the cells in the subthreshold range of membrane potentials, but only if both channels were placed on the dendrites.

Simulated noisy synaptic input could produce discrete up and down states in the model neurons when both potassium channels were included in the dendritic membrane, but not if one or both channels were selectively located in the soma. Voltage sensitivity of state transitions duplicated that seen *in vivo*. The voltage attained in the up state was determined by a dynamic interaction between synaptic input and outwardly-rectifying potassium channels located on the dendrites. Depolarizations resulting from increased synaptic excitation increased potassium conductance and the electrotonic length of the dendrites, while reductions in excitatory input reduced potassium conductance, making the remaining synapses more effective. The relatively constant membrane potential in the up state was thus due to dynamic adjustment of the effective electrotonic length of the striatal cell dendrites by the outward rectifier. ONR N00014-92-J-1113

399.13

ANALYSIS OF BISTABLE-LIKE SPONTANEOUS SHIFTS OF MEMBRANE POTENTIAL REVEALS SIMILAR PATTERNS IN STRIATAL AND CORTICOSTRIATAL NEURONS.

E. A. Stern[1] C. J. Wilson[2] and A. E. Kincaid[2] [1]Neurobiology Dept., Life Sciences Inst., Hebrew University, Jerusalem, Israel, [2]Dept. of Anatomy and Neurobiology, University of Tennessee , Memphis, TN.*

Spontaneous bistable-like shifts of membrane potentials have been recorded in identified striatal and corticostriatal neurons in rats anaesthetized with urethane. These shifts appear to be caused by interactions of synaptic inputs with membrane properties. We have analyzed their occurrence using standard statistical methods.

We find that, for both striatal and corticostriatal cells, the time of occurrence of each depolarized (up) state is independent of the duration of the previous hyperpolarized (down) state, as well as the history of state transitions. This allows us to treat times of occurrence of these states as a Poisson process with a distribution of delays, and develop unbiased estimators of the phenomena.

The times between state transitions were similar in the striatal and corticostriatal cells, as were the amounts of time spent in the two states. However, the variance of the times spent in the two states was significantly greater for the corticostriatal neurons, as was the bandwidth of the membrane potential fluctuations in the depolarized state. These differences are consistent with the more linear membrane properties of the corticostriatal neurons. The voltage dependence of the dwell time in each state was analyzed for both types of neurons. Time in the up state increased in both cell types when the cells were depolarized with constant current.

Noise analysis was performed on the membrane potential fluctuations of striatal neurons in the up state to characterize the underlying unitary events. The parameters of the synaptic inputs, which were modeled with a distribution of Γ functions, changed as a function of membrane depolarization. This suggests that the membrane noise in the up state is not a result of changes in the number of synaptic inputs alone but also membrane properties of the cell that produce a selective amplification of individual synaptic events along the dendritic tree.

399.14

STRIATAL SPINY NEURONS POSSESS TWO TYPES OF INWARDLY-RECTIFYING CURRENTS. E.S. Nisenbaum* and C.J. Wilson. Department of Anatomy and Neurobiology, College of Medicine, University of Tennessee-Memphis, Memphis, TN 38163

An identifying electrophysiological property of striatal spiny neurons recorded *in vivo* or *in vitro* is the pronounced rectification of their membranes in response to hyperpolarizing current pulses. Several types of inwardly-rectifying currents have been identified and include a fast, barium-sensitive K^+ current (I_{Kir}) and a time-dependent, cesium-sensitive mixed Na^+/K^+ current (I_H). The present experiments have investigated the potential contribution of I_{Kir} and I_H to the inward rectification of striatal spiny neurons in an *in vitro* slice preparation.

In control slices, inward rectification was observed as an asymptote in the amplitude of voltage deflections produced by intracellular hyperpolarizing current pulses (0.1-1.0 nA in amplitude, 400 ms duration). Blockade of inward Na^+ and Ca^{2+} currents with TTX (1 μM) and cadmium (400 μM) did not alter the rectification. In contrast, application of barium (100 μM) produced a membrane depolarization and abolished much of the inward rectification in these neurons. In addition, rebound depolarizing responses were evoked at the offset of large hyperpolarizing current pulses in the presence of barium. These rebound depolarizations are indicative of I_H, and accordingly could be blocked by extracellular cesium (3 mM). Cesium also produced a further increase in input resistance and decrease in resting membrane potential. These results indicate that striatal spiny cells possess both I_{Kir} and I_H. In addition to contributing to the resting potential of spiny neurons, these currents account for the low input resistance and short time constant of these cells at hyperpolarized membrane potentials and thus would be predicted to reduce the efficacy of spatially and/or temporally discrete synaptic inputs. ONR: N00014-92-J-1113

399.15

SPACING OF BOUTONS ALONG CORTICOSTRIATAL AXONS DIFFERS IN PATCH VS MATRIX COMPARTMENTS. A.E. Kincaid* and C.J. Wilson. Department of Anatomy and Neurobiology, College of Medicine, University of Tennessee, Memphis, TN 38163.

Cortical neurons that innervate the striatum are thought to preferentially innervate either the patch compartment or the matrix compartment. While we know the laminae and the area of cortex that give rise to the corticostriatal axons that innervate specific compartments, interpretation of axonal population tracing studies requires information about arborization patterns of single axons. We compared the pattern of innervation and the density of axonal varicosities along corticostriatal axons that were found in the patch or matrix compartments.

Corticostriatal axons were labeled with either an extracellular injection of biotinylated dextran amine or an intracellular injection of biocytin, and striatal compartments were identified using calbindin immunocytochemistry. Corticostriatal axons were identified in either the patch or matrix compartment and the distance between boutons was measured using a computer based image analysis program. Comparisons were made between axons that were found in the patch compartment and those found in the matrix compartment in both the axonal population studies and the intracellularly stained axons.

Patch compartments innervated by corticostriatal axons were more densely innervated than equal areas of matrix, in animals that received equivalent injections of extracellular tracer in patch vs matrix parts of cortex. Axons that were located in the patch compartment had a shorter inter-varicose distance, on average, than those axons that were located in the matrix compartment in both the population studies and the intracellularly stained axons. These results suggest that a given length of corticostriatal axon in a patch compartment will make more synapses than an equal length of corticostriatal axon in the matrix. Supported by NS20743.

CEREBELLUM I

400.1

CHANGES OF CAT CEREBELLAR FIELDPOTENTIALS EVOKED BY CONDITIONED LIMB MOVEMENTS FOLLOWING INJECTION OF BOTULINUM TOXIN TYPE A INTO THE TRICEPS MUSCLES. F.P. Kolb*, J.R. Bloedel+, V.Bracha+, E. Wiedemann, W.H. Fischer. Institute of Physiology, University of Munich; W-8000 Munich 2, Germany; +Barrow Neurological Institute, Phoenix, AZ 85013.

By using the operant conditioning paradigm tame cats were trained to stand still on four strain gauges-equipped platforms. Following an auditory stimulus the cat had to perform a step to a fifth platform situated frontally. Ful-filling predefined parameters the animal was rewarded by food. Fieldpotentials from the left cerebellar anterior lobe were recorded from a chronically implanted multielectrode for a period of up to eight weeks. Electromygraphic activity was obtained via chronically inserted wires from biceps and triceps muscles of both forelimbs. Botulinum toxin (12ng) injected into the triceps muscles evoked a transient muscular weakness. It was derived and quantified from a statistical analysis of biomechanical parameters of the step. The resulting impedement caused the animal to transiently change its strategy when performing the step. Changes in some of the fieldpotentials recorded at different depths within the cerebellar cortex could be related to changes in the biomechanical data. This findings suggest that the cerebellar cortex is, beside its well known ability of correcting ongoing and short lasting mismatches between the planned and executed motor programs, also involved in long term and plastic processes within the sensory motor system. Supported by Deutsche Forschungsgemeinschaft. SFB 220, D9.

400.2

EFFECTS OF TEMPORARY INACTIVATION OF THE SPECIFIC CEREBELLAR NUCLEI ON THE ORGANIZATION OF EMG ACTIVITY DURING A COMPLEX FORELIMB MOVEMENT IN CATS. M.S. Milak*, V. Bracha, J.R. Bloedel. Barrow Neurological Institute, Phoenix, AZ 85013.

These experiments were designed to analyze the specific involvement of individual cerebellar nuclei in the organization of muscular activity required for the execution of a complex forelimb movement. Each animal was trained to reach for a manipulandum and move it through a template consisting of 2 straight segments. The effects of muscimol injected into individual cerebellar nuclei ipsilateral to the performing forelimb were assessed. Dentate nucleus injections resulted in an inability to modify the EMG pattern in a task-dependent manner, and the flexor activity related to bar contact was greatly diminished, resulting in the cat often losing its grasp of the bar. Blocking anterior interposed nucleus output produced a dissociation of the EMG activity between the proximal and distal muscle groups. Although the inhibition of the posterior interposed nucleus caused dysmetria, the relative pattern of muscle activity did not show qualitative changes. Inhibition of the fastigial nucleus had no significant effect on the EMG onset time at paw lift-off, but its duration and amplitude increased as the movement through the template was executed. The data demonstrate that the effective performance of the complex forelimb movement is dependent on the functional integrity of all cerebellar nuclei. However, the differential effects of local nuclear inactivation on forelimb EMG patterns suggest that the individual cerebellar nuclei play a selective and specific role in organizing the muscle activity required for a movement and its task-dependent reorganization. NIH NS21958, NS30013.

400.3

UNIT ACTIVITY OF CEREBELLAR NUCLEI NEURONS DURING A CONDITIONAL MOTOR TASK WITH DELAY.
L. Germain* and Y. Lamarre. CRSN, Dept. of Physiology, Univ. de Montréal, Montréal, Québec, Canada.
In order to demonstrate functional differences between the dentate and the interpositus nuclei of the cerebellum one monkey was trained to perform a conditional motor task with delay. Each behavioral trial was composed of a 500 msec control period, an auditory signal of 400 msec duration (1000 Hor 400 Hz tone, 70 dB) a randomized delay period of 500 to 1500 msec duration, a go-signal (small torque pulse to the manipulandum) and finally execution of an elbow flexion or extension according to the auditory instruction (1000 Hz flexion, 400 Hz extension). A sample of 85 cells were recorded from the interpositus nucleus and a sample of 66 cells were recorded in the dentate nucleus.
In our sample of dentate neurons 30% (20/66) showed auditory responses, 41% (27/66) changed their firing rate during the instructed delay, 89% (59/66) were activated before and during movement execution. Among these cells only 27% (16/59) had their activity correlated with peak angular acceleration. In our sample of interpositus neurons none displayed auditory responses, 26% (22/85) changed their firing rate during the instructed period and all of them were activated during movement execution. Among these cells 59% (50/85) had their activity correlated with peak angular acceleration. These data suggest that the dentate nucleus is involved in the processing of directional information during preparation for an intended movement and that the interpositus is involved in the control of movement parameter during its execution (supported by MRC of Canada and FRSQ).

400.4

CLIMBING FIBER ACTIVITY IN THE LATERAL CEREBELLAR CORTEX (CRUS II) DURING VOLUNTARY ARM MOVEMENT IN MONKEY. J-P. Pellerin*, M-T. Parent, C. Valiquette and Y. Lamarre. Centre de Recherche en Sciences Neurologiques, Université de Montréal, Montréal (Qc), Canada, H3C 3J7.

Climbing fiber responses (CFR) were recorded in the cerebellar cortex (crus II) of a monkey trained to perform flexion or extension of the elbow in response to randomly presented visual, auditory and somesthetic cues. Task related CFR were movement related and occur 40 to 100 ms before the onset of movement. Eventhough the movements triggered by the 3 sensory signals were the same, only about 25% of the cells responded with the 3 "go" signals. Responses were seen most frequently with the visual "go" signal only (52%). Other cells responded to the visual and somesthetic cues (10%), visual and auditory (5%) and somesthetic only (8%). The probability of occurence of CFR was highest with modal reaction time. A number of cells were also recorded when the monkey performed spontaneous movements similar to the triggered movements. In all instances, CFR were abolished or strongly reduced and more variable in time with spontaneous movements as compared to triggered movements. Considering the topographical organisation of the olivocerebellar and cerebello-nuclear projections, we suggest that crus II, by its relationship to the Dentate nucleus, may be part of a circuit regulating the initiation of the movement triggered by external stimuli. In self-initiated movements, crus II would not exert the same influence on the movement initiation. These results suggest that the olivocerebellar projections in crus II may provide a more precise timing control of movement initiation in response to external events, particularly those triggered by visual stimuli. Supported by MRC grant of Canada.

400.5

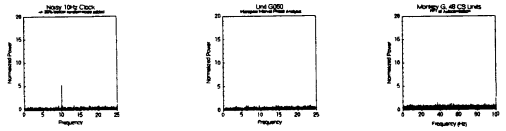
COMPLEX SPIKE ACTIVITY IN THE AWAKE BEHAVING MONKEY: NON-CLOCK-LIKE DISCHARGE. J.G. Keating* and W.T. Thach. Dept. of Anatomy, Washington Univ. Sch. Med. St. Louis, MO 63110.

We have recorded from Purkinje cells in 3 Rhesus monkeys during the performance of up to 16 different visually cued wrist rotation tasks.

Interspike intervals for all complex spikes recorded for a unit were placed in 5ms bins. Autocorrelation analysis was done. As a control, a "noisy" clock was created by moving the times of CS discharge from one cell to the nearest 10 Hz clock beat, and -20 to 20ms was randomly added to each spike. Even with noise over 40% of its cycle time the clock-like discharge is clearly visible on the Fourier transform of the autocorrelogram (fig 1). Analysis of the actual discharge of this unit is shown in fig 2. No periodicity could be seen for any of the 65 units analyzed. Fourier analysis of the autocorrelograms failed to reveal any periodicity up to 100Hz. The results from 48 units from one monkey are overlain in fig 3. Units with large numbers of complex spikes (1000's) gave flatter more even histograms than those with fewer (100's), suggesting a random discharge pattern.

The failure to observe clock-like timing in awake behaving animals, and in particular the random nature of complex spike discharge observed, questions the physiological significance of quasi-periodic IO discharge in other preparations. (support NIH grant NS12777)

Figure 1 Figure 2 Figure 3



400.6

CEREBELLAR NEURON DISCHARGE DURING SIMPLE AND COMPOUND MOVEMENTS. H.P. Goodkin* and W.T. Thach, Department of Anatomy and Neurobiology, Washington Univ. Sch. of Med., St. Louis, MO 63110.

A rhesus monkey (Macaca mulatta) was trained in one set of tasks (constrained finger tasks - "simple") to close microswitches in response to LED display by flexing the thumb (T) or the index finger (I) or the two together in a pinch (P). In another task (unconstrained food well task - "compound"), the monkey reached and pinched food bits from within a food well. The simple tasks required movement at fewer joints than did the compound task, but EMG analysis showed activity throughout the entire upper extremity for both the simple and compound tasks. Inactivation of the cerebellar dentate nucleus by muscimol injection has been shown to impair the performance of reaching and pinching (compound tasks) but negligibly that of the simple tasks (Goodkin and Thach, Soc Neurosci Abs, 218.11, 1992). Correspondingly, some units (8/26) fired in relation to the compound task and not the simple tasks, and of those related to both tasks (17/26) a third fired at much higher frequencies during the compound task. Nevertheless, many units did discharge in relation to the simple tasks (33/76); and although most of these discharged in the same pattern for T, I, and P, 2 related to T and P but not I and 2 related to I and P but not T (none related only to P). The discrepancy between the inactivation and the single unit recording data suggests that the cerebellum controls much activity during simple constrained movement that is not essential for its successful performance which becomes essential for the performance of compound unconstrained movements. This, in turn, suggests that the specific controlled variable may be predominantly or even exclusively antagonists and synergists -- "support" muscles -- rather than the agonist prime movers. (NIH grant NS12777).

400.7

IMPAIRED PINCH AND PRESERVED REACH AFTER LESION OF CEREBELLAR THALAMUS. A.J. Bastian* and W.T. Thach, Dept of Anatomy, Program in Physical Therapy, The IWJ Institute for Rehab. Research, Wash. Univ. Sch. of Med., St. Louis, Mo. 63110.

This 50 y/o man has had postural and action tremor since age 8. MRI showed an old infarct in the left ventral lateral thalamus. Motor impairments were restricted to the right arm; all sensory modalities including proprioception were normal. We examined limb kinematics during reach and pinch tasks.

The seated patient reached to a target at shoulder height in front of him. He was videotaped with markers at the tip of the index finger, shoulder, elbow, and wrist joints. Marker positions were digitized and joint angles and trajectories calculated. Wrist trajectories were normal. There was no target overshoot. 3-6 Hz tremor at the elbow and shoulder occurred during movement and increased while holding at the target. Elbow and shoulder angles changed simultaneously.

The patient was also videotaped pinching a quarter protruding from a narrow slot, with markers on the tip of the thumb, thumb DIP, thumb MCP, wrist, index MCP, index PIP, and tip of the index finger. Position of the markers were digitized and the trajectories for the tip of index and thumb calculated. 3-6 Hz tremor of the tip of index and thumb was marked during this task. The patient successfully retrieved the coin 64% of trials; control successes were 100%. Time between thumb and index contact of the coin was greater for patient vs control, ($p=.05$) indicating decomposition of pinch into separate thumb and index movements.

The ventral lateral thalamus receives overlapping projections from the 3 deep cerebellar nuclei (Asanuma et al 1983). The dorsal column-lemniscal system projects separately and more posteriorly to ventral posterolateral thalamus. Absence of somatosensory deficits would argue for exclusive involvement of the cerebellar thalamus. Kinematic analysis showed an impaired pinch with preserved reach. Goodkin and Thach (Soc Neurosci Abst. 544.14, 1990) reported a dentate lesion resulting in pinch *and* reach deficits. This patient shows that cerebellar output pathways influencing reaching and pinching may be dissociated. (NIH grant NS12777).

400.8

STORAGE OF MULTIPLE GAZE-HAND CALIBRATIONS. T.A. Martin*, J.G. Keating, H.P. Goodkin, A.J. Bastian, and W.T. Thach, Dept. of Anatomy and IWJ Rehab. Res. Inst., Wash. Univ. Sch. Med., St. Louis, MO, 63110

Wearing wedge prism spectacles while throwing at a target results in a re-calibration of the gaze direction and the throwing direction to hit the target. Base right prisms bend the optic path to the right and the eyes and head (gaze) turn to the left to fixate the target. The arm, calibrated with gaze direction, throws to the left of the target. With time, the throws shift towards the right, until they consistently fall on target. Following removal of the prisms, the adaptation is apparent on the first throw -- the gaze is now on-target, but the subject misses the target in the opposite direction by a distance almost equal to the initial error. Previous work demonstrates that the adaptation is impaired in patients with damage of cerebellar cortex, inferior olive, or mossy fibers of the middle cerebellar peduncle (Thach et al., Soc. Neurosci. Abs., 551.2, 1991), and is specific to the throwing arm (right or left) and the type of throw (underhand or overhand) (Thach et al., Soc. Neurosci. Abs., 218.12, 1992).

Following extended training with a specific diopter strength of wedge prism spectacles, subjects stored two eye-hand calibrations (no-prisms and trained-prisms). Trained subjects throw successfully on-target immediately upon donning the trained-prisms and immediately upon removing them. When donning a novel pair of prisms, subjects adapt as in the naive state (above): they throw off-target in the direction of the prism-bent gaze, gradually adapt throws onto target, and then throw off-target in the opposite direction when the novel prisms are removed. This adaptation affects both the no-prisms and trained-prisms calibrations; each must be independently re-adapted. This suggests that 2 (or more) gaze-hand calibrations may be stored simultaneously. Prior work suggests cerebellar cortex as the site of storage (Thach et al., Ann. Rev. Neurosci., 1992) (NIH grant NS12777).

400.9

DISTURBANCES OF KINESTHESIA IN PERSONS WITH CEREBELLAR DISORDERS. Stephen E. Grill[1], Cheryl Marcus[1], Mark Hallett[1], Jau-Shin Lou[1]*, and Lisa M. McShane[2], Human Motor Control Section[1] and Biometry and Field Studies Branch[2], NINDS, NIH, Bethesda, MD, U.S.A.

Voluntary control of movement is likely dependent on perception of kinematic variables. It is possible that deficits in persons with movement disorders are in part due to impaired perception. We compared the perception of kinesthetic stimuli in persons with cerebellar degenerations to normal age-matched volunteers. We also recorded the discharge of muscle spindle afferents and cutaneous mechanoreceptors from the radial nerve during imposition of the stimuli to identify which receptors are able to provide the afferent information.

We evaluated separately perception of duration, amplitude, and velocity by imposing three types of kinesthetic stimuli about the metacarpophalangeal joint of the right index finger. For the duration task, pairs of abrupt displacements of 1 degree but variable durations (100, 125, 150, 175, 200, or 225 msec) were employed. For the amplitude task, abrupt displacements of different magnitudes (0.95, 1.08, 1.21, 1.34, 1.47, or 1.60 deg) were employed. For the velocity task, the finger was moved at one velocity (10, 15, 20, 25, 30, or 35 deg/sec) for 500 msec and the speed of the movement then abruptly changed for another 500 msec. Subjects were instructed to respond by saying which one of the pair of stimuli was longer, larger, or faster. The study design was a randomized permuted blocks design using 10 blocks of size 36. Statistical analysis involved logistic regression techniques. Persons with cerebellar disorders performed significantly worse than normal subjects for the duration ($p=0.018$) and the velocity ($p=0.020$) task but not for the amplitude task ($p=0.053$). Microneurographic recordings during the presentation of the stimuli indicate that muscle spindle afferents provide a more reliable signal than do mechanoreceptors.

Defects of cerebellar subjects in voluntary control may in part to related to a deficit in processing kinesthetic signals. The kinesthetic signals are likely to arise largely from muscle spindle afferents.

400.10

STRETCH REFLEX MODULATION DURING SINUSOIDAL TRACKING IN CEREBELLAR ATAXIA. Johnson, M.T.V., Amrami, K., Kipnis, A.N., Mendez, A., Poppele, R., and Ebner, T.J.* Departments of Neurosurgery, Neurology and Physiology, University of Minnesota and EMPI, Inc., Mpls., MN 55455.

Our previous study showed that patients with Parkinson's disease exhibit a characteristic abnormality in the modulation of their stretch reflexes during volitional movements (Johnson, et al., Brain 114: 443-460, 1991). Extending this original study, we evaluated in subjects with cerebellar ataxia the modulation of reflex activity during volitional wrist movement. Eight subjects with cerebellar ataxia, 14 with Parkinson's disease and 10 normals were studied. Cerebellar ataxia and Parkinson's disease were established by physical examination and history. The subjects were asked to track a visually presented sinusoid with their wrist. Perturbations of the movement with torque transients at random times occurred at 45° and 90° intervals. Flexor and extensor reflex and volitional EMG and wrist displacement were recorded as a function of tracking phase. Subjects with cerebellar ataxia exhibited several abnormalities in the modulation of their reflexes. In some subjects this included increased amplitude of the short latency reflex components and/or delayed long latency reflexes. However, the pattern of the reflex modulation relative to the volitional tracking cycle was relatively normal. In three patients with cerebellar ataxia reflex modulation was virtually absent. In contrast subjects with Parkinson's disease exhibited an abnormal pattern of modulation in which the reflex activity relative to the tracking cycle was shifted by as much as 180°. These results suggest that the cerebellum contributes to the modulation of reflexes occurring during wrist movements and unique deficits in reflex modulation may characterize different movement disorders. Supported in part by NIH Grant R43-NS28633.

400.11

THE CEREBELLUM AND TIMING OVER TWO DIFFERENT TEMPORAL RANGES. J. Grinband, R. B. Ivry*, and S. Roberts. Dept. of Psychology, Univ. of Calif. at Berkeley, Berkeley, CA 94720.

Previous research has indicated that human patients with cerebellar lesions are impaired in judging the duration of intervals shorter than 1 sec. To explore the short-range timing task, rats (rattus norvegicus) were simultaneously trained on two time perception tasks. In the short-range timing task, rats were trained to discriminate intervals between 200 and 850 ms. In the long-range timing task, the intervals ranged from 25 to 40 sec. The durations were signaled by lights, with different lights used for each task. After psychometric functions were established, bilateral electrolytic lesions targeted at the lateral cerebellar nuclei were made. Control animals received sham lesions. Rats with cerebellar lesions were more variable than controls on the short-range timing task with minimal change in the point of subjective equality. In contrast, no differences were observed between the groups on the long-range task. The differential effects of the lesions on the two tasks suggest that the processing of temporal information over these two ranges involves different neural mechanisms. However, the effects of the lesions were transient: after one week of post-surgery testing, most of the experimental animals had returned to pre-surgery levels of performance. It is not clear whether the recovery reflects the extensive training given the animals (approximately 2800 trials per rat per week) or indicates that the cerebellum does not play a critical role in perceptual timing in the rat.

CEREBELLUM II

401.1

TRANSIENTS IN SIMPLE SPIKE ACTIVITY FOLLOWING COMPLEX SPIKES OF FLOCCULAR PURKINJE CELLS DURING COMPENSATORY EYE MOVEMENTS. D.H. Wang*, C.I. De Zeeuw, D.R. Wylie, and J.I. Simpson. Dept. Physiology and Biophysics, NYU Medical School, New York, NY, 10016.

Complex spikes (CS) of Purkinje cells (P-cells) of the cerebellum are followed by a pause (inactivation period) in simple spikes (SS) that can, in turn, be followed by a transient enhancement or reduction of the SS activity for up to 100 msec (McDevitt, Ebner and Bloedel,'82). Using cross-correlation of CS and SS activity, we examined the occurrence of these transients in vertical axis P-cells of the flocculus of the awake, pigmented rabbit during sinusoidal rotation (0.1 Hz) in the light (VOR-light) and dark (VOR-dark). Sinusoidal and constant velocity optokinetic stimuli (OKS) were also used. The SS are modulated under all conditions, but the CS activity is modulated only during VOR-light and OKS. The CS modulation is reciprocal to the SS modulation. In about half the cells a transient enhancement was present during spontaneous activity, VOR-light, VOR-dark and OKS. From inspection of the cross-correlograms, it is apparent that the transient SS modulation, whether an enhancement or a reduction, makes only a small contribution to the overall SS modulation during the VOR-light and OKS. During VOR-dark SS transients are present in the cross-correlogram, but like the CS activity, they are unrelated to the sinusoidal SS modulation. From these data it is not clear what the transient SS modulations, either enhancing or reducing, contribute to the compensatory eye movements, as customarily measured.

401.2

PHASE RELATIONS OF PURKINJE CELLS IN THE RABBIT FLOCCULUS DURING COMPENSATORY EYE MOVEMENTS. J.S. Stahl*, C.I. De Zeeuw, D.R. Wylie, D.H. Wang, and J.I. Simpson. Dept. Physiology and Biophysics, New York University Medical School, New York, NY, 10016.

In previous studies we found that during sinusoidal rotation of the alert, pigmented rabbit the phase of flocculus receiving neurons (FRNs) in the medial vestibular nucleus leads that of the nonFRNs (Stahl and Simpson,'92). If the FRN phase lead is produced by the signal contributed by the floccular Purkinje cells (P-cells), then the P-cells should in effect lead the FRNs. To test this hypothesis, we recorded the simple spikes (SS) of P-cells in the vertical axis zones of the flocculus in alert, pigmented rabbits. Single unit SS activity of P-cells was identified by the presence of a pause of SS after complex spikes. The animals were rotated sinusoidally about the vertical axis in the light (0.05 to 0.8 Hz) and the dark (0.1Hz to 0.4Hz). We calculated the phase of the SS activity using spectral analysis and linear multivariate regression. During rotation in the light the P-cells showed a phase lead re peak contra head position that increased with frequency (median 53° at 0.05Hz; 73° at 0.8Hz). The P-cells led the FRNs significantly at all frequencies except 0.8Hz. The difference was greatest at 0.05Hz (difference of medians = 16°, $p<0.001$) and progressively decreased with increasing frequency (0.1Hz, 14°, $p<0.001$; 0.2Hz, 12°, $p<0.001$; 0.4Hz, 9°, $p<0.01$; 0.8Hz, 1°, n.s.). In the dark, the P-cells had a greater phase lead re contra head position than in the light (median 88° at 0.1Hz; 85° at 0.4Hz). The phase of the P-cells led the FRNs at all frequencies (0.1Hz, 18°, $p<0.005$; 0.2Hz, 21°, $p<0.01$; 0.4Hz, 12°, $p<0.05$). These findings are consistent with the hypothesis that the floccular output increases the phase lead of the net premotor signal. The phase lead of the floccular signal may be created by enhancing the velocity components of signals originating in the vestibular nuclei.

401.3

THE FIFTH WHEEL OF THE FLOCCULUS. J.I. Simpson*[1], D.R. Wylie[1], C.I. De Zeeuw[1,2], P.L. DiGiorgi[1], and J. Tan[2]. (1) Dept. Physiology & Biophysics, NYU Medical Center, NY, NY, 10016. (2) Dept. Neuroanatomy, Erasmus University Rotterdam, 3000DR, Rotterdam, The Netherlands.

The rabbit flocculus can be divided into five zones (zones 1, 2, 3, 4, and C2) using AChE histochemistry. Zones 1 to 4 are involved in compensatory eye movements and the relations of their Purkinje cells with the inferior (IO) and cerebellar or vestibular nuclei (CN/VN) are known. The corresponding anatomy for the fifth floccular zone, C2, is unknown. In the present study, we determined in the rabbit the connections of this zone with the IO and CN/VN by means of anterograde tracing techniques. Small biocytin injections were made into the C2 zone, which was recognized by the absence of a climbing fiber response to visual stimulation. This physiological identification of the C2 zone was anatomically confirmed by AChE staining. The individually labeled axons of the C2 Purkinje cells innervated the interposed posterior nucleus (IPN), either alone or together with a collateral innervation of dorsal group y (Dy). Injections of WGA-HRP into Dy and the adjacent IPN resulted in anterograde labeling in the rostral tip of the contralateral medial accessory olive (rMAO), the ventrolateral outgrowth (VLO), and the rostral dorsal cap (rdc). Injections of WGA-HRP into these different olivary subnuclei showed that the rMAO projected to the C2 zone in the flocculus while both the VLO and rdc projected to zones 1 and 3. It can be concluded that the floccular C2 zone is like the great majority of cerebellar parasagittal zones in that it is part of a three-element loop composed of sequential projections from the IO to Purkinje cells to the CN/VN and back to the corresponding olivary subnucleus. For the non-visual part of the flocculus this loop consists of the C2 zone, IPN and Dy, and rMAO.

401.4

CEREBELLECTOMY REVEALS THAT STORAGE AND EXPRESSION OF VESTIBULO-OCULAR REFLEX ADAPTATION OCCURS IN THE BRAINSTEM. A.M. Pastor*, R.R. de la Cruz & R. Baker. Dept. of Physiology and Biology, Univ. of Seville, Spain, NYU Med. Ctr., NY, NY 10016.

The role of the vestibulo-cerebellum in vestibulo-ocular reflex (VOR) adaptation was determined in normal, adapted and cerebellectomized goldfish. Combined visual and vestibular stimulation was employed to alter the ratio of eye to head velocity (VOR gain). The time course of eye velocity responses to head velocity steps consisted of an early dynamic component followed by a plateau of constant velocity. Control and adapted dynamic VOR responses diverged at the earliest detected latency of 18ms during both high and low VOR gain states demonstrating modification of the shortest latency VOR pathways. Acute cerebellectomy after VOR adaptation revealed that the adapted and post-lesion responses overlapped for 50ms in the low and 70ms in the high gain VOR paradigms. Thus the cerebellum is not the storage site for altered eye velocity since brainstem pathways alone are sufficient for adapted VOR expression. The latency of vestibulo-cerebellar Purkinje cell responses to head and eye velocity steps exhibited a mean of 43ms and 70ms, respectively. Vestibulo-cerebellar activity therefore is not necessary for the expression of altered dynamic responses. However, in chronic cerebellectomized animals, the dynamic component was not modifiable. As a result, we conclude that the vestibulo-cerebellum is necessary for the induction, but not the maintenance, of the adapted VOR dynamic response. By contrast, the plateau of constant velocity could be significantly adapted and hence its brainstem pathways are modifiable in the absence of the cerebellum. Altogether, these findings favor the view that a continuous integration of visual and vestibular sensory signals with an internal representation of eye velocity occurs in the cerebellum to produce a signal that induces VOR adaptation in the brainstem.

401.5

DECREASES IN NITRIC OXIDE (NO) PRODUCTION AFTER INJECTION OF N-MONO-METHYL ARGININE INTO THE VESTIBULO-CEREBELLAR REGION OF GOLDFISH REDUCES VESTIBULO-OCULAR REFLEX GAIN. ADAPTATION. J.G. McElligott[1]*, Jun Li[2] and Sheryl Smith[2]. [1]Dept. of Pharmacology, Temple University School of Medicine, Phila., PA 19140, and [2]Dept. of Anatomy and Institute of Neuroscience, Hahnemann University Phila, PA 19102

The vestibulo-ocular reflex (VOR) has been studied as a model system for investigating adaptive neuroplastic changes in the sensori-motor system. Work in our lab has shown that a non-NMDA antagonist, CNQX, (Soc Neurosci. Companion Abst, 1993) but not a NMDA antagionist, MK-801, (Soc Neurosci. Abstr. 18 #215.3, 1992) will prevent VOR gain adaptation when injected locally into the vestibulo-cerebellum. Other agents, most notably nitric oxide (NO) has also been implicated in playing a role in other forms of neuroplastic changes in the central nervous system. In the work presented here, we investigated the effect of injecting N-mono-methyl arginine (NMMA) which decreases NO production via the NO synthase reaction. Goldfish were restrained in a cylindrical test aquarium which was sinusoidally rotated about the vertical axis at 1/8 Hz ± 20°. Projection of visual stimuli (random dot pattern) onto the wall of the aquarium at the same frequency, but double the amplitude, and 180° out of phase with the vestibular stimuli produced VOR gain increases (towards 3X). The gain of the VOR was assayed by measuring the movements of both eyes using the electro-magnetic search coil technique. Fish were trained to increase VOR gain over several hours during which time the VOR in both the light and the dark was measured. Direct injection of NMMA bilaterally (12.5 µg/0.5 µl/side) into the vestibulo-cerebellum of goldfish had no effect on the normal non-adapted VOR gain in the light and the dark. However, injection of NMMA prevented the robust adaptive VOR gain increase that is normally observed within the first hour of training in control injected goldfish. In addition, after 3 hours of continuous VOR adaptive gain training there was a profound and significant reduction in the adaptive gain increase. Since NO has been shown to be released in the cerebellum, our results would indicate that NO mediates the development of adaptive VOR gain increases within the vestibulo-cerebellum. *(Supported by NIDCD-NIH #DC 01094 & USAF #92-NL-024).*

401.6

VESTIBULO-OCULAR REFLEX GAIN ADAPTATION IS BLOCKED FOLLOWING INFUSION OF CNQX, A NON-NMDA ANTAGONIST, INTO THE VESTIBULO-CEREBELLUM OF THE GOLDFISH. T. L. Carter* and J. G. McElligott

Dept. of Pharmacology, Temple University School of Medicine, Phila., PA 19140.

Antagonists to glutamate receptors (NMDA and/or non-NMDA) have been found to interfere with hippocampal long-term potentiation, cerebellar long-term depression and developmental visual cortex plasticity. Our previous work has shown that systemically administered MK-801, a NMDA antagonist, delays the onset and reduces the rate of adaptive vestibulo-ocular reflex (VOR) gain increases but not gain decreases. In addition, when MK-801 was injected directly into the vestibulo-cerebellum, normal VOR gain adaptation occurred. The study reported here examines the influence of a cerebellar-infused, non-NMDA antagonist, CNQX, on VOR adaptation. Goldfish were restrained in a cylindrical aquarium which was sinusoidally rotated about the vertical axis at 1/8 Hz ± 20°. The fish were trained to increase gain (toward 3x) or decrease gain (toward 0x) by altering the phase and amplitude of the visual stimuli projected onto the wall of the test aquarium. The gain of the VOR was assayed by measuring the movements of both eyes using the electro-magnetic search coil technique. Fish were trained to change VOR gain over a 3 hour period during which time the VOR in both the light and the dark was measured at 15 minute (1st hour) and 30 minute intervals (2nd and 3rd hours). After initial VOR measurements, CNQX was infused bilaterally into the vestibulo-cerebellar region (0.5ul/side @ 0.1ul/min). Post-infusion measurements of the VOR were taken before the initiation of VOR adaptive training. CNQX (145ng/side @ 1.25mM) or a corresponding vehicle (1:5 Tris/artificial CSF) when administered into the vestibulo-cerebellum of the goldfish did not alter non-adapted VOR gain prior to adaptive training. However, CNQX but not the control treated animals experienced a complete inhibition of both VOR adaptive gain increases and decreases during the entire three hour training period. This work suggests that the non-NMDA receptors (AMPA/quisqualate and possibly kainate) located in the vestibulo-cerebellar region of the goldfish brain are involved in VOR neuroplasticity. *(Supported by a grant from NIDCD-NIH # DC 01094)*

401.7

VARIABILITY OF CLIMBING FIBER EFFECTS ON DEEP CEREBELLAR NUCLEAR NEURONS DURING SPONTANEOUS AND EXPERIMENTALLY-INDUCED EYE AND EYELID MOVEMENTS. A. Gruart*, A. Pastor and J.M. Delgado-García. Lab. de Neurociencia. Univ. de Sevilla. 41012-Sevilla, Spain.

Field potentials induced in deep cerebellar nuclei by the electrical stimulation of the contralateral red nucleus (RN), pontine nuclei (PN), restiform body (RB) and inferior olive (IO) were recorded in alert cats during eye and eyelid movements. Upper eyelid and eye movements were recorded with the search-coil technique. Recording sites were selected according to their relationships with spontaneous and/or experimentally-induced eye and eyelid movements, and identified by the antidromic field potentials induced by RN and/or RB stimulations. Control field potentials induced in selected cerebellar nuclear areas consisted of two negative waves at 0.5-1 ms and 2-3 ms followed by a late (4-6 ms) positive wave. It was observed in acute experiments that the two negative waves corresponded, respectively, to the antidromic and the synaptic activation of cerebellar nuclear neurons. The delayed positivity corresponded to the inhibitory effect of Purkinje cells on the subjacent nuclear neurons. The presentation of novel stimuli (flash lights, tones) to the alert behaving animal increased by 5-10 times the amplitude of the second negativity, and less noticeably, that of the delayed positivity. This increase in synaptic field potential amplitude fade out following the repeated presentation of the stimuli. When recording in the fastigial nucleus, the amplitude of the second negativity was modulated 120 deg in advance during sinusoidal rotation of the animal at 0.5 Hz. The increase in the synaptic field potential was experimentally evoked using a 50 µs conditioning stimulus in the PN 5-20 ms prior to the IO stimulation. A similar increase was observed during the acquisition of an eyelid response during a classical conditioning paradigm. These results suggest the involvement of mossy and climbing fiber responses on deep cerebellar nuclei neurons during motor response adaptations to novel stimuli.

401.8

DISCHARGE PROPERTIES OF IDENTIFIED DEEP CEREBELLAR NUCLEI NEURONS RELATED TO EYELID MOVEMENTS IN THE ALERT CAT. J.M. Delgado-García* and A. Gruart. Lab. de Neurociencia, Univ. de Sevilla, 41012-Sevilla, Spain.

The activity of cerebellar nuclear neurons was recorded in the alert cat during blinks induced by corneal air puffs, light flashes and tones. Recorded neurons were identified by their antidromic activation from various projection sites. Upper eyelid movements were recorded with the search-coil technique. Eyelid response to 100 ms air puffs consisted of an early (16.5 ± 2.7 ms) and fast (≤ 1.200 deg/s) downward movement followed by 2-3 downward sags that occurred at constant latencies. Blinks induced by flashes or tones presented longer latencies (52.6 ± 4.8 and 50.1 ± 8.0 ms). Type A neurons (n=90) increased their discharge rate in coincidence with the beginning of the blink, regardless of the stimulus modality. The late downward inflexions were also accompanied by corresponding increases in the neuronal firing rate. Type A neurons projected to the red nucleus (50%), restiform body (25%), pontine nuclei (15%) and through the medial longitudinal fascicle (10%). Type B neurons (n=32) showed a brief and irregular burst of spikes slightly preceding the eyelid response followed by a significant decrease in their firing rate. The firing response of type B neurons was always related to the motor response and not to the sensory modality. Type B neurons projected to the red nucleus (50%), oculomotor complex (25%) and restiform body (15%). No precise temporal coupling was found between the beginning of type A and B neural response and the start of either the stimulus or the motor response. However, significant relationships between mean firing rate of type A and B neurons and eyelid position, velocity and/or acceleration were demonstrated by linear regression analysis. Type A and B neurons seem to be directly involved in the execution of reflex blinks following the smaller details of eyelid displacements. The opposite behavior of type A and B neurons could be related to reciprocal roles of the different muscles involved in eye blinks.

402.1

DENDRITIC MORPHOLOGY OF RAT PHRENIC MOTONEURONS VISUALIZED BY RETROGRADE TRACING WITH CHOLERA TOXIN-B SUBUNIT (CTB). K.G. Smithson*, Y.S. Prakash and G.C. Sieck
Departments of Anesthesiology, Physiology and Biophysics,
Mayo Clinic and Foundation, Rochester, MN 55905

Previous descriptions of rat phrenic motoneuron morphology (Goshgarian, H.G. et al. *J. Comp. Neurol.* 201:441,1981; Lindsay, A.D. et al. *J Comp. Neurol.* 308:169,1991.) have employed horseradish peroxidase (HRP) as a retrograde tracer. Cholera toxin (CTB), however, is a more sensitive tracer particularly with respect to revealing distal dendritic morphology. In the present study phrenic motoneuron morphology has been reinvestigated with CTB to evaluate the fine dendritic ramifications of these neurons, and to provide a framework from which to study dendritic plasticity. The right hemidiaphragm of six adult male rats was injected with 1-2 μl of 1-2% CTB. Following a survival period of 48-72 h animals were fixed, and tissue sections prepared, after which the CTB was detected immunocytochemically. The CTB-label revealed many stained neurons within the cervical spinal cord. In these cells, staining extended to at least third order dendrites and in some cases up to fifth order labeling could be seen. Several features of phrenic motoneuron morphology not previously reported were observed. Frequently, distal dendrites were observed crossing the midline to ramify in the contralateral ventral horn. Also observed were fine varicose processes coursing rostrocaudally between and along motoneuron somata. These processes appeared to end abruptly in bulbous "terminations". These results suggest that adult motoneurons receive afferent input from the contralateral spinal cord–a feature previously thought to be restricted to the developing rat. The presence of bulbous "terminations" and fine processes around phrenic motoneuron somata suggest the possibility of local circuits which may be modulated by these motoneurons. (Supported by NIH grants HL37680, HL34817 and GM08288)

402.2

EXPECTED VALUES ON MOTONEURON DENDROGRAMS W. B. MARKS* LAB OF NEURAL CONTROL, NINDS, BETHESDA, MD 20892

Our stochastic model of motoneuron dendrites (Burke, Marks, and Ulfhacke 1992) generates sample dendrograms by sequentially appending 25 μm (ΔL) segments taken randomly from urns containing pieces only of the appropriate diameter and distance from soma. Each urn contains pieces that continue, terminate, and branch (into various diameters), in proportions that fit observations. Each dendrogram so constructed is a unique statistical sample. Here we calculate the expected shape of averages over these dendrograms, directly from the parameters of the model. The vector $\mathbf{m}(n)$ whose ith component is the expected number of branches whose diameter is in bin i at distance bin n, can be used to compute total dendrite area, volume, or the equivalent cable (with p=1, 2 or 3/2) from $E(\Sigma a_i(n)^p) = \mathbf{m}(n) \bullet \mathbf{a}^p$, where E (.) is expectation and vector **a** is the set of bin-centered diameters. In the differential limit $n\Delta L$ approaches L, the distance from the soma. The parameters of the stochastic model are the diagonal matrices M(L) and B(L), the probabilities of terminating and branching, and D, the daughter-parent diameter array. Given $\mathbf{m}(L=0)$,

$$\frac{\partial \mathbf{m}}{\partial L} = \frac{\partial \mathbf{m}}{\partial a}\frac{\partial a}{\partial L} + (-\mathbf{M} - \mathbf{B} + 2\,\mathbf{D}\,\mathbf{B})\,\mathbf{m},$$

gives $\mathbf{m}(L>0)$. Here "a" represents diameter, and the first term is the effect of taper. By setting taper $\partial a/\partial L$ = const /$\sqrt{a}$, L becomes electrotonic length. The expectation of more complex functions on branching processes can be computed using the generating function g(s, n) of the complex vector **s**. For $Z_i(n)$ = the occupancy of diameter bin i at distance n, $g(s, n) = E(\Sigma Z_j(n) s_j)$. For example, variances of functions of **m** require $E(a_i a_j) = \partial^2 g(s, n) / \partial s_i \partial s_j$. In this we hope to address questions like "What physiological functions are maximized by motoneuron dendrites?"

402.3

DENDRITIC STRUCTURE OF RENSHAW CELLS AND IA INHIBITORY INTERNEURONS IN THE CAT'S SPINAL CORD. M. J. Sedivec*, D. E. Dewey and R. E. W. Fyffe. Dept. of Anatomy, Wright State University, Dayton, Ohio 45435, and Dept. of Biology, Appalachian State University, Boone, N.C. 28608.

The dendritic trees of interneurons that mediate recurrent (Renshaw cells; RCs) and reciprocal (Ia afferent evoked; IaIns) inhibition of motoneurons (MNs) have been analyzed previously (Lagerback & Kellerth, 1985, J. Comp.Neurol. 240:368; Rastad et al, 1990, Anat.Embryol. 181:381), but the sample of fully reconstructed cells is limited. This work analyzes physiologically identified interneurons, intracellularly stained with horseradish peroxidase. Multipolar RCs form a homogeneous population, with an average of 5.4 stem dendrites per cell and total cell surface areas of about 24,000 μm^2. In contrast, IaIns (average of 5.3 dendrites/cell), have greater surface areas (mean 93,000 μm^2) but individually exhibit great variability. For both cell types, dendritic stem diameter correlates with cell surface area, total dendritic length and the number of end branches. The combined dendritic trunk parameter of RCs equals 1.0 for a distance of about 300 μm from the soma, whereas in IaIns this parameter decreased monotonically as a function of distance. In some respects the branching and equivalent cable structures of IaIns resemble those of γ-MNs whereas RCs have some structural features in common with α-MNs. Supported by NIH grant NS25547.

402.4

SEROTONERGIC INNERVATION OF MOTONEURONS IN THE CAT'S LUMBAR SPINAL CORD. J. C. Pearson *, F.J. Alvarez, D.E. Dewey, D. Harrington and R.E.W. Fyffe. Dept. of Anatomy, Wright State University, Dayton, Ohio 45435.

The bistable properties of lumbar spinal motoneurons (MNs) are dependent on intact serotonergic innervation of the spinal cord (e.g. Hounsgaard et al, 1988, J. Physiol. 405:345-367). The L_7 ventral horn receives profuse innervation from serotonergic axons (Arvidsson et al, 1990, Synapse 6:237-270) but it is not known how many serotonergic boutons contact MNs directly, or, where on the complex MN dendritic tree the synaptic contacts are made. We have combined intracellular staining of MNs with horseradish peroxidase and immuno-cytochemical staining of serotonergic axons and terminals to permit quantitative light microscopic analysis of these issues. Some selected appositions between serotonin immunoreactive boutons and identified MN dendrites were confirmed by electron microscopic examination to be genuine synaptic contacts. Each MN received between 900 and 1500 presumed contacts from serotonin containing axons. The contact sites were widely distributed over the soma and dendrites, including dendrites that extended into the white matter. Contact density was highest on 1st-4th order dendrites within 500 μm from the soma, with dendritic contacts tending to be larger than those on the soma. Some presynaptic axons generated multiple contacts along dendritic branches. We conclude that serotonergic systems contribute a major direct input to spinal MNs. Supported by NIH grant NS25547.

402.5

DIFFERENTIAL DISTRIBUTION OF GLYCINE RECEPTORS ON INTERNEURONS IN THE CAT SPINAL CORD. R. E. W. Fyffe*, F. J. Alvarez and D. Harrington. Dept. of Anatomy, Wright State University, Dayton, Ohio 45435

Several types of interneurons in the mammalian spinal cord, including those mediating reciprocal (Ia afferent evoked; IaIns) and recurrent (Renshaw cells; RCs) inhibition of motoneurons (MNs) use glycine as their neurotransmitter. These interneurons are themselves subject to inhibitory control, possibly also mediated by glycine. Using intracellular staining with neurobiotin to label physiologically identified interneurons, and a monoclonal antibody against a 93 kDa glycine receptor (Gly-R) associated protein, we show that ventral horn interneurons, including IaIns, RCs and neurons with contralaterally projecting axons, exhibit a high density of Gly-Rs over their cell bodies and dendrites. The density of Gly-R labelling is higher than observed for MNs using similar techniques. The soma and proximal half of the dendritic tree of RCs is densely covered by large receptor clusters, many of which form perforated disks, but fewer Gly-Rs are seen on the distal dendrites. In contrast, the soma and proximal dendrites of IaIns and other interneurons have a high density of small Gly-R clusters that become progressively larger on distal dendrites, a size gradient that is also observed for Gly-Rs on α-MN dendrites. The dense distribution of Gly-R's on soma and dendrites of all the interneurons suggests that they are subject to very powerful inhibition, probably from a variety of sources. Supported by NIH grant NS25547

402.6

DISTRIBUTION OF GLYCINE RECEPTORS ON SINGLE INTRACELLULARLY LABELED MOTONEURONS OF THE CAT LUMBAR SPINAL CORD. F. J. Alvarez*, D. Harrington, D. E. Dewey, and R. E. W. Fyffe. Dept. of Anatomy, Wright State University, Dayton Ohio 45435

Glycine is recognised to be the neurotransmitter utilized by a variety of inhibitory pathways involved in the regulation of motoneuron firing. Elegant electron microscopic immunocytochemical studies (e.g.Triller et al, 1985, J.Cell Biol. 101: 683-688) have demonstrated the presence of glycine receptors (Gly-Rs) on the soma and proximal dendrites of neurons in the ventral horn of the rat spinal cord. Here, using an antibody (R7A) that recognises the 93 kDa Gly-R associated protein, we have determined the distribution of Gly-R's over the whole soma-dendritic surface of physiologically identified α- and γ- motoneurons. Electron microscopic examination confirms the labelling to be restricted to post-synaptic densities apposed by boutons containing flattened or pleomorphic vesicles. Gly-Rs are present, in α-motoneurons, on the soma, proximal and distal dendrites, including branches in the white matter, and on some dendritic swellings and spines when the latter structures are present. The discrete receptor clusters are smaller on the soma and proximal dendrites than on more distal dendrites, although receptor density is higher proximally. In contrast, γ-motoneurons express relatively few Gly-R's, and they are generally generally absent from the more proximal dendritic segments and the cell bodies. Supported by NIH grant NS25547

402.7

PEPTIDERGIC AND GABAERGIC INNERVATION OF THE VENTROLATERAL DENDRITIC BUNDLE IN THE CAT S1 SPINAL CORD SEGMENT

V. Ramírez-León (1)*, T. Hökfelt (2) and B. Ulfhake (1), Departments of (1)Anatomy, and (2)Histology and Neurobiology, Karolinska Institutet, S-104 01 Stockholm, Sweden.

The motoneurons (MNs) in the ventrolateral nucleus (VLN) of the upper sacral spinal segments in the cat supply the external sphincters and the ischiocavernosii muscles. The dendrites of the MNs in the VLN are arranged into rostro-caudally oriented bundles (ventrolateral dendritic bundle; VLB). In this study we describe the distribution and synaptic arrangement of thyrotropin-releasing hormone (TRH)-, substance P (SP)- enkephalin (ENK)- and GABA-immunoreactive (IR) axonal boutons. This was accomplished using the peroxidase-antiperoxidase (PAP) technique (Sternberger et al., 1970).

IR axonal boutons were not randomly distributed within the dendritic arborizations in the VLB. SP- and TRH-IR boutons had a wide distribution, including both thin distal branches and thick proximal dendrites. ENK-IR and GABA-IR boutons were more restrictedly distributed, apposing mainly medium-sized and large dendrites.

A frequent finding in the VLB, valid for the neuropeptides and GABA, was that one and the same IR-bouton made synaptic contact with 2 adjacent dendrites. The postsynaptic dendrites involved in such arrangements often disclosed also dendro-dendritic contacts.

402.8

DISTRIBUTION OF SYNAPTIC VESICLE PROTEINS IN THE MONKEY (Macaca fascicularis) SPINAL CORD WITH EMPHASIS ON THE 5-HT AND GABA SYSTEM IN THE MOTOR NUCLEI

U Arvidsson*, F Piehl, B Meister, O Shupliakov, L Brodin, T Hökfelt and S Cullheim. Department of Neuroscience and Anatomy, Karolinska Institutet, Stockholm, Sweden.

Spinal cord motoneurons receive information from a large number of different sources and rough calculations indicate that one single motoneuron is contacted by at least 50.000-100.000 nerve terminals. So far, only few inputs to motoneurons have been identified with respect to their content of transmitter substances. One such system, however, the descending 5-HT raphe-spinal system seems to constitute one of the largest inputs to the spinal motor nuclei. 5-HT-immunoreactive nerve terminals in this part of the cord harbor also to a variable degree other neuroactive compounds such as several peptides. In contrast to the 5-HT system the GABAergic innervation of motoneurons seems to have its origin mainly from neurons at spinal cord levels. No coexistence between GABA and other neuroactive compounds including 5-HT has been described. Thus, the serotoninergic and GABAergic systems represent inputs to spinal cord motoneurons which differ with respect to both origin and degree of coexistence with other neuroactive compounds. In order to study possible molecular differences between different types of terminals/synapses on motoneurons we have here analyzed with immunohistochemistry 5-HT- and GABA-containing (analyzed with GAD) nerve terminals in the ventral horn of the grey monkey with regard to their contents of the synaptic vesicle proteins: synapsin, synaptophysin, rab3, synaptobrevin and synaptotagmin. A large number of varicosities contained synapsin-, synaptophysin-, rab3, synaptobrevin- and synaptotagmin-LI in the motor nuclei. In general, immunolabeling of all synaptic vesicle proteins outlined unstained somata and dendrites and crossectioned processes in the neuropil. The distribution pattern and density for each compound was not identical, however. Instead a specific staining pattern and also a variation in density for each compound was seen. Double labeled sections, analyzed with a confocal microscope, indicate that none of the synaptic vesicle protein-LIs could be found in 5-HT-IR terminals, whereas GAD-IR terminals exhibit all synaptic vesicle protein-LIs with the exception of synaptotagmin-LI. The results of this study suggest that 5-HT terminals differ from GABA terminals in the monkey motor nuclei with respect to their content of synaptic vesicle proteins. This could reflect that the exocytotic machinery in 5-HT terminals differs from that of GABA terminals.

402.9

GLUTATHIONE RECEPTORS IN HUMAN SPINAL CORD: A POTENTIAL ROLE IN NORMAL AND ABNORMAL FUNCTION?

R. Wagey*, R.A. Lanius, C. Krieger, and C.A. Shaw. Depts. of Medicine, Ophthalmology, and Neuroscience, University of British Columbia, Vancouver, B.C., Canada.

Glutathione (GSH) is a tripeptide which is widely distributed in the human nervous system and is believed to be an important antioxidant. GSH binding sites have been detected in rodent brain, largely within the white matter where they are localized to glial cells. Since GSH may be involved in diseases associated with free radical production in man, eg. spinal cord trauma, we have characterized GSH binding sites in human spinal cord. Quantitative autoradiography of ^{35}S-GSH binding in spinal cord demonstrates specific binding largely within the grey matter, including both the dorsal and ventral horns. ^{35}S-GSH labelled a high affinity binding site showing a K_d of approximately 2.5 nM. ^{35}S-GSH binding to the high affinity site reached equilibrium by 60 min at 4°C. Competition studies showed that ^{35}S-GSH binding was displaceable by GSH>cysteine> hexyl GSH. Glutamate and N-methyl-D-aspartate were ineffective as displacers. The present results show that GSH receptors are present in human spinal cord. Given the antioxidant and potential neurotransmitter roles of GSH, these data suggest that this peptide may play an important role in normal neural function, and that alterations in GSH or GSH receptor levels may contribute to abnormal function.

402.10

EPILEPTIFORM ACTIVITY INDUCED BY SUDDEN COOLING OF TOAD SPINAL CORD IS INHIBITED BY NMDA ANTAGONISTS.

N.L. Daló, J. C. Hackman and R.A. Davidoff*. Dept. Basic Sci. Universidad Centroccidental, Barquisimeto, Venezuela and DVAMC and Dept. Neurol. Univ. Miami School of Medicine, Miami FL 33101.

Sudden cooling of the spinal cord induces seizures in the South American toad, *Bufo marinus*. The mechanism was investigated in a hemisected spinal cord preparation, mounted in a sucrose gap apparatus, and superfused with HCO_3^--buffered Ringer at 21°C. Recordings were made from dorsal (DR) and ventral roots (VR). A rapid temperature change in the spinal cord caused by the rapid addition of cold Ringer (6-8 °C), produced a large depolarization (up to 18 mV) of afferent terminals and motoneurons. This was followed by a slow decline of the depolarization lasting from 6 to 23 min. During the decline, irregular, spontaneous depolarizing oscillations ("epileptiform" activity) were recorded. The spontaneous activity was more marked in the VR. Adding the NMDA (*N*-methyl-D-aspartate) antagonists, APH (DL-2-amino-7-phosphonoheptanoate, 10 μM, 15 min) or Mg^{2+} (1 mM), to the Ringer suppressed the epileptiform activity without reducing the large slow depolarization. The presence of 10 mM Mg^{2+} or 1 mM kynurenate in the Ringer reduced but did not abolish the cold-induced depolarization. The cold-induced depolarization and the epileptiform activity were not changed by addition of 10 μM dihydro-ouabain, a sodium pump inhibitor. Low temperatures prolonged DR and VR potentials evoked by single DR stimuli. Prolongation was reversed by adding APH (10 μM) or Mg^{2+} (1 mM). In sum, the release of excitatory amino acids and activation of NMDA receptors appear necessary for the generation of the epileptiform activity evoked by sudden cooling of the toad spinal cord. (Supported by USPHS #NS17577 and DVAMC MRIS #1769 & #3369).

402.11

THE INFLUENCE OF TONICALLY ACTIVE VOLTAGE-DEPENDENT POTASSIUM CHANNELS ON THE ELECTROTONIC PROPERTIES OF SPINAL MOTONEURONS.

D. Campbell and P.K. Rose*. MRC Group In Sensory-Motor Physiology, Department of Physiology, Queen's University, Canada K7L 3N6

The electrophysiological properties of spinal motoneurons are consistent with the presence of a leak at or near the soma. The cause of this somatic leak is unknown. Tonically active, voltage-dependent potassium channels located on the soma may be one of the factors which contribute to this leak. The objective of the present experiment was to block these channels using intracellular injections of cesium and thus determine their contribution to the somatic leak. The injection of cesium into neck motoneurons of anaesthetized cats caused a progressive broadening and increase in the amplitude of antidromic action potentials. These changes were taken as evidence that some or all voltage-dependent potassium channels were blocked. This block caused a decrease (30 to 40%) in the "resting" input conductance of the motoneurons. Preliminary estimates indicated that the somatic leak was less than the somatic leak measured in a control population of motoneurons. These results demonstrate that voltage-dependent potassium channels influence the electrotonic properties of motoneurons at "rest" and are partly responsible for their somatic leak. (Supported by MRC of Canada).

402.12

DEVELOPMENT OF REPETITIVE FIRING IN SPINAL MOTONEURONS OF NEWBORN RATS.

B.-X.Gao*, and L. Ziskind-Conhaim. Dept. of Physiology and Ctr. for Neuroscience, Univ. of Wisconsin, Madison WI, 53706.

The development of repetitive firing and the changes in the ionic mechanisms that underly it were studied in spinal motoneurons in a thin slice preparation of embryonic and neonatal rats. Prolonged depolarization (90-300 ms) generated 1-2 action potentials in embryonic motoneurons (E16-E18), but a train of action potentials lasting as long as the depolarizations was produced in neonatal motoneurons (P1-P3). Whole-cell voltage-clamp recordings were used to determine the developmental changes in the ionic currents that contributed to the repetitive firing. Despite the difference in the firing pattern of embryonic and neonatal motoneurons, at both ages only one peak of inward current was recorded during the prolonged depolarization. The repetitive firing in neonatal motoneurons was not due to a shorter refractory period. To determine whether K^+ outward current contributed to the frequency of repeated action potentials, K^+ current was blocked by substituting Cs^+ for K^+ in the recording pipette. Blocking the outward current resulted in motoneuron depolarization which was associated with bursts of spontaneous action potentials. In neonatal motoneurons, which were held at -60 mV, a prolonged depolarization failed to produce a train of action potentials, and similar to embryonic motoneurons, only 1-2 action potentials were generated. Elimination of K^+ current increased the durations of both the total inward current and the refractory period. These findings suggested that K^+ currents contributed to the repetitive firing of neonatal motoneurons. Subsequent studies will determine the developmental changes in inward and outward currents and their role in generating the repeated action potentials. Supported by RCDA (NS01314) and NS23808 to L. Z-C.

402.13

ELECTROTONIC CHARACTERISTICS OF CAT GAMMA-MOTONEURONS. R. E. Burke*, R. E. W. Fyffe and A. K. Moschovakis. Lab. of Neural Control, NINDS, NIH, Bethesda, MD 20892 and Dept. of Anatomy, Wright State Univ., Dayton, OH 45435.

Electrophysiological estimates of cell input resistance (R_N=8.6 and 10.0 MΩ) and tail time constant (τ_{tail} = 13.2 and 14.0 ms) were obtained from two hindlimb γ-motoneurons (MNs) that were then injected with HRP and fully reconstructed. We used these data (+10% shrinkage correction) in compartmental computer models[1,2] to estimate the specific resistance of the somatic (R_{ms}) and dendritic (R_{md}) membrane, using biologically plausible values of specific cytoplasmic resistance (R_i=60 to 200 Ω cm) and membrane capacitance (C_m=0.8 and 1.0 μF/cm^2). Values of $R_{ms} \leq R_{md}$ that matched[1] experimental R_N were used to construct unbranched equivalent cable models[2] to explore the parameters that produced transients with the observed τ_{tail}, using NODUS[3]. Final estimates were then confirmed in models with full branching structure. In both γ-MNs, best fits were found for R_i = 60 - 70 Ω cm, C_m=0.8 or 1.0 μF/cm^2 and $R_{ms} << R_{md}$, implying significant somatic shunts (G_{shunt} >98% of total G_{soma})[1,2]. For C_m=0.8 μF/cm^2, R_{md} was 60 - 75 KΩ/cm^2 (giving average dendritic path L[1] of 0.54 - 0.85). For C_m=1.0 μF/cm^2, R_{md} was 30 - 35 KΩ/cm^2 (average path L: 0.88 - 1.16). Like α-MNs[1,2], γ-MNs appear to have compact electrotonic architectures and large somatic shunts when studied with conventional micropipettes.

[1] Fleshman et al., J. Neurophysiol. 60:60, 1988; [2] Clements and Redman, J. Physiol. (Lond.) 409:63, 1989; [3] De Schutter, TINS 15:462, 1992.

402.14

TWO DIFFERENT FIRING PATTERNS OF TRIGEMINAL MOTONEURONS IN THE GUINEA PIG BRAIN STEM SLICE. F.R. Morales*, P. Castillo, J.K. Engelhardt and M.H. Chase. Departamento de Fisiología, Facultad de Medicina, Universidad de la República, Montevideo (Uruguay), Department of Physiology, Department of Anatomy and Cell Biology and the Brain Research Institute, UCLA School of Medicine, Los Angeles, CA 90024-1746

The firing pattern of a neuron depends on the interplay of a multitude of factors. Among these are the nature of the synaptic input to the neuron and the intrinsic properties of the neuronal membrane itself. In the present work we studied the pattern of discharge of motoneurons of the trigeminal motor pool in the guinea pig brain slice in response to long (typically 1 sec) pulses of depolarizing current. The results presented here are based on observations in 39 motoneurons with membrane potentials of at least -50 mV and action potential amplitudes ≥ 60 mV. Two different firing patterns were observed. In 74% of the neurons studied, the frequency of discharge was greater at the beginning of the current pulse with progressive accommodation toward the end of the pulse. In contrast, in 26% of the neurons, the first action potential of the train appeared with a substantial delay following the beginning of the pulse (delayed excitation) followed by an acceleration of the discharge frequency toward the end of the pulse. Neurons with these two types of firing patterns did not exhibit any statistically significant differences in membrane potential or action potential amplitude. Bath application of 100 μM 4-aminopyridine converted the delayed excitation-accelerating type firing pattern into the accommodating type firing pattern. We attribute these different firing patterns to a type of potassium (A) current that is present in a subpopulation of trigeminal motoneurons at resting potential and is slowly inactivated during the depolarization produced by a long pulse of current. (Supported by NS 09999)

402.15

CHARACTERIZATION OF OUTWARD CURRENTS IN GUINEA PIG TRIGEMINAL MOTONEURONS (TMNs) RECORDED IN VITRO. C.F. Hsiao*, L.J. Goldberg, and S. H. Chandler. Depts. of Physiological Science and School of Dentistry, UCLA, Los Angeles, CA 90024.

The underlying outward currents responsible for spike repolarization and slow AHP in TMNs were examined using sharp electrodes in combination with single electrode voltage clamp techniques (SEVC) from brainstem slices. Three outward currents were identified on the basis of their kinetics and pharmacological sensitivities to 4-AP, TEA and low Ca^{++}/Mn^{++} solutions containing TTX. A transient outward current (TOC) was observed which was activated by depolarizing command potentials between -40 and -55 mV, peaked within 7 ms of the onset the voltage step, inactivated with a time constant of 3-9 ms and was blocked by bath application of 4-AP (5 mM) but persisted in solutions containing low Ca^{++}/ Mn^{++} and 20 mM TEA. Voltage-dependent steady-state inactivation was characterized by a Boltzman function with a slope factor (k) between -4 and -7 and half-inactivation ($V_{1/2}$) occurring between -54 and -76 mV. Two sustained currents were observed in response to long (>1 sec) depolarizing step command potentials from holding potentials between -40 and -45 mV in the absence of the TOC. In the presence of TTX a slowly activating and deactivating outward current (time constant >200ms) was reduced substantially following bath application of low Ca^{++}/Mn^{++} containing solutions. The remaining sustained current activated and deactivated more rapidly and was reduced by 1-10 mM TEA or 1-5 mM 4-AP application.

These data demonstrate the presence of at least 3 pharmacologically and kinetically distinct outward currents which could contribute to spike repolarization and slow AHP production in TMNs. Supported by NIH grants DE06193, DE04166 and DE 07212.

402.16

N-METHYL-D,L-ASPARTATE (NMA) INDUCED VOLTAGE OSCILLATIONS IN GUINEA PIG TRIGEMINAL MOTONEURONS RECORDED IN VITRO. Y.I. Kim and S.H. Chandler*. Dept. of Physiological Science, UCLA, Los Angeles, CA 90024.

NMDA receptors in trigeminal motoneurons (TMNs) have been implicated in the production of rhythmical masticatory-like activity induced by repetitive stimulation of the masticatory cortex (Katakura and Chandler, 1990). Whole cell patch or sharp electrode recordings were obtained from TMNs to study the effects of bath applied NMA on the resting potential and membrane properties of these neurons. In TMNs NMA led to small depolarizations (2-7mV) and a modest increase in apparent input resistance (20-100%) prior to the onset of rhythmic burst discharges. The duration of the burst discharge (2 sec-2 min) and interburst phase (5 sec -3 min) were voltage dependent; the burst phase was longer and the interburst phase was shorter when the cell was depolarized, and eliminated when the cell was hyperpolarized artificially beyond -70 mV. TTX eliminated the spike discharges during the burst phase leaving the underlying rhythmical depolarizing and repolarizing shifts in membrane potential and depolarizing ramp voltage clamp commands produced NMA induced inward rectification. Ringer solution containing 0 Mg^{++} transformed the rhythmic NMA induced burst discharges into continuous discharge or maintained depolarizations, while 0 Ca^{++} containing ringer solutions blocked all rhythmic activity.

These results demonstrate that in TMNs continuous NMDA receptor activation can produce voltage and Mg^{++} dependent rhythmical membrane potential oscillations and burst discharges which are consistent with the known properties of the NMDA channel described in other regions of the CNS. Furthermore, the data suggest that NMDA induced voltage oscillations and burst discharges should be considered when models of central pattern generators for mastication are proposed. Supported by NIH grants DE06193 and DE07212.

402.17

RAT MOTONEURONAL CHOLINE ACETYLTRANSFERASE (ChAT) mRNA LEVELS ARE INCREASED BY EXPOSURE TO SUPRAPHYSIOLOGICAL AMOUNTS OF AN ANABOLIC-ANDROGENIC STEROID. P. E Micevych*, P. Popper, and C. E. Blanco. Dept. of Anatomy & Cell Biology, Laboratory of Neuroendocrinology, UCLA School of Medicine, Los Angeles, CA 90024-1763.

ChAT mRNA levels in motoneurons which innervate the sexually dimorphic and androgen sensitive bulbocavernosus/levator ani muscle complex of the rat are decreased in castrated males and are maintained at intact male levels by testosterone (T) replacement therapy. The purpose of this study was to determine whether the expression of ChAT mRNA in spinal motoneurons could be elevated by supraphysiological levels of the anabolic-androgenic steroid, T propionate (TP). Serum T levels were elevated by subcutaneous implantation of TP containing silastic capsules into gonadally intact males. Four weeks after implantation, blood was drawn for the determination of serum T levels and the animals were killed by transcardial perfusion under pentobarbital anesthesia. The spinal cords were removed and subsequently processed for ChAT *in situ* hybridization histochemistry using a ^{35}S-labelled ribonucleic acid probe. The average serum T levels were 2 ng/ml for intact male rats, 10 ng/ml for animals implanted with a single TP capsule, and 20 ng/ml for those treated with 2 TP capsules. Compared to intact untreated males, ChAT mRNA levels were unaltered in L2-L4 motoneurons, and significantly greater in L5 motoneurons for the males treated at the lower TP dose. At the higher TP dose, motoneuronal ChAT mRNA levels were significantly greater when compared to untreated males and the low TP dose group throughout the lumbosacral spinal cord. These data demonstrate that supraphysiological levels of anabolic-androgenic steroids alter the expression of specific motoneuronal mRNAs. Furthermore, these results also suggest that anabolic-androgenic steroids act to influence skeletal muscles at the level of the motoneuron. Supported by HD 7228 and NS 21220.

402.18

FIRING PATTERNS AND THEIR MODULATION BY NOREPINEPHRINE IN THE NUCLEUS AMBIGUUS NEURONS IN SLICES FROM GUINEA PIGS. Y. Nishimura*, T. Asahara, M. Muramatsu, K. Yoshioka, T. Tanaka and [1]T. Yamamoto. Department of Physiology, School of Medicine Mie University, Tsu, Mie 514 and [1]Department of Physiology,Fukui Medical School , Fukui 910-11, Japan,

Intracellular recording was done in the ambiguus neurons (AMBs) in slices from guinea pigs with a glass microelectrode filled with 3M-KCl or 5% Lucifer yellow (LY) dissolved in 0.1M LiCl. AMBs were identified by intracellular staining with LY. The subthreshold depolarizing current pulse evoked a slow depolarization of 200~300 msec duration, which was insensitive to 1μM tetrodotoxin, but was blocked by replacing Ca^{2+} with Co^{2+}. Injection of the suprathreshold depolarizing current induced a continuous tonic firing in 40% of 86 AMBs (tonic cells). Fifty-two cells (60%) discharged spikes at the onset of the current injection, which were followed by the depolarization without spikes (phasic cells). The afterdepolarization following a train of spikes could be seen more often in the phasic cells. Replacement of Ca^{2+} with Co^{2+} prolonged the duration of the train of spikes to change the phasic pattern to the tonic one, and reduced the afterhyperpolarization (AHP) following a train of spikes. Norepinephrine (NE:100 μM) prolonged the duration of the train of spikes and reduced AHP in both types of AMBs. We suppose that the Ca-mediated K current [IK(Ca)] and Ca-sensitive slow depolarization contributed to the control of the firing behavior and that NE modulated the firing patterns by affecting the IK(Ca)in the AMBs .

402.19

SHORT TERM DEPRESSION AND NON-LINEAR TEMPORAL SYNAPTIC INTERACTIONS IN VAGAL MOTONEURONS. R. Nitzan*, I. Segev, and Y. Yarom. Dept. of Neurobiology, Inst. of Life Sciences, The Hebrew University of Jerusalem, Jerusalem 91904, ISRAEL.

Temporal interactions between evoked synaptic potentials in motoneurons from the Dorsal Motor Vagal Nucleus (DMVN) were studied by conventional intracellular recordings from submerged brain stem slices. Detailed model of DMVN motoneurons (Nitzan et al., *J. Neurophysiol. 63:333-346*, 1990) was used to quantify the non linearity of synaptic interaction expected in passive models.

Synaptic potentials were elicited by nonspecific stimulation in the nucleus surroundings. Short trains of various frequencies and duration were used. In 50% of the neurons, inter-train depression was clearly observed. The maximal potential of the synaptic response to a train of stimuli was reached after the first stimulus in the burst. The amplitudes of the successive synaptic potentials in the burst were smaller than that of the first response. The depression was a function of the duration and the frequency of the stimuli. It reached a maximum value of 60% decrease in amplitude in the 6th response to a 90Hz stimuli. Introducing GABA blockers to the physiological solution (picrotoxin, bicuculline, saclofen) had no affect on these results.

Simulations of electrically proximal or distal synapses activated at frequencies similar to the experimental conditions have shown a 10-50% decrease in the amplitude of successive synaptic potential in the train. Maximal affect was measured when the synapses were activated at high frequency on distal dendrites. The decline in the amplitude of the synaptic potentials expected from the passive model, however, can only partially account for the experimentally observed depression. A good match to the experimental results could be obtained when the maximal synaptic conductance was decreased by 50 to 70% after the first stimulus.

We conclude that high frequency synaptic input to motoneurons of the DMVN is accompanied by a fast decrease in the synaptic efficacy.

402.20

RESONANCE BEHAVIOR OF OLIVARY NEURONS. I. Lampl and Y. Yarom*. Dept. of Neurobiology, Life Science Institute, Hebrew University. Jerusalem ISRAEL.

The ability to generate subthreshold membrane potential oscillations in neurons from the inferior olive (I.O) nucleus has been attributed to the electrical properties of these neurons as well as to the properties of the network. Thus, this oscillatory behavior is independent of the membrane potential, occurs simultaneously in a large number of neurons, is TTX insensitive and is abolished by calcium blockers. In the present *in-vitro* study we implemented the ZAP method to analyze the properties of olivary neurons in the frequency domain in an attempt to characterize those properties that contribute or support the oscillatory activity.

The ZAP function (Puil et al. 1985) is a sine wave of continuously (and linearly) increasing (or decreasing) frequency which lasts for a given duration. This signal was injected, as current, into I.O neurons via an intracellular microelectrode which was also used to measure the voltage responses of the neurons. Following a Fast Fourier Transform (FFT) of both the current wave form and the neural response to such current, we calculated the impedance of the neuron and described it as a function of frequency (Z-F curve).

We found that the Z-F curves of olivary neurons demonstrate a peak impedance (resonance) at a frequency of 4-6 Hz. This peak, which is higher than the DC resistance, is: independent of the membrane holding potential; TTX insensitive; unaffected by K^+ channels blockers; and almost completely blocked in the presence of Co^{++} in the physiological solution. In several experiments, where the ZAP method was applied while the cells oscillated spontaneously, it was found that the peak resonance is similar to the frequency of the spontaneous oscillation. Furthermore, changing the temperature in the recording chamber result in a corresponding change in the resonance frequency as well as the frequency of the spontaneous oscillations.

These results indicate that neurons from the I.O nucleus have resonance behavior that reflects the mechanism that underlies the spontaneous subthreshold oscillations.

SPINAL CORD AND BRAINSTEM IV

403.1

THE EFFECT OF TOOTH DISPLACEMENT ON MASSETER MOTONEURONS. Dean Dessem* and Revers Donga, Department of Physiology, U. of Maryland Dental School, Baltimore, MD 21201

Cats were anesthetized with sodium pentobarbital, paralyzed with gallamine and respired. Recordings were made using microelectrodes filled with 2M KAcetate while displacing the maxillary canine teeth. Forces were applied in a variety of directions and ranges of force (0.3-0.46N). Stable intracellular recordings were made in 48 neurons (MP>50mV) during tooth displacement. Twenty-seven of these were identified as being from masseter motoneurons by fixed, short-latency (0.9-1.1ms), antidromic activation following stimulation (58-160μA) of the masseter nerve. Some motoneurons showed a reproducible, short-latency (3.95-6.68ms) depolarization (0.8-9.5mV), which in some cases was large enough to generate spikes. Typically this depolarization was followed immediately by a brief inhibitory period. Early inhibitory responses in motoneurons impaled with 3M KCl-filled electrodes, were reversed into depolarizing responses after 3 minutes implying a chloride dependent component to the inhibition. In some motoneurons sustained (100ms) tooth displacement produced a prolonged depolarization which generated multiple spiking. These results demonstrate that tooth displacement is capable not only of eliciting a variety of effects in trigeminal motoneurons but that these responses differ from those elicited by electrical stimulation of the alveolar nerves. Supported by NIH DE10132.

403.2

ACTIVITY OF ROSTRAL TRIGEMINAL NEURONES IN THE AWAKE CAT. B.E. Cairns*, M. Fragoso, & P.J. Soja. *Fac. Pharm. Sci, University of British Columbia, Vancouver, BC, Canada V6T 1Z3.*

The present study was performed to record the extracellular unit activity of trigeminal nucleus oralis sensory neurones in the chronic, unanaesthetized cat during the behavioral state of wakefulness.

Three adult cats were prepared for chronic extracellular recording of trigeminal neurones using methodologies developed previously (*J. Neurophysiol., 44: 349-357, 1980*). Glass micropipettes filled with 2M NaCL were lowered in tracts directed to the subnucleus oralis, wherein low-intensity stimuli applied to the ipsilateral inferior alveolar nerve (IAN), maxillary or mandibular canine tooth pulp (TP) afferents evoked an orthodromic field potential (*Sleep Res., 22: 424, 1993*).

Suprathreshold search stimuli (*ibid*) applied to the IAN or TP resulted in a short synchronous burst of action potentials with interspike intervals ranging from of 1.0-1.25msec that were superimposed on the decay phase of the field potential. Such stimuli often resulted in a longer latency (20-40msec) train consisting of 4-6 asynchronous action potentials. The latency-to-onset of a single spike following threshold stimuli ranged from 3-4msec for IAN or maxillary TP and 5-7msec for mandibular TP volleys, respectively. Stimulation of the digastric (Dig) muscle or VII nerve evoked long-latency (8-40msec) asynchronous spike trains indicating that these cells also receive convergent input from diverse orofacial receptive fields. No antidromic spikes could be evoked upon VII or Dig stimulation indicating that the recorded neurones were not VII or V motoneurones. Evoked activity of individual neurones could often be held for greater than 30 minutes. None of the recorded neurones exhibited spontaneous discharge activity during wakefulness.

The present results corroborate data obtained from studies performed in "acute" preparations and demonstrate the feasibility of exploring further the activities of IAN or TP-driven trigeminothalamic tract and V (commisural) premotor neurones across various behavioral states. Supported by grants from the MRC and BCHRF.

403.3

SUBTHRESHOLD SYNAPTIC ACTIVITY INFLUENCING LUMBAR NEURONES IN THE CHRONIC CAT. P.J. Soja*, J.-I. Oka and M. Fragoso. *Fac. Pharm. Sci., U BC, Vancouver, BC, Canada V6T 1Z3.*

Previous studies in our laboratory have demonstrated that sciatic nerve-evoked volleys recorded extracellularly within the spinothalamic and spinoreticular tracts are markedly suppressed during the behavioral state of active sleep (AS) when compared to quiet sleep (QS) or wakefulness (W). (*Soc. Neuroscience Abst. 18: 61, 1992*). The present study was performed to record intracellularly the membrane potential activity of individual lumbar neurons in the chronic, unanaesthetized cat during W, QS, and AS.

Three adult cats were prepared for chronic intracellular recording of lumbar neuronal activity using methodologies developed previously (*Physiol. Behav., 27: 355-362, 1981*; *ibid*). Bevelled glass micropipettes filled with 2M K^+-citrate were lowered into the spinal cord at stereotaxic coordinates corresponding to the intermediate nucleus as evidenced by the presence of a sciatic or sural nerve-evoked orthodromic field potential (*Soc. Neuroscience Abst. 18: 61, 1992*). Neurones impaled exhibited membrane potential and action potential amplitudes exceeding -50mV and 55mV, respectively. Most neurones displayed spontaneous spike activity upon impalement that subsequently subsided or ceased upon the sustained injection of constant hyperpolarizing current (0.5-2.0nA). Three of these neurones were identified as spinal sensory tract neurones since antidromic spikes could be evoked from the lateral medullary reticular formation. During W and QS, membrane potential activity consisted predominantly of frequently occurring large amplitude (2.5-3.5mV) "simple" depolarizing synaptic potentials which varied markedly in their risetime and decay parameters. "Compound" potentials, i.e, those with inflexions on their rising phase, were also recorded. During AS, there was a marked increase in depolarizing and hyperpolarizing synaptic activity.

The present results suggest that individual sensory tract neurones undergo an marked increase in conductance during the state of active sleep. Supported by a MRC Development Grant (DG-399).

403.4

ELECTRICAL STIMULATION OF THE CHOLINERGIC LATERODORSAL TEGMENTAL NUCLEUS (LDT) ELICITS EPSPs IN MEDIAL PONTINE RETICULAR NEURONS IN THE CAT. H. Imon*, K. Ito, L. Dauphin, & R. W. McCarley. Lab Neurosci., Dept. Psychiatry, Harvard Med. Sch./Brockton VAMC, Brockton MA 02401

Our laboratory has described cholinergic projections from LDT/PPT to the pontine reticular formation (PRF) and there is now considerable evidence that such cholinergic projections play a key role in the induction and maintenance of the REM phase of sleep. However, a key element supporting this REM sleep role of mesopontine cholinergic neurons has been missing, namely a demonstration of excitatory synaptic effects of LDT/PPT stimulation upon the presumed effector neurons in PRF. Accordingly we examined the effects of LDT electrical stimulation in acute cats under urethane anaesthesia and with artificial ventilation. After removal of a portion of the cerebellum, surface landmarks and stereotaxic techniques were used to place stimulating electrodes bilaterally in the LDT (location histologically verified). A transverse cut throughout the reticular formation was made 1-2 mm rostral to LDT to diminish the possibility of polysynaptic stimulation from rostral effects. Electrical stimulation parameters were 0.1 mS duration for a single pulse and 300-700 uA intensity. Intracellular recordings were made of pontine reticular formation neurons. LDT stimulation produced two EPSPs, one of very short latency (about 0.6 mS) and presumptively arising from activation of rapidly conducting fibers, perhaps reticulo-reticular and MLF fibers. A second EPSP was of longer latency, 2-3.7 mS, and had a mean conduction velocity of about 2 m/S, within the conduction range reported for cholinergic fibers. This second EPSP was abolished by a cut just caudal to the LDT nuclei and juxtaposed between them and the recording sites of the neurons. These data suggest that cholinergic mesopontine neurons provide excitatory synaptic input to PRF neurons.

403.5

ORDER OF REM SLEEP FIRING RECRUITMENT CORRELATES WITH SOMA SIZE OF PONTINE RETICULAR FORMATION NEURONS: INTRACELLULAR RECORDING AND LABELING IN THE NATURALLY SLEEPING CAT.

K. Ito*, H.Imon, L. Dauphin, & R.W. McCarley. Lab. Neurosci.,Dept.Psych.,Harvard Med. Sch./VAMC, Brockton Ma 02401.

Acute intracellular labeling studies in our laboratory have examined the projections of giant cell field pontine reticular formation (FTG) neurons. Type I neurons had primarily reticulo-spinal projections, relatively few collaterals, and most had soma diameters ≥ 47.5 μm. Type II neurons projected to brainstem reticular sites, often had collaterals, and had soma diameters < 45.7 μm. The present study used intracellular recording and intracellular labeling (PhAL, biocytin, or neurobiotin) in naturally sleeping, chronic cats to determine if the "lead time" of onset of markedly increased discharge rate prior to REM was correlated with soma size. Soma diameter was determined from digitizer measurements of cross-sectional area (inter- and intra-rater variation < 5%), and done blind to the physiology. RESULTS. Diameters of the 20 labeled neurons ranged from 43 μm to 88μm (soma size range: 1500 ≈ 6000 μm^2). Within this range there was a strong positive linear correlation between diameter and "lead time", which ranged from near zero minutes to about six minutes. That larger neurons had longer "lead times" was contrary to our expectation that smaller neurons, which might be Type II neurons with abundant reticular projections, would show earlier recruitment. It was also expected from the inverse size dependence of recruitment order in alpha motoneuronal pools. Earlier recruitment of larger neurons may reflect a stronger cholinergic excitatory input.

403.6

SEROTONIN (5HT) MICROINJECTED INTO THE HYPOGLOSSAL (XII) NUCLEUS REDUCES THE DEPRESSION OF XII NERVE ACTIVITY DURING REM SLEEP-LIKE ATONIA.

L.Kubin*, C.Reignier, G.Woch, H.Tojima, A.I.Pack and R.O.Davies. Department of Animal Biology and Center for Sleep and Respiratory Neurobiology, University of Pennsylvania, Philadelphia, PA 19104.

We have demonstrated that XII motoneurons are under an endogenous excitatory drive mediated by 5HT (Neurosci.Lett.92,139:243) and the 5HT level is reduced in the XII nucleus region during the postural atonia and respiratory depression induced by pontine carbachol microinjections (REM sleep atonia)(Soc.Neurosci.Abstr.92,18:1528). In the present study, we assessed if serotonergic agonists microinjected into the XII nucleus can effectively reduce the depression of the XII nerve activity induced by pontine carbachol. In 7 decerebrate, paralyzed, vagotomized and artificially ventilated cats, 5HT or 5CT (5HT agonist), injected into one XII nucleus (mean:130 nl,1-5 mM), augmented the peak XII nerve activity on the treated side to 193%±50(SD) of control. Then the atonia of REM sleep was induced by pontine carbachol. This reduced the activity on the treated side to 66%±24 of the precarbachol level and that on the untreated side to 19%±11. Both tonic (expiratory) and phasic (inspiratory) activities on the treated side were similarly affected. The agonist-enhanced XII nerve activity could be depressed by lingual nerve stimulation. These studies are consistent with the possibility that locally applied appropriate 5HT receptor agonists act by substituting for the reduced 5HT release during the carbachol induced atonia. (Supported by HL-47600 & HL-42236.)

403.7

RESPIRATORY MODULATION OF MEMBRANE RESISTANCE IN INSPIRATORY HYPOGLOSSAL (XII) MOTONEURONS (Mns).

G.Woch, L.Kubin, R.O.Davies* and A.I.Pack. University of Pennsylvania, Philadelphia, PA 19104.

Genioglossal Mns, the tongue protruders, frequently show an inspiratory modulation of their discharge. The source and the nature of the synaptic inputs that cause this modulation (i.e., whether providing inspiratory EPSPs, expiratory IPSPs, or both) remain unknown. To begin distinguishing among these possibilities, we have measured the changes in membrane resistance of XII Mns with respect to the respiratory cycle. In paralyzed, vagotomized and artificially ventilated cats, we recorded intracellularly from 8 Mns. The mean respiratory modulation of their membrane potential was 5.3 mV ± 2.1(SD). The difference in voltage responses to hyperpolarizing current pulses (1-2 nA) applied during mid-inspiration and late expiration were measured using a computerized data acquisition system. We observed inconsistent changes in membrane resistance, with an expiratory decrease in 4 and an increase in the remaining 4 Mns (mean: 0.3 Mohm ± 0.9(SD) decrease in expiration). We compared these results to the effects of lingual nerve stimulation, which evokes an amino acid-mediated IPSPs in XII Mns. The IPSPs (mean: 4.3 mV ± 2.5; 7 Mns) were consistently associated with a membrane resistance decrease (mean: 1.1 Mohm ± 0.5). These results suggest that a somatic, amino acid-mediated inhibition in expiration, if present, plays a minor role in shaping the membrane potential trajectory of inspiratory XII Mns. (Supported by HL-42236 & HL-47600.)

403.8

IDENTIFICATION OF LINGUAL MUSCLE INTERNEURONS USING PSEUDORABIES VIRUS.

R. Fay* & R. Norgren, Neuroscience Program & Department of Behavioral Science, College of Medicine, The Pennsylvania State University, Hershey, PA 17033.

The lingual musculature is involved in a variety of functions associated with ingestion, swallowing, respiration, and phonation. Movement of the tongue involves the complex coordination among three sets of muscles -- retractors, protruders, and intrinsic tongue muscles. In order to identify the interneuronal connections in the brainstem involved in the control of each of the tongue muscles, we injected pseudorabies virus (PrV) into the hyoglossus and styloglossus muscles (retractors), the genioglossus and geniohyoid muscles (protruders), and into the tongue itself in Sprague-Dawley rats. The animals were allowed to survive between 72 and 90 hours. Prior to viral injection, the superior cervical ganglia were removed bilaterally. In those animals receiving injections into the tongue, the chorda tympani and lingual root of the glossopharyngeal nerves also were sectioned ipsilaterally. Injection volumes ranged from 1-12 μL at an average titre of $5x10^8$ pfu/mL. Primary infections were restricted to the hypoglossal nucleus (Mo 12) in a predictable myotopic pattern. Secondary infections appeared in neurons known to project monosynaptically to Mo 12 including the subcoeruleus and supratrigeminal areas, the principal sensory trigeminal nucleus, and the pontine and medullary parvicellular reticular zones. Nevertheless, for each of the injected muscles, the pattern of labeling differed within these areas. Polysynaptic connections were revealed to the pedunculopontine, laterodorsal tegmental, oral pontine, gigantocellular, lateral paragigantocellular, rostroventrolateral, and paratrigeminal nuclei following the longest survival periods. Labeled neurons also were found within and surrounding the nucleus ambiguus and the nucleus of the solitary tract. Supported by PHS grants DC 00240 and MH 00653.

403.9

INDEPENDENT CIRCUITS INNERVATE PROTRUDER AND RETRACTOR MUSCLES OF THE TONGUE IN ADULT RAT.

E.G. Dobbins* & J.L. Feldman, Systems Neurobiology Lab., Dept. of Physiological Science, UCLA, Los Angeles, CA 90024-1527.

Muscles of the tongue are separated into **protruders** (genioglossus, geniohyoid) innervated by the medial branch of the hypoglossal (XII) nerve and **retractors** (hyloglossus, styloglossus, mylohyoid) innervated by the lateral branch. Protruders, stabilize the pharynx and maintain an open airway during inspiration; retractors aid in the initiation of swallowing. These functional differences are paralleled by segregation of motoneurons (Krammer et al., *Brain Res.* 170 ('79): 533). Innervation of protruder and retractor muscles was evaluated in locally deafferented, ganglionectomized adult rats by transneuronal transport of pseudorabies virus. Consistent with previous reports, injections into the medial (protruder) or lateral (retractor) branch of XII nerve labeled motoneurons in the ventral and dorsal quadrants of XII nucleus, respectively. Premotoneurons were in the tegmental field (TF) lateral to XII nucleus. Neurons premotor to retractor muscles were predominately dorsal to those premotor to protruder muscles. Dorsal neurons in the tegmental field extended from XII nucleus to spinal trigeminal nucleus. A few neurons were in the ventrolateral TF. In contrast, neurons afferent to the protruder motoneurons were concentrated in ventrolateral TF. Nuclei or regions associated with autonomic control (raphe magnus and obscurus, A5 region, Kölliker-Fuse, lateral parabrachial, locus coeruleus and subcoeruleus) project to both motoneuron populations. These results suggest that distinct circuitry underlies the functional differences in activity of tongue muscles. We are grateful to L.W. Enquist, Ph.D., Du Pont Merck Pharmaceutical Co., for his generous gift of PRV and antibody. Supported by NIH Grant NS24742.

403.10

A FUNCTIONAL MAPPING OF THE HYPOGLOSSAL NUCLEUS IN RAT.

E.E. Gilliam*, J.R. McClung, and S.J. Goldberg, Department of Anatomy, Medical College of Virginia-Virginia Commonwealth University, Richmond, VA 23298.

The rat's tongue, like man's, is composed of extrinsic and intrinsic muscles which function to protrude and retract the tongue. Anatomical studies have shown that the hypoglossal nucleus and nerve are basically "compartmentalized" into protrusive and retrusive subdivisions (nucleus) or branches (nerve). The medial branch of the nerve innervates the extrinsic genioglossus muscle and the lateral branch innervates the extrinsic styloglossus and hyoglossus muscles. Both branches innervate the intrinsic tongue muscles. This study was designed to physiologically examine the organization of the hypoglossal nucleus.

The lateral and medial branches of the hypoglossal nerve were stimulated separately. The resulting antidromic field potential was recorded in the ipsilateral hypoglossal nucleus (200 and 400 microns lateral to the midline and at various depths). Localized stimulation of those motoneurons proximal to the field was done with single and tetanic stimulation (20-170 Hz) using a 10 micron tip glass microelectrode. The protrusive and/or retrusive forces produced were measured using a sensitive force transducer attached by silk suture to the tip of the tongue. The geography of the protrusive and retrusive motoneuron areas could then be delineated within the XIIth nucleus.

Protrusive (genioglossus) forces result from stimulation of rostral and ventral areas of the nucleus with the medial branch of the nerve intact. Retrusive (hyoglossus and styloglossus) forces result from stimulation of rostral and dorsal areas of the nucleus with the lateral branch of the nerve intact. There is, however, significant overlap between these protrusive and retrusive extrinsic motoneuron areas.

Forces resulting from stimulation of intrinsic motoneuron areas were observed as generally weak retrusions with either the medial or lateral nerve branch intact. There was considerable overlap of these intrinsic areas of the nucleus, but this overlap was generally found caudally.

We are continuing to analyze the tension and speed related properties of all responses. [Supported by EY-07924]

403.11

NOREPINEHRINE (NE) ACTIONS ON HYPOGLOSSAL MOTONEURON (HM) FIRING BEHAVIOR AND THE PHARMACOLOGY OF THE RESPONSE.
M.A Parkis* D.A.Bayliss, and A.J.Berger. Department of Physiology and Biophysics, University of Washington, Seattle, WA 98195

Previously we showed that HMs in slices from rat brainstem responded to NE with depolarization, increased input resistance (R_N), lowered current threshold for minimum repetitive firing (I_{Thr}), and, in some cells, an increase in the slope of the relation between firing frequency and injected current (*Soc. Neurosci Abstracts*, 18:512, 1992). Here we further characterize NE's effect on HM firing behavior, and determine the adrenoceptor subtype mediating the response.

Bath-applied NE (100μM) caused an increase in the delay to the first spike in response to I_{Thr}, (avg. delay 2.9 msec in control, 79 msec in NE, n=10). This result suggests that NE may increase a depolarization-activated K^+-current similar to I_A. In 5 of 12 cells which showed firing frequency adaptation in response to a 1-second constant-current pulse in control, NE decreased or eliminated that adaptation.

Prazosin (10μM) attenuated the NE response in all cells tested (n=4), whereas propranolol (10μM) had no effect (n=4). Phenylephrine (10-20μM) mimicked the effects of NE on HMs, causing a reversible depolarization and an increase in R_N (n=8). Isoproterenol (20 or 100μM) produced no change in the membrane potential, R_N, or firing behavior of HMs (n=4). These findings indicate that NE acts on HMs via the α_1-adrenoceptor subtype. Because of the similarity of the responses of HMs to NE and to thyrotropin releasung hormone (TRH, *J. Neurophys.*, 68:1733-45), we investigated whether these agents share an intracellular mechanism of action in these cells. When TRH (1 or 5μM) was present in the bathing solution, the depolarizing response to focally-applied NE was either abolished (n=1) or greatly diminished (to an average of 24% of control, n=4). Upon washout of TRH, the response to NE returned to near control levels. These data suggest that the responses of HMs to TRH and NE share a common intracellular pathway. (Supported by HL-49657.)

403.12

THE MECHANISM MEDIATING THYROTROPIN-RELEASING HORMONE (TRH) EFFECTS ON MOTONEURONS INVOLVES G-PROTEINS BUT NOT PROTEIN KINASE C, IP_3 OR INTRACELLULAR CALCIUM. D.A. Bayliss*, F. Viana and A. J. Berger. Dept. Physiol. & Biophys., Univ. of Wash., Seattle 98195.

Effects of TRH on clonal pituitary cells are mediated by GTP-binding proteins (G-proteins), involve activation of protein kinase C (PKC) and increases in intracellular inositol trisphosphate (IP_3) and calcium (Ann NY Acad Sci 553:191, 1989). We used conventional intracellular recording in brainstem slices to test whether a similar mechanism mediates the TRH-induced depolarization and increase in input resistance (R_N) of hypoglossal motoneurons (HMs). TRH-induced depolarization recovered quickly (within ~8-10 min) and could be repeated with modest tachyphylaxis (↓ by ~20%) when HMs were impaled with electrodes containing 3 M KCl (n=12) or 30 mM GTP (in KCl; n=7). However, with GTPγS-containing electrodes (10 mM; n=4) the depolarization induced by TRH was long-lasting (up to 1 h), and with GDPβS-containing electrodes (20 mM; n=5) tachyphylaxis with repeated TRH application was exaggerated (↓ by ~60%). Phorbol dibutyrate (PdBu; 10 μM in perfusate), an activator of PKC, neither mimicked nor occluded the effects of TRH (n=3); in control experiments, as expected, 10 μM PdBu decreased a slow afterhyperpolarization (AHP) in CA1 hippocampal neurons. There was no effect on membrane potential, R_N or the response to TRH in HMs during long recordings with electrodes containing IP_3 at high concentration (60 mM; n=2). The effect of TRH was not blocked in HMs impaled with electrodes containing calcium chelators (0.1 M EGTA, n=3; 5 mM BAPTA , n=2) despite a nearly complete abolition of the calcium-dependent AHP. Together, these results indicate that G-proteins mediate the response of HMs to TRH but that the effects of TRH do not involve activation of PKC or increases in intracellular IP_3 and calcium concentration. Involvement of other intracellular signaling pathways (e.g. cAMP, arachidonic acid) remain to be tested. (Supported by NS14857 and Francis Families Foundation).

403.13

Calcium Currents and Calcium Channels in Visually Identified Hypoglossal Motoneurons (HMs). M. Umemiya and A.J. Berger.* Dept. Physiology and Biophysics, University of Washington, Seattle, WA 98115.

Whole-cell Ca currents and single Ca channel activities were recorded using patch clamp techniques in a thin-slice preparation of the medulla from neonatal rats (P1 to P5). HMs were identified visually by their location within the hypoglossal nucleus, and by their size and shape. Previously this laboratory has shown that neonatal HMs have both low-voltage activated (LVA) and high-voltage activated (HVA) Ca currents (Viana et al., *J. Neurophysiol.*, in press). The purpose of the present study was to characterize these currents at the single-channel level, and to identify components of whole-cell Ca currents.

Using the on-cell recording configuration, three channel types were identified by their single channel conductance. A 7-pS channel was activated at the lowest potential, around -50 mV. The largest channel (22 pS) activated at potentials positive to -5 mV and channels of intermediate conductance (14 pS) were activated around -10 mV.

In whole-cell recording, an LVA calcium current and at least three components of HVA calcium current were identified by their activation and pharmacological characteristics. The LVA Ca current began to be activated around -50 mV and was blocked by 100 μM Cd^{2+}. The peak amplitude of the HVA current was reduced 13±8.2% (mean±SD, n=20) with nifedipine or nimodipine, by 38±15.8% (n=23) with ω-CgTx, and by 45±15.6% (n=9) with ω-AgaTx-IVA. HVA Ca currents were abolished by 50 μM Cd^{2+}.

The results demonstrate that motoneurons from neonatal rats possess a diversity of Ca channel types, including a P-type and N-type channel that are responsible for a large proportion of the HVA current.
(Supported by NS 14857.)

403.14

CHARACTERISTICS AND DEVELOPMENT OF I_h IN RAT HYPOGLOSSAL MOTONEURONS. M.C. Bellingham*, D.A. Bayliss, F. Viana and A.J. Berger, Dept. Physiol. & Biophys., Univ. Washington, Seattle, WA 98195.

Adult rat hypoglossal motoneurons (HMs) recorded from brainstem slices exhibit a slowly activating inward current during prolonged membrane hyperpolarization. Using current and voltage clamp methods, we examined the characteristics of this current to determine if it is the hyperpolarization-activated cationic current (I_h) seen in other neurons. I_h of young adult (P21-65) rat HMs was measured as the difference between the current at the beginning and the end of a long (1-2 sec) negative voltage step from a holding potential of -55 to -60 mV. I_h showed a reversal potential of -38 ± 6 mV (mean ± SD, n = 5), estimated from the intersect of I/V plots from two different holding potentials, indicating the current was carried by a mixture of cations. Bath application of 0.5 or 2 mM Ba^{2+} reduced I_h at half activation by only 15-25% (n = 2) or 40 ± 16% (n = 3) respectively, whereas bath application of 3 mM Cs^+ reduced I_h by 89 ± 4 % (n = 3). These properties are characteristic of I_h in other neurons. Tail current analysis showed voltage-dependent activation of I_h, beginning at -65 to -75 mV and maximal at -95 to -110 mV, with half activation at -80 ± 2 mV (n = 10). Time constants (τ, single exponential fitting of current records) of I_h activation and deactivation showed voltage dependence. As more negative voltages were reached, activation proceeded more quickly (*i.e.*, activation τ decreased) and deactivation was slower (*i.e.*, deactivation τ increased). We also determined the time course for the development of I_h during the neonatal to young adult period. In adult HMs, I_h amplitude at half activation was -1.9 ± 0.6 nA (n = 15), while in neonatal (P2-8) HMs, it was -0.16 ± 0.11 nA (n = 7). As HMs only show a 4-fold increase in resting membrane conductance and doubling of total membrane area (Soc. Neurosci. Abstr., 18:1049) during the same period, the 10-fold increase in I_h is due, at least in part, to an increase in I_h current density during development. Supported by NS 14857, HL 49657 and Francis Family Foundation (D.A.B.).

403.15

AREA POSTREMA ACTIVITY STUDIED IN FERRET BRAIN SLICES. D.O. Carpenter, N. Hori and N.L. Strominger*. Wadsworth Center for Laboratories and Research, NY State Dept. of Health and School of Public Health, Albany, NY 12201 and Dept. of Anatomy, Albany Medical College, Albany, NY 12208.

Brain stem slices (450um), prepared from deeply anesthetized ferrets, were preincubated in oxygenated Krebs-Ringer and then mounted in a recording chamber submerged on a plexi mesh. Extracellular recordings of spontaneous activity of area postrema (AP) neurons were obtained for up to ten hours and responses to bath applied or ionophoresed agents were determined. Most neurons showed a low frequency spontaneous discharge. When β-estradiol was bath perfused at low concentrations (10^{-9}-10^{-8}M), activity increased, while at concentrations ≥10^{-7}M, it diminished. With ionophoresis of glutamate from an independent micropipette, a brief excitatory response was obtained. In contrast, ionophoresis of apomorphine resulted in very long latency, prolonged excitation. These observations are similar to those from in vivo studies in dogs. In some slices, injections of HRP or WGA-HRP were made in attempts to study local AP connections. Reciprocal relations were seen only between AP and the immediately adjacent solitary complex.

403.16

PERIAQUEDUCTAL GRAY (PAG) STIMULATION INCREASES C-FOS EXPRESSION IN SELECTED BRAIN STEM AREAS
P. Room, R. Dantuma and G. Holstege*. Dept. of Anatomy and Embryology, Faculty of Medicine, University of Groningen, Groningen, The Netherlands.

The midbrain periaqueductal gray (PAG) exerts a strong influence on somatic and autonomic motoneurons. Physiological studies have shown that stimulation in the PAG results in motor activities such as defense behavior, vocalization and locomotion. The main projection from the PAG is to the ventromedial tegmentum of caudal pons and medulla (Progr. in Brain Res. 87, p307, 1991). We used *c-fos* immunoreactivity as a marker of neural activity to determine the pattern of activated neurons in the lower parts of the brain stem following stimulation in different parts of the PAG. Rats were anaesthetized (urethane 1- 1.5 g/kg) and the PAG was electrically stimulated for 45 minutes (100 μA, 80 Hz, 1 sec on/9 sec off). During stimulation EMG's, heart rate and blood pressure were monitored. In rats which were surgically prepared for brain stimulation but not stimulated, small numbers of *c-fos*+ cells were observed in the cuneiform nucleus, the nucleus raphe pallidus and raphe magnus, the ventrolateral medulla and the solitary tract nucleus. Following stimulation in the PAG, the number of *c-fos*+ cells was much higher in these areas and *c-fos*+ cells were also observed in the nucleus raphe dorsalis, raphe medianus and raphe pontis, the locus coeruleus, Barrington's nucleus and in the nucleus retroambiguus. We conclude that stimulation in the PAG increased *c-fos* expression in selected parts of the brainstem which in part corresponds with those previously shown in tracing studies.

403.17

THE VENTROLATERAL TEGMENTAL NUCLEUS IN THE RAT: ANALYSIS OF THE AFFERENT AND EFFERENT CONNECTIONS. A. Klepper* and H. Herbert, University of Tübingen, Dept. Animal Physiology, 72076 Tübingen, Germany.

The ventrolateral tegmental nucleus (VLTg), a subdivision of the oral pontine reticular nucleus, is discussed as a part of a neuronal circuit that mediates acoustically elicited behavior. In order to shed light on the potential role of the VLTg as a sensorimotor interface, we analyzed the afferent and efferent connections of this reticular nucleus by using the retrograde fluorescent tracer Fluoro-Gold and the anterograde tracer *Phaseolus vulgaris*-leucoagglutinin, respectively.

The VLTg is reciprocally connected with various nuclei along the neuraxis, including the zona incerta, the superior colliculus, the central gray, and various mesencephalic, pontine, and medullary reticular nuclei. Moreover, the VLTg receives a prominent input from various auditory nuclei: the cochlear root nucleus, the dorsal and ventral cochlear nuclei, the external cortex of the inferior colliculus, and the secondary auditory cortex. Furthermore, the VLTg projects to facial motoneurons which innervate the pinna muscles.

In summary, the VLTg receives projections from a variety of brain areas including prominent inputs from auditory nuclei and, in turn, projects to reticular nuclei containing premotor neurons, as well as to the facial nucleus. We therefore conclude that the VLTg integrates sensory information and participates in mediating audiomotor behavior like the pinna reflex and the acoustic startle response.

Supported by DFG (SFB 307) and Graduiertenkolleg Neurobiologie

403.18

ANOXIC RESPONSE OF MEDULLARY PARAPYRAMIDAL NEURONS. M. Patil, D. Durand, and M.A. Haxhiu.* Applied Neural Control Lab., Dept. of Biomedical Engineering, and Dept. of Medicine, Case Western Reserve University, Cleveland, OH - 44106.

Parapyramidal neurons, located at the ventral surface of the medulla and lateral to the pyramids, have been identified as higher-order neurons within the baroreceptors and chemoreceptor reflex pathway. This identification was based on their c-*fos* protooncogene expression (Erickson & Millhorn, 1991). The present study is being conducted to investigate the electrophysiological effects of anoxia on these parapyramidal neurons. Neonatal (7 to 20 days) and adult rats were used to obtain medullary slices, which were dissected and the ventral half was used. Intra-cellular recordings were obtained *in vitro* from four parapyramidal cells in control conditions, 95% O_2 and after exposure to 95% N_2, in a bicorbonate buffer. Anoxic response was observed within a few seconds after exposure to nitrogen. Depolarization of 10 to 20 mV was observed in both, adult and neonatal cells after the first anoxic insult which lasted for 5 -7 mins. In neonatal cells, which were found to fire spontaneously at a rate of about 3 spikes/sec, anoxia abolished all spontaneous activity. However, electrical stimulus could generate action potentials, whose shape or size did not appear to change significantly compared to control. In comparision, spontaneous activity was absent in the adult neuron impaled. Anoxia decreased the somatic firing threshold. A transient decrease in the input resistance was also observed in anoxia. Complete recovery was obtained by switching to 95% O_2 in both neonates (within 5mins) and adults (within 10 mins). Subsequent anoxic insults did not produce a significant changes in the resting potential. Neonatal cells survived in anoxia for >45 mins, whereas in adults, survival was up to 30 mins. Preliminary results indicate that anoxia produces depolarization and abolishes spontaneous activity.

Supported by NIH Grant # HL 25830

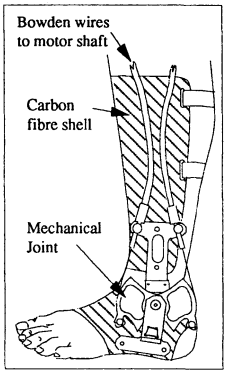
CONTROL OF POSTURE AND MOVEMENT VIII

404.1

A METHOD TO APPLY AN EXTERNAL PERTURBATION TO THE HUMAN ANKLE JOINT DURING ANY POINT OF THE GAIT CYCLE. T. Sinkjær* and J. B. Andersen Dept. of Medical Informatics and Image Analysis, University of Aalborg, Fr. Bajersvej 7D, 9220 Aalborg SO, Denmark.

Previous investigations based on the H-reflex have shown that the short latency spinal reflexes of the human ankle extensors are highly modulated during gait. The H-reflex study is a relatively easy method to achieve results from the spinal reflex system. However, it is only possible to probe central factors since the electrical stimulation bypass the muscle and the fusimotor system, which might contribute to the reflex modulation.

A system able to apply a stretch reflex perturbation to the muscles around the human ankle during gait were developed. The two link system consists of a mechanical joint, strapped to the shin and the foot of the subjects (See Figure). The mechanical joint is revolving around the ankle joint and by means of bowden wires it is connected to a motor placed next to a treadmill where the subject is walking. By a position feedback from the mechanical joint the motor is regulated in such a way that it follows the movement of the ankle without influencing the gait pattern. When wanted, the system is designed to impose an ankle rotation with a displacement of up to 20°, a speed of 150 °/s and a torque of up to 200 Nm during any time of the gait cycle. The weight of the total system attached to the leg of the subject is 0,9 kg.



404.2

KINEMATIC CHANGES AFTER UNILATERAL ANTERIOR CRUCIATE LIGAMENT TRANSECTION IN THE DOG: COMPENSATORY RESPONSES OF THE CNS. J.A. Vilensky*, B.L. O'Connor and K.D. Brandt. Depts. of Anatomy and Medicine (Rheumatology), Ind. Univ. Sch. of Med., Ft. Wayne and Indianapolis, IN 46805/46202

Presumably, subsequent to anterior cruciate ligament transection (ACLT), the CNS uses sensations of pain and/or instability to modify movements to preserve ambulation while protecting the unstable joint from the rapid degeneration that occurs in dogs whose limb is deafferented prior to ACLT (1). This study characterizes the kinematic changes after ACLT. Six dogs underwent left ACLT and were filmed before and 1-26 weeks after ACLT. The dogs showed reduced left knee and ankle joint flexion during stance (less "yielding"), but flexion of the right knee and ankle was increased. Additionally, the left hip was more extended during stance. The forelimb joints showed only slight changes. The ACLT dogs also exhibited greatly increased vertical movements of the sacrum. The CNS apparently partially compensates for knee instability by reducing the stance phase movements of the ipsilateral ankle and knee and increasing those of corresponding contralateral joints, causing increased vertical body movements (limping). The similar responses of the ankle and knee of each side (decreased flexion ipsilaterally and increased contralaterally) suggest biomechanical and/or neurological linkages in the actions of these joints. 1) J Bone Jt Surg 67A:562, 1985 (Supported by PHS P60AR20582).

404.3

1STREPTOMYCIN, VESTIBULOTOXICITY IN PIGMENTED RATS. [1]G. Meza*, [2]N. Daunton, [1]L. López-Griego and [3]M. Salas. [1]Dept. Neurociencias, IFIC, UNAM. Apdo. Postal 70-253, 04510 México, D.F. MEXICO.

To investigate the vestibular target organ of streptomycin (STP), postrotatory nystagmus (PN), righting reflex (RR) and swimming behavior (SB) were studied in STP-300-500 mg/kg -i.m- daily-long period-treated pigmented rats. Audition alteration was followed by auditory evoked brainstem potential (AEP) evaluation. Regardless of treatment length and dose, AEP remained same as controls. PN response was slightly affected only at 500 mg/kg and at the longest timespan. In contrast, even at 300 mg/kg STP, RR and SB were deeply affected, since the time of righting and resurfacing was considerably higher than controls. These results corroborate STP vestibulotoxicity and strongly suggest a specific otolithic organ STP action never reported before in the rat now being checked by other methodology.

Financed partly by DGPA-UNAM (México) IN201791 to G.M. [2]N.D. is at Ames Research Center (NASA) and [3]M.S. at the I.I. Biomédicas (UNAM, México).

404.4

IDIOPATHIC MIRROR MOVEMENTS. L.M.Harrison, M.J.Mayston, J.Gibbs and J.A.Stephens.*Dept. of Physiology, University College, London. WC1E 6BT.

Intense mirror movements are not usually seen in normal adults. We have investigated the neurophysiology of such movements in a 15 year old male with no dysmorphic features. Surface EMG was recorded from the first dorsal interosseous muscle (1DI) during repetitive index finger abduction. EMG commenced at the same time in the 1DI of both the voluntarily abducted finger and the mirroring finger. To investigate whether this simultaneous EMG is due to a common drive to L and R motoneuron pools, cross-correlation analysis of multiunit EMG from co-contracting L and R muscle pairs (1DI, forearm extensors (Fext), triceps and deltoid) was performed. All correlograms exhibited a short duration central peak centred around time zero. The size of this peak showed a distal to proximal gradient, being larger for distal muscle pairs. No central peak was present in correlograms constructed from data recorded from normal 5-6 yr olds with moderate mirroring. Focal magnetic brain stimulation of the L and then the R motor cortex of our subject revealed bilateral responses in 1DI and Fext; the contralateral response was larger than the novel ipsilateral response. Cutaneo-muscular reflexes were recorded from L and R 1DI whilst stimulating the digital nerves of the L and then the R index finger. The reflex E2 component, which is dependent upon the integrity of the pyramidal tract, was recorded contralaterally in addition to the normal ipsilaterally recorded response. Taken together, the results indicate the presence of pyramidal tract fibres that have branched and innervated homologous L and R motoneuron pools.

404.5

TRAJECTORY FORMATION AND INTERJOINT COORDINATION OF DRAWING MOVEMENTS IN NORMAL AND HEMIPARETIC SUBJECTS. M.F. Levin*, M. Horowitz, J. Jurrius, C. Lamothe, A.G. Feldman, Research Centre, Rehabilitation Institute of Montreal, Montreal, Quebec, Canada. H3S 2J4

Movements can be organized at the interjoint level and in terms of extrapersonal space (trajectory planning). We investigated how interjoint coordination is related to trajectory formation. Arm movements were studied in 7 normal and 7 hemiparetic subjects. Data from affected arms of hemiparetic subjects were compared to those from their non-affected arms and to data from normal subjects. Subjects were seated in front of a horizontal surface adjusted to the height of the sternal notch with the trunk immobilized to the back of the chair. In one series of experiments, subjects made planar arm reaching movements (20 and 40 cm) to 4 different targets inlaid on the table. In the other, subjects traced 40 cm diameter circles on the plane with their index finger. Kinematic and electromyographic data from the finger, wrist, elbow and shoulder were recorded with a 3D optical tracking system. Results showed that movement amplitudes were lower and movement times were longer in the affected arms. Trajectories were marked by deviations from smooth lines and characterized by segmentation, hysteresis and abrupt changes in tangential velocity. The degree of disruption of interjoint coordination between the elbow and shoulder was related to spasticity and functional activity scores. Results of these studies may have implications for the rehabilitation of hemiparetic patients.

404.6

THE INFLUENCE OF ATTENTION ON POSTURAL CONTROL W.E.McIlroy* and B.E.Maki, Sunnybrook Health Science Centre, University of Toronto, Toronto, Ontario, CANADA. M4N 3M5

This study addresses the potential relationship between attention and the control of posture in young adults using a dual-task paradigm. Postural responses were measured using EMG's and ground reaction forces. To address concerns about confounding due to arousal, we monitored skin conductance and assessed trait and state anxiety, using S-R GTA and PARQ questionnaires. A first study quantified postural responses during unperturbed stance in 41 subjects while they performed either: 1) no secondary task, or 2) a cognitive task (backward counting by 7's). Results revealed a significant increase in mean anterior-posterior center-of-pressure displacement when performing the secondary task. In the second experiment, subjects tracked either an auditory or visual signal. Use of a continuous, quantifiable secondary task allowed us to document occurrences of attention switching. Significant differences in the performance of the postural tasks (one-foot standing, pseudorandom platform perturbation) were measured within individual subjects as changes in frequency and variability of postural responses. Furthermore, performance on the tracking task was seen to decrease with increasing postural challenge. This appeared to be due, in part, to transient pauses in the tracking behavior associated with brief periods of relative postural instability, possibly revealing transient shifts in allocation of attentional resources. The present results confirm the potential importance of attention in the determination of postural responses, and suggest that use of continuous and quantifiable secondary tasks, such as tracking, may help to reveal details of the dynamic attentional requirements of maintaining upright stance.

404.7

THE EFFECT OF COGNITIVE DEMANDS ON POSTURAL CONTROL IN ELDERLY FALLERS AND NON-FALLERS. A. Shumway-Cook*, M. Baldwin, K. Kerns, M.Woollacott, Northwest Hospital, Seattle, Wa 98133

This study compared attentional requirements of stance postural control in young vs elderly adults. A dual task design measured the effects of cognitive tasks (Judgement of Line Orientation and Sentence Completion) on postural sway in 20 young, 22 elderly non-fallers, and 22 elderly fallers. A forceplate measured center of gravity while subjects stood for 30 seconds on a firm or compliant surface. Repeated-measures was used to analyze change in sway path (mm).

There was a significant main effect of cognitive tasks, and a task x group interaction. In young controls both tasks affected sway on the compliant but not the firm surface. In elderly non-fallers both tasks affected sway on the firm surface but not the compliant surface. In elderly fallers cognitive tasks affected sway on both firm and compliant surfaces.

Results suggest that competing demands for attentional resources by cognitive and postural systems may contribute to instability in the elderly.

404.8

USE OF FUNCTIONAL ELECTRICAL STIMULATION TO ENHANCE PARALYZED CAT HINDLIMB MUSCLES. J. Mao, N. Tyreman, R.B. Stein*, T. Gordon, Div. of Neuroscience, Univ. of Alberta, Edmonton, CANADA T6G 2S2.

An optimal regime is to be established for using functional electrical stimulation (FES) to enhance muscle strength and endurance. FES was applied to cat hindlimb muscles under different conditions. In 8 free-moving cats, the (non-paralyzed) medial gastrocnemius (MG) muscles were stimulated at 20 Hz and 50% of daily time through cuff electrodes implanted around the MG nerves. Once or twice per week the MG muscles were recorded for their forces, speeds, and endurance. These chronic recordings show that fatigue resistance and contraction time both increased while the maximum tetanic force decreased. In 3 free-moving cats which were hemispinalized and (ipsilaterally) deafferented, the paralyzed MG muscles were stimulated with different patterns (20 Hz or 100 Hz, 5% or 50% of daily time). The fatigue resistance increased with both 20 Hz/50% and 100 Hz/5% stimulation but not with 20 Hz/5% stimulation. Still, the maximum tetanic force decreased. In order to prevent the decay in muscle force while maintaining the increased endurance, a metal boot was made for fixing the muscle length and providing resistance while the muscle was stimulated to contract. For 2 hrs/day and 5 days/week, the metal boot fixed the paralyzed hindlimb of one cat so that its tibialis anterior (TA) muscle was lengthened and its MG shortened. During this period 20 Hz stimuli (2 s on and 2 s off) were applied alternately to the paralyzed TA and MG muscles each contracting against the resistance. Initial data of long-term recordings show that the maximum tetanic force of the lengthened TA muscle was not decaying while its fatigue resistance gradually increased. In contrast, the muscle force and endurance of the shortened MG did not increase significantly. These results suggest that muscle length and contraction against resistance should be considered in using FES to enhance paralyzed muscles. (Supported by MRC and NCE of Canada).

404.9

LIMB TRAJECTORY IN NON-DISABLED SUBJECTS UNDER TWO CONDITIONS OF EXTERNAL CONSTRAINT COMPARED WITH THE NON-PARETIC LIMB OF SUBJECTS WITH HEMIPARESIS

CA Giuliani*, PA Genova, JL Purser, KE Light, Motion Analysis Lab., University of North Carolina at Chapel Hill, Chapel Hill, NC 27499-7153

The purpose of this study was to test our hypothesis that weakness and difficulty constraining degrees of freedom were elements underlying arm control deficits in subjects with unilateral brain lesion. To test this hypothesis 8 non-disabled (ND) subjects and 8 subjects with right CVA were instructed to tap vertically on a Fitts board as fast as possible for 10 seconds using a hand-held stylus. All subjects tapped with their right dominant limb. ND subjects performed this task under two additional conditions of wrist immobilization and fatigue (a mass of 6 % body weight attached to the limb). All trials were videotaped and the stylus trajectory was digitized at 60 Hz. Variables chosen for comparison that quantify phase plane characteristics were: up/down velocity ratio, timing ratio of peak velocity, peak amplitude phase, cycle frequency, and the ratio of amplitude/velocity slopes. Significant differences ($p = < 05$) were found between free and fatigued conditions for all variables and no significant differences were found between the free and immobilized conditions for ND subjects. No significant differences were found between ND subjects in the fatigue condition and right CVA subjects for all variables except cycle frequency and slope ratio. That there was no effect of immobilization suggests that ND subjects were using a predominant elbow strategy. Fatigue in ND subjects appeared to alter the dynamics of an otherwise stable motor control system. The similarity between the non-paretic limb of subjects with right CVA and the ND subjects during the fatigue condition provides support for the hypothesis that weakness contributes to deficits in motor control in the non-paretic limb of subjects with unilateral brain lesion.

Supported in part by a grant from the Foundation for Physical Therapy.

404.10

SIGNIFICANT REDUCTIONS IN UPPER LIMB SPASTICITY IN HEMIPARETIC STROKE SUBJECTS USING CUTANEOUS LEVELS OF ELECTRICAL STIMULATION. I.P.A. Dewald*, J.D. Given, D. Yamada and W.Z. Rymer, Sensory Motor Performance Program, Rehab. Institute of Chicago, and Dept. of Physical Medicine and Rehabilitation, Northwestern University Medical School, Chicago, IL.

In order to investigate the effect of different electrical stimulation protocols on spasticity in a hemiparetic test population (n=7), pre-stimulation torque responses of the impaired upper limb were measured during slow ramp perturbations of the elbow and compared with torque responses obtained immediately following stimulation over the antagonistic muscle. The subject's arm was positioned in 120° of elbow extension or 60° of elbow flexion depending on whether the stiffness in flexors or extensors was estimated (180° is designated as full extension). The arm was moved through an arc of one radian (57.3 degrees) at a constant angular velocity, which was determined by the level of spasticity in the arm. In addition, EMG signals of biceps, brachioradialis, and triceps muscles were collected. The electrical stimulation was applied to skin over the biceps muscle for a period of ten minutes at a 20 Hz frequency, pulse duration 0.1 ms, with an intensity level below motor threshold, but above sensory threshold. The joint extensions and/or flexions were performed immediately after electrical stimulation, and subsequently at several intervals up to 30 minutes after cessation of the stimulation. In some cases, subjects were again given electrical stimulation, but at a level which was just above motor threshold.

In all subjects, cutaneous stimulation over the elbow flexors at submotor levels significantly reduced the torque in both elbow flexors and extensors, while stimulation slightly above motor threshold resulted in significant reductions in extensor torques, but significant increases in flexor torques. These effects remained statistically significant for at least 30 minutes following stimulation although the positive effects diminished with time.

The observed reductions in spasticity (by as much as two-thirds of pre-stimulation peak torque levels) were determined to be due mostly to changes in stretch reflex threshold as opposed to gain. These reductions in spasticity hold significance not only by avoiding side effects of drugs such as Dantrolene (i.e., generalized weakness), but also because the most efficacious level of stimulation is the best tolerated.

This work is supported by Washington Square Foundation Grant No. 357

404.11

USE OF A SERVO MOTOR DEVICE TO QUANTIFY SPASTIC PLANTARFLEXOR MUSCLE CHARACTERISTICS. L.D. Abraham*, S.C. Allison, P.A. Anderla and C. Stanford. Kinesiology & Health Education and Institute for Neuroscience, University of Texas, Austin, TX 78712 and Healthcare Rehabilitation Center, Austin, TX 78745.

Objective measurement of spasticity in the ankle plantarflexors is important clinically, given the devastating effect of plantarflexor spasticity on gait and functional mobility in patients with upper motor neuron lesions. We have developed a torque motor device to provide mechanical perturbations to the ankle joint and to measure kinetic, kinematic and physiological responses. The magnetic servo motor receives command strings relayed from a desktop computer, which are processed through the servo controller using a digital PID control algorithm. This system allows for independent control of angular velocity, angular acceleration and angular displacement. The apparatus allows fine adjustment of motor position for alignment with the ankle rotational axis. Patient safety is assured through three safeguards: (1) software positional limits to prevent motion beyond safe ranges, (2) adjustable mechanical stops to prevent unsafe motion and (3) limited (30 N-m) maximum torque-generating capacity of the motor.

The system is designed to produce mechanical perturbations concurrent with procedures such as tendon vibration, H-reflex testing and voluntary muscle activation. LabView (National Instruments, Inc.) software was used to create a customized virtual instrument which controls the servo motor device and also collects the analog data. Using the computer for both control and data collection provides flexibility and allows for a wide variety of measures, both direct and derived. We are currently using the system to measure reflex threshold angles and effects of tendon vibration or antagonist muscle activation on maximum H-reflex amplitudes. The aims of this testing are to reveal potential neurophysiological mechanisms underlying spasticity and eventually to develop a quantitative spasticity measurement scale.

404.12

SENSORIMOTOR GATING IN BOYS WITH TOURETTE'S SYNDROME (TS) AND ATTENTION DEFICIT HYPERACTIVITY DISORDER (ADHD). F.X.Castellanos, E.J.Fine, D.L.Kaysen, P.L.Kozuch, S.D.Hamburger, J.L.Rapoport*, and M.Hallett Child Psychiatry Branch, NIMH, Bethesda MD 20892.

Gating deficits have been ascribed to corticostriatal dopaminergic dysfunction in schizophrenia and obsessive compulsive disorder. Utilizing supraorbital nerve electrical stimulation, we conditioned the blink reflex with a sensory threshold stimulus at baseline, and 30, 60, 90, 120 and 250 ms before an adequate stimulus elicited a blink reflex. At 90 and 120 ms, seven TS + ADHD boys exhibited significantly reduced inhibition of the blink reflex ($p<.003$, and $p<.01$, respectively) compared to 9 age-sex matched, normal controls. There were no statistical differences at intervals of 30, 60 or 250 ms. We have demonstrated the feasibility of this technique in pediatric patients. These data extend Smith & Lees (1989) finding of abnormal blink recovery in TS, suggesting a disturbance of basal ganglia input to brainstem interneurons.

CONTROL OF POSTURE AND MOVEMENT IX

405.1

DISCHARGE PATTERN OF MOTOR UNITS (MUs) DURING MOTOR PREPARATION FOR A TASK PERFORMED AGAINST LOADS. L. Rispal-Padel* and S. Mellah. C. R. "Cerveau et Cognition", CNRS, Faculté de Médecine, Toulouse, France.

Slow biceps MUs become active during the motor preparation for a forearm flexion movement (Mellah et al., 1990). To determine whether this activity is correlated with the forthcoming muscular force to be developed we examined the patterns of activity of MUs during the preparation for and execution of a flexion movement. The monkey had to overcome static loads of various weights during the movement execution but during the preparatory period, an abutment was placed behind the cradle supporting the forearm to compensate for the load.

The discharge frequency of the "slow tonic" MUs which were actived during the preparatory period under normal conditions increased during this period when heavier loads were to be overcome during the forthcoming movement. Changes occurred in the frequency as well as the duration of the preparatory discharge pattern. At very high loads when the discharge frequency was at its maximum, the discharge onset continued to occur more and more early on.

Under anisometric conditions, the discharge frequency plays an important part in the force grading processes. When the movement is preceded by a preparatory period, however, the temporal component of the discharge constitues an additional mechanism which seems to be of importance mainly when the load to be overcome is heavy.

405.2

MOTOR UNIT ACTIVITY DURING HUMAN ARM MOVEMENTS AND MATCHED ISOMETRIC CONTRACTIONS. **S.J. Garland*, T. Ivanova, and K.J. Miller. Dept. of Physical Therapy, University of Western Ontario, London, Ontario, Canada, N6G 1H1**

Most of the studies of human motor unit behavior have been performed using isometric contractions. However, most daily activities executed by humans require movement. The purpose of this study was to determine whether the motor unit activity would be comparable between the conditions of movement and isometric contractions. Surface EMG recordings were obtained from biceps and triceps brachii muscles. Motor unit activity was recorded from the lateral head of the triceps brachii muscle using a subcutaneous fine-wire electrode. Subjects performed 100 alternating flexion and extension elbow movements (50^{o}-130^{o}) in a horizontal plane. Utilizing phase-plane tracking, the arm movements had pre-determined acceleration and deceleration characteristics. After the movements were completed, the wrist was secured onto an isometric force transducer that kept the arm in the horizontal plane. The acceleration and deceleration profiles from the movements could be reproduced in the isometric setting by presenting the kinematic profiles as force pulse templates. Following adjustment of the amplitude of the voluntary isometric contractions of elbow flexor and extensor muscles, the surface EMG activity was equivalent in both the movement and the isometric paradigm. This ensured that the muscular tension produced in both paradigms was comparable. The same motor units could be identified in both paradigms. Thus, these motor units were not "task-dependent". It may be possible to make inferences regarding movement based on isometric paradigms given the appropriate contraction parameters.

Supported by NSERC (Canada).

405.3

INTERACTIONS OF MULTIPLE UNITARY EMG POTENTIALS AT DIFFERENT LEVELS OF MOTOR DRIVE USING AN ACUTE EXPERIMENTAL SIMULATION. S. Day, P. Sjolander, M. Hulliger and G. Melvill-Jones* Dept. of Clinical Neurosciences, Univ. of Calgary, Canada T2N 4N1.

The EMG is a complex interference signal, composed of the unitary action potentials contributed by each active motor unit. As the level of motor drive is increased, by recruitment and/or rate modulation, the contribution of each single unit may change.

An acute cat soleus muscle preparation was used to study the isometric EMG - force relationship, including an analysis of unit EMG contributions to whole muscle EMG. The ventral roots containing soleus motor units were divided into 10 filament units (FU) each unit containing 10-20 motor units (MU). Together, using a recruitment and rate modulation strategy imitating the size principal, these FU were stimulated over the entire physiologic range of soleus MU firing rate using pseudo-random pulse trains with a degree of variability, to simulate normal muscle activation and avoid the problems associated with synchronization. To assess the importance of the level of motor drive on the characteristics of the individual FU muscle membrane potentials recorded by surface and indwelling electrodes, each ventral root filament was stimulated individually and together (combined) with the other filaments. For selected FU's, a spike triggered averaging (STA) technique was used to differentiate the waveform of the FU from an aggregate signal composed of a number of FU's.

The EMG recorded during the stimulation of each individual filament was algebraically summed and the results were compared to the EMG trace of the combined stimulation trial. The results are qualitatively similar, suggesting largely linear summation of EMG potentials, and also an absence of any significant changes in the characteristics of individual muscle membrane potentials, as the level of motor drive increases. Indeed, preliminary evidence from STA data of individual FU's suggests that no systematic changes occur in the shape of the muscle membrane potentials with increased motor drive. This indicates that, as muscle activation increases, any changes in whole muscle impedance are small enough not to alter the individual FU contributions to the EMG, and it suggests that linear summation of single MU potentials for EMG modelling is, after all, justified. Supported by AHMRF & MRC (Canada)

405.4

POSSIBLE RECEPTOR MECHANISMS RESPONSIBLE FOR THE GAIN COMPRESSION OF GOLGI TENDON ORGAN RESPONSE TO FORCE. P. Sjölander, M. Hulliger, Y. Coiton, U.R. Windhorst* and E. Otten[1]. Dept. of Clinical Neurosciences, Univ. of Calgary, Canada T2N 4N1 and Dept. of Medical Physiology[1], Univ. of Groningen, NL-9712 KZ Groningen.

The discharge rate of Ib afferents is known to saturate at relatively small muscle forces. This has led to the suggestion that they are unlikely to provide a reliable estimate of whole muscle force. However, in recent experiments we have demonstrated that staggered stimulation (imitating recruitment) of soleus motor axons produces discharge rates of Ib afferents which increase monotonously, if non-linearly, with muscle force. The present study investigated encoder and transducer properties of the Ib receptors which may contribute to the saturation and summation effects previously demonstrated.

Responses of single Ib's from the soleus muscle were recorded in dorsal root filaments of anaesthetized cats. The L7 and S1 ventral roots were divided into a number of filaments containing soleus motor axons. The tetanic force elicited by each bundle varied between 2 and 25% of the maximum tetanic force. Pairs of filaments which individually excited a Ib afferent were activated under isometric conditions, using a ramp-shaped (5-25/s) stimulation for one and rectangular-shaped patterns (12, 17, 30/s) for the other, either alone or in combination.

Comparisons of the effects of individual and combined filament stimulation showed: 1) for most filament pairs pronounced sub-linear summation of Ib biasing was found; 2) occasionally complete absence of summation (occlusion) was observed; 3) the degree of summation on a given Ib afferent varied considerably between different filament pairs; 4) inverting the stimulation patterns between the filaments a pair could alter the extent of summation significantly; 5) neither the absence of summation nor the degree of summation was related to the force produced by the filaments.

While complete occlusion of single filament biassing effects would be compatible with competition between parallel encoding sites on myelinated terminal branch trees, the variation of the degree of partial occlusion upon inversion of inputs is not, and might arise from non-linear compartmentalized receptor terminal activation.

Supported by AHFMR and MRC (Canada)

405.5

FORCE CODING BY A POPULATION OF CAT GOLGI TENDON ORGAN AFFERENTS: THE ROLE OF MUSCLE ACTIVATION PATTERNS. M. Hulliger, Y. Coiton, P. Sjölander, U.R. Windhorst, E. Otten[1] and T.E. Feasby*. Dept. of Clinical Neurosciences, Univ. of Calgary, Canada T2N 4N1 and Dept. of Medical Physiology[1], Univ. of Groningen, NL-9712 KZ Groningen.

While firing rates of single Golgi tendon organ (GTO) afferents often poorly reflect whole muscle force and even the force generated by the motor units (MU) which excite them, it has long been postulated that ensemble responses might monitor whole muscle force more reliably. This was confirmed for various forms of distributed or temporally dispersed activation of large fractions of the cat soleus MU pool (Soc. Neurosci., Abstr. 18, 1407, 1992): the population response-whole muscle force relations were monotonous, yet revealed different degrees of gain compression non-linearity, with the least non-linear relation resulting from a combination of staggered activation (of subsets of MUs) and rate modulation. The present study was undertaken to determine which of the three, recruitment, rate-modulation, or size of biasing effect on GTOs, were most influential in linearizing the input-output characteristics of the GTO population response.

Subsets of MUs were activated through 8 ventral root filaments of widely ranging size (2-25% of maximum soleus force), using staggered activation (imitating recruitment), distributed stimulation (for pure rate modulation), or a combination of the two.

Least significant was the size of biasing effects elicited by individual filaments, since inversion of filament rank order had minimal effects on the GTO firing vs force relation. Pure rate modulation tended to enhance, while staggered activation reduced gain compression non-linearity. This suggests that temporally dispersed activation (recruitment) of MUs was most significant in linearizing the population response-force relation and in extending the range of operation (in terms of resolving force input) of ensembles of GTO afferents.

Supported by AHFMR and MRC (Canada).

405.6

DYNAMICS OF MUSCLE SPINDLE PRIMARY ENDINGS: MECHANICS AND IONICS. E. Otten, M. Hulliger* and K.A. Scheepstra. Dept. of Medical Physiology, Univ. of Groningen, NL-9712 KZ Groningen, and Dept. of Clinical Neurosciences*, Univ. of Calgary, Canada T2N 4N1.

Modelling the dynamics of spindle primary endings mainly on mechanical events, Schaafsma et al. (J. Neurophys. 65, 1297-1312, 1991) had to infer exaggerated dynamic properties of intrafusal muscle fibres. It is very likely however, that a considerable fraction of the spindle dynamics is of ionic origin. In order to see whether dynamics can arise from specific ionic currents activated by deformation of the ending, a mathematical model of a mechanoreceptor terminal was formulated.

The kernel of this model consists of the Frankenhaeuser-Huxley (FH) equations (describing the generation of action potentials in myelinated axons). Two modifications were implemented: first, the four permeability constants (K^+, Na^+, non-specific and leak currents) were changed simultaneously (using automated tuning), to introduce low-rate repetitive firing capability (down to 15/s), while minimizing the deviations from the original FH values. Second, a slow repolarizing (potassium) conductance was added, to model properties of longer duration (e.g. accommodation) than those required for individual action potentials.

The ionic model reproduced several functional features of primary endings, such as band-pass filter properties with phase advance below and phase lag above 12 Hz and a peak in gain around 20 Hz. Unlike the mechanical model of Schaafsma et al., its dynamics are moderate, comparable to those of spindle secondary endings. However, when the two models were integrated and the ionic (sensory ending) component was activated by the output of the mechanical model component (length variation of the ending), exaggerated dynamics emerged. The mechanical component was therefore retuned, with the result that the dynamic properties of intrafusal fibers were less overrated, i.e. much closer to those of extrafusal fibres.

Experiments are currently under way to determine quantitatively the relative contribution of ionic and mechanical processes to the dynamics and overall behaviour of muscle spindle primary afferents.

Supported by NWO (The Netherlands) and AHFMR, MRC (Canada).

405.7

PROPRIOCEPTIVE TUNING CURVES L.A. Jones*[1] and I.W. Hunter[2], School of Physical & Occupational Therapy[1] and Dept. Biomedical Eng.,[2], McGill University, 3654 Drummond St., Montreal, Canada H3G 1Y5.

In both the tactile and auditory sensory modalities, the ability to detect a particular amplitude of vibration depends on the frequency of the stimulus being presented. The relation between stimulus frequency and the smallest amplitude that is detected at a given frequency is known as a tuning curve. A comparison of these curves for audition and touch reveals that the amplitudes for detection are much higher for the skin than for the ear, and that the auditory system can detect very small movements of the eardrum over a much larger range of frequencies. The tactile system is, however, good at detecting changes in amplitude at low frequencies.

The objective of the present experiment was to determine the tuning curve for the proprioceptive sensory modality using a procedure similar to that employed in audition and touch. Subjects were seated in an apparatus with each forearm coupled to a linear motor that was under computer control. Movements of the same frequency but varying in amplitude were imposed on each arm, and subjects were required to indicate which vibratory stimulus had the larger amplitude. A transformed up–down procedure, which seeks a stimulus level that corresponds to 71% correct performance, was used to track the subject's threshold at each frequency.

The results indicate that the ability to detect changes in the amplitude of a vibratory movement imposed on the forearm depends on the frequency of the movement, with lower thresholds being associated with movements of higher frequencies (i.e. above 5 Hz). These findings are consistent with those obtained using unidirectional movements of varying velocity, for which it has been found that performance is optimal for velocities between 2–80°/s (Hall & McCloskey, J. Physiol. 1983). The proprioceptive tuning curve resembles those derived for touch in that they are clearly U-shaped functions.

405.8

COMPENSATION FOR LOAD INSTABILITY: LIMITS OF JOINT STIFFNESS. T.E. Milner* and C. Cloutier. Institut de Réadaptation de Montréal, 6300 Darlington Ave., Montréal, Canada H3S 2J4.

In order to investigate the practical limit of mechanical stability that could be achieved by the neuromuscular system, an experiment was carried out in which subjects were required to stabilize a wrist manipulandum that was made mechanically unstable by means of positive position feedback to a torque motor (negative stiffness). For 9 of the 10 subjects, the limit was between -0.16 and -0.25 Nm/deg; for the remaining subject it was -0.4 Nm/deg.

At the end of the stabilization period the wrist was rapidly flexed by 3° and held in this position by means of a position servo to measure wrist stiffness. The measured wrist stiffness was almost always equal to or greater than the magnitude of the negative load stiffness. As the magnitude of the negative stiffness increased, wrist stiffness increased as the result of increased co-contraction of wrist flexor and extensor muscles during stabilization. An increase in flexor muscle activity was observed following muscle stretch.

Subjects were also asked to voluntarily co-contract wrist flexor and extensor muscles maximally with no load. The wrist stiffness measured in this situation was often slightly less than that achieved at the limit of mechanical stability. The maximum stiffness achieved by co-contraction of wrist flexors and extensors under any condition was only about half that predicted from the stiffness of maximally activated extensor muscles combined with the stiffness of flexor muscles generating an equal but opposite joint torque. We suggest that this limitation was due to mutual reciprocal inhibition of flexor and extensor muscles.

405.9

IDENTIFICATION OF TIME-VARYING NEUROMUSCULAR SYSTEMS. R.F. Kirsch* and R.E. Kearney. Dept. of Biomedical Engineering, McGill University, Montréal, Québec, Canada H3A 2B4.

The nervous system produces coordinated movements by modulating muscle activation patterns and reflex properties across time, making a time-varying description of neuromuscular dynamics natural and potentially useful. Special techniques must be used to characterize time-varying neuromuscular properties, however, since standard time-invariant methods are unable to separate system dynamics from rapid changes in these dynamics with time.

We have developed a time-varying identification technique which characterizes linear dynamic systems by estimating a set of impulse response functions, one for each time instant during some nonstationary behavior. A sample-to-sample tracking speed is achieved by performing the estimation at each time across an ensemble of many similar trials rather than across time in a single trial; in each trial, an independent stochastic perturbation is imposed upon the input and the evoked output is measured. Digital and analog simulations have verified the accuracy and tracking speed of our algorithm.

We have used this technique to characterize human ankle joint stiffness and the triceps surae stretch reflex during two simple time-varying behaviors. Rapid changes in isometric contraction level were found to produce large changes in the magnitude of the stretch reflex, but its dynamics were unaffected and magnitude changes were tightly synchronized to activation level. Joint stiffness could be described by a 2nd order mechanical system during steady-state conditions, but not during changes in contraction level; furthermore, stiffness initially *declined* as torque increased before increasing to the appropriate steady-state level. A rapid stretch imposed upon the active triceps surae produced a large reflex EMG response, after which stretch reflex magnitude was nearly completely suppressed for 60 - 80 ms. Joint stiffness during the stretch was complex, initially increasing, then abruptly declining until stretch termination, and finally reaching a new, higher steady-state value.

405.10

NEUROMUSCULAR COMPARTMENTS AS FUNCTIONAL MECHANICAL UNITS IN MOTOR CONTROL. J. H. Lawrence,III* T. R. Nichols, and A. W. English. Depts. of Physiology and Anatomy and Cell Biology, Emory University Atlanta, GA 30322.

Traditionally, the force vector of a given muscle has been presumed to be constant over all levels of recruitment: definable by a single line connecting a point of origin with a point insertion. English and Ledbetter (*Anatomical Records* 204, 180-193, 1982) showed that the nerve to lateral gastrocnemius (LG-n) branches to innervate four (4) anatomically discrete regions of the muscle. We stimulated muscles crossing the ankle joints in cats deeply anesthetized with pentobarbital, and used a multiaxis force-moment sensor to measure the resulting isometric torques. The control position for the cat hindlimb was a 120° knee angle with the ankle fixed at 110° extension, 0° adduction and 0° eversion (110,0,0).

We have found that there is a change in the degree of off-sagittal torque within triceps surae when separate regions of MG and LG are denervated. At the control orientation, there was a 10% decrease in off-sagittal (ad/abduction) torque for MG when the proximal portion of the muscle (MG-1) was denervated. Moreover, we saw a 40% increase in off-sagittal torque for LG when the medial compartment (LG-m) was denervated. These torque vector %changes were not constant as the ankle orientation was varied in the ad/abduction direction. Therefore, the force vector output of a muscle is dependent upon the activation state of the neuromuscular compartments and the ankle joint orientation.

(Supported by NIH grant NS20855)

405.11

A SPIKING NEURAL NETWORK MODEL FOR NEURONS CONTROLLING WRIST MOVEMENT. M.A. Maier*, L.E. Shupe and E.E. Fetz. Dept. of Physiology & Biophysics, University of Washington, Seattle, WA, 98195.

Dynamic neural networks of units with time-varying activity can be trained to simulate sensorimotor behavior from examples using backpropagation; this algorithm requires units with differentiable "sigmoidal" input-output functions. The time-varying activity of such units usually represents the firing rates of neural populations. To simulate more realistic neural activity we used "spiking units", which integrate synaptic input (triangular EPSPs and IPSPs) to threshold and fire discrete spikes that elicit delayed PSPs in their target units. Our spiking units also incorporate a refractory period and a mechanism for adaptation to sustained input. To combine the trainability of continuous networks with the realism of spiking networks, we transformed the weights derived by the former to construct the latter. To obtain spiking networks whose output activity resembled desired patterns and had adequate dynamic range, we found it necessary to derive the weights from trained networks whose units had non-standard sigmoid functions that matched the input-output transform of spiking neurons.

This strategy was applied to networks that incorporate anatomical constraints and simulate target tracking with wrist responses: input representations of step changes in target location were transformed into 8 different motor unit firing patterns observed during wrist flexion/extension movements in the monkey. The networks incorporate appropriate connections and activations of several neuronal populations, including cortical and rubral cells and segmental interneurons and afferents. (Supported by NS12542 and the Swiss National Science Foundation)

405.12

SYNCHRONIZATION OF MOTONEURON ACTIVITY AFTER RENSHAW CELL BLOCKADE. R. E. Druzinsky *, M. Maltenfort and W. Z. Rymer. Departments of Physiology, Biomedical Engineering, and Physical Medicine and Rehabilitation, Northwestern University Medical School, Chicago, IL 60611.

Several studies from this laboratory and others have tested the hypothesis that recurrent inhibition functions to desynchronize the output of a motoneuron pool. Recent simulations (see companion abstract: Maltenfort, *et al.*, 1993) have confirmed that Renshaw cells are capable of decreasing synchronization among tonically activated motoneurons. Our current studies are attempting to elucidate the conditions under which active desynchronization is present *in vivo*.

Triceps surae muscles were studied in decerebrate cats, before and after the administration of the anti-cholinergic agents mecamylamine and atropine. Activity of a pair of motor units was recorded along with EMGs, muscle force, and length during ramp and hold stretches.

Force and EMG were averaged over several trials, and time-varying coefficients of variation (V) of these quantities were calculated. Changes in Vs of the EMG and force records were detected even when no comparable changes in unit-to-unit correlations were apparent. Thus, pairwise analysis may not be a sensitive measure of synchronous activity within a large population of active motor units. Preliminary results demonstrated that significant desynchronization of motoneuron output occured at high levels of activation (when forces were high).

Supported by NIH/NINDS NS30295-02.

405.13

SIMULATION OF RENSHAW CELL-MEDIATED EFFECTS ON MOTONEURON SYNCHRONY, FORCE AND EMG. M.G. Maltenfort*, R.E. Druzinsky, C. J. Heckman and W. Z. Rymer. Rehabilitation Inst. of Chicago, and Depts. of Physical Medicine and Rehabilitation, Physiology and Biomedical Engineering, Northwestern University, Chicago, IL 60611.

In our previous study (Maltenfort and Rymer, 1992) simulations of a motor pool firing in the steady-state suggested that a small amount of current from Renshaw cells (RCs) is sufficient to desynchronize motoneurons (MNs). In the new study, the simulation has been revised to include a more realistic number of RCs. Simple models of force and EMG production have been added.

The magnitudes of IPSPs are comparable to those produced by RCs *in vivo*. RC IPSPs reduce the mean firing rate of all MNs by 1 pps, but have little or no effect on recruitment.

Synchronized MN firing was linked to increased EMG amplitude and variability in EMG and force. The force effects only appeared when force production was ≥ 15% maximal, with 80% of MNs recruited, and may have been due to untetanized firing of type FF units. MN desynchronization was present from 40% MN recruitment onward.

Ongoing work will examine the effect of changing RC firing rates on the force level at which force effects appear; transient changes in force development from a step activation; and RC interactions with correlated and uncorrelated inputs to the MNs.

This work was supported by NIH grant T32HD07418-01.

405.14

MOTOR UNIT RECRUITMENT PATTERNS IN HETEROGENIC REFLEXES OF THE DECEREBRATE CAT. S.M. Dacko*, A.J. Sokoloff, and T.C. Cope. Dept. Physiology and Biophysics, Hahnemann Univ., Philadelphia, PA 19102

An interesting divergence of activity among triceps surae muscles in decerebrate cats has been recently reported by Nichols (*J. Physiol.* 410:263, 1989): ramp-and-hold stretches of the medial gastrocnemius (MG) muscle imposed on a background of crossed extension reflex activation produced excitation of the MG muscle but inhibition of its synergist, the slow contracting soleus (SOL) muscle. Here we test the possibility that underlying this behavior is a coordinated inactivation of slow twitch motor units in both MG and SOL muscles. Physiological properties and firing responses were recorded for 6 slow-twitch and 2 fast-twitch motor units in decerebrate cats. Ramp-and-hold stretches (4mm) applied to the MG during activation of a crossed extension reflex produced inhibition of SOL as reported by Nichols. All MG units, both fast and slow, were recruited by stretch even during periods of SOL inhibition. Additionally, the instantaneous rates of stretch-evoked firing for these MG units were not slower during SOL inhibition than they were when there was no inhibition. These findings support our assertion that units are not selectively inhibited on the basis of their slow twitch character, instead, the observed patterns of inhibition represent the separate control of different motor pools. (Supported by NIH Grant NS21023).

405.15

The Computation of Position-Sense From Mono- and Multiarticular Muscle Spindles. S. H. Scott* and G. E. Loeb. MRC Group in Sensorimotor Physiology, Dept. of Physiology, Queen's University, Kingston, Ont. CANADA K7L 3N6

It is known that muscle spindles provide the majority of information about limb position, but little is known about how position-sense is computed from their signals. We have begun to analyze the computational aspects of transforming noisy spindle signals from multi-segmented limbs into different putative reference frames. A musculoskeletal model was developed containing mono- and multiarticular muscles. A fixed number of spindles were distributed amongst the muscles; the signals from each sensor were assumed to contain Gaussian noise. The number of sensors in each muscle were varied systematically and the amount of sensor noise transmitted into different reference frames was computed. The optimal distribution of sensors amongst the mono- and multi-articular muscles depended strongly on the coordinate frame in which the computations were computed. The model was then modified to reflect the actual morphometry of the human upper limb. With this adjusted model, the optimal distribution of spindles computed for each coordinate frame was always close to the actual distribution of spindles in the various arm muscles. We also estimated the relative accuracy of the upper and lower limb joints for estimating joint flexion/extension and the position of the end of the limb based on the actual numbers of spindles spanning each joint. There is a descending gradient in the numbers of spindles spanning proximal to distal joints, which corresponds to a gradient in angular resolution, but which only partially compensates for the fact that angular errors in proximal joints would result in larger uncertainties about end-point position The predicted values based on the spindle counts in the upper limb are surprisingly similar to the psychophysical results of Hall and McCloskey (1983).

405.16

THE INHIBITION OF MAXIMAL FORCE DURING A VOLUNTARY BILATERAL EFFORT : A CENTRAL OR PERIPHERAL EFFECT. L.E. Tremblay*, J. Bélanger, L. Laganière, J. Viau and Y. Lessard. Physical Therapy Dept. Health Sc. Faculty, Ottawa Univ., Ottawa, Canada.

In a voluntary situation, the maximal torque produced during a bilateral (BL) effort of the quadriceps femoris (QS) is less than the sum of the torques recorded by each QS separately (ULR or L, unilateral right or left). This inhibition has always been thought to be dependent on the CNS (supraspinal influence). Electrical stimulation (ES) allows us to re-examine this hypothesis. ES induces the development of muscle tension while bypassing the CNS. The aim of the study was to determine if the torque induced by ES in the BL situation was similar to the sum of the torques induced ULR + ULL. The maximal torques recorded in the QS of 15 male subjects were induced by an electrical muscle stimulator «Respond Select»$_c$ using a biphasic, symmetrical wave with a pulse width of 300 microsec. The torques were measured with the help of the «Cybex 6000» machine. The angular velocity was 0° per sec at an angle where the torque was maximum. All the subjects tolerated the maximal intensity delivered by the stimulator (100 mA). This was applied with silicone-carbon electrodes (5x10cm), applied following a standard procedure. The ratio of BL torques orver ULR + ULL torques during a voluntary effort were 0.89 ± 0.07 ($P < 0.01$). The ratios of BL torques (ES) were 0.91 ± 0.07 ($P < 0.01$). Additional tests using ES were performed in order to demonstrate that the inhibition is probably peripheral and spinal cord in origin. The motor nuclei of the right QS appears to be inhibited by approximately 4.5% during stimulation of the left QS and the same is true for the left QS. We believe that the motor nuclei of both QS appear to be mutually inhibited each by half the amount of the inhibition observed in the BL ES situation. This inhibition could possibly originate from the recruitment of the 1a afferent fibres and the activation of the 1a inhibitory interneurons which make tri or polysynaptic transcommissural relays with contralateral motor neurons.

405.17

THE UNIT-TO-AGGREGATE (UTA) COHERENCE APPROACH FOR ANALYSIS OF SYNCHRONY IN NEURAL POPULATIONS C.N. Christakos*
Dept. Basic Sci., Med. School, Univ. Crete, Heraklion, Greece, and Ctr. Neurobiol., Col. Phys. & Surgeons, Columbia Univ., New York, USA

Theoretical analysis based on the spectral relationships between unitary and population-aggregate activity, as well as extensive computer simulations of a uniform neuron population, indicate that: (1) For a subset of uncorrelated units in a population, the UTA coherence is very low at all frequencies. (2) For a subset of units that are correlated at some frequency f_S, the value of the UTA coherence at f_S reflects the strength and extent of the synchrony within the population, the degree of phase concentration of these units, and the numerical size of the population; and it remains substantial for a wide range of values of these parameters (e.g., the extent can be as low as 10%). (3) The UTA coherence for this subset is very low at other frequencies where there is no synchrony (except for harmonics of f_S).

These properties make the UTA coherence function a useful tool for analysis of population synchrony. Such computations for a sample of recorded units enable: the detection of synchrony and the estimation of its extent within the population, inferences on other characteristics of synchrony, and the study of changes in synchrony as conditions are altered (including pathological conditions). The main advantage of this approach is that from readily recorded neural activities, it efficiently identifies correlated units, and provides information on characteristics of synchrony at every frequency within the range of interest. Applications are presented for rhythmic neural activities.

Supported in part by N.I.H. grants NS-22715 and HL-27300.

405.18

DETERMINANTS OF POST-SPIKE FACILITATION OF EMG ACTIVITY. **P. A. Fortier*, Department of Anatomy and Neurobiology, University of Ottawa, Ottawa, Ontario, Canada, K1H 8M5.**

Several measures are used (e.g. Lemon et al. 1987; Mewes and Cheney 1991) to calculate the size of post-spike or -stimulus facilitation (PSF) of EMG in order to estimate the magnitude of descending effects on motoneuron (MN) pools. The lack of a standard measure is because there is no report of the factors which determine PSF size. These factors include MN-EPSP size, EMG noise, motor-unit action-potential (MUAP) cancellation, and both MUAP height and duration. One cannot examine these factors individually in the animal, but they can be thoroughly tested in computer simulations. The results showed that overlap of MUAPs with opposite polarity caused 25% EMG cancellation, but this did not affect the linear relationship (r = 0.99) between MN-EPSP height and PSF area because cancellation was relatively constant when only MN-EPSP amplitude was varied. Under normal conditions, however, other factors vary. Increases in EMG noise raised the baseline of the spike-triggered average but had minimal effect on the PSF, however, increases in MUAP amplitude and duration had significant effects on the PSF which reduced its reliability as an estimate of the magnitude of the descending effects on MN pools. Several methods of normalizing the PSF were tested. The best method was the "mean bin amplitude of PSF above baseline / baseline standard deviation", yet it was only a moderate estimate of descending effects when wide fluctuations in all factors existed. [Funded by Canadian MRC]

CIRCUITRY AND PATTERN GENERATION III

406.1

SENSORY INDUCED PHASE SWITCHING IN THE MOTOR PATTERN OF THE LOBSTER GASTRIC CIRCUIT *IN VITRO*. D. Combes, J. Simmers, P. Meyrand & M. Moulins* Laboratoire de Neurobiologie et Physiologie Comparées, Université de Bordeaux I & CNRS, Place Peyneau, 33120 Arcachon, France.

We are investigating the nature and mechanisms of mechanosensory feedback from a unineuronal tendon organ, the Anterior Gastric Receptor (AGR), to the gastric mill central pattern generator (CPG) in the stomatogastric nervous system (STNS) of *Homarus gammarus*.

We have found that AGR, which is activated by contraction of the powerstroke muscle of the gastric medial tooth, has access to the gastric CPG via direct excitation of two in-parallel interneurons, one (CG) excitatory, the other (GI) inhibitory. In the spontaneously active STNS *in vitro*, moderately intense bursts (spike frequency <25Hz) evoked in AGR in time with homonymous motoneurons reinforces the ongoing gastric rhythm in which medial and lateral teeth powerstroke motoneurons fire in phase. With intense rhythmic bursts in AGR, however, these phase relations switch so that the medial and lateral powerstroke motoneurons now burst in antiphase. These bimodal effects of AGR can be simulated by direct intracellular stimulation of the two intercalated interneurons : rhythmic gastric-timed bursts in CG alone reinforces the ongoing motor pattern while simultaneous activation of CG and GI causes the phase switch in pattern seen during elevated receptor discharge.

Our results suggest that these effects on the gastric mill rhythm depend on the balance of firing in interneurons CG and GI, and that "selection" of the pathway resides in their different postsynaptic sensitivities to AGR. While high intrinsic firing rates in CG ensure that the excitatory pathway is predominant during low to moderate levels of sensory input, strong synaptic facilitation in GI favours the inhibitory pathway during high levels of receptor activity.

406.2

ACTIVITY OF THE STOMATOGASTRIC NETWORKS IN FREE MOVING CRABS. H.-G. Heinzel*, D. Weigeldt, and H. Böhm. Institute of Zoology, University Bonn, 53115 Bonn, Germany.

Chronically implanted cuff-electrodes were used to monitor gastric and pyloric motor patterns and the activity of the anterior gastric receptor (AGR) from stomatogastric nerves of the crab *Cancer pagurus* for the first time. Recordings were performed for periods of up to one week from the dorsal ventricular (dvn), dorsal gastric (dgn) and medial ventricular nerve (mvn) while the animal was free to move around in its aquarium carrying its leash of electrode wires.

All animals (n=37) showed a continuous pyloric rhythm with a period duration between 1 and 2s. Spontaneous bouts of gastric activity had period durations between 5 and 30s. Gastric activity always caused strong modulation of the pyloric rhythm as the pyloric inferior cardiac (IC) and the ventricular dilator neurons (VD) stopped firing during each burst of the gastric mill motoneurons (GM). Pyloric modulations of the gastric rhythm were found in the bursts of GM, the lateral gastric (LG) and surprisingly in the dorsal gastric (DG) motoneuron. This points out that gastric-pyloric interactions may even play a stronger role than inferred from *in vitro* studies where especially DG did not show any pyloric modulations (Weimann et al. , J. Neurophysiol. 65, 111, 1991).

During quiescence of the gastric system the AGR stretch receptor fired actionpotentials at a constant rate of 2-5Hz which travelled from the stomatogastric ganglion (STG) towards the receptor ending. With regular rhythmic gastric activity AGR bursts were generated during each GM burst with AGR spikes now travelling to the STG. Strong GM bursts led to increased firing of AGR and a subsequent omission of the next GM burst in a series of alternating DG-GM bursts. Our experiments under the closed loop situation in the animal thus prove the realisation of the reflex reversal which has been suggested from *in vitro* studies (Simmers & Moulins, J. Neurophysiol. 59, 757, 1988).

Supported by HFSP and German Research Foundation He1118/7.

406.3

INTERACTIONS OF CENTRAL PATTERN GENERATORS ARE ALTERED BY MULTIPLE MODULATORS. P.S. Dickinson* and C. Mecsas. Dept. of Biology, Bowdoin College, Brunswick, ME 04011.

We previously showed that two neuropeptides, proctolin and red pigment concentrating hormone (RPCH), interact in modulating the cardiac sac motor pattern in the spiny lobster, Panulirus interruptus (Soc. Neurosci. Abst. 16: 724, 1990). When bath applied to the isolated stomatogastric ganglion (STG), proctolin alone had no effect on the cardiac sac pattern, whereas proctolin applied shortly after the effects of RPCH had washed out caused rhythmic activity in the cardiac sac network to be initiated. We also previously showed that bath application of RPCH to the isolated STG caused the cardiac sac and gastric mill patterns to 'fuse', and thus provoked a new conjoint rhythm (Nature 344: 155-158, 1990). A large increase in the amplitude of the post synaptic potentials (psps) from the inferior ventricular (IV) neurons of the cardiac sac network onto the neurons of the gastric mill pattern generator mediated these changes. We have now shown that, when the cardiac sac pattern is induced by the bath application of proctolin after RPCH, 'fusion' of the gastric mill and cardiac sac patterns likewise occurs. As is the case in RPCH alone, this fusion appears to be mediated largely by an increase in the amplitude of the psps from the IV cells to the neurons of the gastric mill network.

406.4

PATTERNS OF CYCLIC GMP IMMUNOREACTIVITY IN THE CRAB STOMATOGASTRIC NERVOUS SYSTEM. N. L. Scholz, L. M. Hurley, J. W. Truman, and K. Graubard*, University of Washington, Dept. of Zoology, NJ-15, Seattle, WA 98195.

Chemical inputs often act on target neurons by activating cyclic nucleotide cascades. In the crustacean stomatogastric nervous system (STNS), modulatory inputs shape the motor patterns produced by central pattern generating networks. However, specific roles for second messengers in mediating these effects are not yet understood.

We have used an antibody that recognizes cGMP (de Vente et al., 1987, Neuroscience 22:361) to map the cellular distribution of this second messenger in the STNS of the crab, Cancer productus. Upon *in vitro* stimulation of the STNS with sodium nitroprusside (SNP) in the presence of isobutylmethylxanthine (IBMX), subsets of cells in the commisural, esophageal, and stomatogastric ganglia (STG) show cGMP immunoreactivity. Neuronal cell bodies and their projections appear to label equally. The immunostaining is blocked by preincubation of the antisera with cGMP (10^{-7} M) but not with cAMP or GMP (10^{-5} M). Labeling is strongest in the STG, where approximately one-third of all neurons (10-12 cells) stain for cGMP. Individual neurons in the gastric and pyloric circuits have been identified by tracing axons to their respective target muscles; e.g. PD and GM cells stain consistently. Also, certain nerves do not contain labeled fibers, suggesting that cGMP is not being activated in cells that project in these tracts (e.g. PY, VD, and IC cells). Preliminary electrophysiological analyses indicate that SNP is not producing a cytotoxic effect, since cells continue to burst in the presence of SNP. We are currently investigating the potential role of cGMP in mediating the effects of known neuromodulators, and the physiological consequences of elevated cGMP in these central pattern generator networks. Supported by NIH grants (NS15697 to K.G. and a NRSA to N.S.), NSF grants (IBN-9242993 to J.T. and a predoctoral fellowship to L.H.) and by the Human Frontiers Science Program (to K.G.).

406.5

SHAB GENE EXPRESSION IN IDENTIFIED NEURONS OF THE PYLORIC NETWORK IN THE LOBSTER STOMATOGASTRIC GANGLION. D.J. Baro,* A.R. Zarrin, T.R. Podleski, and R. M. Harris-Warrick. Section for Neurobiology and Behavior, Cornell University, Ithaca, NY 14850

Identified pyloric neurons in the stomatogastric ganglion of the lobster *Panulirus interruptus* display cellular variation with respect to K^+ currents. We are examining the differences in gene expression which may underlie these electrophysiological differences using *in situ* hybridization on whole mounts of the ganglion. We constructed a probe specific for the lobster *shab* gene, one of the genes that encode delayed rectifier-like channels. The probe was synthesized by transcription with digoxigenin-UTP. Hybridization to mRNA was visualized using anti-digoxigenin antibodies conjugated to alkaline phosphatase followed by a chromogenic reaction. The specificity of the probe was confirmed by Southern blot hybridization. The *in situ* hybridization was carried out under the same stringency conditions as the Southern blot hybridization (T_m - 15°) to prevent detection of other Shaker family members. Differential staining of the cells within the ganglion was observed. Of the six major cell types that comprise the pyloric network, only AB, PD, and some PY cells possess enough *Shab* mRNA to be easily detected by *in situ* hybridization. VD, LP, IC, and some PY cells show low or undetectable levels of *Shab* gene expression under these conditions. We are currently trying to confirm these data with quantitative single cell PCR, as well as expand our study to include other members of the Shaker family.

Supported by NIH grant NS25915

406.6

A SLOWLY INACTIVATING POTASSIUM CONDUCTANCE UNDERLIES DEPOLARIZATION-INDUCED TRANSITIONS BETWEEN TONIC AND BURSTING BEHAVIOR IN REAL AND MODEL NEURONS. L.F. Abbott*, G.G. Turrigiano, and E. Marder. Depts of Physics and Biology, Brandeis University, Waltham, MA 02254.

Many neurons undergo transitions between tonic and bursting states, and the transmission and integration of information is strongly influenced by which state the neuron is in. We have been studying the role of a slowly inactivating potassium conductance in such transitions. Many cultured STG neurons fire tonically at the start of a constant depolarizing current pulse, but over the course of several seconds of depolarization the spikes inactivate and the neuron begins to oscillate. In voltage clamp experiments we have isolated a slowly inactivating outward conductance that inactivates with a time course similar to the time course of the transition from tonic to bursting behavior. This conductance displays cummulative inactivation; that is, it inactivates slowly during repeated short depolarizing pulses, making it succeptible to slow inactivation during phasic activity in STG neurons . We have incorporated this conductance into a conductance-based model of an STG neuron, and have found that it is sufficient to reproduce the transition from tonic to bursting that we observe during prolonged depolarization of the real neurons.While this conductance de-inactivates rapidly at membrane potentials below -60 mV, it de-inactivates only very slowly at membrane potentials above -50 mV. The most hyperpolarized membrane potential of STG neurons can vary from -50 to -70 mV, depending on the neuromodulatory state of the system. This suggests that neuromodulators or other inputs that simply change the resting potential of STG neurons can change their response to rhythmic input from tonic to bursting. We are currently using the model to predict how this conductance will help shape the response of STG neurons to patterned inputs. Supported by MH-46742 to L.F.A. and BNS-9009251 to E.M.

406.7

PHASE-SETTING IN A MODEL OF THE GASTRIC MILL CPG IN THE LOBSTER P.F.Rowat* and A.I.Selverston Biology Department, U.C. San Diego, La Jolla, CA 92093–0322.

We have constructed a simplifying, biologically constrained, network model of the gastric mill CPG which shows that only a few basic mechanisms are sufficient to produce the relatively complex patterns characteristic of the cycling gastric mill. These mechanisms include a cell model having a fast current with an N-shaped I-V curve and slow inward and outward currents with linear steady-state I-V curves; graded synaptic transmission; and "slow" synapses. The cell model captures important characteristic behaviors of gastric mill cells such as plateau potentials, post-inhibitory rebound, and endogenous osillations. The reciprocal inhibitory pair is an essential sub-network of the gastric mill network, which enables the model network to produce a pattern whether or not the individual cells are endogenous oscillators. In the model network, the relative phases of cells were adjusted by manipulating the gains of slow currents in individual cells and by the use of slow synapses. Phase relationships can also be set by changing synaptic strengths. The relative phase of two cells can be modified by approximately 15% by adjusting the slow current gains, and by approximately 5% by changing the ratio of synaptic strengths. Larger changes in phase are obtained by the use of slow synapses. We also present other results on the effectiveness of these mechanisms for manipulating relative phases in cells arranged in pairs, in chains, and in more complex networks of cells.

Supported by NSF:IBN-9122712, ONR:N00014-88-K-0328, and NIMH MH46899

406.8

SLOW SYNAPTIC POTENTIALS MEDIATING INTERACTIONS OF GASTRIC PATTERN-GENERATING NEURONS IN THE STOMATOGASTRIC GANGLION OF SPINY LOBSTERS. R.C.Elson* and A.I.Selverston. Department of Biology, UCSD, La Jolla CA 92093.

We are studying the properties of chemical synapses within the gastric mill central pattern generator, a network of 10 identified motor neurons and one interneuron (Int 1) in the stomatogastric ganglion (STG) of spiny lobsters. Four gastric motor neurons (including the important Lateral Gastric cell) are thought to use glutamate as a neuromuscular transmitter. At their central outputs, these neurons evoke inhibitory synaptic responses of two types. Type (1) are fast, unitary IPSPs (rise-time 10-20 ms) that are mediated by an increased chloride conductance and blocked by picrotoxin. They resemble the glutamatergic IPSPs described elsewhere in the STG (Marder & Eisen 1984). Type (2), however, are slow inhibitory potentials (rise-time 1-2 s) lacking unitary components, mediated by a conductance increase to potassium and resistant to picrotoxin. At some synapses, type (1) or type (2) occur singly; at others they occur together, producing biphasic potentials. Int 1 also has diverse synaptic actions. In some followers, it drives type (1) IPSPs; in others, it evokes slow excitation. In the Dorsal Gastric neuron, the excitation comprises unitary EPSPs that summate slowly and are picrotoxin-resistant.

Slow synaptic potentials seem tuned to the slow bursts and long cycle periods of the gastric motor pattern. We plan further study of their pharmacology and integrative significance.

Supported by NIH grants NS09322, PO1N25916 and PHS07220.

406.9

SPECIFIC MUSCARINIC MODULATION OF IDENTIFIED STOMATOGASTRIC CPG NEURONS IN PRIMARY CULTURE. T.A. Cleland* and A.I. Selverston. Dept. of Biology 0322, U. Calif. San Diego, La Jolla, CA 92093-0322.

The gastric mill network within the stomatogastric ganglion of the spiny lobster drives the movements of three teeth within the stomach. *In vitro*, the network is quiescent without modulatory input; specific modulators initiate and sustain characteristic gastric rhythms. The rhythm initiated by the muscarinic agonist pilocarpine has been shown to be based on a "kernel circuit" of endogenously oscillatory neurons (Elson and Selverston 1992). The muscarinic modulation of isolated, identified gastric neurons was studied in primary culture using voltage clamp techniques. Primary culture offers the twin advantages of unambiguous cellular isolation from the network and a resolution of the space-clamp limitations that hinder such studies on most STG cells *in situ*. We show that one effect of pilocarpine is to reduce neurons' net potassium conductance; preliminary evidence indicates, however, that different identified cells respond quantitatively differently to this muscarinic modulation. Efforts are underway to further elucidate the nature of these differences and how they contribute to the distinct and complex voltage behaviors of these bursting cells and their impact on network motor output.

406.10

QUANTIFYING THE CONTRIBUTION OF THE PSRs IN SENSORY INDUCED GASTRIC MILL ACTIVITY IN THE INTACT LOBSTER. M.E.T.Boyle and A.I.Selverston* Dept. of Biol. UCSD, La Jolla, CA 92093.

The lobster gastric mill (GM) consists of three teeth in the stomach, two lateral and one medial, which masticate food and is under control of the stomatogastric ganglion (STG). Heretofore, GM activation in the intact restrained animal has required injection of neuromodulators. Here we demonstrate the ability to induce GM activity by activation of the posterior stomach receptors (PSRs), one of six groups of sensory receptors in the foregut, and quantify their contribution to the GM response. We use an endoscope to observe, quantify, and compare GM movement patterns produced by sensory stimulation in the intact and deafferented animal.

Intact lobsters were restrained while an endoscope with an irrigation tube was inserted through the esophagus and into the cardiac sac region. Irrigation pump levels between 3 and 8 (10 being the strongest) activated a quiescent GM in 9 out of 10 cases and change GM ongoing activity in 8 of 10 cases. The most striking feature was that there was no predominant chew type as seen with CCK or proctolin. Additionally, there could be up to five different chew types in one minute when GM activity was first initiated. In all cases, GM chewing frequency, mode, and strength changed when the pump was turned on and off.

PSRs are mechanoreceptors which monitor medial tooth activity. Bipolar sensory neurons are located in the posterior stomach nerve (psn). The dorsal psn (d-psn) is the most specific nerve associated with the PSRs. In 5 preparations the d-psns were bilaterally cut while monitoring pressure induced GM activity. Minutes after the d-psns were cut GM activity was substantially lower. No increased GM activity was seen with increased pump levels. These preliminary results suggest that the PSRs may mediate pressure induced chewing. Supported by ONR N00014-91J-1720 and NIH RO1-09322.

406.11

CELLULAR PROPERTIES UNDERLYING PHASE AND DUTY CYCLE REGULATION IN THE LOBSTER PYLORIC NETWORK. S.L. Hooper*. Dept. of Biological Sci., Ohio University, Athens, OH 45701

Rhythmic motor patterns (e.g., walking) are produced over a wide range of cycle frequencies. As frequency changes, the relative time (phase) and length (duty cycle) of the motions must be regulated to maintain motor pattern integrity. I am using the pyloric network to identify the mechanisms underlying this regulation. Current injection into the pyloric pacemaker neuron induces up to a four-fold variation in pyloric cycle frequency. Over most of this range, phase and duty cycle is maintained within 0.1 phase units; the pyloric network is thus a constant phase and duty cycle network.

The pyloric pacemaker neuron group maintains constant duty cycle by as yet unknown mechanisms. This group inhibits all other pyloric neurons; these follower neurons fire as a result of post inhibitory rebound. As cycle frequency changes, both the duration and the amplitude of the inhibition received by the follower neurons change. The follower pyloric neurons maintain constant phase because their delay to firing changes appropriately in response to these changes in inhibitory input.

My results show that some pyloric followers specifically respond to changes in either inhibition duration or amplitude. If hyperpolarizing current pulses of various timings and amplitudes are injected into Lateral Pyloric (LP) neurons isolated from the pyloric network, delay to firing in the neuron appropriately changes as inhibition amplitude changes, but is unaffected by altering inhibition duration. Alternatively, delay to firing in isolated Pyloric (PY) neurons changes appropriately with inhibition duration, but is insensitive to altering inhibition amplitude. Pyloric phase regulation is thus at least partially due to inherent properties of the network's neurons (as opposed to details of the network's synaptic connectivity), and different neurons use different cues (duration *vs.* amplitude of inhibition) to "sense" changes in overall pattern frequency.

406.12

DYNAMIC CLAMP DISSECTION OF THE MECHANISMS OF PROCTOLIN MODULATION OF THE PYLORIC RHYTHM. A.A. Sharp* and E. Marder. Bio. Dept., Brandeis Univ., Waltham, MA 02254.

Bath application of proctolin and stimulation of proctolinergic inputs to the STG have previously been shown to activate or increase the frequency of the pyloric rhythm. Photoinactivation and pharmacological blockade of inhibitory synapses have revealed the pyloric target neurons for proctolin in the STG, and provided insight to the mechanisms underlying the response of the entire network to proctolin (Hooper and Marder, 1987, *J. Neurosci.* 7:2097). Activation of a voltage-sensitive inward current by proctolin underlies the pyloric response to proctolin (Golowasch and Marder, 1992, *J. Neurosci.* 12:810).

We now use the Dynamic Clamp (Sharp et al., 1993, *J. Neurophys.* 69:992) to introduce an artificial proctolin conductance (I_{proc}) into the AB, LP and IC neurons individually and in pairs in order to determine the role each neuron plays in generating proctolinergic modulation of the pyloric rhythm. I_{proc}, which was measured in the LP and IC, is sufficient to increase the amplitude and frequency of AB oscillations without depolarizing the baseline potential. I_{proc} increases the firing rate, burst duration and baseline potential of the LP and IC neurons. The responses seen in the individual neurons are strongly dependent on the characteristics of the activation curve of I_{proc}. I_{proc} in the AB increases the pyloric frequency. I_{proc} in the LP or IC has little to no effect on the pyloric frequency, but it causes marked effects on the phase relationships of the pyloric neurons. In quiescent preparations, I_{proc} in the AB alone can initiate a two phase rhythm (AB/PD with PY), but activation of the LP and IC only occurs if they also receive I_{proc}. This method allows us to separate the frequency and duty cycle effect of proctolinergic modulation.

Supported by NS 17813 and BNS 9009251.

COMPARATIVE NEUROANATOMY II

407.1

HISTOCHEMICAL MAPPING OF NADPH-DIAPHORASE IN THE TURTLE BRAIN. *R. Sarrafizadeh*, T. Braun, and J. C. Houk.* Department of Physiology, Northwestern University Medical Center, Chicago, IL 60611-3008.

Neuronal NADPH-diaphorase is likely to be a nitric oxide (NO) synthase (Hope et al. Proc. Natn. Acad. Sci. 88, 2811-2814), and cells containing NO synthase are selectively spared following hypoxic ischemia (Ferriero et al. Ann. Neurol. 24, 670-676). We have studied the distribution of neurons containing NADPH-diaphorase in the turtle brain as a first step in understanding the unusual resistance of the turtle brain to hypoxia. Anesthetized turtles were perfused with 0.2M phosphate buffered saline (PBS) followed by 4% paraformaldehyde. The brain and rostral cervical spinal cord were then dissected, gelatin embedded, post-fixed for 30 minutes, washed for 24 hours in PBS and cryoprotected overnight in 30% sucrose. Coronal sections 50 µm thick were cut and reacted in PBS containing 0.1 mg/ml nitroblue tetrazolium, 0.1 mg/ml ß-NADPH and 0.3% Triton X-100 for 30 minutes at room temperature, after which the sections were washed in PBS and mounted on gelatin-coated slides.

In the telencephalon moderate label was seen in Area d, nucleus of the diagonal band of Broca, and the lateral nucleus of the olfactory tract, together with lighter label in other sites. The globus pallidus and the central and basal nucleus of the amygdala showed modest label in the basal ganglia. In the diencephalon the lateral geniculate nucleus was moderately labeled. The substantia nigra was heavily labeled in the mesencephalon, whereas more modest label appeared in the red nucleus, the nucleus of the medial longitudinal fasciculus, the periventricular stratum griseum and the nuclei of the torus semicircularis. In the pons the locus coeruleus and the parvicellular nucleus isthmi were heavily labeled, and the lateral and medial cerebellar nuclei, the principal trigeminal nucleus and pontine reticular formation neurons were moderately labeled. The dorsal column nuclei and the vestibulocochlear nucleus showed modest label, while the lateral reticular nucleus and other sites showed light label in the medulla. Labeling was heaviest in lamina VII of C1 spinal cord.

The patterns of NADPH-diaphorase staining in the turtle and rat appear similar, but not identical. Staining of red nucleus, lateral cerebellar nucleus and lateral reticular nucleus suggests a potential role for NO in the turtle rubrocerebellar network.

407.2

ON THE LAMINAR ORGANIZATION OF THE SUPERFICIAL DORSAL HORN OF Alligator mississippiensis. C. Jeffery Woodbury*, Dept. of Ornithology, American Museum of Natural History, 79th and CPW, New York, NY 10024 and Dept. of Anatomy, University of Utah School of Medicine, Salt Lake City, UT 84132.

Two types of laminar organization are found in the dorsal horn (DH) of birds, a truly laminar (i.e., Rexed-type) pattern and a novel (i.e., Brinkman and Martin-type) pattern in which lamina III lies medial to laminae I/II. Recent taxonomic studies in birds revealed that each DH type characterizes a specific subset of higher avian taxa (Woodbury, Soc. Neurosci. Abst. 17, 652, 1991). In the present study of crocodilian DH, horseradish peroxidase (HRP)/HRP-ligand tract-tracing, along with cytoarchitectonic and immunohistochemical techniques, were used to determine laminar topology in order to polarize the character variation in birds using outgroup comparison for phylogenetic systematic analyses of these data.

Unlike birds and mammals, the DH of alligators exhibits only minor elaboration of the substantia gelatinosa and thus appears like that of other reptiles examined (e.g., turtles and lizards) despite the fact that alligators are more closely related to birds. Nevertheless, application of HRP to a cutaneous nerve labels two separate projections across the mediolateral axis; further, each of these separate projections appears to be somatotopically organized, as seen when different nerves are labeled bilaterally. Moreover, wheat germ agglutinnin-HRP and cholera toxin B-HRP preferentially label the lateral and medial projections, respectively, when applied to a cut cutaneous nerve or injected under the skin. These findings suggest, therefore, that lamina II lies lateral to lamina III (supported also by cytoarchitectonic and immunohistochemical data), and that cutaneous afferents form two separate somatotopic maps across the mediolateral axis of the superficial DH, as in chickens and many other birds. As analyses of the DH of dinosaurs is obviously out of the question, the most parsimonious explanation is that the 'novel' DH lamination pattern of birds is not novel at all but was present in the common ancestor of both birds and crocodilians and is therefore primitive to birds. The truly laminar DH appears to have evolved at least two separate times in amniotes, once in mammals and again within birds.

407.3

CONVERGENCE OF THALAMIC AND CHOLINERGIC PROJECTIONS IN THE DENTATE AREA OF LIZARDS.
Hoogland, P.V., and E. Vermeulen-VanderZee, (SPON: European Neuroscience Association) Dept. of Anatomy and Embryology, Free University, Van der Boechorststraat 7, 1081 BT Amsterdam, The Netherlands.

The small-celled part of the medial cortex (Cxms) in lizards is the equivalent of the mammalian hipocampal area dentata. As in mammals most of the afferents to this cortical area are arranged in sharply delimited laminae. In reptiles this lamination pattern is species-specific. In the lizards *Tupinambis nigropunctatus* and *Podarcis hispanica* projections from the multisensory dorsolateral thalamic nucleus (Dla) terminate in the middle one third of the outer plexiform layer throughout the whole rostro-caudal extent of Cxms. In the lizard *Gekko gecko* Dla projects to only the rostral one third of Cxms where the fibers terminate in the superficial half of the outer plexiform layer and in the deep half of the inner plexiform layer. To find out whether the species related variation of thalamic projections to Cxms is a solitary phenomenon or that it is related to variations of other afferents of Cxms, we studied the relationships between the thalamic and cholinergic projections from the basal telencephalon in the medial cortex of the lizards mentioned above. Therefore, we performed acetylcholinesterase and choline acetyltransferase stainings on Cxms of these lizards. It appears that the cholinergic afferents terminate approximately in the same subregion and the same laminae as the Dla projections. Therefore, there seems to be a close association between thalamic and cholinergic afferents in the Cxms of lizards, irrespective of their precise location in the cortex of the various species. This suggests a functional relationship between these two afferents of the dentate area in lizards.

407.4

GAD IMMUNOREACTIVITY IN REPTILIAN THALAMUS. M.B. Pritz* and M.E. Stritzel. Dept. of Neurosurgery, Univ. of Calif. Irvine Med. Ctr., Orange, CA 92668.

Previous experiments in Caiman crocodilus that utilized a polyclonal antibody to GABA or GAD found that certain thalamic nuclei that projected to the telencephalon lacked GABA(+) or GAD(+) neurons. These nuclei were: nucleus dorsolateralis anterior (Dla), nucleus dorsomedialis anterior (Dma), nucleus rotundus (Rt), nucleus reuniens pars centralis (Rc) and pars diffusa (Rd), nucleus diagonalis (D), and the medialis complex (MC). The present study investigated immunoreactivity to monoclonal antibodies to GAD in these same 7 thalamic nuclei in Caiman and in a closely related Crocodilian, Alligator mississippiensis, as well as immunoreactivity to a polyclonal and monoclonal antibodies to GAD in the dorsal part of the lateral geniculate nucleus (Gd) in Caiman.

Immunoreactive puncta to the GAD monoclonal antibodies were observed in the following thalamic nuclei in Caiman and Alligator: Dla, Dma, Rt, Rc, Rd, D, and MC. GAD immunoreactivity in Alligator was more robust than in Caiman. GAD-2 immunoreactivity was more intense than immunoreactivity to GAD-1 or GAD-5. GAD(+) puncta were most intense in Dla and Dma; significant but less intense in MC and D; and less intense but present in Rt, Rc,and Rd. No GAD(+) neurons were identified in any of these 7 thalamic nuclei with any GAD monoclonal antibody epitope in either Caiman or Alligator.

In the Gd of Caiman, GAD(+) neurons, although sparse, were identified with the polyclonal but not the monoclonal antibodies. GAD(+) puncta were found throughout this nucleus with both GAD antibodies.

The findings of GAD(+) neurons and puncta in the Gd of Caiman and the lack of GAD(+) cells in the 7 other thalamic nuclei in Caiman and Alligator are similar to the results of previous reports in other reptiles. The presence of GAD(+) puncta in these 8 thalamic nuclei, coupled with the sparse number of GAD(+) thalamic reticular neurons in Caiman, suggests that this GAD input may arise from an extra-thalamic source.

407.5

COMPARISONS OF FOREBRAIN GROUPS AMONG TETRAPODS. T.J. Neary* and L.L. Bruce. Dept. Biomed. Sci., Creighton Univ., Omaha, NE 68178.

New data on hypothalamic and olfactory connections in reptiles and amphibians, combined with connectional, histochemical, and topological data reveal unexpected similarities between thalamic, amygdalar, and pallial groups in tetrapods. 1) The amphibian medial pallium is comparable to both medial and dorsal cortex of reptiles, which are comparable to the hippocampal formation and subiculum of mammals, respectively. Furthermore, the mammalian isocortex may be derived from the same anlage as the reptilian dorsal cortex, rather than from DVR. 2) Major mammalian pallial amygdalar groups appear to be represented in the reptilian telencephalon. The reptilian DVR and lateral amygdala are comparable to the mammalian basolateral and lateral amygdala, and basomedial amygdalar nuclei, respectively. 3) The amphibian caudal striatum is comparable to the reptilian striatoamygdalar area and mammalian central amygdala. 4) Subdivisions within the lateral amygdala of amphibians are comparable to the medial dorsal, medial ventral, and a ventromedial portion of the external amygdala of reptiles, which are comparable to the anterior dorsal medial, anterior ventral medial, and posterior medial groups of the mammalian olfactory amygdala, respectively.

407.6

DUAL ELABORATION HYPOTHESIS OF DORSAL THALAMIC EVOLUTION AND DUAL EXPANSION HYPOTHESIS OF DORSAL PALLIAL EVOLUTION: CLADISTIC ANALYSIS OF THE FOREBRAIN. A.B. Butler*, I.T.N.I., 4433 N. 33rd St., Arlington, Va. 22207.

A previous cladistic analysis of the dorsal thalamus in jawed vertebrates (*Brain Res. Rev.*, in press) revealed two fundamental divisions: the lemnothalamus (LTh) and the collothalamus (CTh), predominantly in receipt of lemniscal and midbrain roof inputs, respectively. In amniotes, these divisions both comprise multiple migrated nuclei. For the present cladistic analysis of the pallium, the distribution of 27 traits was analyzed among amniotes; outgroup comparisons were made to identify the traits present in ancestral captorhinomorph amniotes, allowing a reconstruction of subsequent dorsal pallial evolution.

In ancestral tetrapods, the LTh projected to medial and dorsal pallia, and the CTh projected to the striatum. In captorhinomorphs, expansions of the medial part (Dm) and lateral part (Dl) of the dorsal pallium occurred in correlation with the elaboration of the LTh and CTh, respectively. Also, CTh inputs to Dl were gained.

In ancestral mammals, marked expansion of both Dm and Dl continued, and an inside-out migration pattern was gained. The Dm pallial field gives rise to cortices that include the subicular, cingulate, entorhinal, prefrontal, primary sensorimotor, and striate cortices, i.e. those cortices that are in receipt of LTh projections. Dl gives rise to the CTh-recipient cortices, including extrastriate cortices and auditory and insular cortices. In ancestral non-synapsid amniotes, continued expansion of Dl was greater than that of Dm. Dm gives rise to the dorsal cortex, including the pallial thickening, and Dl gives rise to the dorsal ventricular ridge.

407.7

HISTOCHEMICAL MAPPING OF NITRIC OXIDE SYNTHASE IN THE QUAIL BRAIN. F. Sánchez†, R. Arévalo^, J.R. Alonso^, N. Aste°, C. Viglietti-Panzica°*, and G.C. Panzica°. ^Dept Cell Biology & Pathology, and †Dept Human Anatomy & Histology, Univ. of Salamanca (Spain); °Dept Human. Anatomy & Physiology, Univ. of Torino (Italy).

The distribution of NADPH-diaphorase activity (ND), an enzyme recently identified as the nitric oxide synthase, was histochemically investigated in the Japanese quail brain. In the telencephalon, the paleostriatal-paraolfactory lobe complex demonstrated the highest presence of both positive cells and processes.They were observed also in several regions of the hyperstriatum, as well as in the archistriatal nucleus taeniae. Some regions were totally lacking of positive elements: i.e. the ectostriatum and the hyppocampus. In the diencephalon, the magnocellular hypothalamic system did not show any particular accumulation of reaction product. On the contrary, retino-recipient areas, such as the visual suprachiasmatic nucleus and the lateral geniculate complex demonstrated a composite structure of both positive neurons and processes. The brainstem revealed a large ND positive population extending from the ventral mesencephalon to the dorsal pons. In the optic tectum fusiform positive elements were distributed within the stratum griseum and superficialis. In the medulla, a dense terminal field within the nucleus of the solitary tract, and scattered neurons in the reticular nuclei were observed. Although the staining of neurons and tracts for ND was highly selective, they did not correspond to any single known neurochemically identified system. The findings of the present study reveal that the ND containing systems in the avian brain are organized according to a pattern comparable, for its complexity, to that observed in mammals. However, important interspecific differences were observed suggesting that this novel neural system could be implicated in diverse tasks.

407.8

ULTRASTRUCTURAL STUDY OF THE TARGETS OF CORTICAL AFFERENTS IN THE AVIAN STRIATUM. C.L. Veenman* and A. Reiner. Dept. of Anatomy & Neurobiology, Univ. of Tennessee, Memphis, TN, 38163.

Previous retrograde and anterograde tracing studies showed that the outer rind of the avian 'neocortex' projects to the caudate/putamen. To determine the targets of these corticostriatal projections at an ultrastructural level, we injected the anterograde tracer biotinylated dextran amine (BDA) in dorsomedial and dorsolateral regions of the outer rind of the pigeon 'neocortex'. Abundant anterograde transport was obtained by placing three pressure injections of 0.1µl 5% BDA spaced 0.5mm apart in each of these separate neocortical areas. The injection sites were marked by numerous filled neurons, while retrograde transport was negligible. Light microscopy showed numerous BDA-labeled terminals in the caudate/putamen appearing as strings of varicosities beaded along labeled axons. Electron microscopy showed that the labeled terminals were characterized by densely packed, unlabeled vesicles embedded in an otherwise uniformly BDA filled cytoplasm. Distinct postsynaptic densities were observed where BDA-labeled corticostriatal terminals contacted the heads of spines. Such postsynaptic densities, however, were not evident at appositions between labeled terminals and dendritic shafts or perikarya. No obvious differences between terminations of the corticostriatal projections from the two different 'neocortical' regions were seen at light or electron microscopic levels. Thus, avian corticostriatal fibers appear to selectively synapse on the heads of spines of striatal neurons. These asymmetrical synapses suggest that avian corticostriatal terminals excite spiny striatal neurons, as is true of mammalian corticostriatal terminals. Supported by NS-19620 & NS-28721 (A.R.)

407.9

BASAL FOREBRAIN PROJECTION TO THE CHOLINERGIC NEURONS OF THE TEGMENTAL PEDUNCULOPONTINE NUCLEUS IN PIGEONS. L. Medina* and A. Reiner. Dept. Anat. & Neurobiol., U.T. Memphis, TN 38163.

The cholinergic neurons of the tegmenti-pedunculopontine nucleus (TPP) have widespread forebrain and midbrain projections, and thereby exert an important modulatory influence on diverse neural functions. In mammals, the TPP has been found to be under the influence of the basal forebrain via projections from the ventral pallidum (VP) and the substantia innominata (SI). Since our previous studies in birds have revealed the existence of a cholinergic cell group in the isthmic tegmentum comparable to the mammalian TPP, in the present study we have investigated the presence of a basal forebrain-TPP projection in pigeons by means of: (1) BDA injections in the basal forebrain combined with immunohistochemistry for choline acetyltransferase (ChAT); and (2) LM and EM analysis of the innervation in the TPP of normal animals and of animals with a lesion in the basal forebrain projection bundle, using single- and double-label immunochemistry for ChAT, substance P (SP), dynorphin (DYN) and glutamic acid decarboxylase (GAD). After BDA injections in the basal forebrain, numerous labeled fibers could be traced to a large terminal area in the midbrain/isthmic tegmentum which includes the ChAT+ cell field of the TPP. Plexuses of SP+, DYN+ and GAD+ fibers were found to overlap the ChAT+ neurons in the TPP of normal animals. EM analysis indicated the existence of synaptic contacts between SP+ terminals and ChAT+ perikarya and dendrites. Unilateral lesions of the basal forebrain projection bundle produced a dramatic loss of the SP, DYN and GAD innervation in the TPP in the lesion side, compared to the normal levels observed in the control side. Our results provide evidence for the presence of a basal forebrain-TPP projection in birds, and suggest that this projection utilizies SP, DYN and GABA.

Supported by NS-19620 to A.R. and the Spanish Ministry of Education to L.M.

407.10

SITES OF GENE EXPRESSION AND RECEPTORS FOR VASOACTIVE INTESTINAL POLYPEPTIDE THROUGHOUT THE CHICK BRAIN. W.J. Kuenzel*, S.K. McCune[1], R.T. Talbot[2], P.J. Sharp[2] and J.M. Hill[1]. *Dept.Poultry Sci.,Univ. Maryland, College Park, MD 20742;[1]Lab.Devel.Neurobiol.,NICHD, NIH, Bethesda, MD 20892;[2]Dept.Reprod.Devel.,AFRC,IAPGR,Roslin, Midlothian, Scotland, U.K.

The gene for vasoactive intestinal polypeptide (VIP) has been cloned in the chick. A synthetic cDNA sequence corresponding to nucleotides that code for nearly half of the VIP peptide was made and labeled with ^{35}S. In situ hybridization was then performed using brain tissue that was frozen immediately after birds were sacrificed. In addition, in vitro autoradiography was performed using ^{125}I-VIP with and without VIP and GMP-PNP. VIP message was found in the lateral septal organ (LSO) pars medialis (contains cerebrospinal fluid contacting neurons), medial preoptic region, bed n. of the pallial commissure (nCPa), anterior hypothalamic (hypo.) n., lateral hypo. n.(displayed most extensive and dense message), periventricular hypo. n., lat. to paraventricular n. and ventromedial hypo. n., stratum cellulare externum, inferior hypo. n., infundibular hypo. n., stratum griseum et fibrosum superficiale(SGFS), hippocampus and area parahippocampalis, area ventralis of Tsai, neurohypophysis, substantia nigra, intercollicular n.(ICo), central gray (GCt), locus ceruleus, parabrachial n.(PB), ventrolateral medulla (VLM) and n. tractus solitarius. Receptors were found in the LSO pars lateralis, hyperstriatum ventrale, paleostriatum augmentatum, archistriatum, lat.ant. thalamic n., base of third ventricle, n.CPa, n. septomescephalic tract, piriform cortex, area corticoidea dorsolateralis, ICo, GCt, SGFS, PB, raphe n. and VLM. VIP is found in structures associated with the visceral forebrain and brainstem respiratory-vocal pathway. Supported by USDA Grant #90-37240-5506.

407.11

DOES PURKINJE CELL COMPLEXITY REFLECT EVOLUTIONARY CHANGE? B.R. Krauss, D.R. Chialvo, A.V. Apkarian* and B.J. Serog. Dept. of Neurosurgery SUNY Health Science Center at Syracuse, NY 13210.

Cerebellar Purkinje cells have the most extensive dendritic arborization in the CNS. These arbors are confined to a single plane, perpendicular to the main axis of the folium. We reexamined a recently postulated (Takeda et al. Neurosci. Res., 13:19, 1992) relationship between phylogeny and the complexity of the Purkinje cell dendritic field by measuring its fractal dimension (D_f).

Fractal geometry provides a basis for the analysis and interpretation of complex forms found in nature. The invariance of shapes over different scales gives statistical self-similarity, quantified by D_f. This quantity indicates the space filling properties of the object. The D_f of Purkinje cells from 12 species with diverging eras spanning 6 million years were measured.

Fractal dimension of Purkinje cells were determined from photocopies of camera-lucida drawn golgi-impregnated cells and digitized video images of human Purkinje cells. The images were scanned with an HP Scannjet Plus and a personal computer used for calculations. Standard box-counting method was used to estimate D_f.

The correlation (y=mx+b) between diverging era (in years$X10^8$) and D_f for water creatures was m=.-0.057, b=1.792, and for land creatures m=-0.096, b=1.779. The slope of the water creatures line was not significantly different from zero (F=0.8, p<0.41) but the slope of the land creatures was (F=10.89, p<0.02).

Purkinje cell complexity and phylogenetic evolution seem related in land creatures, which qualitatively agrees with Takeda et al.. This relationship is not evident in water creatures. Moreover, we found that small variations in data collection and analysis can dramatically change the D_f. This emphasizes the necessity of standardized data processing to obtain meaningful comparisons.

407.12

Consequences of reduced eye size on the visual and photoperiodic systems in the blind mole rat, *Spalax ehrenbergi*.

M. Herbin*, S. Richard, J.P. Rio, J. Repérant, E. Nevo and H.M. Cooper. *INSERM U-371, Cerveau et Vision, 69500 Bron; Lab. Anatomie Comparée, M.N.H.N., INSERM U-106, 75005 Paris, FRANCE; Inst. of Evolution, 31905 Haifa, ISRAEL.*

Although the subterranean mole rat is considered to be completely blind, thermal and circadian activity rhythms are synchronized by the external light cycle. The subcutaneous eye measures 600-700 µm in diameter and the optic nerve (50 µm diam.) contains 900-1000 unmyelinated fibers only (EM study). In vitro application of HRP to the optic nerve labels a population of 850 ganglion cells (RGC), homogeneously distributed in the retina. Cell soma size distribution is unimodal (6-16 µm) and RGC's appear immature with sparsely branched dendritic trees. Intraocular injection of CT-HRP shows that all structures known to be innervated by the retina in other mammals, receive retinal projections in *Spalax*. The vLGN, dLGN, pretectum, superior colliculus and accessory optic system all receive a sparse bilateral retinal projection, are highly reduced in size, and show a poor cytoarchitectural organization. Injection of HRP in the colliculus labels RGC's distributed over the entire surface of both retinas, suggesting a lack of topographic organization. Geniculo-cortical connections, revealed by multiple injection of fluorescent tracers in area 17, were also poorly organized. In contrast to other mammals, the suprachiasmatic nucleus (SCN) and the bed nucleus of the stria terminalis receive a significant proportion of retinal projections (> 30%). Histochemical and immunohistochemical studies show that the SCN is organized similar to that of other rodents, and contains cells immunoreactive to VIP, VP, and SOM as well as 5-HT, MET-ENK and NPY immunopositive fibers. These results demonstrate that adaptive evolution in the subterranean niche has led to a mosaic of regressive and progressive features: structures related to form vision, motion analysis, and visuomotor function, are hypoplasic, whereas structures related to photoperiodic light detection and circadian neuroendocrine regulations are relatively hypertrophied and conserve a normal pattern of intrinsic organization.

407.13

INTER-INDIVIDUAL VARIABILITY OF THE SULCAL PATTERN IN THE ANTERIOR CINGULATE REGION.
F. Tomaiuolo, T. Paus, R. Morris, D. McDonald, M. Petrides* and A.C. Evans. Institute of Human Physiology, University of Verona, Italy and Montreal Neurological Institute, McGill University, Montreal, Quebec, Canada.

Brain magnetic-resonance images (MRI) were obtained from 305 young healthy volunteers and transformed into standardized stereotactic space (Talairach and Tournoux 1988). With an interactive 3-D software, the presence of the paracingulate sulcus (PCS) and the course of the cingulate sulcus (CS) were examined in 238 hemispheres. An uninterrupted PCS was present in 35/121 left and 19/117 right hemispheres. The caudal limits of the PCS were located at the level of Y = 14 ± 12 and Y = 21 ± 12 mm for the left and right PCS. Approximately at the level of the anterior commissure, the CS was either interrupted and/or it gave off a vertically oriented branch (left: 47/102; right: 50/97). The rostrocaudal location of this abrupt change in the course of the CS was Y = -1 ± 6 and Y = 2 ± 8 mm for the left and right CS. In another 16 left and 19 right hemispheres, two vertical branches were observed in the same portion of the CS (rostral branch: Y = 6 ± 6 mm; caudal branch; Y = -8 ± 5 mm). The implication of the above defined variability in the CS pattern for functional neuroimaging was examined in the accompanying PET/MRI study.

407.14

THE CORPUS CALLOSUM IN CHILDREN AND ADOLESCENTS: EFFECTS OF GENDER AND AGE.
J.N. Giedd, P.L. Kozuch, F.X. Castellanos, C.A. King, S.D. Hamburger, A.J. Allen*, and J.L. Rapoport. Child Psychiatry Branch, NIMH, Bethesda, MD 20892

The midsagittal cross-sectional area of the corpus callosum was measured from magnetic resonance images of children and adolescents (41 males and 43 females) age 5 to 18 free of developmental, psychiatric, or medical illness. Subjects were divided into pre (Tanner stage ≤ 1) or post (Tanner stage >1) pubertal groups based on physical examination at the time of the scan. Results indicate significant gender effects (male > female), most pronounced in the anterior midbody and splenium, areas implicated in attention deficit hyperactivity disorder and dyslexia. Age effects were significant for pre-pubertal but not post-pubertal groups.

LEARNING AND MEMORY: SYSTEMS AND FUNCTIONS V

408.1

SINGLE UNIT MAPPING OF THE CEREBELLAR DEEP NUCLEI IN WELL TRAINED RABBITS FOLLOWING NM CONDITIONING.
J. Tracy*& R.F. Thompson. Neurosciences Program, University of Southern California, Los Angeles, CA 90089-2520.

We have previously shown that the lateral interpositus and medial dentate nuclei of the cerebellum receive convergent projections from peripheral auditory and somatosensory stimuli (Tracy et al., 1991, Neurosci. Abs). Convergence of these stimuli (1 KHz tone and a corneal airpuff) is expected if associational mechanisms for classical conditioning of the rabbit nictitating membrane (NM) response are present in these structures. Single cells which respond to either the tone, the airpuff, or both, are congregated near the lateral edge of the anterior interpositus nucleus and the medial dentate. The single units which respond to both types of stimuli are located in the dorsal-most aspect of this region.

Currently, unit recordings in the well training animals, those who have reached a learning criteria of 90% CRs and received one day of overtraining, indicate that specific regions of the cerebellar nuclei respond to the training stimuli with a neural model time-locked to the conditioned behavior. These responsive areas appear to be in the same columnar region of the lateral interpositus/medial dentate nuclei in which convergent responses to peripheral stimuli are found, but tend to be more ventral. These responses tend to be biphasic, with the first part of the neural model preceding the CR and showing an onset latency which predicts the onset of the blink response. (Supported by ONR N0001488K0112 & NSF BNS-8718300 to R.F.T.)

408.2

EFFECTS OF MOTOR CORTEX LESIONS ON ACQUISITION OF TRACE EYEBLINK CONDITIONING.
D. Ivkovich* and R.F. Thompson. Neurosciences Program, University of Southern California, Los Angeles, CA 90089-2520

Rabbits were classically conditioned using an auditory click conditioned stimulus (CS) followed by a 500 ms trace interval and an airpuff unconditioned stimulus (US; 100 ms, 3 psi). Seven rabbits received bilateral motor cortex lesions and 7-10 days recovery prior to training. Six surgically naive control animals underwent identical behavioral training procedures. Each training session consisted of 108 trials, 12 blocks of 1 CS-alone trial followed by 8 paired CS-US trials. There was no significant difference in learning rates of the two groups (average trials-to-criterion= 962). First, middle and criterion days of training were compared using a between groups repeated measures ANOVA. Lesions did not affect percent conditioned responses or measures of conditioned or unconditioned response amplitude. There was a significant increase across days for all these measures. Reflexive eyeblinks to 4 different US intensity levels (1, 2, 3, & 4 psi) measured in lesioned animals exhibited characteristic enhancement over the course of training and were unaffected by motor cortex lesions. A significant effect of lesion was observed, however, for conditioned response latency on CS-alone test trials ($F_{1,10}$=6.5). Onset latencies were consistently slower for lesioned animals. Although motor cortex lesions have been reported to prevent acquisition of short-latency conditioned responses in cats (Woody et al., 1974), the current study suggests these same lesions have no effect on learning of the trace eyeblink response in rabbits, though performance may be slightly affected. (Supported by NSF BNS-8718300 and ONR N0001488K0112 to R.F. Thompson)

408.3

CEREBELLAR CORTICAL STIMULATION AS BOTH CS AND US IN CLASSICAL CONDITIONING. P.G. Shinkman*, R.A. Swain, and R.F. Thompson. Neurosciences Program, Univ. Southern California, Los Angeles, CA 90089-2520.

Previously we reported classical conditioning of discrete motor responses of the facial and neck musculature a) when electrical stimulation of white matter underlying cerebellar lobule HVI is used as the US, and b) when weak electrical stimulation of cerebellar cortex, producing excitation of parallel fibers but no observable behavioral response, is used as the CS in conjunction with the white matter electrical US. These findings point to critical roles for both cerebellar cortex and deep cerebellar nuclei in classical conditioning, and suggest that such conditioning is mediated by an interaction between cortical and subcortical cerebellar circuits. The present experiment was designed to further establish and elucidate the nature of cortical participation in the conditioning process. New Zealand white rabbits were implanted chronically with bipolar stimulating electrodes placed within the cerebellar cortical layers. Threshold measurements were made for the elicitation of discrete motor responses. During subsequent conditioning trials, subthreshold stimulation was used as the CS and suprathreshold stimulation through the same electrode was used as the US. In this preparation, we observed conditioning, extinction upon the introduction of CS-alone trials, and reacquisition with savings. These results, together with previous findings, strongly implicate cerebellar cortical efferent connections to the deep cerebellar nuclei in the neuronal plasticity manifested in classical conditioning. (Supported by NSF BNS 8718300, ONR N00014-88K-0112, and the McKnight Foundation, to R.F. Thompson, and by the BDRC, Univ. North Carolina.)

408.4

BACLOFEN INFUSION INTO THE CEREBELLUM REVERSIBLY BLOCKS RETENTION AND ACQUISITION OF THE CLASSICALLY CONDITIONED EYEBLINK RESPONSE IN THE RABBIT. A. E. NORDHOLM*, O.A. RAMIREZ, D. J. KRUPA & R. F. THOMPSON, Neurosciences Program, University of Southern California, Los Angeles, CA 90089-2520.

It has been recently reported (Krupa, et al., *Science*, 1993) that cerebellar infusion of the GABA-A agonist muscimol reversibly blocks acquisition and retention of the classically conditioned eyeblink response. In the present study we sought to determine what role the GABA-B receptor has in this learning paradigm.

All animals were implanted with cannulae aimed at the dorsolateral anterior interpositus nucleus. In well trained animals, infusions of a GABA-B agonist baclofen completely blocked retention of the conditioned eyeblink response. This effect was eliminated 24 hours later.

During the acquisition phase of the experiment animals received 6 days of training, 3 days of infusion with baclofen(N=5) or saline(N=5) and 3 days of no infusion training. The baclofen group showed no learning on days 1-3 (infusion) and no savings on day 4. The rate of acquisition for the baclofen group on days 4-6(no infusion) was similar to that of the saline group on days 1-3. Infusion of ^{3}H-baclofen revealed that the baclofen remained in the cerebellum. Furthermore, the effect of baclofen was partially reversed with the GABA-B antagonist phaclofen, suggesting that the effect of the baclofen were receptor specific. This result is consistent with a large body of literature that implicates the cerebellum as the site for the formation, storage and retrieval of the classically conditioned eyeblink response. (Supported by ONR N0001488K0112 & NSF BNS-8718300 to R.F.T.)

408.5

EFFECTS OF REVERSIBLE LESIONS ON EXTINCTION OF THE RABBIT'S CLASSICALLY CONDITIONED EYEBLINK RESPONSE. D. J. Krupa*, R. F. Thompson. Neurosciences Program, Univ. of So. Calif., LA, CA 90089.

Reversible inactivation with muscimol was used to identify neural substrates which might mediate extinction of the rabbit's conditioned eyeblink response. Naive rabbits, implanted with cannulae aimed at (i) ipsilateral interpositus (IP), (ii) contralateral red nucleus (RN), or (iii) dorsal aspect of the ipsilateral facial nucleus (FN), were well-trained using tone-airpuff conditioning. Following acquisition, rabbits received injections of muscimol into either the IP, RN, or FN prior to the first 3 or the first 6 days (only IP and FN) of CS alone extinction training. Muscimol infusions completely abolished CRs during these extinction sessions. Controls received saline alone infusions into the IP. Following these sessions, all animals received 4 additional extinction sessions without infusion followed by 1 session of reacquisition training (paired tone-airpuff). Controls extinguished to baseline responding by day 4. Subjects that received muscimol in the IP for 3 days responded with significantly fewer CRs on day 4 than controls on day 1, indicating that some extinction had occurred. Animals infused with muscimol into the RN or FN for 3 days responded (on day 4) at levels equal to day 1 of controls and subsequently extinguished at rates identical to days 1-4 of controls. Animals infused with muscimol in the IP for 6 days were responding (on day 7) at baseline levels, equivalent to day 7 of controls; they appeared to have completely extinguished. In marked contrast, animals infused with muscimol into the FN for 6 days responded (on day 7) at levels equivalent to day 1 of controls and subsequently extinguished at rates equal to days 1-4 of controls; extinction was completely prevented by FN muscimol inactivation. All animals robustly reacquired the CR on the reacquisition day. Disruption of the tone CS by muscimol infusions into the FN is unlikely: infusions of 3H-muscimol do not label structures of the CS pathway and identical infusions have no effect on the animal's ability to acquire the CR. Muscimol infusion into the IP, which has been shown to completely block acquisition of CRs, does not appear to block extinction; whereas infusions into the FN, which do not block acquisition of CRs, do prevent extinction. These results are consistent with the hypothesis that extinction results in the formation of a separate memory and indicate that areas of the brainstem in and around the FN may be critically involved in this form of learning. Support: NSF BNS-8718300, ONR N0001488K0112 & McKnight to RFT.

408.6

INACTIVATION OF MOTOR NUCLEI BLOCKS EXPRESSION BUT NOT ACQUISITION OF RABBIT'S CLASSICALLY CONDITIONED EYEBLINK RESPONSE. J. K. Thompson*, D. J. Krupa, J. Weng, & R. F. Thompson. Neurosciences Program, University of Southern California, Los Angeles, CA 90089.

The facial (FN) and accessory abducens (ACC) nuclei were reversibly inactivated by microinjections of muscimol in order to further study their role in eyeblink conditioning. Naive rabbits, implanted with cannulae aimed at the dorsal aspects of the ipsilateral FN, received injections of muscimol (0.4 µg in 0.4 µl saline) prior to the start of the first 6 days of tone-airpuff conditioning (airpuff: 3 psi; ISI 250 ms). Muscimol completely inactivated facial musculature including eyelid reflexes and also abolished eyeball retraction, indicating that both the FN and ACC were inactivated. Both conditioned (CRs) and unconditioned responses (URs) were completely blocked throughout the 6 infusion sessions (measured via minitorque potentiometer attached to nictitating membrane with suture). On day 7, training was continued but no infusion administered. Rabbits performed CRs at asymptotic levels from the start of training; they had fully learned the CR despite having not performed any CRs or URs during previous training sessions. Control rabbits infused with saline showed no impairment in CR or UR amplitude and had fully learned the CR by day 3. Infusions of 3H-muscimol following training indicated that the drug diffused throughout the FN, the ACC, and neighboring regions of reticular formation. These results demonstrate that the association between the tone and the airpuff cannot be formed or stored in the motor nuclei or surrounding regions of reticular formation, since inactivation of these regions has no effect on the rabbit's ability to learn the CR.

Muscimol injections into 2 other rabbits significantly reduced but did not completely abolish the UR. These rabbits acquired the CR normally, i.e., both reached learning criterion by day 2. Interestingly, the CR amplitude was equal to or greater than the UR amplitude, indicating that the strength of the neural input to the motor nuclei which drives the CR is equivalent to that which drives the UR.

Support:NSF BNS-8718300, ONR N0001488K0112 & McKnight to RFT.

408.7

THE EFFECT OF HIPPOCAMPAL LESIONS ON THE RETENTION OF A TRACE EYEBLINK CONDITIONING. J. J. Kim*, R. E. Clark & R. F. Thompson, Neurosciences Program, University of Southern California, Los Angeles, CA 90089-2520.

Water-deprived New Zealand male rabbits underwent trace eyeblink conditioning. Animals were presented daily with 100 pairings of a tone (250 ms) followed by an airpuff (100 ms) with a 500 ms trace period between the termination of the tone and the onset of the airpuff and an inter-trial interval of 40 ± 10 s. Water deprivation considerably facilitated learning on the trace paradigm compared to non-water deprived subjects. Upon learning, animals received hippocampal aspirations on that day. After 7 postoperative days of recovery, rabbits were retested on the trace paradigm. Our results show that hippocampal lesions completely abolished adaptive conditioned responses (CRs). These same animals, however, acquired CRs on a standard delay eyeblink conditioning. Thus, the hippocampus appears to be important for the retention of trace CRs.

408.8

ABOLITION OF THE RABBIT NICTITATING MEMBRANE CONDITIONED RESPONSE BY REVERSIBLE INACTIVATION OF BRAIN STRUCTURES. Georges Tocco, Oscar Ramirez, Alan Nordholm, Michel Baudry and Richard F. Thompson*. University of Southern California, Neurosciences Program, Los Angeles California 90089-2520.

While the cerebellum is necessary and sufficient for the learning of the classical conditioning of the nictitating membrane in the delay paradigm, the hippocampus is required for the learning in a trace paradigm when at least 300 msec separate the end of the CS and US onset. The relative importance of these two brain structures for the retention of the learned response in a trace paradigm are however not well understood. In an attempt to answer this question, reversible inactivation of the hippocampus or the interpositus nucleus were achieved by infusion of different drugs (lidocaine, muscimol, CNQX, AP5, ...)

Male New Zealand white rabbits were implanted with one cannula in both hippocampi as well as a cannula in the interpositus nucleus ipsilateral to the stimulated eye. Rabbits were then trained on a trace paradigm until they reached criterion (8 out of 9 consecutive trials). Retention of the conditioned responses were subsequently tested after infusion of different drugs. Infusion of lidocaine or CNQX, an AMPA receptor antagonist in both hippocampi abolished the conditioned response, leaving the unconditioned response intact. This effect seem to decrease in time. Abolition of the conditioned response is also obtained by infusion of CNQX, muscimol (a $GABA_A$ receptor agonist) or lidocaine in the interpositus nucleus.

Our results further stress the importance of the hippocampus as well as the cerebellum for the expression of a learned response in a trace paradigm. This work was supported by ONR N00014-91-G-1796 to MB and ONR N00014-88-K-0112 & NSF BNS-8718300 to RFT.

408.9

LEARNING RELATED UNIT ACTIVITY IN THE CEREBELLAR CORTEX IS ABOLISHED WITH REVERSIBLE INACTIVATION OF THE INTERPOSITUS NUCLEUS. R.E. Clark,* E.B. Gohl, and D.G. Lavond. Neuroscience Program, University of Southern California, Los Angeles, CA 90089-2520.

Learning related activity develops in the cerebellar cortex, over the course of classical conditioning of the rabbit eyeblink response, which has a similar shape and time course as the learned behavioral response and precedes this response in time (unit model). This cortical activity could be the source of the essential plasticity associated with this type of learning, or it could receive this information from some other structure. The interpositus nucleus has been implicated as the essential site for this plasticity. A cooling probe was implanted just lateral to the interpositus nucleus to create a reversible lesion of that structure. Rabbits were classically conditioned using our standard paradigm. A microelectrode driver was used to search the contralateral and ipsilateral cerebellar cortex for learning related activity. A reversible lesion was created in the interpositus by activating the cooling probe and the cerebellar cortical activity was then reassessed. Our results show that learning related unit activity developed in cerebellar cortex bilaterally and that interpositus inactivation abolished both the learned behavioral response and the associated unit activity. Some recordings from the anterior ipsilateral cortex showed tone evoked responses followed by a unit model. During cooling the unit model is abolished while the tone evoked response remains unchanged. These results suggest that the cortex receives the learning related activity from the interpositus and cooling does not prevent auditory information from reaching the cortex.

408.10

LEARNING RELATED UNIT ACTIVITY IN THE LATERAL PONTINE NUCLEI IS ABOLISHED WITH REVERSIBLE INACTIVATION OF THE INTERPOSITUS NUCLEUS. E.B. Gohl, R.E. Clark, and D.G. Lavond*. Neuroscience Program, University of Southern California, Los Angeles, CA 90089-2520.

Several studies have implicated the lateral pontine nuclei as a relay for auditory conditioned stimulus (CS) information. However, other studies have shown that learning related activity develops in this area over the course of training. This suggests that the pontine nuclei could be involved with the learned response, or simply "informed" of the learned response by some afferent structure. The interpositus nucleus has been implicated as the essential site of plasticity associated with this type of learning, therefore it could serve as the source of the learning related activity seen in the pontine nuclei. A cooling probe was implanted just lateral to the interpositus nucleus to create a reversible lesion of that structure. Rabbits were classically conditioned using our standard paradigm. A microelectrode driver was used to search the lateral pons for learning related activity. A reversible lesion was then created in the interpositus by activating the cooling probe and the pontine activity was then reassessed. Our results show that in well trained animals, some recordings from numerous regions of the lateral pons showed a clear tone evoked phasic response followed by unit activity that modeled the learned behavioral response. During interpositus inactivation the learned behavioral response and the unit model were abolished, while the tone evoked response remained unchanged. These data lend further support to the notion that the lateral pontine nuclei relay auditory CS information to the cerebellum and provides new evidence that this region is simply informed of the learned response by the interpositus nucleus.

408.11

DIFFERENTIAL EFFECTS OF HEMICEREBELLECTOMY PERFORMED ON POSTNATAL DAY (PND) 10 OR 20 ON EYEBLINK CONDITIONING IN THE DEVELOPING RAT. J.H. Freeman, Jr.[2]*, C.S. Hendrix[2], & M.E. Stanton,[1,2] [1]Neurotoxicology Div., US EPA, RTP, NC 27711; [2]Psychology Dept., UNC, Chapel Hill, NC 27599.

The present study was undertaken to further characterize the role of the cerebellum in the ontogeny of eyeblink conditioning (EBC) in the developing rat (Stanton, *et al.*, 1992, *Behav. Neurosci., 106*, p. 657; Freeman & Stanton, 1992, *Soc. Neurosci. Abs., 18*, p. 1559). In the adult rabbit, lesions of the cerebellum ipsilateral to the trained eye abolish EBC, while leaving conditioning with the contralateral eye intact (Lincoln *et al.*, 1982, *Brain Res., 242*, p. 190). We sought to determine whether cerebellar circuitry supporting EBC in the developing rat is also lateralized. Evidence from other studies has shown that cerebellar projections to the red nucleus are altered by hemicerebellectomy performed around PND10 but not after later lesions. Thus, early cerebellar lesions may result in reorganization of cerebellar projections that are detrimental to EBC performance. In Experiment 1, pups were given sham surgery, lesions of the contralateral or ipsilateral cerebellar hemisphere on PND10, and given EBC training on PND24 (see Freeman & Stanton, *ibid*, for methods). Pups given lesions of either the ipsilateral or contralateral hemisphere were impaired on EBC. This experiment led to the notion that lesions given early in development may produce different effects on EBC than lesions performed in more mature animals. In Experiment 2, lesions of the ipsilateral or contralateral cerebellar hemisphere were performed on PND10 or 20 and pups were trained on EBC on PND34. Pups given ipsilateral cerebellar lesions on either PND10 or 20 were impaired on EBC. In contrasts, pups given contralateral cerebellar lesions on PND10 were impaired on EBC, while pups given the same lesion on PND20 were unimpaired. Early hemicerebellectomy may produce reorganization of cerebellar projections that impairs EBC.

408.12

ONTOGENY OF LATENT INHIBITION OF EYEBLINK CONDITIONING IN THE RAT. C.S. Hendrix[2], J.H. Freeman, Jr.[2], & M.E. Stanton[1,2]*, [1]Neurotoxicology Div., US EPA, RTP, NC 27711; [2]Psychology Dept., UNC, Chapel Hill, NC 27599.

Eyeblink conditioning may reveal how interactions between the developing hippocampus and cerebellum contribute to the ontogeny of learning. The cerebellum appears to subserve delay conditioning of the eyeblink response. While the hippocampus is not critically involved in the acquistion of delay conditioning, it may interact with the cerebellum to modulate the acquisition of "higher order" eyeblink conditioning phenomena such as latent inhibition (LI). This study sought to determine if there is an ontogenetic profile to LI in eyeblink conditioning, e.g., is there a point in development when delay conditioning is present in the absence of LI. Rats aged 20, 24, and 32 days postnatal were selected for testing as this represents ages which precede, coincide with, and follow emergence of delay conditioning in the developing rat (Stanton *et al.*, 1992, *Behav. Neurosci., 106*, p. 657). Rats at each age were randomly assigned to groups which received either 450 trials of preexposure to a 2.8KHz 80dB tone CS, or chamber exposure only, followed by 150 trials of delay conditioning in which a 380ms CS preceded and co-terminated with a 100ms eyeshock US (see Stanton *et al., ibid.*). CR amplitude was compared between the groups at each of the ages to assess strength of conditioning. In 24- and 32-day-olds the degree of LI was equivalent with the CS preexposed group showing impaired conditioning relative to the non-preexposed group. However, the 20-day-olds showed a reversal of this effect as the CS-preexposed group showed facilitation of delay conditioning relative to the non-preexposed group. These results show that LI of eyeblink conditioning can be demonstrated in the developing rat and is present at a point in development when delay conditioning first emerges. Further studies of this kind may help reveal how the developing cerebellum and hippocampus interact in the ontogeny of learning and memory.

408.13

RED NUCLEUS PROJECTIONS TO CEREBELLAR CORTEX: IMPLICATIONS FOR CLASSICAL EYEBLINK CONDITIONING. M.E. Rosenfield and J.W. Moore* Dept. of Psychol., Univ. of Mass., Amherst, MA 01003.

Conditioned eyeblink responses are presumably learned in the cerebellum and relayed to motoneurons via the red nucleus (Thompson, *Science*, 233:941, 1986). Projections from the red nucleus to cerebellar cortex (Larsell's HVI) could be important for shaping temporally adaptive features of conditioned responses (Moore et al, *Biol. Cyber.*, 62:17, 1989). We implanted WGA-HRP (Sigma L3892) unilaterally into HVI in 8 albino rabbits (Mori et al, *Brain Res. Bull.*, 6:19, 1981). The pipette remained *in situ* for 45 hours before sacrifice. Animals were perfused transcardially (descending aorta clamped) with approximately 2 L of .9% saline followed by .5 L of 10% formalin and then 3 L of 12% sucrose solution at 4 degrees C. Brains were blocked immediately on extraction (saving only the brain stem and cerebellum), placed in 30% sucrose in .1 M phosphate buffer (pH = 7.2), and stored at 4 degrees C for 20 h. The cerebellum was embedded in gelatin. Frozen sections were cut transversely at 60 μ, mounted on subbed slides, and reacted with TMB. Implantations met our criterion for inclusion if (a) diffusion did not involve cerebellar deep nuclei; (b) retrogradely labeled neurons were seen in the pontine nuclei, spinal trigeminal nucleus par oralis, and the dorsal accessory olivary nucleus. Four criterion cases showed an average of 16 retrogradely labeled cells in contralateral red nucleus at the level of the 3rd nerve within subregions implicated in eyeblink conditioning by lesioning (Rosenfield & Moore, *Behav. Brain Res.*, 10:393, 1983) and recording (Desmond & Moore *Neurosci. Res.*, 10:260, 1991) studies. These observations are consistent with previous reports (Dietrichs & Walberg, *Exp. Brain Res.*, 50:353, 1983; Rosenfield & Moore, *Soc. Neurosci. Abstr.*, 17:870, 1991) and also with the hypothesis that learning in the red nucleus is precursor to learning in the cerebellum. (Supported by AFOSR grant 92-NL-033)

408.14

CLASSICAL EYELID CONDITIONING IN RABBITS WITH TEMPORAL UNCERTAINTY. J-S Choi*, M.J. Hirl, and J.W. Moore. Neuroscience and Behavior Program, University of Massachusetts, Amherst, MA 01003.

In order to challenge and extend real-time computational models of CR waveforms (e.g., Desmond & Moore, *Biol. Cybern.* 58: 405, 1988), we investigated the timing of conditioned eyelid movement as a function of temporal uncertainty. In two experiments, rabbits were trained with randomly varying ISIs with a mean ISI of 500 ms. The CS was a 300-ms 1-kHz 80-dB tone, and the US was a 2.5-mA 1-ms dc pulse to the periocular tissue of the right eye. There were 100 trials/session at a rate of 3/min, with every 10th trial a CS-alone probe. Independent variables were W, the range of possible ISIs in milliseconds, and m, the number of possible ISIs within W. Dependent variables included CR latency (L), time of peak CR amplitude (P), and movement time (MT = P – L).

In Experiment 1, W = 0, 50, 100, 200, and 400. For W = 0, m = 1; for W $\geq$ 50, m = W/2. Each of 4 animals experienced all Ws. There were 12 sessions under the initial W (W = 0 or 400) and 10 for each subsequent W. The results from the last 5 sessions/W were: (a) probe-trial CR topographies spanned W; (b) MT was constant for W = 0 and 50 but increased linearly with W for W $\geq$ 100, with P increasingly overshooting the mean ISI of 500 ms; (c) Pearson correlation coefficients between L and P, computed for each animal from probe trials, averaged .25 and were unrelated to W.

In Experiment 2, W = 400 and m = 2, 3, 4, or 5 for different animals. Probe-trial CR topographies after 20 sessions spanned W. They were bimodal for m = 2 (ISIs 300 and 700) but unimodal for m = 3-5, and P was an inverted-U function of m. On CS–US trials, CRs began to return to baseline immediately upon the occurrence of the US, suggesting it had become a conditioned inhibitor. (Supported by NSF grant BNS 8810624 and AFOSR grant 92-NL-033)

408.15

THE CEREBELLUM AND RED NUCLEUS ARE NOT REQUIRED FOR CLASSICAL CONDITIONING OF AN IN VITRO MODEL OF THE EYE-BLINK REFLEX. J. Keifer.* Dept. of Physiology, Northwestern Univ. Medical School, 303 E. Chicago Ave., Chicago, IL 60611.

The role of the cerebellum in the acquisition of conditioned responses has been a controversial issue. Lesion data from behaving animals have suggested that the interpositus nucleus is an essential component of the neural circuitry involved in acquisition, while the role of the cerebellar cortex has been more difficult to define. Alternative interpretations view these findings as more consistent with the general involvement of the cerebellum in movement control, rather than in learning. In previous reports we have introduced the in vitro turtle brainstem-cerebellum preparation as a model system in which to study the classically conditioned eye-blink response (Soc. Neurosci. Abs. 16: 763, 1990; 18: 1560, 1992). In the present study, the minimal amount of tissue required to support conditioning was examined. The entire cerebellum, including the cerebellar nuclei, and the red nucleus bilaterally, were surgically removed. The preparation was presented with the standard delay training protocol of a 1 s CS to the auditory nerve immediately preceding a single shock US to the trigeminal nerve. In 3 of 7 preparations tested, acquisition of conditioned abducens nerve responses was recorded. Extinction of CRs was produced by applying control stimuli of alternate CS and US. These preparations were generally unstable, producing highly variable responses and having a shortened period of viability. Interestingly, 2 preparations that demonstrated conditioning also exhibited UR suppression. These data suggest that the cerebellum and red nucleus are not embedded in the *essential* neural pathways that support acquisition of CRs, although they are likely to play some role. The results emphasize the importance of examining the basic eye-blink reflex pathway itself and its connections with the reticular formation as potential sites of synapse modification. Moreover, the results do not support theories that UR suppression is mediated by the red nucleus as this phenomenon was observed in the absence of this neuronal structure. Supported by NSF BNS-9109572.

408.16

THE IMPORTANCE OF EQUATING FLEXION FORCE WHEN STUDYING "INSTRUMENTAL LEARNING" IN SPINALIZED RATS. D.G. Barstow, J.W. Grau, & M.W. Meagher*. Dept. of Psychology, Texas A&M Univ., College Station, TX 77843.

Evidence suggests spinal mechanisms are capable of supporting instrumental conditioning . In these studies, one group of spinalized rats (Exp) can terminate hindlimb shock by exhibiting a flexion response. A "Yoked" group (Yok) experiences the same shock irrespective of leg position. Although we were previously able to obtain an Exp/Yok difference (Parker et al., Neurosci. Abs., 18, 1026) we failed to replicate important details of past studies. These include: 1) an increase in response duration in Exp Ss; and 2) poor learning after non contingent shock (a "learned helplessness" effect). In the present study we show that these effects are observed if response force is matched prior to training.

A day after subjects (N=24) received a spinal transection at T2, they were placed in restraining tubes. For half the subjects, shock intensity was then adjusted so that it elicited a flexion with a force of 0.4 N; the other half experienced a shock intensity required to elicit a flexion of 0.6 N. Subjects then received 30 m of contingent shock (Exp), non contingent shock (Yok), or no shock (Unshk). Subjects in the Exp group, but not the Yok group, exhibited leg flexions that steadily increased in duration across training. At the end of training, response force was again equated across subjects. All subjects then received 30 m of contingent shock. Both the Exp and Unshk groups acquired the flexion response whereas subjects in the Yok group did not. Supported by MH48994 to J.W.G.

LEARNING AND MEMORY: SYSTEMS AND FUNCTIONS VI

409.1

PET STUDIES OF A CUED RECALL EXPLICIT MEMORY TASK. R.L. Buckner*, S.E. Petersen, F.M. Miezin, J. Ojemann, L. Squire†, and M.E. Raichle. Washington Univ. Sch. of Med., Box 8111, St. Louis, MO 63110. †UCSD Dept. of Psychiat., VA Med. Ctr., San Diego, CA, 92161.

Areas of the normal human brain used during an explicit memory task were identified using positron emission tomography (PET). In three independent experiments, scans were obtained during a RECALL task in which subjects explicitly retrieved prescan study words using uppercase visual word stems as cues. A BASELINE task, in which subjects produced the first word that came to mind when presented with word stems, and a FIXATION task were used as control conditions. Across experiments the format of the study words was varied. In the "Case-Same" experiment, study words (COURSE) were presented in the same uppercase visual font as the stem cues (COU). In the "Case-Change" experiment, study words (course) were presented in a lowercase visual font. In the "Auditory" experiment, study words were presented aurally. As compared to the FIXATION task, the BASELINE task activated several regions in response to the reading and production demands of the task including a region in left lateral prefrontal cortex (at or near area 44 or 45). Across all experiments, the RECALL task activated the same regions as the BASELINE task, and in addition activated a region in anterior right frontal cortex (at or near area 10). None of the three experiments produced left medial temporal lobe activation. An activation in the right hippocampal region (including the parahippocampal gyrus) was observed only in the Case-Same experiment where study words and recall cues were visually identical. These findings show that the frontal lobes play a functional role in explicit, cued-recall across three different experiments. Medial temporal lobe activation was detected in only one of the three experiments.

409.2

PET STUDIES OF A WORD STEM COMPLETION IMPLICIT MEMORY TASK. S.E. Petersen*, R.L. Buckner, L. Squire†, F.M. Miezin, and M.E. Raichle. Washington Univ. Sch. of Med., Box 8111, St. Louis, MO 63110. †UCSD Dept. of Psychiat., VA Med. Ctr., San Diego, CA, 92161.

In the preceding abstract, we reported data from three PET experiments which studied cued-recall explicit memory tasks based on word stems. In two of these experiments, an implicit memory version of the stem completion task was also studied (PRIMING). In the PRIMING task, subjects produced the first word that came to mind when presented with uppercase visual word stems (IDE). Half of the stems were the beginnings of words from a study list presented three minutes prior to the scan. In the first "Case-Same" experiment, study words were visually identical to test stems (IDEAL). In the second "Case-Change" experiment, study words were visually different from test stems (ideal). Subjects produced 71% and 53% of the study words, respectively, during the two PRIMING tasks. When the PET data from the two experiments were combined, no regions increased blood flow in the PRIMING task compared to the BASELINE control task described in the previous abstract. Regions in bilateral occipital temporal cortex showed blood flow reductions, which appeared approximately symmetrical in magnitude. When experiments were analyzed independently, right lateralized reductions were larger in magnitude than left reductions for both experiments. Post-hoc inspection of the data revealed that the right posterior reduction localizes to two slightly different regions in the two experiments and thus appeared attenuated when data were combined. Behavioral data indicated that study words were produced more quickly than novel words. These blood flow reductions and behavioral data suggest a neurobiological effect of priming: following exposure to a stimulus, subsequent perceptual processing is more efficient, producing quicker response times and requiring less neural activity.

409.3

REGIONAL CEREBRAL BLOOD FLOW (rCBF) DURING ACQUISITION OF PROCEDURAL MEMORY. J. Metz*, J. Singh, J.D.E. Gabrieli, D. B. Willingham, D. Dooley, M. Jiang, C.-T. Chen, and M. Cooper. University of Chicago, Chicago, IL. 60637.

The serial reaction time (SRT) task measures a pattern-specific form of sensorimotor skill learning and retention. SRT learning is considered to be implicit or procedural and to depend upon a fronto-striatal network. We examined the neural network underlying normal SRT learning by using O-15 labeled water in PET studies to measure rCBF at different stages of SRT learning.

11 young adults performed the SRT while lying in a PETT VI scanner. Subjects pressed buttons in response to boxes changing colors on a computer screen. Initially, subjects saw a block (144 trials) in which the 4 horizontally displayed boxes turned on randomly (R1). This was followed by 7 blocks in which boxes turned on according to a 12-unit repeating pattern (P1 through P7). Finally, there was another random block (R2). rCBF was measured during blocks R1 and R2, and P1, P3, P5, and P7.

RT decreased from R1 through P7 by 154 msec, then increased again at R2 by 96 msec. Subjects reported little awareness that there was any pattern in the blocks. Image data obtained during the performance of each block were masked, normalized, correlated with MRI, transformed into Talairach space, and averaged across subjects. Averaged images from R2 were subtracted from the averages for each of the other blocks. Bilateral regions of the medial and lateral frontal cortex, thalamus, and basal ganglia showed gradual increases in rCBF from P1 through P7. The anterior cingulate was active throughout the pattern scans. Visual cortex was active initially (R1 and P1), then decreased before increasing slightly at P7.

The correlation between SRT learning and sites of apparently progressive neural activity are consistent with patient findings in defining a fronto-striato-thalamic loop as a critical neural substrate for the acquisition and retention of procedural sensorimotor skill in humans.

409.4

CHANGES IN REGIONAL CEREBRAL BLOOD FLOW ASSOCIATED WITH A NON-PATTERNED PROCEDURAL MEMORY TASK. J. Singh*, J. Metz, J. D.E. Gabrieli, D. B. Willingham, D. Dooley, M. Jiang, C-T. Chen, & M. Cooper. The University of Chicago, Chicago, IL 60637.

In a companion abstract, "Regional Cerebral Blood Flow (rCBF) During Acquisition of Procedural Memory;" Metz et al., we reported that the changes associated with the acquisition of a procedural memory skill receive major contributions from the fronto-striatal-thalamic loop. We now report the rCBF changes associated with a non-patterned procedural memory task that does not involve an imbedded pattern.

We used our previous serial reaction time (SRT) paradigm in which subjects see four boxes displayed on a computer screen. Subjects are required to push buttons corresponding to a box which changes color. In that experiment, the subjects were exposed to 1 random and 7 repeating-sequence patterns, and another random block. In the present study, the subjects were exposed only to random blocks (144 trails per block; total number of blocks: 9). We studied 3 healthy normal young subjects (age range, 23-36 yrs). Subjects performed the task while lying in the PET scanner. A bolus of 60-80 mCi O-15-labeled water was injected 20 sec before the start of blocks Ran-1 to Ran-9; 10 sec after injection, a 50-sec PET scan was begun. Image data were normalized, tranformed into Talarirach space, masked, and averaged across all subjects.

Behavioral data indicated that there was a small, but steady decrease in the reaction time (with a slope only about 1/3 as steep as in the patterned SRT paradigm), but in the current experiment, the increase in the RT during the last random block observed in the SRT-patterned paradigm was absent. Imaging results indicated that the thalamus and basal ganglia were active as in the first study, but there was no activation in the frontal cortex. These pilot data raise the possibility of a major contribution of the frontal cortex to the pattern-specific memory, whereas the simple sensori-motor task may depend upon the thalamic-striatal loop.

409.5

FUNCTIONAL ACTIVATION OF RIGHT HIPPOCAMPUS DURING AN OLFACTORY RECOGNITION MEMORY TASK. M. Jones-Gotman*, R.J. Zatorre, A.C. Evans and E. Meyer. Montreal Neurological Institute, McGill University, Montreal, Quebec, Canada, H3A 2B4.

The neural substrate of human olfactory memory was studied by examining cerebral blood flow (CBF) changes with positron emission tomography. In the experimental condition, nine normal right-handed subjects were scanned while they smelled a series of odorants birhinally and indicated, by pressing a key, which ones had been presented earlier. The baseline condition consisted of inhalation and key pressing but with no odor present. Anatomical localization was provided by matched magnetic resonance imaging. Subtraction of the obtained images indicated CBF increases bilaterally in the piriform cortex and unilaterally in the right orbitofrontal cortex, replicating our earlier observations, and suggesting a relative functional asymmetry in olfactory processing. In addition, a region corresponding to the hippocampus and parahippocampal gyrus on the right side also demonstrated increased CBF, despite birhinal presentation. This area, not activated during passive odor inhalation, is likely involved specifically in some aspect of olfactory memory, in agreement with our findings from patients with excision in the right medial temporal region.

409.6

EFFECTS OF SEMANTIC DISTINCTIVENESS ON TEMPORAL ORDER MEMORY IN FRONTAL PATIENTS. J. A. Mangels*, A. P. Shimamura. Dept. of Psychology, University of California, Berkeley, 94720.

Patients with frontal lobe lesions often exhibit deficits on tests of memory for temporal order. However, recent studies demonstrate that memory for temporal order can be improved to the level of control subjects by encoding manipulations aimed at increasing distinctiveness (e.g. subject performed tasks). To date, stimulus distinctiveness has not been manipulated along the semantic dimension.

We compared memory for temporal order in 7 patients with dorsolateral prefrontal lesions and 12 age and education matched control subjects. Subjects were presented with a serial list of 15 concrete words or pictures. After a 30 second distractor task, subjects reconstructed the temporal order of the list from a random array of the same stimuli. Groups did not differ on memory for temporal order when lists consisted of stimulus items from different categories. However, when categorized lists were used (a list of three items from each of five categories, randomly ordered), the temporal order memory of frontal patients was significantly impaired for both pictures and words. Results suggest that the deficit observed in frontal patients on tests of temporal order memory may be attributed to high susceptibility to semantic interference. Supported by NIH grant AG09055 to APS and NSF graduate research fellowship to JAM.

409.7

WORD LIST LEARNING IN PATIENTS WITH FRONTAL LOBE LESIONS. D.T. Stuss*, M.P. Alexander, C. Palumbo, L. Buckle, L. Sayer and J. Pogue. Rotman Research Institute of Baycrest Centre, Toronto, M6A 2E1; University of Toronto, Toronto, Ontario; Braintree Hospital, Braintree, MA; Boston VAMC.

The effect of frontal lobe damage on verbal learning was explored in 32 patients with localized frontal lesions (13 bilateral, 10 left and 9 right). Patients were given three word lists to learn; the lists differed in their intrinsic semantic organisation. Results: 1) Recognition verbal memory was mildly but significantly impaired for left and bilateral cases; 2) Free recall was impaired in the same two groups; 3) All frontal groups had impaired higher order organisational processes; 4) Perseverations were significant only in the right frontal group. Conclusion: While not 'amnesic', patients with frontal lesions have impaired verbal learning, and this deficit is highly multidetermined due to damage to a variety of frontal processes.

409.8

EARLY BLIND'S SPATIAL REPRESENTATIONS: ROUTES OR MAPS? C. Thinus-Blanc* & F. Gaunet. Lab. of Cognitive Neurosciences, CNRS, 31, Ch. J. Aiguier 13402 Marseille France.

Three groups of subjects (early, late and blindfolded sighted subjects) were submitted to an experiment inspired by Rieser, Guth and Hill (1986)'s study: they were guided from a reference starting location in an unfamilair room toward target places according to a specific path. Then the task consisted to point, then, to walk toward each target location and to verbally estimate the distances between them, even if they have not travelled between some of the places (inference). The same estimations were required after 1)having imagined that they had walked to the opposite corner of the room and 2)having actually moved to the opposite corner.

The early blind participants made much more errors in angle estimate during the pointing task but not when they had to actually walk toward the targets. Deep deficits were also recorded in this group when the task required to imagine oneself pointing from the opposite corner of the room whereas no differences were observed between groups after the subjects had been actually guided to the new place. Finally, unlike the late blind and sighted blindfolded groups which improved their spatial memory performance during the course of the experiment, no learning occured in the early blind group.

These results, together with the data collected during the interviews following the experiment, suggest that early blind persons' spatial strategies rely on proprioceptive memory and not on an overall map which would have allowed greater behavioral flexibility.

409.9

REDUCED WORKING MEMORY CAPACITY IN PATIENTS WITH GLOBAL AMNESIA: EVIDENCE FOR A LIMBIC/DIENCEPHALIC CONTRIBUTION TO WORKING MEMORY PERFORMANCE. J.D.E. Gabrieli*, M.M. Keane, and G.T. Stebbins. Department of Psychology, Stanford University, Stanford, CA 94305 and Memory Disorders Research Center, Boston VA, Boston MA 02130.

Working memory (WM) is a multi-component psychological system that supports the temporary processing and storage of information needed to perform complex cognitive tasks. WM is an important component of human cognitive architecture, and is often used as an explanatory concept for understanding individual differences in problem-solving ability, age-related changes in cognition, the usefulness of tests for personnel selection, and cognitive impairment in patients with frontal-lobe lesions, Parkinson's disease, Huntington's disease, and schizophrenia. Animal studies implicate two major neural-systems constituents of WM: a frontal-striatal component and a hippocampal component. Prior studies with patients confirm the participation of a frontal-striatal component in human WM, but there is little evidence concerning the role of medial-temporal and related diencephalic structures. We examined this issue by administering a test of WM capacity to 10 patients with global amnesia (AMN) (5 with Korsakoff's syndrome, 4 with medial-temporal lesions, and 1 with a thalamic lesion), and comparing their performance with 9 age- and education-matched control (CON) subjects (5 normal controls and 4 alcoholic controls). Subjects answered questions about auditorily presented sentences (processing) while trying to remember the final word of each sentence (storage) for a subsequent recall test. Subjects began with 1 sentence before recall, and proceeded with increasingly larger numbers of sentences (2, 3, etc.) until they failed 2 of 3 trials for a given number of sentences. WM capacity was defined as the longest span (i.e., largest number of sentences) at which subjects recalled the words correctly for at least 2 of the 3 trials. AMN patients (mean of 1.3) had an impaired WM capacity that was about half of that of control subjects (mean of 2.3); patients with medial-temporal or diencephalic etiologies of amnesia performed similarly. The digits spans of the AMN patients were unimpaired. These results suggest that brain structures important for declarative memory play an important role in WM performance. It may be that when information demands on a WM task exceed specialized primary memory buffers in frontal and other cortical regions, information is stored temporarily in secondary declarative memory stores. WM deficits in AMN patients may reflect impairment in the use of declarative memory stores. Supported by ONR grant N00014-92-J-184 and NINDS grant 26985.

409.10

DISSOCIATION BETWEEN TWO KINDS OF VISUAL WORKING MEMORY IN PARKINSON'S DISEASE. B. R. Postle*, S. Corkin, and J. H. Growdon. Department of Brain and Cognitive Sciences and the Clinical Research Center, Mass. Institute of Technology, Cambridge, MA 02139.

Physiological and behavioral evidence from monkeys suggests that visual working memory is divided into two dissociable subsystems, one for remembering the spatial location of stimuli (subserved by dorsal areas of prefrontal cortex, including the principal sulcus), and one for remembering the characteristics of shapes (subserved by ventral and orbital areas of prefrontal cortex). We proposed that visual working memory in humans also comprises spatial and shape recognition components, each having different neural substrates within frontal cortex. We selected subjects with PD to test our hypothesis because PD disrupts the circuitry linking dorsolateral prefrontal cortex, basal ganglia, and thalamus, thus creating a functional deafferentation of dorsolateral prefrontal cortex, while sparing other regions of prefrontal cortex. PD subjects (Hoehn & Yahr Stage II) and age-matched control subjects (NCS) performed two working memory tasks, in which subjects learned arbitrary associations between stimulus pairs, relying on spatial cues in one task and shape cues in the other. On the spatial test, PD subjects made more errors than NCS (p=.05) and required more trials to criterion (p=.05); but on the shape test PD subjects did not differ from NCS, either in errors (p=.26) or trials to criterion (p=.12). These results show a dissociation between two kinds of visual working memory: one for spatial location (impaired in PD) and one for shape recognition (normal in PD). We suggest that working memory systems are domain specific, and not dependent upon a central processor.

409.11

HETEROGENEITY IN MEMORY DISORDER AND LESION SITES WITH ACoA ANEURYSM. S.M. August*[1], P.J. Eslinger[3], L.M. Grattan[1], D. Rigamonti[2], & A. Depatri[2]. Depts. of Neurology[1] and Neurosurgery[2] Univ. of MD Med. Sch., Baltimore, MD, 21201 & [3]Penn State Univ. College of Med., Hershey, PA 17033

Significant learning and memory disturbances have been associated with rupture and clipping of anterior communicating artery (ACoA) aneurysms. Commonly referred to as "basal forebrain amnesia" these acute learning and memory impairments have been ascribed to distortions in the spatial-temporal processing of information. The purpose of this investigation was to prospectively examine a consecutive series of patients with ACoA aneurysms (n=12) during acute recovery (within 1 month). Patients with ruptured aneurysms in different vascular distributions (n=10) comprised the comparison group. Neuropsychological measures included standardized assessments of learning and memory supplemented by experimental procedures designed to probe the spatial-temporal components of memory. Neurobehavioral findings indicated that the ACoA group produced significantly more confabulatory responses than the comparison group. However, there were no significant differences on standard and experimental measures of learning, memory and spatial-temporal ordering for verbal or visual information. Results of neuroanatomic studies indicated that 75% of the ACoA group had lesions outside of the basal forebrain region. The lack of clear cognitive differences between the groups in acute recovery may be related to marked heterogeneity among the ACoA patients with respect to neuroanatomic lesion and performance on cognitive measures. Defining memory disturbance from medical etiology of vascular patients may be problematic in studies of learning and memory.

409.12

THE POSSIBLE CONTRIBUTION OF THE SEPTAL REGION TO HUMAN MEMORY. H. J. Markowitsch[1], D. Y. von Cramon[2] and U. Schuri[2] (SPON: European Neuroscience Association*). [1]Physiolog. Psychology, University of Bielefeld, D-33501 Bielefeld, [2]Dept. of Neuropsychology, City Hospital, Munich-Bogenhausen, D-8000 Munich, Germany.

A particularly well documented, intelligent patient (H.I.) with very selective, minute, but most likely bilateral damage of the basal forebrain including the septal region is presented. Though behavioral progress was found for a number of areas, she remained deficient, especially in long term memory. The severest and largely modality-nonspecific deficits were observed in recall (as opposed to recognition) situations. As a peculiar finding which we would attribute to septal damage, H.I. was mainly involved and affected in tests containing emotional (especially emotionally negative) stimuli, or certain flavors. While this involvement might have helped her in memorizing material judged as positive, it was of negative influence under other circumstances. The septal area may serve as an interface contributing a specific combination of emotional flavor and evaluating (feedback) judgement to a larger (septo-hippocampal-amygdalar) memory and learning processing network.

409.13

THE LATE NEGATIVE SLOW ERP IS ENHANCED PRIOR TO CORRECT SYNONYM RECOGNITION MEMORY PERFORMANCE. D.M. Rice*, R. Bella, A. Pham Andrus Gerontology Center and Program in Neural, Information and Behavioral Sciences, University of Southern California, Los Angeles, CA 90089-0191.

We reported at last year's meeting that a postresponse late negative slow ERP is selectively enhanced by the repetition of words as opposed to pronounceable nonwords in a short term visual recognition memory task (Rice and Locke, SNA, 1992). This suggested that this late negative slow wave enhancement at retrieval relative to encoding reflects a short term reflective memory process related to stimulus meaning. We now present further evidence to support this hypothesis. Sixteen young adults had recognition performance and nose-referenced ERPs recorded during a short term synonym recognition memory task. All words were presented and then followed by their synonyms within 6-18 seconds in a continuous recognition memory paradigm. Subjects made repeat-novel responses on the basis of stimulus meaning. Across subjects, the late negative slow wave over the average of the 19 leads was significantly ($p<0.01$) enhanced at retrieval relative to encoding. This enhancement began at approximately 700 msec poststimulus and continued until about 1100 msec poststimulus; the onset of this enhancement was about 400 msec prior to the average synonym recognition response. Thus, unlike a word recognition memory task, a synonym recognition memory task is associated with a late negative slow wave enhancement prior to the correct recognition memory response. This is consistent with our interpretation that the retrieval-related enhancement of the late negative ERP in a recognition memory task reflects a short term conscious recollective memory process associated with stimulus meaning.

409.14

SPATIO-TEMPORAL DYNAMICS OF HUMAN CORTICAL EEG DURING SOMATOSENSORY PERCEPTION. C. Barczys1* and W.J. Freeman2. 1Biophysics Group and 2Neurobiology Division, Univ. of California, Berkeley, CA 94720.

Previous research in the rabbit olfactory bulb and monkey visual cortex demonstrated that EEG segments recorded from 64-electrode arrays during a discrimination task could be sorted by stimulus type by using the spatial patterns of amplitude of a global carrier waveform. To test whether these results could be extended to human somatosensory perception, a neurosurgical patient fitted for diagnostic purposes with an 8x8 subdural electrode array at spacings of 1 cm on the somatosensory cortex was trained to perform a somatosensory discrimination task. The EEG traces were digitized at 256 Hz, filtered (20-56 Hz), and edited. Segments were classified by stimulus type after Fourier decomposition with a Euclidean-distance measure in 64-space. Classification accuracy of the training sets was 83 ± 4% for the post-stimulus "perceptual" segments versus 66 ± 3% for the pre-stimulus segments, but at chance levels in cross-validation with test sets. A Genetic Algorithm for detecting outliers increased the accuracy on the training sets to 96% but did not improve the cross-validation results. Animated computer graphics of the 8x8 traces and phase portraits showed differing domains of EEG activity in the array with transitions to less chaotic activity during the 200-400 msec post-stimulus time period for perception. The results show that perceptual patterns found in 4x4 mm arrays in animals are not revealed in 8x8 cm arrays in humans.

Supported by grant MH06686 from the National Institute of Mental Health.

409.15

MEMORY IMPROVEMENT BY AN AYURVEDIC CNS-RASAYANA-MENTAT. V. Patel, A. Agarwal,G.P. Dubey, and B. Garg* Department of Pathology and Neurology, I.U.School of Medicine, Indianapolis, IN 46202 and Psychosomatic Biofeedback Laboratory,Banaras Hindu Univ. Varanasi, India.

Ayurveda, a dynamic ancient medical system of India, continues to provide healthcare to over 600 million people of India. The CNS-Rasayana "Mentat" is a mixture of Ayurvedic herbal preparation traditionally used for memory enhancement, relieving anxiety and management of depression and behavioral disorders. We carried out a preliminary double-blind placebo-controlled study to investigate these effects of Mentat. Twenty normal subjects were selected in each of the three age groups (Table), 10 receiving placebo and other 10 receiving mentat. Change in short-term memory quotient (MQ) was measured by word memory test before the initiation and after 3 months of oral treatment. The ingestion of Mentat (4 grams:2 grams morning and evening) brought about significant ($P<0.05$) increase in short-term MQ (Table). There also was significant ($P<0.05$) reduction in anxiety score (determined by Hamilton's scale) and neuroticism index (Eysenck's Inventory Index).

Table: Change in MQ of Adult Subjects Before and After 3 months of Treatment

Treatment	Placebo		Mentat	
Age groups	Before	After	Before	After
15-25 yrs	88.4_3.3	90.1+2.8	89.1+2.6	98.3+1.8*
35-45 yrs	82.4+3.1	83.1+3.4	84.2+2.4	93.9+1.9*
55-65 yrs	82.3+3.6	83.1+2.8	80.75+1.8	89.5+1.6*

These preliminary findings warrant further studies on the use of Mentat in memory dysfunction disorders.

410.1

SPATIAL LEARNING IN FORNIX TRANSECTED AND SEPTAL DAMAGED RHESUS MONKEYS. M.E. Weber, D.L. Rosene*, and M.B. Moss. Department of Anatomy & Neurobiology, Boston University School of Medicine, Boston, MA 02118.

The fornix (Fx) is the principal subcortical efferent pathway from the hippocampal formation (HF). It also carries afferents to the HF including axons from the cholinergic cells of the medial septum/diagonal band (MSDB). Previous studies in monkeys have demonstrated that the hippocampus is important in spatial learning and memory (Mahut, 1972; Parkinson et.al., 1988). In the current study, we attempted to dissociate the effects of Fx transection which disrupts both HF afferents and efferents from an ibotenic acid lesion of the MSDB that removes only the cholinergic input to the HF.

A total of thirteen animals were used for this study. Three sustained transection of the Fx (N=3) and three received ibotenic acid lesions of the MSDB (N=3). Their performance on the spatial condition of the Delayed Recognition Span Task (DRST) and on a spatial reversal task was compared to unoperated control monkeys (N=7, DRST; N=5, spatial reversal). On the DRST, both MSDB and Fx animals were significantly impaired relative to controls ($p<0.05$) but were not different from each other. On the spatial reversal task, Fx animals were significantly impaired relative to controls on reversals 1 and 2 ($p<0.01$) but not on reversal 3. While the MSDB animals obtained elevated error scores, the difference did not reach significance on any of the three reversals. These results indicate that the cholinergic input to the HF is critical for performance on the recognition span test, a memory loading task, but may not be critical to performance on spatial reversals, a set shifting "executive function" task. Furthermore, these data suggest that either HF efferents in the Fx or non-septal Fx afferents are important for reversal performance. Supported by NIH grants AG04321 and NS16841.

410.2

EFFECTS OF COMBINED MEDIAL TEMPORO-PREFRONTAL LESIONS ON VISUAL RECOGNITION IN MONKEYS. D.M. Kowalska, J. Bachevalier*, and M. Mishkin. Lab. Neuropsychology, NIMH, Bethesda, MD 20892.

Large bilateral lesions of either the medial temporal lobe (MT) or the ventromedial prefrontal cortex (VMPF) yield a severe, and almost equal, loss in visual recognition, whereas damage to only half of these large brain areas, i.e. anterior vs posterior medial temporal lobe (A vs P) or ventral vs medial prefrontal cortex (V vs M), yield a much milder impairment (Mishkin, Nature, 1978, 273:297; Bachevalier & Mishkin, Behav. Brain Res., 1986, 20:249). Because each of the MT components was thought to be anatomically connected mainly with one of the two VMPF components (A with V and P with M), it was hypothesized that crossed lesions (i.e A+M and P+V) should produce a visual memory loss greater than uncrossed lesions (i.e. A+V and P+M) and equal to that found after either complete MT or complete VMPF lesions. To test this proposal, we prepared four groups of 3-4 monkeys each, in which lesions of each of the two components in MT was combined with each of the two components in VMPF. These monkeys were tested preoperatively on delayed nonmatching-to-sample with trial-unique objects, retrained on the task postoperatively, and then given a performance test with increasing delays (30, 60, 120 sec) or lists (3, 5, 10 objects). Monkeys in each of these four groups (A+M, P+V, A+V, and P+M) were significantly impaired across the 6 conditions of the DNMS performance test (averaging 76%, 70%, 69%, and 79%, respectively), as compared to unoperated controls (97%) and to monkeys with lesions of only one of the components in the MT and VMPF regions (range: 80% to 90%). Nevertheless, in none of these four groups was the loss in recognition memory as severe as the memory loss that follows the complete MT (59%) or VMPF (65%) lesions. Moreover, the losses after the crossed lesions were not greater than those after the uncrossed lesions. The results indicate that each of the two components in the medial temporal lobe must interact functionally with each of the two components in the ventromedial prefrontal cortex.

410.3

EFFECTS OF RHINAL CORTICAL LESIONS COMBINED WITH HIPPOCAMPECTOMY ON VISUAL RECOGNITION MEMORY IN RHESUS MONKEYS. M. Meunier*, W. Hadfield, J. Bachevalier and E.A. Murray. Lab. Neuropsychology, NIMH, NIH, Bethesda, MD 20892.

Murray and Mishkin (J. Neurosci., 1986, 6:1991) reported that monkeys with rhinal cortex (Rh) ablations combined with hippocampectomy were only mildly impaired in visual recognition memory as measured by the delayed nonmatching-to-sample (DNMS) task with trial-unique objects, whereas monkeys with Rh lesions combined with amygdalectomy were severely impaired. More recently, however, monkeys with Rh lesions alone have been found to have a severe loss in visual recognition memory. Consequently, the results of the former study have been reevaluated: in that study, the Rh ablations sustained by the monkeys also receiving hippocampectomy (but not those receiving amygdalectomy) spared the rostralmost portion of the rhinal cortex, and resulted in removal of only about ¾ of the rhinal cortex, hence we will refer to them as H+¾Rh. To determine whether the good performance of Group H+¾Rh was due to the incomplete rhinal cortex removal or, paradoxically, to the combination of rhinal and hippocampal damage, we prepared a new group of monkeys with H+complete Rh lesions (H+Rh), and assessed their visual recognition memory using DNMS. Group H+Rh scored 76% correct responses across the 6 conditions of the DNMS performance test (which employed longer delays between sample and choice, and longer lists of items to be remembered) as compared with 93% for their controls, which corresponds to an average loss, in terms of d' (see Ringo, Behav. Brain Res., 1991, 42:123), of 0.82. Group H+¾Rh scored 84% (d' loss = 0.49), and Group Rh scored 67% (d' loss = 1.08). Analysis of the memory loss in terms of d' indicates that Group H+Rh performed significantly worse than Group H+¾Rh, but significantly better than Group Rh. Thus, whereas the comparison of Groups H+Rh and H+¾Rh indicates that sparing of the rostralmost portion of the rhinal cortex contributed to the relatively good performance of Group H+¾Rh, the comparison of Groups H+Rh and Rh suggests that other factors contributed as well.

410.4

LONG-TERM EFFECTS OF NEONATAL HIPPOCAMPAL LESIONS ON VISUAL RECOGNITION MEMORY. M. Beauregard * and J. Bachevalier. Dept. Neurobiology and Anatomy, Univ. Texas Science Center, Houston, Tx 77225.

We examined the performance of three adult monkeys (7-8 years of age) that had sustained early lesions of the hippocampal formation (Group H) and four adult normal controls (Group N) in delayed nonmatching-to-sample with trial unique objects, and compared it with the performance of the same animals obtained when they were tested at 10 months of age in the same recognition task. In the performance test, including delays of 10, 30, 60, and 120 sec, there were no differences within groups between scores obtained at 10 months versus those obtained at 7 years of age. Likewise, at the two ages, there were no differences between Groups H and N. However, there was a significant Group and Delay interaction, indicating that, whereas scores of animals in Group N did not change across all delays, performance of animals in Group H decreased with increasing delays. Overall, the data suggest that lesions of the hippocampal formation, done either in infancy or in adulthood (Clower et al., 1991), do not yield a visual memory loss, at least when the delays are short (i.e. 2 min or less).

410.5

A NOVEL TEST OF SPATIAL WORKING MEMORY IN PRIMATES: EFFECT OF PREFRONTAL DOPAMINE DEPLETION. P. Collins, A.C. Roberts, B. J. Everitt* and T.W Robbins. Departments of Experimental Psychology and Anatomy* University of Cambridge, Cambridge, CB2 3EB, U. K. (SPON:European Brain and Behaviour Society).

Patients with Parkinson's disease have impaired performance on a self-ordered search task designed to measure spatial working memory. This frontal-like deficit may result from degeneration of the mesocortical dopamine pathway associated with Parkinson's disease. The present study is concerned with the development of a primate analogue of this task and an assessment of the role of prefrontal dopamine in its performance. The task is run on a touch sensitive visual display unit which, on any given trial, presents a pre-defined number of identical blue squares at random sites across the screen. On each trial a marmoset has to touch each square, once and once only, in a self determined sequence to obtain reward. In control animals performance declined from 90% correct on sequences of one and two squares to 40% correct on sequences of 5 squares. Injection of 6-hydroxydopamine into the prefrontal cortex of marmosets pretreated with specific noradrenaline (NA) and serotonin (5-HT) uptake blockers, resulted in selective depletion of dopamine (DA) (90% loss of DA, < 50% loss of NA, <10% loss of 5-HT) and impaired performance on a classical manual spatial delayed response task (SDR). The effect of the lesion on the self-ordered search task will be compared to that on SDR, the traditional test of spatial working memory in non-human primates, and to the performance of patients with Parkinson's disease and frontal lobe excision.

410.6

PRIMATE ANALOGUE OF THE WISCONSIN CARD SORT TEST (WCST): A BEHAVIOURAL AND MICRODIALYSIS STUDY OF DOPAMINERGIC MECHANISMS IN THE MARMOSET. A. C. Roberts*, M. A. De Salvia, L. S. Wilkinson, P. Collins, J. L. Muir, T. W. Robbins and B. J. Everitt†

Departments of Experimental Psychology and Anatomy†, University of Cambridge, Cambridge, CB2 3EB, U.K.

Degeneration of the dopamine projection to prefrontal cortex may contribute to the frontal-like cognitive impairments associated with Parkinson's disease (PD) and schizophrenia. The present study investigated the effects of 6-hydroxydopamine lesions of the prefrontal cortex, in the marmoset, on two tests of prefrontal cognitive function, spatial delayed response (SDR) and attentional set-shifting. The latter test provided a componential analysis of the WCST, a commonly used test of frontal lobe function in man. The lesion produced a marked depletion of dopamine restricted to the prefrontal cortex and led to a long term adaptive change in the striatum such that extracellular dopamine in the caudate nucleus, as measured by *in vivo* microdialysis, was elevated in response to potassium stimulation. This was accompanied by an improvement in the ability to *shift* attention from one dimensional property of a stimulus to another whilst the ability to *maintain* attention towards one particular dimension was unchanged. In contrast, SDR performance was impaired in agreement with previous results. It is proposed that attentional set-shifting is mediated by a balanced interaction between prefrontal and striatal dopamine and that elevated dopamine contributes to the improvement in attentional set-shifting ability. This interpretation is consistent with the impaired attentional set-shifting ability of patients with PD using the same test as used here for non-human primates.

410.7

HIPPOCAMPAL AND CORTICAL ACETYLCHOLINE LEVELS IN CONSCIOUS, BEHAVING PRIMATES AS MEASURED BY *IN VIVO* MICRODIALYSIS.

T. D. Smith,* Y. L. Hurd, D. R. Weinberger and R. C. Saunders. NIMH Neuroscience Center at St. Elizabeths, Clinical Brain Disorders Branch, Washington, D.C. 20032.

In vivo microdialysis was adapted for measurement of acetylcholine (ACh) and choline levels in discrete brain regions of awake, behaving primates. Rhesus monkeys (*Macaca mulatta*) were trained to perform a series of behavioral tasks in an automated testing apparatus. Microdialysis probes were positioned into hippocampus and prefrontal cortex (principalis sulcus) of unanesthetized monkeys through specially designed probe guides previously fixed to the skull. Probes were perfused with artificial CSF containing 10 µM or 30 µM neostigmine at a rate of 1.1 µl/min. Dialysate was collected every 20 min beginning either 1 hr or 20 hr after probe placement and was analyzed for ACh and choline concentrations using HPLC coupled with electrochemical detection. A stable baseline for cortical and hippocampal ACh levels was established 3 hr after acute implantation and 1 hr following overnight placement of microdialysis probes and maintained for at least 5 hr in both preparations. Infusion of 10 µM tetrodotoxin (TTX), a sodium channel blocker, reduced ACh overflow by 50-75% within 1 hr in both brain regions. In contrast, choline levels remained unaffected by 1 hr of TTX infusion. Systemic administration of the muscarinic antagonist, scopolamine hydrobromide (5.0-60.0 µg/kg, IM), dose-dependently increased ACh overflow 0-400% within 1 hr. Choline levels were only slightly enhanced. Thus, extracellular levels of ACh are (1) detectable and stable for many hours (2) from neuronally derived pools and (3) responsive to systemic and local pharmacological manipulation. Following initial characterization of ACh and choline overflow, neurochemicals were measured during performance on the behavioral task, delayed-nonmatch-to-sample. Hippocampal and cortical ACh levels increased 2-18% above baseline during performance and continued to rise (16-28%) following completion of the task. ACh overflow declined to baseline values within 60-100 min post-task. Choline levels were unaffected by task performance. These data demonstrate the feasibility of characterizing dynamic neurochemical events in discrete brain regions and correlating these changes with performance on behavioral tasks.

410.8

A PRIMATE MODEL OF SCHIZOPHRENIA-LIKE NEUROPHYSIOLOGICAL DYSFUNCTION: EVIDENCE FOR THE PCP/NMDA MODEL OF SCHIZOPHRENIA.

Javitt DC*, Shelley AM, Schroeder CE, Steinschneider MS, Arezzo JC and Vaughan, HG, Jr. Departments of Psychiatry and Neuroscience, Albert Einstein College of Medicine, Bronx, NY 10461.

Schizophrenic subjects show severe impairments in cognitive functioning and information processing that are resistant to pharmacotherapy and persist despite overall clinical improvement. P300 (P3) is a long-duration cognitive event-related potential (ERP) component that indexes cortical response to unexpected, behaviorally relevant stimuli and provides a "window" into the cognitive dysfunction associated with schizophrenia. P3 is elicited most commonly in an "oddball" paradigm in which a sequence of repetitively presented standard stimuli is interrupted by an unexpected, physically deviant stimulus. In an auditory oddball paradigm, P3 is preceded by two negative components that reflect earlier stages of information processing, mismatch negativity (MMN) and N2. MMN indexes preattentive, automatic detection of stimulus deviance within auditory cortex and so represents the earliest stage of cognitive information processing within cortex, whereas N2 indexes attention-dependent stimulus classification events. Monkeys generate cognitive ERP components that closely resemble human MMN, N2 and P3 in terms of latency, scalp distribution and sensitivity to stimulus and task manipulations. The present study investigates the generation of MMN and N2 in schizophrenics in order to determine the earliest stages at which cortical information processing are impaired. Parallel studies in monkeys demonstrate that abnormalities of cognitive ERP generation similar to those seen in schizophrenics can be induced by focal infusion of phencyclidine (PCP)-like antagonists of NMDA receptor-mediated neurotransmission into primary auditory cortex, suggesting that NMDA receptor dysfunction might be pathophysiologically implicated in the cognitive information processing abnormalities associated with schizophrenia. (supported by MH49334 and MH06723)

LEARNING AND MEMORY: PHYSIOLOGY V

411.1

CAUDATE ACTIVITY IN THE EXECUTION OF OCULOMOTOR HABITS

P.F. Dominey[a,b], J-P Joseph*[b] and M.A. Arbib[a]

a. CNE, NIBS, University of Southern California, Los Angeles, CA 90089-2520
b. Vision et Motricité, INSERM Unité 94, 69500 Bron, France.

We recorded 60 task-related cells from the head of the caudate nucleus in a primate trained to associate centrally-fixated visual cues with saccades to one of two peripheral targets (choice). A delay between cue onset and saccade dissociated cue- from saccade- related activity. After a correct saccade and peripheral fixation, the cue re-appeared at the correct peripheral location (peripheral cue), and the reward was given. We also studied a saccade-with-overlap task in which onset of a single peripheral target indicated the direction of the saccade (no choice).

Purely cue-related responses were observed in 4 (7%) cells. Sustained peri-cue activity that reliably predicted saccade direction (oculomotor set) was seen in 16 cells (27%). Cues associated with the same saccade evoked similar activity in all 7 of these cells tested. Peri-saccadic activity was recorded in 34 cells, with 17 (28%) spatially selective. 13 of 17 displayed sustained discharge until the reward. Of the 17 non-spatially selective cells, 15 showed sustained discharge until the reward, suggesting activity related to expectation of peripheral cue and reward, respectively. Six cells (10%) showed peri-saccade inhibition. Few task-related cells clearly distinguished cue-guided from visually guided saccades.

The small number of purely cue-related cells might suggest that caudate does not participate in this primarily cortical task. Alternatively, we could consider that oculomotor set demonstrates that IT corticostriatal projections convey cue-related activity to saccade-related caudate cells via plastic synapses that are strengthened by learning. We present neural network models for both mechanisms and compare them with our data in consideration of the view that striatum participates in the formation and execution of S-R habits via corticostriatal plasticity.

411.2

DEVELOPMENTAL STUDIES OF EYEBLINK CONDITIONING AND RELATED NEURONAL ACTIVITY IN MOTOR CORTEX.

C. Woody* and E. Gruen. UCLA Med. Ctr., MRRC, BRI, Los Angeles, CA 90024.

Some forms of Pavlovian conditioning are not acquired in mammals such as cats until after three weeks of age (Spear,N.E. and Campbell,B.A., The Ontogeny of Learning and Memory, Hillsdale, N.J., Erlbaum, 1979). We determined if this was so for short and long latency eyeblink conditioning, and tested the hypothesis that failure of development might depend on inability of neurons of the motor cortex to learn. Each of three cats tested at 14-17 days of age failed to learn a blink CR after pairing click with glabella tap and electrical stimulation of the lateral hypothalamus (Aou et al. J. Neurosci. 1992). Each of three cats tested at 4-5 weeks of age developed blink CRs. The 14-17 day old cats responded to the UCS with blink URs and also showed increased cortical spike activity to click CS.

When tested for conditioning by pairing click as CS with local ionophoretic application of glutamate as UCS, single cortical units developed spike activity CRs in cats of both 2 and >4 weeks of age. The "inability to learn" the behavioral CR may thus reflect an inability to integrate cortical outputs in such a way to permit expression of the short latency components of the CR that require the cortex for their development. Other forms of Pavlovian conditioning can be learned and expressed prenatally. (Supported by HD05958; we also thank S. Soltysik and E. White for their contributions to these studies.)

411.3

INVOLVEMENT OF THE CEREBELLAR NUCLEI AND THE RED NUCLEUS IN THE CONTROL OF THE EYEBLINK REFLEX IN THE RABBIT: THE ROLE OF GABA-A RECEPTOR MEDIATED NEUROTRANSMISSION.

N.K. Winters, M.L. Webster, K.B. Irwin, V. Bracha* and J.R. Bloedel. Barrow Neurological Institute, Phoenix, AZ 85013.

The purpose of the present study was to examine the effects of modifying the GABA-a receptor mediated neurotransmision in the cerebello-rubral system on the performance of the classically conditioned (CR) and unconditioned (UR) eyeblink reflexes. The deep cerebellar nuclei and the red nucleus were injected with either muscimol or bicuculline on separate days and the effects of both drugs on the previously learned CRs and on the URs were compared in 20 trained rabbits. Injections of the GABA-a agonist muscimol in the dentate-interposed cerebellar region as well as in the red nucleus disrupted the performance of CRs. This effect was in most cases paralleled by reduced amplitudes and increased latencies of the URs. Interestingly, the application of the GABA-a antagonist bicuculline into identical injection sites in the cerebellar nuclear region led to qualitatively analogous behavioral effects - depression of both CRs and URs. Although similar behavioral effects of bicuculline were observed in most animals after infusion in the red nucleus, in some rabbits the GABA-a antagonist enhanced performance of both responses.

These observations indicate that the GABA-a synaptic transmission within the cerebello-rubral circuits is involved in the control of both conditioned and unconditioned eye-blink reflexes.

NIH Grants NS 30013 and NS 21958.

411.4

HEART RATE CONDITIONING AND THE CEREBELLAR VERMIS IN RATS.

R. N. Leaton* and J. M. Kelso. Dept. of Psychology, Dartmouth College, Hanover, NH 03755.

Lesions of the cerebellar vermis block Pavlovian fear-conditioned heart changes in the rat (Supple & Leaton, 1990). The superior cerebellar peduncle (SCP) provides the major ascending projection from the cerebellum, providing a vermal- thalamic-hypothalamic pathway of possible significance in these conditioning changes. However, a preliminary analysis (Leaton & Kelso, 1992) found no significant effect of lesions of the SCP on conditioned bradycardia.

The present experiment compared conditioned bradycardia in 3 groups of rats. Group VER (n=10)--aspirated lesions of the cerebellar vermis; Group SCP (n=10)--knife-cut lesions of the SCP; and Group Sham--sham surgery. Animals were tested in a Plexiglas restraining tube. They were adapted to the restraint in 2 daily 20-min sessions, baseline heart rate was measured in 3 daily 30-min sessions, unconditioned response to the CS was measured during 3 daily sessions of 8 trials on a variable 3-min ITI, and conditioning was assessed over 6 daily sessions of 8 trials on a variable 3-min ITI. The compound CS (10-s duration)was the onset of the 7.5-W house light and a 90-dB, 1-kHz tone. The UCS was a 0.5 ma, 1-s tail shock.

Groups SCP and Sham showed significant conditioned bradycardia over the 6 training days and were not statistically distinguishable on any measure. Group VER showed impaired acquisition and the impairment was significantly related to the size of the vermal lesion. The effects of vermal lesions on conditioned bradycardia are not mediated by the ascending projection through the SCP.

411.5

Changes in Conditioning-Related Neuronal Activity in Rabbit Lobule HVI of Cerebellar Cortex and Interpositus Nucleus During Discrimination and Reversal Conditioning. T. J. Gould* & J. E. Steinmetz, Dept. of Psych., Prog. in Neural Science, Indiana Univ., Bloomington, IN 47405.

At least two areas of the cerebellum (i.e., lobule HVI of cerebellar cortex and interpositus nucleus (INP)) are believed to be involved in acquisition of a classically conditioned response (CR). Discrimination conditioning is a classical conditioning procedure requiring animals to differentiate between 2 conditioned stimuli (CSs). Single units have been recorded from both HVI and INP after discrimination training (Berthier & Moore, Exp. Brain Res: 63, 1986; 83, 1990) but changes in activity that occur throughout discrimination and subsequent reversal conditioning have not been examined in these areas. Twelve animals received discrimination training until they performed for 2 days at criterion (i.e., 70% or more CRs to CS+ and 30% or less CRs to CS-) and then the CSs frequencies were reversed (i.e., reversal training). Animals were again trained until they reached 2 days at the discrimination criterion. Multiple unit activity in HVI or INP was monitored during training. The INP was lesioned after the last day of training in 3 animals and they were tested with both CSs to see if CRs remained. Both HVI and the INP developed CR-related multiple unit activity evoked by CS+ during discrimination and reversal conditioning but no CR-related activity to CS-. During reversal conditioning, however, INP CR-related activity was somewhat attenuated. The INP lesions prevented CRs to both CSs. These results suggest HVI and INP are involved in discrimination and reversal conditioning but that the two areas are not activated equally during both phases of the learning. (Research supported by NIMH grant MH44052)

411.6

MULTIPLE-UNIT ACTIVITY IN THE PARABRACHIAL NUCLEUS DURING CLASSICALLY CONDITIONED BRADYCARDIA IN THE RABBIT.
W.E. Supple, Jr*. and L. Sebastiani.Dept of Psych, Univ. of Vermont, Burlington, VT 05405*. Dept of Physio & Biochem, Univ. of Pisa, Italy.

The amygdala central nucleus (ACE) and anterior cerebellar vermis (ACV) are both importantly involved in conditioned bradycardia. The pontine parabrachial nucleus (PBN) is anatomically connected to both the ACE and ACV, and stimulation of the PBN modifies heart-rate. This study recorded the evoked activity from neurons in the PBN during the acquisition of differentially conditioned bradycardia. Multiple unit electrodes were implanted in the PBN in 10 rabbits. Training consisted of two phases: evocation and habituation of the HR orienting response (OR) (unreinforced tone presentations) and differential classical conditioning. The initial HR OR was bradycardia that subsequently habituated. The differentially conditioned HR CR consisted of bradycardia during the CS+ with minimal HR change to the CS-. Recordings of PBN unit activity during the OR found some placements with phasic excitatory responses that rapidly habituated. There was evidence of conditioning-related activity in the PBN with some placements showing differential evoked responses consisting of a short-latency (~20 ms) phasic excitation followed by a sustained inhibition of discharge to the CS+ but not to the CS-. Other placements showed simple, short-latency increases in activity to CS+ but not CS-. These data suggest that neurons in the PBN change their activity as a function of Pavlovian differential conditioning, and indicate that the PBN may be part of a larger neuroanatomical circuit mediating Pavlovian fear conditioning in the mammalian brain. Supported by NIMH grant MH47307.

411.7

DEVELOPMENT OF A RABBIT ANESTHETIZED CLASSICAL CONDITIONING PREPARATION. B.J. Anderson*, D.P. Miller, M.T. Allen, & J.E. Steinmetz, Dept. of Psych. and Prog. in Neural Sci., Indiana Univ., Bloomington, IN 47405

The interpositus nucleus and cerebellar cortex appear to be important sites of plasticity for the classically conditioned eyeblink response. Single-unit and multiple-unit recordings in these structures have revealed neuronal activity that precedes and models the CR. To better understand the development of CR-related activity over phases of conditioning, and to use intracellular recording techniques to study the mechanisms that underlie the development of the CR-related activity across conditioning, an anesthetized conditioning paradigm would be beneficial. We are currently investigating parallels between neuronal activity in conditioned animals in the awake and anesthetized state. Animals are being trained with a click as the CS and airpuff or inferior olive stimulation as the US. Neuronal activity from the abducens nucleus, interpositus nucleus and red nucleus in the awake rabbit is being compared to activity induced by the click in anesthetized animals given CS presentations.

An acute anesthetized conditioning preparation is also being investigated. This work involves giving paired or unpaired presentations of pontine stimulation as a CS and inferior olive stimulation as an US in anesthetized rabbits. Baseline evoked CS and US activity in the cerebellum, red nucleus and abducens nucleus are being compared with evoked activity recorded after 400-700 presentations of pontine and inferior olive stimulation. Early results show that training-related changes can be seen in the anesthetized animals given paired training but not in rabbits given unpaired training. These preparations should prove valuable for future intracellular recording studies.
Supported by NIMH grant MH44052

411.8

MODULATION OF NEURONAL ACTIVITY IN THE MEDIAL GENICULATE DURING AUDITORY TRACE CONDITIONING.
K.N. O'Connor*, T.L. Allison, M.E. Rosenfield and J.W. Moore.
Dept. of Psychol., Univ. of Mass., Amherst, MA 01003.

In classical trace conditioning the acquisition of a CR is dependent on neural coding of the CS following its termination. There is evidence that the medial or magnocellular region of the medial genicualate nucleus (mMGN) is one area where this coding may occur; it is essential to the acquisition and maintenance of successful discrimination of auditory stimuli in the conditioning of bradycardia in rabbits (Jarrel et al., *Brain Res.*, 382:199, 1986), and multi-unit reponses to reinforced tones in this region increase during conditioning in comparison to non-reinforced tones (Disterhoft & Olds, *J. Neurophys.*, 35:665, 1972).

Single cell activity was recorded from the MGN during differential trace conditioning of the rabbit nictitating membrane response (NMR). Rabbits were first trained to discriminate between a reinforced CS+ and nonreinforced CS- (75 dB tones of 600 or 1200 Hz) 150 ms in duration followed by a 200-ms trace interval. The US was electrostimulation to the periorbital region of the eye. The rabbits were then prepared for subsequent recording; under anesthesia the dura was exposed, and a recording chamber cemented in place.

Most cells in the mMGN displayed changes in activity during both the CS and trace interval. These changes were largest for CS+ trials in which a CR was made. Examination of probe CSs of short (50 ms) and long (600 ms) duration suggests that changes in activity in the trace interval is time-locked to CS-offset rather than onset and may be needed for appropriate CR timing and topography during trace conditioning.

(Supported by AFOSR grant 92-NL-033 and NSF grant BNS 88-10624)

411.9

FAST HABITUATION OF AUDITORY EVOKED POTENTIALS TO PAIRED TONE STIMULI IN THE RAT. C.M. Specht* and D.W. Shucard Department of Neurology, SUNY @ Buffalo, 100 High Street (D-6), Buffalo, NY 14203.

Fast habituation of the auditory evoked potential (AEP) in humans was first described by Callaway (1973) as a general reduction in evoked potential amplitude that occurs to the second of a pair of auditory stimuli when both stimuli are presented with an interstimulus interval (ISI) of no more than 10 seconds. When auditory stimuli are presented in pairs with an ISI of 2 seconds and an interpair interval (IPI) of approximately 10 seconds, reduction in amplitude to the second tone occurs by as much as 30 to 50 percent. In addition, it has been shown that fast habituation may depend somewhat on a subject's anticipation of the stimulus. Studies in our laboratory have demonstrated this decrement in amplitude to the second tone of a pair in human infants, children and adults and have explored the implications of this finding with respect to attentional processes. In this investigation we describe an animal model of the fast-habituation methodology and subsequent findings of AEPs associated with paired tone stimuli delivered to adult male Sprague Dawley rats chronically implanted with skull electrodes. AEPs were recorded in awake restrained rats using a method previously developed in our laboratory (Church and Shucard, 1987). Rats were presented with pairs of auditory tones (1000Hz, 100msec duration, approximately 90dB, 2 second ISI) with an IPI less than 10 seconds. Findings showed 1) an AEP waveform with four to six distinct peaks occurring between 25 and 400 milliseconds after stimulus onset, and as for humans, 2) a marked amplitude decrement from tone 1 to tone 2 in recordings obtained from left and right cerebral hemispheres. This methodology may prove useful for studying the development of attention, attentional systems, and the mechanism(s) of fast habituation using techniques not possible in humans.

411.10

RECOVERY OF VISUAL DISCRIMINATION IN LESION RATS AFTER COMPOUND OR CROSS-MODAL CONDITIONING. E.R. Delay* and T.D. Tran. Dept. Psychology, Regis Univ., Denver, CO 80221.

Postoperative compound training with haptic and visual cues hampers relearning of visual decorticate rats in a brightness discrimination maze task (LeVere & LeVere, *Physiol. Psychol.*, 14, 165-174, 1982), whereas postop training with auditory cues aids relearning of a visual avoidance task (Delay, *Neuropsychologia*, 26, 661-671, 1988). To directly compare these training procedures, rats were trained on a brightness avoidance task before visual decortication. Six days postop, lesion rats were given either auditory intensity or compound auditory and visual intensity training. The next day, all rats were retrained on the brightness discrimination. Results showed that lesion rats given cross-modal training with the auditory intensity cue relearned the brightness discrimination more effectively than lesion control rats. However, deficits in relearning the brightness discrimination were observed for visual decorticate rats after compound conditioning. Depending upon the temporal contiguity of the cues from each modality, postoperative training can facilitate or hinder behavioral recovery. Compound conditioning biased the lesion rat away from cues requiring the damaged visual system, whereas cross-modal training produced a learning set which aided the rat when it had to use the damaged system.

411.11

GLUTAMATE AND GABA ROLE IN RAT BASAL FOREBRAIN NEURON RESPONSES TO A VISUAL CONDITIONED STIMULUS. J.H. Pirch*, Department of Pharmacology, Texas Tech University Health Sciences Center, Lubbock, TX 79430.

Extracellular recordings were obtained from single neurons in the basal forebrain of rats during classical associative conditioning. Medial forebrain bundle (MFB) stimulation was paired with a 2-sec light stimulus (CS+) to one eye, or a similar light stimulus (CS-) was presented to the other eye without MFB stimulation. Event-related slow potentials were recorded from the frontal cortex to monitor development and maintenance of discrimination between CS+ and CS-. Recordings were obtained while the animals were anesthetized with urethane (1.3-1.5 g/kg with supplementation). Neurons in the substantia innominata, medial globus pallidus, and nucleus basalis magnocellularis responded differentially to CS+ and CS-, demonstrating significantly larger responses to CS+ (Pirch, Brain Res. Bull. 31:73-83, 1993). Some neurons were excited by CS+, and others were inhibited. Microiontophoretic application of the non-selective excitatory amino acid antagonist, kynurenic acid, significantly reduced the excitatory response to CS+ in 8 of 14 cells that demonstrated discriminative conditioning. Iontophoretic application of the selective NMDA receptor antagonist, AP-5, significantly reduced the excitatory response to CS+ in 5 of 8 cells tested in separate experiments. Bicuculline, an antagonist at $GABA_A$ receptors, attenuated the inhibitory response to CS+ in 7 of 11 neurons. These studies provide preliminary evidence that glutamate and GABA are involved in conditioning-related responses of basal forebrain neurons. The glutamate role appears to be mediated via NMDA receptors, and GABA's influence is mediated at least partly through $GABA_A$ receptors. Demonstration of an effect of kynurenic acid and AP-5 on these conditioning-related neural responses indicates that an action in the basal forebrain may contribute to the effect of systemically-administered NMDA antagonists on memory tasks. (Supported by a Seed Grant from Texas Tech University Health Sciences Center)

411.12

VASOPRESSIN CONTENT IN THE CNS CHANGES DURING MAINTENANCE OF CONDITIONED TASTE AVERSION

K.C. Chambers*, E. A. Brownson and R. D. Brinton. Neurobiology, Psychology & Mol Pharmacology & Toxicology, Univ of Southern California, Los Angeles, CA

The rate of extinction of a conditioned taste aversion (CTA) is significantly faster than normal in fluid deprived male rats. Because vasopressin (AVP) is involved in fluid balance and in enhanced memory function, we hypothesized that changes in AVP content in the CNS modulate the extinction rate of a CTA.

Male Fischer 344 rats were assigned to one of two treatment groups: non fluid deprived and 23hr fluid deprived. Each group received the same CTA paradigm. Animals from both groups were sacrificed following 23 hr of fluid deprivation, following 10 days of fluid deprivation + acquisition of the CTA + one day of extinction trial during which animals demonstrated a strong aversion, and 50-75% extinction of the CTA in the fluid deprived animal. A nondeprived animal was always sacrificed with a deprived animal. Micropunches of 8 different brain regions were made and AVP levels determined by quantitative RIA.

Following 23 hrs of fluid deprivation, a significant ($p < .0000$) increase in AVP content occurred in the paraventricular nucleus (PVN). AVP content was significantly lower in the PVN of deprived animals when compared to nondeprived animals three days following acquisition of the CTA ($p < .03$) and at the time of extinction ($p < .0000$). AVP levels in deprived animals was also significantly lower in the medial amygdala at the time of extinction ($p < .05$). Surprisingly, in the nondeprived-nonextinguished animals, AVP levels increased significantly over time in the PVN ($p < .007$), in the medial amygdala ($p < .04$) and in the bed nucleus of the stria terminalis ($p < .0004$). These data support the hypothesis that AVP regulates maintenance of a CTA and demonstrate that when AVP levels do not increase animals more rapidly extinguish a CTA whereas increases in AVP content in select brain regions are associated with long-term maintenance of the CTA.

Supported by NIH grant MH46036 to R.D.B.

411.13

TRAINING CHICKS ON A PASSIVE AVOIDANCE TASK TRANSIENTLY INCREASES IN VITRO CALCIUM FLUX IN A SPECIFIC FOREBRAIN NUCLEUS, THE IMHV. M.P. Clements and S.P.R. Rose*, Brain & Behaviour Research Group, Open University, Milton Keynes, MK7 6AA, U.K. (SPON: Brain Research Association)

Day-old chicks which peck spontaneously at a bright bead coated in the bitter-tasting methylanthranilate (MeA) learn on a single trial to avoid similar but dry beads subsequently. This learning paradigm initiates a molecular cascade in a specific forebrain region, the Intermediate Medial Hyperstriatum Ventrale (IMHV). Within 30min of the aversive experience NMDA glutamate receptor activity is upregulated and there are changes in the phosphorylation state of the presynaptic membrane PKC substrate B50 (GAP43). Memory for the avoidance is blocked by MK801, PKC inhibitors and the nitric synthase inhibitor nitroarginine, implying that NO may function as a retrograde messenger in this learning task. This cascade, initiated *in vivo*, continues *in vitro* in tissue prisms prepared from the IMHV; thus there are changes in inositol phosphare labelling compatible with NMDA up-regulation. These findings suggested that the early sequelae of the learning experience could include changes in synaptic calcium flux. To test this hypothesis, chicks were trained on either water or MeA coated bead, and tested 5 and 30min, 3 and 24hr subsequently; birds trained on water pecked, and on MeA avoided on test. Tissue prisms were immediately prepared from left and right IMHV and incubated with $^{45}Ca^{2+}$ for 5min in an oxygenated, glucose-containing, phosphate-bicarbonate buffered medium, pH 7.4, 37°. There was no effect of MeA training in prisms cut 5 min after the training experience. Ca^{2+} iuptake was significantly elevated in both hemispheres in the 30min slices and in left IMHV after 3hr. This elevation was transient however and was at control levels by 24hr.

We suggest that this enhancement of calcium flux consequent on training activates the next step in the cascade: IEG expression.

411.14

THE NMDA-RECEPTOR ANTAGONIST MK-801 BLOCKS NAVIGATIONAL LEARNING IN HOMING PIGEONS. L.V. Riters* and V.P.Bingman.Bowling Green State University, Bowling Green, OH 43402.

The present study employed the NMDA receptor antagonist MK-801 to begin to investigate the possible importance of NMDA-mediated LTP for naturally occurring spatial learning in birds by exploiting the navigational ability of homing pigeons. Control pigeons released from two unfamiliar release sites displayed vanishing bearings that were poorly oriented. However, when released a second time from the same sites they displayed good homeward orientation. The control birds apparently learned something about the spatial relationships of stimuli at the release sites on the first releases and used that information to orient better when released a second time from the same locations. Experimental pigeons given the NMDA receptor antagonist MK-801 initially behaved as controls, orienting poorly when released for the first time from the two sites. In contrast to controls, the experimental birds failed to show significant improvement in orientation when released again from the same sites without MK-801. Results of a simple operant chamber task suggest the impairments observed are specific to learning rather than non-specific drug effects. The data indicate that blocking NMDA receptors can disrupt navigational learning in homing pigeons. As such, the results are consistant with the hypothesis that NMDA mediated LTP plays an important role in spatial learning in birds.

411.15

P3-LIKE POTENTIALS RECORDED FROM THE CORTICAL SURFACE OF RATS IN PASSIVE AND ACTIVE ODD-BALL TASKS. E. Jodo, S. Hoshino* and Y. Kayama. Dept. of Physiol., Fukushima Med. Col., Fukushima 960-12, Japan.

This study examined whether P3-like event-related potentials could be recorded through an electrode placed on the frontal cortex of awake rats in passive and active auditory odd-ball tasks. In both tasks either 1000 Hz infrequent target tone (30%), or 2000 Hz frequent standard tone (70%), was pseudo-randomly presented at interstimulus intervals of 2-5 sec, with the constraint that the target tone did not occur consecutively. The tone duration was 1 sec (in passive task) or 0.8 sec (in active task). In the passive task an intracranial electrical stimulation (ICS) was given to the medial forebrain bundle (a reward area) only after the cessation of target tone regardless of behavior, while in the active task the ICS was given only when rats pressed the key within 1 sec after the cessation of target tone. In both tasks a P3-like positive slow deflection at a peak latency around 300 msec was elicited, with a larger amplitude to the target than to the standard tone; the P3-like potential in the active task was much more distinct than that in the passive task. These results suggest that a P3-like potential similar to the human P3b can be recorded from the cortical surface of awake rats.

411.16

AUDITORY ERPs DISSOCIATE EARLY AND LATE MEMORY PROCESSES

L. Nielsen-Bohlman*, L.L. Chao & R.T. Knight: Dept. of Neurology & Ctr. for Neuroscience; U.C. Davis, VAMC 150 Muir Road; Martinez, CA 94553-4695.

Working memory has been dissociated into rapid and delayed processes in the visual modality. We examined whether similar mechanisms were engaged during auditory recognition. Event-related potentials (ERPs) recorded from 15 subjects (age 21±1) were used to examine changes in neural processing during early and late stages of recognition memory. Digitized environmental sounds were presented binaurally in 4 blocks (111 to 114 sounds per block, 700 msec duration, 1200 msec ISI). Twenty percent of the sounds were presented once and 80% were presented twice. The repeated sounds occurred at delays of either 1.9 seconds (short delay) or 4 to 12 seconds (long delay). Subjects indicated whether they had heard the sounds before by pressing a 'yes' or 'no' button.

In all conditions, auditory stimuli generated a long duration negative potential (628 msec) over frontal sites. This negativity was larger for the initial presentation and long delay stimuli than for short delays (Fpz: initial presentation=-4.8μV, long delay=-4.5μV, short delay=-2.9μV,$p>0.001$). Short delay repetitions generated an early positive component (404 msec) with a broad posterior scalp distribution. Initial presentations and long delays generated a reduced early positivity (Pz: initial presentation=1.9μV, long delay=3.9μV, short delay=7.3μV , $p>0.001$) and an additional late positivity (544 msec). An N400 component was generated only at initial presentation and long delays (Cz: initial presentation=-3.1μV, long delay=-2.1μV, short delay=0.9μV,$p>0.001$). These results parallel those obtained in the visual modality. The findings indicate that the early positivity indexes a rapid working memory process and the late positivity reflects delayed processes. Increased reaction times and a drop in accuracy at the long delay condition suggest that subjects had greater difficulty with this condition than with the short delay condition. The longer latency of the late positivity may be due the duration of stimulus evaluation. The N4 component was generated only during initial presentation and long delays and may index a search of long term memory. These data support the idea that working memory processes can be dissociated into early and late components and this dissociation may be modality independent.

412.1

EFFECTS OF PHARMACOLOGICAL MANIPULATIONS OF THE GABA-ERGIC SYSTEM ON CONFLICT LEARNING AND MEMORY IN THE MOUSE. M.E. Judge*. Pharmaceuticals Division, Novo Nordisk A/S, DK-2760 Måløv, DENMARK.

Memory processes are profoundly influenced by enhancing GABA-ergic neurotransmission, the main action of anxiolytics, sedatives, and anticonvulsants. The effects of these drugs were evaluated in a conflict memory test in mice. Thirsty NMRI mice (9 wk ♀) were injected i.p. and trained 30 min later. They were given 5 min to drink for 5 sec. Side effects at high doses inhibited drinking. Few compounds affected the next phase, delivery of mild shocks until drinking ceased. Memory was tested the next day (latency to drink). Pre-training treatment with benzodiazepine (BZ) agonists did not impair learning, but did impair memory. Diazepam and midazolam produced antrograde amnesia at 2-3 mg/kg; alprozolam and lorazepam were moderately potent and triazolam was over 10 times more potent. This corresponds well with what is seen in human testing, as does the fact that BZ's given pre-test did not impair retrieval. Similar effects were seen with the GABA-uptake inhibitor NNC 05-0711 and the $GABA_A$ agonists muscimol and THIP, as well as the barbiturates (at very high doses). The barbiturates differed from the other compounds in producing learning and retrieval impairment at doses lower than anterograde amnesia producing doses. These results indicate that approach-avoidance conflict memory testing in mice can be used to model the cognitive impairing effects of pharmacological enhancement of GABA-ergic function in man.

412.2

THE EFFECT OF GABA AGONISM AND UPTAKE INHIBITION AND BENZODIAZAPINE AGONISM AND BLOCKADE ON AVOIDANCE LEARNING IN THE RAT. A. D. Kastello, L. Rajachandran* and J. V. Cassella. Neurogen Corporation, Branford, CT 06405.

It is well known that benzodiazepines (BZ) modulate GABA's effects in the central nervous system and that actions at both of these receptors influence learning and memory processes. In animals, as well as humans, BZ agonists interfere with the acquisition and retention of information acquired under the influence of the drug. In addition, GABA agonism and antagonism are known to influence memory in certain tasks. This study examined the interaction of the $GABA_A$/BZ receptor complex, as well as the $GABA_B$ receptor, on learning and memory in rats. In dose response studies, drugs were administered 15 min prior to the first acquisition trial. Retention was measured 24-hours later under drug free conditions. The $GABA_B$ agonist Baclofen (2.0-8.0 mg/kg,IP) impaired both acquisition and retention of a repeated-trial step down passive avoidance task in adult male Sprague Dawley rats. Muscimol (1.0-2.0 mg/kg,IP), a $GABA_A$ agonist, impaired retention only. The BZ agonist Diazepam (0.625-5.0 mg/kg,IP) dose dependently impaired acquisition and retention. The deficits produced by Diazepam (2.5 mg/kg,IP), given 30 min prior to training, were reversed by pretreatment (40 min) with either the BZ antagonist Ro 15-1788 (10.0 mg/kg,IP) or the BZ partial inverse agonist CGS 8216 (5.0 mg/kg,IP). Administration of a GABA uptake inhibitor 30 min prior to training produced both acquisition and retention deficits, while pretreatment (40 min) with Ro 15-1788 partially reversed the retention deficits. These studies demonstrate the involvement of $GABA_A$ and $GABA_B$ receptors in learning and memory processes and suggest a modulatory influence of the BZ receptor on $GABA_A$'s effect on learning and memory.

412.3

THE ELEVATED T MAZE, A NEW ANIMAL MODEL OF ANXIETY AND MEMORY. **F.G. Graeff, M.B. Viana and C. Tomaz*. Lab. of Psychobiology, Univ. of São Paulo, 14040-901 Ribeirão Preto, SP, Brazil.**

In rats placed in a T maze, consisting of an enclosed arm at a right angle with two open arms elevated 50 cm above the ground, diazepam (DZP; 1-4 mg/kg, ip) abolished the delay of withdrawal from the enclosed arm towards the open arms, measured by retesting in the presence of the drug soon after training, as well as by further retest 72 h later, in the absence of drug. In rats reinjected with 2 mg/kg of DZP before retest no evidence of state dependency was found. Thus, DZP had both an anxiolytic and an amnestic effect in this inhibitory avoidance task. However, in the same animals DZP did not affect the latency of withdrawal from one of the open arms towards the closed arm on the first day. Moreover, the latency of this escape response decreased in the retest performed 72 h later irrespective of drug treatment, indicating that memory of this task was resistant to DZP. These results support the view that the anxiolytic and the amnestic effects of DZP are closely related, and suggest that the T maze model may be useful for simultaneous measurement of drug effects on anxiety and memory. [Supported by FAPESP 90/3474-0].

412.4

CHLORDIAZEPOXIDE IMPROVES PERFORMANCE OF SEPTAL LESIONED ANIMALS IN A MORRIS MAZE H.T. Farber*, E. Thomas, H. Grishkat, and S.E. Choi. Dept. of Psychology, Bryn Mawr College, Bryn Mawr, PA 19010.

Human studies have demonstrated an inverse relationship between state anxiety and performance on memory tasks. We investigated the effects of lateral septal lesions (shown to be anxiogenic) upon a spatial memory task in rats. In addition we examined the effects of chlordiazepoxide (CDP) in septally lesioned animals in the same task.

Forty-six rats were subjected to lesions of the lateral septum or to sham lesions. One-half of each group was given CDP (2 mg/kg) or saline and tested in a Morris water maze, 5 trials/day over 3 days.

When compared to their sham controls, animals which received septal lesions showed significantly impaired learning in the Morris maze. Lesioned animals given a low dose of CDP were superior to those given saline. CDP itself in non-lesioned animals had no statistically significant effect upon performance.

The fact that CDP improves performance in lesioned animals suggests that this improvement may be due to emotional factors, such as anxiety, rather than direct effects upon memory. The Morris maze task may be useful in the study of emotional effects on cognitive performance.

412.5

ENHANCED SENSITIVITY TO THE AMNESIC EFFECTS OF DIAZEPAM IN AGED RATS. R.W. Skelton*, R.K. McNamara, & T.M. Davis, Dept. Psychology, Univ. Victoria, Victoria, BC, Canada, V8W 3P5.

The elderly are primary consumers of benzodiazepine drugs and yet may be hypersensitive to many of their side-effects. The present study examined the amnesic effects of diazepam (DZP) on young (~6 month old) and old (18-24 month old) hooded rats, using the Morris water maze.

Rats were trained undrugged using 4 trials per day, first to a stationary visible platform (2 days), then to a submerged platform in the same location (5 days), and then to a reversed platform location (3 days). Probe trials were given post-acquisition and post-reversal. Rats were then tested on alternate days in an 8 trials/day spatial-learning-set procedure in which the location of the submerged platform was varied between days. On alternate test days, each rat received vehicle or one dose of DZP (1, 2, 5 mg/kg in ascending order) ½ hr before testing. Rats were also tested with vehicle and DZP (5 mg/kg) with a visible platform, and finally, with a submerged platform 1 full hour after vehicle or DZP (5 mg/kg) injections.

Undrugged old rats showed slower initial acquisition, but comparable probe performance, reversal learning, and learning-set performance. At 1 mg/kg, neither age was impaired. At 2 mg/kg, old rats were impaired but young rats were not. At 5 mg/kg, both groups were impaired when injected ½ before testing, but only the old rats were impaired when injected 1 hr before testing. Neither group was impaired on the visible platform test. These results suggest that DZP impairs spatial learning at lower doses and for longer periods in old rats relative to young rats. (Supported by grants from NSERC CANADA and BC Health Research Foundation).

412.6

MIDAZOLAM IMPAIRS RETENTION OF A REWARD INCREASE. J.A. Salinas*, H. Dickinson-Anson & J.L. McGaugh. Center for the Neurobio. of Learning & Memory and Dept. of Psychobio., U. of Calif., Irvine, CA 92717-3800.

We reported previously that midazolam (MDZ) administered immediately prior to a reduction in reward magnitude impairs the retention of the aversive consequences of such a decrease. Such findings are consistent with other evidence indicating that benzodiazepines induce anterograde amnesia. An alternative interpretation of this result is that MDZ's anxiolytic properties decreased the aversiveness of the reward reduction. To clarify the basis of the effect the present experiment examined benzodiazepine effects on memory of a nonaversive change in reward. Male Sprague-Dawley rats (175-200g) that were food deprived and maintained at 80% of body weight were trained to run a straight alley (six trials per day) for either ten 45 mg food pellets or one 45 mg food pellet until asymptote was reached. Half the animals in the high reward condition were then trained for one day at the low reward level. These animals displayed a sharp increase in response latencies on that day. Twenty minutes prior to the next day's training session, half of all animals received 1 mg/kg MDZ or saline (i.p.). On the training session animals that had previously had their reward reduced were returned to the high reward condition and the reward conditions were maintained for an additional day of training. No further injections were given. On the day following injection, the latencies of shifted animals given saline were comparable to those of unshifted controls. Comparable recovery in performance was not displayed by the shift/MDZ animals on that day. Thus, MDZ injected immediately prior to an increase in reward magnitude impaired the retention of such an increase. The findings indicate that the memory impairing effects of midazolam are not due to the drug's anxiolytic properties.

Supported by NSF fellowship RCD-9054728 (JAS) and PHS MH12526 (NIMH and NIDA) (JLM).

412.7

LACK OF EFFECT OF BENZODIAZEPINE RECEPTOR LIGANDS ON SIMULTANEOUS VISUAL DISCRIMINATIONS OF VARIABLE DIFFICULTY. L. DiNardo*, P. Dudchenko, B. Gordon, M. Keckley, and M. Sarter. Dept. Psychology, Ohio State Univ., Columbus, OH 43210.

Benzodiazepine receptor (BZR) agonists produce impairments in tasks measuring attentional abilities. As these tasks typically involve demands on discriminative abilities, BZR agonists have been proposed to disrupt these abilities. However, previous studies were frequently based on go-no go paradigms and therefore failed to dissociate between effects on discriminative abilities and response inhibition. Rats were trained to discriminate between two simultaneously flashing lights in an operant chamber. One group of animals was trained to respond to the faster flashing light in each of 5 pairs of stimuli (1.25, 1.67, 2.5, 3.75, 4.17 Hz vs. 5 Hz); while a second group was rewarded for responding to the slower flashing light in each of 5 pairs (1.25, 1.46, 1.67, 2.5, 3.75 Hz vs. 5 Hz). Decreasing differences between the frequency of both stimuli resulted in decreasing accuracy. The BZR-agonist CDP (1.56, 6.25, 9.38 mg/kg) produced a dose-dependent increase in errors of omission, but did not result in an increase in incorrect responses. These data do not support the hypothesis that BZR agonists affect simultaneous discriminative abilities. Thus, drug-induced impairments in signal detection tasks may not be due to a general disruption of sensory abilities.

412.8

CROSSMODAL DIVIDED ATTENTION IN RODENTS. J. Turchi, J. McGaughy, B. Givens* and M. Sarter. Dept. Psychology, Ohio State Univ., Columbus, OH 43210.

"Divided attention" is a psychological construct that hinges on assumptions about a fixed finite capacity of subjects to simultaneously process multiple sets of information. A model of a crossmodal divided attention task was developed in rats. Initially, rats were trained consecutively in operant auditory and visual conditional discrimination tasks. The final task consisted of two successive blocks of 20 trials per modality (modality certainty), followed by 60 trials consisting of a semi-randomized sequence of stimuli of both modalities (auditory or visual) and qualities (flashing/pulsing or constantly turned on; modality uncertainty). In comparison to unimodal blocks of trials, performance in the mixed condition was assumed to reflect the demands on the parallel processing of two sets of stimulus-response rules. Response latencies were generally longer in the bimodal condition. Administration of scopolamine (SC; 0.03, 0.06, 0.1 mg/kg) or chlordiazepoxide (CDP; 1, 3, 5, 8 mg/kg) increased response latencies, and this effect was greater in the mixed condition. Both drugs produced qualitatively similar effects; however, SC was more potent in increasing the absolute divided attention costs than CDP. These data support hypotheses about the comparable effects of benzodiazepine receptor agonists and muscarinic antagonists on brain information processing capacity.

412.9

ATTENTIONAL EFFECTS OF INFUSIONS OF BENZODIAZEPINE RECEPTOR LIGANDS INTO THE SUBSTANTIA INNOMINATA OF THE BASAL FOREBRAIN. J. Travers*, J. McGaughy, and M. Sarter. Dept. Psychology, Ohio State Univ., Columbus, OH 43210.

The attentional impairments produced by benzodiazepine receptor (BZR) agonists are hypothesized to be mediated via basal forebrain GABA-cholinergic interactions. Accordingly, studies have demonstrated that the behavioral effects of intrabasalis infusions of the GABA-agonist muscimol are attenuated by systemic co-administration of physostigmine (Dudchenko & Sarter 1991; Muir et al. 1992). To determine the role of endogenous GABA, the effects of intrabasalis infusions of BZR ligands on attentional abilities were tested in animals trained to detect visual stimuli presented for 25, 50, 100, or 500 ms. Guide cannula (26 gauge) were implanted to allow for repeated bilateral infusions of drugs into the basal forebrain. Infusions of the BZR agonist chlordiazepoxide (CDP; 20, 40 μg/0.5 μl/hemisphere) or of the inverse agonist ß-CCM (1.5, 3 μg/0.5 μl/hs) impaired and facilitated, respectively, the animals' performance in this task. These data support the hypothesis that attentional abilities are correlated with increases in activity in basal forebrain GABAergic inputs, or in their target neurons. In addition, decreases in GABAergic transmission (as a result of infusions of ß-CCM) result in the facilitation of attentional abilities.

412.10

BASAL FOREBRAIN-LESION INDUCED BLOCKADE OF THE EFFECTS OF BENZODIAZEPINE RECEPTOR LIGANDS ON VIGILANCE. P. Dudchenko*, C. Apple, T. Conti, and M. Sarter. Dept. Psychology, The Ohio State Univ., Columbus, OH 43210.

Based on the general hypothesis that the attentional effects of benzodiazepine receptor (BZR) ligands are mediated via basal forebrain GABA-cholinergic interactions, it was predicted that BZR-agonists no longer interact with the residual attentional abilities of basal forebrain-lesioned rats. Animals were trained in a task that required the animals to detect visual stimuli presented for 25, 50, 100, or 500 ms. Following training to a specified criterion, animals were assigned to one of three groups: 1) quisqualate-induced lesions of the region of the nucleus basalis/substantia innominata of the basal forebrain; 2) lesions of the head of the nucleus caudatus; 3) sham lesions of both the basal forebrain and the caudate. Unexpectedly, the performance of basal forebrain lesioned animals largely recovered. In both sham-lesioned and caudate-lesioned animals, the administration of the BZR-agonist chlordiazepoxide (CDP; 1.56, 3.13, 6.25, 9.38, 12.5 mg/kg) dose-dependently impaired the animals' performance in this task. However, the potency of CDP to impair performance in basal forebrain-lesioned animals was greatly reduced. These data suggest that basal forebrain neurons are critically involved in the mediation of the attentional effects of BZR-ligands. The neuronal basis of the behavioral recovery in lesioned animals remains unsettled.

LEARNING AND MEMORY: PHARMACOLOGY—EXCITATORY AMINO ACIDS

413.1

MK-801 BLOCKS ACQUISITION AND EXPRESSION OF CLASSICALLY CONDITIONED RESPONSES IN THE RABBIT NICTITATING MEMBRANE PREPARATION. E.J. Kehoe, J. Cox, R. Guthrie, M. Macrae, and I. Gormezano*. School of Psychology, University of New South Wales, Kensington, NSW 2033 Australia.

MK-801, an antagonist of neurotransmission via N-methyl-D-aspartate receptors, was given (0.1 mg/kg IV) to rabbits either before or after sessions of classical conditioning. The conditioned stimuli (CSs) were a pure tone and a flashing light. The unconditioned stimulus (US) was a 100-ms, 3-mA, 50-Hz AC current to the skin near the right eye. The measured response was closure of the nictitating membrane. The animals that received the MK-801 before the session showed little acquisition of a conditioned response (CR). When MK-801 administration was suspended, responding remained at a low level. In contrast, the animals that received MK-801 after the session showed high levels of CR acquisition. When they were tested by administration of the MK-801 before a single session, they showed a large deficit in responding. Responding returned to its former level when MK-801 was suspended. In all animals, tests for motor deficits caused by MK-801 proved negative.

413.2

D-CYCLOSERINE ENHANCES HIPPOCAMPALLY-DEPENDENT EYEBLINK CONDITIONING IN AGING AS WELL AS YOUNG RABBITS. J.F. Disterhoft, L.T. Thompson, G.J. Halperin, and T. Lanthorn*, CMS Biology, Northwestern University Medical School, Chicago, IL 60611 & *NDR, G.D. Searle & Co, Skokie, IL 60073.

Previous work demonstrated that daily treatment with D-cycloserine (DCS; 6 mg/kg) facilitated acquisition of hippocampally-dependent trace eyeblink conditioning in young rabbits (Thompson et al., *Nature, 359*, 638-641). Aging rabbits exhibit severe deficits in acquiring the same task as compared to young controls. DCS crosses the blood-brain barrier readily, and its behavioral facilitation is hypothesized to be mediated at least in part by its effects on hippocampal neurons. The present study evaluated the effects of a broad range of doses of DCS on acquisition by both young and aging subjects.

Immediately after treatment with 0.75, 1.5, 3.0, 6.0, or 12.0 mg/kg of DCS, daily training occurred, with 80 trial presentations of a 100 ms tone CS followed after a 500 ms ISI by a 150 ms airpuff US. Aging (37.4 ± 0.3 mo) and young (3.4 ± 0.2 mo) rabbits were trained in pairs in the trace eyeblink conditioning task in a soundproofed chamber until a criterion of 80% conditioned responses (CRs) was attained. CR and UR amplitudes, latencies, and other parameters were scored by a PC-based data acquistion system.

Aging control subjects required up to three times as many trials on average to acquire hippocampally-dependent trace eyeblink conditioning. DCS, a partial-agonist of the glycine coagonist binding site on the NMDA receptor/channel protein, significantly improved acquisition in both young and aging rabbits. Optimal doses improved acquisition by more than 50% as compared to controls, without effects on the UR. The improvement in aging subjects was particularly dramatic, restoring acquisition at optimal doses to levels essentially indistinguishable from that of young control subjects. Activation of neuronal NMDA receptors plays a critical role in many forms of associative learning, including eyeblink conditioning, and is greatly facilitated by treatment with DCS. SUPPORTED BY 1 R01 AG08796, 1 R01 DA07633 AND G.D. SEARLE & CO.

413.3

ANESTHETIC-DEPENDENT ENHANCEMENT OF TASTE AVERSION LEARNING *IN UTERO*. G.A. Mickley*, J.D. Lovelace, S.T. Farrell, and K.S. Chang. Armstrong Laboratory, Brooks AFB, TX 78235-5324.

Rat fetuses (E18) can learn a taste aversion *in utero* when exposed to a sweet flavor (10.0 μL of 0.3% saccharin = SAC) followed by an i.p. injection of lithium chloride (5.0 μL of 0.19M solution = LiCl). Here we report that this phenomenon can be significantly modulated by the type of anesthesia administered to the pregnant dam before the conditioning procedure.

Dams were anesthetized with either Sodium Pentobarbital (50 mg/kg, i.m.) or a combination of Ketamine Hydrochloride (100 mg/kg, i.m.) and Xylazine (10 mg/kg, i.m.). Fetuses received pairings of SAC+LiCl or one of the following control combinations: SAC+Saline, H_20+LiCl; H_20+Saline. At age 15 days neonatal rats were given a taste preference test by allowing them to select nipples painted with either saccharin or vehicle (H_20). After weaning, rats were given an additional taste preference test where they were allowed to drink from bottles filled with either 0.15% saccharin or water.

Neonates that received SAC+LiCl injections under the influence of Ketamine (KET group) showed a stronger conditioned taste aversion than did those from dams injected with Sodium Pentobarbital (NaPent group). The taste aversion measured in the KET group was also detectable later in development (i.e., during the bottle test); whereas, the taste aversion could not be detected later in the NaPent group. Thus, *in utero* taste aversion learning is a more-potent phenomenon in KET rats than NaPent rats. Since Ketamine blocks N-methyl-D-aspartate (NMDA) glutamate receptors, and these receptors have been implicated in neural plasticity during development, our data suggest the testable hypothesis that NMDA antagonism can potentiate fetal learning.

413.4

NMDA ANTAGONIST IN THE BASOLATERAL AMYGDALA IMPAIRS TASTE-POTENTIATED ODOR AVERSION LEARNING. T. Hatfield* and M. Gallagher, Department of Psychology, University of North Carolina, Chapel Hill, NC 27599.

The present study examined the effects of direct infusion of the NMDA antagonist, d-APV, into the basolateral amygdala (ABL) on taste-potentiated odor aversion learning (TPOA). TPOA is a form of learning that relies on information processing in two sensory modalities, taste and odor. Unlike taste aversion learning, odor aversions are not readily acquired in conditioning paradigms using delayed illness as the unconditioned stimulus. However, presenting the odor and taste together as a compound conditioned stimulus potentiates learning of the odor aversion. ABL neurotoxic lesions impair this form of learning (Hatfield et al., Behav. Neurosci, 106, No. 2, p.286-293).

Cannula were surgically implanted in rats to target the ABL. D-APV (1.0μg) was directly infused into the ABL just prior to TPOA conditioning. Infusion of d-APV caused a significant impairment in odor potentiation learning as compared to both surgical and vehicle control groups. At the same dose, the inactive stereoisomer, l-APV, did not produce an impairment in odor potentiation. All groups, including those rats infused with d-APV, showed equivalent learning of the taste aversion. These results suggest that the particular associative demand of the TPOA task requires the integrity of NMDA function in the basolateral amygdala. [Supported by NIMH-35554 to M.G. and T.H.]

413.5

IDENTIFICATION OF THE POSTTRAINING PERIOD WHEN GLUTAMATE RECEPTOR BLOCKADE IMPAIRS OLFACTORY LEARNING IN RAT PUPS. D. A. Weldon* and G. G. Fedorcik. Dept. of Psychology, Hamilton College, Clinton, NY 13323.

Glutamate receptors appear to play an important role in early olfactory conditioning in neonatal rats. Impairment of olfactory learning in rat pups can be produced by immediate posttraining injections of the noncompetitive N-methyl-D-aspartate (NMDA) receptor antagonist MK-801 (Weldon & Lorusso, *Soc. Neurosci. Abstr.*, **16:** 486, 1991). The purpose of the present experiment was to investigate the specific time period when NMDA receptor blockade would have this effect.

Six day old Sprague-Dawley rat pups were exposed to peppermint odor paired with tactile stimulation (stroking the skin with a paint brush) for 20 10-sec conditioning trials over a 60 min training session. Pups received an injection of MK-801 (0.1 mg/kg, i.p.) 0, 10, 30, or 60 min after the training period. The next day, the pups were placed in a testing chamber for a total of 6 min, and the amount of time that they spent over the conditioned odor was recorded. In comparison with the performance of pups treated with saline immediately after training, there was a statistically significant reduction in the preference for the conditioned odor in the animals receiving MK-801 immediately following the training period. Treatment with the drug at the other intervals did not produced an impairment in performance. The data indicate that immediate posttraining activation of NMDA receptors is required for normal olfactory learning in neonatal rats.

413.6

SPATIAL LEARNING DEFICIT IN EPILEPTIC RATS MAY BE DEPENDENT ON THE LOSS OF HIPPOCAMPAL NMDA RECEPTORS. H. Lahtinen*, A. Ylinen, J. Sirviö, R. Miettinen and P.J. Riekkinen Sr. Dept. of Neurology, University of Kuopio, P.O.B. 1627, SF-70211 Kuopio, Finland.

Sustained electrical stimulation (60 min, 2 mA) of the perforant pathway (PP) was used to induce hippocampal seizures in conscious rats. PP-stimulation lead to pyramidal cell degeneration of hippocampal CA1 and CA3c regions, loss of hilar somatostatin-immunoreactive (SOM-IR) neurons and impaired spatial learning, as reported earlier. CGP 39551, a competitive NMDA receptor antagonist administered as a single dose (10 mg/kg, i.p.) 4.5 h prior to the stimulation totally reversed the water maze learning deficit of the stimulated animals but only partially protected from the pyramidal cell damage and had no effect on the SOM-IR cell loss. Furthermore, contrary to the saline group, the CGP 39551 pretreated rats did not show a significant loss of NMDA-sensitive [^{3}H]glutamate binding in the strata oriens and radiatum of the CA1 area. Acquisition in the spatial learning and memory test (water maze swimming latencies) were dependent on the NMDA receptor binding in CA1 (ANOVA: $F_{1,7}$=8.0, p=0.025) but not on the degree of pyramidal cell degeneration ($F_{3,7}$=1.3, p>0.05). However, the loss of receptor binding correlated with the degree pyramidal cell damage (r=-0.69, p=0.004). Thus, our results suggest that while the hippocampal pyramidal cell degeneration in the PP-stimulated epileptic rats is likely to account for the loss of NMDA receptors in the dendritic field as well, the learning ability of these animals may ultimately be dependent on the adequate NMDA receptor functioning in the CA1 region.

413.7

APV AND CNQX DISRUPT BOTH WATER MAZE ACQUISITION AND SENSORIMOTOR PERFORMANCE ABILITIES RELATED TO THE WATER MAZE TASK. **D.P. Cain,* D. Saucier, E.L. Hargreaves, F. Boon, J. Hall, J. DeSouza, and E. Wilson. Dept. Psychology, Univ. Western Ontario, London, CANADA, N6A 5C2.**

The effects of intraventricular APV (10 or 30 μg) and CNQX (2, 10, 30 μg) were tested on the acquisition of the Morris water maze (hidden and visible platforms), and on walking along a narrow wooden beam (balance task). Behaviors incompatible with adequate maze performance were documented: thigmotactic swimming (swimming >80% of a trial within 15 cm of the wall), and platform deflections/walk-overs (encountering but failing to mount or remain on the platform). APV elevated escape latencies in both the hidden (10 & 30 μg) and visible (30 μg) versions. However, post-acquisition probe trials revealed that the 10 μg rats spent as much time in the vicinity of the platform as the control rats. The poor maze performance of rats receiving either dose of APV was correlated ($p < .05$) with a high incidence of thigmotactic swimming, platform deflections, and poor performance on the balance beam task. Data for CNQX were similar. These results suggest that sensorimotor abilities related to behaviors required to perform the task were disrupted and may account for a portion of the observed water maze deficit. Thus, putative blockade of glutamate transmission (NMDA or AMPA receptor subtypes) may induce a more global dementia and not a specific learning deficit. Supported by NSERC to DPC.

413.8

MILACEMIDE TREATMENT IN C57BL/6J MICE: EFFECTS ON THE ACQUISITION OF A MORRIS WATER MAZE TASK. J.E. Finkelstein[1], H.L. Petri[2], E.L. Bresnahan[3]*, and D.K. Ingram[1]. [1]Gerontol. Res. C, NIH-NIA; [2]Towson St. U.; [3]Essex Comm. College, Baltimore, MD 21224.

The N-methyl-D-aspartate (NMDA) subtype of the glutamate receptor appears to be involved with processes of learning and memory. A neutral amino acid binding site is known to exist on the NMDA complex. Glycine binds with high affinity to this site and has been found to potentiate NMDA activity (Bonhaus et al., *Molec. Pharm., 36:* 273, 1989). Milacemide (2-N-pentylaminoacetamide HCL) is a glycine agonist which has been found to enhance performance of rodents in passive and active avoidance tasks (Quartermain et al., *Pharmacol., Biochem., Behav., 39:* 31, 1991). and has improved the performance of humans in several word retrieval tasks (Schwartz et al., *Clin. Neuropharm., 15:* 114, 1992). We evaluated the effects of milacemide on the performance of male C57BL/6J mice in a complex spatial task, the Morris water maze. Because NMDA receptor activation appears involved in induction of long-term potentiation, it was hypothesized that milacemide administration would be involved in task acquisition. Therefore, mice were treated with either milacemide (10 mg/kg) or vehicle 1 hr prior to training on each of 4 consecutive days. Results indicated that mice treated with milacemide learned the task significantly faster than controls over 4 days of training, as measured by mean distance (cm) to reach the goal platform. Therefore, agonism of the glycine site on the NMDA receptor appears to facilitate performance of learning in a spatial memory task.

413.9

PHENCYCLIDINE (PCP) IMPAIRS TEMPORAL ORDER MEMORY FOR SPATIAL LOCATIONS IN RATS. Long, J.M.*, and Kesner, R.P. Dept. of Psychology, Univ. of Utah, Salt Lake City, UT. 84112

Previous research has shown that hippocampal lesions in the rat disrupt memory for temporal order information for spatial location (Chiba, Kesner,& Reynolds, 1993). In some, but not all tasks in which hippocampal lesions disrupt memory, administration of NMDA antagonists (ie. AP5, MK-801, PCP) result in similar impairments. The present study was designed to assess the role of the NMDA receptor complex in memory of temporal order information for spatial location. In the study phase Long Evans rats were trained on an eight arm radial maze to visit each of eight arms in a randomly selected order. In the test phase the rats had to choose which of two arms presented occurred earlier in the study phase sequence. The arms presented as test arms varied according to temporal lag. Rats were given IP injections of saline or PCP (3-4 mg/kg) on a double alternation schedule. The results indicated that with PCP injections rats were impaired across all temporal lags relative to saline injections. A second experiment showed that PCP had no effect on the ability of rats to discriminate 3-dimensional objects, a task that is not sensitive to hippocampal dysfunction. The data suggest that NMDA receptors, probably located in the hippocampus, are important in mediating temporal order memory for spatial location, but not visual discrimination learning.

413.10

EFFECTS OF PHENCYCLIDINE ON LEARNING OF A 12-ARM SPATIAL CONTINUOUS RECOGNITION MEMORY TASK.
M. Dakis, R. P. Kesner, F. Matsuo* Dept. of Psychology, Univ. of Utah, Salt Lake City, UT 84112

In order to test the role of NMDA receptors in the hippocampus on the acquisition of a spatial continuous recognition memory task, rats were injected bilaterally with 1µl of 36 or 54nM concentrations of PCP (a non-competitive NMDA antagonist), or isotonic saline directly into the CA-1/dentate gyrus region of the hippocampus. While under the influence of the drug, the rats were each allowed sequential access to 12 arms of the maze from the central platform. Access required that the rat orient to a cue on a clear Plexiglass door of the designated arm; upon orientation, the door was opened and latency to reach the end of each arm was measured. Of the 12 presentations, three or four of the arms were repeated, but did not contain reinforcement. Repeated arms were presented with lags ranging from 0 to 6 (from 0 to 6 different arm presentations between the first and the repeated presentation). The dependent variable consisted of the difference in latencies between the first and the second presentation of a repeated arm. All animals received 16 sessions for a total of 8 presentations of each lag. Results indicate that compared with saline and 36nM, the 54nM PCP group could not learn the task. The acquisition deficit is not likely due to any sensory-motor effects, since none was observed with 54nM injections. The results support the hypothesis that the NMDA receptor mediates the consolidation of spatial information in the CA-1/dentate gyrus region of the hippocampus.

413.11

MK801 DISRUPTS LONG-TERM MEMORY FORMATION IN THE 2-DAY OLD CHICK. D. R. Smith, S. C. Fromont, M. R. Rosenzweig*, and E. L. Bennett. Department of Psychology, University of California, Berkeley, CA 94720.

MK801 is a non-competitive antagonist of the NMDA receptor which acts by blocking the influx of Ca^{2+} via the NMDA recpetor complex. The onset of amnesia produced by MK801 was determined using 2 day-old chicks trained on a 1-trial passive avoidance task. Chicks were given bilateral injections into the intermediate medial hyperstriatum ventrale 30 minutes pre-training, of either saline or 0.15mM MK801, and tested either 90 min, 2 h, 3 h, 4 h, or 24 h, post-training. Our results show that 0.15mM produced significant amnesia 4 h and 24 h post-training compared to saline controls ($p<.01$). MK801 did not produce amnesia at any other test time, and previous tests showed that amnesia was not apparent before 90min post-training. Whereas it had been reported that amnesia produced by MK801 starts at some time between 30 min and 3 h post-training (Burchuladze & Rose, 1992), our finding demontrates that MK801 causes an onset of amnesia significantly later than do protein synthesis inhibitors. These results suggest that NMDA receptor activation, along with Ca^{2+} and Ca^{2+}-depentent processes, are important for long-term memory formation in the chick, and that these processes are important for the retrieval of a passive avoidance task.

413.12

A DIFFERENTIATION BETWEEN THE EFFECTS OF MK-801 ON SENSORY PROCESSING VERSUS MOTORIC ACTIVATION: IMPLICATIONS FOR THE ROLE OF GLUTAMATE IN LEARNING & MEMORY. **Huiliang Dai* and Robert J. Carey**
Res. & Devel. Serv. -151, VA Medical Center, Syracuse, NY 13210

A frequently used pharmacological tool for understanding the behavioral functions of glutamate is the drug MK-801 which is a non-competitive NMDA antagonist at the ion channel complex. Increasingly, the interaction of glutamate and dopamine systems in the striatum have been investigated and the induction of a drug related hyperlocomotion by MK-801 has been suggestive of an inhibitory modulatory effect of glutamate on dopamine activity. This focus on the locomotor effects of MK-801, however, fails to consider the sensory gating role of the striatum. In order to assess the impact of MK-801 on this function of the striatum, a behavioral test was developed in which sensory-motor effects could be distinguished using a video tracking system. A zone composing 1/9 of the floor area was monitored independently from the rest of the area. Intermittently, a 2"x2" styrofoam cube was placed in the small zone. The presence of the object reliably increased the duration in the small zone but did not affect either the number of visits to the small zone, or overall locomotion behavior. MK-801 0.01 to 0.3 mg/kg dose range produced a dose dependent decrease in object zone time and an increase in locomotion. Importantly, the interference with the investigation of the stimulus object occurred at 0.03 mg/kg whereas hyperlocomotion did not develop until a 0.1 mg/kg dose level was administered. The interference in sensory processing by MK-801 at a dose well below that required for a locomotion behavioral change point to an important function for the glutamate system in attentional mechanism which would impact substantially upon tests involving learning and memory.

413.13

EFFECTS OF INTRA-HIPPOCAMPAL NMDA BLOCKADE AND KINASE INHIBITION ON SPONTANEOUS ALTERNATION AND PERFORANT PATH – DENTATE GYRUS EVOKED POTENTIALS. D.L. Walker* and P.E. Gold. Dept. Psychology, University of Virginia, Charlottesville, VA, 22903.

We previously reported that spontaneous alternation (SA) is disrupted by systemically administered NMDA antagonists. Others have suggested that spatial learning deficits produced by NMDA blockade are attributable to a direct impairment of endogenous hippocampal long-term potentiation (LTP)–a view consistent with evidence that intra-hippocampal infusion of NMDA antagonists disrupts spatial learning and also interferes with LTP induction. In the present study, we have attempted to determine if SA is also disrupted by the circumscribed blockade of hippocampal NMDA receptors, and have examined the effects of a second class of compounds which disrupt LTP, protein kinase inhibitors, on this behavior.

When injected into the rat hippocampus 15 min prior to the 8 min test, the NMDA antagonists CPP and D,L-AP5 each decreased alternation rates. The specific protein kinase C inhibitor, NPC 15437, also disrupted alternation behavior whereas the more general kinase inhibitor, PMXB, did not. Interpretation of these data was complicated by the additional findings that intra-hippocampal administration of L-AP5 (which is inactive with respect to NMDA receptors) also disrupted SA (albeit at a higher dose), and that both the D- and L- isomers of AP5 as well as each kinase inhibitor dramatically disrupted evoked responses (manifest as a decrease of population spike amplitude and EPSP slope, and an increased spike latency).

These data indicate that behaviorally effective doses of AP5 may have effects which extend beyond NMDA blockade. Moreover, the effects of these compounds on hippocampal transmission, in general, suggest that attribution of the amnestic consequences of their administration to impaired LTP may be unwarranted. [Supported by NIA (AG 07648), NSF (BNS 9012239), and by an NIMH training grant (MH 1811)].

413.14

INTRACAUDAL INJECTION OF L-PYROGLUTAMIC ACID IN RATS IMPAIRS REVERSAL OF A LIGHT DISCRIMINATION TASK. Jennie C. Johannesen, Jennifer L. Geelmuyden and Richard E. Musty*. Department of Psychology, Univ. of Vermont, Burlington, VT 05405

The effect of bilateral, intracaudal injections of L-pyroglutamic acid (L-pga) on the ability of rats to acquire and reverse an appetitive light discrimination task was evaluated. Seven female Wistar rats received bilateral, intracaudal injections of 3 µL of L-pga (25.8 mg/ml), while 7 others received 3 µL injections of 0.9% saline. Eight female Wistars received sham lesions. All rats were trained to traverse a Y-maze for food reinforcement; in the acquisition phase, they entered a randomly alternated lit arm for a food reward and in the reversal phase, they entered the dark arm to obtain the reward. All subjects were trained to a criterion of 5 out of 6 trials correct on two consecutive days. Data analysis revealed that whereas rats injected with L-pga acquire the task as rapidly as the control animals, they are significantly impaired on the reversal phase ($p < .01$). The results obtained resemble those of Kirkby (1969, Physiol and Beh, 4, 451-454) who reported similar findings in rats with electrolytic lesions of the caudate. This deficit and the fact that L-pga acts at glutamate receptors, supports the theory that L-pga may be a useful model of neurodegenerative diseases like Huntington's disease.

414.1

AMPHETAMINE FACILITATES RECOVERY OF SOME ASPECTS OF MOTOR FUNCTION FOLLOWING UNILATERAL CORTICAL LESIONS IN THE RAT. T.D. Schmanke*, R. A. Avery and T.M. Barth, Department of Psychology, Texas Christian University, Ft. Worth, TX 76129.

Previous studies suggest that amphetamine facilitates recovery of hindlimb sensorimotor function following cortical damage, if the animal receives task-specific experience while under the influence of the drug (amphetamine + practice). The majority of these studies used a beam walking task where the rat traversed a narrow wooden bridge. The present study was designed to determine if the amphetamine + practice effect generalizes to forelimb sensorimotor function. Rats received unilateral electrolytic lesions in the sensorimotor cortex (SMC) that included the caudal forelimb representation . Beginning twenty-four hours following surgery, the animals received a regimen of amphetamine (2 mg/kg) and experience on forelimb tactile placing tasks as well as the bilateral tactile stimulation test. During the period immediately following drug administration, amphetamine intoxicated animals were not significantly different from saline treated control animals. For example, amphetamine treated animals failed to exhibit tactile placing with the forelimb contralateral to the lesion. However, amphetamine facilitated recovery on this task and had no effect on recovery from somatosensory asymmetry. In experiment 2, rats received large SMC lesions that included both the forelimb and hindlimb representations. The aim of this study was to replicate the effects on the beam walking task as well as test the same animals on a task requiring negotiation of an elevated grid floor (foot-fault test). During the period of drug intoxication, amphetamine treated animals negotiated the narrow beam significantly better than saline treated animals. The amphetamine treated animals continued to show a facilitation of recovery on subsequent test days. In contrast, there was no amphetamine effect on the foot-fault test. These data suggest that amphetamine does not affect recovery on all sensorimotor tasks and that performance during the period of drug intoxication does not predict subsequent recovery (i.e. tactile placing).

414.2

THE EFFECTS OF CUEING ON THE SENSORIMOTOR ASYMMETRY FOLLOWING UNILATERAL LESIONS OF RAT ANTEROMEDIAL CORTEX: MANIPULATIONS OF CUE SIZE AND CUE-TRIAL INTERVAL. B.B. Marks* and T.M. Barth, Department of Psychology, Texas Christian University, Ft. Worth, TX 76129.

Following unilateral lesions of the anteromedial cortex (AMC), rats exhibit an ipsilateral sensorimotor asymmetry as measured by a bilateral tactile stimulation test (e.g. contacting an adhesive patch placed on the forelimb ipsilateral to the lesion before contacting a simultaneously applied contralateral patch). A previous experiment demonstrated that a tactile cue presented 5s prior to the bilateral tactile stimulation test neutralizes this asymmetry. These data support the view that the sensorimotor asymmetry observed after unilateral AMC lesions may reflect an impairment in the allocation of attention to stimuli presented on the side contralateral to the lesion. The present study examined the effects of manipulating the size of the cue and the time interval between cue presentation and the test trial (cue-test interval).

Rats received unilateral electrolytic lesions of the AMC and were tested one or two days following surgery. First, the animals received the bilateral stimulation test without a cue, in order to determine the presence of an ipsilateral asymmetry (contacting the ipsilateral patch before the contralateral patch on at least 80% of the trials). Animals exhibiting an ipsilateral asymmetry were then given a series of trials with a cue presented prior to the bilateral stimulation test. Cue size was varied by increasing or decreasing its size by 75% of the standard patch. The cue-test interval was either 5s, 30s, or 60s. The results were that the size of the cue had little effect in neutralizing the asymmetry. In contrast, the cue-test interval proved to be very important, with cues having the largest effect at 5s, a mild effect at 30s, and no effect at 60s. These data support previous findings that suggest the neutralizing effects of the cue are transitory.

414.3

EFFECTS OF CORTICAL LESIONS ON APOMORPHINE-INDUCED ROTATIONAL BEHAVIOR . A. Herranz*, R.C. Heim, M. Poltorak, M. Giordano, H.E. Cannon-Spoor and W.J. Freed, NIMH Neuroscience Center at St. Elizabeths, Washington, DC 20032.

The afferents to the neostriatum from the cerebral cortex and the substantia nigra (SN) are largely coincident on the same neurons. We evaluated the effects of corticostriatal deafferentation on a behavior mediated by striatal dopaminergic synapses. Apomorphine-induced rotation in rats with unilateral lesions of the SN was used as a behavioral measure, to avoid the complicating influence of presynaptic effects on dopamine release. Right SN lesions were performed by stereotaxic administration of 4 µg of 6-OHDA. The animals were then screened for rotational behavior after administration of 0.1 mg/kg of apomorphine HCl. After obtaining stable baseline rotational behavior over 8 weeks, cortical lesions were performed by aspiration ipsilateral to the SN lesion. Lesions extended from 3mm anterior to 3mm posterior to the bregma. "Small" lesions extended 2-3 mm laterally from the midline and "large" lesions extended laterally to 5-7 mm. Animals were tested over a period of 28 weeks following the cortical lesions. The large cortical lesions reduced rotation by approximately 55%, while a 35% reduction was induced by the small lesions. The decreases were stable over the 28 week testing period. We conclude that the cortical input to the striatum can modulate the behavioral manifestations of activation of the nigrostriatal dopamine system, even in the absence of presynaptic dopamine terminals.

414.4

N-METHYL-D-ASPARTATE ANTAGONIST MK-801 BLOCKS AMPHETAMINE INDUCED BUT NOT PHENCYCLIDINE INDUCED BEHAVIORAL SENSITIZATION. X. Xu and E.F. Domino*. Dept. of Pharmacology, University of Michigan, Ann Arbor, MI 48109-0626.

Recent studies have demonstrated that behavioral sensitization to amphetamine and cocaine requires activation of the N-methyl-D-aspartate (NMDA) class of glutamate receptors because sensitization is blocked by MK-801, a non-competitive NMDA receptor antagonist. The present study reexamined the effects of daily injection of phencyclidine on locomotor activity and stereotypy, and investigated whether MK-801 blocked behavioral sensitization to phencyclidine. Female Sprague-Dawley rats were injected i.p. once daily with phencyclidine, *d*-amphetamine, 0.9% NaCl, or MK-801 followed 30 min later by phencyclidine or *d*-amphetamine. Locomotor activity and stereotypy were measured automatically with the Digiscan system. The results confirmed an earlier finding that four daily injections of phencyclidine induced sensitization to both locomotor activity and stereotypy. Moreover, MK-801 did not block behavioral sensitization to phencyclidine although it blocks behavioral sensitization to *d*-amphetamine. The present research suggests that the development of behavioral sensitization to phencyclidine may be mediated by neuronal mechanisms different from those underlying sensitization to amphetamine and cocaine. (Supported in part by NIDA Grant DA-01531.)

414.5

MULTIPLE INJECTIONS OF MK-801 AFTER RECOVERY FROM LESIONS IN THE SOMATIC SENSORIMOTOR CORTEX REINSTATE FORELIMB PLACING DEFICITS IN THE RAT. S. Barbay* and T.M. Barth, Department of Psychology, Texas Christian University, Ft. Worth, TX 76129.

Unilateral lesions in the rat somatic sensorimotor cortex (SMC) produce a syndrome that includes impairments in tactile placing with the forelimb contralateral to the lesion and an ipsilateral sensorimotor asymmetry on a bilateral tactile stimulation test. These symptoms eventually recover. Following the recovery, a single injection of the noncompetitive NMDA antagonist MK-801, reinstates the contralateral impariment in tactile placing for 7-14 days (reinstatement effect). However, a previous study demonstrated that a regimen of MK-801 beginning 16 hrs after the lesion prevented the reinstatement effect. It was suggested that MK-801 administered shortly after the lesion prevents the degeneration of neurons distant from the lesion site. Thus, the transneuronal degeneration may be necessary for the reinstatement effect to occur. An alternative to the "neuroprotective hypothesis" is that multiple injections of the drug desensitize the animal to the later postrecovery adminstration of MK-801. The present study investigates this alternative hypothesis by beginning the regimen of MK-801 after recovery is complete.

Rats received unilateral SMC lesions, allowed to recover, and then received injections of MK-801 (1 mg/kg) on three consecutive days. This produced a profound reinstatement of the contralateral placing deficits which endured for 14-28 days. Following recovery from the reinstatement, the rats were given a single injection of MK-801 (1 mg/kg). Once again, contralateral forelimb placing deficits were reinstated. In some cases the second reinstatement appeared to be permanent (at least 60 days). These data fail to support the idea that multiple injections of MK-801 desensitize the animal to future treatments of the drug. Moreover, the working hypothesis (that neuroprotective effects of early MK-801 treatment prevent later reinstatement), appears to be tenable.

414.6

SCOPOLAMINE FACILITATES RECOVERY OF FORELIMB PLACING BEHAVIOR FOLLOWING UNILATERAL CORTICAL LESIONS IN THE RAT. R. M. Saponjic*, S. Barbay, M. R. Hoane, S. L. Irish and T.M. Barth, Department of Psychology, Texas Christian University, Ft. Worth, TX 76129.

Following brain injury there is an excessive nonspecific release of excitatory neurotransmitters (i.e. N-methyl-D-Aspartate [NMDA]; acetylcholine) that may lead to secondary cell death proximal and distal to the site of trauma. Although much research effort has focussed on the excessive release of excitatory amino acids, acetylcholine is also released in excess. Recent studies have suggested that excitatory neurotransmitter receptor antagonists may be effective neuroprotective agents (i.e. MK-801) and facilitate recovery of function. The present experiment was designed to determine whether admistration of scopolamine (a muscarinic receptor antagonist) could facilitate behavioral recovery following unilateral lesions of the rat somatic sensorimotor cortex (SMC).

Rats received unilateral electrolytic lesions in the SMC and a regimen of scopolamine (1mg/kg) or saline beginning 15 min after the damage. The behavioral tests included tasks sensitive to forelimb motor and sensory function. Rats treated with saline showed the expected severe contralateral impairments in forelimb placing as well as an ipsilateral somatosensory asymmetry on the bilateral tactile stimulation test. In contrast, the magnitude of the initial forelimb placing deficit was significantly reduced in animals treated with scopolamine, and the rate of subsequent recovery was facilitated when compared to saline treated controls. Scopolamine had no effect on the somatosensory asymmetry. These data support the idea that muscarinic receptor stimulation may play a role in the pathophysiological responses associated with brain injury. Future studies will compare the relative effectiveness of MK-801 and scopolamine as neuroprotective agents.

414.7

THE AMELIORATIVE EFFECT OF REPEATED INJECTIONS OF SCOPOLAMINE HYDROBROMIDE ON RECOVERY FROM BRAIN DAMAGE: AN ANALYSIS OF AGE. A.M. Schneider*, E. Thomas, E. Cardemil, C. Carr, M. Jacobs, M. Criden, Depts. of Psychology and Biology, Swarthmore College, Swarthmore, PA 19081 and Bryn Mawr College, Bryn Mawr, PA 19010

In neurodegenerative diseases such as Parkinson's disease and perhaps Alzheimer's and Huntington's disease, synaptic adaptations are the likely explanation for the symptoms not appearing until the degenerative process is almost complete. Thus, the symptoms appear to be "masked" by synaptic compensation.

We present an animal model of the "masking" effect in the cholinergic system and evidence indicating that the effect declines with age. We found in younger (3 month) and older (10-12 month) rats that repeated injections of scopolamine hydrobromide (a procedure that produces upregulated muscarinic receptors), if given prior to surgery, "masked" the amnesic effects of nucleus basalis magnocellularis (NBM) lesions. Specifically, in the younger rats repeated injections of a low dose of scopolamine (4 mg/kg) reduced the amnesic effects of lesions of NBM in a passive-avoidance test. In the older rats the lower dose was ineffective, but a higher dose (8mg/kg) was effective in ameliorating the amnesic effects of the lesions.

These findings suggest that there is greater plasticity in younger rats than in older rats in the mechanisms that may compensate for the effects of brain damage.

Supported by grants from the Howard Hughes Medical Institute and the Pew Foundation.

414.8

ACCELERATION OF RECOVERY FROM CORTICAL HEMIPLEGIA BY TWO GINKGO BILOBA EXTRACTS. S. Brailowsky*, T. Montiel and L. Medina-Ceja . Instituto de Fisiología Celular, U.N.A.M., 04510 México D.F., MEXICO

In a previous study (Restor. Neurol. Neurosci., 3:267, 1991) we reported on the beneficial effects of a Ginkgo biloba extract (EGb761-IPSEN) on two models of cortical hemiplegia in rats. Being interested in identifying the active principle responsible for these effects, we studied the motor behavior (elevated beam test) of rats having sustained unilateral motor cortex aspiration. Two extracts were assayed: EGb-761, 100 and 50 mg/kg p.o., and a EGb extract containing no terpenes (100 mg/kg p.o.). Both treatments were given 24 h after the lesion and for 7 days. Both extracts produced a faster recovery than that observed in lesion-only rats. The treated groups also showed smaller ventricles on the lesion side, suggestive of reduced edema.

We conclude that the active substance(s) participating in this beneficial effect of EGb-761 extract are non-terpenes and that a dose 50% smaller than the one used previously is sufficient to accelerate recovery from a cortical lesion.

Supported in part by DGAPA-UNAM and by IPSEN-Paris.

414.9

INHIBITION OF PERSISTING RAT HIND-LIMB FLEXION (SPINAL FIXATION) BY PREVIOUS HIND-LEG STIMULATION. M.M. Patterson*, K. Perkins, M. Feizelli and M.J. Bartelt, College of Osteopathic Medicine and Department of Psychology, Ohio University, Athens, OH 45701

Previous work has demonstrated that 40 minutes of 2-4 ma, 100 Hz stimulation to a spinalized rat's hind leg generates a spinal flexion alteration (spinal fixation) which is readily measurable as persisting hind leg flexion following stimulus termination (Steinmetz, et.al., 1981, *JCPP*). In the present study, we explored the influence of prestimulating unspinalized rats' hind legs on the amount of hind leg flexion induced by restimulating the leg 72 hours later, immediately following spinal section.

Sixty-nine rats were anesthetized with Nembutal (50 mg/kg, ip) and then stimulated on the upper right hind leg with either 1.5 or 2.5 ma, 100 Hz, 7 msec pulses for 30 minutes or were simply placed in the experimental apparatus for the equivalent time. They were then returned to their home cages. Seventy-two hours later, all rats were reanesthetized, spinalized at T7 and stimulated with either 1.5 or 2.5 ma for 40 min. Following the stimulation, the spinal fixation was assessed by determining the amount of weight required to pull the stimulated (flexed) leg even with the unstimulated leg.

The study resulted in 4 experimental groups, plus controls. The 1.5 ma control (0, 1.5 ma stimulation) had 23.2 gms of flexion as opposed to 47.0 gms for the 2.5 ma control group (0, 2.5 ma stimulation), values usual for these parameters. The 1.5, 1.5 ma stimulation group had 14.2 gms, the 1.5, 2.5 had 11.5 gms, while the 2.5, 1,5 had 9.0 gms, and the 2.5, 2.5 ma group had 49.0 gms. Except for the 2.5, 2.5 ma group, the prestimulation reliably reduced the amount of persisting hind limb flexion to the following fixating stimulus. This result is somewhat surprising since it might have been expected that the initial stimulation period would summate with the later stimulation to produce additional fixation. It is possible that the intitial stimulation caused alterations of the spinal interneurons which made them less plastic to further alterations, except for the combination of higher stimulus intensities with both stimuli. Alternatively, the effects of stimualtion on cortical function may influence the effects of later stimulation through descending influences. The effects of time between stimulation periods, and stimulus intensity parameters are now being explored. Supported by American Osteopathic Association Grant 92-08-319.

414.10

ENERGY METABOLISM CHANGES ACCOMPANYING RECOVERY FROM UNILATERAL NEGLECT IN THE RAT AS MEASURED BY CYTOCHROME OXIDASE. M.F. Novotny*, L.J. Cudmore, C.G. Ellard, and D.P. Crowne. Department of Psychology, University of Waterloo, Waterloo, Ontario, Canada, N2L 3G1.

Contralateral neglect induced by unilateral lesions to the parietal or frontal cortex eventually recovers. The deficits can be reinstated following transection of the corpus callosum suggesting a possible role of the intact contralateral hemisphere in recovery. In this study, cortical activity changes were assessed during recovery from neglect in rats using cytochrome oxidase. Adult Long Evans male rats underwent either unilateral medial frontal or posterior parietal lesions. They were tested for unilateral neglect with a modified version of the polysensory test for neglect. The animals were tested prior to and 3 times per week following surgery . At either 2 days or 7 days postoperatively (PO), animals were perfused and tissue was reacted for cytochrome oxidase using a modified version of the Wong-Riley technique. The pattern of cytochrome oxidase staining appeared to be different for parietal and frontal lesions. In the medial frontal group, the cortex ipsilateral to the lesion showed a decrease in cytochrome staining compared to the intact contralateral hemisphere. However, in the parietal lesion group the cortex adjacent to the lesion site was more darkly reacted, much like the contralateral hemisphere. Unilateral lesions to the frontal and parietal cortex both produce unilateral neglect, but have differential cortical activity changes during recovery.

This research was funded by grants to DPC and CGE from the Natural Sciences and Engineering Research Council of Canada.

414.11

METABOLIC CHANGES SCANNED BY MR IMAGING IN THE HYPOTHALAMUS OF RATS WITH L-LYSINE DEFICIENCY AFTER L-LYSINE ADMINISTRATION INTRAPERITONEALLY. T.Yokawa[1], E.Tabuchi[1], T.Inubushi[2], T.Ono[3] and K.Torii[1,4]*. ERATO, JRDC, Japan[1]. Mole. Neurobiol. Res. Center, Shiga Med. Univ., Japan[2]. Dept. Physiol., Toyama Med. & Pharmaceu. Univ., Japan[3]. Central Research Lab., Ajinomoto Co. Inc., Japan[4].

Each L-amino acid (AA) in plasma and brain remains unchanged all day long while normal diet is available. Once L-lysine (Lys) deficient diet offered to rats, Lys in plasma and brain declined. When solutions of AAs were offered, they selected the Lys solution and their food intake and growth normalized. The single neuron activity in the lateral hypothalamic area of these rats suggested that the neural plasticity occurred, specifically responding to Lys, iontophoretic application and during ingestion of AA. Non-invasive magnetic resonance imaging (MRI) has been developed to monitor changes in cerebral blood flow (CBF) and oxygenation. MRI signal intensity changes in T2* weighted images of the brain of rats with Lys deficiency were studied using MR Imager (4.7 tesla, 40 cm bore in diameter) with a handmade volume coil. Wistar strain male rats (6 weeks of age, N=6 in each group) fed with Lys deficient diet for 4 days were used. When they received a Lys injection intraperitoneally (0.2 M, 10 ml/kg) higher signals in the medial and lateral hypothalamus were appeared in T2* weighted images. This higher signal in the hypothalamus caused by the Lys treatment lasted for 30 min, and then gradually decreased. There were no signal changes in the case of saline injection as control. These results suggest that the medial and lateral hypothalamus in an essential AA deficiency may play an important roles to response to particular deficient nutrient intake during recognition processes.

414.12

RESPONSES OF LATERAL HYPOTHALAMIC NEURONS IN LYSINE DEFICIENT RATS DURING INGESTION OF AMINO ACIDS AND NaCl. T. Kondoh[1], E. Tabuchi[1], T. Voynikov[1], T. Yokawa[1], T. Ono[2], A. Niijima[3]* and K. Torii[1] [1]Torii Project, ERATO, JRDC, Yokohama, Japan, [2]Dept. Physiol., Toyama Med. & Pharmaceu. Univ., Toyama, Japan, [3]Dept. Physiol., Sch. of Med., Niigata Univ., Niigata, Japan.

Rats fed the L-lysine (Lys) deficient diet selectively ingest Lys solution, after learning and memorizing the taste. In the present study, we first confirmed that Lys-deficient rats learned to selectively ingest Lys, and then recorded single neuron activity from the lateral hypothalamus (LHA) of these rats during discrimination of conditioned cue tone stimuli associated with amino acids (0.2M Lys, 0.15M monosodium L-glutamate (MSG), 0.05M L-arginine, 0.5M L-glycine), 0.15M NaCl (saline) and water, and their subsequent ingestion. More LHA neurons responded to Lys ingestion, its cue tone, and to saline; and fewer responded to arginine ingestion during Lys deficiency than in control. Typically, during Lys deficiency, neurons that responded to Lys or to MSG also responded to saline. Neurons that differentiate cue tone and/or ingestion of the solutions were located more in the dorsal and lateral LHA. The results suggest that preference for a deficient amino acid and NaCl during Lys deficiency and for MSG in normal protein nutrition might be mediated through the LHA, and learning and memory could affect these processes. Chronic recordings of LHA neuron activity in freely behaving rats are currently under investigation to demonstrate possible neural plastic changes.

414.13

ESTROGEN-INDUCED ACCELERATED SPECTRIN PROTEOLYSIS IN THE RAT ARCUATE NUCLEUS. M-C de Lacoste* & C.E. Lewis Dept. Ob. Gyn., Yale University Medical School, New Haven, CT 06510.

Reportedly, estrogen can induce synaptic plasticity in the central nervous system (e.g., Frankfurt & McEwen, 1992; Naftolin et al., 1990). However, the mechanisms underlying this plasticity remain to be deciphered. This study was undertaken to determine if post-translational modification, i.e., calpain cleavage, of the spectrin-based neuronal cytoskeleton may serve as a central molecular mechanism underlying some of the morphological changes seen in association with estrogen-induced synaptic plasticity. Methods: 22 adult normal cycling Sprague-Dawley rats were used: 18 of the animals received a single subcutaneous injection of 1.0mg estradiol valerate(.1cc of 10mgEV/ml in sesame oil) at 70-75 days of age (ET animals) while 4 served as untreated controls. Animals were sacrificed 4,6, 8 and 32 weeks later and perfusion- fixed. Blocks of tissue containing the rostral hypothalamus were vibratome-sectioned and processed for both light (LM) and electron microscopic (EM) immunocytochemistry using standard ABC immunoperoxidase techniques and the RA150 antibody. The RA150 antibody, an antibody made to a synthetic peptide characterizing the C-terminus of the calpain-I cleavage site of alpha fodrin, serves as a specific marker of spectrin proteolysis. Results: Little or no immunostaining was seen throughout the arcuate nucleus of untreated animals. In contrast, at the LM level RA150 immunodecoration of arcuate neuropil could be discerned in the 4,6,8 and 32 wk ET animals. At the EM level, the immunoreactive neurites could be easily identified as dendrites. Hence, it appears that within the arcuate nucleus, estrogen induces proteolysis of the spectrin neuronal cytoskeleton. These findings parallel results obtained in other laboratories which indicate that long-term potentiation is associated with spectrin breakdown. *The RA150 antibody was kindly provided by Dr. Jon Morrow.*

414.14

DENDRITIC BRANCHES OF A RAT ABDUCENS MOTONEURONE FORM CLUSTERS WITH SIMILAR SOMATOFUGAL SPREAD OF ELECTROTONUS. H.Bras[1], S.M. Korogod[2], V.N. Sarana[2], P.Gogan[1] and S. Tyc-Dumont[1*]. [1]CNRS UPR 418 13009 Marseille France, [2]State University, Dniepropetrovsk 320625, Ukraine.

Following high spatial resolution description of 3D geometry of the dendrites of an abducens motoneurone intracellularly labelled with HRP, we simulated the distribution of electrotonic voltage over the whole dendritic tree in response to steady state depolarisation of the soma. Stochastic local non-uniformities of the branch diameter and asymmetries of the branchings led to corresponding local non-monotonous voltage decay along the somatofugal dendritic pathways. Part of dendritic branches had similar electrotonic behavior over a relatively long pathway at a given distance from soma, resulting in their grouping. A cluster analysis (K-means clustering method) was performed to estimate quantitatively this grouping. Each of the 63 studied branches (> 50 μm) were characterized by 4 parameters: electrotonic voltage and gradient, mean and standard deviations. The results revealed good distinction between 4 clusters, which corresponded to the 4 observed groupings. In the parametric Euclidean space, distances between the clusters ranged from 1.66 to 5.68. Within clusters, the distance from the center varied between 0.087 and 0.85. The less compact cluster was formed by 8 of the most proximal branches of 7 dendrites. Two other clusters contained 18 and 24 branches respectively, corresponding mainly to 5 dendrites. The fourth cluster was formed by 13 of the most distal branches of the same dendrite. The clustering of the branches survived under variation of the specific membrane resistivity ranging from 1 to 10 kOhm.cm^2.

414.15

HYPERAMMONEMIA DECREASES PROTEIN KINASE C-DEPENDENT PHOSPHORYLATION OF MICROTUBULE-ASSOCIATED PROTEIN 2 AND INCREASES ITS BINDING TO TUBULIN. V. Felipo*, M. D. Miñana and E. Grau. Instituto de Investigaciones Citológicas. Amadeo de Saboya, 4. 46010 Valencia. Spain.

Chronic hyperammonemia increases the tubulin content in brain. The synthesis of tubulin is regulated by the level of free tubulin and its polymerization in brain is mainly controlled by the binding of microtubule-associated protein 2 (MAP-2) and Tau proteins, which stimulate polymerization. The ability of these proteins to bind tubulin is in turn controlled by their phosphorylation. In studies to clarify the mechanism by which high ammonia levels induce tubulin synthesis we found that chronic hyperammonemia increases the polymerization of microtubule and that changes in polymerization precede those in tubulin synthesis.

We show now that the binding of MAP-2 to microtubules as well as the capacity of MAPs to stimulate polymerization of tubulin is increased in hyperammonemia and that protein kinase C-dependent phosphorylation of MAP-2 is markedly diminished. This could explain the increased binding of MAP-2 to tubulin and the enhanced polymerization of brain microtubules in hyperammonemic rats. The results reported also indicate that physiological or pathological changes in vivo can lead to alterations in cytoskeletal function which, in turn, could act as mediators in neurological alterations such as those reported in hyperammonemia.

414.16

GEL ELECTROPHORESIS OF PROTEINS TRANSPORTED BY FAST AXOPLASMIC FLOW IN THE SCIATIC NERVE OF EXERCISE-TRAINED RATS. C.-M. Kang, P.-A. Lavoie* and P. F. Gardiner. Départements d'Éducation Physique et de Pharmacologie, Université de Montréal, Montréal, Québec, Canada, H3C 3J7.

A previous study in our laboratory showed that endurance training increased the quantity of protein subjected to fast axonal transport in the sciatic motor axons of rats. The aim of the present study was to determine whether it is an overall increase of all proteins or a selective increase of some protein(s), as in nerve regeneration. Rats were trained on treadmill for 11 to 13 weeks (27 m/min with 10% grade, 2 h/d, 3 days out of 4 by the 7th week). ^{35}S-methionine (450 μCi) was injected bilaterally into the ventral horn of the L_4-L_5 spinal cord segments, and a ligature was placed on both sciatic nerves about 70 mm from the cell body 5½ h later. Three hours after ligation, the nerve outside the spine was removed and sliced into 3-mm segments. The segment most proximal to the ligature was prepared for gel electrophoresis, and the other 8 segments were processed for liquid scintillation counting. The proteins with molecular weight between 15 kd and 190 kd were analyzed on 7.5% or 12.5% polyacrylamide gels. The percentage of each protein did not differ significantly between trained and control. It seems that, unlike nerve regeneration, exercise training produces a uniform increase among the transported protein species. (Supported by NSERC Canada and FCAR Québec).

414.17

MICRODIALYSIS REVEALS DOPAMINE RELEASE IN THE NUCLEUS ACCUMBENS OF NEONATAL DOPAMINE DEPLETED RATS RECEIVING CAUDAL LINEAR NUCLEUS STIMULATION AS ADULTS. **Kulraj Sidhu*, Janet Nunnally, James R. Stellar, Daniel Fisher, Megan Fisher. Departments of Psychology and Pharmacy, Northeastern University, Boston MA 02115**

Previously we had demonstrated that adult rats receiving 6-OHDA treatment (ICV 50 ug, each side) at neonatal day 3, have a novel dopamine (DA) projection from the caudal linear nucleus (CLi) of the Raphe to the nucleus accumbens core (ACC) that is not seen in normal adult rats (Sidhu et al NS Abstracts, 1992) or vehicle treated controls. In this study, day 3 6-OHDA or vehicle treated rats were subjected as adults to ACC microdialysis (collinear probe design, 2.0mm active collection area, 5.0ul samples with 1.0ul/minute CSF flow). Samples were analyzed for DA and DOPAC with microbore HPLC-ED methods. Rats were conscious and unrestrained. Animals received 10mg/kg, IP, of the DA reuptake blocker GBR12909. Also, direct CLi stimulation through a chronic electrode was imposed using parameters that were within normal self-stimulation ranges. ACC DA and DOPAC was detected at up to 50% above basal levels after combined GBR12909 and CLi stimulation treatment. Unlike vehicle treated rats GBR12909 had little or no effects on its own. These results reinforce the notion that the novel Cli-ACC pathway may play a role in sparing of reward function in these rats.

414.18

NEONATAL CASTRATION BLOCKS THE CHOLINE INDUCED INCREASE IN SIZE OF SEPTAL NEURONS IMMUNOREACTIVE FOR NGFR. E. Gorry[1], R. Loy[2], W.H. Meck[3], & C.L. Williams[4*]. College of Physicians and Surgeons[1], Depts. of Psychology, Barnard College[4] & Columbia University[3], NY, NY 10027 and Canandaigua VAMC and Dept. of Neurology, University Rochester[2], Rochester, NY 14620.

Recent work has shown that choline chloride administered during ED 12-17 and PD 16-30 produces a long-term enhancement of visuospatial memory in male rats. This organizational effect of perinatal choline on memory appears to be dependent upon exposure to neonatal steroids; castration at birth, but not at 50 days of age, prevents the improvement in memory following perinatal choline supplementation. We have also found that hippocampal NGF is elevated in choline-treated rats castrated as adults, while neonatally castrated rats do not show the choline-induced increase in NGF (Gorry et al., Soc. Neurosci. Abstr., *18*, 1992). In addition, perinatal choline supplementation increases basal forebrain cell size (Loy & Choe, Soc. Neurosci. Abstr., *17*, 1991). We now report that the size of medial septal neurons immunoreactive for nerve growth factor receptor (NGFRir) is influenced by both neonatal steroids and perinatal choline supplementation. Choline administered from ED 12-PD 30 increased both the perimeter and the area of NGFRir medial septal neurons in adult (7 mo) castrated rats. This choline-induced increase in cell size was blocked by neonatal castration. These data suggest that there is an interaction between organizational effects of gonadal steroids and organizational effects of supplemental choline on the developing basal forebrain cholinergic system and visuospatial memory. (Supported by PO1 AG09525)

415.1

DOES THE PIGEON HAVE A NUCLEUS UVAEFORMIS (UVA), OR THE SONGBIRD A NUCLEUS DORSOLATERALIS POSTERIOR THALAMI, PARS CAUDALIS (CDLP)? I.M. Wild*, Dept. of Anatomy, School of Medicine, University of Auckland, Auckland, New Zealand.

Uva in songbirds is thought to be involved in song learning or song control circuitry primarily by virtue of its direct and indirect projections upon the High Vocal Center (HVC), which is only two synapes away from vocal motoneurons. Uva is composed of cells of similar size and location to those of cDLP in pigeon, both nuclei lying within the crook of the elbow of the habenulo-interpeduncular tract and dorsal to the nucleus spiriform medialis. The sources of input to cDLP in pigeon are known to be lamina 13 of the optic tectum, and the dorsal column nuclei (DCN). cDLP projects upon the "neostriatum", where the region of its terminal field anterolateral to the auditory Field L contains a somatosensory representation of the body. To determine whether Uva has similar inputs to those of cDLP, injections of cholera toxin B-chain conjugated to HRP were made into either the DCN or the deep tectum in greenfinches, zebra finches, and Java sparrows. Both DCN and tectal injections produced dense and specific anterograde labelling of Uva, predominantly contralaterally and ipsilaterally, respectively. Injections centered on Uva produced retrogradely labelled cells in DCN, tectal lamina 13, and in the rostro-ventrolateral medulla. They also produced a terminal field within the "neostriatum" anterolateral to Field L which has been previously identified as nucleus interfacialis (NIf). Somatosensory evoked potentials were recorded from NIf, and injections of retrograde tracers were made at the recording site. Retrogradely labelled cells were found in only two thalamic nuclei: Uva, particularly its ventral portion, and ventrolateral parts of nucleus ovoidalis. These results confirm precisely those found previously in the pigeon somatosensory system, but they are difficult to interpret in terms of a supposed role of Uva in the control of song. One of their implications could be, however, that HVC - to which NIf projects - is functionally heterogeneous. Supported by Whitehall Foundation, Inc.

415.2

HIERARCHICAL ORGANIZATION OF BRAIN AREAS MEDIATING ZEBRA FINCH LEARNED VOCALIZATIONS. E. T. Vu*, M. E. Mazurek, and Y. Kuo. Division of Biology, Caltech, Pasadena, CA 91125.

Although several lines of evidence suggest that the stereotyped sequencing of syllables in adult zebra finch song arises from a central motor representation of song, the neural basis for this is not well understood. Using chronically implanted stimulating electrodes, we probed for the existence and location of a neural representation of this feature of song behavior, syllable sequencing. Individual nuclei in the song motor pathway were briefly stimulated during singing (7 pulses at 400 Hz, 0.4 ms duration). We hypothesized that if the precise sequencing of song syllables required peripheral feedback or required precisely timed neural activity in all parts of the motor pathway then experimentally altering the firing pattern at any point of the motor pathway would change the syllable sequence. Alternatively, if a localized central representation of syllable sequence existed then stimulating some nuclei but not others would result in altered syllable sequencing. Stimulating song nucleus RA distorted an ongoing syllable without changing the order or timing of ensuing syllables, whereas stimulating nuclei higher up the motor pathway (i.e. HVc and NIf) altered both ongoing syllables and the ensuing song pattern. Specifically, birds suspended a song phrase they were singing soon after the stimulus was applied and rapidly resumed at the beginning of the phrase. HVc stimulation in a deafened bird had the same effect. These findings suggest that syllable sequencing during singing is organized in nuclei above RA, and that the resulting pattern is imposed on and unaffected by activity in lower structures of the motor pathway.

415.3

AUDITORY RESPONSE PROPERTIES OF FIELD L IN THE ZEBRA FINCH D. Lim*, Division of Biology, California Institute of Technology, Pasadena, CA 91125.

The connections of field L and its auditory response properties to various kinds of stimuli were studied in adult male zebra finches *(Poephila guttata)*. Biotinylated dextran amine was injected in three subdivisions--L1, L2, and L3, for both anterograde and retrograde tracing. In all three layers, most of afferent projections were found in the shelf area around HVc. Layer L3 projected to the neighboring area of RA. There were also reciprocal connections between L1 and L3. To study the physiological response properties of each layer, a total of 417 units were collected from 39 animals. These recordings showed a relatively simple stereotyped responses in L2 while L1 and L3 layers exhibited widely varying responses. Different kinds of stimulus selectivities were identified in these two layers, such as preference for song, white noise, and FM. These subdivisions also contained most of the neurons that responded selectively to the direction in which song or FM signals were presented. The responses of neurons in L1 and L3 to signals composed of two harmonic frequencies differed from the linear sum of their responses to individual components, whereas the neurons of L2 showed no significant interactions. L2 neurons occurred in tonotopic sequences, whereas such orders were not found in L1 and L3. The contributions of L1 and L3 to the selectivity of HVc neurons for song were examined by injection of a local anesthetic into these layers while HVc song-selective neurons were monitored. The results show that HVc needs L1 and L3 for the maintenance of song selectivity. This work was supported by International Human Frontier Science Program.

415.4

EFFECT OF DEAFENING ON THE CONTACT CALL OF ADULT BUDGERIGARS. J.T. Heaton and R.J. Dooling*. Dept. of Psychology, Univ. of Maryland, College Park, MD. 20742-4411.

Auditory feedback is necessary for the development of normal song and contact calls in budgerigars. Here we report that auditory feedback is also required for the maintenance of species typical contact calls in adult budgerigars.

Six adult budgerigars were deafened by bilateral extirpation of the basilar papillae after removal of the columella and supportive structures. Deafened birds vocalized less frequently than normal birds, and their calls showed abnormalities in acoustic structure within a few weeks of surgery. Within several months, nearly all contact calls produced by deafened birds were strikingly abnormal, and by 5 months none of the birds in our sample produced normal contact calls. Several birds, however, continued to produce alarm calls that were very similar to those produced prior to deafening. It was also noted that some of the deafened birds would assume a singing posture, which included head and throat movements indicative of vocalization, without making a sound. These data raise interesting questions about whether different neural mechanisms are involved in the production of different call types in the budgerigar vocal repertoire.

Supported by grants NIH DC-00198(R.J.D), and NIH MH-40698 (S.E. Brauth)

415.5

FREE-FIELD RELEASE FROM NOISE MASKING IN PARAKEETS. O.N. Larsen*, M. Dent and R.J. Dooling, Dept. of Psychology, Univ. of Maryland, College Park, MD 20742.

The auditory system is constantly faced with the task of detecting biologically important sounds against a spatially diffuse background of environmental noise. In a few cases, such as close to running water, the environmental noise is dominated by a single source. In humans, speech intelligibility and signal detection is generally improved as such a masker and the signal are spatially separated. We wished to learn to what extent signal detection is improved with spatial separation of signal and masker in birds with small head widths and consequently with little sound diffraction in the biologically important frequency range.

Two adult male parakeets (*Melopsittacus undulatus*) were trained using operant conditioning techniques to peck keys for food reinforcement in response to the presentation of pure tone stimuli (frequencies 2.86 and 4.00 kHz, duration 400 ms) embedded in continuous, broadband noise (1.5 to 10 kHz) with a spectrum level of 10 dB/Hz. Trained birds were tested using the method of constant stimuli (step size 3 dB) at seven sound pressure levels with the noise source fixed in front of the bird (azimuth = 0°) and the tone source in one of 12 azimuth positions (step size 30°). As a control absolute thresholds for these tone signals (i.e. no masking noise) were measured in all 12 azimuth positions. Additional experiments were conducted with the noise located at 90°, 180°, and 270° and the tone source at either 0°, 90°, 180°, or 270°.

Results show that detection of signals in noise improves when noise and signal sources are spatially separated. The improvement is about 6 dB at a signal and noise separation of 90° with less improvement at other azimuth angles. This pattern of masking release is qualitatively similar to that found in mammalian species.

Supported by grants DNSRC 11-7765-3 (O.N.L.) and NIH DC-00198 (R.J.D.)

415.6

USE OF A PARTICLE-BASED MAGNETORECEPTOR BY THE BOBOLINK. R.C. Beason*, N. Dussourd, M.E. Deutschlander and C.A. Augonis. Biology Dept., State Univ. of New York, Geneseo, NY 14454.

The effect of a brief magnetic impulse on magnetic perception was tested on the bobolink (Dolichonyx oryzivorus), a transequatorial migratory bird. The effect of the treatment on the magnetoreceptor was assayed based on the migratory orientation of each bird. Birds were tested in a planetarium without any visible cues available. Each bird served as its own control before treatment, and the effect of the treatment for each bird was computed as deviations in orientation away from its control direction. Birds treated such that the tip of the bill (if it were a magnetic material) would attract the south end of a compass (north-anterior birds) had a deviation of 105° CCW and those treated in the opposite direction (south-anterior) showed a deviation of 51° CW. Birds treated with the polarity vertical through the head and all birds magnetized a second time opposite their original polarity were axially bimodal or were not significantly oriented. These results indicate that treatment with a strong (0.5 T) magnetic impulse affects a magnetic receptor in this species. The material most likely involved in this process is the magnetite which has been reported previously in this species. These results also indicate that the sensory information from the modified receptor is being interpreted by the bird and not ignored. Funding provided by NSF (BNS 9011840).

415.7

BLOCKADE OF NMDA RECEPTORS IN RA DISRUPTS LEARNED VOCALIZATIONS IN ADULT MALE ZEBRA FINCHES. A. Lombardino* and F. Nottebohm. Rockefeller Univ. Field Research Ctr., Millbrook, NY 12545

Male zebra finches learn their song and long call by reference to external models. Their nucleus robustus archistriatalis is a key telencephalic nucleus for the production of learned song. RA neurons have NMDA receptors that respond readily to excitatory amino acid stimulation from lMAN. lMAN is necessary for song learning, but electrolytic lesion of lMAN in adulthood does not affect the production of learned vocalizations. Consequently, D,L-APV and MK-801, which are NMDA receptor antagonists, should have little effect on the song and long call of adult birds when administered to RA. To our surprise, this is not the case. Bilateral microinfusion of nanomolar concentrations of D,L-APV or MK-801 into RA reversibly blocked directed song and disrupted the morphology of the long call; song introductory notes were still produced, and depending on the dosage their morphology was unchanged or degraded. The first two or three notes of song were given unchanged and repeated monotonously at drug concentrations below those able to block the whole song motif. There was no effect of these treatments on the unlearned long call of female zebra finches. Control infusions of saline or L-APV had no effect on male vocalizations. Electrolytic lesion of LMAN days or weeks prior to treatment did not abolish these effects. Similarly, lidocaine (8%) microinfusion into lMAN did not interfere with the production of learned vocalizations, nor did it alter the effect of the NMDA receptor antagonists in RA. Taken together, our results suggest that NMDA receptor-mediated glutamatergic transmission in and/or around RA and from non-lMAN sources-- HVC, RA interneurons, or other unidentified afferents-- is important for the production of learned vocalizations in the adult zebra finch.

415.8

CONTRIBUTIONS OF PHOTOPERIOD AND TESTOSTERONE TO SEASONAL CHANGES IN SONG CONTROL NUCLEI. G.T. Smith*†, E. A. Brenowitz†‡, and J.C. Wingfield†. Depts. of †Zoology and ‡Psychology, Univ. of Washington, Seattle, Washington 98195.

Bird song, like many reproductive behaviors, changes seasonally. Brain regions controlling song also change seasonally in several songbird species. We independently manipulated photoperiod and testosterone (T) in castrated male Gambel's white-crowned sparrows to determine the roles of these variables in mediating seasonal changes in the song control regions (SCRs).

Sixteen adult males were captured during fall migration and kept initially on short days (SD, 8L16D). All males were castrated. Half of the males received implants containing T, and the other half received blank (BL) implants. Half of each group was transferred to long days (LD, 20L4D), while the other half was kept on SD. This created 4 groups (LD+T, LD+BL, SD+T, SD+BL) of 4 males each. We collected blood samples during the study to determine plasma concentrations of T. Birds were sacrificed after 6 weeks. Nissl-defined volumes of the SCRs HVC, RA, X, LMAN, and nXII and of 3 regions not involved in song (SpM, Rt, and Pt) were measured.

HVC, RA, and X were larger in T males than BL males, regardless of photoperiod treatment. T-treated males also had larger neurons and less-densely spaced neurons in RA than BL males. T had no significant effects on the volumes of any other brain region. Photoperiod did not have a significant effect on the volume of any of the brain regions measured. These results suggest that testosterone plays the dominant role in mediating seasonal effects on the SCRs. GTS is an HHMI Predoctoral Fellow.

415.9

OPIOID BINDING SITES IN A PASSERINE SONG SYSTEM. C.C. Gulledge* and P. Deviche, Inst. Arctic Biology, Univ. Alaska Fairbanks, Fairbanks, AK 99775.

Specific brain regions, known as the song system, control avian vocalizations. These regions include Area X, n. intercollicularis (ICo) and the Higher Vocal Center (HVC). Some song nuclei contain immunoreactive opioid peptides, but the presence of opioid receptors in these regions has not been examined. We employed *in vitro* autoradiography to localize specific opioid receptors in brain tissues of adult male dark-eyed juncos (*Junco hyemalis*). Tritiated DAMGO, pCl-DPDPE and EKC were used to label μ, δ and κ receptors, respectively. Receptor densities were measured by computerized microdensitometry. Our data show song regions containing high opioid receptor densities, the relative density of binding sites in each region being receptor type-specific. Area X has high μ receptor densities. ICo has relatively high μ and δ receptor densities. The HVC also has relatively high μ and δ receptor densities, but binding is not limited to the region, as adjacent areas of the neostriatum have similar levels. κ receptor densities were low in all the song regions analyzed. Previous studies indicate that opioids have an effect on vocalizations in mammals and chicks. Our results suggest that in passerines this influence depends on direct opioid effects on song nuclei. Further, each opioid receptor type may control different aspects of vocal behavior. Supported by NSF Award BNS-9121258.

415.10

SYNAPTIC ACTIVITY OF NEURONS IN ZEBRA FINCH SONG NUCLEUS HVc IN RESPONSE TO AUDITORY STIMULI. M.S. Lewicki and A.J. Doupe*, Division of Biology, Caltech, Pasadena, CA 91125

Neurons in the caudal nucleus of the ventral hyperstriatum (HVc) of the zebra finch have responses that are sensitive to the temporal structure of the bird's own song. Extracellular studies have shown that the responses can depend on the order of song syllables. Furthermore, the neurons are capable of preserving auditory information over periods greater than several hundred milliseconds after the offset of the stimulus. To investigate the mechanisms underlying these computations, we used *in vivo* intracellular and whole cell patch recordings of adult male HVc neurons in response to a variety of auditory stimuli. The intracellular records exhibit response patterns similar to those observed extracellularly. Some neurons show a strong preference for the bird's own song over both the reversed song and over the normal syllables presented in reverse order. We observed both excitatory and inhibitory potentials with time scales lasting from tens of milliseconds to several seconds following the stimulus. Single song syllables can evoke both excitatory and inhibitory post-synaptic potentials which are present in the absence of spiking and have voltage-dependent amplitudes. Synaptic responses to a single syllable can outlast the stimulus by more than a second. We have observed a variety of synaptic responses which in concert provide a basis for understanding the characteristic sensitivity of HVc neurons to the temporal structure of song.

415.11

THE VOCAL CONTROL PATHWAYS IN BUDGERIGARS ARE STRIKINGLY DIFFERENT FROM THOSE IN SONGBIRDS G.F. Striedter*. Div. of Biology, California Institute of Technology, Pasadena, CA 91125

Vocal learning has evolved in both oscine songbirds and parrots, but the neural circuits underlying vocal learning are only superficially similar in the two taxa. Injections of biotinylated dextrans into nuclei "MAN", "HVc" and "RA" of budgerigars revealed that "RA" projects bilaterally to the hypoglossal nucleus, that "HVc" projects topographically to "RA", and that "MAN" projects only weakly to both "HVc" and "RA", but heavily to an area immediately surrounding "HVc" and "RA". Nuclei "MAN", "HVc" and "RA" receive inputs from three apparently distinct populations of cells within nucleus "DLM" in the rostrodorsal thalamus. All of these connections differ from those observed in songbirds. Most importantly, none of the above-mentioned nuclei receive inputs from the principal telencephalic auditory area, Field L, which provides the auditory input to the vocal control system in songbirds. However, dextran injections into the physiologically identified auditory portions of nucleus basalis revealed that nucleus basalis projects to the overlying frontal neostriatum, which in turn projects to a shelf area surrounding the dorsal portion of "HVc". Some neurons in this shelf area project into "HVc", and some neurons in "HVc" extend dendrites into the shelf area. These data suggest that "HVc" in budgerigars may receive the auditory feedback required for vocal learning via an auditory pathway through nucleus basalis. Physiological recordings from "HVc" in freely moving, vocalizing budgerigars reveal multiunit bursts of activity preceding each vocalization by an average of 39 ms (sd=21ms), providing the first neurophysiological evidence that nucleus "HVc" in budgerigars may be involved in generating natural vocalizations.

415.12

LESIONS OF mMAN PRODUCE SLIGHT DISRUPTIONS IN VOCAL BEHAVIOR OF JUVENILE MALE ZEBRA FINCHES. E.F. Foster* and S.W. Bottjer. University of Southern California, Los Angeles, CA 90089-2520.

The medial magnocellular nucleus of the anterior neostriatum (mMAN) is a small nucleus of the songbird forebrain which sends an axonal projection to the Higher Vocal Center (HVC), a nucleus known to be critical for normal song production in adult birds. mMAN also receives a single afferent projection from neurons in the dorsal thalamus. In order to assess the role of mMAN in vocal behavior, it was lesioned bilaterally using ibotenic acid in adult and juvenile zebra finches. Complete lesions of mMAN in adult birds did not produce any disruption of vocal behavior. Two juvenile males that received lesions at 42 days of age showed slight disruption of their adult song behavior. The overall stereotypy of note sequencing was maintained, however, lesioned birds produced fewer notes than did normal controls and individual notes tended to be either omitted or repeated within a song bout. The quality of some individual notes tended to be poor and notes were of longer duration compared to notes typical of normal birds. The lack of a behavioral effect of mMAN lesions in adults is especially interesting because lesions of HVC, the only known efferent target of mMAN, dramatically disrupt song behavior in adults. The effect of mMAN lesions in juveniles may correspond to the time when HVC axons are establishing synapses with one of its main targets, RA, which in turn projects to the motor neurons innervating the vocal organ.

415.13

CHANGES IN CATECHOLAMINE LEVELS AND TURNOVER IN THE BRAINS OF MALE ZEBRA FINCHES DURING DEVELOPMENT. S.R. Barclay, C.F. Harding*, and S. Waterman. Biopsychology Program, Hunter College, CUNY, New York, NY 10021

In adult zebra finches, catecholamine (CA) levels and turnover in hypothalamic and vocal control nuclei (VCN) are modulated by gonadal hormones. In adults, initiation of courtship singing is dependent on norepinephrine (NE) levels in particular VCN. The primary goal of the current project was to quantify CA levels and turnover in ten behaviorally-relevant brain nuclei (6 VCN, 2 hypothalamic, 2 auditory) at four critical points during development of the VCN and the sensitive period for vocal learning. Aviary-housed finches were sacrificed at 25, 35, 55, and 90 days of age. Some birds were pretreated with alpha-methyl-para-tyrosine to allow estimation of CA turnover. Preliminary study showed that this method worked as well in juveniles as in adults. Brain nuclei were microdissected and CA levels quantified by HPLC-EC. In most nuclei, CA function varied profoundly over development. In most VCN, NE function was highest at days 25 and/or 35, while in hypothalamic nuclei, NE function was maximal at day 90. In most brain nuclei, dopaminergic (DA) function showed clear peaks early in development. In a given nucleus, peak NE and DA function did not coincide. Further research is necessary to determine whether these changes in CA function are related to sexual differentiation of these brain areas and/or to vocal learning.

Supported by NSF Award IBN-9212441 & NIH RR08176

415.14

ORGANIZATION OF SELECTIVE AUDITORY RESPONSES IN A VOCAL NUCLEUS OF THE ZEBRA FINCH. D.S. Vicario* and S.J. Chew. The Rockefeller University, New York, NY 10021.

Songbirds learn their songs by imitating models that they hear. In the zebra finch, the long call is also learned through imitation. Production of these learned vocalizations depends on a specialized vocal control pathway in the songbird brain. Nuclei on this pathway, including nucleus robustus archistriatalis (RA), are known to respond to auditory stimuli, and to respond preferentially to the bird's own song (BOS). We wanted to further characterize the effective features of auditory stimuli, including calls, and to compare the auditory preferences of neurons located in subregions of RA known to have different brainstem projections.

Single and multi-unit recordings were carried out in adult male zebra finches, either awake or anesthetized with urethane. The birds heard BOS, the bird's own call (BOC), degraded versions of BOS, and the songs and calls of conspecifics. Of the 43 units at 15 recording sites analyzed so far, 81% had strong selective responses to specific components of BOS, consistent with earlier reports. Most units also responded vigorously to BOC. At each site, we were able to discriminate 2-6 (median=3) single units; however, not all units at a given site responded to the same stimulus component(s). Response patterns also differed between recording sites.

These results confirm earlier reports of song-selective responses in RA, and extend them to the learned long call, which is acoustically similar to song syllables. The spatial data suggest that RA is not topographically organized into local zones specific to a given syllable. **(Supported by MH40900 and BNS-9109598)**

415.15

EUROPEAN STARLINGS SHOW DEVELOPMENTAL CHANGES IN RECEPTOR DENSITY AND VOLUME OF SONG CONTROL NUCLEI AS DEFINED BY AUTORADIOGRAPHY FOR MUSCARINIC CHOLINERGIC RECEPTORS AND α_2-ADRENERGIC RECEPTORS J.M. Casto*, D.J. Bernard and G.F. Ball Dept of Psychology, Johns Hopkins University, Baltimore, MD 21218, USA

Four song control nuclei, the hyperstriatum ventralis pars caudalis, the nucleus robustus archistriatalis, the magnocellular nucleus of the anterior neostriatum, and area X (HVc, RA, MAN, and X respectively) in adult European starlings can be discerned from the surrounding neural tissue based on the density of both muscarinic cholinergic and α_2-adrenergic receptors. In adults, area X and HVc have high muscarinic and α_2-adrenergic receptor densities relative to surrounding structures and MAN and RA are defined by low muscarinic receptor density but high α_2 receptor density relative to surrounding structures. We used *in vitro* receptor autoradiography for muscarinic cholinergic (defined by N-methyl scopolamine binding) and α_2-adrenergic receptors (defined by p-amino clonidine binding) in order to compare the distribution of these receptor sub-types in 20-day old and adult starlings. All four nuclei in 20-day old starlings are characterized by a high density of α_2-adrenergic receptors relative to surrounding tissue. Juvenile and adults had similar α_2 receptor densities in RA. The volumes of RA and HVc as assessed from the density of α_2 receptors were 2-5 times larger in adults than in juveniles. In 20-day olds, unlike adults, RA is characterized by high muscarinic receptor density relative to the surrounding archistriatum. Mean muscarinic receptor density in RA is 6-7 times higher in juveniles than in adults. Thus, during starling development muscarinic receptor density in RA decreases, α_2 receptor density remains constant, and the volumes of RA and HVc increase. Such developmental changes in the song system may provide insight concerning the neurochemical prerequisites for song learning and behavior.

415.16

TWO INDEPENDENT MARKERS DEMONSTRATE AN INCREASED VOLUME OF HVC IN SHORT-DAY PHOTOSENSITIVE RELATIVE TO LONG-DAY PHOTOREFRACTORY EUROPEAN STARLINGS D.J. Bernard* and G.F. Ball Department of Psychology, Johns Hopkins University, Baltimore, MD 21218, USA

Seasonal changes in the volume of several song control nuclei observed in song birds are believed to arise as a result of fluctuating levels of circulating testosterone (T). Although T is elevated in "spring" birds, there may also be extra-gonadal effects of long days. For example, T treatment results in greater masculinization of nucleus robustus archistriatalis (RA) of female canaries on long than on short days (DeVoogd et al., 1985). In this experiment we dissociated the effects of long day lengths and elevated T levels. We placed photosensitive male European starlings (*Sturnus vulgaris*) on 11L:13D or 16L:8D photoperiods for 5 months. On 11L:13D (a day length characteristic of shortening days in late October), there is gradual recrudescence of the gonads such that after 3 to 4 months they approach maximal size. Thus, starlings in this condition have elevated T titres even though they are experiencing short days. In contrast, starlings maintained on 16L:8D initially show marked gonadal growth. However, after about 4 weeks the birds become photorefractory and the gonads regress. These starlings have low T titres even though they are on long days. At sacrifice the 11L:13D and 16L:8D birds had mean left testis volumes of 303.18 and 6.59 mm^3, respectively. Comparison of the high vocal center (HVc) from Nissl stained sections revealed that the volume was approximately 40% larger in the 11L:13D than in 16L:8D birds. Previous work (e.g., Gahr, 1990) has indicated that "seasonal" changes in HVc volume evident in Nissl stained tissue may not be apparent when investigated with independent cytoarchitectural criteria. The density of α_2-adrenergic receptors as determined by in vitro receptor autoradiography with [^{3}H] p-amino-clonidine (PAC) is higher in HVc than in the surrounding neostriatum, clearly defining the boundaries of the nucleus. We reconstructed the volume of HVc using PAC autoradiography on adjacent sections. The results were identical to those from the Nissl stained tissue. While these results do not rule out the possibility of T-independent effects of long days, they indicate that the effects of T on HVc volume are more dramatic than the effects of long days alone.

415.17

BRAIN SPACE FOR LEARNED PRODUCTION BUT NOT PERCEPTION OF SONG IN MARSH WRENS. E. Brenowitz*, B. Nalls, C. Horning, and D. Kroodsma. Depts. of Psychol. & Zool., Univ. of WA, Seattle, WA 98195 & Dept. of Zool., Univ. of MA, Amherst, MA 01003.

The song nuclei HVC and RA are larger in male birds that produce larger song repertoires. These nuclei act both in song production and perception. In singing birds it is difficult to determine the extent to which the size of these nuclei reflects a commitment of brain space to producing or perceiving song repertoires. We tested the hypothesis that species differences in the size of song nuclei are related to the need to perceive conspecific song by comparing HVC and RA in nonsinging females of two marsh wren species (Cistothorus palustris eastern & western). Western male wrens have larger song repertoires than eastern males.

Using Nissl-stained sections, we measured the absolute volumes of HVC and RA, as well as their volumes relative to the thalamic nucleus Rt and to the entire telencephalon of males and females. We also measured density, number, and somal area of HVC neurons. To determine whether Nissl stain accurately identified the borders of HVC, we incubated sections with the H222Py monoclonal antibody to the human estrogen receptor (Abbott).

Western and eastern female wrens did not differ in any measured attributes of HVC or RA. The volumes of HVC and RA, and cellular attributes of HVC, however, were greater in western males than in eastern males. Also, these nuclei were much larger in males than in females. Female wrens thus do not provide evidence of anatomical specializations for perceptual processing of conspecific male song.

415.18

SONG LEARNING IN ISOLATED ZEBRA FINCHES: ADULT LEARNING AND CONVERGENCE OF SIBLING SONG. S.F. Volman* and H. Khanna. Dept. of Zoology, The Ohio State University, Columbus OH 43210.

Zebra finches reared in isolation from an adult male tutor develop an isolate song that is less stereotyped than the song of normal adults. It has been reported that such birds can learn as adults when exposed to a song tutor (Eales, 1985). We have repeated these experiments and found that isolate birds did, sometimes, learn new song within 2-3 weeks after a tutor was introduced after at least 90 days of song isolation. However, adult learning occurred under only some circumstances. In our experiments, male birds (N=10) isolated with male siblings were never successfully tutored as adults, and only some birds that were isolated and then tutored alone changed their songs. Furthermore, the song of lone-male birds was, on average, less stereotyped at 90 days than that of males reared together, and the songs of group-reared birds were often more similar to their sibling's songs than expected from previous reports, although they remained less stereotyped than the songs of normal adult males. These results suggest that lack of song stereotypy is not necessarily correlated with a zebra finches' ability to modify its song as an adult. They also suggest that siblings can, to some extent, learn song from each other during the normal sensitive period if an adult song tutor is not present.

Supported by NIH and the Deafness Research Foundation

415.19

FUNCTIONAL PARTITIONING OF SYRINGEAL HALVES DURING SONG. S.E. Allan and R.A. Suthers*. Medical Science Program and Center for the Integrative Study of Animal Behavior, Indiana University, Bloomington, IN 47405.

Bronchial airflow and thoracic air sac pressure were recorded in brown-headed cowbirds (*Molothrus ater ater*) during song. The right and left syringeal halves control the production of different aspects of the song. The left side of the syrinx produces the low constant frequency (0.5-1.5 kHz) syllables of the introductory notes. The right side of the syrinx produces higher frequency (1-6 kHz) syllables with more pronounced frequency modulation within the introductory notes, as well as, the final whistle (5-13 kHz). Inspiration during song is predominantly controlled by the left side of the syrinx. The correlation coefficients of pressure and flow patterns provide a measure of stereotypy in the motor dynamics, permitting song matching within and across individuals. The partitioning of the frequency range and inspiratory contributions is consistent across all birds tested. The functional significance of this partitioning is unknown, but it may facilitate the production of the wide dynamic range typical of cowbird song (0.5- 13 kHz). Supported by NIH #NS29467 & NS09229

415.20

ESTROGEN-INDEPENDENCE OF NEUROGENESIS IN THE ADULT CANARY FOREBRAIN A. Hidalgo*, S. A. Goldman, Dept. of Neurology and Neuroscience, Cornell Univ. Medical College, New York, NY 10021.

The vocal control nucleus HVC of the songbird brain exhibits persistent neurogenesis in adulthood. In females, the rate of neurogenesis is not affected by testosterone (Goldman and Nottebohm, PNAS 80:2390-94, 1983), despite androgen induced neuritic arborization, angiogenesis and gliogenesis. We asked whether estrogens might influence adult neurogenesis, by assessing the effect of ovariectomy (OvX) upon neuronal production in the canary HVC. Fifteen adult females were separated into groups of ovariectomized (OvXed; n=4), estradiol silastic-replaced OvXed (n=8), and sham-operated birds (n=3). In order to label dividing cells and their progeny, the birds were injected bidaily for 8 days with ^{3}H-thymidine, sacrificed 32 days later, and their brains autoradiographed. We found no difference in the HVC neuronal labeling indices (LI) between untreated OvXed birds (LI=8.1±4.18%) and estrogen-replaced OvXed birds (LI=8.0±3.00%). Interestingly, a small but significant fall in the NLI was noted in the sham-operated birds (LI=3.9±2.94%), relative to their ovariectomized counterparts. Among estrogen-replaced OvXed birds, the LIs were no different between birds given estradiol before (7.7±2.95%) and after (8.4±3.05%) ^{3}H-thymidine. RIA confirmed that serum estrogen levels were reduced in the castrated (25 pg/ml), compared to the estrogen-replaced (269 pg/ml) and gonadally-intact birds (88 pg/ml). Since cells within the HVC exhibit estrogen receptor immunoreactivity (ER-IR), we asked whether the new neurons or their precursors expressed ER-IR. Two female canaries were given ^{3}H-thymidine for either 4 or 14 days, then sacrificed, probed for ER-IR, and autoradiographed. ^{3}H-thymidine$^+$ cells displayed no detectable ER-IR within their first 2 weeks of postmitotic life. Rather, during migration from the ventricular zone, the new neurons appeared to traverse a layer of mitotically-quiescent, ER$^+$ subventricular cells. These results suggest that estrogen does not influence the rate of neuronal production or the early postmitotic survival of the newly generated neurons of the adult canary brain. (Supported by NINDS K08NS01316, R29NS29813, the Mathers and Lookout Foundations).

415.21

DIFFERENCES BETWEEN MOTOR RECRUITMENT AND AUDITORY RESPONSE PROPERTIES IN ZEBRA FINCH HVc NEURONS. C.-H. Yu*, D. Margoliash, Com. Neurobiology, Dept. Organismal Bio. & Anat., University of Chicago, Chicago, IL 60637.

We have characterized the motor and auditory properties of single HVc neurons in male zebra finches (*Taeniopygia guttata*). Birds were chronically implanted with microwire bundles. Single units were extracted from multiunit recordings using spike waveform template matching techniques. Auditory stimuli included the bird's own song (BOS), modifications of BOS, and conspecific songs.

When birds sang, all neurons analyzed were active throughout the song. Neuronal activity increased 60-70 msec preceding the onset of each song, and terminated 30-70 msec before the end of each song. Motor recruitment histograms were unique for each syllable type and independent of the position of the syllable in the song.

In contrast, excitatory auditory responses to playback of BOS tended to be phasic, and correlated with specific syllable types of BOS. Auditory responses were dependent on the position of the syllable in BOS, and were weaker in response to the time reversed BOS or to conspecific songs. There was no apparent correlation between auditory response and motor recruitment histograms.

The spontaneous activity immediately preceding and following song production was higher than the spontaneous activity during non-singing periods. We observed a gradual transition of the spontaneous activity level, lasting 2-5 sec, from non-singing periods to pre-singing activity level. Thus, the same single units may be recruited with different network properties during production and perception of song.

415.22

ACUTE BLOCKADE OF N-METHYL-D-ASPARTATE (NMDA) RECEPTORS DURING SONG EXPOSURE PREVENTS SONG LEARNING IN ZEBRA FINCHES. S.M. Aamodt*, E.J. Nordeen and K.W. Nordeen. Psychology Dept., Univ. of Rochester, Rochester, NY 14627.

During avian vocal learning, birds memorize a conspecific song model and then use auditory feedback to match their vocalizations to that model. Since experience-dependent behavioral and synaptic plasticity have been linked to activation of N-methyl-D-aspartate (NMDA) receptors, recent studies have addressed their role in avian vocal learning. In zebra finches, NMDA receptors are present in song control nuclei, and NMDA receptor binding declines with age in one brain region specifically implicated in song learning (Aamodt et al., 1992). We report here that blocking NMDA receptors during song memorization impairs song development.

From 21 to 50 days, all birds were housed with a singing tutor for two hours every other day. No other song model was available at any time. Experimental birds (MK-exp, N=7) were injected with the noncompetitive NMDA receptor antagonist MK-801 (0.1 mg/kg) just before tutoring and with saline on nontutoring days. One control group (N=4) received saline before tutoring and MK-801 on nontutoring days, while another control group (N=4) received saline on all days. Finally, to control for motoric side effects of MK-801, some birds (N=3) received the dopamine receptor antagonist haloperidol (0.08 mg/kg) before tutoring and saline on nontutoring days.

In adulthood, no MK-exp bird shared any song notes with the tutor. In contrast, most control birds had copied elements of the tutor's song. Songs produced by MK-exp birds contained abnormal notes that resembled those of birds never exposed to a song model. Moreover, at 120 days, over a month after normal birds develop stereotyped adult song, the songs of MK-exp birds were still changing. We are now determining if these same birds can learn a new song model in adulthood.

415.23

EARLY ISOLATION FROM CONSPECIFIC SONG DOES NOT SUSTAIN ENHANCED MK-801 BINDING IN AN AVIAN SONG NUCLEUS. K.W. Nordeen*, S.M. Aamodt and E.J. Nordeen. Psychology Dept., Univ. of Rochester, Rochester, NY 14627.

In many avian species, birds are most likely to reproduce songs heard during a restricted developmental period. In male zebra finches, this sensitive learning period correlates with an age-related decline in NMDA receptors within the lateral magnocellular nucleus of the anterior neostriatum (lMAN), a brain area necessary for song learning. In the lMAN, binding of the NMDA receptor antagonist [^{3}H]MK-801 is twice as high in 30 day males within the sensitive period as in adults (Aamodt et al., 1992). To test the hypothesis that the sensitive period for song learning is linked to the high NMDA receptor binding typical of juveniles, we measured [^{3}H]MK-801 binding in young adult male zebra finches (80 days) that had been isolated from an appropriate song model from 10 days posthatch. This early isolation interferes with normal song development and reportedly extends the sensitive period for song learning beyond its normal closure around 60 days of age (Eales, 1987).

Early isolation from song did not affect NMDA receptor binding at 80 days of age. [^{3}H]MK-801 binding within lMAN of controls (0.106 + 0.003 pmoles/mg tissue, N=11) was not significantly different from that in isolates (0.107 $\pm$ 0.005 pmoles/mg tissue, N=10). If isolates, but not controls, are capable of acquiring new songs at 80 days of age (a prediction we are now confirming), our data suggest that the sensitive period is not defined by levels of NMDA receptor binding within lMAN.

415.24

THALAMIC AUDITORY INPUT TO VOCAL CONTROL NUCLEI IN THE BUDGERIGAR. S. E. Durand, W. S. Hall and S. E. Brauth*. Dept. of Psychology, Univ. of MD., College Park, MD. 20742.

Auditory projections derived from cell groups surrounding core neuronal populations of the auditory midbrain and thalamus may provide parallel input to vocal control nuclei in the budgerigar. Neurons along the medial margin of the inferior colliculus project axons to peri-ovoidal cell groups in the thalamus that contain acoustically responsive units. These include the n. dorsomedialis (DMP) and dorsolateralis posterior and the external shell of n. ovoidalis. All three cell groups are afferent to the ventral paleostriatum which projects directly to the magnocellular nucleus of the anterior neostriatum (MAN) in the budgerigar. In addition, DMP projects directly to MAN. In the oscine song system, MAN is critical to the acquisition of vocal patterns, a capacity exhibited by adult budgerigars in response to changes in their social environment. A second pathway from the peri-ovoidal thalamus appears to directly target the archistriatal (motor) nuclei of the budgerigar vocal system.

Lesions that involve the ventral paleostriatum and peri-ovoidal thalamus disrupt vocal learning in young budgerigars who have not yet learned to produce contact calls. These results argue that direct thalamotelencephalic pathways to paleostriatal and vocal motor nuclei may play a critical role in the acquisition of vocalizations by auditory experience. Supported by MH40698 (S.E.B.) & Whitehall Foundation J91-17 (W.S.H.).

415.25

EFFECTS OF HYPERSTRIATAL LESIONS ON IMPRINTING IN GENETICALLY VARIABLE QUAIL J.K. Kovach*, Dept. of Research, Menninger Clinic, Topeka, KS. U.S.A.

Imprinting has been long recognized as an outstanding model system in which to study early attachment and the related mechanisms of learning and memory (Hess, 1959, Science, 130: 133). The present study tested earlier indications that the medial region of the hyperstriatum ventrale (IMHV) may play a critical role in avian imprinting (Bateson, et al., 1973, Science, 181: 576; Horn, G., 1985, Memory, imprinting, and the brain, Oxford: Clarendon Press). Post-imprinting performances were compared in genetic control and artificially selected Japanese quail chicks (C. coturnix japonica) of variable imprintabilities, with and without bilateral ablation of the medial hyperstriatum by series of stereotaxic radio-frequency lesions. Learning was examined by effects of 1st post-hatch day lesions on subsequent imprinting. Retention was studied by effects of 3rd day lesions in subjects that exhibited strong stimulus preferences due to prior imprinting. Data indicated highly significant and genetically variable learning in all preparations. Systematically variable small deficits in Hi imprintability selected subjects indicated stimulus specific lesion effects in learning, and differential mediation of the learning and retention of imprinted memory. Earlier inferences about hyperstriatal functions are examined in the light of the data, and the uses of genetic variability for modeling the mechanisms of learning and memory are discussed.

415.26

LOCAL INTRACEREBRAL IMPLANTS OF ESTROGEN MASCULINIZE SOME ASPECTS OF THE ZEBRA FINCH SONG SYSTEM. W. Grisham*, G.A. Mathews, & A.P. Arnold. Dept. of Psychology and Brain Research Institute, UCLA, Los Angeles, CA. 90024-1563.

Estrogen administration during an early critical period has long been known to masculinize the morphology of sexually differentiated brain areas in both song birds and mammals. This study investigated sites of estrogen action on morphology using localized microimplants of estradiol benzoate (EB).

Female zebra finch nestlings 10-13 days old were implanted with Silastic pellets containing 2 µg EB near the higher vocal center (HVC) (n=7), in the brain distant from HVC (n=12), or in the periphery under the skin of the breast or in the peritoneal cavity (n=9). Controls were either unimplanted (n=5) or implanted near HVC with blank Silastic pellets without hormone (n=7). The brains were fixed by perfusion at 60 days, removed, sectioned at 30 µm, and thionin-stained. Cross sectional areas of HVC, RA, and lMAN neurons were measured.

HVC neurons were larger (more masculine) in the HVC-implanted group than in the other groups, which did not differ among themselves. This result suggests that implants near HVC were at or near the site of estrogen action. The distance of the EB pellet to HVC was inversely correlated with the degree of masculinization of RA and lMAN soma size across brain-implanted groups. To our knowledge, this is the first demonstration of localized implants causing morphological masculinization. Supported by NIH NRSA 1 F32 NS09040 to W.G. and USPHS DC002179-09 to A.P.A.

HORMONAL CONTROL OF REPRODUCTIVE BEHAVIOR: IMMEDIATE EARLY GENE EXPRESSION

416.1

A D1 ANTAGONIST DECREASES COPULATION-INDUCED C-FOS EXPRESSION IN MPOA. L. A. Lumley*[1], T. J. Bazzett[2] and E. M. Hull[1]. [1]Department of Psychology, SUNY at Buffalo, Buffalo, NY 14260; [2]Department of Biopsychology, University of Michigan, Ann Arbor, MI 48104

Copulation increases the expression of the immediate-early gene c-fos in several steroid-concentrating areas, including the MPOA, in male rodents (Baum & Everitt, 1991; Kollack & Newman, 1991; Robertson et al., 1991). Dopamine agonists, such as cocaine or amphetamine, also increase immunoreactivity to Fos, the protein product of c-fos, in the striatum; this effect was blocked by a dopamine D1 antagonist, suggesting that stimulation of D1 receptors may be an obligatory step in this process (Graybiel et al., 1990; Young et al., 1991).

Copulation-induced expression of Fos in the MPOA may also depend on stimulation of D1 receptors. Copulation increases dopamine release in the MPOA (Blackburn et al., 1992; Hull et al., 1993). We administered a D1 antagonist (SCH-23390), a D2 antagonist (raclopride) or vehicle to male rats 30 min before they copulated to one ejaculation. Control animals were injected with vehicle, but were not allowed to copulate. Animals were sacrificed one hour after ejaculation, or 90 min after injection of controls.

Copulation significantly increased the number of Fos-immunoreactive cells in the MPOA. Pretreatment with the D1, but not the D2, antagonist decreased the number of labeled cells, compared to the vehicle-copulation animals. Thus, stimulation of D1 receptors in the MPOA contributes to the copulation-induced increase in Fos reactivity.

416.2

C-FOS IN THE SEXUALLY DIMORPHIC AREA (SDA) OF THE GERBIL HYPOTHALAMUS, AND RELATED AREAS, DURING SEXUAL ACTIVITY. M. M. Heeb *and P. Yahr. Dept. of Psychobiology, Univ. of California, Irvine, CA 92717.

The SDA is necessary for male sexual behavior but may also serve other functions. It projects to 70+ areas of the brain and is reciprocally connected to most of them. One way we are identifying the pathways that mediate sex behavior is to determine which areas express c-fos during sexual activity. Groups of 4 male gerbils matched for sexual experience are prepared for immunocytochemistry after exposure to a female and ejaculating, intromitting, mounting or only investigating. Sexually experienced males were also studied without testing. The mating environment induced c-fos in the SDA, nucleus accumbens, caudomedial bed nucleus of the stria terminalis, posterodorsal medial amygdala, ventral premammillary nucleus, paraventricular nucleus of the hypothalamus, central tegmental area and retrorubral field (RRF). Ejaculation produced the most labeling and investigation the least. Two areas, the posterodorsal preoptic nucleus (PDPN) and the projection field of the RRF in the ventral pallidum (VP), expressed c-fos only after minimum levels of sexual activity are attained. To date, only males that intromit and/or ejaculate have labeled cells in VP; only those that ejaculate have labeled cells in the PDPN.

Supported by MH 26481, MH 00478 and MH 14599.

416.3

CASTRATION DECREASES c-FOS LABELLING IN THE MPN mgn FOLLOWING EXPOSURE TO FEMALE HAMSTER VAGINAL SECRETIONS (FHVS). J.M.Swann*, Department of Biological Sciences, Rutgers University, Newark, N.J.07102.

In male Syrian hamsters exposure to vaginal secretions from females stimulates anogenital investigation and copulatory behavior. This behavior appears to be mediated by three central areas of the vomeronasal pathway. Using c-fos as a marker for neuronal stimulation, we found an increase in the number of cfos neurons in the posteromedial bed nucleus of the stria terminalis (BNSTpm), posterior medial nucleus of the amygdala (Mp) and the magnocellular medial preoptic nucleus (MPN mgn) of gonadally intact male hamsters following exposure to FHVS. Copulatory response to FHVS is dependent on circulating gonadal steroids. To determine if neuronal stimulation in response to FHVS is dependent on testosterone we compared c-fos levels in the vomeronasal pathway of castrated males to that of intact males and castrated males treated with testosterone.

Male Syrian hamsters that were intact (n=3), castrated for 15 weeks (n=3), or castrated for 9 weeks and treated with testosterone for 6 weeks (n=3) were given a cotton swab with FHVS and perfused one hour later. Brains were then removed, sectioned and processed for c-fos immunocytochemistry The number of cfos labelled neurons does not differ among all treatment groups in rostral and caudal M, BNSTpm, BNST posterior intermediate, and the medial MPN. However, exposure to FHVS stimulated fewer neurons in the MPN mgn of castrated animals than that of intact or testosterone treated castrates ($p < .05$). These data suggest that castration prevents exposure to FHVS from stimulating neurons in the MPN mgn. Previous studies indicate that this nucleus regulates copulatory behavior in male hamsters. Thus, our results suggest that testosterone regulates the expression of copulatory behavior by regulating chemosensory input to this nucleus. Supported by NIH R29HD28467.

416.4

REDUCED MOUNTING RATES IN MALE MICE WITH A NULL MUTATION OF THE *c-FOS* PROTO-ONCOGENE. M.J. Baum*[2], J.G. Brown[3], E. Kica[2], B.S. Rubin[4], R.S. Johnson[5] and V.E. Papaioannou[3]. Dept. of Biology[2], Boston University, Boston, MA 02215, Dept. of Pathology[3] and Dept. of Anatomy[4], Tufts University School of Medicine, Boston, MA 02111 and Dana-Farber Cancer Institute[5], Harvard Medical School, Boston, MA 02115.

Mount latencies were longer and subsequent mounting rates with estrous females were significantly lower in male mice homozygous for a null mutation of the *c-fos* proto-oncogene than in heterozygous mutant or wild-type controls. Even so, an equivalent percentage of mounts led to penile intromission, an equivalent number of intravaginal thrusts was displayed, and the incidence of ejaculation was similar among males of the three genotypes. The nuclear protein product (FOS) of *c-fos* was visualized immunocytochemically in the brains of heterozygous male mutants which were killed 1h after they ejaculated. Compared with unmated controls, increased FOS immunoreactivity was present in neurons of the medial amygdala, the bed nucleus of the stria terminalis, the medial preoptic area (mPOA), the paraventricular nucleus, and the midbrain central tegmental field. Thus, in male mice, as in several other rodent species, c-fos expression increases in limbic and midbrain circuits which convey olfactory/vomeronasal as well as genital/somatosensory information to the mPOA following contact with an estrous female. These increments in neural *c-fos* expression may normally contribute to the initiation and maintenance of masculine sexual arousal during a series of mounts and intromissions leading to ejaculation. Supported by HD21094, MH00392 (MJB) and HD27295 (VEP).

416.5

CELL BODY LESIONS OF THE VENTRAL PREMAMMILLARY NUCLEUS DISRUPT MALE COPULATORY BEHAVIOR WITHOUT ALTERING BEHAVIORAL ACTIVATION OF HYPOTHALAMIC C-FOS. S.G. Truitt*, T. R. Akesson, and C. Ulibarri. Dept of VCAPP, WSU, Pullman, WA 99164

Afferent and efferent connections of the ventral premammillary nucleus (PMv) interact with other hypothalamic nuclei to make up a system that is involved in the regulation of male copulatory behavior. After male copulatory behavior the early response oncogene, c-fos, is activated in nuclei that project to and receive projections from the PMv. If some of the behavioral deficits that result from PMv lesions were due to decreased interactions with other hypothalamic nuclei, after PMv lesions, we would expect reduced c-fos activation in hypothalamic nuclei that are efferent to the PMv.

To test this hypothesis, sexually experienced male Long Evans rats were castrated and implanted with Silastic capsules containing testosterone. Lesions were created by bilateral infusion of NMDA into the PMv (n = 13). Control rats received sham surgeries (n = 9). Rats were tested weekly for five weeks for male sexual behavior with receptive females. Rats with lesions showed increased intromission latency, ejaculation latency, postejaculatory interval, mount and intromission number as compared to control rats. Copulatory rate and efficiency were decreased relative to control rats. Mount latency was unaffected.

After the last test, rats were killed by aldehyde perfusion either 1 (n = 10) or 1.5 (n = 12) hr after ejaculation. Coronal sections were processed for c-fos immunoreactivity (fos-ir). In comparisons between control and treated rats, similar patterns of fos-ir were seen in the preoptic area, bed nucleus of the stria terminalis, and medial amygdala. These findings suggest that reductions in male copulatory behavior produced by PMv lesions did not affect activation of other nuclei to produce c-fos after copulation. Alternatively, PMv lesions affected cells that did not show c-fos activation after copulation. Supported by HD22869.

416.6

C-FOS EXPRESSION IN FEMALE HAMSTER BRAIN FOLLOWING LORDOSIS AND AGGRESSIVE BEHAVIORS. M. A. Ioppa* and R. L. Meisel. Department of Psychological Sciences, Purdue University, West Lafayette, IN 47907.

In this experiment we determined the expression of c-fos immunoreactivity in areas of the female hamster brain which concentrate estrogen and progesterone, and are critical for lordosis and aggression. Ovariectomized hamsters were given 1) estradiol and progesterone treatment (E+P), but no behavior test, 2) E+P plus a lordosis behavior test, 3) oil treatment plus an aggressive behavior test, or 4) oil treatment, but no behavior test. Immunocytochemistry for c-fos protein was conducted using a primary antibody to human c-fos protein (Oncogene Sciences) and a peroxidase-based Vector ABC Elite kit. Preliminary results show that within the lateral ventromedial hypothalamus (VMH), c-fos expression appeared to be higher in animals given no hormones and tested for aggression than in all other groups. No differences were found in c-fos immunostaining in the central VMH between the 4 groups. Animals treated with E+P and tested for lordosis expressed significantly more c-fos labeled cells in the medial preoptic nucleus than any other group. Cellular activation in forebrain nuclei depend upon the specific social behavior displayed, rather than the female's hormonal status.

Supported by NIH grant HD-21478.

416.7

CHEMOSENSORY FACTORS FROM MALE PRAIRIE VOLES INCREASE C-FOS EXPRESSION IN FEMALE CONSPECIFICS C.A. Moffatt, G.F. Ball, and R.J. Nelson* Department of Psychology, Johns Hopkins University, Baltimore, MD 21218, USA

Unlike the majority of laboratory rodent species, female prairie voles do not exhibit regular estrous cycles; rather, female prairie voles are induced into estrus by chemosensory stimuli contained in the urine of male conspecifics. Female prairie voles come into contact with these chemosensory factors when they groom the ano-genital region of unfamiliar males. Contact with male urine elicits a surge in luteinizing hormone (LH) release that reaches a peak approximately 30 min after initial contact with the urine.

In the present study, c-*fos* immunoreactivity was measured to determine which areas of the brain were stimulated in response to exposure to the estrus-inducing stimuli found in male urine. We hypothesized that c-*fos* immunoreactivity would be greatest in regions of the brain important for the mediation of mating behavior and control of LH release. To test this hypothesis, female prairie voles had a single drop of either male urine or water placed on their external nares. Thirty minutes after exposure to the urine or water, the females were killed and perfused. The brains were processed for immunocytochemistry of c-*fos* antigen. Females exposed to male urine had a greater amount of c-*fos* immunoreactivity in nuclei associated with control of LH release than females exposed to water. Specifically, females exposed to male urine had greater numbers of c-*fos* immunoreactive neurons in the accessory olfactory bulbs, medial preoptic area, lateral preoptic area, and lateral septum than females exposed to water. Exposure to urine also increased c-*fos* expression in the main olfactory bulbs, anterior olfactory nucleus, and primary olfactory cortex. These results indicate that exposure to male urine stimulates regions of the brain important for the regulation of LH release.

416.8

C-FOS EXPRESSION IN NEUROCHEMICALLY-IDENTIFIED, ESTROGEN RECEPTOR-CONTAINING NEURAL POPULATIONS IN THE FEMALE RAT AFTER MATING. A. E. Herbison (SPON: Brain Research Association) Laboratory of Neuroendocrinology, AFRC Babraham Institute, Cambridge CB2 4AT, U.K.

Recent work in the female rat has demonstrated that estrogen receptors (ERs) are located within 70-80% of the sexually dimorphic calcitonin gene-related peptide (CGRP)-containing neurones in the preoptic area (POA; Herbison et al, Neuroendo,56,761)) and approximatelty 60% of somatostatin (SOM) neurones in the ventrolateral division of the ventromedial nucleus (VMN; Herbison, unpublished) The functional roles of these neural populations are not understood. This study, using immunocytochemical detection of FOS as a marker of neuronal activation, has examined whether these SOM and CGRP populations are part of the neural network involved in regulating female sexual behaviour.

Female rats were ovariectomized, treated with estradiol and progesterone, and either placed alone in a testing chamber or paired with a male rat until 10-15 intromissions were observed. In the POA of mated females, a marked increase in FOS immunoreactivity was detected predominantly in the medial preoptic nucleus, as reported previously (Erskine et al., Soc.Neurosci.1992). Double-labelling of sections revealed that over half of all LHRH neurones were FOS-positive in both female groups while 12$\pm$3% of CGRP neurones contained FOS in control animals compared with 30$\pm$5% in mated animals ($P < 0.05$). In the ventrolateral VMN, only a small increase in the numbers of FOS-positive cells was observed. However, in preliminary double-labelling experiments, no SOM neurones were found to contain FOS in non-mated rats compared with approximately 15% of SOM neurones in mated animals.

These results show that neurones in the ER-containing CGRP and SOM cell populations in the POA and VMN respectively, are immunoreactive for FOS after mating and suggest that both neurochemically-defined cell groups may be part of the steroid-sensitive neural network controlling sexual behaviour in the female rat.

416.9

REDUCED OLFACTORY AND SOMATOSENSORY INPUT DOES NOT DECREASE FOS-LIKE IMMUNOREACTIVITY IN THE MEDIAL PREOPTIC AREA (MPOA) OF MATERNALLY ACTIVE POST-PARTUM FEMALE RATS. A. Fleming*, C.J. Walsh, A. Lee, & M. Korsmit, Dept. of Psych., Erindale College, Univ. of Toronto, Mississauga, ONT L5L 1C6

The expression of the immediate-early gene c-*fos* in neurons is regulated with great anatomical specificity by a variety of pharmacological and environmental stimuli. The distribution of Fos protein in the brain can be used as a marker to help map functional neural systems involved in sensory processing and behavior.

Previous research shows that postpartum animals who receive a one hour interactive experience with pups exhibit higher density of cells showing Fos-lir in medial preoptic area (MPOA) and medial and cortical amygdala than do other groups receiving a variety of other social and olfactory experiences (Fleming, Korsmit, Suh, and Rusak, Neuroscience, 1992; see also Numan & Numan, Neuroscience, 1992).

This study explores the effects on MPOA and limbic Fos-lir of removing or reducing olfactory and somatosensory input to dams during a post-partum maternal experience. Pregnant females underwent either 1) olfactory desensitization: olfactory bulbectomy or zinc sulfate treatment, 2) ventral somatosensory desensitization: thelectomy (nipple removal) or ventral anaesthetizations, or 3) sham treatments.

Results replicate earlier findings that indicate that dam-litter interactions produce elevated Fos labelling in MPOA and amygdala and show further that a reduction of olfactory or ventral somatosensory stimulation does not significantly decrease Fos-lir in the MPOA. We are currently investigating the effects of both single and combined olfactory and somatosensory desensitization on Fos-lir not only in MPOA, but also in olfactory, limbic, and cortical structures.

416.10

FOS PRODUCTION IN PREOPTIC NEURONS CORRELATED WITH DIFFERENT ASPECTS OF MATERNAL BEHAVIOR IN RATS. M. Numan* and M.J. Numan. Dept. Psychology, Boston College, Chestnut Hill, MA 02167.

Lesion and hormone implant studies have shown that the preoptic area (POA) is essential for maternal behavior. These procedures, however, are not capable of identifying specific POA neurons. In this study we use Fos immunocytochemistry to detect neurons whose activation is associated with maternal behavior. Lactating rats received a 2-hr exposure to the following. Full maternal behavior (FMB): females were allowed to retrieve and nurse pups. Partial pup stimulation (PPS): females were exposed to pups suspended from their cages in a nylon mesh bag. These females could see, hear, and smell (primary olfactory input) the pups but could not perform maternal activities. Candy control (CC): females were exposed to candy pieces in nylon bag. Females were perfused and the presence of Fos-like immunoreactivity was determined. FMB females had more cells labeled with Fos in the medial POA and ventral bed nucleus of stria terminalis than did females in the remaining groups. Fos labeling in the PPS and CC females was low. These results suggest that direct interactions with pups, which involve both maternal performance and pup stimulation (suckling; vomeronasal input) is necessary to activate the POA. Subsequent studies are examining Fos labeling in the POA of females that are only allowed to retrieve or nurse, and in maternally behaving bulbectomized and thelectomized rats.

Supported by Whitehall Foundation.

416.11

C-FOS EXPRESSION AND NEUROTRANSMITTER RELEASE IN BRAIN STRUCTURES INVOLVED IN THE INDUCTION OF MATERNAL BEHAVIOUR IN SHEEP. A.Da Costa*, K.Broad, R.Guevara+ & K.M.Kendrick, AFRC Babraham Institute, Cambridge CB2 4AT, UK; Fac Med. +UNAM, Mexico 04510

In order to identify the neural substrates involved in controlling the induction of maternal behaviour in sheep, the changes in the expression of the immediate early gene, c-fos, were mapped. In addition, neurochemical changes occurring during the induction of maternal behaviour and the formation of the ewe/lamb bond were monitored in two areas found to express high levels of c-fos mRNA, using microdialysis. The effects of lamb separation followed by the exposure to the odours of an alien or own lamb were also investigated. A ^{35}S labelled oligonucleotide probe was used to map c-fos mRNA *in situ*. High expression was found in medial preoptic area, supraoptic nucleus, septum (S) bed nucleus of the stria terminalis, paraventricular nucleus (PVN) and ventromedial hypothalamus of ewes that had maternal behaviour induced by parturition or by artificial stimulation of the vagina and cervix (VCS). Microdialysis employed CMA-10 probes placed over the S and PVN. Ringer was pumped through these probes at 5µl/min and samples collected every 5 min. Amino-acids and monoamines were measured by HPLC. In both the S and PVN, aspartate (Asp), glutamate (Glu) and GABA release increased during the period 60 to 30 minutes pre-partum or after artificial VCS. Release of noradrenaline (NA), serotonin (5-HT) and dopamine (DA) also peaked at parturition. Similar increases in the release of these neurotransmitters occurred after VCS. The release of NA, adrenalin (AD), DA and 5-HT in the S, and of NA, DA and 5-HT in the PVN increased 5-15 minutes after the removal of the ewe's own lamb. Exposure of the ewe to the odours of an alien lamb specifically increased the concentration of AD in the S and NA in the PVN, whereas similar exposure of the ewes to the odours of their own lambs increased the release of AD in the PVN. Release of NA and DA in the S, and of AD, DA and 5-HT in the PVN, occurred irrespective of whether the lamb presented to the ewe was its own or an alien one. The PVN may be acting through oxytocin release and the S through its connections with the olfactory bulb, hippocampus and preoptic region. (Supported by JNICT, Portugal and MAFF, U.K.)

416.12

METABOLIC VERSUS HORMONAL REGULATION OF HAMSTER GnRH NEURONAL ACTIVITY. T.L. Thomas* and G.N. Wade. Dept. of Psychology and Neuroscience and Behavior Program, Univ. of Massachusetts, Amherst, MA 01003.

Separate "pulse-associated" and "surge-associated" GnRH neurons have been posited to exist in Syrian hamster forebrain. These two populations have been hypothesized to be differentially responsive to fuel availability and estrogen levels, respectively. Food deprivation on estrous cycle days 1-2, when LH secretion is pulsatile, interrupts ovulatory cycles, while food deprivation on days 3-4, just prior to the LH surge, does not. *Fos* expression in "pulse-associated" caudal POA GnRH neurons is suppressed following food deprivation on days 1-2 and remains suppressed after 2 days refeeding. Since hamsters do not increase their food intake following deprivation, we speculated that *Fos* activity remains suppressed due to enduring metabolic effects of food deprivation. Experiment 1 found some support for the hypothesis that two days of food deprivation while estrogen levels are high (days 3-4) also results in suppressed *Fos* expression in "pulse-associated" GnRH neurons after two days of refeeding. In addition, we report whether food deprivation while estrogen levels are high differentially suppresses GnRH *Fos* expression in the two populations. Also, since an LH surge can be restored by estradiol (EB) replacement on day 3, following two days food deprivation, we report whether EB differentially restores *Fos* expression in the two populations of GnRH neurons.

416.13

SEXUAL STIMULATION INDUCES *FOS* PROTEIN WITHIN GnRH-CONTAINING NEURONS OF THE FEMALE RAT PREOPTIC AREA. J.G. Pfaus*[1,2], S.P. Kleopoulos[2], A.L. Jakob[1], R.B. Gibbs[2], & D.W. Pfaff[2]. [1]Center for Studies in Behavioral Neurobiology, Department of Psychology, Concordia University, Montréal, Québec, Canada; and [2]Laboratory of Neurobiology and Behavior, Rockefeller University, New York.

We and others have shown that sexual stimulation (copulation with intromission, or vaginocervical stimulation) induces *c-fos* mRNA and Fos-like immunoreactivity (IR) within estrogen-concentrating and non-concentrating regions of the female rat forebrain, including regions that contain GnRH cells in the septum and anterior preoptic area. These effects do not appear to require treatment with estrogen or progesterone. Because vaginocervical stimulation facilitates lordosis and increases the release of luteinizing hormone, the present study examined whether hormone treatment or sexual stimulation increase Fos-like IR specifically within GnRH-containing neurons. Ovariectomized rats were administered estradiol benzoate (10 µg) 48 hr and progesterone (500 µg) 4 hr (N=30), or the oil vehicles (N=30), before either 1 hour of copulation (n=5/group), 50 vaginocervical stimulations with a glass rod distributed over 1 hour (n=5/group), or no stimulation (n=5/group). Fos-like IR was found within a significant number of GnRH neurons largely in the anterior preoptic area following copulation with intromission or vaginocervical stimulation compared to no stimulation. Few GnRH cells coexpressed Fos following hormone treatment alone; however, this treatment enhanced the number of GnRH neurons that coexpressed Fos following vaginocervical stimulation, suggesting that estrogen and progesterone may augment the responsiveness of certain GnRH neurons to sexual stimulation.

DRUGS OF ABUSE: OPIOIDS AND OTHERS—OPIOIDS: NEUROCHEMISTRY

417.1

A MORPHINE METABOLITE MAY MEDIATE EFFECTS IN THE CENTRAL NERVOUS SYSTEM. J. Mørland*, T. Aasmundstad, R. Paulsen, Å. Fallgren and F. Fonnum. Natl Inst Forensic Toxicol and NDRE-TOX, Oslo, Norway.

Morphine (M) is rapidly metabolized by glucuronidation in the liver and gut of several species. The glucuronidation may take place in the '3'-or'6'-position of M. Morphine-6-glucuronide (M6G) has been demonstrated to be a more potent agonist than M. Since M6G is rapidly formed in vivo and reaches concentrations higher than M, we wanted to investigate the ability of M6G to cross the blood-brain barrier. We studied freely moving rats implanted with microdialysis tubing in the striatum. After s.c. administration of 1 mg M6G per 100g rat, the concentration of M6G peaked in serum after approximately 20 min at 15 uM, and was about 5 uM after 60 min. The microdialysate concentration of M6G peaked after 60 min at about 0.02 uM and decreased gradually to about 0.003 uM during the subsequent 3 hours. No free M was detected in serum or microdialysate. The dialy- sate M6G concentration time curve roughly paralleled respiratory depression and analgesia. It is concluded that M6G may cross the bloodbrain barrier and mediate some of the central nervous actions of M.

417.2

MICRODIALYSIS OF ENKEPHALIN RELEASE IN THE GLOBUS PALLIDUS/VENTRAL PALLIDUM AFTER SYSTEMIC MORPHINE ADMINISTRATION. M.F. Olive*, M. Bertolucci, C.J. Evans and N.T. Maidment. Neuroscience Ph.D. Program & Dept. of Psychiatry, NPI & BRI, UCLA School of Medicine, Los Angeles, CA 90024.

We have previously demonstrated the feasibility of monitoring opioid peptide release *in vivo* using microdialysis linked to sensitive solid-phase radioimmunoassay procedures (*Neuroscience* 33:549-557). Preliminary experiments on unanesthetized freely moving rats have shown that acute injection of morphine (10 mg/kg i.p.) produces a 3-fold increase in recovered enkephalin levels over a 2-hour period from probes implanted in the globus pallidus/ventral pallidum. This effect was dose-dependent, with no effect being observed with lower (2 mg/kg i.p.) or higher (40 mg/kg i.p.) doses, and also site-specific with negative results being obtained in the caudate and nucleus accumbens. Pretreatment of rats for 16 days with morphine (10 mg/kg i.p.) resulted in tolerance to the effect of a subsequent challenge of the drug.

Experiments designed to examine the time-course of the tolerance phenomenon showed that this was not a rapid effect. Naive rats (n=5) implanted with dialysis probes into the globus pallidus/ventral pallidum were injected with morphine (10 mg/kg i.p.) at 3-hr intervals for a total of 4 injections, and the effect of the 4th administration was not significantly different from that of the 1st. Rats injected with saline (n=6) i.p. showed no elevated levels of enkephalin release.

These data demonstrate the potential of this methodology for studying both the basic pharmacology of opioid peptide release regulation and the role of these peptides in reward pathways.

Supported by NIDA Center Grant DA 05010 and the W.M. Keck Foundation.

417.3

SPECIFIC EFFECTS OF MORPHINE AND NICOTINE ON NEURONAL RESPONSES TO VENTRAL PALLIDUM AND FIMBRIA STIMULATION IN THE NUCLEUS ACCUMBENS. C. Eyl, and R.L. Hakan*, Dept. Psych., Univ. of North Carolina at Wilmington, NC 28403

The effects of systemic nicotine and morphine on neuronal activity in the nucleus accumbens (NAS) of halothane anesthetized rats were examined. Normally inactive NAS neurons antidromically activated by ventral pallidum (VP) stimulation, had mixed responses to nicotine: 8 were inhibited, 5 excited and 2 unaffected. However, NAS units orthodromically activated by VP stimulation were consistently inhibited by nicotine (n=7) while saline injections had no effect (n=6). Similarly, morphine had mixed effects on NAS-VP projection neurons (6 inhibited, 3 excited and 3 unaffected) but reliably inhibited orthodromic responses to VP stimulation (9/10).

Previous studies (SNS, #229.2,1992) have demonstrated that systemic nicotine inhibits orthodromic responses of NAS units evoked by fimbria stimulation (fimbria-driven) but has no effect on spontaneous NAS activity. In contrast, morphine inhibits spontaneous NAS activity but does not affect fimbria-driven responses. The present study also re-assessed the effects of nicotine and morphine on fimbria-driven and spontaneously active NAS units. Again, fimbria driven NAS units were inhibited by nicotine (7/8) but unaffected by morphine (12/13) while spontaneous activity was unaffected by nicotine (8/10) but inhibited by morphine (6/8). These results demonstrate specificity in the pattern of NAS neuronal responses to drugs of abuse. Moreover, until recently the VP-NAS feedback pathway had been unacknowledged. These results begin to describe the neuropharmacology of this NAS afferent.

417.4

INTERACTION OF MORPHINE AND THE 5-HT_3 ANTAGONIST, GRANISETRON, ON DOPAMINE AND NON-DOPAMINE CELLS IN THE VENTRAL TEGMENTAL AREA. A.N. Gifford* and R.Y. Wang, Dept. Psychiatry, SUNY at Stony Brook, NY 11794.

5-HT_3 antagonists have been found to be effective in suppressing morphine induced place-preference. Moreover, injection of the 5-HT_3 antagonist, tropisetron, into the VTA reverses the morphine-induced increase in extracellular DA levels in the nucleus accumbens. The present study was undertaken to examine whether the latter effect of 5-HT_3 antagonists is mediated by a blockade of the morphine-induced increase in A10 DA cell firing rate. In chloral hydrate anesthetized rats acute granisetron (0.1 mg/kg or 0.5 mg/kg i.v.), failed to attenuate the increase in DA cell firing rate to morphine (cumulative dose 2-4 mg/kg i.v.). Both granisetron and tropisetron were also unable to reverse the rate-increasing effects of morphine when given in exponential increasing doses (up to 0.8 mg/kg, i.v.) immediately after morphine (2 mg/kg, i.v.), whereas naloxone (0.1 mg/kg, i.v.) induced a rapid reversal. These results thus do not support a blockade of morphine's excitation of A10 DA cells as an explanation for the suppression of morphine reward by 5-HT_3 antagonists.

In a second study in which rats were treated chronically for 4-6 weeks with granisetron (0.1 mg/kg s.c. per day) there was also no attenuation in the A10 DA cell firing rate increase to morphine, applied iontophoretically. However, preliminary findings indicated that there appeared to be a shift the response of non-DA VTA cells to iontophoresed morphine from primarily inhibition in control rats (15 cells inhibited, 2 excited, 2 no response) to either no response or excitation in the granisetron treated rats (2 cells inhibited, 11 excited, 15 no response; $P<0.001$, Chi-square test comparing proportion cells inhibited in control versus treated rats).

417.5

REGION SPECIFIC INCREASE IN BRAIN ADENYLYL CYCLASE mRNA IS INVOLVED IN MORPHINE DEPENDENCE

I. Matsuoka[1], R. Maldonado[2]*, J. Hanoune[1] and B.P. Roques[2]. [1]Dpt. de Pharmacologie et Physiologie Hépatique. U99 INSERM. Hôpital Henri Mondor. 94010 Créteil, France. [2]Dpt. de Pharmacochimie Moléculaire et Structurale. U266 INSERM - URA D1500 CNRS. Faculté de Pharmacie. 75006 Paris, France.

Acute administration of opioids decreases adenylate cyclase activity by acting on Gi-proteins coupled receptors. It has been therefore proposed that during chronic exposure to morphine the neurons could dramatically increase their adenylate cyclase activity to overcome the constant inhibition of the enzyme leading to an enhancement in neuron excitability. In this study, we examined the effects of chronic infusion of morphine on "in situ" hybridization signals of adenylyl-cyclase and calmodulin in the mouse brain. The present results provides the first direct evidence that chronic morphine treatment induces a selective increase in adenylyl-cyclase mRNA transcript in the locus coeruleus, the amygdala and the thalamus. These changes were observed as early as 12h after morphine chonic treatment. Interestingly, locus coeruleus and amygdala are respectively involved in the expression of physical signs and motivational reactions of morphine abstinence. In addition, the overexpression of adenylyl-cyclase mRNA in thalamic nuclei could participate to morphine tolerance. Moreover, the time course of morphine-induced change in adenylyl-cyclase mRNA is correlated with the severity of morphine withdrawal syndrome. These results are all the most relevant as there was not change in another marker, calmodulin. These findings offer the first mechanistic explanation of morphine dependence at the molecular level.

417.6

CHRONIC MORPHINE TREATMENT INDUCES c-*JUN* mRNA EXPRESSION IN RAT DORSAL ROOT GANGLION NEURONS. D. Besse, K. Ren and M.A. Ruda* NAB, NIDR, NIH, Bethesda, MD 20892.

In order to assess the molecular basis of morphine tolerance, the effects of chronic intrathecal (i.t.) morphine on transcription factor regulation were examined in dorsal root ganglia (DRG).

In one study, a cannula was inserted into the spinal subarachnoid space in anesthetized adult male rats. The next day, an osmotic pump (Alzet® 2001) filled with either saline (SAL) or morphine (MOR, 10μg/hr) was subcutaneously implanted and connected to the i.t. canula of rats exhibiting no motor impairment (n=3 per group). L2 to L6 DRG were collected bilaterally after 4 days. Total RNA was extracted and Northern blots were performed using a ^{32}P-labeled 51-mer oligonucleotide probe complementary to bases 2810-2861 of rat c-*Jun*. The characteristic c-*Jun* hybridization bands at 2.7 and 3.1 Kb were found in each experimental group (naive, SAL and MOR). For both bands, the level of mRNA expression was similar in the SAL and naive groups. However, in the MOR group, c-*Jun* mRNA levels were increased for both the 2.7 (100%) and 3.1 (59%) Kb bands as compared to the SAL group. In a parallel study, 2 days after repetitive subcutaneous injections of morphine (40mg/kg, every 12 hrs), the c-*Jun* mRNA induction was 57% and 47% for the 2.7 and 3.1 Kb bands, respectively.

The morphine-induced increase in c-*Jun* mRNA may be related to behavioral tolerance, since the initial morphine-induced increase in paw withdrawal latency from radiant heat had returned to control (naive or SAL) values by 4 days. Furthermore, since μ opioid receptors are numerous on spinal Aδ- and C- afferent fibers, the long-term binding of morphine to spinal μ opioid receptors may produce a modification in the second messenger cascade leading to a transcription factor regulation in the DRG.

417.7

MORPHINE INDUCTION OF FOS IN STRIATUM IS MEDIATED BY NMDA AND D1 DOPAMINE RECEPTORS.

J.Liu*, J. Nickolenko, F.R. Sharp, Dept. Neurology, Univ. of California and SFVAMC, San Francisco, CA 94121.

Immediate early genes (IEGs) form transcription factors that produce long-term changes in gene expression. Since morphine associated tolerance and addiction can be blocked by MK801, and since NMDA receptor activation can induce IEGs, this prompted a study of morphine.

Morphine (10mg/kg) induced c-fos mRNA, assessed using in situ hybridization, and Fos protein, assessed using immunocytochemistry with the LA041 monoclonal antibody, in medial striatum and nucleus accumbens of adult rats. AP-1 activity was increased in striatum within 1h post morphine. Morphine induction of c-fos mRNA and Fos protein was blocked by MK801 (2mg/kg) and morphine induction of Fos protein was blocked by SCH23990 (1mg/kg), a D1 antagonist. This data suggests that morphine activation of striatal mu receptors cannot induce Fos without the coordinate activation of both D1 and NMDA receptors. If Fos is induced in neurons with mu, D1, and NMDA receptors, the fact that NMDA should act on the SRE in the Fos promoter, and D1 should act on the CRE element in the Fos promoter, suggests that combined activation of SRE and CRE might be required in vivo to induce the Fos gene. Alternatively, the D1 and NMDA receptors might be located on separate neurons both of which would need to be activated in order to induce the Fos gene.

417.8

INJECTIONS OF METHYLNALOXONIUM INTO THE LOCUS COERULEUS PRODUCE CEREBRAL HYPERMETABOLISM IN MORPHINE-DEPENDENT RATS. A.S. Kimes*†, R. Maldonado‡, G.F. Koob§, E. Ambrosio†¥, and E.D. London†¶✦. †Addiction Res. Ctr., NIDA, NIH, Balto., MD, 21224; ‡Pharmacochimie Moléculaire, INSERM U266, Paris, France; §Dept. Neuropharmacol., The Scripps Res. Inst., La Jolla, CA.; ¥UNED, Madrid, Spain, ¶Dept. Radiology, Johns Hopkins Sch. Med., Balto., MD 21204; and ✦Dept. Pharmacol. Exp. Ther., Univ. of Maryland Sch. Med., Balto., MD 21201.

Opioid withdrawal (OW), produced by systemic naloxone, induces a typical behavioral syndrome and cerebral hypermetabolism, particularly in the central amygdaloid nucleus, the locus coeruleus (LC) and thalamic and hypothalamic nuclei (Kimes & London, *J. Pharmacol. Exp. Ther.* 248:897). Behaviors characteristic of the classic OW syndrome are produced by intracerebral injections of methylnaloxonium (MN), an opioid antagonist, into the LC (Maldonado *et al., J. Pharmacol. Exp. Ther.* 261:669). The present work tested whether MN injection into LC produces cerebral hypermetabolism comparable to that produced by systemic naloxone. The purpose of the work was to clarify the role of LC in OW.

Intracerebral cannulae were implanted bilaterally, directed toward the LC of anesthetized male Fischer-344 rats. Within 4 weeks of cannulation, rats were made morphine-dependent by s.c. implantation of 75 mg morphine pellets (1 pellet on day 1; 2 pellets on day 4). On day 8, rats (n = 7/group) received 0.5 μl/min of saline or MN (1 μg/μl), infused into LC for 1 min. Immediately thereafter, metabolic rates for glucose were determined in 42 brain regions by the deoxyglucose method (Sokoloff *et al., J. Neurochem.* 28:897). MN stimulated glucose metabolism (21-45% > control) in the n. accumbens, caudate putamen, central and medial amygdaloid nuclei, thalamic and hypothalamic nuclei, hippocampal areas, substantia nigra pars reticulata, dorsal and ventral tegmental areas, and the cerebellar vermis. As local injections of MN into the LC produce cerebral hypermetabolism and behaviors that are characteristic of OW in dependent rats, it appears that the LC is a primary site of OW.

417.9

ANTAGONIST ACTION OF BUPRENORPHINE ON LC UNIT ACTIVITY IN MORPHINE DEPENDENT RATS. S.J. Grant*, G. Sonti. Dept. Psychology and Neuroscience Prog., Univ. Delaware, Newark, DE. 19716.

Buprenorphine, a synthetic opioid proposed as a potential treatment for opioid and cocaine craving, has potent agonist actions on the impulse activity of noradrenergic and dopaminergic neurons in drug naive rats. We have now extended these studies to opioid dependent subjects.

Rats were chronically treated with either buprenorphine (0.5 mg/kg s.c.) or morphine pellets (75mg) for 5 days. Saline injections and sham pellets were used as controls. Two days after the last drug treatment extracellular recordings were obtained from noradrenergic neurons in the Locus Coeruleus (LC) *in vivo*. Either buprenorphine (BUP: 0.025-8 mg/kg)), morphine (MORPH: 0.5 - 32 mg/kg), or naloxone (NAL: 0.001-64 mg/kg) were administered systemically (i.v.)

LC neurons exhibited tolerance to both MORPH and BUP after chronic BUP treatment, but the tolerance to BUP was greater than to MORPH. NAL produced a slight increase in impulse activity, but only at high doses (>10 mg/kg) that produced similar increases in firing rate in control animals.

In contrast, **BUP acted as an antagonist in MORPH dependent subjects.** In fact, BUP (0.05-1 mg/kg) produced as much hyperactivity as NAL (<0.05 µg/kg). On the other hand, there was tolerance to MORPH challenge.

These results suggest that **BUP is a partial agonist** whose intrinsic efficacy is less than MORPH. The agonist action of BUP in drug naive animals may therefore be due to the high degree of receptor reserve on LC neurons.

417.10

INCREASED DOPAMINE EFFLUX IN THE NUCLEUS ACCUMBENS DURING MANUAL AND SELF-ADMINISTRATION OF HEROIN AS MEASURED BY *IN VIVO* CHRONOAMPEROMETRY. GW Hubert*, AG Phillips, HC Fibiger, and CD Blaha. Dept. Psych., Univ. British Columbia Vancouver, BC, Canada V6T 1Z4.

The effects of heroin (HER) intravenous self-administration (IVSA) on dopamine (DA) efflux in the N. accumbens (Nac) was examined using chronoamperometry (CA; 1s pulse/120s) with stearate-modified graphite paste recording electrodes. Rats trained to bar press for food (FR2-schedule) were implanted with a jugular catheter and bilateral electrodes. IVSA sessions began with a 5s flash of cage-lights and priming dose of HER (0.03mg/0.1ml). After drug-prime, cage-lights remained on over the entire IVSA session with the exception of 30s time-out (lever-inactive) periods after each HER infusion. Rats were then allowed to bar press on an FR2 schedule for 12 additional HER infusions. The CA signal due to the oxidation of DA consistently increased to a maximum level (12.4 ± 1.7nA) after an average of 8 infusions of HER. A second experiment was performed in which doses of HER were manually administered i.v. Four different doses of HER (30, 60, 120, and 180 mg/kg) induced dose-related increases of the CA signal (2.8, 5.6, 7.8, and 10.3nA), respectively. These data are consistent with a role for DA in the rewarding properties of opiate drugs.

417.11

EFFECTS OF PEDUNCULOPONTINE NUCLEUS (PPN) LESIONS ON THE ACQUISITION OF IV HEROIN SELF-ADMINISTRATION. M.C. Olmstead,* E.M. Munn and R.A. Wise. Dept Psychol McGill Univ (M.C.O) and Ctr Stud Behav Neurobiol and Dept Psychol, Concordia (E.M.M. and R.A.W.), Montreal, Canada H3G 1M8.

The PPN is a brainstem nucleus projecting to and receiving projections from the mesolimbic structures implicated in opiate reward. PPN lesions have been reported to block opiate-conditioned place preferences, and we studied their effect on a drug-reinforced lever-pressing task. Adult male Long-Evans rats were prepared with chronic intravenous catheters and excitotoxin (0.5 ul of 0.1M NMDA injected bilaterally over 10 minutes) lesions. Two weeks later they were given the opportunity to lever-press for intravenous heroin (0.1 mg/kg/inj infused at a rate of 0.25 ml/28 sec) on an FR-1 schedule of reinforcement for 4h per day. Three animals with good bilateral lesions failed to learn to lever-press for IV heroin within 15 days, whereas animals with sham lesions learned in the first week. While lesioned animals showed no such responses, sham-lesioned animals subsequently increased responding when saline was substituted for heroin and returned to normal self-administration patterns when drug was reinstated. Asymmetrical lesions and lesions of adjacent structures had variable effects. While additional animals must be tested, our initial results suggest that PPN plays a role not only in the stimulus-learning of the place preference paradigm but also in the response-learning of the instrumental paradigm.

417.12

DECREASES IN EXTRACELLULAR NUCLEUS ACCUMBENS DOPAMINE DURING INTRAVENOUS HEROIN SELF-ADMINISTRATION IN RATS. S.E. Hemby*, T.J. Martin, C.Co, S.I. Dworkin, & J.E. Smith. Center for Neurobiology of Drug Abuse, Dept. of Physiology and Pharmacology, Bowman Gray Sch. of Med., Wake Forest Univ, Winston-Salem, NC, 27157

Acute administration of mu opiate receptor agonists increase extracellular nucleus accumbens (NACC) dopamine concentrations. The present study determined whether acute response-independent intravenous (IV) heroin infusions and chronic IV heroin self-administration would also increase extracellular dopamine in the NACC. Drug-naive male Fisher (F-344) rats were randomly divided into three groups to receive two IV infusions of saline (n=3) or heroin (60 or 100 µg/kg/infusion; n=8 & 6, respectively) spaced one hour apart. Extracellular dopamine concentrations in the NACC were not significantly increased from baseline following saline or 60 µg/kg heroin; however, 100 µg/kg of heroin significantly increased extracellular dopamine to approximately 180% above baseline values. A second group of subjects were trained to self-administer 60 or 100 µg/kg/infusion (n=5 & 7, respectively) on a fixed ratio 10 (FR10) schedule of reinforcement. Microdialysis samples were collected in five minute intervals 15 minutes prior to the session (baseline), during the 2 hour self-administration session, and 30 minutes after the session (rebaseline). Furthermore, IP naloxone (10 mg/kg) administered one hour after the session did not induce signs of opiate withdrawal, suggesting that this dose did not induce physical dependence. For the 60 µg group, extracellular dopamine decreased approximately 10-25% from baseline, while this reduction was approximately 50% in the 100 µg group. These results suggest that response-independent administration of abused drugs may not be the most appropriate model for investigating the neural substrates mediating the reinforcing effects of these compounds (research by DA-01999, DA-00114).

417.13

RESPONSE PROPERTIES OF VENTRAL TEGMENTAL NEURONS DURING HEROIN SELF-ADMINISTRATION IN THE RAT. H.Zhang* and E.A. Stein. Depts. of Pharmacology & Psychiatry, Medical College of Wisconsin, Milwaukee, WI 53226.

The mesocorticolimbic dopamine (DA) system, with cells of origin within the mesencephalic ventral tegmental area (VTA), is believed to play a critical role in the mediation of behaviors reinforced by both natural (eg food) and artifical reinforcers such as drugs of abuse. Few studies, however, have examined the activity of the system with techniques capable of sufficient temporal resolution in order to follow neuronal activity during the behavior. A recent voltammetric study demonstrated an apparent increase in nucleus accumbens DA concentration immediately preceeding heroin self administration (SA) and a decrease after drug administration (Kiyatkin et al, Synapse 1993). The present study directly measured VTA neuronal activity during heroin SA behavior. Changes in cell activity before SA behavior may be reflective of an animals arousal/motivational state, while response following drug delivery should reflect a combination of the reinforcing and pharmacologic drug effects. Rats were prepared with a chronic jugular catheter and four modified "ear bar" recepticles, constructed of dental acrylic, were mounted on their skull for subsequent atraumatic fixation in the correct stereotaxic plane. Bilateral burr holes over the VTA were sealed with silicone rubber between recording sessions. Rats SAed heroin by breaking a photocell beam across a licking spout. Extracellular recordings from both DA projection and non DA interneurons were made through NaCl filled glass microelectrodes. DA cell identification was based on such standard criteria as waveshape, firing rate and pattern and histologic reconstruction. Rats rapidly learned to SA heroin (60 or 100 µg/kg/inj) during the first session. Approximately 40% of recorded neurons to date have been identified as DA cells. Preliminary data indicates most identified DA cells responded to heroin with an increase in activity while non-DA cells decresed their activity; these drug-induced alterations generally returned to baseline prior to each SA. Clear firing changes in DA cells immediately prior to heroin SA have also been observed (Supported in part by grant DA 05012).

417.14

MICRODIALYSIS ASSESSMENT OF NUCLEUS ACCUMBENS DOPAMINE AND METABOLITES DURING IV HEROIN SELF-ADMINISTRATION: CHANGES WITH UNIT DOSE . R. Rivest,* K. Leeb, P. Leone, and R.A. Wise. Ctr Stud Behav Neurobiol, Concordia University, Montreal, Quebec, Canada, H3G 1M8.

Self-administered IV heroin has been found to increase dopaminergic cell firing and to elevate dopamine (DA) turnover in nucleus accumbens (NAS). At unit dosages of 100 or 200µg/kg/injection, rats adjust their response rate to maintain a relatively constant hourly drug intake; at these unit doses relatively constant elevations of NAS DA are seen. However, Hemby et al. (SN Abstr 1992) reported that at 60 µg/kg/inj self-administration was maintained in the absence of elevated DA. Our aim was to explore the regulation of heroin intake and the correlated levels of DA across a more extended range of unit dosages. Rats with NAS guide cannulae were trained to self-administer heroin HCl at one of four unit dosages: 50, 100, 200 or 400 µg/kg/inj. NAS DA and metabolites were sampled at 10- or 20-min intervals by microdialysis and measured using HPLC with electrochemical detection. Rats tested at the 50µg/kg/inj dosage simply took multiple injections each time they went to the lever, giving themselves the same amount of drug and elevating NAS DA levels to the same degree as did animals working for the 100 and 200µg/kg/inj dosages. However, hourly drug intake was greater in animals given the 400µg/kg/inj dosage and DA levels were increased to as much as 500% in this case. These data suggest that compensatory response adjustments for changes in unit dosage may characterize self-administration of only a narrow range of low dosages. The high dosage data make it clear that such low-dosage "regulation" is not due to a ceiling on the ability of heroin to elevate NAS DA..

417.15

A GAS CHROMATOGRAPHY- MASS SPECTROMETRY (GCMS) METHOD FOR IBOGAINE. S.M. Keefner, L.B. Hough, C.A. Gallagher, A. Seyed-Mozaffari[1], S. Archer[1] and S.D. Glick*, Dept. Pharmacology and Toxicology, Albany Medical College, Albany, NY 12208 and [1]Dept. Chemistry, Rensselaer Polytechnic Institute, Troy, NY 12180.

A sensitive method for measuring ibogaine, an agent claimed to be effective in treating opiate and stimulant addiction, has been developed. Supernatant aliquots from brain homogenates (0.4 N $HClO_4$) containing ibogaine standards (50-500 ng) or unknowns were spiked with O-[CD_3]-ibogaine (250 ng), alkalinized (10 N KOH), and extracted into hexane. Following back extraction into HCl (0.01 N) and evaporation to dryness, residues were derivatized in trifluoroacetic anhydride (50 µl, 30 min, 60°), evaporated to dryness and resuspended in toluene (50 µl). Analysis by capillary GCMS (splitless mode, 5% phenyl methyl silicone, 90-250°) in SIM mode detected ibogaine (m/e 406,391) and O-[CD_3]-ibogaine (m/e 409,394). The 406/409 ratios were linear with ibogaine standards from 0 to 500 ng, with an approximate lower limit of sensitivity of 50 ng. One hr after administration in rats (40 mg/kg, i.p.), brain ibogaine levels were 1.3-4.7 µg/g, considerably lower than previously reported by others using less accurate spectrofluorometric methods. This method allows for greatly improved detection and quantification of ibogaine in biological samples and may lead to a better understanding of its mechanisms of action (supported by DA03817).

417.16

STRUCTURE-ACTIVITY RELATIONS OF NEUROPEPTIDE FF RECEPTOR BINDING IN RAT SPINAL CORD MEMBRANES. K. Payza* and H.Y.T. Yang, Laboratory of Biochemical Genetics, NIMH Neuroscience Center at St. Elizabeths, Washington, DC 20032.

Neuropeptide FF (FLFQPQRFamide, NPFF) modulates opiate analgesia, tolerance, and withdrawal. An NPFF antagonist could be useful in augmenting morphine analgesia and attenuating tolerance and withdrawal. The development of NPFF antagonists first requires information on the structure-activity relations of NPFF receptor binding. Therefore we characterized the ligand binding site of NPFF receptors with a radioligand binding assay. We found that the Arg-Phe-amide was critical for binding activity, and that tetrapeptides bound to NPFF receptors with high affinity if they contained this C-terminal, such as PQRFamide (K_i=12 nM), FMRFamide (1.8 nM), PMRFamide (0.54 nM), FFRFamide (0.25 nM), and FWRFamide (0.42 nM); compare to NPFF (0.26 nM). The potent binding of FMRFamide explains the similar bioactivities of FMRFamide and NPFF. Substitutions at the first and second positions of FMRFamide, particularly with aromatic residues, were tolerated much better than substitutions at the third and fourth positions. These findings are expected to aid the development of high affinity NPFF antagonists.

417.17

CHRONIC OPIOID RECEPTOR ACTIVATION RESULTS IN NEURONAL SUPERSENSITIVITY IN CULTURED CATECHOLAMINERGIC NEURONS. E. Ronken*, A.H. Mulder and A.N.M. Schoffelmeer, Dept. of Pharmacology, Free University, Van der Boechorststraat 7, 1081 BT Amsterdam, The Netherlands.

Central catecholaminergic neurotransmission in the rat brain is known to be modulated by activation of opioid receptors. Thus, in brain slices stimulated release of [^{3}H]dopamine (DA) is inhibited exclusively by activation of κ-opioid receptors whereas that of [^{3}H]noradrenaline (NA) is subject to inhibition exclusively through µ-opioid receptors. In this study, we investigated the adaptive changes upon sustained activation of opioid receptors in cultured DAergic neurons from the ventral mesencephalon (VM) and in NAergic neurons from the locus coeruleus (LC). Chronic activation of µ-opioid receptors on LC by morphine (1 µM) for 4 days induced a 55-150% increase in [^{3}H]NA release by K^+ (25 mM)- or NMDA (100 µM)-induced depolarization upon opioid withdrawal. Moreover, µ-opioid receptors on LC were found to be resistant to desensitization as no change in release inhibiting properties of DAMGO were found (EC_{50} 10-12nM, maximal inhibitory effect about 80-90%). Similarly, sustained activation of κ-opioid receptors for 4 days in VM by the κ receptor agonist U69593 (1 µM) resulted in a 70% increase in K^+- and NMDA-stimulated [^{3}H]DA release without κ receptor desensitization (EC_{50} 3-6 nM; maximal inhibitory effect 50%). Neuronal supersensitivity was not found upon chronic autoreceptor activation with clonidine (1 µM, LC, α_2) and with LY171555 (1 µM, VM, D_2) suggesting that observed adaptive changes in neuronal excitability upon chronic opioid receptor activation are a characteristic feature of opioid receptors. Therefore, as µ- and κ-opioid receptors appear to be resistant to desensitization upon chronic activation, its tonic activation in vivo by endogenous opioid peptides may play a role in regulation of long-term neuronal excitability towards various excitatory stimuli such as EAA. Moreover, these phenomena may play a role in the acquisition and maintenance of drug abuse.

Supported by the Dutch Organisation for Scientific Research (grant 900-543-121).

417.18

µ AND κ- OPIATE RECEPTOR BINDING CHARACTERISTICS AND ADENYLATE CYCLASE ACTIVITY IN RAT SPINAL CORD. Poluru L. Reddy* and Hemendra N. Bhargava, Dept. Pharmacodyn., Univ. Ill., Chicago, IL 60612.

Considerable evidence suggests that the spinal cord is an important site for the mediation of the antinociceptive action of opiates. Although opioid receptor binding sites have been well characterized in spinal cord, the effector systems coupled to these receptors to produce their responses are less well understood. The present study was undertaken to examine whether there is any correlation between the number of binding sites (B_{max}) of µ and κ- opioids and their effect on adenylate cyclase (AC) activity in spinal cord of adult male Sprague-Dawley rats. ^{3}H-DAMGO and ^{3}H-EKC were used to label µ- and κ- opiate receptors respectively. AC activity was determined using radioligand binding assay. The B_{max} values (fmoles/mg protein) of ^{3}H-DAMGO and ^{3}EKC in spinal cord were 51.68 ± 3.98 and 36.65 ± 3.64 respectively. At 10µM concentration, k-agonist (U-50,488H), µ-agonist (DAMGO) inhibited the AC activity by 35 and 11%, respectively. Thus, compared to µ agonist. k-agonist is more potent inhibitor of AC activity in spinal cord despite the less number of bindings sites. It is concluded that there may be no correlation between the number of opiate receptor binding sites and the degree of inhibitory effect on AC activity in spinal cord of the rat (Supported by a grant DA-02598 and a Research Scientist Development Award K02-DA-0130 from the NIDA).

DRUGS OF ABUSE: OPIOIDS AND OTHERS—OPIOIDS: BEHAVIOR

418.1

LACK OF BRAIN REWARD FACILITATION BY THC BEFORE OR AFTER SUBACUTE MORPHINE IN LEWIS RATS. B. Hine* and M. Borrero. U. of Puerto Rico Sch. Pharmacy, San Juan, P.R. 00936.

Male Lewis rats, implanted with platinum bipolar electrodes in the ventral tegmentum, received training in a brain stimulation reward (BSR) procedure similar to that reported by Gardner (Psychopharm.96:142,1988;Pharmacol.Biochem.Behav.48:571, 1991) to demonstrate BSR threshold decreases to a 1.5 mg/kg acute dose of Δ^9-tetrahydrocannabinol (THC) 15 min after injection. After three months of stabilization of BSR thresholds and stimulation-delivery response rates, calculated over 10-min periods of the 40-min training and THC vehicle injection sessions, rats were given THC doses of 0.25-2.0 mg/kg in separate sessions, interspersed with vehicle sessions. After a two-week period of daily s.c. morphine sulfate (MS) injections (cumulative dose = 960 mg/kg), these rats were challenged with THC (0.25-1.0 mg/kg) over a period of 5-13 days after termination of MS injections. Consistent with previous reports from this laboratory (FASEB J.5:703,1991;Soc.Neurosci.Abstr. 17:1433,1991), no evidence of reliable BSR threshold decreases or response-rate increases by THC was observed before MS, and 2 mg/kg produced marked rate depression, relative to vehicle data. Subacute MS also produced no BSR facilitation to THC challenge, except for slightly increased rates at 0.25 mg/kg. Rate depression at 1 mg/kg was profound. The reason for the discrepancy between these data and the report of BSR facilitation by THC are unknown. Supported by GM 08224.

418.2

INTRA-ACCUMBENS DPDPE LOWERS THE THRESHOLD FOR BRAIN-STIMULATION REWARD. C.L. Duvauchelle,* S.M. Fleming and C. Kornetsky. Lab. of Behavioral Pharmacology, Boston University School of Medicine, Boston, MA 02118.

Intraventricular application of delta receptor specific agonists have been shown to induce conditioned place preferences and increase response rates for brain stimulation reward (BSR). However, neither delta agonists or antagonists have been reported to affect intravenous self administration of heroin. In order to more specifically determine the role of a delta agonist on the brain reward system the present study examines the effects of intra-accumbens microinjections of the delta agonist, DPDPE on BSR to the ventral tegmental area. The thresholds for BSR were determined 45 minutes after the infusion of DPDPE (0.0, 2.5, 5.0 and 7.5 µg/.5 µl/side) into the nucleus accumbens. DPDPE significantly lowered the threshold for BSR while vehicle infusions had no effect. These findings indicate that DPDPE increases sensitivity to rewarding electrical stimulation of dopamine cell bodies, supporting the hypothesis that delta opiate receptor ligands have rewarding effects. (Supported by grant DA02326 and DA00099 to CK).

418.3

ACQUISITION OF INTRAVENOUS HEROIN SELF-ADMINISTRATION IN LEWIS AND FISCHER RATS.

K.Leeb* and R.A. Wise. Center for Studies in Behavioral Neurobiology, Concordia University, Montreal, Quebec, Canada, H3G 1M8.

Lewis (LEW) and Fischer 344 (F344) rats have been reported to differ in their sensitivities to and preferences for a variety of drugs. Among other findings, orally self-administered cocaine, etonitazine and morphine are reported to be stronger reinforcers for LEW than for F344 rats. The aim of the present study was to determine if these strain differences in drug reinforcement would be apparent when the more effective intravenous (IV) route of administration was used. Male LEW and F344 rats with chronic intravenous catheters were trained to lever-press for 0.1mg/kg infusions of heroin in 4h daily sessions. LEW and F344 rats did not differ in the rate at which they learned to self-administer IV heroin. Preliminary data indicate that Sprague-Dawley and Long-Evans rats acquire self-administration at similar rates. In order to compare drug intake, we switched the animals to a VDI paradigm after 15 days of acquisition testing. In this paradigm the dosage of heroin (0.025, 0.05 or 0.1mg/kg/infusion) is irregularly varied from trial-to-trial and inter-response times are correlated with the preceding dosage. LEW and F344 rats did not differ in regulation of drug intake; the two strains responded with similar inter-response times across the range of tested dosages. Thus it appears that when heroin is given by intravenous injection it is a potent reinforcer for both Lewis and Fischer strains. Differences in response to oral opiates may be unique to doses or routes of administration that are marginally reinforcing and not likely to be subject to abuse.

418.4

RESPONSES OF NUCLEUS ACCUMBENS NEURONS TO NOVELTY STIMULI AND HEROIN SELF-ADMINISTRATION IN THE FREELY-MOVING RAT

R.-S. Lee*, S. C. Steffensen, G. F. Koob, R. L. Howard, R. Lintz, G. Berg and S. J. Henriksen. Dept. of Neuropharmacology, The Scripps Research Institute, La Jolla, CA 92037.

The nucleus accumbens (NAcc) has been hypothesized to function as an integrator of motor and complex limbic information. Other evidence indicates that the NAcc may be critical for reward-seeking behaviors. To further explore NAcc physiological correlates of several classes of motivated behaviors, we have employed two paradigms: (1) Naive rats were placed and allowed to move freely in an open field (opaque Plexiglas box fitted with nosepoke holes), while novelty stimuli (food and non-food items) were presented to the subjects; (2) Rats were further trained to self-administer heroin intravenously (0.06 mg/kg/injection through an indwelling catheter) by bar pressing (FR-1 schedule) in another operant chamber. NAcc neuronal activity was recorded using stainless steel microwires (62 μ) implanted into the NAcc under anesthesia. The spontaneous firing rate of sampled NAcc neurons (N=34) ranged from 0.21 to 18.23 spikes/sec (median=1.21 Hz). In the open field, NAcc neurons (N=4) responded during prolonged nosepoking events by a decrease in spontaneous activity. Just prior to entry into a hole the firing rate of most cells was briefly elevated above baseline. Some neurons (N=3) repeatedly decreased their discharge rate during focused attention as well as during feeding with a favorite novel food morsel (popcorn), supplied by the experimenter. The inhibitory response was strong and long-lasting. In addition, similar to our earlier report (Soc. Neurosci. Abstr., Vol 18: 373, 1992) drug-related bar-pressing events were followed by decreased NAcc neuronal activity lasting up to one minute (N=7). These data suggest that NAcc neuronal discharge is actively modulated during specific novel exploratory behaviors, during consummatory events and during heroin self-administration behavior. The direction in the change of neuronal activity (decrease) is similar during each of these behavioral events. (Supported by DA-03665 and TRD 1RT 183 to SJH).

418.5

BLOCKADE OF KAPPA OPIATE TOLERANCE BY MK-801, AN NMDA RECEPTOR ANTAGONIST, IN THE MOUSE.

Sanjay N. Thorat* and Hemendra N. Bhargava, Dept. Pharmacodyn., Univ. Ill., Chicago, IL 60612.

The effect of MK-801 (0.25 to 1.0 mg/kg, ip) on tolerance to the analgesic and hypothermic effects of U-50,488H, a k-opiate receptor agonist, was determined in the mouse. Male Swiss Webster mice were injected twice daily with either saline or MK-801, 30 min prior to the injection of U-50,488H (25 mg/kg, ip) for 9 days. The tail-flick latency and colonic temperature of mice were determined 60 min after the injection of U-50,488H on days 1, 3, 5, 7 and 9. Tolerance to the analgesic and hypothermic effects of U-50,488H was evident on day 5 of the treatment. Concurrent injections of MK-801 in all doses used prevented the development of tolerance to the pharmacological effects of U-50,488H as evidenced by reappearance of the prolongation of tail-flick latency and hypothermic response. MK-801 by itself either on acute or chronic administration did not affect the analgesic and hypothermic actions of acutely administered U-50,488H. It is concluded that NMDA receptor antagonist can block the tolerance to the pharmacological actions of k-opiate agonist in the mouse (Supported by grant DA-02598 and a Research Scientist Development Award K02-00130 from the National Institute on Drug Abuse).

418.6

Conditioned Tolerance To Intravenous (iv) Morphine: Demonstration and Spinal Mediation.

J.E. Grisel*, E.P. Wiertelak[1], R.J. Frohardt, H.-Y.T. Yang,[2], S.F. Maier & L.R. Watkins. Dept. of Psychology, University of Colorado, Boulder, CO 80309; Macalester College, St. Paul, MN 55105[1]; NIMH, Washington, D.C. 20032[2].

Tolerance to morphine can be divided into two classes. The present experiments distinguish between "non-associative" or dispositional tolerance and "associative" or conditioned tolerance using 3 groups of rats: those tested for morphine analgesia in a context they have been conditioned to associate with morphine (5mg/kg daily for 5 consecutive days), a second group given identical morphine injections but conditioned to associate the test context with saline injections, and a third group of pharmacologically naive but similarly handled rats. Exper. I found only dispositional tolerance following sc morphine, but both dispositional and associative tolerance following iv drug. Exper. II suggested a compensatory mechanism underlying conditioned tolerance using iv morphine since a hyperalgesic state resulted in animals conditioned to expect morphine, but given saline. The remaining experiments investigated spinal mechanisms of condtioned morphine tolerance. Antagonists of putative endogenous anti-opiates were injected intrathecally prior to analgesia testing (tailflick) on the test day after 5 days of conditioning. Three doses of the CCK_B antagonist L-365,260 given prior to morphine had no effect on conditioned tolerance. In contrast a neurotensin antagonist (D-Trp11) abolished conditioned tolerance without effecting dispositional tolerance. Studies on FMRFamide are in progress.

418.7

EFFECTS OF NOVEL KAPPA OPIOIDS ALONE AND IN COMBINATION WITH OPIOID ANTAGONISTS ON SHOCK TITRATION IN SQUIRREL MONKEYS.

R. C. Pitts and L. A. Dykstra*. Dept. of Psychology, Univ. of N. Carolina, Chapel Hill, NC 27599-3270.

Effects of three novel kappa-selective compounds, spiradoline, CI-977, and U69,593, were assessed alone and in combination with the opioid antagonists quadazocine and β-funaltrexamine (β-FNA) in squirrel monkeys responding under a shock-titration procedure. In this procedure, shock intensity increased every 15 s from .01 mA to 2.0 mA in 30 increments. Five lever presses during any given 15 s shock period produced a 15 s shock-free period after which shock resumed at the next lower intensity. When given alone, spiradoline, CI-977, and U69,593 produced dose-dependent increases in the intensity below which the monkeys maintained shock 50% of the time (median shock level, MSL) and decreases in the rate of lever pressing. When given in combination with these kappa agonists, quadazocine produced rightward shifts in the dose-effect functions (both MSL and rate) for spiradoline, CI-977, and U69,593. In contrast, β-FNA failed to shift the dose-effect functions of any of the kappa agonists. Apparent pA_2 values obtained for quadazocine on MSL ranged between 6.12 and 6.68 and were similar to those obtained previously for quadazocine in combination with the kappa agonists U50, 488 and bremazocine in squirrel monkeys responding under the shock-titration procedure (Dykstra & Massie, 1988). Supported by grants DA02749 and DA00033 from NIDA.

418.8

MORPHINE INDUCED ANALGESIA, ABSTINENCE, REWARD AND AVERSION IN THE FAWN HOODED RAT.

G.S. Borszcz* and C.P. Johnson, Department of Psychology, Dartmouth College, Hanover N.H. 03755

The Fawn Hooded (FH) rat strain has been shown to possess altered central serotonergic function. Because many of the behavioral effects of morphine treatment are partially mediated by central serotonergic pathways the present study was designed to characterize the FH rat's sensitivity to this drug.

Morphine analgesia was assessed by determining the thresholds of motor reflexes and vocalizations elicited by tailshock. Morphine abstinence was evaluated by scoring the behavioral syndrome elicited by naltrexone (5 mg/kg, sc) administered 1 hr following repeated morphine injections. Separate groups received either low or high doses of morphine. The schedule of morphine administration was: 0 hr = 10 mg/kg, 4 hr = 20 mg/kg, 24 hr = 20 mg/kg, 28 hr = 20 or 30 mg/kg, 48 hr = 20 or 30 mg/kg, 52 hr = 20 or 40 mg/kg, 72 hr = 20 or 40 mg/kg. Morphine reward was determined using the conditioned place preference paradigm. On alternate days for 8 days morphine (5mg/kg, sc) or saline injections were paired with one of two distinctive sides (horizontal vs vertical stripes) of a shuttle box. On the 9th day place preference was scored by recording the amount of time animals spent on each side of the shuttle box during a 15 min free choice period. Aversion generated by morphine withdrawal was assessed using the conditioned place aversion paradigm. Animals were given repeated injections of morphine on the schedule previously described for induction of morphine abstinence (low dose group). Following naltrexone (5 mg/kg, sc) administration they were place on one side of the shuttle box for 1 hr. The following day place aversion was scored as the time spent on each side of the shuttle box during a 15 min free choice period.

Compared to Long-Evans (LE) rats, FH rats exhibited reduced sensitivity to the analgesic and rewarding properties of morphine. Alternately, no differences were observed between strains in the capacity of morphine to induce abstinence or aversion. These results indicate that the FH rat may be a useful model for evaluating the mechanisms by which narcotics influence behavior.

418.9

MORPHINE-INDUCED CONDITIONED ANALGESIA USING A TASTE CUE IN RATS. J. M. Valone* and M. T. Bardo. Department of Psychology, University of Kentucky, Lexington, KY 40506.

In a Pavlovian paradigm, morphine was paired with a taste which served as the conditioned stimulus (CS) in rats. Conditioned taste aversion and conditioned analgesia were assessed using either 0, 1, 3, 10 or 30 mg/kg morphine. Results showed that 3, 10 or 30 mg/kg morphine produced a taste aversion, whereas only 30 mg/kg produced conditioned analgesia as measured by a hot plate test. Another experiment was designed to assess the effect of a delay between CS presentation and morphine administration. Results showed that delaying morphine for six hours after CS presentation eliminated both the conditioned taste aversion and the conditioned analgesia. A third experiment tested conditioned analgesia using either a 50°C or 54°C hot plate. This experiment found a conditioned analgesic response at both temperatures but less variability at 54°C. The analgesic response to acute doses of lithium (0, 3, 10 and 30 mg/kg) were compared to an acute dose of morphine (15 mg/kg). Results showed that, in contrast to morphine, none of the lithium doses produced an analgesic response. When paired with saccharin, lithium produced a conditioned taste aversion but no conditioned analgesic response. Taken together, these results indicate that a conditioned taste aversion is not sufficient to produce a conditioned analgesic response. (Supported by USPHS Grants DA-05312 and DA-07746).

418.10

BUPRENORPHINE DRUG DISCRIMINATION IN OPIATE-DEPENDENT ANIMALS: AN ASSESSMENT OF THE KAPPA ANTAGONIST PROPERTIES OF BUPRENORPHINE. S. Pournaghash* and A. L. Riley. The American University, Washington D.C. 20016

In work on drug discrimination learning with buprenorphine (see Pournaghash & Riley Neurosci. Abst., 41.4, 1991), stimulus control by buprenorphine appears to be mediated by its activity at the mu receptor. Specifically, animals trained to discriminate buprenorphine from distilled water generalized this control to the mu agonist morphine but not the kappa antagonist MR2266. Given that opiate tolerance has been reported to alter the involvement of various receptors in mediating the effects of drugs on behavior (see Negus, Picker & Dykstra Psychopharmacology 98:141-143, 1989), the present experiment assessed the effects of opiate tolerance on the receptor bases of the discriminative control by buprenorphine. Specifically, opiate-tolerant rats were trained to discriminate buprenorphine from distilled water and then tested for the generalization of this control to the mu agonist morphine and the kappa antagonist MR2266. Exposure to morphine shifted the morphine generalization function to the right (indicating tolerance), yet MR2266 continued to fail to substitute for buprenorphine. The present study finds no evidence for the ability of buprenorphine's kappa activity to support drug discrimination learning, even in opiate-tolerant subjects.

418.11

QUININE BLOCKS THE BEHAVIORAL AND NEUROCHEMICAL EFFECTS OF HYDROCODONE BUT NOT HYDROMORPHONE. B. Gomez-Mancilla*, S. Cheung, S.V. Otton, E.M. Sellers. Addiction Research Foundation, Departments of Pharmacology, Medicine and Psychiatry, University of Toronto, Toronto, Ontario M5S 2S1.

Several opiates of abuse are converted by cytochrome P450 2D6 (CYP2D6) to metabolites of much greater pharmacological potency than the parent compound (i.e. hydrocodone to hydromorphone, codeine to morphine). The activity of human CYP2D6 is genetically variable (absent in about 7% of Caucasian) and can be inhibited by several compounds (quinine, quinidine). Using Sprague-Dawley rats, we compared the locomotor activity and dopamine release in the n. accumbens produced by hydrocodone and hydromorphone given alone and 60 minutes after quinine (40 mg/kg i.p.). The administration of hydrocodone (0.01, 0.1 and 1 mg/kg s.c.) and hydromorphone (0.001, 0.01, and 0.1 mg/kg s.c.) produced a dose-related increase in locomotor activity. Hydrocodone (1 mg/kg s.c.) and hydromorphone (0.1 mg/kg s.c.) increased dopamine release 38 ± 8.8%, and 78 ± 13%, respectively. Quinine pre-treatment reduced locomotor activation 70% and dopamine release in the n. accumbens to 11 ± 4.9% compared with the administration of hydrocodone given alone. When hydromorphone was given, there was no effect of quinine on locomotor activity or dopamine release in the n. accumbens. These result suggest that CYP2D6 activity may be an important determinant in the pharmacologic effects of hydrocodone (including abuse liability) mediated through hydromorphone, its active metabolite.

Supported by NIDA #RO1 DA06889.

418.12

MORPHINE, CHLORDIAZEPOXIDE, AND PENTOBARBITAL AFFECT APPETITIVE PAVLOVIAN CONDITIONING OF MOTOR ACTIVITY IN RATS. J.D. Valentine*, P. Tollison and L.L. Hernandez Univ. of S. Carolina, Dept. of Psychology, and V.A. Medical Center, Columbia, SC, 29201.

Rats were subjected to 15 daily appetitive Pavlovian conditioning sessions while under the influence of a relatively low dose of morphine, chlordiazepoxide, or pentobarbital. Morphine dose-dependently increased baseline motor activity (BMA), whereas chlordiazepoxide and pentobarbital dose-dependently decreased BMA. All 3 of the drugs dose-dependently enhanced the magnitude of the conditioned motor-activity (CMA) response, an effect that developed as conditioning proceeded.

The rats were then subjected to 10 daily extinction sessions that were not preceded by any treatments. The group differences in BMA and CMA that were observed during conditioning did not carry over into extinction training, and no other significant group differences developed during these 10 extinction sessions.

Finally, an additional extinction session was conducted before which each rat was administered the same treatment that it had received before each of the conditioning sessions. The chlordiazepoxide and pentobarbital treatments dose-dependently decreased BMA, but did not affect CMA; however, re-administering morphine completely reinstated the dose-dependent increases of BMA and CMA observed during conditioning. Re-administering saline had no effect on either BMA or CMA.

This work was supported by USPHS Grant RO1-DA06676 and DVA Medical Research Funds.

418.13

CONDITIONED PLACE PREFERENCE WITH BUPRENORPHINE: INTERACTIONS WITH NALTREXONE, MORPHINE AND AMPHETAMINE

J.K. Rowlett*, T.R. Gibson, J.M. Valone & M.T. Bardo, Department of Psychology, University of Kentucky, Lexington, KY 40506.

The rewarding effect of the opioid mixed agonist-antagonist buprenorphine alone and combined with various drugs was assessed using conditioned place preference (CPP). In one experiment, rats were given one of four doses of buprenorphine (0.001, 0.01, 0.1, 1.0 mg/kg) or saline paired with the white compartment of a CPP chamber. Significant CPP was obtained for 1.0 mg/kg buprenorphine after four trials and for 0.1 and 1.0 mg/kg buprenorphine after eight trials. The effects of combined treatments during conditioning of buprenorphine with the opioid receptor antagonist naltrexone, the opioid receptor agonist morphine, and the psychostimulant amphetamine also were assessed. Pretreatment with 1.0 mg/kg naltrexone blocked CPP produced by six pairings of 0.1 mg/kg buprenorphine. Buprenorphine treatment (1.0 mg/kg) produced additive effects on CPP produced by three pairings of either 0.3 mg/kg morphine or 0.5 mg/kg amphetamine. Taken together, these results indicate that buprenorphine, similar to full opioid agonists, produces CPP that follows a linear dose-response function and is reversed by naltrexone. Buprenorphine elicited additive effects when combined with morphine or amphetamine, also consistent with full agonist effects on reward. (Supported by USPHS grants DA05312 and DA07746).

418.14

ASSOCIATIVE FACTORS IN STRESS-INDUCED SENSITIZATION TO THE BEHAVIORAL ACTIVATING EFFECTS OF MORPHINE. Y. Shaham*, J.E. Kelsey, and J. Stewart. Center for Studies in Behavioral Neurobiology, Department of Psychology, Concordia University, Montreal, Quebec, Canada H3G 1M8.

A recent report (Shaham, 1993) showed that associative factors may contribute to the stress-induced increase in oral opioid consumption. We examined whether similar learning factors might also be involved in the stress-induced sensitization to the behavioral activating effects of morphine. Forty-eight male rats were divided into 6 groups in a 2 (drug condition: morphine/saline) x 3 (stress condition: Control [C], Paired-Stress [PS] and Unpaired-Stress [UPS]) factorial design. Prior to tests for sensitization to a low dose of morphine (7 sessions, 48 h apart), PS rats were restrained for 15 min prior to the morphine (10 mg/kg) or saline injections and were then placed in the locomotor activity boxes for 90 min. UPS rats were restrained in the animal colony 24 h after each session. Three separate tests for morphine or stress sensitization of locomotor activity were conducted with 1) a low dose of morphine (3 mg/kg), 2) morphine+paired stress, and 3) saline+paired stress. C groups were not exposed to stress in any of the tests for sensitization. On these tests, the behavioral activating effects of morphine were enhanced in animals previously exposed to paired-stress, but not in animals previously exposed to unpaired-stress. Exposure to stress did not affect locomotor activity in the absence of morphine. These results indicate that the learned association between exposure to stress and opioid drugs may contribute to the stress-induced changes in the behavioral activating effects of these drugs.

418.15

CONDITIONED TASTE AVERSIONS TO COMPOUNDS WITH OPIATE AGONIST/ANTAGONIST ACTIVITY AT SPECIFIC OPIATE RECEPTOR SUBTYPES. C. M. Ferrari*, P. M. Melton and A. L. Riley. Psychopharmacology Laboratory, The American University, Washington, D.C., 20016.

Given Self and Stein's (Pharmacol. & Toxicol. 70:87-94, 1992) recent conclusion that the reinforcing and aversive properties of the opiates are mediated by different receptor subtypes (mu and kappa, respectively), the present study examined the ability of compounds with various activity at opiate receptor subtypes, e.g., naloxone (mu), MR2266 (kappa), nalorphine (mixed mu/kappa) and buprenorphine (mixed mu/kappa) to condition taste aversions in rats, an index of the aversive properties of drugs (Riley & Tuck, ANYAS 443:272-292, 1985). Specifically, rats were given a novel saccharin solution to drink followed within 15 min by an injection of one of a range of doses of the aforementioned compounds. None of the opiates conditioned aversions to the associated saccharin solution, even with high doses and repeated conditioning trials. Although it might be expected that the compounds with mu agonist (buprenorphine) and kappa antagonist (buprenorphine and MR2266) activity would be ineffective in conditioning aversions, it was surprising that compounds with kappa agonist (nalorphine) and mu antagonist (nalorphine and naloxone) activity were without effect in this design.

EPILEPSY: HUMAN STUDIES AND ANIMAL MODELS III

419.1

^{1}H MRS AND T2 RELAXOMETRY OF THE CONTRALATERAL TEMPORAL LOBE FOLLOWING EPILEPSY SURGERY. D.G. Gadian, A. Connelly, C.L. Johnson, G.D. Jackson, C.E Polkey[1], A. Incisa della Rocchetta and F. Vargha-Khadem. (SPON: European Brain and Behaviour Society). Inst. of Child Health and Hospital for Sick Children, Gt. Ormond Street, London WC1N, and [1]Maudsley Hospital, London, UK.

Resection of temporal lobe structures can cure cases of intractable temporal lobe epilepsy. Several types of operation are possible; for example, if preservation of the maximal amount of neural tissue is considered important, then selective amygdalohippocampectomy may be performed. Magnetic resonance imaging (MRI) and spectroscopy (MRS) can be used post-operatively to define the degree of surgical removal and to assess the integrity of the contralateral mesial temporal structures.

^{1}H spectra were obtained at 1.5T from 2x2x2cm cubes within the mesial temporal lobes contralateral to the side of temporal lobe resection, using a water-suppressed spin echo sequence with TE = 135ms and TR = 1600ms. The dominant signals are from N-acetylaspartate (NAA), creatine + phosphocreatine (Cr), and choline-containing compounds (Cho). Since NAA is believed to be present primarily within neurons, a reduction in NAA or in the NAA/Cho + Cr ratio is interpreted in terms of neuronal loss or damage. T2 relaxation times, which are sensitive to abnormalities of the hippocampus, were measured from 16-echo T2 maps.

NAA/Cho + Cr ratios in the temporal lobe contralateral to the resection were abnormally low in five of the eight patients examined, suggesting the presence of diffuse neuronal damage. This damage can be detected independently of hippocampal abnormalities, which were seen by T2 relaxometry in only two of the patients. The prognostic implications of these findings will be evaluated by following the outcome in this patient group.

419.2

RELATIONSHIP OF COGNITIVE DYSFUNCTION TO ^{1}H MRS ASSESSMENT OF TEMPORAL LOBE PATHOLOGY. F. Vargha-Khadem, A. Connelly, J.H. Cross, G.D. Jackson, E.B. Isaacs and D.G. Gadian. (SPON: European Brain and Behaviour Society). Inst. of Child Health and Hospital for Sick Children, Gt. Ormond Street, London WC1N, UK.

Children with intractable epilepsy may demonstrate impaired cognitive function with deterioration over time, but the pathological basis of this process remains unclear. Magnetic resonance techniques can be used to investigate the underlying pathology.

Magnetic resonance and neuropsychological investigations were carried out on 22 right-handed children with intractable complex partial seizures. The neuropsychological measures included the assessment of performance and verbal IQ using Wechsler intelligence scales. ^{1}H spectra were obtained at 1.5T from 2x2x2cm cubes within the left and right mesial temporal lobes using a water-suppressed spin echo sequence with TE = 135ms and TR = 1600ms. The dominant signals are from N-acetylaspartate (NAA), creatine + phosphocreatine (Cr), and choline-containing compounds (Cho). Since NAA is believed to be present primarily within neurons, a reduction in NAA or in the NAA/Cho + Cr ratio is interpreted in terms of neuronal loss or damage.

The performance IQ was 96 ± 24 (mean ± SD; range 50-142), and the verbal IQ was 87 ± 18 (range 51-117). The difference between performance and verbal IQ measurements was calculated for each subject, and plotted against the difference between the NAA/Cho + Cr ratios obtained from the right and left mesial temporal lobes. A highly significant correlation was observed ($r = 0.65$, $p = 0.001$), suggesting that neuronal loss or damage is associated with cognitive dysfunction in these children.

419.3

CORRELATION BETWEEN AMYGDALOID AND HIPPOCAMPAL VOLUME AND MEMORY REPRESENTATION IN TEMPORAL LOBE EPILEPSY (TLE). **Imad M. Najm*, Youssef G. Comair and Hans O. Luders. Depts of Neurology and Neurosurgery, Cleveland Clinic, Cleveland, OH 44195.**

Impaired memory performance during the intracarotid amobarbital procedure (IAP) may reflect severe hippocampal damage. Moreover, pathological studies from patients with Temporal Lobe Epilepsy (TLE) have demonstrated the existence of a relationship between hippocampal volume and neuronal damage. In view of the role of the hippocampus in memory we investigated the relationship between the hippocampal volume and the IAP memory scores in patients with TLE. Volumetric MRI studies of hippocampal formation (HF) and amygdala from 15 patients with TLE were performed. As previously shown, the difference between the right and the left HF volume was considered evidence of lateralized HF atrophy if the difference was below -0.2 cm^3 or above + 0.6 cm^3 and indeterminate if the difference was between - 0.2 and + 0.6 cm^3. These results were compared to IAP memory scores. In 8 patients, the atrophic side was correlated with an impaired ipsilateral IAP memory performance. In 4 patients, an indetermined atrophy was associated with a bilateral memory representation. In 3 patients, no correlation was found between the memory representation and the HF volume. No significant correlation was found between amygdala volume and memory representation. These results show a good correlation between HF volume and memory representation in 12/15 patients evaluated for TLE. Thus, HF volume measurement may be used as a non-invasive method to predict or to cofirm memory dominance in TLE.

419.4

CONSERVATION OF GROSS MOTOR FUNCTION FOLLOWING FUNCTIONAL HEMISPHERECTOMY IN HUMAN. H. Sveistrup[1*], T.B. Hoshizaki[1], S. Brien[2], E. Spyro[1], and J.G. Villemure[3]. [1]McGill University, [2]Jewish General Hospital, [3]Montreal Neurological Institute, Montreal, Canada.

Functional hemispherectomy is used as an alternative intervention to anatomical hemispherectomy for the treatment of seizures. It consists of complete callosotomy, complete disconnection of the frontal and parieto-occipital lobes in the coronal plane, and a temporal lobectomy (Villemure, In: Epilepsy Surgery, Ed. H. Luders, Raven Press: NewYork, 1991, pp 569-578). We studied the effect of this surgery on kinematic and kinetic parameters of three gross motor tasks: leg extension-flexion, arm extension-flexion, and locomotion (walking) in a 33-year-old female injured from birth.

Differences between ipsi- and contralateral side movements were recorded pre-surgery with greater motor dysfunction (i.e., decreased range of motion, decreased leg extension-flexion torque) observed on the side contralateral to the surgery. Following functional hemispherectomy, no changes were recorded in the kinetic and kinematic variables studied. The contralateral side remained more dysfunctional than the ipsilateral side. These data suggest that the surgical intervention did not alter the ability of the patient to perform these specific tasks.

It is possible that during development, the control of gross motor behaviors was integrated into the ipsilateral hemisphere. Alternatively, the specific movements studied may not require contributions from the neural structures functionally removed.

419.5

NEUROACTIVE AMINO ACIDS IN SYNAPTOSOMES FROM FOCAL AND NONFOCAL TEMPORAL LOBE TISSUE BIOPSIED FROM PATIENTS WITH INTRACTABLE COMPLEX PARTIAL SEIZURES. D. Labiner[1*], P.-L. Lieu[2], M. E. Weinand[3] and R. J. Huxtable[2], Departments of Neurology[1], Pharmacology[2] and Surgery[3], University of Arizona College of Medicine, Tucson, Arizona 85724.

Temporal lobe tissue was obtained from 8 patients undergoing surgery for medically intractable complex partial seizures. Tissue from the epileptic focus was removed by suction, and nonfocal tissue was removed *en bloc*. Synaptosomes (P_2 fraction) were prepared from both tissues and analyzed for the major neuroactive amino acids. Concentrations (μMol/g protein) and percent changes in P_2 from the focus (compared to nonfocal P_2) were: Glu 2.99* (67%); Asp 2.39 (91%); Gly 1.16 (108%); GABA 0.57 (63%); Ala 0.27* (39%); Tau 0.26* (34%); and Gln 0.28* (14%) [*p<.05]. Total concentrations of the 7 amino acids were 12.47±2.56 μMol/g protein in nonfocal P_2 and 7.91±2.40 in focal P_2. A larger decrement was seen in the neuroinhibitory amino acids, GABA+Tau (to 41% of nonfocal P_2), than in the neuroexcitatory amino acids, Asp+Glu (to 71% of nonfocal P_2). This resulted in a marked elevation in the ratio of excitatory to inhibitory amino acids in P_2 from the focal area (the ratio being 7.12 in focal and 4.69 in nonfocal P_2). The other marked change was a large decrease in Gln in focal P_2, the Gln/Glu ratio dropping from 0.47±0.13 in nonfocal synaptosomes to 0.08±0.07 in synaptosomes from focal tissue. The significance of these findings for the neurochemistry of intractable complex partial seizures await study of the effects of the differing surgical treatments of focal and nonfocal tissue. However, the implications are that, in this well-defined group of patients with medically intractable complex partial seizures, there are underlying changes in neuroactive amino acids consistent with increased excitability in the focal region.

419.6

SELECTIVE NEURONAL LOSS IN LAYER III OF THE MEDIAL ENTORHINAL CORTEX IN THREE RAT MODELS OF EPILEPSY. T. Eid, F. Du, E.W. Lothman[1], C. Köhler[2], R. Schwarcz*. Md. Psych. Res. Ctr., Baltimore, MD 21228, [1]Dept. Neurology, Univ. Virginia Med. Ctr., Charlottesville, VA 22908 and [2]Preclin. CNS Res., Hoffman-La Roche, Basel, Switzerland.

We recently showed that neurons in layer III of the medial entorhinal cortex (EC) are preferentially vulnerable to the convulsant aminooxyacetic acid (Neurosci. Lett. 147: 185, 1992). Since neurons in layer III of the EC also preferentially degenerate in human temporal lobe epilepsy (cf. Du et al., this meeting), we have examined the EC, by light microscopy in Nissl-stained sections, in 3 models of experimental epilepsy. Adult, male rats were either electrically stimulated in the ventral hippocampus for 90 minutes to produce limbic seizures or injected with kainic acid (10 mg/kg, s.c.) or pilocarpine (325 mg/kg, i.p.). After 24 hours, the brains of all rats that had acute behavioral seizures showed neuronal degeneration in the EC in addition to lesions in other limbic areas. In the EC, neurodegeneration was almost exclusively restricted to layer III. Typically, this laminar lesion was particularly pronounced in the medial part of the EC, though the lateral EC was often affected as well. Notably, surviving neurons were frequently seen in the lesioned layer. These data suggest a possible involvement of exquisitely vulnerable EC neurons in the mechanisms underlying limbic seizures.

Supported by USPHS grant NS 16102.

419.7

PREFERENTIAL LOSS OF NEURONS IN LAYER III OF THE ENTORHINAL CORTEX IN PATIENTS WITH TEMPORAL LOBE EPILEPSY. F. Du*, W.O. Whetsell, Jr[1]., B. Abou-Khalil[1], B. Blumenkopf[1], E.W. Lothman[2] and R. Schwarcz. Maryland Psychiatric Research Center, Baltimore, MD 21228, [1]Vanderbilt Univ. School Med., Nashville, TN 37232 and [2]Univ. Virginia Med. Ctr., Charlottesville, VA 22908.

Experimental and human clinical studies have suggested that the entorhinal cortex (EC) may participate in the generation and/or propagation of limbic seizures. Therefore, we have examined, by light microscopy, surgical specimens containing the EC obtained from 6 patients with complex partial seizures during temporal lobectomy. In Nissl-stained sections, a loss of neurons was observed in the anterior portion of the EC. Cell loss was particularly evident in layer III, but was also observed in layer II. This pattern of neurodegeneration was consistent in all specimens studied, though the degree of cell loss varied between patients. The laminar lesion described here was mainly seen in the medial EC, but was also noticed in the lateral EC. Ammon's horn sclerosis was also detected in all 6 patients. These results provide neuropathological evidence for a role of the EC in temporal lobe epilepsy. Since the EC occupies a pivotal position in gating hippocampal input and output, our results support previous suggestions that dysfunction of this region may contribute, either independently or in concert with Ammon's horn sclerosis, to epileptogenesis in humans.

Supported by USPHS grant NS 16102.

419.8

ACCUMULATION OF β-AMYLOID PRECURSOR PROTEIN (βAPP) AND INTERLEUKIN-1α (IL-1α) IN HUMAN TEMPORAL LOBE EPILEPSY. J.G. Sheng, C.R. Rovnaghi, F.R. Boop, R.E. Mrak, and W.S.T. Griffin*. Depts. of Anatomy, Neurosurgery, and Pathology, Univ. of Arkansas for Med. Sci., Little Rock, AR 72202.

Excessive expression of βAPP is not only a neuropathological feature of Alzheimer's disease (AD) but also of heart disease, head trauma, and HIV infection. Elevation of βAPP levels is associated with excessive growth of neurites and neuronal degeneration, both of which occur in focal epilepsy. IL-1 stimulates excessive expression of βAPP. We measured the expression of βAPP and IL-1 in resected temporal lobes from a group of patients with intractable epilepsy. By SDS-page immunoblot analysis, we identified three distinct βAPP immunoreactive (βAPP^+) bands (MW 120-135 kD). Epileptic tissue showed 3 fold ($P<0.05$) and 4.5 fold ($P<0.001$) elevations of βAPP^+ product in the 135 and 130 kD bands, respectively, over analogous regions of autopsy tissue from non-epileptics of similar age (AMC); the amounts in the βAPP^+ 120 kD band were not different. In immunoreacted tissue sections from epileptics, the number of βAPP^+ neurons was higher than in analogous sections from AMC (133 ± 12/mm² *vs* 8 ± 3/mm²; $P<0.001$), and the levels of βAPP^+ product in these cells were greater than in βAPP^+ neurons in AMC. In addition, clusters of βAPP^+ dystrophic neurites were observed in some epilepsy cases, but not AMC. IL-1α$^+$ microglia ($GFAP^-/S100\beta^-$) were more numerous in tissue sections from epileptics than AMC (80 ± 8/mm² *vs* 25 ± 5/mm²; $P<0.001$). IL-1α$^+$ microglia in epileptic tissue were activated and had elevated levels of IL-1α$^+$ product. IL-1α$^+$ and βAPP^+ cells in temporal lobe from epileptics were similar in appearance to those observed soon after acute head injury, a risk factor for development of the neuropathology and dementia of AD. These findings may improve our understanding of the neuropathophysiological changes observed in epilepsy. Supported in part by NS27414 and AG10208.

419.9

PROLONGED GABA RESPONSES IN DENTATE GRANULE CELLS OF PATIENTS WITH TEMPORAL LOBE SCLEROSIS. A. Williamson*, G.M. Shepherd and D.D Spencer. Section of Neurosurgery, Yale University School of Medicine, New Haven, CT.

Human medial temporal lobe sclerosis is characterized by extensive anatomical and neurochemical reorganization throughout the entire hippocampal formation as well as by hyperexcitability in both the granule cell and pyramidal cell populations. In order to assess other changes in the excitable properties of these cells, we have compared the sclerotic hippocampi with those of patients with temporal lobe tumors (TTLE) in which there is no neurochemical or anatomical reorganization, and in which the cells do not exhibit hyperexcitability.

We have compared the electrophysiological effects of focally applied GABA (1-10 mM) on granule cells from both types of hippocampi maintained in the slice preparation using intracellular recording techniques. In both types of tissue, GABA produced large depolarizations associated with an approximately 60% conductance increase when measured at rest. However, the GABA responses in the sclerotic tissue were significantly prolonged relative the those seen in the TTLE tissue. The mean duration of the GABA responses were 46.7 ± 11.6 sec (n= 11) in the sclerotic tissue but only 9.08 ± 3.6 sec (n=7) in the TTLE tissue. The duration of the GABA response in the TTLE tissue is comparable to that seen in rat granule cells.

This variation in the duration of the responses could be seen in a single case. Cells which were not hyperexcitable and which were found in more posterior sections of the hippocampus had shorter GABA responses than the more excitable cells found in anterior slices from the same patient. Thus these changes may be quite local and specific.

We hypothesize that this prolongation of the GABA response in the sclerotic tissue may be due to a reduction in the GABA uptake system. This change in the cellular responsivness to GABA may represent an adaptation to neuronal injury associated with granule cell hyperexcitability.

419.10

SUBPOPULATIONS OF SMI32-IMMUNOREACTIVE PYRAMIDAL CELLS ARE DIFFERENTIALLY INNERVATED BY PARVALBUMIN-IMMUNOREACTIVE CHANDELIER CELL AXONS IN THE HUMAN NEOCORTEX. M.R Del Río and J. DeFelipe* Instituto Cajal (CSIC). 28002-Madrid, Spain.

Chandelier cells are considered to be one of the most powerful types of cortical inhibitory interneuron. These cells form synapses exclusively with the axon initial segment of pyramidal cells and are the major source of these synapses. Immunocytochemical studies in the primate neocortex have shown that particular populations of pyramidal cells and chandelier cells are labeled, respectively, using the antibody SMI32 that recognizes a non-phosphorilated epitope of neurofilament proteins and an antibody directed against the calcium-binding protein parvalbumin (PV). Therefore, we studied the relationships between these pyramidal cells populations and chandelier cells in the human neocortex. Cortical tissue was obtained during surgical treatment of epileptic patients. After resection, the tissue was immersed in a solution of 4 % paraformaldehyde in phosphate buffer, then cut at 100 μm, and processed for immunocytochemistry using the above two antibodies sequentially in the same sections. We found four pyramidal cells subpopulations with regard to the immunocytochemical staining for SMI32 and the innervation of their axon initial segments by chandelier cell axons (Chax): (1) SMI32-positive pyramidal cells/ PV-positive Chax; (2) SMI32-positive pyramidal cells/ PV-negative Chax; (3) SMI32-negative pyramidal cells/ PV-positive Chax; (4) SMI32-negative pyramidal cells/ PV-negative Chax. Furthermore, there were differences in the concentration and proportion of the different subpopulations across cortical layers. (Supported by DGICYT grant PM92-0021).

419.11

ANTIEPILEPTIC EFFECTS OF ORGANIC CALCIUM CHANNEL BLOCKERS ON EPILEPTIFORM ACTIVITY IN HUMAN NEOCORTEX (*IN VITRO*). E.-J. Speckmann [*1,2], H. Straub, [1] R. Köhling[1], A. Lücke[1], D. Moskopp[3], H. Wassmann[3]. [1] Institut für Physiologie, Universität, Robert-Koch-Str. 27a, 48149 Münster, Germany; [2] Institut für Experimentelle Epilepsieforschung, Universität, Hüfferstr. 68, 48149 Münster, Germany; [3]Klinik und Poliklinik für Neurochirurgie, Universität, Albert-Schweitzer-Str. 33, 48149 Münster, Germany.

Epileptic activity in neuronal populations is accompanied by typical field potentials (EFP). In several animal experiments it has been demonstrated that a calcium ion inward current is essentially involved in the generation of epileptic events. Consequently, epileptic activity was suppressed by application of organic calcium channel blockers. The present investigations aimed to clarify whether this antiepileptic calcium antagonism can also be found in human neocortical tissue.

The experiments were performed on 23 slices (400-500 µm) of human neocortex. Tissue used was a small portion of that which is normally removed for the treatment of brain tumor. EFP were induced by superfusion with Mg^{2+}-free artificial cerebrospinal fluid (CSF) or bicuculline (10µmol/l)-containing CSF. Verapamil and flunarizine were added to the superfusate. All values of time represent means.

EFP appeared spontaneously upon superfusion of Mg^{2+}-free CSF and stimulus-triggered upon application of bicuculline. Low Mg^{2+}-induced EFP were suppressed by 40 µmol/l verapamil within 126 min and by 60 µmol/l verapamil within 65 min. During wash-out of verapamil, EFP reappeared within 10 min. With superfusion of 10 µmol/l flunarizine, EFP were irreversibly abolished within 175 min. Bicuculline-induced EFP were suppressed by 60 µmol/l verapamil within 190 min and returned with wash-out of verapamil within 40 min.

The observations indicate that calcium currents and calcium-dependent currents are essentially involved in human epileptogenesis.

419.12

PHASE RESETTING OF NEURONAL ACTIVITY IN THE HIPPOCAMPUS

D. Durand*, E. Warman, Kate Greene and Paul Kammermier, Dept of Biomedical Engineering, Case Western Reserve University, Cleveland, OH. 44106.

The theory of phase resetting can predict the response of spontaneous oscillators to external perturbations. Some oscillators have the property known as strong resetting in which a perturbation applied during the period of oscillation can shift the occurrence of the next period and thereby reset the oscillation. It has been mathematically shown that systems with this property also present a singularity in the perturbation response. At the singularity, the response on the system is unpredictable and the perturbation often leads to annihilation of the response or induces a chaotic state.

We have applied the theory of phase resetting to quasi-periodic signals generated by epileptogenic agents in the hippocampus in-vitro. The purpose of the study was to determine whether the resulting oscillatory systems had the property of strong resetting and whether a singularity could be found. Orthodromic stimulation of the CA1 region in the hippocampal slice, in the presence of penicillin, generates interictal-like activity with 4 to 7 population spikes at a frequency of 200Hz. By stimulating the CA1 region extracellularly, we have shown that this quasi-periodic system does display strong resetting. Moreover, we also have been able to detect the presence of a singularity for which complete annihilation of the extracellular response is observed. In another set of experiments, perfusion of the hippocampal slice with high potassium solutions generates quasi-static periodic activity with a period of 1 to 2 Hz. This spontaneous interictal-like activity was also tested by stimulating the slice with an electrode located within the CA1 layer. The results indicate that this system also displays the property of strong resetting. However, we have not yet been able to find the singularity.

This analysis of two types of epileptiform activity has shown that the oscillatory waveforms generated in two animal models of epilepsy are vulnerable and could potentially be annihilated with electrical stimulation. Supported by NSF grant # BNS 8809504.

419.13

GREATER SYNCHRONY OF NON-BURST THAN BURST FIRING IN HUMAN EPILEPTIC TEMPORAL LOBES. B.W. COLDER*, R.C. FRYSINGER, C.L. WILSON, AND R.M. HARPER, Dept. of Anatomy and Cell Biology, Dept. of Neurology, and Brain Res. Inst., UCLA School of Med., Los Angeles CA 90024.

The synchronous bursting of neurons in an epileptic focus is hypothesized to generate seizures by recruiting additional interconnected neurons to also fire synchronous bursts. We reported that cells more likely to fire in bursts and cells in the same hemisphere as the seizure onset have a greater tendency to show cross-correlations indicating synchronous discharge. Most spike trains, however, include both burst and non-burst firing patterns. It is unclear how each of these discharge components participates in the formation of cross-correlations showing synchrony and how that participation is affected by proximity to an epileptogenic region. We examined recordings of inter-ictal activity from temporal lobes of patients undergoing chronic depth electrode recording for diagnostic purposes. Cross-correlations were calculated between all pairs of simultaneously recorded cells. Spike trains of cells that took part in a cross-correlation showing synchrony were decomposed into "burst" and "singleton" trains. Burst trains contained all bursts, while singleton trains contained all non-burst firings. Cross-correlations were then calculated between all burst trains and between all singleton trains. In the epileptogenic hemisphere, synchrony was more often observed between singleton trains than burst trains, while the opposite was true for the contralateral hemisphere. These findings suggest that while synchronous firing near the epileptic focus may be a feature of both ictal and inter-ictal activity, the synchronization of bursts may be an ictal phenomenon. Supported by NS 02808

419.14

BURST PATTERNS IN SINGLE CELL DISCHARGE AS A FUNCTION OF PROXIMITY TO EPILEPTOGENIC AREAS IN HUMAN TEMPORAL LOBE. R.C. Frysinger*, B. Colder, C. Wilson and R.M. Harper. Depts. of Anatomy and Cell Biology, Neurology, and the Brain Research Institute, UCLA Med. School, Los Angeles, CA 90024.

Synchronization of action potential bursts has been proposed as part of the mechanism of epileptogenesis, and burst patterning has been suggested as a marker of cells involved in seizure propagation. Hippocampal neurons are known to show burst patterns in normal animals, and the relationship of "normal" to "pathogenic" burst patterns is of considerable theoretical interest. We recorded spike trains from 310 cells in 18 patients with epilepsy undergoing depth recordings of mesial temporal EEG. Bilateral recordings were performed in amygdala, anterior and mid pes hippocampi, anterior and posterior perihippocampal cortex, and transitional cortex. Spike trains producing a statistically significant peak in the autocorrelation within 40 ms of the origin were considered "bursters," and the area beneath the peak and the duration of the burst were recorded and categorized by side (ipsilateral or contralateral to seizure onset) and region. Mean burst area and duration was considerably larger for cells recorded on the contralateral side of all regions, corresponding to a larger number of spikes per burst. The percentage of bursting cells was somewhat lower on the ipsilateral side of pes hippocampi, but higher in transitional cortex. These results suggest that mesial temporal neurons on the epileptogenic side of humans with complex partial seizures have fewer spikes per burst and are generally less likely to show a bursting pattern. Supported by NS 02808.

419.15

QUANTIFICATION OF DENDRITIC DEGENERATION AND ITS RELATION TO AXONAL REGENERATION IN SINGLE DENTATE GRANULE CELLS OF EPILEPTIC HIPPOCAMPUS. M. Isokawa*. Brain Research Institute, CHS, University of California, Los Angeles, CA 90024-1761.

Dendritic degeneration is one of morphological changes in human epileptic hippocampus (Scheibel et al. 1974). Intracellular recordings from human epileptic dentate granule cells (DGCs) suggested that an increased excitatory synaptic action, mediated by the NMDA receptor, was associated with this degeneration (Isokawa et al. 1991). In the present study, dendritic degeneration was quantified by injecting biocytin intracellularly and measuring spines, swellings, summed dendritic lengths (SDL), and the height of dendritic trees (H) in 16 DGCs. When spines were regularly present (N=5), their density was amazingly constant: 0.457 spines/µm ± 0.007 SEM, and the density of swelling was low: 0.045/µm ± 0.005 SEM (SDL: 6024.7 µm ± 642.2 SEM; H: 1164.9 µm ± 129.1 SEM). In 2 neurons, spine density declined to 0.290/µm ± 0.010, which coincided with the shortening of dendrites (SDL: 3018.0 ± 195.4; H: 406.3 ± 65.6). However, swelling density was not different (0.04/µm ± 0). When spines were largely lost (N=2), dendrites consisted of a series of swollen compartments (7.7 to 24.2 µm in size; 0.17 swellings/µm ± 0.06; SDL: 1666 ± 1117.9; H: 368 ± 92.0; 0.009 spines/µm). In the latter two groups of neurons, no aberrant axon collaterals were detected. In 3 neurons in the first group, aberrant collaterals were detected among dendrites. In these neurons, swelling density increased 3-fold (0.12/µm ± 0.09) without affecting spine density (0.45/µm ± 0.006). This result suggests that swelling can occur independent of spine loss in epileptic DGCs, and axonal regeneration may be involved in this process. Dendritic degeneration may not simply be a consequence of a general decline of viability in epileptic hippocampus. Supported by a grant from NIH (NS02808).

420.1

ENHANCED SEIZURE SUSCEPTIBILITY YET IMPAIRMENT OF KINDLING DEVELOPMENT IN α-CALCIUM-CALMODULIN KINASE II MUTANT MICE. L. Butler[1], A. Silva[2], S. Tonegawa[3] and J.O. McNamara[1]*. [1]Departments of Neurology and Neurobiology, Duke University Medical Center, Durham, NC 27710. [2]Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724. [3]Center for Cancer Research, Massachusetts Institute of Technology, Cambridge, MA 02139.

Kindling is an animal model of epilepsy produced by focal electrical stimulation of the brain. Available evidence suggests that formation of long term potentiation (LTP) contributes to kindling development. Since formation of LTP is impaired in transgenic mice carrying a null mutation for the α-subunit of Calcium Calmodulin Kinase II (α-CaMKII), we compared kindling development in wild type (+/+), heterozygous (+/-), and homozygous (-/-) mutants. The development of kindling did not occur in -/- mutants despite large numbers (60) of stimulations. This result is consistent with the idea that LTP is necessary for the development of kindling. Unexpectedly, -/- mutants also exhibited profound hyperexcitability; that is, a tiny, normally subconvulsive stimulation of the amygdala triggered prolonged seizures and/or repeated and sometimes fatal seizures. Combining genetic manipulations with phenotypic analyses of mouse behavior and the underlying cellular and molecular correlates provides an opportunity to elucidate the mechanisms of these striking yet paradoxical phenotypes. Our findings underscore the value of gene targeting strategies for understanding nervous system disorders.

420.2

A DEFECTIVE HERPES SIMPLEX VIRUS VECTOR EXPRESSING THE GLUCOSE TRANSPORTER GENE PROTECTS NEURONS FROM METABOLIC DECLINE AND CELL DEATH DURING GLUCOSE DEPRIVATION. D. Y. Ho*, M. S. Lawrence and R. M. Sapolsky. Dept. of Bio. Sciences, Stanford Univ., Stanford, CA 94305.

Defective herpes simplex virus (HSV) vector system can be used for gene transfer into neurons. We have previously shown that vIE1GT, a HSV vector carrying the glucose transporter (GT) gene (Glut-1 isoform), can deliver the GT gene into various cell types in vitro (including rat hippocampal neurons and astrocytes), and into the rat hippocamus in vivo. Furthermore, GT gene delivery is associated with enhanced GT expression and glucose uptake. In this study, we investigate the effects of vIE1GT infection on cell survivorship and metabolism during glucose deprivation. Ten day-old mixed hippocampal cultures were maintained with various glucose concentrations and surviving neurons were identified by immunocytochemical staining against MAP-2 antigen. Significantly more neurons survived low glucose conditions in vIE1GT-infected cultures as compared to cultures infected with vIE1bgal, a similar HSV vector carrying the E. coli lacZ gene in place of GT. Neuronal metabolic response to varied glucose levels was measured by the use of a microphysiometer. Five day-old mixed hippocampal cultures were maintained in DMEM containing 20 mM glucose, and their metabolic rates were measured. When the glucose concentration was dropped to 0.2 mM, a concomitant decrease of metabolic rate was observed within 5 minutes. vIE1GT infection significantly lessened the decrease in metabolic rate as compared to vIE1bgal-infected or uninfected controls. Thus, the increased glucose transport resulting from vIE1GT infection can protect neurons from the decline in metabolism and the death that follows glucose deprivation.

420.3

VIRAL VECTOR SYSTEMS--NEUROTOXICITY OF THEIR COMPONENTS Sheri L. Fink, Matthew S. Lawrence, Dora Y. Ho, Jeremy R. Tompkins* and Robert M. Sapolsky Dept. of Biological Sciences, Stanford University, Stanford, CA 94305

Viral vectors can be used to import genes into neurons, with the potential to protect against neurodegenerative insults. Reports using such vectors generally note no cytopathicity, often citing as evidence the lack of behavioral changes in subjects, or lack of ventricular enlargement. However, we have investigated the cytopathic effects of components of viral preparations in greater detail, and find evidence for cytopathicity.

In Experiments I and II, one of the following was infused unilaterally into the hippocampus or striatum: PBS; a herpes simplex virus type 1 (HSV-1) deletion mutant [*dl*120, lacking an essential immediate early protein, ICP4; at low (2.5x106 PFU/ul) and high (5.0X107 PFU/ul) titer]; an HSV-1 temperature sensitive virus (ts756, with a mutation in ICP4, minimally permissive at 37 C; same titers); and debris from the E5 and Vero cells used to propagate viral amplicons. In Experiment II, one day after these manipulations rats were microinjected with kainate (.33 nmol in the hippocampus) or quinolinic acid (7.9 nmol in the striatum). Rats were killed five days later and neurons were counted to determine whether any of these components caused loss of neurons (Exp. I), or increased the damage caused by excitotoxin injection (Exp. II). We also studied the effects of the vector system components on neuronal survival in hippocampal culture.

ts756 at high titer caused neuron loss in CA4 and dentate gyrus near the injection site. Cell debris caused cell loss in dentate gyrus only and *dl*120 at high titer lead to near significant cell loss in CA1 and CA4. There was no striatal damage, or worsening of exitotoxic damage in either area. Both ts756 and *dl*120, at MOI's > 1, caused neuron loss in vitro, as did cell debris and cell supernatent. Thus, caution should be given in selecting the mutant viral vector and its titer; experimental and control vectors should be matched for titer, and viral preparations should be devoid of contaminating cell debris and supernatant.

420.4

OVEREXPRESSION OF THE GLUCOSE TRANSPORTER GENE WITH A HERPES VIRAL VECTOR PROTECTS AGAINST A HIPPOCAMPAL EXCITOTOXIC INSULT IN VIVO. M. S. Lawrence, D. Y. Ho and R. Sapolsky*. Dept. of Biological Sciences, Stanford University, Stanford, CA 94305.

Due to their postmitotic nature, neurons are not addressable by conventional gene therapy techniques. The use of a defective herpes simplex virus (HSV) offers an attractive alternative. HSV is both neurotropic and capable of packaging large amounts of DNA for delivery to CNS neurons. We have generated a herpes simplex vector, designated vα4GT, bearing the rat brain glucose transporter gene (GT) under the control of the HSV α4 promoter. In previous studies microinfusion of such vectors into the rat hippocampus results in localized overexpression of the glucose transporter as assessed by *in situ* hybridization, and enhanced [^{14}C]2-deoxyglucose uptake (Ho, et al., PNAS, in press). When vα4GT was microinfused into the hippocampus 12 hours before a microinfusion of the excitotoxin, kainate, at the same location, cell damage (as quantified by size of lesion) was reduced by 27% relative to a contralateral uninfected cell field also exposed to kainate. Under varied glucose regimes, vα4GT was most protective against kainate in hypoglycemic rats, less so in normoglycemic rats and least in hyperglycemic rats. Control rats receiving unilateral infusions of a ß-galactosidase-bearing HSV vector demonstrated no reduction in kainate-induced damage. These results suggest that metabolic distress is an important contributor to the neuropathology of excitotoxic damage in the hippocampus. From a broader therapeutic perspective, they suggest that HSV vectors offer a promising means of delivering neuroprotective genes to the CNS.

420.5

ELEVATED BDNF mRNA EXPRESSION IN THE HIPPOCAMPUS OF AN EPILEPTIC MUTANT MOUSE, STARGAZER *X. Qiao* and J.L. Noebels.* Developmental Neurogenetics Laboratory, Section of Neurophysiology, Department of Neurology and Division of Neuroscience, Baylor College of Medicine, Houston, TX 77030.

We have previously discovered hippocampal mossy fiber sprouting in the inner molecular layer of the dentate gyrus and in the stratum pyramidale of the CA3 region associated with a selective reduction of the hilar cell population following the onset of inherited spike-wave seizures in the mutant mouse *stargazer* (*stg*). To test the hypothesis that the neurotrophic factor BDNF may participate in the mechanisms underlying granule cell synaptic reorganization, *in situ* hybridization of BDNF ^{35}S-cRNA was performed in +/+ and *stg/stg* mouse brain at different ages. The overall pattern of the labelled neurons in +/+ mouse CNS is very similar to that described in rat brain. At 4 weeks of age (10 days after the seizure onset), *stg/stg* mice express higher levels of BDNF mRNA in the hippocampal CA3 pyramidal cells and dentate granule cells than those observed in the control mice. However, in adults, there is no obvious difference in the cellular distribution of BDNF mRNA between the two genotypes. Compared to other acutely-induced convulsive seizure models, the increase of BDNF mRNA in the *stg* mutant with chronic non-convulsive seizures is less intense. These initial results indicate that BDNF mRNA upregulation is associated at an early stage with spike-wave seizure activity and subsequent mossy fiber sprouting in the hippocampus.

420.6

IMMEDIATE-EARLY GENE PROTEIN EXPRESSION IN A MUTANT MOUSE MODEL OF SPIKE-WAVE EPILEPSY, STARGAZER W.K. Nahm , J.L. Noebels* Developmental Neurogenetics Laboratory, Dept. of Neurology, Div. of Neuroscience, Baylor College of Medicine, Houston, TX 77030.

Abnormal patterns of depolarization and bursting enhance the transcriptional activation of immediate-early genes (IEGs) in neurons. To determine whether the IEG protein expression profile differs with the absolute pattern of synaptic synchronization, we compared the nuclear staining pattern of polyclonal antibodies to Fos and Jun induced by kainic acid to that expressed in a genetic model of generalized non-convulsive epilepsy that shows frequent (mean 130/hour) intermittent bursts of 6/sec spike-waves. Two hours after seizures induced by kainic acid (30mg/kg i.p.), +/+ mice show intense Fos staining in all hippocampal subregions and in neocortex, entorhinal cortex, thalamus, and septum. In contrast, *stg/stg* and +/+ control brains showed no hippocampal Fos staining. To exclude the possibility that spontaneous seizures were too infrequent to elicit IEG proteins, we examined *stg/stg* mice treated with CPP (40μM/kg) that displayed a continuous pattern of spike-wave bursting for 1-2hrs. CPP treated *stg/stg* mice showed IEG protein staining patterns identical to untreated *stg/stg*. To determine whether refractory mechanisms contributed to the lack of IEG protein response in the mutants, their chronic seizures were blocked for 30 hours with ethosuximide, followed by immunohistochemistry 2 hours after seizures returned. There was no consistent difference compared to untreated *stg/stg* brains. Additionally, kainic acid induced strong IEG protein expression in *stg/stg* mice. These experiments show clear differences in IEG protein expression between two distinct patterns of abnormal neuronal synchronization, and demonstrate that the two seizure types differentially regulate neuronal gene expression in the brain. Since IEG protein expression is dependent on free intracellular Ca^{2+} levels, these data may reflect underlying differences in Ca^{2+} mobilization and Ca^{2+}-activated mechanisms between convulsive and spike-wave seizures.

420.7

***IN VITRO* ELECTROPHYSIOLOGY OF SPONTANEOUS AND INDUCED EPILEPTIFORM DISCHARGES REVEALS INCREASED CORTICAL EXCITABILITY IN THE MUTANT MOUSE, *STARGAZER*.** K.D.Keegan* & J.L.Noebels, Developmental Neurogenetics Laboratory, Dept. of Neurology, Div. of Neuroscience, Baylor College of Medicine, Houston, TX 77030.

Extra- and intracellular current-clamp recordings were performed in the hippocampus, thalamus and cortex of adult *stargazer* mice (*stg/stg*) and their coisogenic controls (+/+). The aim of this study was to investigate the excitability defect underlying the generation of inherited spike-wave discharges in mice homozygous at the *stg* locus. Field recordings from oblique hemisected thalamo-cortical brain slices maintained at 32° C in normal ACSF [3.5mM K^+] revealed the presence of low frequency (≤ 1/min) abnormal spontaneous network discharges with an irregular rhythm in all layers of the mutant cortex (8/10 mice) but not in +/+ cortex (0/33). Bursting was present in cingulate, neo, and prepyriform cortices. Intracellular record-ings from layer 4-5 neurons showed a giant epsp (PDS) during each discharge. No spontaneous field activity was observed in other regions of the mutant slice. In 0 Mg^{++} saline, similar field discharges and associated PDS's were observed in the cortex and hippocampus of both +/+ and *stg/stg* slices; the most obvious difference between the genotypes was the less regular burst pattern associated with more pro-nounced afterdischarge activity in the cortex of the mutant. Both spontaneous and 0 Mg^{++} induced cortical bursting persisted following surgical isolation of the *stg/stg* cortex from the remainder of the slice. Bath application of 50μM APV in 0 Mg^{++} produced a 90% reduction in the extracellular burst amplitude in both +/+ and *stg/stg* slices, consistent with the involvement of NMDA receptors. These initial studies point to a diffuse increase in cortical network excitability in the *stargazer* brain.

420.8

LACK OF MOSSY FIBER SPROUTING IN A RODENT MODEL OF PETIT MAL EPILEPSY. A.Kandel*, M.Hsu and G.Buzsáki. CMBN, Rutgers, The State University of New Jersey, Newark, NJ 07102

Generalized non-convulsive epilepsy is believed to result primarily from excessive population oscillation of the thalamocortical system, although neurons of several other systems are recruited during the spike and wave discharges. Several recent works reported on the involvement of the hippocampus and it was suggested that physiological and/or anatomical alterations in the hippocampus are either causally related to the occurrence of spike and wave episodes or that the pathological changes in the hippocampus occur parallel with the development of spike and wave activity.

In this study we sought to quantify the amount of mossy fiber sprouting, using Timm staining, and compare it to the estimated lifetime duration of petit mal seizure activity. The rats in the study represented different animal models for generalized non-convulsive epilepsy (WAG/Rij, F344, and Brown Norway x F344 offspring).

The main finding was that mossy fiber sprouting increased with age but was not correlated with the amount of seizures. The oldest rat (F344: 28 months) had the densest Timm staining in the inner molecular layer of the dentate gyrus but had a relatively low amount of seizures. WAG males (11 months) and BNxF344 females (24 months) had the same amount of Timm staining but the WAG group had significantly more seizures. The older WAG group (11 months) had denser Timm staining than the younger WAG group (8 months) even though both groups had comparable amounts of seizures We conclude that mossy fiber sprouting increases with age in all strains tested but no reliable relationship exists between mossy fiber sprouting and the duration of petit mal seizures.

420.9

THE SPATIAL DISTRIBUTION AND MORPHOLOGICAL CHARACTERISTICS OF SPROUTED MOSSY FIBER AXONS IN THE DENTATE GYRUS OF KAINATE-TREATED RATS P. Zhang, G. Golarai, T. Sutula* The Neuroscience Training Program, and Dept. of Neurology, Univ. of Wisconsin, Madison, WI 53792

The anatomical features and spatial distribution of sprouted mossy fiber collaterals in the dentate gyrus were directly demonstrated by study of the axonal arbors of granule cells individually filled *in vitro* with biocytin, and *in vivo* with the anterograde lectin tracer PHA-L. Sprouted axon collaterals extended from the hilus into the supragranular layer in 10 of 12 biocytin filled granule cells from 3 kainate-treated rats. The sprouted collaterals had small diameters, and formed an extensive supragranular plexus with numerous terminal boutons that were smaller than the giant mossy boutons in the hilus. In 4 of the 10 granules cells, sprouted collaterals crossed the hilus and extended as far as 400 microns to the supragranular layer of the opposite blade. The axonal projection of granule cells filled *in vivo* with PHA-L included a dense plexus of axons in the hilus and CA3, with sprouted collaterals of small diameter which extended into the supragranular layer as far as 600 microns from the injection site along the septotemporal axis. The recurrent circuits formed by sprouted mossy fiber collaterals project along the transverse and septotemporal axes of the dentate gyrus, and possess anatomical features that could have powerful synchronizing effects.

420.10

AXONAL SPROUTING OF ADULT RAT NEOCORTICAL PYRAMIDAL CELLS IN CHRONIC EPILEPTOGENIC LESIONS. P. Salin*, J. Parada, S.N. Hoffman, G.F. Tseng, D.A. Prince, Dpt. of Neurology & Neurological Sciences, Stanford University School of Medicine, Stanford, CA 94305.

Sprouting of mossy fibers is prominent in dentate gyrus of epileptogenic temporal lobe in animals and humans and has been suggested as a mechanism underlying the development of epilepsy. We performed experiments to determine whether similar sprouting occurs in chronic neocortical epileptogenetic lesions. Partially isolated somatomotor neocortical slabs prepared in vivo generated epileptiform discharges when studied 2-6 wks. later in vitro (Prince and Tseng J. Neurophysiol. 69:1276, 1993). 39 layer V pyramids were labeled intracellularly with biocytin in slices of injured epileptogenic cortex and 52 cells from the same cortical area and lamina in unlesioned cortex served as controls. 43 of the most completely filled neurons (17 injured and 26 control) were reconstructed and analyzed. Somata of injured cells were significantly smaller (200 ±15.5μm^2 SEM) than those of controls (266 ±19.8 μm^2, $p<0.025$). Axonal aborizations revealed several features suggesting significant reorganization: 1) In a subgroup of cells (6 injured and 7 control) the number of axon collaterals was significantly higher in epileptogenic slices (59 ±1.5) than in control (35 ±4.3, $p<0.02$). 2) The total axonal length was increased in neurons of injured slices (5.58 ±1.3mm) versus control (3.26 ±0.5mm, $p<0.05$). 3) The number of boutons was also significantly increased in injured cells (1090 ±281) versus control (459 ±83, $p<0.05$). 4) The area of distribution of boutons was larger in lesioned slices (0.313 ±0.08mm^2) than in control (0.156 ±0.03mm^2), suggesting that pyramids in injured cortex may contact more distant neurons than in control cortex. These results suggest that sprouting of axon collaterals occurs in adult neocortical neurons after injury. If such reorganization provides enhanced functional recurrent excitation, it would be a powerful factor in development of post-traumatic neocortical epileptogenesis. The relative contribution of axotomy, deafferentation and epileptic discharges per se to this anatomical remodeling remains to be explored. Supported by NIH grant NS12151 from the NINDS, the Morris Research Fund and a Pimley Fellowship.

420.11

MORPHOMETRIC AND ELECTROPHYSIOLOGICAL ANALYSIS OF HIPPOCAMPUS IN RATS WITH LONG-TERM PILOCARPINE SEIZURES T.Nagao, Z.Liu, C.Desjardins, M.Avoli, and P.Gloor*. MNI and McGill University, Montreal, QC, Canada H3A 2B4

Systemic administration of pilocarpine (380 mg/kg, i.p.) to adult male Sprague-Dawley rats induced an acute, prolonged state of seizures (status epilepticus) lasting several hours followed by complete neurological recovery. Spontaneous recurrent seizures of shorter duration appeared 2-2.5 weeks later and continued up to the sacrifice of the animals. Cell loss in the dorsal hippocampus was measured by an unbiased stereological technique. In areas CA1 and CA3, significant neuronal loss was observed 3 weeks after pilocarpine injection. However no progressive neuronal loss was detected when cell numbers were compared 6 and 12 weeks after pilocarpine injection after the rats had displayed recurrent seizures. There was no neuronal loss in the granule cell layer of the dentate gyrus. We used extracellular field potential recordings to analyze the spontaneous activity generated by hippocampal slices obtained from these rats during perfusion with 4-aminopyridine (4AP, 50 μM). The rate of occurrence of interictal discharges induced by 4AP was significantly lower in rats that had received pilocarpine 3 and 12 weeks earlier than in normal rats. Cutting the connections between CA1 and CA3 caused interictal discharges in CA1 area to disappear in both normal and 3 weeks post-pilocarpine rats. By contrast, 12 weeks after pilocarpine, there were interictal discharges in CA1 following the cut, though at a lower rate than before.

Our data indicate that the neuronal loss in CA1 and CA3 areas primarily results from the initial status epilepticus and that further recurrent chronic seizures do not produce any additional cell loss in these sectors. However, our electrophysiological data suggest that some neuronal network rearrangement might occur during the period of chronic recurrent seizures.

420.12

EPILEPTIFORM ACTIVITY IN MODEL OF PARTIAL EPILEPSY IN RAT PIRIFORM CORTEX SPREADS TO HIPPOCAMPAL, AMYGDALOID, AND ENTORHINAL CORTEX. K.L.Ketchum* and L.B.Haberly. Dept. of Anatomy, Univ. of Wisc., Madison, WI 53706.

An anesthetized preparation has been developed that reproduces features of partial (focal) epilepsy including a slow spreading recruitment of adjacent cortex into the generation of epileptiform activity and self-sustaining electrographic seizures (Ketchum & Haberly, Soc. Neur. Abs. 18:910). In this model shocks to afferent fibers pace interictal events in a disinhibited focus created by picrotoxin injection into ant. piriform cortex (PC) of urethane anesthetized rats. At 1 Hz a large stereotyped epileptiform response develops in successive trials which spreads over the full extent of PC at a low rate (~0.001 m/s). Previous study (Ketchum & Haberly, Soc. Neur. Abs. 15:1033) suggests that, after this induction phenomenon, PC outside the disinhibited focus becomes involved in self-regenerative activity mediated by intrinsic associational fibers. It is proposed that this induction process is involved in the generalization of seizure activity evoked by injection of picomolar quantities of convulsant drugs into the deep part of ant. PC in unanesthetized rats (Piredda & Gale, Nature 317:623). If the induction phenomenon has this role then it must spread to other cortical areas to which the PC projects by mono- or multi-synaptic pathways. Simultaneous multiple site recordings revealed that the induction process continued into amygdaloid and entorhinal cortex and subsequently reached the CA1 region of the hippocampus. By manipulating the stimulation rate these areas could be made to repeatedly induce and recover during a single focal application of picrotoxin within the PC. Large abnormal potentials that were time-locked to epileptiform events in PC were also recorded in neocortex. These results indicate that the induction phenomenon can continue into cortical regions with direct projections from the PC (entorhinal and amygdaloid cortex) and in the case of hippocampus, an area that is polysynaptically removed. Supported by NINDS grant NS19865 to LBH and NRSA grant NS08328 to KLK.

420.13

THE ROLE OF GLUTAMATE RECEPTOR SUBTYPES IN THE INDUCTION AND PROPAGATION OF SEIZURE ACTIVITY IN THE HIPPOCAMPUS AND OLFACTORY CORTEX OF THE GUINEA-PIG ISOLATED WHOLE BRAIN. P. Federico* and B.A. MacVicar. Neuroscience Res Group, Univ of Calgary, CANADA T2N 4N1.

Unilateral stimulation of the lateral entorhinal cortex (5 or 10 Hz; 5 or 10 sec) of the guinea-pig isolated whole brain evokes tonic-clonic-like seizure activity that can be recorded bilaterally for 1 min in the ventral hippocampus and entorhinal cortex using electrical and intrinsic imaging techniques (measurements of reflectance changes at 450 nm; $\Delta R/R_f$=4-10 %). This seizure activity propagates consistently to the posteromedial cortical amygdaloid nucleus. Since glutamate is the main excitatory neurotransmitter of hippocampal and entorhinal afferents, we examined the role of glutamate receptor subtypes in the induction and/or propagation of this seizure activity. When the non-NMDA receptor antagonist CNQX (20 μM) or the metabotropic receptor agonist trans-ACPD (150 μM) were added to the brain perfusate for 10-20 min, all seizure activity measured optically or electrically was completely or significantly suppressed. Perfusion of the NMDA receptor antagonists MK-801 (20 uM), AP-5 (100 μM), or AP-7 (200 μM), on the other hand, did not affect the electrophysiological or optical recordings of the seizure activity. These results suggest that activation of non-NMDA receptors is necessary for the generation of tonic-clonic seizure activity in the hippocampus/entorhinal cortex and that activation of metabotropic receptors depresses seizure activity. Activation of NMDA receptors, however, does not have a role in the induction or propagation of this seizure activity. Supported by MRC (Canada).

420.14

MULTIFOCAL SPONTANEOUS EPILEPTIC ACTIVITY IN THE LIMBIC SYSTEM OF THE ISOLATED IN VITRO GUINEA PIG BRAIN. Marco de Curtis,* Gerardo Biella, Matteo Forti, Ferruccio Panzica. Dept. Neurophysiology, Ist. Nazionale Neurologico, Milano, Italy.

An acute model of partial epilepsy (Piredda and Gale, Nature, 317:623, 1985; Ketchum and Haberly, Soc. Neurosci. Abstr. 378:12, 1992) has been utilized to study spontaneous epileptiform activity in the olfactory cortex of the in vitro isolated guinea pig brain (de Curtis, Pare' and Llinas, Hippocampus, 1:341, 1991). Local bicuculline applications at the anterior piriform cortex (APC) induced an increase in neuronal excitability restricted to the site of ejection. The APC epileptogenic focus generated spontaneous "interictal" epileptic potentials, while prolonged self-sustained "ictal" discharges were never observed. Current source density analysis of the spontaneous events recorded over the cortical depth at different antero-posterior sites in the piriform cortex demonstrated that epileptic potentials propagated from the APC focus throughout the piriform lobe along associative fibers. Secondary foci of spontaneous epileptiform activity were observed in the posterior piriform cortex within one hour from the APC bicuculline ejection. The independence of the secondary foci from the primary APC focus was demonstrated after surgical isolation of the latter. Propagated spontaneous and evoked epileptiform activities were abolished at the site of the isolated primary cortical focus after cutting LOT and associative fibers, while spontaneous epileptiform potentials persisted in secondary foci.

420.15

PROPAGATING EPILEPTIC AFTERDISCHARGES IN THE DISINHIBITED HIPPOCAMPAL SLICE R.D. Traub*, J.G.R. Jefferys & R. Miles. IBM Watson Res. Ctr., Yorktown Heights, NY 10598, St. Mary's Hospital Med. Sch., London W2 1PG, U.K. and Institut Pasteur, Paris 15, France.

A model has been proposed for the generation of synchronized afterdischarges (ADs) during $GABA_A$ blockade (Traub, Miles & Jefferys, *J. Physiol.* 461: 525-547, 1993): a population burst develops via spread of firing along recurrent excitatory connections; NMDA currents then elicit dendritic oscillations to give a train of 2° bursts. Phasic AMPA inputs maintain the synchrony of the bursts. Both initial bursts and later bursts are known to propagate along slices at about 0.15 m/s, slower than axon velocity, and propagation of the initial burst appears to depend on the spatial restriction of recurrent axons (Knowles et al., *Neuroscience* 21: 441-455, 1987; Miles et al., *J. Neurophysiol.* 60: 1481-1496 , 1988). Our model of the temporal pattern of ADs predicts that the spatial pattern of 2° bursts can behave differently than for the initial burst, because the dendrites are depolarized during the former. We tested this in a model of 8,000 compartmental neurons, with statistically localized interconnections containing AMPA and NMDA receptors. Under uniform baseline conditions, initial and 2° bursts propagated with the same pattern at about the same velocity, as in Knowles in al. Other observed patterns were replicated by changing the conductance of NMDA synapses or by introducing a gradient in some synaptic property (NMDA or AMPA conductance, connection density): the failure of 2° bursts to propagate along the whole array, the existence of a preferred direction of propagation for 2° bursts, or propagation of 2° bursts in different directions during the same afterdischarge. In all cases, the initial burst propagated uniformly away from the stimulus, as in experiments. In conclusion, our model of afterdischarges in the disinhibited slice predicts the voltage pattern in time and in space.

420.16

OPTICAL RECORDINGS REVEAL THAT EPILEPTIFORM ACTIVITY IN NEOCORTICAL SLICES SPREADS PREFERENTIALLY THROUGH UPPER CORTICAL LAYERS. B. Albowitz and U. Kuhnt*. Dept. of Neurobiol., Max-Planck-Inst. Biophys. Chem., 37018 Göttingen, FRG.

A characteristic feature of epileptiform activity is the spread of synchronous discharge across large areas of neuronal tissue. The pathways responsible for horizontal spread in the neocortex were investigated in sensory neocortical slices by use of an optical recording technique. Coronal slices (350µm) from sensory neocortex of guinea pigs were stained with the fluorescence voltage-sensitive dye RH795. Epileptiform activity was induced in a bath medium containing 20µM bicuculline by single pulse electrical stimulation of the white matter or layer I. Voltage dependent fluorescence changes were monitored by a 10x10 photodiode array. The system provided a spatial resolution of 94µm and a temporal resolution of 0.4ms. Two vertical cuts (parallel to the axis of pyramidal neurones), one sectioning upper layers (I-IV), the other lower layers (V, VI, and the white matter), were made at different positions in the slice.

In intact slices, the latency of epileptiform potentials at specific medio-lateral positions was always shortest in upper cortical layers as compared to lower layers if epileptiform activity was evoked from layer I. If activity was evoked from the white matter, no consistent pattern was found. In sectioned slices, regardless of the point of stimulation, the pattern of spread was not significantly different from that in intact slices if epileptiform activity had to spread across a section through lower cortical layers. However, epileptiform potentials either did not at all spread past a cut through upper cortical layers or spread with significant jumps in latency.

The detailed analysis of spatio-temporal activity patterns in intact and sectioned slices revealed that epileptiform potentials spread to a certain degree independently through upper and lower cortical layers. However, the pathway through upper cortical layers appears to be more powerful and usually predominates.

420.17

MULTI-SITE OPTICAL RECORDINGS OF EPILEPTIFORM BURSTS IN ORGANOTYPIC CULTURES OF RAT CEREBRAL CORTEX. T.S. Donta* and J.A. London. Center for Neurological Sciences, and the Dept. of Biostructure and Function, The Univ. of Connecticut Health Center, Farmington, CT 06030.

In an epileptic seizure, groups of cortical neurons that are normally independently active fire in synchronous bursts. Voltage-sensitive dyes and a 122-element photodiode array were used to record the spread of this excitation across cortical layers in organotypic cultures of rat brain slices. Long-Evans hooded rat pups were anesthetized by hypothermia and their brains removed for sectioning in either the transverse, sagittal, or horizontal planes. Sections (300 μ thick) were transferred onto tissue culture membrane inserts (Millipore) in 6-well dishes containing 1 ml of media, and incubated at 37°C. Cultures were used for recording after 2 weeks to 2 months *in vitro*. The fluorescent styrl dye RH795 (Molecular Probes) was applied to the cultures at 0.2 mg/ml for 1 hour. Optical signals have been recorded after application of bicuculline methiodide, a GABA antagonist that elicits synchronous, epileptiform bursting. Bursting was observed to travel from the deep to the shallow cortical layers, and from the agranular cortex to the granular cortex at speeds of approximately 0.2 m/s. Ongoing studies will analyze seizure origin and spread with greater spatial and temporal detail.

This work was supported by a Klingenstein Fellowship for Neuroscience and USPH Grant 2P01-NS16993-09.

420.18

MULTIPLE PACEMAKERS IN THE CA2-CA3 REGION OF THE GUINEA PIG HIPPOCAMPUS INITIATE EPILEPTIFORM ACTIVITY. L.V. Colom* and P. Saggau. Division of Neuroscience, Baylor College of Medicine, Houston, TX 77030.

Groups of neurons generating abnormal bursts of action potentials can act as pacemakers for interictal-like epileptiform activity. The localization of such aggregates is critical for understanding the origin and spread of epileptiform discharges. Voltage-sensitive dyes and fast multi-site optical recording techniques were used to monitor spatiotemporal patterns of spontaneous epileptiform activity in transverse guinea pig hippocampal slices. Three *in vitro* animal models of epilepsy were studied: 1) high bath potassium concentration (8 mM K^+), 2) 4-aminopyridine (100 µM 4-AP), and 3) the $GABA_A$ antagonists bicuculline and picrotoxin (20 µM BIC, 50 µM PTX). When BIC-PTX was employed, discharges consistently started in the CA2-CA3a region. However, with high K^+ or 4-AP, activity originated in both the CA2-CA3a and CA3c region. Surgical lesions separating these regions demonstrated that both can act as independent pacemakers, showing spontaneous discharges of different frequencies. The slower pacemaker discharged more frequently when separated from the faster. In the CA2-CA3a region, two independent pacemakers were occasionally observed. Although BIC-PTX only generated discharges in the CA2-CA3a region, a subsequent increase in K^+ resulted in additional discharges in the CA3c region, revealing a latent pacemaker. In summary, we demonstrated: 1) the existence of multiple pacemaker areas capable of generating epileptiform discharges in the guinea pig hippocampus, 2) interaction between those pacemakers, and 3) the expression of such pacemakers depending on the epilepsy model used. Supported by a grant of the Cain Foundation to P. Saggau.

420.19

OPTICAL IMAGING OF CARBACHOL-INDUCED SPONTANEOUS EPILEPTIFORM OSCILLATIONS IN GUINEA PIG HIPPOCAMPAL BRAIN SLICES. S.S. Patel*, L.V. Colom and P. Saggau. Division of Neuroscience, Baylor College of Medicine, Houston, TX 77030.

Cholinergic epileptiform activity in hippocampal brain slices frequently shows oscillations in the theta frequency range. Spatio-temporal characteristics of spontaneous oscillatory activity induced by carbamylcholine chloride (carbachol, 50 μM) were studied. Voltage-sensitive dyes and fast multi-site optical recording techniques were used to monitor these events in transverse guinea pig hippocampal slices. A versatile PC-based optical workstation was developed, to handle multiple photodetectors of different spatio-temporal resolution, providing high-speed data acquisition, processing and visualizing of neural activity in a windows-based graphical environment. Here, a 10x10 photodiode array coupled to a low-noise multichannel amplifier unit together with a CCD camera was used. During experiments, neural activity was displayed as a matrix of temporal traces overlaid on a video image of the preparation, allowing one to form spatially averaged functional or anatomical clusters. Spatio-temporal dynamics of activity were examined by viewing continuous sequences of interpolated high resolution images (200x200 pixels). Epileptiform discharges lasting 100 ms to several seconds were recorded. Such activity originated at multiple sites within the CA2-CA3 region. Oscillatory activity with an average frequency of 9.06±2.88 Hz was observed in 44% of the cases. The degree of synchrony of such discharges differed among hippocampal regions. Typically, the CA2-CA3a region showed pronounced oscillations, while area CA3c was less synchronized. A computational neural network is being developed to model the spatio-temporal system dynamics.

420.20

METABOTROPIC GLUTAMATE RECEPTORS MODULATE AND PRODUCE EPILEPTIFORM ACTIVITY IN THE HIPPOCAMPUS. P.A. Rutecki* and Y. Yang. Dept. of Neuro., Neurosci. Training Prog., U. of Wisc., Madison, WI 53792.

Depending on the preparation, activation of glutamate metabotropic receptors by *cis*-(±)-1-aminocyclopentane-1,3-dicarboxylic acid (ACPD) results in stimulation of different second messenger systems and modulation of various ion channels. We studied the effect of ACPD on epileptiform activity produced by 4-aminopyridine (4-AP, 25 μM in 2.5 mM $[K^+]_o$) or bicuculline methiodide (BMI, 10μM in 2.5 or 5 mM $[K^+]_o$) in hippocampal slices prepared from adult rats. Using extracellular recording techniques, the rate of spontaneously occurring epileptiform discharges in the CA3 region of the hippocampus was monitored before and after bath application of ACPD. At 30 μM, ACPD increased the BMI-induced burst rate by 87% (0.15±0.02 vs 0.28±0.02 Hz) and increased the 4-AP-induced burst rate by 44% (0.42±0.04 vs 0.59±0.05 Hz). At 100 μM, ACPD resulted in a 390% increase in rate for BMI (0.59±0.09 Hz) and 83% increase for 4-AP-induced discharges (0.77±0.07 Hz). The discharge rate returned to control values after a 20-30 min wash-out. The acceleration of BMI-induced bursting was not blocked by 50 μM D,L-APV or 1μM timolol. In the absence of convulsants, 100 μM ACPD generated spontaneously occurring epileptiform discharges in the CA3 region of the hippocampus that did not readily wash out. The acceleration of epileptiform discharge rate is consistent with a decrease in potassium currents, a recognized effect of ACPD on CA3 neurons. ACPD-induced epileptiform activity may result from blockade of potassium channels and a reduction in $GABA_A$ inhibition. Supported by the NIH and Veterans Administration.

DEGENERATIVE DISEASE: ALZHEIMER'S— β-AMYLOID VII

421.1

CLONING OF A PROTEIN THAT BINDS TO A RECOGNITION SEQUENCE IN THE APP PROMOTER. A.A. Vostrov[1], W.W. Quitschke[1]*, A.L. Schwarzman[1], A. Blangy[2], F. Cuzin[2], U.V. Wesley[1], N.G. Hagag[1], D. Goldgaber[1]. [1]School of Medicine, SUNY at Stony Brook, Stony Brook, NY 11794-8101. [2]Unite 273 de l'INSERM, Universite de Nice Sophia Antipolis, 06108, Nice FRANCE.

There is a very strong similarity between a specific DNA recognition sequence in the proximal human APP promoter (Quitschke and Goldgaber, J. Bio. Chem., 267:17362;1992) and the murine CDEI-like element. Recently, a murine DNA binding protein that recognizes the murine CDEI-like sequence was cloned (Vidal et al., BBRC, 189:1336;1992). This protein showed extensive local similarities to APP.

Oligonucleotides that contained the APP promoter element and oligonucleotides that contained the murine CDEI-like element were analyzed by a mobility shift assay. Each oligonucleotide successfully competed with the other but not with the control altered oligonucleotides. We found that the cloned murine DNA binding protein that binds to the murine CDEI-like element also binds to the APP promoter element.

Using the mouse cDNA clone as a probe, we identified and sequenced several clones from the human brain cDNA library. Comparison of the human and murine sequences showed in a very high level of conservation. Work is currently in progress to determine the chromosomal localization of the human gene which codes for this DNA binding protein.

421.2

REGULATION OF THE PROMOTER ACTIVITY OF THE APP GENE IN PC12 CELLS BY GROWTH FACTORS AND RETINOIC ACID. D. K. Lahiri* and C.Nall. Lab of Molecular Neurogenetics, Institute of Psychiatric Research, Indiana University School of Medicine, Indianapolis, IN-46202

Abnormalities in gene regulation of ß-amyloid precursor proteins (APP) might be an important factor in the neuropathology of Alzheimer's disease We have studied the effects of nerve growth factor (NGF), basic fibroblast growth factor (bFGF) and all trans-retinoic acid (RA) on the promoter activity of the APP gene. Recombinant plasmid containing a 1.2 kb fragment of the promoter part of the APP gene linked to a reporter gene, chloramphenicol acetyl transferase (CAT), was constructed. Then the promoter activity of the APP gene was tested by transient expression in PC12 cells. The transfection of DNA into cells was done by the electroporation method which minimizes and in some experimental sets eliminate the variation of transfection efficiency among different plates. We have set up an efficient procedure for transfection in differentiated cells. Transfection of DNA was done into cells which were part of three types of treatment groups. The first group was pretreated with factors, a second group was treated with factors only after transfection and a third set was treated with factors before and after transfection. Transfected cells were assayed for the CAT activity at various days of post transfection.

We have found that the promoter activity was increased when cells were treated with either NGF or bFGF and the promoter activity decreased when cells were treated with RA. The degree of increase or decrease of the promoter activity depends on the number of days of treatment with the factors prior to transfection. The promoter activity was also found to be dependent on the duration of treatment with the factors after cells were transfected. Our results suggest a possible participation of a growth-factor (s) mediated transcription element in the regulation of the gene expression of APP. Supported by the N.I.H. grant PHS R01 AG10297-01A1.

421.3

APP mRNA ISOFORMS WITHOUT EXON 15 (L-APP mRNA) ARE UBIQUITOUSLY EXPRESSED IN RAT TISSUES INCLUDING BRAIN, BUT NOT IN NEURONS. R. Sandbrink, C. L. Masters and K. Beyreuther.* Center for Molecular Biology Heidelberg, Univ. of Heidelberg, Germany.

The ßA4-amyloid protein precursor (APP), a glycoprotein with a single transmembrane spanning domain, is the source of ßA4-amyloid, which is deposited in the brains of individuals with Alzheimer´s disease. APP constitutes a family of different isoforms that are generated by alternative splicing of the 19 exons encoding APP gene. While exon 7 and exon 8 are well known to be alternatively spliced, APP mRNA isoforms without exon 15 were only recently identified in leukocytes and rat brain microglial cells and therefore denoted as L-APP mRNA. Since a detailed analysis of the individual L-APP mRNA isoforms has not been performed yet, we designed a quantitative polymerase chain reaction from reverse transcribed RNA allowing us to analyze the complete region between exon 6 and 16 of the APP gene. This strategy enabled us to distinguish between most of the APP splice isoforms generated by alternative splicing of exons 7, 8 and 15. To answer the open question of L-APP expression *in vivo*, we then applied this method to perfused rat tissues. In all peripheral tissues examined, L-APP mRNA isoforms were detected comprising between 25% (skeletal muscle) and about 70% (aorta, pancreas) of total APP transcripts. All four possible APP mRNA isoforms lacking exon 15 could be shown to exist, i. e. L-APP752, L-APP733, L-APP696 and L-APP677. L-APP expression in the central nervous system (about 4% of all APP mRNAs in total brain) was then studied in more detail by analyzing different brain regions and tissues and correlated with data from primary cell cultures. The only cell type, which was shown not to express L-APP mRNA to a detectable level, is the neuronal cell. Therefore, we suggest that the functional consequence of L-APP mRNA expression may be studied in neurons.

421.4

RELATION BETWEEN β/A4 SECRETION AND A 12 kDa COOH-TERMINAL DERIVATIVE OF THE ALZHEIMER'S AMYLOID PRECURSOR PROTEIN. J.T. Durkin*, S. Murthy, S. Mistretta, E.K. Hoffman, T. Levins, B.D. Greenberg, R.W. Scott, and R. Siman. Cephalon, Inc., 145 Brandywine Parkway, West Chester, PA 19380.

The amyloid protein of Alzheimer's disease, β/A4, is produced by one of several possible pathways for processing the β-amyloid precursor protein (APP). Among the COOH-terminal derivatives of APP (CTDs) is a 12 kDa derivative that co-migrates with APP(652-751) (numbered after APP751), and therefore likely includes the intact β/A4 domain (see also Cheung et al., 1992, *Soc. Neurosci. Abstr.*, **18**(1), 764). Only the 12 kDa CTD, and not larger or smaller derivatives, is recognized on immunoblots by an antibody raised against β(1-9). The antibody likely recognizes its epitope only with a free NH_2-terminus, suggesting that the 12 kDa CTD begins precisely at β/A4. We have shown by metabolic labelling and immunoprecipitation that different clones of 293 cells stably overexpressing APP751 secrete different amounts of β/A4 protein. Immunoprecipitation of cell lysates shows that the clone secreting more β/A4 protein also accumulates more 12 kDa CTD at steady-state. The same correlation holds for clones of CHO cells stably overexpressing APP751:K651N/M652L, the double mutation associated with Alzheimer's disease in a Swedish family. The correlation, coupled with the antibody labelling suggesting that β/A4 protein and the 12 kDa CTD have the same NH_2-terminus, suggests that the 12 kDa CTD may be an intermediate in the production of β/A4 protein.

421.5

DECREASED SOLUBLE AMYLOID PRECURSOR PROTEIN IN FAMILIAL ALZHEIMER'S BRAIN(APP717 VAL-ILE MUTATION) N.Nukina*, K.Hashimoto, I.Kanazawa and H.Mizusawa Department of Neurology, University of Tokyo, Tokyo,Japan 113

The amyloid core in senile plaques of Alzheimer's disease is composed of beta protein. Recent discovery of familial Alzheimer's diseases which are linked to APP mutations suggests that the beta protein accumulation is a primary pathology. It is important to clarify whether the processing of APP in those cases with the mutation is different from the processing in other cases. In this study we investigated the APP and beta protein amount in AD with mutation using western blot.

Antibodies used in this study were the following:monoclonal antibody against beta protein (A61) which reacts with beta 1-17,monoclonal antibody 22C11(Boehringer Mannheim), polyclonal antibodies aginst APP synthetic peptides 666-695(anti-C). Tissue from 2 cases with the mutation(APP717 Val-Ile) , 4 sporadic casese and 4 control casese were used.

Tris Saline soluble fraction, Triton soluble fraction, SDS soluble fraction and SDS insoluble fractions were examined by ECL western blot kit(Amarsham) using antibodies. We could not detect specific bands in AD with mutation. The amounts of beta protein in SDS insoluble fractions and membrane bound APP of AD with mutation were not different from those of sporadic ADs and controls. However APP amount of TS soluble fraction was decreased in AD with mutation. The result suggests that the amount of secretory APP is decreased and there is abnormal metabolism of APP in AD with mutation.

421.6

MUTATIONS ASSOCIATED WITH FAD ALTER THE PROTEOLYTIC PROCESSING OF CYTOSOLIC APP ISOFORMS. A. Weidemann, C. Czech, T. Hartmann, R. Prior*; C. L. Masters° and K. Beyreuther.

Center for Molecular Biology, University of Heidelberg (ZMBH), Im Neuenheimer Feld 282, D-6900 Heidelberg, F.R.Germany. °Department of Pathology, University of Melbourne, Parkville, Victoria 3052, Australia.

The ßA4 protein, which forms amyloid aggregates in Alzheimer's disease is released from its cognate precursor (APP) by at least two proteolytic cleavage events, one in the ectodomain of APP the other within the transmembrane domain. Here we report that initiation of APP translation is not restricted to the first AUG start codon preceding the signal peptide sequence which specifies membrane insertion of APP. To a minor extent, further downstream AUG codons are also used for initiation of translation leading to APP derivatives targeted to the cytosol. The latter are not membrane embedded and are therefore at the putative transmembrane domain accessibel for proteases. The catabolism of these cytoplasmic APP derivatives includes proteolysis around the C-terminus of the ßA4 sequence. Additionally, the catabolism is affected by APP mutations described for hereditary forms of Alzheimer's disease.

421.7

Apolipoprotein E and Alzheimer's β-amyloid fibril formation. Thomas Wisniewski, Adam Golabek, Jorge Ghiso and Blas Frangione.* New York University Medical Center, New York, New York, 10016

Apolipoprotein E (Apo E) and Alzheimer's β-protein (Aβ) show high affinity binding both with Aβ in senile plaques and the normal soluble Aβ (sAβ) found in biological fluids. Apo E is highly expressed in the brain and is the major lipoprotein in the cerebrospinal fluid (CSF), where it is an acute phase reactant. Apo E belongs to a group of amyloid-associated proteins, or "pathological chaperones"; by this we mean a group of biochemically unrelated proteins that mediate fibril formation but are not themselves part of the final fibril. Apo E is coded for by a single gene on chromosome 19 at a locus that has been linked with disease in some familial Alzheimer's disease (AD) families. Nine different Apo E alleles are known. Homozygosity for $\epsilon 4$ is associated with both sporadic and familial AD. When synthetic peptides homologous to sAβ are bound to either nylon membranes or cyanogen bromide activated Sepharose and CSF is passed through, Apo E, as well as another apolipoprotein, Apo J, bind. We show with an assay for amyloid formation in solution that Apo E can promote fibril formation; this was not seen with Apo J under the conditions tested. Therefore Apo E may influence whether Aβ remains in solution or forms an amyloid fibril.

421.8

A REL-RELATED REGULATORY PROTEIN CONTROLS EXPRESSION OF THE AMYLOID PRECURSOR PROTEIN (APP) GENE. M. Grilli*, A. Alberici and P.F. Spano Pharmacology Sec., Dept. of Biomed. Sci. & Biotech., Univ. of Brescia, Italy.

Several reports in the literature suggest the potential role for increased or dysregulated APP gene expression in the pathogenesis of Alzheimer's disease. For this reason it becomes very important to address questions about the participants in the transcriptional control of the APP gene and in particular to identify: i) cis regulatory elements in the gene; ii) transcription factors able to bind these sequences; iii) physiological signals they respond to.

We report the finding that a protein belonging to the well-known family of NFκB/Rel transcription factors is involved in regulating expression of the APP gene. Members of this family (referred to as p50, p65, c-Rel, p49, RelB) show high homology to the Rel oncogene and are able to form homo- and hetero-dimers which process and integrate a wide variety of extracellular signals and transmit the information to the transcriptional machinery by directly binding to a range of different DNA sequences in gene control regions.

We have identified two identical sequences located in the APP gene regulatory region which can bind a nuclear factor which is present in various rat brain regions, in primary cultures of cerebellar granule cells and in PC12. By several criteria (competition analysis, affinity properties, immunogenicity) this factor seems very similar to the p-50 homodimer belonging to the NFκB/Rel family. To further prove the molecular nature of the factor recombinant p50 protein expressed by bacteria was obtained and proved to bind the oligonucleotide sequences contained in the APP region.Since in other systems (i.e. IL-2 gene) the p50-p50 homodimer acts as a repressor of gene transcription we are currently investigating the functional role of the APP-κB sequence by transfecting various cell lines with plasmid constructs in which this sequence has been linked to the reporter gene CAT.

421.9

TRANSCRIPTIONAL ACTIVITY AND REGULATION OF THE RAT AMYLOID PRECURSOR PROTEIN (APP) PROMOTER . I. M. Chernak* and P. W. Hoffman . Molecular Neurobiology Unit, National Institute on Aging, Gerontology Research Center, Baltimore, MD 21224

ß-amyloid plaques are present in the brains of Alzheimer's patients, Down's Syndrome patients and to a lesser extent in apparently healthy older humans, but not in the brains of aged or memory-impaired rodents. Expression of the APP gene which gives rise to ß-amyloid may therefore be regulated differently in rodents than humans. Since Down's patients carry an extra copy of the gene and have elevated levels of APP mRNAs, increased transcriptional expression may contribute to the appearance of plaques in humans. Thus, it is of great interest to understand the transcriptional regulation of the APP gene in different species. The 5' upstream regulatory region of the rat APP gene has been cloned and investigated by sequence analysis and by assays of transcriptional activity from extracts of cells transfected with wild type and mutant promoter/reporter gene fusions. This region lacks a TATA box and a CAAT box, has a G+C content of 68%, and is 80-90% homologous to the corresponding nucleotide sequence of the mouse and human APP genes. It contains a variety of putative regulatory DNA elements, including SP1, AP1, AP2, AP4, and GCF sites, GC-rich boxes, and six potential stem-loop secondary structures near the probable transcriptional start point. While a series of elements which are well-conserved between rat, mouse and human may serve similar functions in all species, a variety of species-specific differences are present which could be relevant to the production of amyloid plaques in humans but not in rodents. We have shown that the rat upstream region can act as a functional promoter in both rat PC12 cells and human SHSY5Y neuroblastoma cells, and that its activity is at least ten-fold that of the SV40 promoter in PC12 cells. A series of mutations deleting or altering putative regulatory sites has been constructed and analyzed for transcriptional activity. Additional mutagenesis, gel mobility shift assays and footprinting experiments will be used to identify specific sites on the DNA which interact with age-specific, species-specific, and/or tissue-specific nuclear proteins. We are also investigating the effects of nerve growth factor and retinoic acid on the transcriptional activity of the rat APP promoter in different cell lines.

421.10

TRANSGENIC RAT AND IN-VITRO STUDIES OF ß-AMYLOID PRECURSOR PROTEIN PROCESSING Kevin M. Felsenstein, Allison Treloar*, Janet M. Boome, Lisa W. Hunihan, Kim M. Ingalls, and Susan B. Roberts CNS-Drug Discovery, Bristol-Myers Squibb Pharmaceutical Research Institute, Wallingford, CT 06492

We have previously reported the use of a novel recombinant reporter system for ß-amyloid precursor processing which utilizes the human placental alkaline phosphatase (HPLAP) gene fused to various C-terminal regions of the ß-amyloid precursor protein (ß-APP). The fusion of the C-terminal 164 amino acids of ß-APP has been characterized as being proteolytically processed in a similar manner to actual ß-APP. We have used this system to characterize the proteolytic activities involved in the processing of ß-APP, including the biochemical characterization of the activities and mutational analysis of the processing events including analysis of the FAD mutations. An advantage afforded by this system is the ability to measure both qualitatively and quantitatively (immunologically or enzymatically). In the effort to develop animal models for AD pathology the use of such a fusion protein may provide an alternative approach.

We are currently attempting to generate transgenic rat models based on the overexpression of ß-APP, C-terminal protein derivatives, e.g. APPC-100, and the HPLAP•ß-APP fusion protein. Transgenic rat lines have been established and the status of these studies will be reviewed. Expression of the transgenes has been measured by RNA analysis and in the case of HPLAP•ß-APP transgene by direct enzymatic measurements. Pathological and behavioral analyses are currently underway. These rat transgenics may potentially provide useful models to study the effects of chronic overexpression of potentially amyloidogenic protein fragments and the development of an Alzheimer-like pathology.

421.11

ALZHEIMER-LIKE DIFFUSE AMYLOID PLAQUES CAN BE INDUCED IN TRANSGENIC MICE EXPRESSING HUMAN α1-ANTICHYMOTRYPSIN. R.O. Kuljis*, R.D. Beech, S.R. Ross and C-Y. Yeung. Dept. Neurology, University of Iowa and V.A.M.C., Iowa City, IA 52242-1053 and Depts. of Genetics and Biochemistry, University of Illinois at Chicago, IL 60612.

Large numbers of amyloid-containing plaques are an important histopathological feature of Alzheimer's disease (AD). Some have felt that these may be the initial lesions that lead to senile plaques, neurofibrillary tangles, neuronal loss and reactive gliosis. Three independently derived outbred ICR transgenic mouse strains carrying a human α1-antichymotrypsin (ACT) transgene were generated. Expression of the transgene in each strain was verified by a reverse transcriptase/polymerase chain reaction technique, *in situ* hybridization and immunolabeling. The murine homolog of the ACT gene is not normally expressed in the brain. However, *in situ* hybridization revealed expression of the human ACT transgene throughout the CNS. In 8-month-old specimens, the silver impregnation method of Campbell et al. (Soc. Neurosci. Abstr. 13:678) and a panel of antibodies to ß/A4 amyloid, its precursor protein and ACT reveal massive numbers of deposits that co-contain these proteins throughout the CNS in all three transgenic mouse strains. These lesions are morphologically and immunocytochemically indistinguishable from diffuse amyloid plaques in AD. Thus, constitutive overexpression of ACT alone is sufficient to induce the formation of amyloid plaques in the brain, and further aging or additional experimental manipulations may result in additional Alzheimer-like pathology.

Support: Tobacco Council for Research, Inc., American Federation for Aging Research, Alzheimer's Association and PHS NS29856.

421.12

YET MORE TRANSGENIC MOUSE STUDIES OF ALZHEIMER AMYLOID PRECURSOR (APP). Greenberg,B.D.[1,2], Schwartz,D.[1], Savage,M.[1], Pinsker,L.[1], Howland,D.[1], Ali,S.M.[2*], Gonzalez-DeWhitt,P.A.[2], Altman,R.A.[2], Siedlak,S.[3], Perry, G.[3], & Scott,R.[1] [1]*Cephalon, Inc., West Chester, PA, USA.;* [2]*Upjohn Labs, Kalamazoo, MI, USA;* [3]*Institute of Pathology, Case Western Reserve University, Cleveland, OH, USA*

As an attempt to develop an animal model for Alzheimer-type pathology, we have produced transgenic mice which express the following human APP derivatives:

Line	Promoter	Signal Seq.	cDNA	3'-flank
NAN	mMt-I	bGH	hAPP C-104	bGH
SAR	mMt-I	rGH	hAPP-695 (18-695); (18-639)	rGH
MP-SAR	mAPP	mAPP	hAPP-695 (18-695); (18-639)	rGH
LBM-SAR	mAPP	mAPP	hAPP-695 (18-695); (18-639)	mAPP

Legend: ***bGH*** = bovine growth hormone; ***C-104*** = C-terminal 104 amino acids of the APP; ***hAPP*** = human APP; ***mAPP*** = mouse APP; ***mMt-I*** = mouse metallothionein-I; ***rGH*** = rat growth hormone; **numbers in parentheses** = range of APP segment.

NAN mice express the transgene mRNA at levels up to 6-fold higher than the endogenous APP gene. This expression appears to be linked to an accumulation of endogenous APP within pyramidal neurons of the cortex and hippocampus. SAR mice expressed minuscule quantities of transgene mRNA. Expression was only slightly better in the MP-SAR mouse brains, despite very high levels of MP-SAR RNA and protein in transfected cells. The LBM-SAR transgene, however, appears to be expressed at high levels within transfected cells *and* transgenic mouse brains. Hence, the 3'-flanking sequence plays a critical role in determining the expression level of APP transgenes in mouse brains.

421.13

NEURON - SPECIFIC EXPRESSION OF HUMAN BETA-AMYLOID PRECURSOR PROTEIN (APP) IN TRANSGENIC MICE. D.S. Howland*[1], D.A. Schwartz[1], M.J. Savage[1], F.A. Huntress[2], R.E. Wallace[2], L.R. Pinsker[1], R. Coffee[2], D.J. Kiehn[2], L.J. DeGennaro[3], B.D. Greenberg[1], R. Siman[1], M.E. Swanson[2], and R.W. Scott[1] [1]Cephalon Inc., West Chester, PA 19380, [2]DNX Inc., Princeton, NJ. 08540, and [3]University of Massachusetts Medical School, Department of Neurology, Worcester, MA 01655.

Accumulation of ß/A4 protein in brain is a hallmark of Alzheimer's disease. In attempting to develop an animal model for ßA/4 protein processing and deposition, we have produced transgenic mice employing transgenes which fused the neuron-specific rat synapsin I promoter to native APP751, APP751 containing the V to I or KM to NL familial Alzheimer's disease (FAD) mutations, and the E to Q mutation for hereditary cerebral hemorrhage with amyloidosis, Dutch type. APP695 transgenes containing native and FAD mutation KM to NL were also constructed. DNA-positive F1 mice from each founder analyzed to date by both RNA PCR and quantitative RNase protection assays indicate that transgene mRNA expression is brain-specific. Furthermore, *in situ* hybridization analysis on transgenic brain sections has shown that the transgene is expressed exclusively in neurons. Transgene expression levels in brain varied up to 400-fold between lines and, therefore, animals were prioritized for further analysis starting with those exhibiting highest expression. Comparative RNase protection analysis utilizing mouse probes for both synapsin I and APP have indicated that transgene mRNA levels in brain from the highest expressing lines were equivalent to endogenous synapsin I mRNA but approximately 3 to 5-fold lower than total endogenous APP mRNA. Western blot analysis has revealed that human APP751 protein is overexpressed in transgenic brain relative to endogenous APP751 (see adjacent abstract, Savage et al.). Several transgene templates are currently being microinjected for transgenic rat production.

421.14

HUMAN AMYLOID PRECURSOR PROTEIN EXPRESSION IN TRANSGENIC MICE AS A MODEL OF ALZHEIMER'S DISEASE: SEARCH FOR PATHOLOGY. M.J. Savage[1], L.R. Pinsker[1], D.S. Howland[1], D.A. Schwartz[1], F.A. Huntress[2], R.E. Wallace[2], B.D. Greenberg[1*], R. Siman[1], M.E. Swanson[2], and R.W. Scott[1]. [1]Cephalon, Inc., West Chester, PA 19380, [2]DNX Inc., Princeton, NJ 08540.

In an attempt to generate an animal model of Alzheimer's disease (AD), transgenic mice were constructed containing various forms of HuAPP751 (see previous abstract, Howland, et al.). RNase protection was used to demonstrate a range of HuAPP751 message levels in the transgenic animals. In situ hybridization was used to localize the message to neurons. Using R7, a polyclonal antibody specific for the KPI domain (obtained from N. Robakis), overexpression of APP751 protein was detected in temporal cortex of transgenic mice using Western blots. Overexpression of APP on frozen tissue sections was more variable than the Western blot data, as demonstrated by immunohistochemistry using antibodies to the KPI region of APP. Heterozygous mice ranging in age from 3-11 months were examined for amyloid-related pathology using methods developed in AD tissue. Mice were perfusion-fixed in 4% paraformaldehyde. Brains were split down the hemisphere and each half was processed further for either frozen or paraffin sections. A panel of antibodies generated against regions of the ß/A4 protein was used for immunohistochemistry. In addition, silver-based periodic acid methenamine and Reusche methods were used. To date, we have seen no evidence of amyloid deposition either intracellularly or extracellularly. In conclusion, mere overexpression of HuAPP751 in mouse brain neurons is insufficient to produce amyloid deposition. Selected high expressing lines are being bred to homozygosity to provide large numbers of animals for aging studies and for examination of other markers of neurodegeneration.

421.15

Expression of Human Alzheimer's Amyloid Precursor Protein in Transgenic Mice

I. Lieberburg, L. McConlogue, J. Zhao, L. Paganini, L. Rickerstaff, T. Oltersdorf, and L. Refolo*, Athena Neurosciences, Inc.

Alzheimer's disease (AD) is a neurodegenerative disorder characterized histopathologically by neuronal loss and two lesions, namely neurofibrillary tangles(NFTs) and senile plaques (SPs). An animal model which mimics the AD associated neuropathology would facilitate our understanding of the pathophysiological mechanisms leading to AD and aid in the development of AD therapeutics. SP formation is believed to be an early and critical event in the pathophysiology of AD. The A/b peptide is thought to be the pathophysiologically active component of the SP. This peptide is proteolytically derived from a family of proteins known as the Alzheimer's amyloid precursor proteins (APPs). Recent data indicate that mutations in APP are responsible for some forms of familial AD, suggesting that APP is central to the disease process. Our approach to developing an animal model for AD has been to construct APP transgenic mouse lines which express the familial AD mutations . Using the NSE promoter we have constructed lines expressing wild type , Dutch and Swedish APP_{751}. Data on human APP expression will be presented .

421.16

INTESTINAL β-AMYLOIDOSIS IN TRANSGENIC MICE. K. Fukuchi*, D.D. Kunkel[1], C.E. Ogburn, A.C. Smith, N-T. Dang, T. Ohman, C.E. Furlong[2], S.S. Deeb[2], D. Nochlin, S.M. Sumi and G.M. Martin. Depts. of Pathology, Neurosurgery[1], and Medicine[2], Univ. of Washington, Seattle, WA 98195.

Cell culture experiments support the hypothesis that a C-terminal fragment ofthe β-amyloid precursor protein (βPP) is amyloidogenic and neurotoxic. A cDNA for a signal sequence of human βPP plus the 99 amino acid sequence from the C-terminus was placed under the control of a CMV enhancer and a chicken β-actin promoter. Very high levels of expression of mRNA from the transgene were observed in virtually all tissues examined of transgenic mice. On the Western blots, approximately 10- to 15-kDa protein products from the transgene were observed in lung, muscle, intestine, liver, and kidney of the transgenic mice, but no corresponding fragments were found in control mice. A 3- to 4-fold increase of the C-terminal fragments was found in brains of the transgenic mice compared to the controls. Six-, 9-, 13- and 16-month-old transgenic mice were sacrificed for immunohistochemical and histopathological analyses. Using antibodies to β-amyloid protein (Aβ), extensive Aβ deposits were found in the lamina propria of small intestine in transgenic mice older than 12 months. These deposits were positive for Congo red staining (dichroic birefringence). The deposits were also stained with antibodies to mouse serum amyloid P component and to α1-anti-chymotrypsin. In spite of a 3- to 4-fold increase of the C-terminal fragments (Western blots) in the transgenic animals, immunohistochemical analyses using antibodies to βPP and Aβ revealed no obvious differences between the brains of the transgenic mice and those of control nontransgenic mice. Although the transgenic mice have not developed pathological regions in the brain (up to the age of 16 months), the animals can be used for discovering factors that modulate the progress of β-amyloidosis.

This work was supported by the NIH and the Alzheimer's Association, Inc.

421.17

NMDA AND AMPA RECEPTORS IN TRANSGENIC MICE EXPRESSING HUMAN β-AMYLOID PROTEIN

Richard H. P. Porter*, Faheem A. Sandhu, Robert V. Eller, Mohammad Salim, Sayeeda B. Zain & J. Timothy Greenamyre
University of Rochester, Rochester, NY 14642

The human β-amyloid protein may play an important, possibly primary, role in the pathogenesis of Alzheimer's disease (AD), and it appears to potentiate the susceptibility of neurons to excitotoxicity. Alzheimer's disease is associated with alterations in the N-methyl-D-aspartate (NMDA) and α-amino-3-hydroxy-5-methylisoxazole-4-proprionic acid (AMPA) subtypes of glutamate receptors, and it has been suggested that excitotoxicity may play a role in neuronal damage in AD. In this study, we have used quantitative receptor autoradiography to examine NMDA and AMPA receptors in transgenic mice that contain the gene for the carboxyl-terminal 100 amino acids of the human amyloid precursor protein, beginning with the β-amyloid region, which is under the control of the JC viral early region promoter. Reverse transcriptase polymerase chain reaction confirmed that the brains of transgenic mice expressed β-amyloid mRNA and that control mice did not. NMDA receptors, assessed with [^{3}H]MK-801, were unchanged in the transgenic compared to the control mice. In the transgenic mice, there were no significant changes in [^{3}H]AMPA receptor binding compared to controls. This study represents the first attempt to evaluate in transgenic mice the *in vivo* interaction between β-amyloid expression and excitatory amino acid receptors. *(NS01487, AG02126, T32 GM07356, CA11198 and the American Academy of Neurology.)*

421.18

CHARACTERIZATION OF A 33KD PROTEIN FROM STABLE TRANSFECTED PC12 CELLS, TRANSGENIC MICE AND HUMAN BRAIN.

T. Honda[1], F. Sandhu[2], S. Zain[2], A. Pope[1]* and R.A. Nixon[1]. [1]McLean Hospital, Harvard Medical School, Belmont, MA 02178 and [2]Department of Biochemistry and Cancer Center, University of Rochester, School of Medicine, Rochester, NY 14642.

To examine the processing of APP, two cell lines, which stably express an A4-C100 fragment under the control of SV40 promoter, were created. Antibodies to the amyloid region and intracellular domain of APP stained these cells more strongly than untransfected control PC12 cells. Transfected cell lines overproduced a 16kD protein stained with the amyloid antibodies. The antibody TC2, which was raised against the synthetic peptide C2(APP644-676), intensely stained 33kD bands in the lysates from transfected cells; this same band in control PC12 cell was faintly stained. Staining of the 33kD band was diminished when the antibody was preabsorbed with C2 peptide. After CNBr cleavage of the 33kD protein, the smaller 21kD band was stained with two antibodies to the amyloid region of APP. The purified 33kD protein was stained with the antibody to C-terminal of APP. Moreover, sequence analysis of this protein revealed that the major constituent of this band starts just after the first methionine of the predicted gene product. Although the size of this protein was unexpectedly large, neither glycosylation nor ubiquitination could be detected. These data suggested that this 33kD protein may be an aggregated form of A4-C100. The increased levels of the same size of protein were found in transgenic mice brains, which express A4-C100 specifically in brain (Sandhu, *et al., J. Biol. Chem.*, **266**, 21331-34, 1991). This protein was also present in human brain homogenates, where it was found in increased levels in the insoluble fraction of Alzheimer brains.

421.19

ß-AMYLOID PROTEIN-INDUCED NEUROTOXICITY IN WILD TYPE MICE AND IN TRANSGENIC MICE WITH ELEVATED CuZn SUPEROXIDE DISMUTASE ACTIVITY.

A.L. Friedlich*[1], T.W. Farris[1], E. Carlson[2], C.J. Epstein[2], and L.L. Butcher[1]. [1]Dept. of Psychology, UCLA, Los Angeles, CA 90024 and [2]Dept. of Pediatrics, UCSF, San Francisco, CA 94143.

The *in vivo* neurotoxicity of the Alzheimer ß-amyloid protein (ßAP) remains controversial. Because of the possible involvement of free oxygen radicals in ß-amyloidosis (Friedlich and Butcher, *Neurobiol. Aging*, in press), we investigated the effects of the superoxide radical scavenger, CuZn superoxide dismutase (SOD), on ßAP-induced neurodegeneration. ßAP (ß1-40, 7.5 µg) in water or acetonitrile was injected into the hippocampus of wild type mice and mice homozygous or heterozygous for the CuZn SOD transgene 218/3. One week after surgery, ßAP deposits were identified with anti-ßAP immunocytochemistry and thioflavin S staining. In Nissl-stained material from each mouse type, the necrosis produced by ßAP in acetonitrile was similar to that produced by vehicle injections. No neuronal Alz-50 reactivity was observed with ßAP in acetonitrile. With the water vehicle, ßAP-induced necrosis was significantly greater than that produced by vehicle or reverse peptide injections in wild-type ($p<.001$) but not transgenic animals. Also with water vehicle, control injections did not induce neuronal Alz-50 immunoreactivity, but dendritic and somal Alz-50 positivity juxtaposed ßAP deposits in 88% of the wild type, 50% of the heterozygous, and 15% of the homozygous mice. We conclude that ßAP can induce a specific neuropathology *in vivo* to which increasing CuZn SOD activity confers resistance. (Supported by USPHS NS-10928 to L.L.B. and AG-08938 to C.J.E.).

421.20

THE ROLE OF β/A4-AMYLOID IN THE NEURODEGENERATION OF TRISOMY 16 MICE.

J.A. Rabin, W.C. Mobley, D.M Holtzman,* Depts. of Neurology, Pediatrics, and the Neuroscience Program, UCSF, San Francisco, CA 94143 and Scios Nova, Inc., 2450 Bayshore Parkway, Mountain View, CA 94043.

Mouse trisomy 16 (Ts 16) is an animal model of Down Syndrome (DS). We have previously shown that cholinergic neurons from fetal Ts16 basal forebrain (BF) transplants undergo time-dependent atrophy similar to that observed in DS and Alzheimer's disease (AD). Despite cholinergic atrophy, there was no evidence of β/A4 deposition. The lack of β/A4 deposition in this and other rodent models may be secondary to differences between the rodent and human β/A4 sequence. We therefore asked whether Ts16 BF and hippocampal transplants that are transgenic for the human APP_{751} gene would produce β/A4 deposition and enhance neurodegeneration. We transplanted BF and hippocampus from transgenic or nontransgenic Ts16 and control fetuses into the brains of young adult mice. Grafts were then examined for evidence of β/A4 deposition, neurofibrillary tangle formation, and neurodegeneration at 1 and 6 months. Nontransgenic transplants have been examined at one and six months and show good graft survival but no evidence of plaques or tangles. Transgenic transplants are currently being assessed.

421.21

ELEVATED IL-1ß AND IL-6 mRNAs AND ß-AMYLOID PRECURSOR PROTEIN (ß-APP) LEVELS DURING BRAIN DEGENERATION PROCESS IN THE CEREBELLUM OF *STAGGERER* MUTANT MICE.

B. Brugg[1], Y. Lemaigre-Dubreuil[1], G. Huber[3], E. Wollman[2], N. Delhaye-Bouchaud[1] and J. Mariani[1]* [1]Université P.& M. Curie, Institut des Neurosciences CNRS URA 1488, Paris. [2] Hopital Cochin, INSERM U 283, Paris. [3]Hoffman La Roche, Basel.

Recent findings indicate that cytokines, well known immuno-modulating factors, have important functions in developmental, pathological and regenerative processes in the brain. We are using the *staggerer* mutant mice as a model system to investigate the role of cytokines and ß-APP in neuronal degeneration processes. The homozygous *staggerer* mutant shows a severe deficit in the numbers of Purkinje cells associated with a fast and almost complete degeneration of granule cells and olivary neurons during the first postnatal month. We used polymerase chain reaction (RT-PCR) to detect and quantify IL-1ß and IL-6 mRNAs levels in different brain regions of *staggerer* mutant and control mice. In 30 day-old homozygous *staggerer* mutant, in which the degeneration is very pronounced, we observed a strong increase of IL-1ß and IL-6 mRNAs in the cerebellum. In the cerebral cortex, IL-1ß mRNA levels were not different in the mutant and in the age matched controls. IL-6 mRNA was only detected in *staggerer* cerebellum but not in cerebral cortex or equivalent brain tissues of controls. These results showed that IL-1ß/IL-6 induction parallels the degenerative process and is restricted to brain regions where neuronal degeneration is observed. Using antibodies against synthetic peptides of different regions of the ß-APP protein, we showed by western blot analysis that the amount of ß-APP is increased during the degenerative process in the cerebellum of *staggerer* mutant mice. Further investigations are on the way to characterize at the mRNA and protein levels the different APP forms induced during the degenerative process, and to look for a potential link between cytokines and ß-APP expression.

422.1

INTERLEUKIN-1 (IL-1) INDUCED EXPRESSION OF ALZHEIMER DISEASE-RELATED PROTEINS IN RAT BRAIN. J.H. Wu, J.G. Sheng, R.A. Skinner, L.C. Stanley, P.T. Wall,* and W.S.T. Griffin. Depts. of Pediatrics, Physiology, and Anatomy, Univ. Arkansas for Med. Sci., Little Rock, AR 72202

Excessive expression of interleukin-1 (IL-1) and S100β and β-amyloid-precursor-protein immunoreactive (βAPP$^+$) dystrophic neurites in plaques are features of both Down's syndrome (DS) and Alzheimer's disease (AD). Interleukin-1 stimulates endothelial cell synthesis of β-APP and induces astrocyte proliferation and activation *in vitro*; activated astrocytes synthesize and release elevated amounts of a neurite growth factor S100β. Whether IL-1 induces expression of these proteins in the brain *in vivo* is unknown. S100β^+ astrocytes surround the shell of overgrown dystrophic neurites in neuritic plaques in AD, and biologically active S100β is present in temporal lobe of patients with AD. The present experiment was performed to determine if the increase in IL-1 expression in AD may be related to β-APP and S100β expression. Two μl of either IL-1β (1 U/μl) or PBS were injected into the right hemisphere of rats (n=10 and 7, respectively); hemispheres from un-operated rats (n=3) served as controls. Using SDS-page immunoblot analysis, we identified two distinct βAPP$^+$ bands (120-140 kD) and an S100β^+ band that co-migrated with synthetic S100β in extracts collected 3 days post injection. Relative to PBS injection, IL-1β injection resulted in a 40 percent increase in the level of S100β ($p \leq 0.05$). By similar comparison, there was a 100 percent increase in the level of the lower molecular weight β-APP ($p \leq 0.001$) and a 30 percent ($p \leq 0.003$) increase in the level of the higher molecular weight β-APP. We suggest that excessive expression of IL-1 in DS and AD is related to the excessive expression of S100β and the overgrowth of β-APP$^+$ dystrophic neurites in these diseases. Supported in part by NIH AG10208 and NS27414.

422.2

INCREASED SERINE PHOSPHORYLATION OF APP ON THE EXTRACELLULAR DOMAIN ACCOMPANIES PHORBOL ESTER MEDIATED STIMULATED SECRETION J. Knops, S. Gandy#, P. Greengard#, I. Lieberburg, J. Anderson* & S. Sinha Athena Neurosciences, South San Francisco, California 94127; #The Rockefeller University, New York, NY 10021

The secretion of APP, which involves the cleavage of the full-length holoprotein (FL-APP) to release most of the large ectodomain (s-APP) into the conditioned medium (CM), can be strongly stimulated by activation of protein kinase C either by phorbol esters or activation of specific receptor-mediated signaling pathways. In order to investigate whether this stimulation was accompanied by a change in the putative phosphorylation status of FL-APP, 293 cells stably transfected with APP751 were labeled with ^{32}P *o*-phosphate. Stable ^{32}P incorporation was detected in the mature FL-APP either in the absence or presence of 50 nM PMA, with increased ^{32}P incorporation into [^{32}P]-APP in the presence of the drug. Surprisingly, ^{32}P-phosphate is primarily incorporated in the secreted ectodomain, and this incorporation in s-APP is stable to treatment with a large excess of PNGase F, suggesting that N-linked oligosaccharide sites do not account for phosphate incorporation. Phosphoamino acid analysis of the [^{32}P]-s-APP resulted in the recovery of [^{32}P]-phosphoserine as the preponderant species. PMA treatment increased the total amount of [^{32}P]-s-APP secreted, by direct stimulation of total s-APP released, and also apparently by increased stoichiometry of phosphorylation, but did not otherwise alter the phosphorylation pattern. Brefeldin A completely inhibited the release of [^{32}P]-s-APP, but did not inhibit the incorporation of ^{32}P into the FL-APP, suggesting that the phosphorylation events occur early in the Golgi. It is possible that increased serine phosphorylation of FL-APP in the ectodomain region provides a signal for stimulated cleavage and secretion.

422.3

INCREASED IONIC CONDUCTANCE ELICITED WITH ß AMYLOID PEPTIDE IS DEPENDENT UPON PEPTIDE SECONDARY STRUCTURE. W. Y. Li and L. K. Simmons* Lilly Research Labs, Eki Lilly & Company, Indianapolis, IN 46285.

One of the pathological hallmarks of Alzheimer's disease (AD) is the extracellular deposition of amyloid ß peptide (Aß) in the brain . Aß is believed to be involved in the neurodegeneration that occurs in AD, and we recently showed that Aß (1-40) is maximally toxic *in vitro* when it adopts a ß-sheet conformation. We have investigated the effects of different conformations of Aß (1-40) on ion channel activity in primary cultures of embryonic rat hippocampal cells.

Freshly prepared Aß is weakly toxic and has a random coil conformation. Aging the peptide in water at 37°C for several days initiates a conformational change from random coil to ß-sheet structure, and significantly enhances Aß neurotoxic potency. Using the whole-cell voltage-clamp paradigm, we tested the effects of freshly prepared and aged Aß on currents elicited with voltage step protocols that did not activate voltage-dependent currents in rat hippocampal neurons. Extracellular application of 1 - 10 µM aged Aß elicited a slow decrease in cell input resistance as determined by an increase in leakage current. The reversal potential of the leakage current remained unchanged, suggesting that Aß might be acting as an ionophore. Similar concentrations of freshly prepared Aß or lower concentrations of aged peptide(100 nM) did not induce any changes in cell input resistance. Ion substitution experiments are underway to characterize the ionic selectivity of Aß in more detail. Our findings suggest that increased membrane permeability to ions is a possible mechanism underlying Aß-dependent neurotoxicity.

422.4

RELEASE OF AMYLOID β-PROTEIN PRECURSOR DERIVATIVES FROM VARIOUS BRAIN REGIONS IS STIMULATED BY ELECTRICAL DEPOLARIZATION. J.G. Schulz, R.M. Nitsch, S.A. Farber, A.M. Graybiel*, J.H. Growdon and R.J. Wurtman. Dept. of Brain & Cognitive Sciences, M.I.T., Cambridge, MA 02139 and Dept. of Neurology, Mass. General Hospital, ACC 830, Boston, MA 02114.

We have shown that electrical depolarization increases secretion of large N-terminal amyloid β-protein precursor derivatives (APPs) from hippocampal slices in a tetrodotoxin-sensitive and frequency-dependent manner, indicating that action potentials regulate APP processing. In cell culture, activation of cell-surface receptors linked to phosphatidylinositol turnover also increases APPs secretion. These results suggest that APP processing may be coupled to neurotransmission in the brain. Individual brain regions vary with respect to the neurotransmitters they contain. In order to determine whether electrical depolarization affects APPs secretion differently in individual brain regions, we prepared rat brain tissue slices from cortex, striatum and hippocampus. Slices were superfused (Warner Inst.) and depolarized by electrical field stimulation (30Hz, 1ms pulses, 4.95mA/mm^2). Unstimulated control slices were run in parallel. Secreted proteins were purified by ultrafiltration and APPs was measured by Western blot (mAb 22C11), ECL, and densitometry. Basal, unstimulated APPs release from cerebral cortex was 1.7 times higher than that from striatum. Electrical depolarization increased APPs secretion from cerebral cortex 2.5 fold; APPs release from hippocampus and striatum were increased 1.7-2.0 fold by stimulation. These results show that APPs is released throughout the brain and that individual brain areas may vary in the amount of APPs released basally. They also show that basal APPs release can be stimulated throughout the brain by electrical depolarization, and suggest that the magnitudes of these effects vary among brain regions. Supported by NIMH, NIA, CBSMCT.

422.5

COMPARATIVE DISTRIBUTION OF STRIATAL DIFFUSE PLAQUES AND SELECTED NEUROPEPTIDE MARKERS IN ALZHEIMER'S DISEASE (AD). M. Gearing* and S. S. Mirra. Veterans Affairs Medical Center and Emory University School of Medicine, Atlanta, GA 30322.

Although neuritic and diffuse plaques generally coexist in AD neocortex, the overriding predominance of diffuse plaques in striatum has prompted interest in environmental factors which may affect amyloid deposition and plaque formation. Could regional differences such as those existing within the striatal mosaic play a role? To investigate the relationship between diffuse plaques and regional neuropeptide distribution, we performed a series of double immunohistochemical labeling procedures using an antibody to ß-amyloid (kindly provided by H. Wisniewski) in combination with antibodies to met-enkephalin (INCSTAR) or somatostatin (Chemicon). As expected, the striatum in all the neuropathologically-confirmed AD cases showed numerous ß-amyloid immunoreactive plaques. Colabel with anti-met-enkephalin, a patch marker, did not reveal any obvious association between the striatal mosaic and diffuse plaque distribution. Similarly, striatal diffuse plaques did not colocalize with somatostatin-immunoreactive neurons. Comparison of "pure AD" cases (n=12) with those showing coexistent Parkinson's disease (PD) neuropathology, i.e. nigral degeneration with Lewy bodies at any site, ("AD+PD"; n=5) revealed no differences between these two groups in the distribution of diffuse plaques or the above neuropeptide markers. These preliminary observations suggest that the striatal mosaic appears to be preserved in AD, regardless of the presence or absence of coexistent PD changes. Moreover, the apparent lack of topographic relationship between diffuse plaques and the above neuropeptide markers is true for both the "pure AD" and "AD+PD" groups. These results further suggest that striatal amyloid deposition in AD is not influenced by potential environmental factors associated with the striatal mosaic, or by the somatostatin-immunoreactive neurons which are present in both patch and matrix compartments. Supported by AG10130 and VA Merit Award.

422.6

NEURITIC (NP) PLAQUES IN DIFFERENT AREAS OF ALZHEIMER DISEASE (AD) NEOCORTEX SHARE COMMON MOLECULAR FEATURES M.L. Schmidt, G. DiDario, L. Otvos, Jr., V.M.-Y. Lee, & J.Q. Trojanowski* Dept. Path. & Lab. Med., Univ. Of Penn. Sch. Of Med., Phila., PA 19104

β-amyloid (β/A4) accumulates in plaques in AD and non-demented elderly patients. Diffuse plaques (DPs) contain extracellular non-fibrillar β/A4, lack dystrophic neurites, do not elicit gliosis and occur in similar numbers in AD and control brains, while NPs are dominated by fibrillar accumulations of β/A4, contain dystrophic neurites rich in neuronal cytoskeletal proteins or amyloid precursor protein (APP) domains flanking the β/A4 peptide, and are associated with gliosis and neuron loss. To determine if NPs in diverse areas of the AD neocortex contain similar or distinct molecular components, we analyzed the composition of NPs in six different neocortical areas of AD and non-demented control brains using quantitative immunohistochemistry and antibodies to proteins (β/A4, APP domains outside β/A4, neuronal cytoskeletal and synaptic proteins) found in subsets of amyloid plaques.

The molecular composition of NPs in diverse neocortical areas was similar and the density of NPs was more AD specific than that of DPs. Thus, the elucidation of mechanisms leading to the convergence of β/A4 with cytoskeletal proteins in NPs may clarify the role of NPs in neuron dysfunction and death in AD.

422.7

THE AMYLOID β PROTEIN IN CEREBROSPINAL FLUID FROM ALZHEIMAER'S DISEASE PATIENTS. T. Oosawa, M. Shoji, Y. Harigaya, T.T. Cheung, L. Shaffer, L.H. Younkin*, S.G. Younkin, S. Hirai, Dept. of Neurology, Gunma Univ. Sch. of Med., Maebashi, Gunma 371, Japan. *Case Western Reserve University, Cleveland, OH 44106.

The 4-kilodalton amyloid β protein (βAP), which is deposited as amyloid in the brains of patients with Alzheimer's disease, is produced by normal proteolytic processing of the amyloid β protein precursor. The βAP is also released into living human cerebrospinal fluid (Shoji M. et al., Science 258: 126-129). Here we report the amount of βAP in CSF from 59 subjects, which include 27 patients with sporadic Alzheimer's disease (AD), 14 patients with other CNS disease, and 11 normal volunteers. we examined 3-ml samples of CSF using immunoprecipitation by SGY2134 and detected βAP on 4G8 immunoblots. The amount of βAP is 3.18 ± 1.52 pmol/ml (mean ± S.D.) in AD group, 4.49 ± 3.14 pmol/ml in normal control group. There is no significant difference between AD and normal control groups. There is considerable interindividual variation in the amount of βAP in CSF from all groups. Both clinical stage and progress of dementia are not correlated with the amount of βAP in CSF. Our study suggests that the amount of βAP in CSF is not directly associated with brain pathology in sporadic AD.

422.8

AUTOMATED DETECTION AND MEASUREMENT OF SENILE PLAQUES IN ALZHEIMER'S DISEASE. *L.S. Hibbard* and D.W. McKeel, Jr., Departments of Neurology and Neurological Surgery, and Pathology, Washington University School of Medicine, St. Louis, MO 63110.*

Senile plaques (SP) are the most characteristic neuropathologic lesions of Alzheimer's Disease (AD) and many studies of plaque cortical distribution, density, and morphology have been undertaken to gain new information about the origin and pathogenesis of AD. We have developed an automated computer image analysis program to detect SP, and to measure SP size, shape, population density, and fractional area or load in digital micrographs of silver and β/A4 immunostained tissue sections. Candidate SP features are detected by adaptive thresholding, and size and shape measures are used to discriminate SP from other stained features. *This program requires no user interaction.* Measures of SP density, and load are readily calculated from the pixel values of the detected SP features, and in comparisons with manual counts, the program produced false positive and false negative error rates of 4% and 2%, respectively. All the SP size and shape properties formed skewed, monomodal frequency distributions with maxima near the origins of the property axes. This program was used to study the SP density and load variation in one AD case examining 220 images (16,000 SP) from nine neocortical sections spaced 60 μm apart.

(Support: NIH 5P50-AG05681)

422.9

SELECTIVE LOCALIZATION OF THE AMYLOID PRECURSOR LIKE PROTEIN (APLP) IN THE BRAIN POSTSYNAPTIC DENSITY (PSD) OF RAT AND HUMAN CEREBRAL CORTEX. T.W. Kim*[1,2], K. Wu[1,2], W. Wasco[3], R.E. Tanzi[3], J.L. Xu[1], S. Zhong[4] and I.B. Black[1,2]. [1]Dept. of Neurosci. and Cell Biol., UMDNJ/RWJ Med. Sch, Pisacatway, N.J. 08854; [2]Graduate Program in Physiol. and Neurobiol., Rutgers-The State Univ. of N. J., Piscataway, N.J. 08854; [3]Molec. Neurogenetics Lab., Mass. Gen. Hosp. East, Charlestown, MA. 02129; [4]Dept. of Cell and Dev. Biol., Rutgers-The State Univ. of N.J., Piscataway, N.J. 08854

Senile plaques and neurofibrillary tangles are hallmarks of Alzheimer's disease. The plaques contain amyloid ß-peptide (ßA4), which is generated from the larger ß-amyloid precursor protein (APP) encoded by four different transcripts. In addition to the APPs, several APP-related genes have been recently identified in *Drosophila* and rat. Deficiency of the *amyloid precursor protein-like (Appl)* gene product causes behavioral deficits in *Drosophila*, implicating a role in brain function. Moreover, a mouse cDNA clone encoding amyloid precursor-like protein has been identified and exhibits extensive sequence similarity to the *Appl* and APP genes. To define the potential role of the APLP in the mammalian brain, we sought to directly localize the APLP within the complex cortical synaptic structure. We now report that the 90 kDa APLP protein is localized to the PSD isolated from adult rat cerebral cortex, using an antibody against a specific APLP peptide. During cortical development, the 90 kDa APLP selectively increased significantly from postnatal day 7 to 14, suggesting a role in synaptogenesis or synaptic maturation. The anti-APLP antibodies also recognized a 30 kDa protein which was expressed predominantly at early postnatal stages, but was undetectable in the adult PSD. Moreover, the 90 and 30 kDa proteins exhibited differential regional expression in adult rat brain, implying regionally specific roles. Our observations raise the possibility that the homologue of *APPL*, which appears to be necessary for normal behavior in *Drosophila*, also participates in brain synaptic function in mammals.

422.10

LOW CONCENTRATIONS OF ^{125}I-ßA4 DEPOSIT ONTO BOTH PLAQUES AND A NON-PLAQUE COMPONENT IN ALZHEIMER DISEASE CEREBRAL CORTEX

P.W. Mantyh, J.R. Ghilardi, S.D. Rogers, E.R. Stimson, C.J. Allen, H.V. Vinters, M.W. Dysken*, J.E. Maggio. Mol. Neurobio. Lab (151) and GRECC, VA Med. Cntr., Mpls., MN 55417; Dept. Pathol., UCLA, LA, CA 90024; Dept of Biol. Chem. and Mol. Pharm., Harvard Med. School, Boston, MA 02115

A salient pathological feature of Alzheimer disease (AD) is the presence of a high density of amyloid plaques in the brain tissue of victims. The plaques are predominantly composed of beta amyloid peptide (ßA4), a 40-mer whose neurotoxicity is related to its aggregation. Recently several reports have suggested that normal cells synthesize and release ßA4 and that low concentrations (6.0×10^{-10} M) of ßA4 are present in normal human CSF. To determine the sites at which low concentrations of ßA4 deposit in the human brain, we radioiodinated human ßA4 and developed an *in vitro* assay using 10^{-10} M ^{125}I-ßA4 and thin sections of human cerebral cortex. Low concentrations of ^{125}I-ßA4 clearly bind to plaques (diffuse, neuritic and compact) and cerebrovascular deposits in AD brain. In addition, ^{125}I-ßA4 shows significant binding to a non-plaque component present within specific laminae of the grey matter of AD cerebral cortex. Currently, we are characterizing the chemical nature of this non-plaque component. These studies suggest the sites at which physiological concentrations of ßA4 deposit onto both plaque and non-plaque components in AD brain. While the functional significance of this non-plaque component is currently unknown, it may provide an initial site which promotes ßA4 aggregation and deposition in the AD brain. Supported by the NIH and the VA.

422.11

NON-β REGION APP ANTIBODIES DETECT A SUBSET OF Aβ-POSITIVE DIFFUSE PLAQUES AND BLOOD VESSELS IN AD AND DS CEREBELLUM. C.A. Lemere, J.J. Schildkraut* and D.J. Selkoe. Harvard Medical School, Brigham and Women's Hospital and *Mass Mental Health Center, Boston, MA 02115.

We examined the presence of non-β region APP epitopes in Aβ–bearing diffuse plaques and blood vessels of briefly-fixed, paraffin-embedded AD and DS cerebellum. Because APP antibodies can detect dystrophic neurites in cortical plaques, cerebellum was selected for its near absence of neuritic plaques. Cerebellar sections from 10 cases (6 AD, 2 DS, 2 Ctrl) were serially immunostained with antibodies to Aβ and non-β APP epitopes. A small fraction of R1280 (Aβ)-positive diffuse plaques in cerebellar molecular layer were also recognized by a non-β region $APP_{444-592}$ antibody, B5, and by a Mab (1G5) to this region. A subset of the B5+ plaques were also detected by two Mabs to the APP ectodomain and by an antiserum to APP_{18-33} (N-terminus). Some B5+ plaques and some B5- plaques showed ubiquitin immunoreactivity. However, other neuritic markers (tau and NF) failed to detect any cerebellar plaques. In addition, a moderate percentage of R1280+ blood vessels were positive with mid-region antibodies, B5 and 1G5, and with the N-terminal antiserum. Both plaques and blood vessels were all negative with the C-terminal antibody, C7. Thioflavin detected vascular amyloid but not plaques, with the exception of a small subset of plaques in one DS case. We interpret the non-β region APP reactivity of blood vessel walls to indicate the presence of part or all of APP_S at local sites of Aβ deposition. Similarly, a small minority of plaques contain these epitopes in the apparent absence of dystrophic neurites. Our findings suggest that metabolic derivatives of βAPP besides Aβ can accumulate at sites of Aβ deposition.

422.12

AMYLOID PLAQUES IN CEREBELLAR CORTEX AND THE INTEGRITY OF PURKINJE CELL DENDRITES Y.-T. Li*[1], D.S. Woodruff-Pak[1], J.Q. Trojanowski[2] Lab. of Cognitive Neuroscience[1], Phila. Geriatric Ctr., Phila., PA 19141, Dept. of Psychology[1], Temple Univ., Phila., PA 19122, & Dept. of Path. and Lab. Medicine[2], Univ. of Pennsylvania School of Medicine, Phila., PA 19104.

To investigate the pathological consequences of ß/A4 deposits and their relationship to Purkinje cells (PC) in the postmortem cerebellum of 13 AD, 10 older DS patients, and 9 age-matched, non-AD controls, we probed serial and near serial sections of cerebellum using single and double labeling immunohistochemistry and a panel of antibodies to defined amino acid sequences in peptides and proteins found in neuritic amyloid plaques. Antibodies to ubiquitin, synaptic proteins, and other polypeptides that are perturbed by or incorporated into neuritic plaques also were used. Data confirmed that ß/A4 deposits in cerebellum of AD and older DS adults form diffuse plaques, and the density of ß/A4 lesions per unit area of molecular layer correlated with the number of PC per unit length of subjacent PC layer in double immunostained sections ($r = .85$; $p < .001$). About 65% of ß/A4 immunoreactive deposits in these preparations were in physical contact with 1st, 2nd, and 3rd order PC dendrites. Notably, no ß/A4 plaques were found in the cerebellum of controls, while ß/A4 deposits were most abundant in the cerebellum of older DS patients. Despite the abundance of ß/A deposits in the cerebellar cortex of AD and older DS patients, neither PC bodies nor PC dendrites in physical contact with ß/A4 lesions showed evidence of structural abnormalities of PC. Finally, none of these ß/A4 lesions induced astrogliosis, a hallmark response of astrocytes to nearly all CNS injuries. This study demonstrates that the deposition of ß/A4 peptides in cerebellum of AD and older DS patients does not alter the integrity of PC, but frequent association between ß/A4 plaques and PC dendrites suggests that these neuronal processes could be a source of extracellular ß/A4 in the cerebellar molecular layer. Supported by AG9752 and IIRG-91-059 from the Alzheimer's Assoc. to DSW-P and AG09215, AG09399 and NS-18616 to JQT.

422.13

ALZHEIMER'S DISEASE PATHOLOGY IN THE AMYGDALOID SUBNUCLEI. L. -F. Lue*, S. Byttner, L. Sue, and J. Rogers. Sun Health Research Institute, Sun City, AZ 85351.

We have examined the amygdaloid complex in Alzheimer's disease (AD) and nondemented elderly control (ND) patients in an effort to elucidate potential relationships among various pathologic phenomena and among connectionally related amygdaloid subnuclei. Pathologic phenomena studied were compacted plaques, diffuse plaques, neurofibrillary tangles, and early (C4d) and late (C5b-9) stages of complement activation. Amygdaloid subnuclei examined were the lateral nucleus, basal nucleus (parvicellular division), and periamygdaloid cortex. In general, connectional hypotheses of AD pathology were not supported by the data: there were no meaningful correlations of any of the pathologic indices in one amygdaloid subnucleus with those in the other amygdaloid subnuclei. Although tangles were highly correlated among the subnuclei, it is difficult to construct any simple connectional basis for such a result. Likewise, across nuclei there were no obvious relationships between the expression of any one pathologic entity and the development of another. For example, there was no significant correlation of tangles with compacted plaques, and only a weak (P = .075) correlation of tangles with diffuse plaques. Among the subnuclei, the lateral nucleus exhibited the lowest densities of plaques (diffuse and compacted) and tangles, as well as significantly reduced immunoreactivity for complement proteins. Taken together, these data suggest that there is no causal association between the development of neurofibrillary tangles and neuritic plaques in AD, and that pathology in one brain area does not necessarily dictate pathology in a closely connected brain area. By contrast, complement immunoreactivity appears to be a reasonable predictor of the development of neuritic plaque and neurofibrillary tangle pathology in a structure.

422.14

THE AGE ONSET OF AMYLOID PLAQUE FORMATION IN THE DOG. M. Bobik[1], S. A. Benjamin[2], L. S. Chubb[2], M. J. Russell[1]*. [1]Dept. of Anesthesiol., Univ. Calif. Davis, Davis, CA 95616, and [2]Dept. of Pathol., Univ. of Colo., Fort Collins, CO 80523.

Aged beagles exhibit neuropathological changes similar to those seen in Alzheimer's patients. As part of an ongoing effort to develop the canine as an animal model, we have examined the age at onset of amyloid accumulation in a group of lab-raised beagles of known age and history. We have previously reported that between the ages of 13 and 17 years there is no relationship between amyloid accumulation and age. In this study we examined two age groups of beagles. The younger group were 12 dogs ranging from 8.2 to 8.9 years, and the older group were 16 dogs from 11.1 to 12.7 years old. Formalin fixed and paraffin embedded tissues were sectioned at 10μm and deparaffinized. The sections were stained by the modified Bielschowsky method and examined for amyloid formation in the hippocampal and parahippocampal regions. Amyloid accumulation was found in one of the 12 animals in the younger group and in 5 of the 16 animals in the older group. The one animal in the younger group was found to have diffuse amyloid plaques in the hippocampal region. One of the five dogs in the older group showed compact, possibly neuritic, plaques. None of the animals examined had tangles. Taken together these data indicate that plaque formation may occasionally occur before the age of eight in the beagle, but that for most beagles plaque accumulation occurs between the age of 8.3 and 12.

422.15

THE INCIDENCE OF CEREBRAL β-AMYLOIDOSIS IN AGED CAPTIVE RHESUS MONKEYS. H. Uno* and L.C. Walker. Primate Research Ctr. and Dept. of Pathology, University of Wisconsin, Madison, WI 53715, and The Johns Hopkins University, Baltimore, MD 21205.

During the past 12 years, the population in our aging colony of rhesus monkeys (aged over 20 years) had an annual average of 93 live animals and 15 deaths. By 30 years of age 50 to 70% of each cohort group had died, and maximum longevity was 36 years. We examined the presence of senile plaques and cerebral angiopathy in 51 brains of rhesus monkeys aged 25 to 36 years. The sections of the basal prefrontal, hippocampal, front-parietal gyri, and the amygdala were examined by immunocytochemistry with amyloid-β protein antibody (10D5) and amyloid precursor protein. Twenty of 51 aged brains had no detectable plaque formations and angiopathy in examined brain regions. Among 31 plaque-positive cases, 21 were found in brains aged 25 to 30 years (56% positive) and 10 in ages over 31 years (71% positive). Overall sexual differences were 7 positive cases in 10 male (70%) and 22 positive cases in 41 female brains (53%). The density of plaques per 1 mm^2 area of section varied greatly in each region and individual. The basal prefrontal gyri (area 10, 11, 25) generally contained the highest incidence and density of plaques; approximately 30% of brains showed an average of 12 plaques per 1 mm^2. The amygdala was the next highest region and the hippocampal and pre- and post-central (area 4.3-1.) gyri contained sporadic plaques. The formation of senile plaques in the aged macaque brain has been widely reported. Although the neurofibrillar tangles are generally absent in the monkey brain, a homology and immuno cross-reactivity of beta-amyloid and its precursor proteins between monkey and human brains suggests that aged macaques are a good model for human Alzheimer's disease.

DEGENERATIVE DISEASE: ALZHEIMER'S—NEUROPHARMACOLOGY AND NEUROTRANSMITTERS III

423.1

PROBING THE STATUS OF MUSCARINIC RECEPTOR SUBTYPES IN ALZHEIMER'S DISEASE WITH SUBTYPE-SELECTIVE ANTISERA. D.D. Flynn*, A.I. Levey, G. Ferrari-Dileo, and D.C. Mash. Depts. of Pharmacology & Neurology, Univ. of Miami School of Medicine, Miami, FL., 33101 and Dept. Neurology, Emory Univ. School of Medicine, Atlanta, GA 30322

Subtypes of muscarinic receptors have been identified on the basis of the unique pharmacological profiles of certain antagonists. To date, most of the pharmacological approaches for characterizing muscarinic receptor subtype alterations in Alzheimer's disease have been inadequate because the overlapping drug affinities do not permit the selective detection of any one receptor subtype. We have measured the relative levels of m1 - m5 receptor subtype proteins using quantitative immunoprecipitation with subtype-selective antisera (Levey et al., 1990). Immunoprecipitation of receptor proteins by subtype-specific antisera was examined in the frontal, temporal, parietal and occipital cortices, the dentate gyrus of the hippocampus, and the nucleus basalis in patients with Alzheimer's disease and age-matched control subjects. In agreement with previous studies, the m1 receptor subtype was found to be abundant throughout the human cerebral cortex comprising 35% of the total muscarinic receptor density estimated from [^{3}H]-NMS binding. The m2 receptor subtype was enriched in the occipital cortex and nucleus basalis. High densities of m4 receptors were measured in the nucleus basalis, hippocampus and parietal cortex. The m3 and m5 receptor proteins were detected at significantly lower levels in all brain regions examined. In Alzheimer's disease, the levels of immunoprecipitated m1 receptors were found to be significantly decreased in frontal and temporal cortices. This observation is in contrast with the normal densities of M1 receptor subtype measured with [^{3}H]-pirenzepine. The levels of m2 receptors were decreased in the frontal cortex and nucleus basalis, while there was a trend toward elevated densities of the m4 receptor subtype in frontal and parietal cortices. These results demonstrate that subtype-specific antisera are suitable for studies of receptor structure and regulation in Alzheimer's disease. (Funded by NS19065 & NS30454).

423.2

IMPAIRED CHOLINERGIC MUSCARINIC RECEPTOR-STIMULATED PHOSPHOINOSITIDE HYDROLYSIS IN ALZHEIMER'S DISEASE. Xiaohua Li*, M.D., Ph.D., Ling Song, M.D., Richard S. Jope, Ph.D., and Richard Powers, M.D. Dept. of Psychiatry and Behavioral Neurobiology, Univ. of Alabama, Birmingham, AL 35294.

The effect of Alzheimer's disease on the activity of the second messenger-producing system of receptor-coupled phosphoinositide hydrolysis was studied by measuring the hydrolysis of [^{3}H]phosphatidylinositol (PI) incubated with membranes prepared from postmortem human prefrontal cortex. This second messenger system consists of receptors coupled with G-proteins which activate phospholipase C. The activity of phospholipase C, assessed in membranes by stimulation with calcium, was similar in Alzheimer's disease and control tissue. Activation of G-proteins with GTPγS, a stable analog of GTP, and activation of cholinergic muscarinic receptors with carbachol resulted in less [^{3}H]PI hydrolysis in Alzheimer's disease than control membranes. Western blots demonstrated that the concentration of Gq, the G-protein most likely functional in phosphoinositide metabolism, was unchanged in Alzheimer's disease compared with controls, indicating that the function of the receptor-G-protein complex was the site of the impairment in Alzheimer's disease. These results indicate that postsynaptic muscarinic receptor responses are impaired in Alzheimer's disease, a finding that may explain in part the limited therapeutic responses achieved by administration of cholinomimetics to patients with Alzheimer's disease. Also, this assay provides a means to identify cholinomimetics that are most efficacious in activating muscarinic receptor-coupled phosphoinositide hydrolysis in Alzheimer's disease brain, agents which should have the greatest potential for providing therapeutic responses.

423.3

SUBTYPE SELECTIVE MUSCARINIC AGONISTS: 'IN VITRO' PHARMACOLOGY OF A SERIES OF 1-AZABICYCLO-[2.2.1]HEPTAN-3-ONE OXIMES. R.E. Davis*, P.D. Doyle, J.C. Jaen, D.J. Lauffer, C. Raby, R. Schwarz, H. Tecle and A.J. Thomas, Parke-Davis Pharmaceutical Research, Warner-Lambert Co., Ann Arbor, MI 48105.

The 'in vitro' biochemical profile of a series of novel 1-azabicyclo-[2.2.1]heptan-3-one oximes was assessed. These compounds bind with agonist-like properties to muscarinic receptors in membranes from rat neocortex. In genetically engineered CHO cells selectively expressing a single muscarinic receptor subtype, these compounds exhibit between 2 and 18 fold higher affinity for m1 relative to m2 muscarinic receptor subtypes. In most cases these agents bind with equal affinity to m_1, m_3 and m_4 receptor subtypes. However, some compounds from this series stimulate accumulation of phophatidylinositides in cells selectively expressing the m_1 receptor (CHO_{m1}) but not in cells expressing the m_3 and m_5 receptor subtypes. Further, these agents do not inhibit forskolin-stimulated accumulation of c-AMP in cells selectively expressing m_2 (CHO_{m2}) and m_4 receptors subtypes. Several compounds from this series also increase cellular metabolic activity in CHO_{m1} but not CHO_{m2} cells. Based on these 'in vitro' properties, selected compounds from this series appear to be functional agonists in CHO_{m1} cells and antagonists or weak partial agonists in CHO_{m2-4} cells.

423.4

SUBTYPE SELECTIVE MUSCARINIC AGONISTS: 'IN VIVO' PHARMACOLOGY OF A SERIES OF 1-AZABICYCLO-[2,2,1]HEPTAN-3-ONE OXIMES. M. J. Callahan*, W. J. Lipinski, J. C. Jaen, D. J. Lauffer, H. Tecle, A. J. Thomas and R. E. Davis, Parke-Davis Pharmaceutical Research Div., Warner-Lambert Co., Ann Arbor, MI 48105.

A series of novel subtype selective m_1 muscarinic agonists were assessed for effects on peripheral and central cholinergic activity in rodents and rhesus monkeys. In rats at high doses (>32.0 mg/kg) core body temperature and total power in neocortical EEG activity was decreased in a manner similar to nonselective muscarinic agonists. Unlike other muscarinic agonists however, gastrointestinal motility was decreased rather than increased at these high doses. In addition, overt increases in salivation, lacrimation and diarrhea were not seen at any dose up to 320.0 mg/kg. No changes were seen in neocortical EEG activity in rhesus monkeys at doses up to 3.2 mg/kg. At low doses (1.0-10.0 mg/kg), selected compounds from this series improved the water maze performance of C57/B10j mice. These data suggest that compounds from this series have unique pharmacologic profiles that are divergent from non-selective muscarinic agonists. Selected compounds from this series improve cognitive performance without increasing muscarinic activity in the periphery. At low doses these compounds appear to act as m_1 agonists while at higher doses these compounds possess muscarinic antagonist-like properties.

423.5

WITHDRAWN

423.6

THE MEMORY ENHANCER LINOPIRDINE INCREASES C-FOS EXPRESSION IN CEREBRAL CORTEX OF AGED RATS. G. Dent*, S.W. Tam and R. Grzanna, CNS Diseases Research, The Du Pont Merck Pharmaceutical Company, Wilmington, DE 19880-0400.

The induction of the immediate-early gene c-fos has become a widely used tool to identify the sites of action of pharmacological agents in the central nervous system. This study was conducted to determine the response of the c-fos gene in neurons of cerebral cortex to the memory enhancer linopirdine in 2 month and 30 month old rats. Rats received i.p. injections of either vehicle (distilled water) or 10 mg/kg of linopirdine. Two hours later animals were perfused and brain sections were processed for immunohistochemistry using an antibody that recognizes Fos but not Fos related antigens.

In 2 month old vehicle-injected rats, Fos positive nuclei were present in several cortical areas including anterior cingulate, piriform, auditory and entorhinal cortex. Linopirdine did not noticeably increase Fos staining in this age group. Compared to Fos staining in young rats, staining in 30 month old vehicle-injected rats was decreased in all cortical regions and nearly undetectable in anterior cingulate and piriform cortex. Linopirdine dramatically increased Fos staining in cerebral cortex in this age group. The effect was most pronounced in anterior cingulate, somatosensory, piriform, visual and auditory cortex.

The results reveal substantial changes in c-fos gene expression with aging. The striking effect of linopirdine on c-fos in cortical neurons of aged rats may be related to the memory-enhancing effects of this drug.

423.7

Effects of Linopirdine (DuP 996) on the KCl, Veratridine, NMDA and Electrically Induced Release of [^{3}H]Acetylcholine from Superfused Brain Slices. R. Zaczek*, C. Maciag and W. J. Tinker The Du Pont Merck Pharmaceutical Co., Wilmington, Delaware.

Linopirdine, a drug which improves the performance of rodents in several learning paradigms, has been shown to enhance the K^+-stimulated release of [^{3}H]acetylcholine ([^{3}H]ACh), [^{3}H]dopamine and [^{3}H]serotonin from slices prepared from several rat brain areas without affecting their basal efflux. The present series of studies was performed to assess the ability of the drug to enhance the release of [^{3}H]ACh stimulated by means other than high K^+ concentration. In contrast to the effects of linopirdine to enhance K^+ stimulated release, 10 µM linopirdine had no effect on [^{3}H]ACh release from striatal slices exposed to 10 to 100 µM NMDA. The drug also failed to enhance electrically induced [^{3}H]ACh release from cortical slices under a variety of conditions such as different stimulating currents, durations of stimulation and linopirdine concentrations. In experiments run in parallel with linopirdine, the potassium channel blocker 4-aminopyridine (10 µM) enhanced release by 165% over control demonstrating that, under the conditions used, electrically induced [^{3}H]ACh release can be enhanced by potassium channel blockade. The effects of linopirdine on cortical [^{3}H]ACh release stimulated by the sodium channel agonist veratridine were similar to those observed in experiments using 25 mM K^+ to stimulate release. Linopirdine enahanced the release with an EC_{50} of approximately 5 µM and gave rise to a maximal effect of approximately 300 % of control values. The linopirdine dose response curves for enhancing K^+- and veratridine-stimulated release were somewhat different at high concentrations of the drug. While the enhancement of K^+-stimulated [^{3}H]ACh release decreased as the concentration of linopirdine was raised above 10 µM, no decrease in release enhancement was observed when using veratridine as the stimulus. The results of the present study suggests that linopirdine enhances the release of [^{3}H]ACh evoked by agents giving rise to a sustained depolarization (high K^+ and veratridine). The drug was not effective when the stimulus was pulsatory as in the case of electrically induced release or when the stimulus involved opening channels that allow the direct entry of calcium as is the case with NMDA.

423.8

Effects of Linopirdine (DuP 996) on Hippocampal Extracellular Levels of Acetylcholine in Freely Moving Animals M. Marynowski*, C. Maciag and C.M. Rominger, D. Rominger, S.W. Tam and R. Zaczek, The Du Pont Merck Pharmaceutical Co., Wilmington, Delaware.

Linopirdine , a drug which improves the performance of rodents in several learning paradigms, has been shown to enhance the release of several transmitters in vitro. While epidural cup experiments have described increases in extracellular acetylcholine (ACh) levels after the administration of linopirdine, the central effects of the drug on brain ACh levels in vivo have not been described. In the present study, microdialysis was utilized to determine the effect of linopirdine on apparent ACh release in vivo. Dialysis probes were placed into the dorsal hippocampus and were perfused with artificial CSF containing 100 µM physostigmine. ACh levels were measured using on-line HPLC with EC detection at a platinum electrode. Doses of 1, 5 and 10 mg/kg were injected peripherally into freely moving rats. Preliminary data indicated that 1 mg/kg linopirdine had no effect on extracellular ACh levels. At 5 mg/kg, linopirdine produced a maximum 42% increase in extracellular ACh levels 40 min after drug administration. ACh levels remained elevated (~ 20% over basal release) up to 100 min, but were back to control levels by 2 hours after dosing. A 59% increase in ACh levels over basal was observed 40 min after the administration of 10 mg/kg linopirdine; extracellular ACh levels plateaued at 25-30% over basal and remained elevated for the duration of the experiment (2 hours). Brain concentrations of linopirdine following peripheral administration were measured using [^{3}H]linopirdine. Levels of linopirdine equivalent to 1.1 µM were observed in the hippocampus 40 min after a 10 mg/kg, i.p. dose. This concentration is consistent with that at which release enhancement has been observed in in vitro release assays (EC_{50} ~5 µM). In summary, linopirdine at doses of 5 and 10 mg/kg produced increases in hippocampal extracellular ACh in freely moving rats. The concentrations of hippocampal linopirdine resulting in increased extracellular ACh levels in vivo are consistent with those resulting in enhanced release in vitro.

423.9

The role of Ca^{2+} Channels, Adenosine, and Ca^{2+} Stores on KCl Evoked Acetylcholine Release and Linopirdine (DuP 996) Release Enhancement in Rat Hippocampal Slices. J.A. Saydoff* and R. Zaczek. The Du Pont Merck Pharmaceutical Co., Wilmington, DE 19880.

We used a paradigm of K^+-stimulated [^{3}H]acetylcholine release from superfused rat hippocampal slices to test for possible interactions between the neurotransmitter release enhancer linopirdine and known Ca^{2+} channels, adenosine receptors or intracellular Ca^{2+} stores. Linopirdine (10 μM) increased (> 200% of control) 25 mM K^+-evoked acetylcholine (ACh) release. The nonspecific Ca^{2+} channel antagonist +/-verapamil (5-200 μM) dose-dependently blocked ACh release and linopirdine release enhancement completely. The N type Ca^{2+} channel antagonist ω-conotoxin GVIA (250 nM) and the P type Ca^{2+} channel antagonist, ω-agatoxin IVA (400 nM) each blocked ACh release by 50% and 30% respectively and were additive. However, ACh release enhancement by linopirdine was unaffected by N and/or P type Ca^{2+} channel blockade. The L type Ca^{2+} channel antagonist nitrendipine (5 μM) did not affect ACh release or linopirdine release enhancement. While adenosine agonists acting through type 1 receptors inhibited ACh release by 30%, blockade of adenosine 1 and/or 2 receptors did not alter ACh release. Adenosine receptor blockade or stimulation or phosphodiesterase inhibition (100 μM IBMX) did not influence the release enhancing effects of linopirdine. Presumed depletion of caffeine sensitive intracellular Ca^{2+} stores with 20 mM caffeine decreased ACh release 25% and attenuated the release enhancing effects of linopirdine by 35%. These results suggest that K^+ evoked release of hippocampal ACh is under the control of N and P, but not L type Ca^{2+} channels and that linopirdine does not interact with these channels. Adenosine 1 receptor stimulation can inhibit K^+ evoked ACh release. Linopirdine does not enhance ACh release via adenosine receptors or phosphodiesterase inhibition. Further studies are required to establish whether caffeine sensitive Ca^{2+} stores may contribute to K^+ evoked hippocampal ACh release. The effects of caffeine and verapamil in combination with linopirdine on $[Ca^{2+}]_i$ are under study.

423.10

EFFECTS OF LINOPIRDINE AND DUP 921 ON AGE-RELATED IMPAIRMENTS IN MEMORY AND ON THE CHOLINERGIC SYSTEM. M. G. Baxter[1], K. W. Rohrbach*[2], S. W. Tam[2], R. Zaczek[2], and D. S. Olton[1]. [1]Department of Psychology, The Johns Hopkins University, Baltimore, MD 21218, and [2]CNS Diseases Research, The DuPont Merck Pharmaceutical Company, Experimental Station, Wilmington, DE 19889-0400.

Linopirdine (DuP 996) enhances learning and memory, which may be due to its ability to enhance potassium-stimulated release of acetylcholine, dopamine, and serotonin. The present study assessed the ability of linopirdine and a related compound, DuP 921 (5,5-bis(4-pyridinylmethyl)-5H-cyclopenta-[2,1-b:3,4-b'] dipyridine), to ameliorate age-related memory deficits. A parallel study assessed the effects of both compounds on *in vitro* potassium-stimulated acetylcholine release, acetylcholinesterase activity, and muscarinic and nicotinic receptor binding in cerebral cortical tissue from young rats. Aged male Fischer-344 rats (24 months old) were given either vehicle or one of 5 doses (0.085-8.5 mg/kg) of linopirdine or DuP 921 prior to testing; young rats (4 months old) served as controls and received vehicle prior to testing. Place discrimination and repeated place acquisition were tested in the water maze. Sensorimotor skills were assessed in separate tests. Age-related impairments occurred in all tasks. Linopirdine and DuP 921 moderately improved place discrimination performance at some doses, but were ineffective in repeated acquisition or sensorimotor tasks. Both linopirdine and DuP 921 enhanced potassium-stimulated release of acetylcholine from rat cerebral cortical tissue. Neither compound produced significant acetylcholinesterase inhibition, muscarinic or nicotinic binding. These results support the hypothesis that linopirdine and DuP 921 ameliorate age-related memory impairments through an enhancement of acetylcholine release. These compounds have potential therapeutic uses in treating disorders involving cognitive impairment.

423.11

IN VIVO MICRODIALYSIS AND PHARMACOKINETIC STUDIES WITH DuP-996. T.M. Smith*, A.D. Ramirez, S.D. Heck, V.J. Jasys, R.A. Volkmann, J.T. Forman, D.R. Liston Pfizer Central Research, Groton, CT 06340.

A reduction in cholinergic function is a consistent neurochemical finding in Alzheimer's disease (AD). Neurotransmitter release enhancers such as DuP-996 (3,3-bis(4-pyridinylmethyl)-1-phenylindolin-2-one) may restore cholinergic function by increasing the extracellular pool of acetylcholine (ACh). *In vitro*, DUP-996 enhances the potassium-evoked release of ACh, dopamine and serotonin with an EC50 of 1-2 μM. To assess whether this activity is sufficient to stimulate neurotransmitter release *in vivo*, brain exposure to DuP-996 was determined in conjunction with *in vivo* microdialysis studies. Rats were injected with DuP-996 (10 mg/kg, s.c.); at 10, 30 and 60 minutes plasma and whole brain were sampled and analyzed by HPLC. Drug levels were as follows:

time (min)	plasma (μM)	Brain (μM)	B/P ratio
10	3.8	1.2	0.33
30	3.6	0.9	0.25
60	1.6	0.6	0.37

For *in vivo* microdialysis, samples were collected every 30 min; after a 2 hr baseline period, DuP-996 was administered (10 mg/kg, s.c.) and dialysis continued for an additional 3 hr. ACh levels were determined by HPLC. DuP-996 produced a 30-40% increase in the extracellular concentration of ACh. These studies show that the peripheral administration of DuP-996 (10 mg/kg) produces detectable brain levels of drug resulting in an increase in ACh release, suggesting that these agents have potential for the treatment of AD.

423.12

SYSTEMATIC REGIONAL VARIATIONS IN THE LOSS OF CORTICAL CHOLINERGIC INNERVATION IN ALZHEIMER'S DISEASE. C. Geula* and M.-M. Mesulam. Department of Neurology, Harvard Medical School, Boston, MA 02215.

The relative density of AChE- and ChAT-positive cortical cholinergic fibers was determined in whole hemispheric sections of brains from Alzheimer's disease (AD) patients and controls. The areas with the greatest loss (>75% reduction) of cholinergic innervation were all in the temporal lobe and included areas 20, 21, 22 and 28 of Brodmann. The frontal and parietal association areas as well as the insula and temporal pole showed an intermediate magnitude of loss (40-75%). The anterior cingulate gyrus, primary motor, primary somatosensory and primary visual cortex displayed less than 30% loss of cholinergic fibers. Within the CA1 sector of the hippocampal formation and the subiculum, fiber density was reduced by 41-48%. In the amygdala, the lateral nucleus displayed a marked depletion of ChAT-positive neurites (88%) whereas the basolateral (41%) and particularly the central nucleus (21%) displayed relatively little change. These results indicate marked and consistent regional variations in the loss of cortical cholinergic fibers in AD.

423.13

DISTRIBUTION OF NEUROFIBRILLARY TANGLES IN ALZHEIMER'S DISEASE STRIATUM. N. Selden*, C. Geula and M.-M. Mesulam. Harvard Medical School, Boston, MA 02215.

The distribution of neurofibrillary tangles (NFT) was surveyed in the striatum of Alzheimer's disease (AD) cases (n=9) using the fluorochrome, Thioflavin-S. NFT were more densely distributed in the nucleus accumbens (175/cm^2), olfactory tubercle (249/cm^2) and tail of the caudate nucleus (323/cm^2) than in the head of the caudate nucleus (54/cm^2), body of the caudate nucleus (81/cm^2) or putamen (44/cm^2). Senile plaques (SP) with a prominent amyloid core showed a similar pattern of distribution to NFT. By contrast, non-cored SP were densely distributed in all striatal territories. The concentration of NFT in the nucleus accumbens and olfactory tubercle was not likely to result from contraction atrophy: the area of the nucleus accumbens and olfactory tubercle in AD cases was 99%, of the caudate nucleus 92%, and of the putamen 88% of control values. In aged, neurologically normal controls and a case of Pick's disease, no NFT and few SP were observed in the striatum. The nucleus accumbens, olfactory tubercle and tail of the caudate nucleus all receive dense projections from temporolimbic cortical regions which are selectively vulnerable to the pathology of AD. Our findings suggest that the 'limbic' striatum is preferentially vulnerable to damage in AD.

423.14

RETROGRADE DEGENERATION OF BASAL FOREBRAIN CHOLINERGIC NEURONS FOLLOWING HIPPOCAMPAL IMMUNOTOXIN INJECTIONS. T. Ohtake, S. Heckers, C. Geula, S. Weintraub*, R.G. Wiley+ and M-M. Mesulam. Dept. of Neurology, Harvard Medical School, Boston, MA 02215; +Dept. of Veterans Affairs, Medical Center, Nashville, TN 37212.

Intracerebroventricular injection of the 192 IgG antibody against the p75 nerve growth factor receptor (NGFr) conjugated with saporin destroys the NGFr-containing cholinergic neurons of the basal forebrain. We injected this immunotoxin directly into the hippocampus and studied its retrograde effect upon the cholinergic neurons of the medial septum and diagonal band region (MS-DB).

After 7 days, there was a nearly total depletion of cholinergic axons within the hippocampus and a marked ipsilateral decrease in the number of cholinergic neurons of the MS-DB. At longer survival times, these changes were more pronounced and seemed to have spread to the contralateral side. These findings are consistent with those of Kudo et al. (Neurosci. Lett. 102:125; 1989).

These observations suggest that injected 192 IgG-saporin is transported retrogradely from the hippocampus to the cholinergic neurons in the MS-DB and provide a model for the retrograde degeneration of basal forebrain cholinergic neurons following cortically based pathological processes. Such retrograde degeneration may be responsible for the involvement of cortically projecting subcortical cell groups in Alzheimer's disease.

423.15

NEUROGLIAL CHOLINESTERASES IN THE NORMAL BRAIN AND IN ALZHEIMER'S DISEASE: RELATIONSHIP TO PLAQUES, TANGLES AND PATTERNS OF SELECTIVE VULNERABILITY. C.I. Wright, C. Geula and M-M. Mesulam*. Department of Neurology, Beth Israel Hospital, Harvard Medical School, Boston, MA 02215.

Butyrylcholinesterase (BChE) and an altered form of acetylcholinesterase (AChE) accumulate in the plaques and tangles of Alzheimer's disease (AD). The sources for these plaque and tangle-bound cholinesterases have not been identified. We now report that AChE and BChE activities with pH preferences and inhibitor selectivities identical to those of plaque and tangle-bound cholinesterases are found in the astrocytes and oligodendrocytes of control and AD brains. In non-AD control brains, the ratio of BChE to AChE glia was higher in entorhinal and inferotemporal cortex, two regions with a high susceptibility to the pathology of AD, than in primary somatosensory and visual cortex, two areas with a relatively lower susceptibility to the disease process. In comparison to age-matched control specimens, AD brains had a significantly higher density of BChE glia and a lower density of AChE glia in entorhinal and inferotemporal regions but not in the primary somatosensory or visual areas. These results suggest that glia constitute a likely source for the cholinesterase activity of plaques and tangles, and that a high ratio of BChE to AChE glia may play a permissive or causative role in the neuropathology of AD.

423.16

COMPARATIVE INHIBITION BY TACRINE AND RELATED AMINOACRIDINES ON ACETYLCHOLINESTERASE AND BUTYRYLCHOLINESTERASE. K.A.Skau*. U. Cincinnati College of Pharmacy, Cincinnati, OH 45267.

Aminoacridine derivatives are undergoing clinical trials to alleviate symptoms of Alzheimer's disease. Although the effectiveness of these drugs is believed to be due to inhibition of acetylcholinesterase (AChE), they have a number of actions on other enzymes and receptor proteins. We have investigated the inhibitory action on the related enzyme butyryl-cholinesterase (BuChE). Rat brain AChE and BuChE were purified on trimethyl-m-phenylenediamine and procainamide affinity columns. Enzyme activity was estimated with a colorimetric assay using acetylthiocholine as substrate. Tacrine, SM10888 and HP029 exhibited the same rank order of inhibition on both enzymes. IC50's for AChE vs BuChE were (nM): tacrine 67 vs 38; SM10888 190 vs 100; HP029 640 vs 330. Kinetic analysis showed a mixed inhibition by these drugs on both enzymes. Replots indicated that the non-competitive inhibitory constant was lower than the competitive constant for both enzymes. A tacrine affinity column bound AChE but not BuChE. These results suggest that aminoacridines are slightly more potent inhibitors of BuChE than AChE indicating that tissues with a significant BuChE content (such as heart and liver) may exhibit adverse effects from these drugs. Furthermore, although the potencies are similar, the structural requirements for inhibition of the two enzymes are quite different.

DEGENERATIVE DISEASE: ALZHEIMER'S—OTHER IV

424.1

INCREASED GLIAL REACTIVITY TO BRAIN INJURY IN AGING RATS. M.N. Gordon*, L.A. Holcomb, W.A. Schreier and D.G. Morgan. Dept. of Pharmacology and Therapeutics, Univ. South Florida College of Medicine, Tampa, FL 33612-4799.

Although clinical literature suggests that brain injury in the aged is associated with a poorer prognosis, little research has examined this phenomenon at a cellular or molecular level. We examined astrocyte and microglial reactivity after 6-hydroxydopamine (6-OHDA) lesions of the nigrostriatal system in male F344 rats aged 6, 15 or 24 mo. Rats were killed 2,4,7,10 or 14d after lesioning. Sections through the substantia nigra (SN) and neostriatum were immunocytochemically stained for tyrosine hydroxylase (TH) to quantify lesion severity, for the astrocyte specific marker glial fibrillary acidic protein (GFAP), and for the microglial specific markers complement C3b receptor (OX42) and MHC class II antigen (OX6). Unlesioned rats served as controls.

In control rats, the area immunostained for GFAP, OX42 and OX6 were all increased with aging. In lesioned rats, substantial loss of TH+ neurons in the SN was observed 2d after 6-OHDA injection and was maximal at 14d, producing virtually complete loss of TH immunoreactivity in the SN and neostriatum in rats of all ages. A lesion-induced elevation of neostriatal immunostaining for GFAP, OX42 and OX6 was observed at all ages, but the magnitude of this response over control levels was greatest in the oldest rats. These data confirm our earlier work concerning exaggerated GFAP reactivity to fornix transections in aged mouse hippocampus, and extend this finding to dopamine depleted rats, and to microglia as well. Exaggerated glial reactivity in the aged after brain injury may have deleterious consequences. These findings support the inflammation/complement theories of Alzheimer's pathogenesis. Technical assistance by David G. Berg, Xiaorong Ou and Chun Wang is gratefully acknowledged. Current affil. for WAS: Baxter Diagnostics, Inc., Clinical Chemistry Controls R&D, Miami, FL. Supported by AG07892 (astrocytes) and AFAR (microglia). DGM is an Established Investigator of the American Heart Assn.

424.2

EXPRESSION OF GFAP AND S-100β GENES IN LYMPHOCYTES OF DOWN SYNDROME (DS) PATIENTS. H. Riol*, G. Lévesque, M. Tardy, L. Demers and M.R.V. Murthy. Departement de biochimie, Faculté. de Médecine, Université Laval, Québec, Qué, G1K 7P4 Canada.

After 40 years of age, persons with DS develop neuropathological features of Alzheimer's disease (AD) which suggest that DS may constitute a precocious model for AD. We have previously shown that glial fibrillary acidic protein (GFAP) and S-100β genes were expressed in human lymphocytes and that their mRNA levels were perturbed (respectively increased and decreased) in lymphocytes of AD patients with moderate to middle mental deterioration. In this study, we are interested to test if perturbations of these brain specific gene expressions occur also in DS lymphocytes, and to analyse the relationship between the level of their expression and the age of the patients. Therefore, we have investigated the GFAP and S-100β mRNA levels in lymphocytes of 11 DS patients (25-47 years old), in comparison to normal controls.

Total RNA was extracted from lymphocytes and after a reverse transcription (RT) step, the mRNA sequences were amplified by polymerase chain reaction (PCR). We have developped a semi-quantitative method of RT-PCR to quantify their relative amounts, using endogenous aldolase C mRNA level as internal standard.

As expected, a surexpression of the S-100β gene was found in lymphocytes of DS patients, reflecting trisomy of chromosome 21 containing this gene. However, the resulting amount of S-100β-mRNA was three times the level in normal individuals ($p=0.0001$), suggesting that mechanisms other than gene dosage were also implicated. A similar level of S-100β-mRNA was found whatever the age of the DS patients, indicating that no further change in the expression of this gene occurs from the age of 25 years to 47 years. In contrast, some increase in the GFAP-mRNA level was observed in lymphocytes of DS patients after the age of 36 years and may be associated with an early stage of AD in older DS patients, since such increase has been previously observed in lymphocytes of AD patients. However, this increase is presently under further verification with a larger group of DS patients to check if it is statistically signifant.

424.3

MICROGLIAL DISTRIBUTION IN NORMAL CEREBRAL CORTEX CORRESPONDS TO REGIONAL LOCALIZATION OF ALZHEIMER'S DISEASE (AD) PATHOLOGY. L.G. Sheffield and N.E.J. Berman*. Dept. of Anatomy and Cell Biology, Univ. of Kansas Med. Ctr., Kansas City, KS 66160-7400.

Microglia function as macrophages during development, injury and pathological processes such as AD. This role may extend to direct involvement in the disease process as microglia have been found in association with neuritic plaques (NPs). Regional localization of neurofibrillary tangles (NFTs) and NPs is the hallmark of AD pathology. Those areas most vulnerable to NFTs are the entorhinal cortex, hippocampus and amygdala, while the motor, visual and auditory cortices are largely spared (Arnold et al., Cerebral Cortex, 1:103-116, 1991).

Our study focused on the distribution of microglia in the normal brain. Samples were taken from those cortical areas with the highest and lowest density of NFTs and NPs. Microglia were visualized using the lectin *Ricinus communis* agglutinin-1 (RCA-1; Hutchins et al., Developmental Brain Research, 55:95-102, 1990). Our initial results reveal a higher density of microglia in cortical areas most susceptible to AD pathology and fewer microglia in areas least affected by AD. Interestingly, an area of low microglial density, i.e. motor cortex, had a high density of blood vessels, but an area of high microglial density, i.e. temporal lobe, had a low density of blood vessels. One explanation for the regional variability of AD pathology may be the larger microglial presence in susceptible areas. Supported by MH38399 and HD02528.

424.4

EXPRESSION OF GLUT5 ON MICROGLIA IN NORMAL AND AD BRAIN. J. Payne*,[1] L.A. Mattiace,[1] F. Maher,[2] I.A. Simpson,[2] P. Davies[1]. [1]Dept. of Pathology, Albert Einstein College of Medicine, Bronx, NY 10461; [2]NIDDK, NIH, Bethesda, MD 20892.

GLUT5 is a member of a group of structurally related facilitative glucose transporter proteins that are involved in enhancing transportation of glucose and/or fructose across plasma membranes. Although some isoforms are present in non-CNS tissue, GLUT5 is one of three isoforms present in human brain. RaGLUT5, a commercially available (East Acres Biologicals) affinity purified polyclonal antibody raised to a 12 amino acid synthetic peptide specific for the C-terminal of the human GLUT5 sequence, has been used to determine the cellular localization of GLUT5 protein in tissue sections. Formalin-fixed brain tissue was obtained postmortem from both AD and normal individuals. Using the ABC (Vector) procedure, fifty micron consecutive free floating vibratome sections were stained with 1) RCA-1, a lectin that reacts with β-galactose residues that labels microglia in addition to blood vessels, 2) RaGLUT5, 3) GFAP, an antibody that immunolabels astrocytes, 4) Alz-50, an antibody that immunolabels AD-type pathology. In a peptide competition experiment, RaGLUT5 was pretreated with a saturating concentration of the GLUT-5 C-terminal peptide before incubation with tissue sections. Preliminary results suggest that RaGLUT5 immunolabels microglia in both the cortical grey and subcortical white matter in older adult normal and AD brain. Although the intensity of immunoreactivity and number of microglia is consistent in the older normal individuals, considerable variability exists in the AD cases. Morphologically, RaGLUT5 appears to consistently immunolabel the fine tertiary processes of microglia particularly evident in the cortical grey matter. No staining was seen in peptide competition or in the control without antibody. Our results suggest that GLUT5 may mediate glucose uptake by microglial cells in the brain and that GLUT5 is an excellent microglial marker in formalin-fixed human brain tissue.

424.5

MICROGLIAL-INDUCED CYTOTOXICITY OF HIPPOCAMPAL NEURONS BY REACTIVE OXYGEN INTERMEDIATES: SELECTIVE VULNERABILITY TO NITRIC OXIDE L. Torgersen*, C.E. Nolan, and R.B. Nelson, Department of Neuroscience, Pfizer Central Research, Groton, CT.

Neurodegenerative pathologies such as Alzheimer's disease (AD) are characterized by the sparing of superoxide dismutase-containing neurons, suggesting a role for reactive oxygen intermediates (ROIs) in neuronal loss. A likely source of ROIs in AD are activated brain macrophages (microglia), a prominent feature of dense-core β-amyloid plaques. Activation of cultured rat microglia with opsonized zymosan causes the release of high levels of both nitric oxide (NO, measured by formation of the NO breakdown product NO_2) and superoxide (measured by luminol-enhanced chemiluminescence). The relative contribution of individual ROIs in killing hippocampal neurons was examined by establishing a co-culture system of freshly-plated primary hippocampal neurons and microglia plated in 0.4 μm filter insert chambers. Activation of the microglia caused the death of neurons situated immediately under the insert chambers, but not of neurons outside the perimeter of the insert chambers (indicative of a short-lived toxin). This death could be completely blocked by the NO synthase inhibitors N-monomethyl-L-arginine and N-nitro-L-arginine; the relative potencies of these inhibitors indicated targeting of the inducible macrophage NO synthase rather than the constitutive neuronal NO synthase. Inhibitors of other ROIs, including superoxide dismutase (target species O_2^-), catalase (H_2O_2), mannitol ($OH^\bullet$), and sodium azide (HOCl) were ineffective at preventing neuronal death beneath the chamber inserts. While activated microglia also release large amounts of glutamate, freshly plated neurons are insensitive to this type of toxicity, and the NMDA receptor antagonist MK-801 was unable to protect against neuronal loss in this culture system. These results indicate that microglia are able to release levels of NO toxic to neurons, and that hippocampal neurons may have greater sensitivity to NO than to other reactive oxygen intermediates.

424.6

ACTIVATED MICROGLIA CAUSE NEURONAL DEATH BY RELEASE OF GLUTAMATE AND NITRIC OXIDE C.E. Nolan, L. Torgersen, M. Prochniak, I.A. Shalaby, and R.B. Nelson*, Department of Neuroscience, Pfizer Central Research, Groton, CT.

Dense-core β-amyloid plaques, each associated with multiple activated microglia (brain macrophages), are a major hallmark of Alzheimer's disease (AD) and indicate a massive inflammatory component of AD. Because chronically activated macrophages in peripheral inflammatory diseases release a number of cytotoxins and cause extensive local tissue damage, we investigated whether activated microglia release substances directly toxic to neurons. Activation of cultured rat microglia with complement-opsonized zymosan caused basal release of glutamate (measured by HPLC) to increase from subtoxic (5 μM) to toxic levels (150 μM) in the culture media by 24 h. Exposure of >2-week-old primary hippocampal neurons (glutamate-sensitive) to activated microglial supernatants for 15 min caused extensive neuronal loss which could be blocked with the NMDA receptor antagonist MK-801. Activated microglia also released high levels of nitric oxide (NO, measured by formation of the NO breakdown product NO_2) *versus* undetectable release from non-activated microglia. NO-mediated neurotoxicity was detected in co-cultures of freshly-plated primary hippocampal neurons (glutamate-insensitive) and microglia plated in 0.4 μm filter insert chambers. Activation of the microglia caused the death of neurons situated immediately under the insert chambers, but not of neurons outside the perimeter of the insert chambers (indicative of a short-lived toxin). This death could be completely blocked by the macrophage NO synthase inhibitor N-monomethyl-L-arginine. These results indicate that: 1) microglia are able to release toxic levels of recognized neurotoxins; and 2) activated microglia associated with dense-core β-amyloid plaques may cause AD-associated neurodegeneration by local neurotoxin release.

424.7

BRAIN CLUSTERIN (SGP-2): SECRETION BY CULTURED ASTROCYTES AND NEURONS

T. Oda*, G. M. Pasinetti, T. H. Hogan, S. A. Johnson and C. E. Finch. Andrus Gerontology Center, Neurogerontology Division and Dept. of Biological Sciences, University of Southern California, Los Angeles, CA 90089-0191

Clusterin is a sulfated glycoprotein (SGP-2) that is found in brain and many other peripheral tissues. Several functions have been proposed for clusterin such as inhibiting complement mediated cell lysis, cell aggregation, lipid transport and apoptosis (P. C. May and C. E. Finch, TINS, 1992). Elevation of clusterin expression in the brain is associated with experimental lesioning in rodent, responses to injury and Alzheimer's disease.

We have found clusterin mRNA and secretion of protein from the primary cultures of rat astrocytes, human astrocytoma (HTB 14) and human neuroblastomas (HTB 10 and HTB 11). To further elucidate the role of clusterin in brain, we purified clusterin from the conditioned media of human astrocytoma. The molecular weight was about 70KDa under non-reducing conditions and about 41KDa under reducing conditions, respectively. Ongoing experiments examine the effect of glia-derived clusterin on complement mediated cytotoxicity and transformed neuronal cell lines and primary neuronal culture. Supported by grants to CEF from NIA AG-7909 and Sankyo Co.

424.8

INCREASE IN NADPH-d NEURONS WITHIN ALZHEIMER BASAL FOREBRAIN: COMMENTS ON FIXATION SENSITIVITY OF NADPH-D HISTOCHEMISTRY. W.C. Benzing* and E.J. Mufson, Dept. of Neurol. Sci., Rush Presb. St. Luke Med. Ctr., Chicago, IL 60612.

NADPH-d, a cofactor in the formation of nitric oxide, colocalizes with nitric oxide synthase (NOS) containing CNS neurons. Excess nitric oxide synthesis by NADPH-d/NOS neurons may be neurotoxic within the CNS. Interestingly, these neurons are spared in the caudate and hippocampus, areas of severe degeneration in Huntington's and Alzheimer's (AD) disease, respectively. We determined whether NADPH-d histochemically stained neurons were similarly spared within the cholinergic basal forebrain, which undergoes extensive neuronal loss in AD compared to normal controls (NC). AD (n=8) and NC (n=8) subjects were matched for age, sex, brain weight, post-mortem interval and fixation length. Paraformaldehyde fixation periods greater than 36-48 hours greatly reduces the NADPH-d reaction product. Numbers of light, medium and darkly stained NADPH-d neurons were quantified using a computer assisted system. Quantitative analysis revealed a statistically significant increase in the number of medium and dark stained NADPH-d neurons within the anterolateral and posterior divisions of the nucleus basalis (NB) in AD as compared to NC ($F[2,42] = 4.95$; $p \leq .05$). Interestingly, the cholinergic neurons within these NB subfields are the most severely depleted in AD (Mufson et al., '89). Moreover, many NADPH-d containing fibers were in close apposition to cholinergic magnocellular neurons in AD and NC. Increased numbers of NADPH-d neurons within the basal forebrain may be a mechanism underlying the severe cholinergic cell death seen in AD.

424.9

THE MECHANISM OF LIPID PEROXIDATION MAY VARY DEPENDING UPON THE BRAIN REGION: POSSIBLE IMPORTANCE FOR NEURODEGENERATIVE DISEASES. A.C. Andorn*, M. Franko and B.R. Bacon. Depts. of Psychiat. and Human Behavior, and Med. St. Louis Univ. Schl. of Med., and St. Louis VAMC, St. Louis, MO 63125.

We have previously reported that ascorbate stimulated lipid peroxidation (LP_{asc}) in postmortem human caudate and putamen (C/P) is apparently not mediated by hydroxyl radicals (Mol. Pharm.33:155-162,1987). We, and others, have previously reported that basal and stimulated LP is increased in postmortem prefrontal cortex (PFC) from Alzheimer's disease patients and in C/P of Parkinson's disease patients (Dexter, et al., J. Neurochem.52:381-389,1989;Subbarao, et al. J. Neurochem.55:342-345, 1990; Hajimohammadreza and Brammer, Neurosci. Lett.112:333-337,1990). We now report that LP_{asc} may occur by a different mechanism in cortical areas as compared to C/P. In the C/P, hydroxyl-radical scavengers, such as mannitol, were without effect on LP_{asc}, while in PFC and temporal cortex (TC), 10 μM mannitol inhibits 28.0±15% and 37.2±8.5% of LP_{asc} (using 0.1 mM ascorbate) respectively. Similarly, in the C/P, indomethacin (10μM) was without effect on LP_{asc}. But in PFC and TC, this indomethacin inhibited 47.7±22% and 46.9±19.3% of LP_{asc} respectively. These findings suggest that hydroxyl radicals and possibly the prostaglandin pathway play a more marked role in LP in cortical areas than in the basal ganglia.

424.10

DOES URATE PROTECT ASCORBATE FROM METAL-CATALYZED OXIDATION? M. Romanas, F.E. Samson, S.R. Nelson* and T.L. Pazdernik. Ralph L. Smith Res. Ctr., Univ. Kansas Med. Ctr., Kansas City, Kansas 66160.

Oxygen radicals are implicated in a variety of neuropathologies including ischemia-reperfusion, trauma, ALS, and Alzheimer's disease. Ascorbate (ASC) and urate play major roles in neutralizing these radicals. Ascorbate, on the other hand, can reduce transition metals enabling them to participate in oxygen radical generation. Urate may limit this metal-catalyzed oxidation of ascorbate by forming an inactive metal complex. This was studied by following ASC (100 μM) oxidation at 265 nm in Chelex-treated PO_4 buffer (50 mM, pH 7.3) with the addition of aliquots of preincubated solutions containing urate with Cu^{2+}, Fe^{3+}, or Fe^{3+}-EDTA. The rate of ASC oxidation with 12 μM Fe^{3+} was 5 milli-absorbance units/min (mABS U/min), but the rate with 12 μM Fe^{3+}-EDTA was ten-fold higher, 50 mABS U/min. Neither rate was affected by urate (6, 12, 24 μM). Cu^{2+} (1.3 μM) markedly activated ASC oxidation (165 mABS/min) which was inhibited 56, 67, and 84% with 6, 12, and 24 μM urate, respectively. These results indicate that urate, at levels in cerebrospinal fluid (ca. 20 μM), may preserve ascorbate from oxidation catalyzed by Cu^{2+} but not necessarily from that catalyzed by Fe^{3+} or Fe^{3+}-complexes. Supported in part by NIH 1-P30-AG10182.

424.11

Decreased Ratio of Transferrin to Iron in Alzheimer's and Parkinson's Disease Brains.

D. A. Loeffler[1], J. R. Connor[2], B. S. Snyder[2], A. J. DeMaggio[1], P. L. Juneau[3], and P. A. LeWitt*[1], [1]Sinai Hospital, Detroit, MI 48235, [2]M.S. Hershey Medical Center, Hershey, PA 17033, and [3]Warner Lambert Co., Ann Arbor, MI 48106

Brain iron (Fe) metabolism is abnormal in both Alzheimer's disease (AD) and Parkinson's disease (PD), and may contribute to free radical generation in both disorders. The objective of this study was to investigate the relationship between Fe and the Fe transport protein transferrin (TF) in AD, PD, and aged normal brain. Our hypothesis is that there is a decrease in the TF/Fe ratio, suggesting a decrease in brain iron mobility, in some neurological diseases. TF/Fe was highest in frontal cortex, intermediate in caudate and putamen, and lowest in substantia nigra and globus pallidus. TF/Fe was decreased in all AD and PD brain regions, particularly caudate and putamen, where TF/Fe ratios were 47-55% of normal. These results support the hypothesis that iron mobility is decreased in specific brain regions and specific disease states, and imply a close association but potentially significant differences in iron homeostasis between AD and PD. (Supported in part by United Parkinson Foundation)

424.12

FERRITIN ISOFORMS IN NORMAL AND DISEASED HUMAN BRAIN TISSUE. J.R. Connor*, S.L. Menzies, B.S. Snyder, D.A. Loeffler and P.A. LeWitt, Dept. of Neurosci & Anatomy, PSU Sch. of Med., Hershey, PA and Sinai Hospital, Detroit, MI.

Ferritin is the major iron storage protein and as such plays a significant role in regulating the intracellular availability of iron. Because of the ability of iron to generate free radicals, alterations in the synthesis or function of ferritin could result in enhanced susceptibility of cells to oxidative damage. In this study, we use monoclonal antibodies (provided by P. Arosio) to investigate the cellular distribution of the ferritin isoforms, and to quantitate the ratios of the ferritin isoforms in selected regions of human brain tissue (tissue provided by E.D. Bird, McLean hospital). Immunohistochemical analysis revealed specific cellular distributions for the different isoforms. Microglia stain intensely for L-chain ferritin, whereas oligodendrocytes contain both H and L isoforms. Neurons (specifically pyramidal neurons) contain only H-chain ferritin. Astrocytes normally do not contain either ferritin isoform except in the basal ganglia where they stain robustly and specifically for L-chain. In Alzheimer's Disease, considerable L-chain cellular staining occurs around neuritic plaques, while H-chain is relatively uninvolved. Quantitatively, H-chain ferritin was higher than L-chain in each of the brain regions studied with H:L ratios ranging from ~1.1 (globus pallidus; GP) to 2.8 (substantia nigra; SN). The H:L ratio is altered from normal in the caudate and putamen in Parkinson's Disease, but not in frontal cortex, SN, or GP. In AD tissue, the H:L ratio is only altered in the frontal cortex. These results reveal cellular specificity in ferritin distribution and region specific, disease specific changes in H:L ratios.

424.13

AUTORADIOGRAPHIC EXAMINATION OF NADH: UBIQUINONE OXIDOREDUCTASE (COMPLEX I) IN HIPPOCAMPUS AND CEREBELLUM IN ALZHEIMER'S DISEASE DS Higgins*, JT Greenamyre University of Rochester, Rochester, NY 14642

Accumulating evidence implicates impaired energy metabolism in AD, with abnormalities of ferrocytochrome *c* oxidase (complex IV) and the pyruvate dehydrogenase complex having been described. [^{3}H]Dihydrorotenone (DHR) autoradiography identifies the NADH dehydrogenase subunit 1 of NADH:ubiquinone oxidoreductase (complex I) specifically and with high affinity. NADH enhances DHR binding and provides a measure of the interaction between the proximal NADH binding site and the distal rotenone inhibition site. This technique was employed to examine complex I of the electron transport chain in a series of Alzheimer's Disease (AD) and control postmortem samples of hippocampus and cerebellum. [^{3}H]DHR binding was reduced in all regions of the AD hippocampus (48-61% of control) when examined in the absence of exogenous NADH. The granular and molecular layer of the cerebellum contained equivalent amounts of DHR binding in AD and control samples. NADH (500 μM) enhanced binding in all regions 2 - 3 fold. Under enhanced conditions, binding was normal in AD cerebellum and significant decreases were only apparent in CA1 and dentate gyrus (42 and 52% of control, respectively). No association between [^{3}H]DHR binding and age or postmortem delay was identified. Abnormalities of complex I may significantly alter neuronal function as demonstrated in the mitochondrial encephalomyopathies. Whether the changes identified in AD are pathogenic or secondary in nature remains to be determined. *(Supported by USPHS grants NS01487 and T32 AG107)*

DEGENERATIVE DISEASE: ALZHEIMER'S—OTHER V

425.1

Genotypic/Phenotypic Analysis of Apolipo-protein E in Alzheimer's Disease. G.W.Rebeck*, J.S.Reiter, and B.T.Hyman Dept. of Neurology, Mass. General Hospital, Boston, MA, 02114.

We have recently shown that variability in the number of senile plaques between Alzheimer's disease (AD) cases cannot be accounted for by duration or severity of dementia. We have now initiated a study to determine whether expression of specific isoforms of Apolipoprotein E (ApoE) can account for some of this phenotypic variation. A disproportionate number of patients with late-onset AD express the E4 isoform of ApoE (Strittmatter et al., PNAS (1993) 90:1977-1981), suggesting that ApoE may be a risk factor in the development of AD. Furthermore, we have confirmed that anti-ApoE immunostains both diffuse and compact senile plaques, as well as amyloid angiopathy and extraneuronal neurofibrillary tangles. We have determined the ApoE genotype of neuropathologically examined individuals with sporadic AD (n=17) and age-matched controls (n=14) by DNA amplification and restriction digestion with the HhaI enzyme. We found disequilibrium between the ApoE-E4 isoform and AD (62% of AD patients, 22% of controls). In addition, we examined whether AD patients expressing ApoE-E4 differed neuropathologically from patients expressing only ApoE-E2 or ApoE-E3. Those expressing ApoE-E4 did not have a higher number of senile plaques in temporal neocortex (100 vs. 159 plaques/mm2), or a greater amyloid burden (4.7% vs. 6.1%). We are continuing to genotype additional control and AD cases for these analyses. Supported by NIHAG05598 and NIHAG08487.

425.2

QUANTITATIVE ANALYSIS OF NEUROPATHOLOGIC CHANGES IN THE CEREBRAL CORTEX OF CENTENARIANS. C. Bouras * [1,2], P. Giannakopoulos [4], M. Surini [1], J.P. Michel [4], J. Richard [1] and P.R. Hof [2,3] [1]Dept of Psychiatry, Univ. of Geneva, CH-1225, Geneva-Switzerland, [2]Fishberg Research Center for Neurobiology and [3]Dept. of Geriatrics, Mt Sinai Sch. Med., New York, NY 10029, and [4]Institutions Universitaires de Gériatrie, CH-1226, Geneva-Switzerland.

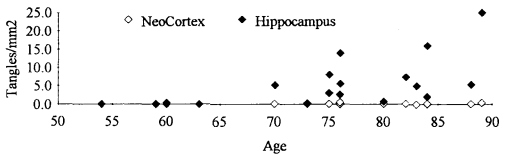
We performed analysis of 67 nonagenarians and centenarians brains (96-104 years old). The patients included 10 men (97.2 ± 1.5 years old) and 57 women (97.5 ± 1.8 years old). Clinical diagnosis was based on review of the medical records and neuropsychological testing. Twenty four patients presented with preserved intellectual abilities and 43 with dementia (15 with senile dementia of the Alzheimer type and 28 with mixed dementia). We assessed quantitatively the localization and distribution of neurofibrillary tangles (NFT) and amyloid deposits (AMLD) using antibodies to Tau and β-A4. In all cases, analysis was performed in the CA1 field of the hippocampus (CA1), the subiculum, in layers II and V of the entorhinal cortex (EC) and in layers II-III and V-VI of the inferior temporal (ITC) and superior frontal cortex (SFC). NFT were present in 100% of the cases in the CA1 and in layers II and V of the EC, 95.2% in subiculum, 95% in layers V and VI of the ITC but only in 44% of the cases in the SFC. AMLD were not always observed. For instance, AMLD were found in 78% of all cases in CA1, 71% in subiculum, 75% in the EC, 80.8% in the ITC and 80% in the SFC. Extensive NFT formation was restricted to the anterior part of the CA1 in demented subjects, whereas the SFC was relatively spared in severaly demented cases as compared to the ITC. Significant differences in NFT counts between demented and non-demented cases were found only in the CA1 and in the SFC. The AMLD densities were significantly higher in the demented patients in all areas. These sugest that NFT formation involve different cortical structures in very old people compared to younger individuals. The NFT densities in the CA1 and in the SFC could be crucial for the neuropathological diagnosis of dementia in this particular group.

425.3

THE DISTRIBUTION AND DENSITY OF TANGLES AND PLAQUES IN NON-DEMENTED AGING. J.L. Price*, M.M. Rundle and J.C. Morris. Depts of Anatomy & Neurobiology and Neurology, Washington Univ. Sch. Med., St. Louis, MO 63110

Tangles and plaques were studied in 24 brains from non-demented subjects aged 54 to 89, with the Bielschowsky method and immunohistochemical stains for paired helical filaments or β-amyloid. The cognitive status of each subject was determined by pre-mortem clinical assessment, and/or by a retrospective post-mortem interview with a close relative; in all cases. The distribution and density of tangles and plaques was mapped from serial sections with a microscope digitizer system.

At least a few tangles were found in all cases, especially in the hippocampal formation. Few tangles were found in the neocortex. The density of tangles in the hippocampus increased progressively with age (Spearman rank correlation = 0.69, $p<0.01$). In contrast, many of the cases had no detectable plaques. Where present, plaques were primarily or exclusively found in the neocortex, with few if any in the hippocampus. The density of plaques showed no relationship to age. This indicates that tangles develop with aging, especially in the hippocampal formation, independent of detectable amyloid deposition. Plaques develop in the neocortex without direct relation to age or to tangles, although amyloid may subsequently exacerbate tangle formation in the hippocampus and elsewhere. NIH AG03991



425.4

CORRELATES OF SUBCORTICAL NEURONAL LOSS IN ALZHEIMER AND PARKINSON DISEASES. C. Zarow*, J.A. Mortimer, S.A. Lyness, H. Chui. *Department of Neurology, Univ. of Southern California, Los Angeles, CA 90242.*

In AD and PD, selective neuronal loss occurs in several subcortical nuclei including the nucleus basalis of Meynert (NB), the locus ceruleus (LC) and the substantia nigra (SN). This histopathologic study was designed to assess: 1) the relationship between severity of subcortical neuronal loss and age at symptom onset and duration of illness, and 2) the relationship between neuronal loss in each of the three subcortical nuclei. The sample was comprised of 70 cases with AD (50% male), 20 PD (90% male) and 20 elderly controls (50% male). In both patient groups there was significant neuronal loss in all three nuclei. The mean number of nucleolated neurons (expressed as a percentage of controls) in AD was 50.75% (NB), 33.5% (LC) and 78.8% (SN); and in PD, 55.7% (NB), 16.4% (LC) and 15.0% (SN). In both patient groups, age at symptom onset was inversely correlated with duration (AD: $r=-0.42$, $p<0.0004$; PD: $r=-0.79$, $p<0.005$). In PD, the only significant correlation was between duration and numbers of neurons in SN ($r=-.66, p<0.02$).In AD, stepwise-multiple regression showed a significant correlation between age at onset and numbers of neurons in the NB ($r=0.34$; $p<0.01$) and LC ($r=0.39$, $p<0.01$), but not in the SN ($r=0.06$). In addition, numbers of neurons in NB and LC were correlated with each other. In AD, the degree of neuronal loss in the SN was highly variable: in 60% of cases counts were similar to controls; in 40% of cases loss was similar to PD. Lewy bodies were found in 1 of 20 AD cases with normal numbers of SN neurons, while these inclusions were found in 7 of 18 cases with significant SN loss (Fisher exact test: $p<0.02$). These findings suggest two factors that may define subgroups of AD: 1) early symptom onset - which is associated with more severe neuronal loss in NB and LC, and 2) Lewy bodies - which are associated with more severe neuronal loss in SN. (NIH 2P50AG05142 and the Veterans Administration)

425.5

A COMPARATIVE ANALYSIS OF CORTICAL AND NUCLEUS BASALIS PATHOLOGY IN ALZHEIMER'S DISEASE USING SEVERAL METHODS INCLUDING A NOVEL NICKEL-ENHANCED PEROXIDASE STAIN K.M.Cullen, G.M.Halliday and J.M.Freeman* University of Sydney, NSW, Australia 2006

We examined the brains of 8 prospectively studied patients with Alzheimer's disease (diagnosis confirmed postmortem (pm)) and 4 age-matched, non-demented controls in order to test a correlation between lesions in the nucleus basalis (NB) and pathology in its putative cortical terminal fields. Blocks containing the NB and specific gyri from 7 cortical regions were taken from formalin-fixed brains (pm delay <26 hrs). For the quantitation of plaque areal fraction and the number of neurofibrillary tangles (NFT), several staining methods were applied to serial (1/15) 50μm frozen sections of the blocks. These techniques included anti-GFAP, -calbindin, -ubiquitin, -BA4 and -Alz90 immunohistochemistry, the Garvey silver stain (Garvey, 1991 *J Histotech* **14**, 39-42) and a novel nickel-enhanced peroxidase method (NEP). Briefly, for the NEP, free-floating sections were incubated with Avidin-Biotin Complex followed by the application of the chromogen, 3,3'diaminobenzidine, in 0.16% nickel ammonium sulfate. NB neurons were counted in a complete series of cresyl violet-stained sections. Three-dimensional reconstructions of NB neurons and NFT were assembled to visualize the topography of lesions. **Results:** The NEP most clearly discerned NFT as no cell nuclei were stained. A considerably higher plaque areal fraction was obtained with the NEP compared to the Garvey silver stain and the BA4 and Alz90 immunostains, thus the NEP may be useful as a sensitive marker for an extensive range of AD pathology. Astrocytic and microglial changes were profound in the cortex and forebrain although not seen within the NB in every AD case. The NB of patients with AD contained numerous globose NFT but no plaques; however, NFT were rarely seen in control NB. Cell loss in the NB varied (30%-88%) and did not correlate with the number of NFT seen in either the cortex or the NB itself. Neither cell loss nor NFT in the NB correlated with cortical plaque density, nor did plaque areal fractions correlate with NFT counts in any cortical region. These results suggest that a) NFT and plaque formation may be independently regulated, b) NB pathology is not directly related to cortical plaque or NFT formation, and c) the presence of globose NFT in the NB is diagnostic for AD.

425.6

A QUALITATIVE AND QUANTITATIVE IMMUNOHISTOCHEMICAL STUDY OF THE MICROVASCULATURE IN AGING AND ALZHEIMER'S DISEASE. L. Buée[1]*, C. Bouras[2], P.R. Hof[3], M. Surini[2], J.H. Morrison[3], H.M. Fillit[3] and A. Delacourte[1]. 1) INSERM U156, 59045 Lille, France; 2) Psychiatry, Univ. of Geneva, CH-1225, Switzerland; 3) The Mount Sinai Medical Center, New York NY 10029, U.S.A.

Pathological changes of the microvasculature in normal aging and Alzheimer's disease (AD) were investigated by immunohistochemistry using the monoclonal antibody 7E12 directed against vascular heparan sulfate proteoglycan protein core. Rare atrophic cortical microvessels were observed in control brains. In AD cases, microvascular pathology was more severe than in control cases. These vascular changes were primarily found in layers III and V of frontal and temporal cortex demonstrating that the alterations display both regional and laminar distribution. Some vascular pathologic changes, such as twisted and coiling vessels also displayed regional and laminar distributions in AD cases. Furthermore, in AD patients, extracellular 7E12-immunoreactive deposits, consistent with thioflavine S-positive senile plaques, were observed. Cortical atrophy and vascular density (VD) were quantified by image analysis and compared between control cases and AD patients. VD was assessed as a relative ratio of "vascular surface"/"cortical surface". Vascular surface was markedly decreased in AD (0.27 ± 0.06 mm^2) compared to control cases (0.31 ± 0.06 mm^2; $p = 0.008$). Cortical thickness was also severely decreased in AD patients (1.17 ± 0.20 mm) compared to control cases (1.83 ± 0.46 mm; $p = 0.0001$) demonstrating a 34.6% atrophy. Even with this significant cortical atrophy, significantly lower relative vascular density ($VD = 16.59 \pm 3.64\%$) was found in AD patients compared to control cases ($VD = 19.48 \pm 3.77\%$; $p < 0.01$).

Thus, these data suggest that vascular abnormalities are heterogeneous in aging and Alzheimer's disease. VD is likely to be related to the neurodegeneration process in selected regions and cortical layers in AD.

(Supported by the France Alzheimer Association, the Florence J. Gould Foundation; the Brookdale Foundation and NIH grant AG05138).

425.7

SPATIAL MEMORY LOSS WITHOUT MORPHOLOGICAL DAMAGE TO CA1 NEURONS. J.C. de la Torre*, T. Fortin, G. Park, B. Pappas. University of Ottawa, Faculty of Medicine and Carleton University, Ottawa, Canada KIH 8M5.

Evidence indicates that the hippocampal formation and specifically the CA1 pyramidal neurons, is involved in spatial memory input. Experiments in animals and humans show that selective damage to CA1 neurons results in spatial and recent memory deficits.

In the present study, aging male Sprague-Dawley rats 9 and 19 months old were subjected to chronic cerebrovascular insufficiency (CVI), which consisted of occluding both carotids and 1 subclavian artery (Group 3-VO) or both carotids (Group 2-VO) for 9 weeks.

After 9 weeks, all rats were tested in the Morris water maze test and compared to age-matched intact controls (Group C). Hippocampal and cortical cerebral blood flow was measured prior to sacrifice. Following perfusion-fixation, brains were prepared for histological evaluation of the hippocampus and cortex. Results show that both Group 3-VO and Group 2-VO showed significant memory impairment with respect to Group C. However, while Group 3-VO showed a mean damage to 36% of its CA1 neurons, Group 2-VO showed no neuronal damage in the hippocampus or cortex. CVI resulted in significant reduction of hippocampal and cortical blood flow but no micro-infarcted brain vessels were seen in any animal. These findings suggest that spatial memory impairment (which is the first important clinical sign in Alzheimer's disease) does not depend on the structural integrity of CA1 or other hippocampal neurons. It is reasonable to assume from this data that such memory deficits in aging rats can result from chronic CVI which could provoke neurochemical changes within the neuron or its synaptic contacts even before neuronal cell loss or neuropathological damage is detected. It is tempting to conjecture whether the evolution of Alzheimer's disease follows a similar course.

425.8

DIFFERENTIAL IMPAIRMENT OF SPATIAL LEARNING IN TWO ANIMAL MODELS OF ALZHEIMER'S DISEASE. G.L. Dunbar*, L.S. Janis, and C.L. Weaver. Psychology Dept., Central Michigan University, Mt. Pleasant, MI.

Degeneration of basal forebrain cholinergic neurons has been linked to Alzheimer's disease (AD) and has provided the basis for animal models of AD using lesions of the nucleus basalis (NB) and the medial septum (MS). We compared the effects of these lesions on the ability of rats to use different strategies for solving a radial-arm maze task. In the first study, both rats given electrolytic MS lesions or sham surgery could solve the task, but only the sham rats could solve the task when forced to use a "nonstereotypic" strategy (i.e., when the rats were prevented from using a stereotypic response pattern, such as entering each adjacent arm in a clockwise fashion). In a second study, rats given either ibotenic acid lesions of the NB or sham surgery were able to solve the same task, even when forced to use a "nonstereotypic" response strategy. Our results indicate that damage to additional neuronal systems (e.g., GABAergic neurons, which also were destroyed by the electrolytic MS lesions) may be critical to produce severe memory deficits, or that the MS-hippocampal system is more critical for spatial learning than the NB-cortical system. In either event, our work suggests that ibotenate NB lesions is an inadequate model of AD, because it doesn't mimic the profound memory loss observed in AD patients. Supported by NSF-ILI grant USE - 9051323.

425.9

COGNITIVE FUNCTION AND ALZHEIMER'S-LIKE PATHOLOGY IN THE AGED CANINE: I. SPATIAL LEARNING AND MEMORY.

E. Head*[1], G. Ivy[1], B.J. Cummings[2], C.W. Cotman[2] and N.W. Milgram[1].
[1]Life Sciences Division, Scarborough Campus, U. Toronto, Scarborough, Ontario, Canada, M1C 1A4. [2]IRU in Brain Aging, Dept of Psychobiology, U.California, Irvine, CA 92717.

A spatial non matching to sample paradigm was developed in order to study the effects of age on spatial working memory in the dog (E. Head *et al*, Soc. Neurosci. Abst. 376.3, 1992). Animals were rewarded for responding to the side opposite to the one where a sample stimulus was presented. At delays of 10 seconds, this was a far easier task to learn for a dog than a visual recognition memory task. Acquisition at short delay was not a good predictor of an animals ability to respond at long delays. Old animals showed little deficit in learning , but were deficient in their capacity to perform above chance at long delays. Within the sample of aged animals, there was considerable variability in both acquisition and in performance over long delays. There was a subpopulation of aged dogs which were unable to achieve the acquisition criterion. On anatomical analysis, the animals in this subpopulation had abnormal accumulation of beta amyloid protein throughout the brain (see accompanying abstract, B.J. Cummings *et al*) and an apparent loss of CA1 pyramidal neurons. These results suggest that spatial memory provides a measure of age-dependent cognitive deterioration while spatial learning may be a predictor of abnormal amyloid accumulation.

425.10

COGNITIVE FUNCTION AND ALZHEIMER'S-LIKE PATHOLOGY IN THE AGED CANINE: II. NEUROPATHOLOGY.

B.J. Cummings[1]*, P.E. Honsberger[1], A.J. Afagh[1], E. Head[2], G. Ivy[2], N.W. Milgram[2] and C.W. Cotman[1] IRU in Brain Aging[1], Dept. of Psychobiology, University of California, Irvine, CA 92717 and Life Sciences Division[2], University of Toronto, Scarborough, Ontario M1C 1A4

We have previously reported that the hippocampal region in aged beagles exhibits neuropathological changes similar to those found in Alzheimer's disease (Cummings *et al*. Neurobiol Aging). Due to the archival nature of this tissue, no behavioral data was available on these animals. We have now examined 17 aged canines (beagles and mixed-breeds) both behaviorally (see accompanying abstract, E. Head *et al.*) and with a variety of AD related neuropathological markers. After behavioral testing, animals were perfused with 4.0% paraformaldhyde on sacrifice. The hippocampus, entorhinal and frontal cortices, and cerebellum were examined with antibodies to ß-amyloid, the amyloid precursor protein, and cytoskeletal markers. In addition, routine Bielschowsky's, thioflavine, and Congo Red staining were utilized. In agreement with previous reports, canine pathology consisted primarily of amyloid angiopathy and diffuse early stage plaques containing intact neurons. No neurofibrillary tangles were detected. When plaques were detected in the hippocampus, they were also present in entorhinal and frontal cortex; however, little pathology was observed in the cerebellum regardless of plaque density in the hippocampus. After the animals were ranked with regard to the extent of ß-amyloid accumulation by an observer blind to the behavioral results, ß-amyloid pathology and spatial task performance were compared. The four animals with the most severe ß-amyloid accumulation were the poorest performers on a spatial task, while the top five performers had no ß-amyloid accumulation. These data suggest that poor performers have an accompanying accumulation of ß-amyloid.

425.11

LONGITUDINAL STUDY ON CONSTRUCTIONAL DISABILITY IN ALZHEIMR'S DISEASE

M.Fujii*, S.Murakami, R.Fukatsu, N.Nakano, K.Utsumi, N.Takahata
Dept.of Neuropsychiatry,Sch.of Medicine,Sapporo Medical Univ.060,Japan

Constructional disabilities are one of the most prominant clinical feature in the patients with Alzheimer's disease. We have demonstrated characteristic eye movement associated with constructional apraxia ,which is similar to lt. unilateral spatial neglect and Balint syndrome , using a vision analyzer. We presented these results indicated that there might be disconnection between visual cognition of objectsand visuo-spatial language function in the last conference.

In this study, we have examined the behavior of the eye movements using a vision analyzer and constructional ability based on verbal instruction, when drawing geometrical figures for 3 years .

The results obtained indicated that gazing points of focus located mainly on copying figure in light visual field, and dislocated gazing point of focus became prominent as the disease progressed. Then, they showed constructional disabilities. In their constructional behavior, closing-in phenomenon was observed often and finally they overwrote copying figure on the original ones.

These findings indicate that the sterategy on copying figures was disturbed first and abstruct thinking (categolizing process) from visual presentation was involved later in in the early stage in Alzheimer's disease.

425.12

ESTROGEN REPLACEMENT AND ALZHEIMER'S DISEASE IN WOMEN.

A. Paganini-Hill, J.G. Buckwalter, C. G. Logan, and V.W. Henderson*. Departments of Preventive Medicine, Neurology, and Psychology, and Schools of Medicine and Gerontology, University of Southern California, Los Angeles, CA 90033-1084

In a retrospective analysis of women who volunteered for a research program on aging and dementia, we found that cases meeting criteria for "probable" Alzheimer's disease (AD) (n = 143) were significantly less likely than elderly nondemented subjects (n = 92) to be using estrogen replacement at the time of their enrollment (7% versus 18%, $P < 0.01$), but groups did not differ with regard to the total number of prescription medications. AD cases using estrogen did not differ significantly from cases not using estrogen in terms of age, education, or duration of dementia symptoms, but they performed significantly better on a brief cognitive screening instrument (the Mini-Mental State examination — $P < 0.005$).

Separate analyses from a large retirement community (Leisure World) surveyed in 1981 used a case-control study nested within a prospective cohort study. 127 of 2418 female cohort members who died between 1981-1992 had AD or related diagnoses mentioned on the death certificate. Four controls were matched to each case by birth and death dates. The risk of AD and related dementia was significantly less in estrogen users relative to non-users (RR = 0.61, 95% CI = 0.40–0.94), and the risk decreased with increasing dose of the longest used estrogen and with increasing duration of use.

Results of these two separate studies support hypotheses that estrogen replacement therapy decreases the risk of AD in women and may also improve cognitive performance of women with AD.

425.13

SINGLE VOLUME 1H SPECTROSCOPY IN ALZHEIMER'S DISEASE.

J.P. Kesslak*[1], D. Atkinson[2], M. Brant-Zawadski[3] and C.W. Cotman[1].
[1]Dept Neurol, IRU Brain Aging, Univ Calif, Irvine, CA,[2] Siemens Med Systems, [3] Dept Radiol, Hoag Hospital, Newport Beach, CA.

Alzheimer's disease (AD) is characterized by progressive neural degeneration that clinically produces a variety of behavioral deficits. Hydrogen magnetic resonance spectroscopy (MRS) provides a novel means to detect biochemical changes in AD brain pathology *in vivo*. This study examined the relative amounts of N-acetyl aspartate (NAA) as an index of neural density, and the metabolites creatine (Cr) and choline (Cho) as neural and non-neural components.

Thirty AD patients (69±8 years of age) diagnosed according to NINCDS-ADRDA guidelines were compared to 10 age matched controls (70±6 years of age). MRS was done within 4 weeks of clinical evaluations on a 1.5 Tesla Siemens Magnetom 63/84 SP system and head coil. Placement of a 2x2x2 cm^3 volume of interest in the parietal and temporal corticies was done from coronal, sagittal and axial images. Peak area for NAA, Cho and Cr was integrated and ratios calculated.

Analysis of variance indicated significantly lower levels of NAA, Cr and Cho for the AD group in the parietal lobe. In the temporal lobe the levels of NAA and Cho were significantly lower in the AD group. The ratios of NAA/Cho, NAA/Cr and Cho/Cr did not indicate any significant differences between groups in either area. There did not appear to be any age related differences in this small sample of normal-aged controls, however, it should be noted that variability did increase with age. These results may indicate cell loss characteristic of AD pathogenesis.

426.1

NIGRAL NEURON NUMBERS IN NORMALS MAY BE RELATED TO PREDISPOSITION TO DEVELOP PARKINSON'S DISEASE. U.B. Muthane*, Ramsay K.A., Jackson-Lewis V, Jiang H, Przedborski S. Dept. of Neurology, Columbia University, 650 West 168th St., Black Bldg. Rm 310, New York, NY 10032.

The reason why some individuals develop Parkinson's disease (PD) and some do not is unclear. Similarly, in the mouse 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) model of parkinsonism it is also unclear as to why mice belonging to C57/bl strain have a greater loss of nigral dopaminergic neurons compared to the CD-1 strain. To examine whether the strain-dependent sensitivity to MPTP is related to the difference in the number of tyrosine hydroxylase (TH) and calbindin (Cal) positive neurons in the substantia nigra, we quantified these neurons in C57/bl and CD-1 mice. In normal mice of the same age and sex, consecutive sections of the midbrain were stained by immunohistochemistry and the number of TH- and Cal-positive nigral neurons quantified. We observed that C57/bl mice had significantly lower number of TH- and Cal-positive neurons ($p<0.005$) compared to CD-1 mice. We hypothesize that a greater postnatal loss of both TH- and Cal- positive nigral neurons occurs in the C57/bl compared to CD-1 mice. Because the more sensitive C57/bl mice have lesser TH positive nigral neurons to begin with and also lesser protective mechanisms like Calbindin it may be possible that humans predisposed to develop Parkinson's disease also may have fewer TH-positive nigral and lesser protective mechanisms compared to those who do not develop the disease.

Supported by the Parkinson's Disease Foundation, NY

426.2

THE LEVELS OF TYROSINE HYDROXYLASE AND DOPA DECARBOXYLASE ARE REDUCED IN STRIATUM OF PATIENTS WITH DOMINANTLY INHERITED OLIVOPONTOCEREBELLAR ATROPHY (OPCA). X.H. Zhong*, J.W. Haycock, and S.J. Kish. Clarke Institute of Psychiatry, Toronto, Canada, and LSU Medical Center, New Orleans, Louisiana, U.S.A.

We have reported a 60%-80% striatal dopamine reduction, but without substantia nigra cell loss, in seven patients with OPCA, a hereditary cerebellar ataxia disorder (Neurology 42:1573, 1992). The selective deficit was suggestive of a "dying-back" phenomenon, in which nerve terminal loss was preceding retrograde degeneration of cell bodies.

We have now measured two additional dopaminergic markers in these patients. Tyrosine hydroxylase (TH) and DOPA decarboxylase (DDC) levels were quantitated by Western blot analysis. TH levels were 66% and 64% lower in the caudate and putamen (respectively) of patients with OPCA. DDC levels were reduced 31% (caudate) and 45% (putamen). The smaller decrement in DDC levels resulted, at least in part, from the appearance of an additional DDC-immunoreactive band of slightly different M, in 6 of the 7 OPCA cases but in none of the controls (n=15). Notably, multiple isoforms of DDC mRNA have been identified in humans, rat and *Drosophila*, but the mammalian DDC mRNAs that have been reported to date do not encode different proteins. Thus, the present findings of reduced TH and DDC levels in striata from OPCA patients provide further support for a "dying-back" phenomenon in the progression of this disorder. In addition, the appearance of multiple M, DDC bands in 6 of the 7 OPCA cases may be indicative of disease-induced changes in alternative splicing. (Supported by USPHS grants NS26034 and NS25134)

426.3

DOPAMINE TRANSPORTER mRNA NEURONAL CONTENT IN THE HUMAN MIDBRAIN DOPAMINERGIC NUCLEI. C. Casan, V. Blanchard, R. Raisman, S. Vyas, G. Uhl, F. Javoy-Agid*
U289 INSERM, Hopital de la Salpêtrière, 75013 PARIS, and Molecular Neurobiology, NIDA/ARC, PO BOX 5180 BALTIMORE.

Loss of midbrain dopaminergic (DA) neurons is a major neurochemical characteristic of Parkinson's disease. However, not all DAergic neurons are equally vulnerable, the most severe neuronal loss always occurs in the substantia nigra (SN) in comparison to the ventral tegmental area (VTA) or central gray substance (CGS), the latter area preserved in the disease. Thus, DA neurons are not equally vulnerable to the disease. In a search for the etiology of the disorder, the involvement of dopamine uptake process has been considered. The expression of plasma membrane DA transporter (DAT) was studied at cellular level in human midbrain post mortem by in situ hybridization using an antisense ^{35}S labelled riboprobe. Throughout the midbrain sections, positively hybridized cells identifiable as TH positive neurons were observed in the various DA areas. The cellular abundance order of this transcripts was: highest in the SN followed by VTA and then by CGS neurons. These data provide evidence of the phenotypic distinctions among DAergic cell groups and suggest that the expression of DAT gene is relatively high in the neurons which are most vulnerable to the disease. This characteristic might provide a greater susceptibility to the neurotoxic factors, or contribute to the severity or progression of neuronal death.

426.4

CALCIUM BINDING PROTEINS DIFFERENTIATE MIDBRAIN DOPAMINERGIC SYSTEMS IN HUMANS. D.A. McRitchie, G.M. Halliday and D.J. Tracey*. Neuropathology Unit, Department of Pathology, University of Sydney, 2006, *School of Anatomy, University of New South Wales, Kensington, 2033, Australia.

Previous studies have reported that substantia nigra neurons containing calbindin-D28K (Cal) are selectively spared in patients with Parkinson's disease (Yamada *et al.* Brain Res. 1990;526:303-307). The aim of this study is to determine the distribution of Cal and parvalbumin (Parv) in the human ventral midbrain using immunohistochemistry. Formalin-fixed midbrains from 11 cases (aged 37-88yrs) with no evidence of neurological or neuropathological abnormalities were used. The tissue was taken with consent at routine hospital autopsies (mean post mortem delay = 19hrs) and the study was approved by the Human Ethics Committee. 50µm sections were cut in the transverse or horizontal planes. Three parallel series of sections were stained with cresyl violet, mouse anti-Cal or mouse anti-Parv. The distribution of Cal- and Parv-immunoreactive (Cal+ or Parv+) neurons was quite exclusive. Cal+ neurons overlapped in their distribution with some pigmented and presumably dopaminergic midbrain neurons while Parv+ neurons were restricted to non-pigmented regions. Many Cal+ neurons were present in the A10 (paranigral and parabrachial pigmented nuclei) and A8 (retrorubral fields) dopaminergic cell groups, but were virtually absent from the A9 dopaminergic cell group (substantia nigra pars compacta). Cal+ neurons were both pigmented and non-pigmented and were concentrated caudally. In contrast, Parv+ neurons were non-pigmented and dispersed throughout the rostral substantia nigra pars reticulata. In contrast to past reports, A9 dopaminergic neurons do not contain Cal in humans. In addition, Parv appears to be exclusively contained in reticulata neurons.

426.5

^{3}H-HEMICHOLINUM-3 AUTORADIOGRAPHY IN DEGENERATIVE PARKINSONISM. J. Pascual, R. Rodríguez-Puertas, M. Lafarga* and A. Pazos. Depts. of Physiol. and Pharmacol., Anat. and Cell. Biol. and Service of Neurology, Univ. Hospital "Marqués de Valdecilla" and Faculty of Medicine, Santander, Spain.

The high-affinity choline uptake carrier (HACU), a selective marker of presynaptic nerve terminals, was labeled by using 5 nM ^{3}H-hemicholinium-3 (^{3}H-HC) in tissue sections of 3 representative brain areas from 7 patients who had died from levodopa-responsive, degenerative parkinsonisms (DP) and from 10 matched controls.

In the striatum ^{3}H-HC binding sites were clearly increased in DP brains as compared to control brains, both over caudate (mean±SD controls vs DP cases= 224±88/333±85; % change= +49%; $p<0.05$) and putamen (178±99/326±177; +83%; $p<0.05$). No significant changes could be found in the frontal cortex of DP cases as compared to controls, both in layers I-III (32±7/34±11; +7%) and in layers IV-VI (28±9/35±31; +24%). On the other hand, in the hippocampus of DP brains as compared to control brains ^{3}H-HC binding sites tended to decrease along all the layers, though this reduction only reached statistical significance in the hilus of the dentate gyrus (105±55/51±17; -52%, $p<0.05$).

Our data help to clarify the state of the presynaptic cholinergic compartment in levodopa-responsive parkinsonism, demonstrating first the striatal cholinergic hyperfunction compensating dopamine deficiency, and second a slight to moderate loss in the presynaptic cholinergic tone in parkinsonian hippocampal formation. These neurochemical changes very probably play an important role in the pathophysiology of some motor and mental symptoms observed in DP patients.

426.6

AUTORADIOGRAPHIC DISTRIBUTION OF [^{125}I]-FERROTRANSFERRIN BINDING SITES IN THE MESENCEPHALON AND THE STRIATUM OF CONTROL SUBJECTS AND PATIENTS WITH PARKINSON'S DISEASE. B.A. Faucheux[1,3], E.C. Hirsch[1], J. Villares[1], F. Javoy-Agid[1], J.J. Hauw[2], Y. Agid[1]*. [1]Lab. Physiopathol. Pathogenèse Maladies Syst. Nerveux, INSERM (U289) and [2]Lab. Neuropathol., Hôpital Salpêtrière; [3]Ctre Gérontol. Ass. Cl. Bernard; Paris, France.

Parkinson's disease (PD) is characterized by a progressive degeneration of dopaminergic (DA) neurons, which is severe in substantia nigra (SN) and moderate in other DA areas of the mesencephalon (ventral tegmental area, cell group A8, central grey substance). Iron levels are increased in the SN of patients with PD. This metal can promote the formation of a variety of cytotoxic reactive oxygen species and membrane lipid peroxidation. A dysregulation of its intracellular uptake by neurons and glial cells in the SN may thus be involved in neuronal death. Localization and number of receptors for transferrin (Tf), the likely most important pathway for iron to gain access to neurons, have been studied by quantitative autoradiography on autopsy mesencephalon and striatum tissue from 7 control subjects and 7 PD patients. In the mesencephalon, densities of [^{125}I]-Tf(Fe)$_2$ binding sites were highest in central grey substance (about 10fmol/mg tissue equivalent), lowest in SN (about 1fmol/mg tissue equivalent), and intermediate in ventral tegmental area and peri- and retro-rubral fields. There were no statistically significant differences between PD and control brains. In the basal ganglia, levels were highest in caudate nucleus and putamen, and lowest in globus pallidus. These results show an heterogeneous distribution of Tf binding. Regional density of Tf binding among brainstem cell groups was highest in the areas which are the least affected by DA neurons losses in PD. The results suggest 1) that the presence of Tf receptors on surrounding glial cells or neurons may protect DA neurons from toxic effects of excess iron, and 2) that iron does not accumulate through a large increase of Tf receptors density in the SN. [Research supported in part by INSERM, ACB and FdF (grant No 91-5716)]

426.7

ALTERATED NUMBER OF [125I] BH-SUBSTANCE P BINDING SITES IN PALLIDUM AND SUBSTANTIA NIGRA IN PARKINSON'S AND ALZHEIMER'S DISEASE. L. Rioux*, Q. Huang and J.N. Joyce . Depts. of Psychiatry and Pharmacology, Univ. of Pennsylvania SCh. Med., Philadelphia, PA, USA.

Previously, we observed a significant increase in the number of NK1([^{125}I] Bolton-Hunter labeled substance P sites in the striatum of Parkinson's disease (PD) patients when compared to non-neurologic controls and to Alzheimer's (AD) patients (Rioux et al, 1993; J. Neurotrasm.). This may reflect responses to altered release of substance P in striatal neurons in response to losses of dopaminergic input. The present study examined possible changes in the density of NK1 sites in striatal output structures. We quantified by receptor autoradiography the number of NK1 receptors, in substantia nigra (SN) and pallidum [internal (GPi) or external (GPe) segment] of patients suffering from AD and PD. Preliminary results show a trend toward an elevation of NK1 sites in the GPi (162 %) andGpe (49%) of AD patients and of patients co-affected with AD and PD (Gpi, 75%; Gpe, 19%) when compared to non-neurologic controls. The results also show a trend toward a decrease of these sites in Gpi (32%), Gpe (54%) and SN (33 %) in PD patients and in SN (17%) of patients co-affected with AD and PD. This study suggests that the expression of NK1 receptors in the basal ganglia is differently affected in PD and AD. As with studies exploring changes in DA systems in PD, AD and AD coexistent with PD it appears that alterations in NK-1 sites are more similar for AD and AD with PD as compared to PD. Thus, AD with PD is not simply two co-existing diseases.

Funded by AG09215 to JNJ, and a Pilot grant from Alzheimer's Association and FRSQ fellowship to LR.

426.8

EFFICACY OF D_1 AGONISTS IN HUMAN CAUDATE: RELATIONSHIP TO PARKINSONISM. J.H. Gilmore[1*], V.J. Watts[1], C.P. Lawler[1], E.P. Noll[1], D.E. Nichols[2] and R.B. Mailman[1]. University of North Carolina[1], Chapel Hill, NC 27599 and Purdue University[2], W. Lafayette, IN 47907

The full efficacy D_1 agonist dihydrexidine (DHX) has profound antiparkinsonian activity in MPTP treated monkeys (Taylor et al., 1991). Although we had hypothesized that these effects were related to its full efficacy at stimulating the enzyme adenylate cyclase, another purported full efficacy D_1 agonist (SKF82958) has recently been shown to have only weak antiparkinsonian activity. One explanation for this discrepancy is that the biochemical efficacy studies were conducted in rodents; it is possible that the D_1 receptor affinity and efficacy of these two compounds are similar in rodent brain, but differ significantly in primate brain. To examine this possibility, we compared the D_1 receptor affinity and functional activity of DHX, SKF82958 and another purported full efficacy compound (A68930) in human brain. Results from in vitro binding studies with membranes from human caudate [n = 4; age = 36 ± 2 yr (SD); post mortem interval = 19 ± 8 hrs (SD)] indicated that these D_1 agonists competed for [^{3}H]-SCH23390 labeled sites with a rank order of potency similar to that in rat striatum [IC50 = 36.8 nM (DHX); 18.6 nM (SKF82958); 3.9 nM (A68930)]. We also examined the ability of these compounds to stimulate the enzyme adenylate cyclase in tissue homogenates of human caudate. DHX and A68930 were of full efficacy (relative to 100 μM dopamine), whereas the prototypical partial D_1 agonist SKF38393 induced less than 50% maximal stimulation. Interestingly, the purported full efficacy D_1 agonist SKF82958 exhibited efficacy lower than that of dopamine or DHX (albeit greater than that of SKF38393). The difference in biochemical efficacy between DHX and SKF82958 in human brain may underlie the dramatically different clinical responses elicited by these drugs in primate models of parkinsonism. (Supported by MH 40537 and MH42705, and the Foundation of Hope)

426.9

APOMORPHINE INHIBITS DOPAMINE (DA) METABOLISM IN HUMANS. M. Levivier[1], A. Naini*[,3], C. Raftopoulos[1], J. Hildebrand[2] and S. Przedborski[2,3]. Depts of [1]Neurosurgery and [2]Neurology, Univ. Libre de Bruxelles - Hôp Erasme, Brussels, Belgium and [3]Dept of Neurology, Columbia Univ., New York, NY 10032.

Apomorphine is a potent DA post-synaptic receptor agonist increasingly used in Parkinson's disease (PD). In animals, apomorphine inhibits DA metabolism through a potent agonistic effect on pre-synaptic DA receptors. Because the pre-synaptic effect of DA agonists may be of interest in neuroprotective therapeutic strategies for PD, we examined the apomorphine plasma and CSF pharmacokinetics as well as its effects on the brain DA metabolism in humans.

Six patients (1 woman, 5 men, mean age 79.5 yr.) with presumed normal-pressure hydrocephalus, who underwent 48-hours intracranial pressure monitoring, were injected with 50 μg/kg apomorphine, s.c. Blood and ventricular CSF samples were collected from 0 to 120 min. after injection and were processed for HPLC.

Maximal plasma apomorphine concentration (24.92 ng/ml) was found 20 min. after injection (area under the curve: 1438.65 ng/ml/120 min.). Maximal ventricular CSF apomorphine concentration (1.07 ng/ml) was found 60 min. after injection (area under the curve: 101.37 ng/ml/120 min.). Apomorphine caused a progressive reduction in ventricular CSF concentrations of DA and its metabolites that started after 10 min., was maximal after 30 min. (free DA: 30%, conjugated DA: 28%, HVA: 21%, DOPAC: 31%) and was still present (5-10%) after 120 min.

Our study reports plasma and CSF pharmacokinetics of apomorphine in humans without evidence of PD. We also show, for the first time in humans, that a dose of apomorphine commonly used in PD, causes significant inhibition of DA metabolism that last for more than 120 min. Thus, in addition to its symptomatic effects, apomorphine may play a role in preventing or slowing down the progression of neurodegeneration in PD.

Supported by the F.N.R.S., Belgium and the Parkinson's Disease Foundation, NY

DEGENERATIVE DISEASE: PARKINSON'S—FUNCTIONAL MORPHOLOGY

427.1

LOCALIZATION OF CATECHOLAMINE SYNTHETIC ENZYMES IN LARVAE OF CRASSOSTREA GIGAS. P.L. Levantine* and D.B. Bonar. University of Maryland, College Park, MD 20742.

Larvae of the Pacific oyster, Crassostrea gigas, were examined for catecholamine synthetic enzymes. Serial sections of staged larvae were probed with antibodies to dopamine beta-hydroxylase (DBH), phenylethanolamine N-methyltransferase (PNMT), and tyrosine hydroxylase (TH). Two classes of anti-DBH cells are evident. The primary anti-DBH cells contain tightly packed vesicles and surround the ciliated opening found at the "heel" of the foot. Secondary anti-DBH cells have more dispersed vesicles and are found around the edge of the ciliated opening, and in the mid- and distal foot. Anti-TH staining appeared to be co-localized with anti-DBH staining. In all 6 stages anti-PNMT stained a ring of vesicles around the velum. In later stage larvae anti-PNMT staining is also evident in the proximal portion of the byssus gland. No catecholamine-like immunoreactivity was localized in any ganglion cells, or in the neuropil or connectives. Norepinephrine levels, as measured by HPLC, increase at class 4, as do cross-sectional area of anti-DBH staining, settlement behavior, and metamorphosis.

427.2

MK-801 PREVENTS MPTP-INDUCED REDUCTION OF CATECHOLAMINES IN GOLDFISH BRAIN AREAS. A. Poli*, M. Virgili, R. Lucchi, O. Gandolfi. and O. Barnabei Dept. of Biology, University of Bologna, 40126-Bologna, Italy.

Previous study from this laboratory demonstrated that treatment with MPTP caused a marked disappearance of tyrosine hydroxylase immunoreactivity and degeneration of neurons in several areas of goldfish brain, with concomitant depletion of DA and NA levels. Except for a marked darkening of the skin , MPTP effects appeared not different from those observed in higher vertebrates. Since the involvement of excitatory amino acids in the meccanism of MPTP toxicity has been demonstrated in mammals, we have examined the effects of the neurotoxin on amino acids release in synaptosomes prepared from discrete goldfish brain areas and the effect of MK-801, a noncompetitive antagonist for the NMDA receptors, on MPTP catecholamines depletion. Although MPTP ($10^{-5} \div 10^{-3}$ M) did not alter the basal amino acids release in synaptosome preparations from telencephalon and hypothalamus, i.p. injection of the toxin (10 mg/kg for 3 days) caused a strong loss of DA and NA in the afore mentioned brain regions. Pretreatment of fish with MK-801 (1 mg/Kg every 6 h for 48 h) significantly reduced DA (31% in thelencephalon; 46% in hypothalamus) and NA (29% in telencephalon; 55% in hypothalamus) MPTP-induced depletion. These results indicate that NMDA receptors appear to be involved in mediating MPTP catecholamine depletion, at least in goldfish brain areas.

427.3

NEUROFILAMENTS MAINTAIN THE STRUCTURAL AND FUNCTIONAL INTEGRITY OF DOPAMINERGIC NEURONS IN CULTURE. R. Hao, R.B. Norgren, Jr., J.F. Rodriguez-Sierra, M. Ebadi and R.F. Pfeiffer*. Sec. of Neurol.; Dept. of Cell Biology and Anatomy; and Dept. of Pharmacol., Univ. Neb. Coll. Med., Omaha, Nebraska 68198-6260.

The major histological changes in brains of Parkinson's patients include the aggregation of neurofilaments and the formation of Lewy bodies in dopaminergic neurons of corpus striatum. In order to delineate the etiology of parkinson's disease, mesencephalic cells of 14 day embryonic rats were cultured in a serum-free medium, and the integrity of dopaminergic neurons was assessed by tyrosine hydroxylase immunocytochemistry and by the presence of high affinity [^{3}H]dopamine uptake and release mechanisms. Cytochalasin B (5 μg/ml) but not colchicine inhibited the dopamine uptake mechanism in a time-dependent fashion without influencing the release of dopamine. Florescence immunochemical analysis of cells exposed to cytochalasin B but not colchicine, revealed shortened axons and dendrites and disappearance of the microspikes. The results of these studies suggest that normal organization of neurofilaments is necessary in order to maintain the morphological and functional integrity of dopaminergic neurons.

427.4

ADVERSE EFFECTS OF L-DOPA AND DOPAMINE RECEPTOR AGONISTS ON CULTURED RAT MESENCEPHALIC DOPAMINE NEURONS. P.J. Kontur*, K.L. Marek, D.E. Redmond, Jr. and R.H. Roth. Neural Transplant Program, Depts. of Pharmacology, Psychiatry and Neurology, Yale U. Sch. of Med., New Haven, CT 06510.

Continued administration of L-DOPA after treatment of Parkinson's disease by transplantation of fetal brain tissue may adversly affect developing dopamine (DA) neurons in the graft. Cell cultures prepared from the ventral mesencephalon of 13 to 13.5 day old rat embryos were used to study the potential toxicity of L-DOPA, the D1 receptor agonist dihydrexidine (DHX) and the D2 receptor agonist (±)-4-propyl-9-hydroxynaphoxazine (PHNO) on the survival and function of DA neurons. A single addition of 25, 50, or 100μM L-DOPA to the culture media resulted in general cellular degeneration, and concentration-dependent decreases in ^{3}H-DA and ^{14}C-GABA uptake and numbers of tyrosine hydroxylase (TH) immunoreactive cells. Daily administration of 1, 5 or 10μM L-DOPA resulted in concentration-dependent decreases in ^{3}H-DA and ^{14}C-GABA uptake and numbers of TH immunoreactive cells. A single addition of DHX resulted in concentration-dependent changes; severe cell loss and decreased ^{3}H-DA uptake after 100μM DHX and increased uptake after 1μM DHX. PHNO did not affect ^{3}H-DA or ^{14}C-GABA uptake except after daily treatment with a 1ng/ml concentration. The demonstration of morphological and biochemical changes in cultured DA neurons after short-term administration of L-DOPA and DA receptor agonists suggests that continued administration of agents used for treatment of Parkinson's disease may affect the development of transplanted fetal DA neurons. *Supported by the G. Harold and Leila Y. Mathers Charitable Foundation and the National Parkinson Foundation.*

427.5

THE PRESENCE OF DOPAMINE-IMMUNOREACTIVE STRIATAL CELLS IN 6-HYDROXYDOPAMINE-LESIONED RATS TREATED WITH L-DIHYDROXYPHENYLALANINE. A. Mura, D. Jackson, M.S. Manley, S.J. Young, and P.M. Groves* Dept. of Psychiatry, Univ. of California at San Diego, La Jolla, CA, 92093.

The dopamine (DA) precursor, L-dihydroxyphenylalanine (L-DOPA), alleviates symptoms of Parkinson's disease despite major degeneration of nigrostriatal DA afferents. Though these afferents contain the vast majority of aromatic amino acid decarboxylase (AADC) which converts L-DOPA to DA, AADC has also been detected in a small number of striatal neurons. A greater number of such neurons is unmasked by destruction of nigrostriatal afferents. Disclosure of AADC in striatal neurons leaves open the possibility that exogenous L-DOPA may be converted to DA in these cells. We have examined striatal DA-like immunoreactivity in DA-depleted rats treated with exogenous L-DOPA. Male Sprague Dawley rats (150-200 g) received unilateral medial forebrain bundle injections of 6-hydroxydopamine (8 μg). Five days after surgery, some animals were given the peripheral decarboxylase inhibitor, benserazide hydrochloride (50 mg/kg, i.p.) followed two hours later by L-DOPA (100 mg/kg, i.p.). Rats exhibiting at least 20 contralateral rotations per min, indicative of large DA lesions (>90%), were perfused with a 5% glutaraldehyde fixative two hours after L-DOPA injections. Untreated lesioned rats were also perfused. Brains were removed, cryoprotected, then cut with a freezing microtome (40 μm). Tissue was processed for DA immunoreactivity with a rabbit anti-DA polyclonal followed by avidin-biotin peroxidase staining methods. Immunoreactivity in striatum ipsilateral to the lesion was notably less than that observed in the contralateral striatum. L-DOPA increased immunoreactivity in striatum ipsilateral to the lesion and we observed DA-like immunoreactivity in some dorsomedial striatal cells. These cells exhibited labeled processes and soma (area; 45-55 μm^2). They were infrequently encountered in lesioned striatum in the absence of L-DOPA or in striatum contralateral to the lesion. Collectively, our results demonstrate the formation of DA in striatal cells, a process which may have consequences related to the therapeutic as well as possible neurotoxic effects of L-DOPA. Supported by grants NIDA DA 02854 and NSF BNS 9006155

427.6

Age-related changes in nigrostriatal dopamine System in Squirrel Monkeys. I. Irwin, L. E. DeLanney*, P. Chan, D. A. DiMonte, M. Sandy and J. W. Langston. California Parkinson's Foundation, San Jose, CA. 95128.

Changes in regional brain neurotransmitter systems are often considered as factors that underly the cognitive and behavioral alterations associated with normal aging. Normal age-related decreases in brain neurotransmitter concentrations have also been implicated as possible contributory factors in neurodegenerative disorders known to affect the elderly. Nowhere has the hypothetical connection between age-related neurotransmitter loss and neurodegenerative disease been more evident than in the case of nigrostriatal (NS) dopamine (DA) and Parkinson's disease (PD).

Non-human primates provide a useful animal model to determine age-related changes in NS-DA across many years in animals closest to humans. We studied changes in DA, serotonin (5HT) and the metabolites DOPAC, HVA and HIAA in young (3 yr), middle aged (10 yr) and old (19 yr) squirrel monkeys (n=7/group) previously characterized for their motoric activity.

Significant age-related loss of DA occured only in the substantia nigra (70%) and the putamen (30%). These changes in DA correlated with reduced motoric activity. No age-related changes in DA were observed in the caudate or globus pallidus nor were any changes in 5HT or HIAA observed in any of these areas. Although regional age-related changes in DOPAC and HVA were detected these did not correlate with the loss of DA, nor was there a consistent pattern of increased turnover in areas selectively subject to DA loss.

These results suggest that the aging squirrel monkey may serve as a useful model to investigate the effects of age related NS-DA loss and to determine the relationship between normal age related DA loss and PD.

427.7

A QUANTITATIVE ANALYSIS OF THE DISTRIBUTION OF TYROSINE HYDROXYLASE-POSITIVE CELLS COLOCALIZED WITH CALRETININ IN THE RAT SUBSTANTIA NIGRA K.R. Isaacs* and D.M. Jacobowitz Lab. Clinical Sciences, NIMH, Bethesda, MD 20892.

Calretinin (CR) and tyrosine hydroxylase (TH) were reported to be colocalized in the substantia nigra (SN) (Rogers, 1992) but a detailed mapping of the distribution of the cells and an estimate of the percent co-localization was needed establish baseline values. CR and TH were colocalized within the same section by immunofluoresence microscopy using antibodies to CR (rabbit polyclonal) and TH (monoclonal) and secondary antibodies conjugated with FITC or Texas Red. Sections were photographed to make black and white montages. CR was found in 55% of the TH cells. Colocalized cells were more frequent in the rostral sections (approx. 55% of total cells counted) and dropped to approx. 33% in caudal sections. Areas of colocalization were most frequent in the medial and lateral portions of the SN compacta. CR-only cells were rare (10%) in rostral sections while TH-only cells accounted for approximately half of the total counted cells. In more caudal sections, CR-only and TH-only cells each accounted for 33% of the total count. Because of the possible Ca^{2+} buffering capacity of calcium binding proteins, the presence of CR within dopamine containing cells may be relevant for neuroprotection in Parkinson's disease.

427.8

IN VIVO ELECTROCHEMICAL STUDIES OF DOPAMINE OVERFLOW AND CLEARANCE IN NORMAL AND MPTP-LESIONED STRIATUM OF RHESUS MONKEYS. G.A. Gerhardt*, J. Hudson, W. A. Cass, B. J. Hoffer, Z. Zhang, A. Ovadia, L. Ketonen, and D.Gash, Univ. of Colo. Health Sci. Ctr., Denver, CO, and Univ. of Rochester, Rochester, NY.

Rapid chronoamperometric recordings using Nafion-coated carbon fiber electrodes (30-90 microns O.D.), combined with pressure ejection of drugs from micropipettes, were used to investigate striatal dopamine (DA) fibers in 3 normal and 5 MPTP-treated Rhesus monkeys. The MPTP was administered from 1 to 18 months prior to recording and all treated monkeys exhibited stable Parkinsonian features. For recording, monkeys (20-24 years old) were anesthetized with isofluorane and placed in a stereotaxic apparatus. MRI-guided sterile stereotaxic procedures were used for implantations of the electrochemical recording arrays. Pressure ejection of potassium from micropipettes, which were positioned approx. 300 microns from the electrochemical sensor, was used to evoke the overflow of DA in the putamen and caudate of both normal and MPTP-treated monkeys. In addition, DA clearance was evaluated in normal and MPTP-treated striatum. Potassium was seen to evoke robust overflow of DA into the extracellular space in the unlesioned putamen and caudate nucleus of the Rhesus monkey. In contrast, potassium did not produce any detectable changes (sensitivity 25-50 nanomolar) in extracellular levels of DA in the MPTP-lesioned striatum. In addition, DA clearance was markedly diminished in the lesioned caudate and putamen as compared to unlesioned striatum, supporting that MPTP produces a loss of high-affinity uptake of DA through its destruction of DA nerve endings. These data demonstrate that the residual dopaminergic fibers remaining after MPTP lesions are incapable of supporting the potassium-evoked overflow of DA, and suggest that the remaining DA fibers following MPTP lesions are incapable of properly releasing DA. (Supported by USPHS NS-09199, AG06434, and NS25778.)

427.9

Evidence of the Presence of a Potent Dopamine-Releasing Protein (DARP) in Rat and Human Serum. D.A. Llano and V.D. Ramirez*
Department of Physiology, University of Illinois, Urbana, Illinois. 61801.
Work from our laboratory has revealed the presence of a potent dopamine (DA)-releasing protein (DARP) in rat and bovine adrenal extracts as well as in the media of primary mesencephalic cell culture from 17 day-old rat embryos. In this report, we demonstrate the presence of this factor in the serum of rats and humans. Serum glycoproteins were isolated by treatment with 50% ammonium sulfate and concanavalin A. Both human and rat extracts had potent DA-releasing activity when used in an *in vitro* superfusion assay in which fragments from the rat corpus striatum (CS) were superfused with extract diluted in Krebs-Ringer phosphate. The activity was attenuated by preincubation of the extract with pronase E (maximum increase in DA release = 19.70 ± 4.41 (n=8) vs. 5.75 ± 1.76 (n=9) pg DA/mg wet tissue/min. for the controls and pronase-treated samples, respectively) and with a DARP-specific monoclonal antibody (maximum increase in DA release = 23.09 ± 8.08 (n=8) vs. 8.73 ± 4.87 (n=8) pg DA/mg wet tissue/min. for the controls and antibody-treated samples, respectively). This DA-releasing activity was not seen in similarly prepared extracts from the spleen. Western blot analysis and immunoaffinity chromatography indicate that it has a molecular weight of about 60kD, which corresponds to the molecular weight of adrenal-derived DARP. In addition, preliminary data using serum extracts from two 15-day adrenalectomized (ADX) animals demonstrate elevated DA-releasing activity relative to that of two sham-ADX animals (maximum increase in DA release = 19.70 ± 4.41 (n=8 superfusions) vs. 49.79 ± 10.53 (n=7) pg DA/mg wet tissue/min. for the controls and 15-day ADX samples, respectively). Furthermore, dopaminergic neuronal activity (determined by analyzing the DOPAC:DA ratio using HPLC-EC) in the hypothalamus and the CS is enhanced in 15-day ADX animals. This ratio increased significantly in the hypothalamus (DOPAC/DA =.860 ± .131 (n=8 rats) and 1.651 ± .286 (n=5) in the sham ADX and 15-day ADX animals, respectively) and the CS (DOPAC/DA =.138 ± .008 (n=8) and .183 ± .023 (n=5)). These data demonstrate the presence of a DARP-like substance in the sera of rats and humans and suggest that the removal of the adrenal glands leads to an increase in basal dopaminergic activity in the hypothalamus and CS.

DEGENERATIVE DISEASE: PARKINSON'S—HUMAN PERFORMANCE AND PRIMATE MODELS

428.1

FURTHER CHARACTERIZATION OF COGNITIVE AND MOTOR DEFICITS IN CHRONIC LOW DOSE MPTP-TREATED MONKEYS. Z.-Q. Sun*, A. Pope, J. S. Schneider, and D. P. Roeltgen. Dept. of Neurology, Hahnemann University, Philadelphia, PA. 19102.

We have previously reported that macaque monkeys exposed chronically to low doses of the neurotoxin MPTP develop cognitive and behavioral deficits similar to those reported in early Parkinson's disease (PD), in frontal lobe patients, and in people with attention deficit hyperactivity disorder (ADHD). We now further examine the chronic low dose MPTP syndrome in 8 monkeys by examining effects of this treatment on the following tasks: delayed matching-to-sample (DMS), object retrieval (OR) (with cognitive and motor components), persistence (during a difficult or impossible task), simple or difficult visual pattern discrimination (VPD), discrimination reversal (DR), and food retrieval tasks (with easy or difficult motor requirements). Monkeys received MPTP (0.05-0.20 mg/kg) on a variable schedule for approximately 3 to 6 months. All monkeys eventually had difficulty performing DMS, the cognitive component of OR, and DR. The first deficits to appear were deficits in persistence tasks and DMS. VPD remained intact throughout the study. Some motor slowing was eventually observed during OR and food retrieval tasks, but this did not appear to significantly impair the animal's ability to correctly perform the tasks. On cognitive tasks, errors of omission or comission were made. Dopamine D-2 agonists reduced the the number of omission errors but not comission errors while adrenergic alpha-2 agonists had opposite effects. These results furhter demonstrate the "frontal" nature of cognitive deficits in monkeys with a primary neurochemical lesion affecting the striatum and further underscore the importance of these animals as models for early PD and ADHD. Supported by MH46531.

428.2

PROGRAMMING OF A MOVEMENT SEQUENCE IN PARKINSON'S DISEASE. P. Weiss[1], G.E. Stelmach[1]*, H. Hefter[2], H.-J. Freund[2] [1]Motor Control Lab., Arizona State Univ., Tempe, AZ 85287 -0404, [2]Department of Neurology, Univ. of Duesseldorf, Germany.

A group of Parkinsonian (age: 59 ± 13 yrs.), elderly (age: 57 ± 9 yrs.) and young (age: 27 ± 3 yrs.) subjects performed sequential movements with a stylus on a digitizer board (100 Hz sampling frequency, 0.1 mm accuracy). After an imperative auditory stimulus, subjects had either to draw a 10 cm horizontal line from a starting point into a square (side length: 2 cm), or draw the 10 cm line and proceed to a second square (side length: 2 cm, Index of difficulty (ID): 2.3), or draw the 10 cm line and proceed to a second smaller square (side length: 0.7 cm, ID: 3.3). 50 trials in each condition were digitally recorded and the movement kinematics were analyzed; movements were compared by examining the peak velocity (PV), time to peak velocity (TPV) and deceleration time (DT) for the first and second movement components.

The Parkinsonian group showed a lengthening of movement time and a decrease in velocity under all three task conditions. In the Parkinsonian group, the longer DT and total time in the first movement component revealed that the patients had difficulties in stopping their actions and that they performed movements faster, when there was a second movement. The significant group by task interaction [$F(1,17) = 11.74$, $P = 0.003$] for the peak velocity in the second component of the movement (ID 2.3 versus ID 3.3) showed that Parkinson patients did not modulate movement speed. We interpreted these findings to indicate that patients with Parkinson's disease have problems in scaling the velocity of their movements under accuracy constraints.

428.3

SPEECH AND VOICE CHARACTERISTICS OF EARLY PARKINSON'S DISEASE. L. Carol Gracco, K. L. Marek, and V. L. Gracco*, Haskins Laboratories and Yale University, New Haven, Ct 06511.

Movement of the speech articulators and vibration of the vocal folds are two important processes for human communication. To be effective, the actions of the lips, jaw, tongue, and larynx must be appropriately scaled and coordinated to produce the aerodynamic and acoustic patterns for speech. Damage to the basal ganglia often produces disruptions in speech and voice resulting in a variety of perceptual and movement symptoms such as hoarse voice, imprecise articulation, and slowed speaking rate. One objective of the present investigation was to examine the breakdown in speech and voice production to obtain insight into the role of the basal ganglia in human communication. Secondly, the speech and voice impairments were compared to limb characteristics, to determine the consistency in degree and severity across motor systems. It is conceivable that speech and voice difficulty in individuals with Parkinson's disease may be more readily apparent or occur earlier in the disease process than the more traditional limb signs.
Twenty subjects with early Parkinson's disease (based on standardized neurological exam) were used. A number of instrumental measures were obtained allowing analysis of the movements of individual articulators and their coordination. In addition, videostroboscopic laryngeal imaging and detailed acoustic analysis of the voice signal were correlated with perceptual ratings of each subjects speech and voice capability.
In these mildly involved subjects, some difficulties were noted in the scaling and coordinating of the speech articulators. Qualitatively, the degree and character of the speech movement deficits were not consistent with those observed in the limbs. Moreover, deficits in larygneal vibration and subsequent acoustic analysis proved to be more sensitive than the standardized neurological exam and may contribute significantly to early evaluation and diagnosis.
Supported by NIH grants DC00121, DC00594, DC00044, and the National Parkinson's Foundation.

428.4

OBJECTIVE ANALYSIS OF SIMPLE REACTION TIME IN PARKINSONIAN AND CONTROL SUBJECTS FOR FIXED AND RANDOM PREPARATORY INTERVALS. A.S. Mandir*, J.F. Paulsen, C.W. Schultz and L.A. Flashman, The Johns Hopkins Univ Sch of Med, Dept of Neurology, Baltimore, MD 21205, UCSD, VA Med Ctr, Depts of Psychology and Neurology, San Diego, CA 92161 and Univ of Iowa, MHCRC, Iowa City, IA 52242.

Preparatory cues may enhance reaction time (RT) by allowing the advantage of premovement "set" phenomena. Simple RT is prolonged in Parkinson Disease (PD) in part from dysfunction of preparatory "set" processing. A paradigm was employed which objectively examined the effect of preparatory intervals on reaction time in PD and control subjects. A computer RT task presented "GO" signals at 1,2,4,7.5,15 and 25 second intervals from a ready state (S1-S2 preparatory interval). Trials were performed in blocks of fixed intervals (allowing for "set") and random intervals. Preliminary data were obtained for 9 PD and 12 control subjects. PD subjects demonstrated prolonged RT compared to controls at all preparatory intervals (Mann-Whitney;p<.01). All subjects demonstrated reduced RT at shorter preparatory intervals for fixed compared to random block trials (Mann-Whitney;p<.01). However, as the preparatory interval lengthened, 7 of 9 PD subjects compared to 1 of 12 control subjects failed to maintain reduced RT for fixed versus random blocks. Those PD subjects eventually demonstrated prolonged RT for fixed compared to random blocks at the longer preparatory intervals ("crossed over"). A propensity for "set" premovement programming dysfunction in PD may occur as the preparatory interval is increased and serve to hinder performance of simple RT tasks.

428.5

POSTURAL TREMOR OF MIDDLE FINGER IN PARKINSON PATIENTS AND AGE-MATCHED CONTROLS. Suzanne S. Palmer*[1] and J. Thomas Hutton[2]. Dept. of Physical Therapy, Texas Tech Univ. Health Sciences Center, Lubbock, TX 79430[1] and Neurology, St. Mary of the Plains Hospital, Lubbock, TX 79410[2].

If a central mechanism were the primary cause of enhanced finger tremor in Parkinson's disease, the peak frequency in spectral analysis of rectified electromyographic activity (EMG) of finger extensor muscle would be correlated with tremor frequency. Age-matched control subjects would not show correlated peak frequencies of EMG and movement acceleration because several other causal factors might also contribute to their tremor.

EMG was recorded from extensor digitorum, and acceleration from the proximal phalanx of the middle finger of 14 Parkinson patients (χ = 69.7 yrs) and 14 age-matched control subjects (χ = 63.8 yrs). They extended the middle finger of their nondominant hand off the table top for 10 min at 20-30° with their hand placed flat. Spectral analysis was done of a 5.44 min epoch of time after the first two min. A multifactor ANOVA test showed peak frequency of tremor was related to type of subject (normal or Parkinson) and peak frequency of EMG, but not to the power of either tremor or EMG. A linear regression analysis of data from Parkinson patients showed peak frequency of tremor was correlated with peak frequency of EMG (correl. coeff. = 0.983, $p < 0.001$). Controls showed a lack of correlation of peak frequency of tremor with peak frequency of EMG (correl. coeff. = 0.389, $p = 0.169$). These results provide evidence central mechanisms enhance activity in the motoneuron pool and muscle and contribute to Parkinson tremor.

428.6

NALTREXONE DECREASES DOPAMINE D_2 BUT NOT D_1 RECEPTOR MEDIATED ROTATIONAL BEHAVIOUR IN UNILATERALLY MPTP-LESIONED MONKEYS. B. Drukarch*[1], R.J. Vermeulen[1,3], A.N.M. Schoffelmeer[2], E.Ch. Wolters[1], C. Goosen[3], and J.C. Stoof[1]. Depts of Neurology[1] and Pharmacology[2], Free University Amsterdam, [3] Medical Biological Laboratory TNO[3], Rijswijk, The Netherlands.

Reportedly, in the unilateral 6-hydroxydopamine rodent model of Parkinson's disease L-Dopa induced rotational behaviour is attenuated by μ-opioid antagonists. However, no information is available whether this effect of μ-opioid receptor blockade on rotational behaviour also occurs upon selective D1 or D2 receptor stimulation. In order to investigate this question and to extend the findings also to other kinds of dopamine receptor induced behaviours, we used the unilateral MPTP monkey model of Parkinson's disease.

The lesion was made by an unilateral infusion of MPTP in the left internal carotid artery. All animals (n=4) showed a loss of handuse at the right side. The D1 agonist SKF 81297 (0.3 mg/kg, i.m.) and the D2 agonist LY 171555 (0.01 mg/kg, i.m.) induced a statistically significant stimulation of both rotational behaviour (away from the lesion) and right hand use. The μ-opioid antagonist naltrexone (0.5 mg/kg, i.m.) alone did not induce any effect on rotations or hand use. However, naltrexone caused a statistically significant (46%, $P<0.05$) reduction of LY 171555 induced rotational behaviour whereas no effect was seen on LY 171555 induced handuse. Interestingly, no effect of naltrexone was observed on D_1 agonist stimulated rotational behaviour and handuse.

These preliminary data indicate that, in models of Parkinson's disease, the effect of μ-opioid receptor blockade on dopamine receptor induced behaviours is limited to D2 receptor stimulated rotations.

428.7

CORRELATION BETWEEN DOPAMINE D_2 RECEPTOR INDUCED BEHAVIOR AND IN VIVO RECEPTOR OCCUPANCY IN A MONKEY MODEL OF PARKINSON'S DISEASE. R.J. Vermeulen[1,3], B. Drukarch[1], M.P. Witter *[2], N.P.L.G. Verhoeff[4], E.A. van Royen[4], E.Ch. Wolters[1], C. Goosen[3] and J.C. Stoof[1]. Depts of Neurology[1] and Anatomy & Embryology*[2] Free University, Dept. of Nuclear Medicine[4], Academic Medical Center Amsterdam, Medical Biological Laboratory TNO[3], Rijswijk, The Netherlands.

Dopamine D_2 receptors are a pivotal target in the pharmacotherapy of Parkinson's disease. Unfortunately, almost no data are available linking clinical effects of drug treatment with the extent of D_2 receptor occupancy in the brain. Therefore, we investigated the correlation between motor behavioral effects of the selective D_2 agonist LY 171555 and in vivo D_2 receptor occupancy in a monkey model of Parkinson's disease using Single Photon Emission Computerized Tomography (SPECT). Four male rhesus monkeys (Macaca mulatta) were unilaterally (i.e. by left intracarotid infusion) lesioned with MPTP. The MPTP-lesioned monkeys consistently showed signs of unilateral Parkinsonism. Administration of LY 171555 (0.01 or 0.3 mg/kg i.m.) induced a significant increase of contralateral rotation (away from the lesion). In the SPECT studies intravenous infusion of ^{123}I-IBZM (3-iodo-6-methoxybenzamide) was used to label D_2 receptors. In the MPTP-lesioned monkeys the ^{123}I-IBZM binding in the left (lesioned) striatum was significantly higher as compared to that in the right (non-lesioned) striatum (approximately 13 % increase, $P<0.05$). Upon administration of LY 171555 (0.01 or 0.3 mg/kg) a significant displacement of ^{123}I-IBZM at both sides (left and right) was detected only at the highest dose. We conclude that LY 171555 induces behavioral effects in unilaterally MPTP-lesioned monkeys at relatively low doses. However, using 123I IBZM SPECT scanning, no direct correlation could be demonstrated between these behavioral effects and D_2 receptor occupancy in vivo.

428.8

ABNORMAL DYNAMIC MODULATION OF PUTAMEN NEURONAL ACTIVITY FOLLOWING MPTP. Erwin B. Montgomery, Jr.,* The Univ of Arizona Col of Med, Department of Neurology, Tucson AZ

The dynamic modulation of neuronal activity associated with movement generation was compared in 26 putamen neurons recorded before MPTP injection and 25 neurons after. The monkey was parkinsonian in cage behavior and had prolonged reaction times in a wrist task after MPTP. Wave-forms representative of dynamic modulation were constructed from peri-event histograms of neuronal activity from 500 ms before to 500 ms after movement onset. Wave-forms were compared by calculating the Pearson correlation coefficients between all possible pairs. The table of Pearson correlations was represented in a three-dimensional space by principal component analysis. Wave-forms of neurons recorded after MPTP were distributed over a smaller region of the similarity space implying a reduction in the dynamic range of neuronal activity modulation. The reduced range may reflect a simpler or reduced repertoire of movement programs that would be most evident when complex movements are required. This may account for the greater impairment of complex vs. simple movements in humans with Parkinson's disease. New models of pathophysiology are needed to explain these changes in dynamic modulation.

428.9

GABA DELIVERY TO THE SUBTHALAMIC NUCLEUS ALLEVIATES MPTP INDUCED HEMIPARKINSONISM IN NON-HUMAN PRIMATES. A.P. Signore[1], M. Goddard[1], P.A. Tresco[2]*, R. Burrill[1], P. Aebischer[1,3]. [1]Division of Biology and Medicine, Brown University; [2]Department of Bioengineering, University of Utah; [3]Division of Surgical Research, CHUV, University of Lausanne Medical School, Switzerland.

Parkinson's disease is characterized by the degeneration of the dopaminergic nigral neurons projecting to the striatum, resulting in altered levels of neurotransmitters in several basal ganglia nuclei. Overactivity of the subthalamic nucleus (STN) occurs in Parkinson's disease as a result of a decreased γ-aminobutyric acid (GABA) innervation to this nucleus, secondary to a decreased dopaminergic innervation of the striatum. To attempt to achieve normalization of STN activity, polymer rods slowly releasing GABA were implanted near the STN in two unilaterally MPTP-lesioned primates. The first primate showed a significant improvement of parkinsonian symptoms, including scoring on an object retrieval task, whereas no significant improvement was observed in the second primate. Morphological evaluation revealed a correct placement of the rod only in the first primate. Extensive lesioning of the substantia nigra was seen in both primates as assessed by tyrosine hydroxylase immunohistochemistry. An additional primate was also implanted with a rod releasing the specific $GABA_B$ receptor agonist, baclofen in the vicinity of the STN. This primate also shows a significant improvement in the object retrieval task. Delivery of GABA or its agonists to the STN through a controlled release process may be a viable strategy in the treatment of Parkinson's disease as this structure is significantly smaller than the dopamine depleted striatal target.

428.10

ALLEVIATION OF BRADYKINESIA AND RIGIDITY BY HIGH FREQUENCY STIMULATION OF THE SUBTHALAMIC NUCLEUS IN MPTP-TREATED MONKEY. Ch. Gross, A. Benazzouz, T. Boraud, J. Féger[1] and B. Bioulac*. Laboratoire de Neurophysiologie, CNRS URA 1200, Université de Bordeaux 2, 33076 Bordeaux Cedex, France. [1]Laboratoire de Pharmacologie, Université de Paris VI, France.

Parkinsonian bradykinesia and rigidity are the consequence of degeneration of dopaminergic neurons in the substantia nigra (SN). This leads to the abnormal hyperactivity in the basal ganglia output structures : internal part of globus pallidus (GPi) and pars reticulata of substantia nigra (SNr). The main driving force of this hyperactivity appears result from the afferent excitatory glutamatergic pathway of the subthalamic nucleus (STN). The lesion of this structure alleviates the parkinsonian motor signs but induces dyskinesia and hemiballismus in the monkey.

In the present work, we have studied the effect of high frequency stimulation (HFS) of the STN in two unilaterally MPTP-treated monkeys. Rigidity was mesured using EMG recordings of biceps and triceps during passive repetitive flexion and extension movements of the forearm. Bradykinesia was quantified by mechanographic and EMG recordings associated to voluntary trained movements. The results demonstrate that HFS of the STN alleviated the hemiparkinsonian motor symptoms in both monkeys. Neither dyskinesia nor hemiballismus was observed during HFS of the STN.

This work confirms the involvement of subthalamic nucleus in the genesis of parkinsonian signs and suggests that this technique may have a therapeutic potentiality for treating parkinsonism.

428.11

STIMULATION OF SUBTHALAMIC NUCLEUS ACUTELY CHANGES CLINICAL STATUS IN PARKINSON'S DISEASE. A.L. Benabid[1], P. Pollak[1], C. Gross[2], D. Hoffmann[1], A. Benazzouz[2], D.M. Gao[1], A. Laurent[1], M. Gentil[1], J. Perret[1] and C. Feuerstein[1]*. 1: Dept of Neurosciences and INSERM U 318, Grenoble. 2: CNRS URA 1200, Bordeaux, France.

In animal models of Parkinson's disease (PD), it is postulated that the excessive output from the subthalamic nucleus (STN) plays a critical role. Selective lesions or high frequency electrical stimulation of STN can alleviate parkinsonian symptoms in MPTP-treated monkeys. We decided to carry out STN stimulation in patients suffering from severe akinetic forms of PD. After approval from ethical committees, we operated a 51 old PD patient, suffering since 8 years from a strongly disabling akineto-rigid form of PD, with on-off effect (Hœhn & Yahr stage 5). Stereotaxic surgery was done under local anaesthesia on one side. The theoretical target was chosen according to stereotaxic atlases. Final position of the chronic electrodes was optimized using electrophysiological recording and stimulation altogetther with clinical assessment and surface EMG. Increase in neuronal activity was recorded in the subthalamic area. In the same place, 130 Hz stimulation induced acute and reversible akinesia alleviation mainly on the contralateral limbs, comparable to that obtained with dopaminergic drugs. No dyskinesia, such as hemiballism, was induced by surgery. Studies of the effects of chronic stimulation were extensively performed before and after connection of electrodes to implantable programmable Medtronic Itrel II stimulators. Mechanisms of stimulation which can induce similar effects than destruction are still unknown. Nevertheless, the interest of stimulation versus neural grafts must be discussed in patients with highly disabling forms of PD.

428.12

A NEW ANIMAL MODEL OF DYSTONIA. Joel S. Perlmutter*, Lee W. Tempel, & Lennis Lich. Depts of Neurology and Radiology, Washington University School of Medicine, St. Louis, MO 63110.

An animal model of dystonia may permit evaluation of the underlying pathophysiology as well as assessment of new treatments. We found that intracarotid (i.c.) administration of MPTP in a baboon may produce a transient hemidystonic syndrome followed by hemiparkinsonism. Six male baboons were studied. Each was fasted overnight, anesthetized with ketamine and kept asleep with nitrous oxide and oxygen. A Hanafee angiographic catheter was advanced under fluoroscopic control from the femoral artery high into either the right or left common carotid artery. Placement of the catheter was confirmed with injection of contrast and a permanent record was made by radiograph. MPTP (about .4 mg/kg dissolved in normal saline, .1 mg/ml) was infused through the catheter no faster than 1 ml per minute. Blood pressure, pulse and temperature were monitored and remained constant throughout the procedure. After completion of MPTP infusion, the position of the catheter was re-confirmed by fluoroscopy. Animals recovered quickly from the sedation and usually appeared normal that day. By the next day, most animals displayed spontaneous ipsilateral turning. Later that day or within a few days, they developed varying severity of contralateral hemidystonia with the arm and leg held in an extended and externally rotated posture. Postural tremor was variably present at that time. This dystonic phase lasted from 1 week to 2 months and was followed by a more typical hemiparkinsonian phase with flexed posture and bradykinesia. Some animals also had postural or resting tremor. The parkinsonian phase remained constant in these animals for as long as 1 1/2 years. We propose that i.c. administration of MPTP can produce a clinical syndrome of hemidystonia that appears similar to the human condition. In particular, this appears directly analogous to dystonia in a lower extremity that may be the presenting, and frequently a transient, manifestation of Parkinson's disease in humans.

428.13

REVERSAL OF EXPERIMENTAL PARKINSONISM IN PRIMATES WITH GM1 GANGLIOSIDE: QUANTITATIVE AUTORADIOGRAPHIC AND IMMUNOHISTOCHEMICAL STUDIES. A. Pope* and J. S. Schneider. Dept. of Neurol., Hahnemann University, Phila. PA. 19102.

We have previously reported that chronic GM1 ganglioside treatment could significantly improve motor and cognitive functioning in monkeys made parkinsonism by MPTP as well as increase striatal dopamine levels and the density of striatal tyrosine hydroxylase (TH)-positive fibers (Science (1992), 256, 843). This study further examines effects of GM1 on the density of striatal dopamine uptake sites ([3H] mazindol binding) D2 ([3H] spiperone binding), and D1 ([3H] SCH23390) receptors as well as the number and distribution of TH-positive ventral mesencephalic neurons in MPTP-exposed monkeys. MPTP caused more extensive losses of [3H] mazindol binding in dorsal than ventral striatal regions. Animals that received GM1 treatment for 6 weeks subsequent to the induction of parkinsonism had significantly increased [3H] mazindol binding, compared to MPTP + saline-treated animals, in most dorsal and ventral striatal regions examined. D2 receptor density was not increased following MPTP exposure and was not influenced by GM1 treatment. Striatal D1 binding was increased in MPTP + saline animals but not in MPTP + GM1-treated animals. Examination of TH-positive ventral mesencephalic neurons revealed more and healthier appearing neurons in GM1-treated monkeys. These results suggest that GM1 ganglioside treatment, instituted within 48 to 60 hours of the last of several MPTP injections, and during a period of active degeneration of dopaminergic neurons, has the ability to stimulate the survival of neurons that perhaps would otherwise have died and promote the sprouting of dopaminergic terminals in the denervated striatum. These data add further support for the use of GM1 ganglioside as a potential therapeutic agent for Parkinson's disease. Supported by Fidia Research Laboratories and the F. M. Kirby Foundation.

428.14

INCREASED POTENCY OF ORALLY ACTIVE SEMISYNTHETIC SPHINGOLIPIDS COMPARED TO GM1 GANGLIOSIDE: IN VIVO AND IN VITRO STUDIES. J. S. Schneider*, N. Stull, L. DiStefano and L. Iacovitti. Dept. of Neurol., Hahnemann University, Phila., PA. 19102.

Although GM1 ganglioside has been shown to exert reparative effects on the MPTP-damaged dopamine (DA) system in vivo and in vitro, its clinical testing has been limited due to its availability only as an injectable. We have investigated the reparative potential of 2 orally administered semisynthetic sphingolipids, LIGA 4 and LIGA 20, on the MPTP-damaged DA system in vivo. C57 black mice were administered MPTP-HCl once daily for 5 days (20 mg/kg) and given either LIGA 4 or LIGA 20 (50 mg/kg daily) or water by oral gavage for 2 weeks beginning 24 hrs. after the last MPTP injection. Other animals received GM1 ganglioside (30 mg/kg, i.p.) for 2 weeks. LIGA 4 and LIGA 20 increased striatal DA levels by 84-95% above lesion controls while GM1 increased DA levels by only 68%. In a dose response study, GM1 was found to have an optimal dose range of 15-30 mg/kg, with decreased efficacy at low and high doses. In vitro, LIGA 20 was shown to be effective at increasing tyrosine hydroxylase (TH) activity levels in E15 rat ventral mesencephalic neurons previously exposed to MPP+ at much lower concentrations than GM1 ganglioside. The ability of GM1 to promote recovery of DA neurons in vitro depends in large part on the extent to which the cultures have been damaged by the neurotoxin. LIGA 20 produced a moderate recovery of TH activity in severely MPP+-damaged cultures where GM1 had no or minimal effects. These results suggest that the semisynthetic sphingolipids LIGA 4 and LIGA 20 are more potent in exerting reparative effects on the damaged DA system than the parent natural GM1 ganglioside. This, combined with the ability of these compounds to maintain activity after oral administration, make them attractive potential therapeutic agents for Parkinson's disease. Supported by AG 10280 and Fidia Research Laboratories.

DEGENERATIVE DISEASE: PARKINSON'S—TRANSPLANTATION AND GLIA

429.1

EVALUATION OF A NEW STEPPING TEST TO MONITOR LIMB FUNCTION IN THE RAT PARKINSON MODEL: LESION, DRUG, AND TRANSPLANT EFFECTS. M. Olsson, G. Nikkhah[1], C. Bentlage and A. Björklund*. Department of Medical Cell Research, Biskopsgatan 5, S-223 62 Lund, Sweden and (1) Neurosurgical clinic, Nordstadt hospital, D-3000 Hannover 1, Germany.

To further evaluate functional effects of nigral transplants as well as dopaminergic drugs in the rat Parkinsons disease (PD) model, there is a need for quantitative tests of spontaneous, i.e. non-drug induced behaviors that are analogous to the deficits seen in PD patients. Schallert et al. (Soc. Neurosci. Abstr. 451:5, 1992) have recently introduced a test of stepping movements to study forelimb akinesia in rats with 6-hydroxydopamine (6-OHDA) lesions of the nigrostriatal pathway. In the present study we have used a modification of this test to monitor changes in forelimb stepping, ipsilateral and contralateral to a unilateral 6-OHDA lesion, after administration of dopamine receptor agonists, or amphetamine, and after transplantation of fetal nigral neurons to the denervated caudate-putamen. Two parameters were measured : (1) Time to initiate stepping with either forelimb when the other limb is immobilized by the experimenter and the hind part of the rat is raised (initiation time); (2) Number of adjusting steps when the rat is passively moved sideways by the experimenter over a distance of 1 m. Both initiation time and adjustment stepping are severly impaired in the forelimb contralateral, but not ipsilateral, to the 6-OHDA lesion. Preliminary results indicate that multiple intrastriatal nigral transplants can significantly improve stepping movements with the impaired limb, and that a similar improvement can be obtained by a low dose of apomorphine (0,0125 mg/kg) but not amphetamine (0.05 mg/kg).

429.2

TRANSPLANTATION OF PRIMARY FIBROBLASTS GENETICALLY MODIFIED TO EXPRESS TYROSINE HYDROXYLASE IN A PARTIAL 6-OHDA RAT LESION MODEL OF PARKINSON'S DISEASE (PD): EFFECTS OF SYSTEMIC TETRAHYDROBIOPTERIN. R.J. Mandel*, D.G. Clevenger, C.K Unruh, S.K. Spratt, M. Schinstine, K. Bankiewicz, and M.V. Sofroniew. Somatix Therapy Corp., 850 Marina Village Pkwy., Alameda, CA 94501.

Primary fibroblasts genetically modified to express tyrosine hydroxylase (TH) have been shown to release L-dopa *in vitro* and have a positive effect on apomorphine (apo)- induced rotation in unilateral 6-hydroxydopamine (6-OHDA) lesioned rats (Fisher *et al., Neuron* 6:371-380, 1991). This study was undertaken to determine whether systemic administration of the co-factor for TH, tetrahydrobiopterin (BH_4), could improve the function of TH^+ fibroblast grafts in a rat model of PD (Uchida *et al., Dev. Neurosci.* 14:173-180, 1992). Ten rats received specific A-9 6-OHDA lesions. Four wks post-lesion, 5 rats received 5 x 10^5 TH^+ cells in 10 µl spread equally over 4 intrastriatal injection sites on the lesioned side, while the 5 control rats received identical transplants of primary fibroblasts expressing the *lac-Z* gene. One wk following transplantation, the rats were tested in cross-over designs for apo- (0.1 mg/kg, sc) and d-amphetamine- (2 mg/kg, ip) ± BH_4 (50 mg/kg, ip) induced rotational behavior. Transplantation of TH^+ fibroblasts significantly reduced apo-induced rotational behavior (-25%) relative to rats which received *lac-Z* expressing fibroblasts ($p = 0.05$) regardless of BH_4 treatment but had no effect on amphetamine-induced rotations. BH_4 treatment significantly **increased** apo- and significantly **decreased** amphetamine-induced rotations regardless of graft treatment ($p < 0.05$).

These data indicate that exogenous BH_4 treatment modifies drug-induced behavior in the rat PD model while the blood brain barrier is disrupted. In contrast, BH_4 treatment did not augment the positive functional effects of TH^+ grafts in this preliminary study.

429.3

PURIFIED BOVINE CHROMAFFIN CELL GRAFTS DO NOT REDUCE AMPHETAMINE-INDUCED ROTATION IN HEMIPARKINSONIAN RATS

M. Bresjanac*†, Sagen†, G. Seigel, Jeffrey H. Kordower and D. M. Gash, Dept. of Neurobiol. and Anat., U. of Rochester Sch. of Med. Rochester, NY 14642; U. of Illinois, Chicago, IL 60612*; Rush Presbyterian/St. Lukes Hospital, Chicago, IL 60612**

Recently, adrenal medullary grafts have been used in animal and clinical studies of treatment for parkinsonism. Survival of such grafts has not been satisfactory. Reported concomitant functional recovery has been variable. The present study aimed to (a) ensure optimal survival of adrenal chromaffin cells in the striata of partially hemiparkinsonian rats, and (b) determine if the presence of viable chromaffin cells leads to a reduction in amphetamine-induced rotation of the recipient animals.

Adult male Fisher 344 rats were rendered partially hemiparkinsonian by intranigral injection of 4 µg of 6-hydroxydopamine. Four weeks later, they were selected for the study if they rotated ipsilateral to the lesion in response to amphetamine (5 mg/kg), while not responding to a low dose of apomorphine (0.025 mg/kg). The selected animals were rank-ordered into three treatment groups. The experimental group received dissociated, purified bovine chromaffin cells. One control group received dissociated non-chromaffin cells from the same glands. All intrastriatal implants contained 100,000 cells in 4 µl of Hank's buffered salt solution. The third group were non-implanted hemiparkinsonian rats. All animals received daily injections of cyclosporine A (10 mg/kg). In the fourth week postimplantation, they were again challenged with amphetamine. At 28 days, they were sacrificed and the brains were processed for histological evaluation.

Viable grafts were found by immunohistochemistry to tyrosine hydroxylase, chromogranin A and bovine fibronectin in 14 out of 16 animals. Nevertheless, there was no reduction in amphetamine-induced rotation in any of the groups during our observation period.

Supported by UPF Individual Research Grant and the NIH NS25778 and NS25054.

429.4

USE OF THE ANTI-APOPTOTIC GENE *BCL-2* IN NEURAL TRANSPLANTATION. R. Anton*, D.J. Kane, J.S. Manaster, J.H. Kordower,[1] S.B. Schueler,[1] C.H. Markham, D.E. Bredesen. Dept. of Neurol., UCLA, L.A., CA 90024 & Univ. of Ill. Sch. Med. Dept. of Neuro. Sci., Rush Presbyterian Med. Ctr.[1], Chicago, IL 60612.

Long-term survival of grafted neural cells is a major goal of neural transplantation, but typical survival rates of grafted fetal neurons are in the range of 1-10%. Whether the death of transplanted neural cells is apoptotic or necrotic is unknown. We recently reported that the expression of the proto-oncogene *bcl-2* inhibits both apoptotic and necrotic neural cell death (Zhong *et al.*, 1992, 1993; Kane *et al.*, submitted). Therefore, we questioned whether the expression of *bcl-2* in a subclone of conditionally immortalized nigral neural cells (Durand *et al.*, 1990) would enhance survival and efficacy of grafts of these cells. In a 6-OHDA induced rat model of Parkinson's disease, Hoechst 33258 prelabelled cells were stereotactically transplanted into the striatum ipsilateral to the lesioned nigrostriatal pathway. Six rats received *bcl-2* transfected cells, 5 received cells transfected with vector alone, and 6 were sham operated. Four weeks following transplantation, the rats with grafts containing *bcl-2* expressing cells showed a stable 30% decrease in apomorphine-induced rotational behavior. In contrast, no improvement occurred in the rats with transplanted cells containing vector alone or in rats with sham injections. Histological examination showed the presence of Hoechst 33258 labelled cells in the striatum of rats which received the cells expressing *bcl-2*. No tumor formation was observed in these rats. No immunofluorescence was detectable in the other two groups of rats at the transplantation site.

429.5

RESTORATION OF DOPAMINE OVERFLOW AND CLEARANCE FROM 6-OHDA LESIONED RAT STRIATUM REINNERVATED BY FETAL VENTRAL MESENCEPHALIC GRAFT. S.D. Wang*, J.C. Liu and Y. Wang, National Defense Medical Center, Taipei, Taiwan.

The purpose of this experiment is to investigate the electrochemical functions of the mesencephalic dopaminergic grafts in the rat striatum. Sprague Dawley rats were injected unilaterally with 6-hydroxydopamine (6OHDA) into the anterior forebrain bundle and screened by measuring apomorphine- induced rotation. These unilaterally lesioned rats were later transplanted with fetal ventral mesencephalon (VM). Only animals received VM transplantation showed significant decreases in rotation postgrafting. A high-speed chronoamperometric recording techniques using Nafion-coated carbon fiber electrode was used to evaluate dopamine turnover in the striatum of urethane- anesthetized rats. We found that 6OHDA lesions resulted in loss of KCl-induced DA overflow and clearance. These electrochemical functions were regenerated after VM graft. Furthermore, immunohistochemical studies using tyrosine hydroxylase confirmed graft survival and outgrowth from the graft into the lesioned striatum. In conclusion, these findings suggested that the behavioral improvements by grafts of fetal mesencephalic tissue correlated with the morphological nerve reinnervation and the restoration of DA turnover.

429.6

HOST COMPENSATORY RESPONSE TO NEURAL GRAFTING IN THE UNILATERAL 6-OHDA PARTIAL LESIONED RAT. K. Sakai*, S. Moran, and J.T. Hansen. Department of Neurobiology and Anatomy, University of Rochester School of Medicine and Dentistry, Rochester, NY 14642.

Evidence suggests that the host brain can respond to neural transplantation, even with poor graft survival, by mounting a sprouting response presumably from spared elements of an existing neuroanatomical pathway. To test the hypothesis that a host sprouting response is an important compensatory mechanism for restoring damaged or degenerated input to a brain region, we used a selective unilateral 6-OHDA lesioned rat model in which the dopamine (DA) neurons of the ventral tegmental area (VTA; A10) were spared but the DA neurons of the substantia nigra, pars compacta (A9) were lesioned. Tyrosine hydroxylase-like immunoreactivity (TH) and Lucifer yellow (LY) tract tracing were used to examine the host response following co-grafts of adrenal chromaffin cells and peripheral nerve into the partially denervated rat neostriatum. Two weeks following grafting, an increase in TH stained fibers was observed adjacent to the ventromedial aspect of the implant site. LY retrograde tracing confirmed that these fibers originated, in part, from spared A10 and medial A9 DA neurons. This apparent increase in TH staining in striatal fibers occurred regardless of grafted chromaffin cell survival. At least two mechanisms may account for this striatal neural plasticity: (1) an absolute increase of striatal TH axons and terminals in response to grafting, and/or (2) an upregulation of DA synthesis in spared fibers. Studies currently are underway to examine these two mechanisms and to determine the role that neurotrophic factors and cytokines play in this host response. This host response may have significant implications for ameliorating deficits in patients with neurodegenerative disorders. (Supported by NS 25778)

429.7

IN VIVO PROLIFERATION OF A7 GLIAL CELLS IS REDUCED AFTER GRAFTING INTO ADULT RAT STRIATUM. G.S. Okoye*, R.S. Nowakowski#, W.J. Freed+ and H.M. Geller. Neurosurgical Research Laboratory and Dept. of Pharmacology, #Dept. of Neuroscience and Cell Biology, UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ 08854, and +NIMH Neurosciences Center at St. Elizabeths, Washington,DC 20032.

The SV40 large T-antigen immortalized A7 glial cell line is currently being evaluated for potential use as an *in vivo* implant to promote functional recovery in animal models of neurodegenerative diseases, especially Parkinson's disease. A major concern for the use of immortalized cells for somatic therapy for neurodegenerative diseases is their potential to give rise to intracerebral tumors. This study was designed to evaluate the degree of proliferation of A7 cells after grafting into adult rat striatum. Cultured A7 cells were dissociated, labeled with the fluorescent dye DiI and resuspended at 500 cells/µl. 5.5 µl of A7 suspension were injected into adult rat striatum. Twenty four hours prior to each time point (7 and 14 days, respectively), 4 animals received 6 equally spaced serial injections of 5-bromo-4-deoxyuridine (BrdU) at 50 µg/gm, i.p. Twelve hours following the last BrdU injection, animals were transcardially perfused and cryostat sectioned. The surviving A7 cells and the BrdU positive cell some of the sections were determined. The results demonstrated that while the total surviving A7 cells remain approximately constant, the relative percentage of proliferating (% BrdU positive) A7 cells, decreased from 36% at 7 days to 9.8% at 14 days after grafting. Thus, *in vivo* implantation may reduce the rate of proliferation of immortalized cells. Supported in parts by: NIMH Grant T32 MH 19547 and NIH PO1 NS 21469.

429.8

A7 GLIAL CELLS MIGRATE AFTER GRAFTING INTO ADULT RAT STRIATUM. H.M. Geller, G.S. Okoye, M. Marone, W.J. Freed#, X.R. Zhou, Paul Manowitz*. Neurosurgical Research Laboratory, and Department of Pharmacology and *Department of Psychiatry and Neurology, UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ 08854 and #NIMH Neurosciences Center at St. Elizabeths, Washingtion, DC 20032.

The A7 cell line represents an alternative cell source for transplantation to promote functional recovery in animal models of neurodegenerative diseases, especially Parkinson's disease. This study was designed to evaluate the ability of A7-AP cells grafted into the adult rat striatum to migrate away from the implant site. We have transfected A7 cells with the gene for human placenta alkaline phosphatase to permit cellular localization after implantation into the rat brain; these cells are called A7-AP. Cultured A7-AP cells were dissociated and resuspended at 500 cells/µl. 5.5 µl of A7-AP suspension were injected into the left striatum. After 1, 2, 4, 8 and 12 week(s) respectively, animals (4 at each time point) were transcardially perfused and cryostat sectioned; every fifth section was histochemically stained for alkaline phosphatase. The results indicated that, while most A7-AP cells remained within 40 µm of the graft site, other cells were found as far as 70 µm. Many of the migrated A7-AP cells were located in areas apparently free of damage caused by implantation procedure. Several of the A7-AP cells appear to integrate with rat host brain cells, suggesting possible cell-cell communication between the A7-AP and the host brain cells. Immunofluorescence histochemical staining revealed enhanced expression of certain extracellular matrix molecules (fibronectin and laminin) at the graft site. Supported in part by: NIMH Grant T32 MH 19547 and NIH PO1 NS 21469.

429.9

FGF-2-MEDIATED NEUROPROTECTION IN THE MPTP-MODEL OF PARKINSON'S DISEASE: FOCUS ON ASTROGLIAL CELLS.
D. Otto and K. Unsicker*, Dept. Anatomy and Cell Biology, University of Heidelberg, D-69120 Heidelberg, Germany.

We have previously shown that FGF-2 causes marked protection of DAergic parameters in the MPTP-mouse model of Parkinson's disease (Otto and Unsicker, J. Neurosci. 10:1912 1990). Since FGF-2 has been shown to (i) exert its neurotrophic effect on cultured DAergic neurons via astroglial cells (Engele and Bohn, J. Neurosci. 11:3070,1991) and (ii) cause reactive gliosis after injection into the CNS, we have investigated the astroglial cell response to MPTP and FGF-2. MPTP (3x30mg/kg) induced a transient 20% increase in striatal GFAP at day 4. Morphologically, MPTP induced a marked increase in number, size, arborization, and stainability of GFAP-immunoreactive cells at d4, which persisted in milder forms until d18. Administration of 4µg of either FGF or cytochrome C, soaked into a piece of gelfoam, to the right striatum in MPTP- or saline-injected mice increased striatal GFAP-levels bilaterally about 2- to 2.5-fold at 14d, when FGF-2 showed marked protection of DAergic parameters. GFAP-positive cells were increased in numbers under any experimental situation. Subtle differences in their morphologies were only noted at greater distances away from the site of application. The lack of a marked FGF-mediated gliotic reaction may limit concerns regarding potential applicability of FGF-2 to the Parkinsonian striatum.

429.10

CRITICAL ROLE FOR ASTROCYTES IN EXPERIMENTAL PARKINSONISM.
T.J. Langan*, R.J. Plunkett, N. Razack and K. Kelly. Depts. of Neurology and Neurosurgery, School of Med. and Biomed. Sci., S.U.N.Y., Buffalo, N.Y., 14222.

In rat parkinsonism (nigral injection of 6-OH-dopamine), surgical cavitation causes transient improvement, and gelfoam implants into the cavities contain factor(s) that promote dopaminergic re-sprouting. We investigated the composition of these implants. Trypsinization (0.25 g%) for 10 min. was followed by DNAse (0.1 mg/ml), trituration, and plating in calf serum/DMEM (10%) on to polylysine- coated coverslips (10^4 cells/cm^2). In 3-4 d, the cultures consisted of 100% vimentin positive (VIM +) cells and only rare GFAP(+) cells. By 7 d, they were dividing actively, and were 93 ± 14 % (GFAP +), 100% (VIM +) and 67 ± 6 % (RAN 2 +). At confluence (14 d), <10% were (VIM +) or (GFAP +); most still were (RAN 2 +). The 7 d cultures were synchronized by 48 h of serum depletion followed by readdition: After a 12 h lag, S phase nuclei increased from 16 ± 4 to 74 ± 12 %. The gliosis caused by cavitation included dramatic, parallel increases in GFAP and VIM staining which also peaked at 7 d.

Therefore, type 1 astroglia are the apparent source of trophic factors that improve parkinsonism in this animal model, and their in vitro differentiation resembles the gliosis occurring in situ. In addition, a system is provided for examining cell cycle regulation in these cells that potentially are critical in recovery.

SYMPOSIA WEDNESDAY PM

430

SYMPOSIUM: THALAMOCORTICAL MECHANISMS UNDERLYING GENERALIZED ABSENCE SEIZURES. D.A. Prince (Chairperson), Stanford Univ., J. Huguenard, Stanford Univ., D. McCormick, Yale Univ., J. Noebels, Baylor College of Medicine, D.A. Hosford, Duke Univ.

Clinical and basic neurophysiologic data emphasize the importance of abnormal oscillatory burst activity within the thalamocortical circuit during generalized absence seizures. Recent advances in our understanding of the intrinsic membrane conductances of thalamic neurons that promote rhythmic activities, the modulation of these currents by the neurotransmitters of ascending brainstem, descending cortical and intrinsic thalamic circuits have allowed a more complete understanding of the normal regulation of thalamocortical excitability. This symposium will provide an overview of the neuroanatomic and neurophysiologic basis for normal and abnormal thalamocortical oscillations, their modulation by transmitter systems and pharmacologic agents, and the use of animal models of absence seizures to test the validity of predictions regarding basic mechanisms of dysfunction within the thalamocortical circuit. Furthermore examples will be provided of how pharmacological predictions based on this neurophysiological/neuroanatomical framework can be tested in animal models.

431

SYMPOSIUM. CORTICAL OSCILLATORY RESPONSES AND FEATURE BINDING.

J. Anthony Movshon, Howard Hughes Medical Institute and New York University (Chairperson); Christoph von der Malsburg, Ruhr-Universität Bochum and University of Southern California; Wolf Singer, Max-Planck-Institut für Hirnforschung; Ralph D. Freeman, University of California, Berkeley; David C. Van Essen; Washington University.

In recent years, several groups have reported that neurons in visual cortex can respond to visual stimuli with an oscillatory discharge whose frequency is in the range of 20 -70 Hz; these oscillations can be synchronized over substantial distances in cortex. These findings have been linked to theoretical work on the possible role of synchronized oscillatory responses in the "binding" of perceptually-unified features across a scene. This area has not been without controversy, and a number of investigators have found that oscillatory responses in cortical neurons are rather rare, and have raised doubts about the relationship of these responses to perceptual phenomena.

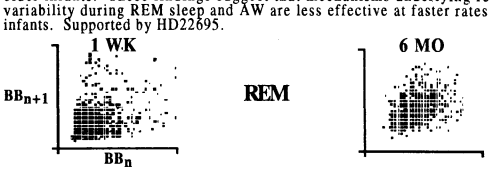
Freeman, Movshon, and Singer will present experimental evidence on the nature, prevalence, origins and perceptual significance of neuronal oscillations and synchrony; von der Malsburg and Van Essen will consider the implications and possible utility of these signals in the context of theories of visual binding and attention.

BIOLOGICAL RHYTHMS AND SLEEP III

434.1

DEVELOPMENT OF BREATH-TO-BREATH PATTERNING DURING SLEEP AND WAKING STATES IN NORMAL INFANTS.
S.L. Raetz*, V.L. Schechtman & R.M. Harper. Brain Research Institute and Dept of Anatomy & Cell Biology, UCLA, Los Angeles, CA 90024-1763.

The number of breathing pauses, amount of periodic breathing, and mean respiratory rate vary substantially with maturation and behavioral state. We used nonlinear procedures to describe moment-to-moment respiratory patterning in developing infants. Twelve-hour, nighttime polygraphic recordings, consisting of electroencephalogram, electrooculogram, electromyogram, electrocardiogram, nasal air flow, abdominal movement and expired CO_2 were obtained from 6 normal infants on 2 occasions, at 1 wk and at 6 mo of life. Each 1-min epoch was defined as waking (AW), quiet sleep (QS), rapid-eye-movement (REM) sleep, or indeterminate state by two trained observers. For every recording, approximately 60 min of data from each behavioral state throughout the night were selected for analyses, and breath-to-breath (B-B) intervals were calculated from the expired CO_2 signal. Each B-B interval within a particular behavioral state was plotted as a function of the previous interval, resulting in a BB_{n+1} vs. BB_n (Poincaré) scatter plot. The most marked breath-to-breath changes occurred during both REM sleep and AW. At 1 wk of age, as respiratory rates increased, the extent of change from 1 breath to the next decreased, resulting in a V-shaped scatter. At 6 mo of age, interbreath variability was no longer a function of rate (no V-shape in scatter plot). Overall variation was comparable across the 2 ages; however, interbreath intervals increased with age. These data confirm our finding from a data set with a different population of younger infants that during AW, the V-shape is not apparent in older infants. These findings suggest that mechanisms underlying respiratory variability during REM sleep and AW are less effective at faster rates in young infants. Supported by HD22695.



434.2

THE TEMPORAL DISTRIBUTION OF SLOW-WAVE EEG ACTIVITY ACROSS THE NIGHT IN NORMAL INFANTS.
V.L. Schechtman*, R.K. Harper and R.M. Harper. UCLA Brain Research Institute and the Dept. of Anatomy and Cell Biology, Los Angeles, CA 90024.

Adults show a decline in slow-wave (delta) EEG activity across the night. This decline is thought to reflect a sleep-dependent reduction in the need for slow wave sleep following a period of sleep deprivation. In the present study, we examine the ontogeny of this pattern of delta activity in normal infants from 1 week to 6 months of age.

Twelve-hour overnight recordings of EEG, eye movements, digastric EMG, EKG, and respiration were obtained from 25 normal infants at 1 week, and 1, 2, 3, 4, and 6 months of age. EEG was band-pass filtered leaving only activity ranging from 0.5 to 2.5 Hz, and the filtered EEG traces were full-wave rectified and integrated over 1-minute periods. The night was divided into four 3-hour segments, beginning at sleep onset, and the mean integrated 0.5-2.5 Hz EEG activity during all minutes of quiet sleep was determined for each segment of the night.

At 1 week and 1 month of age, infants showed no significant decay in delta activity across the night. In infants 2 to 4 mo of age, integrated delta declined significantly from the first to the second period of the night, and in 6-mo-old infants, integrated delta amplitude declined over three segments of the night. Thus, delta activity declines with sleep time in infants as young as 2 months of age. The duration of this decline increases (from 6 hours to 9 hours) as infants establish longer consolidated waking periods.

This research was supported by HD-22695.

434.3

THE DYNAMICS OF PHASE RELATION BETWEEN DIFFERENT FREQUENCY BANDS DURING SLEEP. J. Röschke*, K. Mann, Department of Psychiatry, University of Mainz, Germany

The conventional scoring procedure (Rechtschaffen & Kales) enables a quantitative assessment of the sleep profile. The value of this method is limited in order to determine dynamical attributes of the sleep EEG.

Therefore we extended computerized EEG analysis by applying a special method of spectral analysis. In this approach the sleep process was characterized as a dynamic and continuous cycle. Different EEG rhythmicities in the delta (0.5-3.5 Hz), theta (3.5-7.5 Hz), alpha (7.5-15 Hz), beta (15-35 Hz) and gamma range (35-45 Hz) has been taken into account. Following digital filtering of sleep EEG data in these frequency bands the RMS values of successively digitized (f_s=100Hz, bipolar registration C_z/P_z, 12 bit ADC) EEG epochs, each consisting of 2048 data points, have been performed. After artifact rejection, data reduction and smoothing the RMS values versus time represent the temporal course of EEG activity in certain frequency bands during the night. For assessment of relationship between these different EEG rhythms the cross correlation coefficient has been calculated.

Our results, based on the evaluation of 11 healthy male subjects (21-31 years, mean 24.8 years), revealed a highest RMS value in the delta range. In the fast frequency bands the RMS values decreased continuously. Considering the relationship between different frequency bands a reciprocal oscillation of the slower rhythmicities and the faster frequency components were observed (r(delta, beta)= -0.57).

Taken together it can be expected that this approach seems to be a suitable procedure to give deeper insight into the dynamics and the microstructure of the sleep process.

434.4

COHERENT SPONTANEOUS 40-HZ OSCILLATIONS AND A LACK OF RESET BY SENSORY STIMULI DURING DREAM-STATES IN HUMANS. U.Ribary*, R.Llinás, M.Joliot, R.Jagow, and J.Volkmann. Center for Neuromagnetism, Dept. of Physiology and Biophysics, New York University Medical Center, New York, N.Y, 10016, USA.

A 37-channel MEG system (BTi) was used, in order to determine the spatio-temporal dynamic properties of 40-Hz oscillatory activities in the human brain during different sleep stages.

Earlier MEG data recorded on humans, indicated synchronized spontaneous 40-Hz oscillations and a reset of activity by auditory stimuli. This reset was observed during averaging procedures and resulted in a relative increase in power of 40-Hz activity during auditory stimulation. In addition, by filtering the data in different frequency bands, we demonstrated a spatio-temporally organized pattern of activity at around 40-Hz. Recent magnetic recordings, obtained from five healthy adults, demonstrated large spontaneous 40-Hz coherent magnetic activity in the awake and in rapid-eye-movement (REM) sleep states but very reduced such activity during delta sleep. This 40-Hz magnetic oscillation had not been shown to be reset by sensory stimuli during REM or delta sleep, as observed in the awake state. The spontaneous 40-Hz activity in REM sleep was characterized, as that in the awake state, by a fronto-occiptal phase shift over the head having a duration of approximately 12-13 msec. Because spontaneous 40-Hz oscillations were seen in wakefulness and in dreaming we propose it as a correlate of cognition, probably resultant from coherent 40-Hz resonance between thalamocortical specific and nonspecific loops. As such, consciousness may arise by the coactivation of at least these two systems, which would temporarily conjoin cerebral cortical sites specifically activated at or around 40-Hz frequency. In this manner the specific system would provide the content, and the nonspecific system the temporal binding of such content into a single cognitive experience evoked either by external stimuli or, intrinsically, during dreaming.

434.5

EVENING VS. MIDDAY BRIGHT LIGHT-INDUCED rCBF CHANGES IN HUMAN SUBJECTS USING PET: DEVELOPMENT OF A PROBE OF CIRCADIAN TIMING SYSTEM FUNCTION. D.J. Diehl, M.A. Mintun* and D.J. Kupfer. Dept. of Psychiatry and Radiology, University of Pittsburgh, Pittsburgh, PA 15213.

The circadian timing system (CTS) is most vulnerable to bright light-induced phase shifts during early or late night, while it is relatively nonresponsive to bright light during midday. Bright light initiates its CTS effects via the retinohypothalamic tract which projects directly to the suprachiasmatic nucleus (SCN). The objective of our study was to use the positron emission tomography (PET) activation method to map the regional cerebral blood flow (rCBF) changes induced by an evening, but not by a midday, bright light stimulus. Such evening-specific rCBF changes are likely to be mediated by the SCN via its efferent systems, reflecting a functional response of the CTS.

We conducted evening (9pm) and midday (12N) ^{15}O-water rCBF PET scanning sessions with five normal human subjects. Each scanning session consisted of six rCBF scans at three different light intensities (50, 2500, and 7000 lux). Significant changes in rCBF between the 7000 lux stimulus-state and the 50 lux control-state were identified using the statistical parametric mapping method developed by Friston et al ($p<.001$). The evening 7000-50 lux comparison demonstrated bright light-induced rCBF increases in the visual cortex and right orbitofrontal cortex, and rCBF *decreases* in the pineal gland, medial temporal lobe bilaterally, left superior and middle temporal gyri, and right putamen. In contrast, the midday 7000-50 lux comparison revealed *only* a rCBF increase in the left putamen.

Our results demonstrate an extensive circadian variation in rCBF response to bright light in normal human subjects. The evening-specific rCBF decrease in the pineal gland is almost certainly mediated by the SCN via the well described SCN-pineal gland multisynaptic pathway. The other evening-specific rCBF changes may also be mediated by the SCN via as yet uncharacterized efferent pathways. Thus, our PET activation paradigm may have utility as an in vivo probe of CTS function in normal and pathological conditions.

434.6

ACUTE PLASMA MELATONIN SUPPRESSION IN COLOR-BLIND MALES. F. L. Ruberg, J. R. Gaddy, J. P. Hanifin, M. D. Rollag and G. C. Brainard*. Dept. of Neurology, Jefferson Medical College, Philadelphia, PA 19107; Dept. of Anatomy, USUHS, Bethesda, MD 20815.

Most demonstrations of acute plasma melatonin suppression in humans have involved test subjects with normal trichromatic photopic visual systems. Previously, we observed a male with a red-green color vision deficiency who failed to manifest melatonin suppression after exposure to 10,000 lux of white light from 2:00AM to 3:00AM. This study sought to determine conclusively whether a retinal deficiency in the color perceptual system could effect light mediated melatonin suppression. Male recruits were screened for color vision defects using Ishihara's test for color-blindness (24 plate edition), the Farnsworth-Munsell D-15, and the Farnsworth-Munsell 100-Hue test. Color deficient volunteers (n=5) were identified, diagnosed (2 protanopes, 1 deuteranope, 1 deuteranomalous, and 1 unspecified dichromat) and differentiated from normally sighted controls (n=5). Subjects were exposed to 90 minutes of 200 lux polychromatic light via a uniform illumination system from 2:00AM to 3:30AM. Pupils were dilated to assure full retinal exposure. Blood samples taken by venipuncture before and after light exposure were assayed for melatonin content by radioimmunoassay. Preliminary analyses of the data clearly reveal melatonin suppression after light exposure in both experimental groups ($p<0.05$), with no significant difference in the degree of suppression between the normal and color-blind volunteers. Specifically, melatonin suppression was noted in the 4 dichromatic subjects--that is, in the 2 protanopes (lacking the "red" cone pigment), the deuteranope (lacking the "green" cone pigment), and the unspecified dichromat (with an unknown deficiency). These findings suggest that a normal trichromatic photopic system is not necessary for light mediated neuroendocrine regulation. (Supported by NASA#NAGW1196, LRI#91SP1, and USUHS#RO7049).

434.7

INFLUENCE OF THE SITE OF IMPLANTATION ON THE RESTORATION OF CIRCADIAN RHYTHMICITY BY DISSOCIATED CELL GRAFTS OF THE SUPRACHIASMATIC NUCLEUS. M.N. Lehman*, K.A. Zimmer and W.N. Strother. Dept. Anat. & Cell Biol., Univ. Cincinnati Coll. Med., Cincinnati, OH 45267-0521.

Dissociated cell suspensions of the suprachiasmatic nucleus (SCN) implanted into the medial hypothalamus can restore free-running locomotor rhythms to SCN-lesioned hamsters (Silver et al., Brain Res, 1990). In order to examine the potential range of host targets for the recovery of circadian function after grafting, we implanted freshly dissociated fetal (E13) hamster anterior hypothalamic cells (100,000 cells/2 µl injection) bilaterally into either the medial hypothalamus (n=4), medial preoptic area (n=3), midbrain periaqueductal gray (n=4), or dorsal cortex (n=2) of arrhythmic SCN-lesioned adults. In order to unambiguously attribute restored rhythms to the presence of the grafted SCN, donor tissue was taken from heterozygous *tau* mutant hamsters whose free-running period is 22 hrs, while hosts were wild type animals with free-running periods of 24 hrs. Injections into the hypothalamus restored free-running locomotor rhythms in two of four recipients; recovery was correlated with the presence of SCN peptidergic cells and fibers in the middle and caudal hypothalamus and/or midline thalamus. In contrast, none of the other placements restored rhythmicity despite the presence in some instances of robust SCN peptidergic cells and fibers at the sites of implantation. Hosts with restored rhythms exhibited free-running periods (21.2, 20.9 hrs) shorter than that observed after whole tissue grafts from *tau* heterozygote donors (Ralph et al., Science, 247:975). The results suggest that proximity of donor SCN cells to a subset of normal efferent targets of the SCN, specifically those in the medial hypothalamus and/or midline thalamus, may be required for restoration of rhythmicity. [Supported by NIH R01 NS28175 to M.N.L.]

434.8

TRANSPLANTATION OF GOLDEN HAMSTER SUPRACHIASMATIC NUCLEI INTO SCN LESIONED HOSTS: EFFECT OF DONOR AGE ON RHYTHM RESTORATION, CM Kaufman* and M Menaker, Dept. Biology, University of Virginia, Charlottesville, VA 22903.

Suparchiasmatic nucleus (SCN) transplants using hamsters previously have been conducted with embryonic day (E) 13 to E 15 donor SCN tissue (gestation is 16 days in duration). Rat tissue has been used over a 7 day age span ending at postnatal day (P) 1 (gestation lasting 22 days). We investigated the age range of donor hamster SCN tissue that yielded functional transplants in SCN-lesioned hosts (*i.e.*, restored activity rhythms with the circadian period of the donor—in this case a 20 hr period). SCN from hamsters as young as E 11 and as old as P 12 became functional grafts. The success of transplanted tissue from older animals was surprising (*e.g.*, P 10 SCN were successful in 3/5 transplants). Neither the time between transplantation and the appearance of a rhythm nor the robustness of the restored rhythm correlated with donor age. The presence or absence of at least one patch of VIP immunoreactive cells was a good indicator of transplantation success or failure, respectively, in 48/50 cases. The findings reported here suggest that the SCN may be capable of developing in a host animal and that the SCN exhibits what may be an extended duration of neural plasticity. The span of 18 days during which hamster SCN tissue can be harvested for transplantation should enabled the expansion of applications for which SCN transplantation can be used.

434.9

SCN TRANSPLANTS ARE SUFFICIENT TO SUSTAIN CIRCADIAN LOCOMOTOR RHYTHMS IN HAMSTERS. J. LeSauter*[1], M.N. Lehman[2] and R. Silver[1]. [1]Barnard College of Columbia U., New York, NY 10027, [2]U. of Cincinnati, Coll. Med., Cincinnati, OH 45267.

Though the mammalian suprachiasmatic nuclei (SCN) are known to be the dominant pacemaker controlling circadian rhythmicity, several lines of evidence leave open the possibility that important oscillators lie outside of the SCN. Ablation of the SCN results in a loss of most but not all circadian rhythms. Furthemore, while circadian rhythms are restored by intraventricular perinatal SCN transplants, these grafts invariably contain extra-SCN hypothalamic tissue. Together, this evidence suggests the possibility that oscillators outside the SCN may be necessary or sufficient to sustain circadian rhythmicity.

In order to better define the hypothalamic region that controls circadian rhythms, we made 500μm vibratome sections of postnatal day 1 (PN1) brains (shown to be effective in restoring circadian rhythms in SCN-lesioned adult hamters; *Romero et al., Dev. Brain Res.,1993*), and made hypothalamic punches (500μm diameter) of the SCN, sub-PVN, and SON for implantation. Host animals were partially SCN-lesioned *tau* mutant hamsters with free-running circadian periods of 20 or 22 hours while donor animals were wild type with free-running periods of about 24 hours. Circadian locomotor rhythms with the period of the donor tissue were restored in 6/9 animals bearing SCN punches, indicating that the SCN are sufficient to sustain circadian rhythmicity. Neither sub-PVN nor SON punches restored rhythmicity. Staining for NP and VIP suggests that the latter grafts were not as robust as the SCN grafts, possibly because PVN and SON mature earlier, and harvesting at PN1 was too late for optimal survival of grafts from these regions. Supported by NIH grants NS-24292 to RS and MNL.

434.10

ESTABLISHMENT AND CHARACTERIZATION OF AN IMMORTAL CELL LINE DERIVED FROM THE RAT SCN. D.J. Earnest*[1], S. DiGiorgio[1], M.J. Gallagher[1], R.L. Gannon[2], and M.A. Rea[2]. [1]Dept. of Neurobiology/Anatomy, Univ. of Rochester Sch. of Med., Rochester, NY 14642 & [2]Circadian Neurobiol. Res. Group, Armstrong Lab., Brooks AFB, TX 78235.

Development of an experimental strategy enabling the study of individual cells or types of cells derived from the suprachiasmatic nucleus (SCN) could yield insight into the cellular organization of the circadian pacemaker mechanism in mammals. Consequently, the present study was conducted to determine whether mitotic, immortal cell lines could be established from progenitors of the SCN and whether the immortalized lines retained phenotypic properties of the parent cells.

Primary cultures derived from mitotic progenitors of the rat SCN were established and targeted for transfer of an immortalizing oncogene using a retroviral vector. After drug selection, expansion of infected colonies yielded continuous cell lines with dynamic growth rates in which cells doubled in number once every 24 hours. The growth potential of these cell lines has remained stable even after extensive passage for over 2 years. Although growth-stimulated, immortalized cells did not express properties associated with tumorigenicity. Morphological observations indicate that the cell lines exhibit both glial and neuronal phenotypes. The neuronal component of the cell lines was confirmed by the consistent expression of immunostaining in individual colonies for neuron-specific antigens and peptides that distinguish SCN neurons. Further increases in the expression of immortalized cells with peptidergic phenotypes of SCN-like neurons were observed in response to differentiating agents. Experiments are currently in progress to determine whether immortalized cells also retain the unique functional capacity of the SCN *in situ* to endogenously generate circadian rhythms. Supported by AFOSR 90-0182 (D.E.) and 2312W6 (M.R.).

434.11

OPTICAL RECORDINGS WITH INFRARED DARK-FIELD MICROSCOPY IN HYPOTHALAMIC SLICES CONTAINING THE SUPRACHIASMATIC NUCLEI. L. Trachsel*, H.U. Dodt and W. Zieglgänsberger. *Max Planck Institute of Psychiatry, Clinical Institute, 8000 Munich 40, FRG.*

A novel type of infrared video microscopy visualizes cellular activity of the CNS (Dodt et al.). We applied this technique in the suprachiasmatic nuclei (SCN), the putative circadian pacemaker in mammals. During the light phase, frontal hypothalamic slices (300-400 μm) were prepared from adult male Wistar rats kept in a 12 h light-dark cycle. Spatial resolution of the video microscopy was 1-4 μm/pixel, potentially allowing analyses on a cellular level. Time resolution of image processing was 2.5 frames/s, intensity resolution 8 bits/pixel. Electrical stimulation (2 s, 50 Hz, 8-13 V) in the optic chiasm resulted in a transient response of the ventral SCN cell population, as seen in the surge of image intensity. Image intensity peaked 3-5 s (control level = 100%) after stimulation onset. Slices were pharmacologically treated during 1 h at subjective midday. Post-stimulation 30-s recordings were done before, during and after treatment. The elicited response was reversibly depressed to 27.3% (n=12) by D-AP5 (50 μM) + CNQX (10 μM), by CNQX alone to 68.3% (n=19), by octanol (2 mM) to 20.3% (n=9), by Mg^{++} (10 mM) + Ca^{++} (0.5 mM) to 58.3% (n=18), and elevated to 151% (n=6) by mecamylamine (10 μM). The response was elevated by bicuculline (50 μM) at subjective midday to 204% (n=15) and at midnight to 156% (n=18). The results suggest that afferent input from the optic tract leads to cellular excitation in the ventral SCN, possibly mediated by excitatory amino acids. Its daytime $GABA_A$-ergic inhibition may be different from its nighttime level. The nicotinic acetylcholine system, as well as electrotonic transmission through gap junctions may be involved in the transient response in the SCN to tetanic stimulation of the optic chiasm. (Supported by the BMFT.)

ISCHEMIA III

435.1

ANOXIC LTP : SELECTIVE PERSISTENT POTENTIATION OF THE NMDA RECEPTOR-MEDIATED CURRENTS INDUCED BY ANOXIA IN CA1 HIPPOCAMPAL NEURONS. C. Hammond, V. Crépel and Y. Ben Ari*. INSERM U29, 123 Bd Port Royal 75014 Paris, France.

Global ischemia induces a delayed degeneration of CA1 pyramidal neurons. Since ischemia induces a transient (10-20 min) increase of glutamate release yet glutamate receptors antagonists have protective effects when administered several hours after the insult, we have tested the hypothesis that brief anoxic episodes induce long term changes in glutamate transmission. In presence of normal Mg^{2+} concentration, a brief (1.30-2min) anoxic-aglycemic (AA) episode (n=13) persistently (1h) potentiated the APV-sensitive (NMDA) component (+43±6%, p≤0.0001) but not the CNQX-sensitive (non-NMDA) component (-1.2±3.5%) of the glutamatergic EPSP recorded at Vm=-65 mV. Furthermore, at Vm=-100mV, in contrast to the control glutamatergic EPSP which was exclusively mediated by NMDA receptors, after anoxia more than 50% (58±18%, n=9) was APV-sensitive and thus mediated by NMDA receptors. This novel form of LTP to which we refer as anoxic LTP was also observed in experiments in which the NMDA receptor-mediated EPSP was pharmacologically isolated (+50±7%, p≤0.0001, n=12). Induction of anoxic LTP is NMDA-dependent since it is sensitive to NMDA receptor antagonists given before during and shortly after the AA episode (15-21 min, n=8). It is also voltage dependent since it was prevented by clamping the cell membrane to resting potential during anoxia (n=6). The maintenance of anoxic LTP has a postsynaptic locus since the current evoked by focal pressure application of NMDA (300 μM) was also persistently potentiated (+59 ± 7%, p≤0.001, n=6). Therefore after anoxic episodes, large NMDA receptor-mediated currents will be generated even at -100 mV in presence of physiological Mg^{2+} concentration.

Crépel V et al, J. Physiol (1993), 459, 201P and J. Neurophysiol (1993) in press

435.2

REDUCTION IN FOCAL ISCHEMIC INFARCTIONS BY CEREBELLAR STIMULATION IS NOT ATTRIBUTABLE TO CHANGES IN CEREBRAL BLOOD FLOW (rCBF) OR METABOLISM (rCGU). S. Yamamoto*, E.V. Golanov, D.V. Wilson and D.J. Reis. Div. of Neurobiol., Dept. of Neurol. & Neurosci., Cornell Univ. Med. College, New York, NY 10021.

Stimulation of the fastigial nucleus (FN) decreases, by over 50%, the infarction produced by occlusion of the middle cerebral artery (MCAO) in rat (Reis et al. *JCBFM* 11:810, 1991). The zone of salvage corresponds to the hypermetabolic hypoperfused ischemic penumbra. We investigated if neuroprotection was attributable to increasing rCBF and/or decreasing rCGU in the penumbra. Rats were anesthetized, the MCA exposed (controls) and occluded (MCAO). The FN was stimulated for 1h without (FNstim) or with MCAO (FN+MCAO). rCBF and rCGU were measured autoradiographically simultaneously with ^{14}C-IAP or ^{14}C-2-DG in the ischemic core and penumbra and contralateral cortex. In summary (Table): (a) MCAO reduced rCBF and increased rCGU in penumbra ipsilaterally; (b) FNstim alone globally increased only rCBF; (c) FNstim + MCAO did not differ from MCAO alone. We conclude: the neuroprotection elicited from FN does not result from increased rCBF or decreased rCGU. The mechanism of salvage may relate to molecular events conceivably affecting channel or receptor functions across brain.

Group	Core		Penumbra		Cont. cortex	
	CBF[a]	CGU[b]	CBF[a]	CGU[b]	CBF[a]	CGU[b]
Control	129±18	134±15	123±21	130±14	124±15	121±5
FN	221±24*	149±13	250±43*	151±14	224±46*	154±9
MCA	4±1	35±12	29±5	170±16	139±9	163±12
MCA+FN	11±8	75±30	20±9	178±18	254±30*	182±15

For each group n=5; * - p<0.05; [a] - ml/100g/min; [b] - μM/100g/min.

435.3

CGS-19755 PREFERENTIALLY DISTRIBUTES TO AREAS OF ISCHEMIC BRAIN IN A RABBIT MODEL OF FOCAL CEREBRAL ISCHEMIA. E. Yoon*, G.H. Sun, D.M. Kunis, A. Kotake, G.K. Steinberg, Department of Neurosurgery, Stanford University School of Medicine, Stanford, CA. 94305.

We have measured the regional brain uptake of CGS-19755 in a rabbit model of focal cerebral ischemia. Eighteen New Zealand white rabbits underwent occlusion of the left middle cerebral, internal carotid and anterior cerebral arteries for 2 hours. Ten minutes after occlusion, animals were given a bolus of 40 mg/kg (100 μCi) CGS-19755, and were sacrificed at 1 hour (n=5), 2 hours (n=8) or 4 hours (n=5) after occlusion. Animals sacrificed at 4 hours were unoccluded at the end of 2 hours of ischemia to allow reperfusion. Somatosensory evoked potentials were used to confirm ischemia. Technetium 99-m pyrophosphate was also administered i.v. to account for the brain vasculature contribution of ^{3}H-CGS-19755. While blood levels of CGS-19755 declined between 1 and 4 hours (1 hour, 40.29 μg/ml; 2 hours, 18.07 μg/ml; 4 hours, 8.31 μg/ml), brain and CSF levels remained stable (brain 1 hour, 1.13 μg/g; 2 hours, 1.13 μg/g; 4 hours, 1.60 μg/g; CSF 1 hour, 2.65 μg/ml; 2 hours, 3.79 μg/ml; 4 hours, 2.83 μg/ml). Unexpectedly, in the 1 hour group, the concentration of CGS-19755 in left frontal cortex (2.46 μg/g) was significantly higher than in areas of non-ischemic brain, such as the left cerebellum (.80 μg/g, $p < .01$). Concentrations of CGS-19755 in ischemic left frontal cortex for the 2 hour group (3.16 μg/g), and 4 hour group (3.99 μg/g), also were higher than in non-ischemic regions ($p < .01$, $p < .10$, respectively). Local ischemic damage to the blood-brain barrier may explain the preferential uptake of CGS-19755, a drug whose distribution is primarily limited by its low permeability coefficient.

435.4

SHOULD GLUCOSE BE ADDED TO THE DIALYSATE FLUID WHEN PERFORMING INTRACEREBRAL MICRODIALYSIS?

E. Ronne-Engström *[1], H. Carlson[1], L. Yansheng[3], U. Ungerstedt[2], L.Hillered [1].1.Dpt of Neurosurg University Hospital Uppsala, Sweden, 2.Dpt of Pharmacology Karolinska Institute3Dpt of Neurosurg Karolinska Hospital Stockholm Sweden.

Introduction: Intracerebral microdialysis is a method to measure, in vivo, substances in the extracellular fluid (ECF), during e.g. ischemia and seizures. It has been argued that the method may drain substances e.g. glucose, which could influence the results.

Methods: In this study microdialysis was performed with two probes, placed bilaterally in rat parietal cortex. The probes were perfused with artificial CSF with (CSF +G) or without (CSF -G) 3 mM glucose respectively. After obtaining basal levels hypoxia was induced. The extracellular concentrations of aspartate (ASP), glutamate GLU), lactate (LACT) and pyruvate (PYR) were measured. The results obtained with CSF+G and CSF- G were compared before, 10 and 40 minutes after induction of hypoxia.

Results: Twenty animals were investigated. Mean pO2 during the hypoxic period was 3,66, and during this period electroencephalography showed decreased amplitudes. There were no significant differences between basal levels or changes induced by hypoxia in dialysate concentrations of ASP, GLU, LACT or PYR in samples obtained with CSF -G or CSF+G.

Conclusion: The results suggest that microdialysis does not drain glucose from the surrounding tissue to an extent that influence short term measurements of energy related metabolites and excitatory amino acids.

435.5

ENERGY METABOLISM AND EXCITOTOXICTY: GLUCOSE PREVENTS GLUTAMATE OVERFLOW IN ISCHEMIC BRAIN. R.A. Swanson*, J. Chen, and S.H. Graham. Dept. of Neurology, Univ. of California and V.A.M.C., San Francisco, CA 94121.

Acute excitotoxic neuronal injury probably cannot occur in brain as long as energy-dependent reuptake mechanisms are functional. Astrocytes in culture can maintain glutamate uptake during hypoxia if glucose is available. To determine whether this capacity is shared by astrocytes in situ, glutamate levels were measured in ischemic brain during exogenous glucose delivery.

Microdialysis probes were placed bilaterally in caudate nuclei of rats and perfused with artificial CSF containing either 30mM or 0mM glucose. Ischemia was induced by cardiac arrest. Dialysate collected from probes without glucose showed a prompt rise in glutamate to 17 times baseline values, while dialysate collected from probes perfused with 30mM glucose showed only a 4-fold increase in glutamate at up to 60 minutes of ischemia.

These results show that glycolyic metabolism alone can fuel efficient uptake of glutamate in brain. The findings have implications for several disorders, particularly incomplete ischemia, in which impairment of energy metabolism has been linked to excitotoxic neuronal injury.

435.6

INCREASED CYTOKINE RELEASE AND EXPRESSION IN BLOOD VESSELS OF RATS WITH RISK-FACTORS FOR STROKE. A.-L. Sirén*, T. Liu, Y. Liu, M. Spatz, R. McCarron, M. Grojec, G. Feuerstein, and J.M. Hallenbeck. Dept. of Neurology, USUHS, Bethesda, MD 20814; Dept. of Pharmacology, SmithKline Beecham, King of Prussia, PA 19406; and Stroke Branch, NINDS, Bethesda, MD 20892 .

We have proposed that risk-factors for stroke promote a perivascular accumulation of monocyte/macrophages and an intensified cytokine-mediated interaction between monocyte/macrophages and endothelial cells which prepares local segments of blood vessels for subsequent thrombosis or hemorrhage. Therefore, the cytokine release and mRNA expression were studied in isolated carotid arteries and aortas of rats with and without the stroke-risk factors hypertension or advanced age. Spontaneously hypertensive rats (SHR), normotensive Wistar-Kyoto (WKY) as well as 2-year-old (2Y) and 16-week-old (16W) normotensive Sprague-Dawley (SD) rats were used. Incubation with lipopolysaccharide (LPS, 30-300 ng/ml) increased in a concentration-dependent manner the release of tumor necrosis factor alpha (TNF-α) and mRNA for interleukin-1β (IL-1β) in carotid arteries of SHR. IL-1β mRNA was also increased in the aortas of SHR. In WKY rats a significant increase in TNF-α release was found only after the highest LPS dose. The LPS-stimulated TNF-α release in carotid arteries of aged (2Y) SD rats (762 ± 243 units/mg tissue) was significantly greater as compared to young (16W) controls (387 ± 173 units/mg, $n=12$, $p<0.05$). The results indicate that hypertension and aging are associated with an increased cytokine production in the vascular cells which could increase the tendency for the endothelium to convert from an anticoagulant to a procoagulant surface.

435.7

IMMUNE RESPONSES IN PHOTOCHEMICALLY INDUCED CORTICAL ISCHEMIA IN THE RAT. S. Jander, M. Kraemer, O.W. Witte and G. Stoll (SPON: European NeuroscienceAssociation). Dep. of Neurology, Heinrich Heine Univ., Duesseldorf, Germany.

The mechanisms leading to secondary cell damage after ischemia are not well understood. In this study, focal cerebral ischemia in rats was induced by intravenous application of Rose bengal and subsequent illumination of the motor cortex (photothrombosis). To examine the contribution of the immune system in this model we stained paraffin and cryosections at various stages after ischemia with the following antibodies: a rat pan-T-cell marker, antibody (ab) ED 1 for macrophages, and ab OX-6 and OX-18 for MHC Class II and I antigens, respectively. 4 hours after ischemia no cells were stained, on day 1 T-cells predominated, up to 3 days increasing numbers of *T-cells and macrophages* appeared which persisted for 14 days. At day 30, only a few T-cells were left in the lesion, macrophages concentrated at the bottom of the lesion. Concomitantly, increased MHC class II expression was found which was not restricted to the lesion in contrast to MHC class I antigens. Our data indicate that the immune system strongly responds to photochemically induced ischemia and point toward a pathogenetic role of infiltrating cells for secondary cell damage.

435.8

LEUKOCYTES AND LEUKOTRIENE B_4 (LTB_4) RECEPTORS IN STROKE. F.C. Barone*, L.M. Hillegass, M.N. Tzimas, D.B. Schmidt, R.F. White, W.J. Price, G.Z. Feuerstein, R.K. Clark, D.E. Griswold and H.M. Sarau. Division of Pharmacology, SmithKline Beecham Pharmaceuticals, King of Prussia, PA 19406.

In previous studies, we validated the use of a myeloperoxidase activity assay (MPO) to quantitate increased polymorphonuclear leukocytes (PMN), and also identified increased LTB_4 receptor binding (LTB_4-RB) as another marker for inflammatory cell infiltration following MCAO. In the present study we determined the time-course for these effects following middle cerebral artery occlusion MCAO) made permanently (PMCAO) or transiently (160 min; TMCAO) in spontaneously hypertensive rats. Ischemic and contralateral (control) cortical samples were removed from isptonic saline perfused animals and assayed for MPO (Units/g wet weight) and ^{3}H-LTB_4-RB (fmol/mg protein). Data were analyzed by ANOVA (*$p<0.05$ vs control cortex; N=4-7/timepoint). Results were as follows:

Time (Days)	0.08	0.25	0.5	1	5	15
PMCAO-MPO	.12±.02	.17±.02	.27±.02*	.30±.09*	4.7±1.4*	.12±.03
PMCAO-LTB_4-RB	2.2±0.2	2.9±0.2	5.5±0.5*	4.6±1.0*	11.7±2.3*	11.5±1.4*
TMCAO-MPO	.12±.03	.20±.04*	.44±.12*	.93±.21*	3.2±1.1*	.11±.07
PMCAO-LTB_4-RB	2.6±0.3	4.0±0.2*	7.3±0.8*	11.1±1.6*	10.7±1.1*	*15.5±1.9**

No changes occured due to sham surgery. These results correspond well to our immunohistochemical data that characterized leukocyte infiltration and active gliosis under these conditions and demonstrated that TMCAO produces a significantly larger early influx of PMN than PMCAO. LTB_4-RB correlates well with early MPO changes but remains elevated when MPO has returned to baseline and PMN are no longer present. This persistent increase in ischemic cortex RB appears to be on the LTB_4 receptors of activated macrophages and astrocytes that increase in response to tissue injury.

435.9

INTRAVENTRICULAR INSULIN & IGF-1 IN GLOBAL ISCHEMIA IN RATS. Chang Z. Zhu, Roland N. Auer* Department of Neuroscience, University of Calgary, Canada, T2N 4N1

A beneficial effect of insulin in reducing cerebral ischemic necrosis has been recently shown. A portion of the neuroprotective effect in some ischemia models may be due to a direct central mechanism of insulin action, possibly via a growth factor effect. To test this hypothesis of the neuroprotective mechanism of insulin action, we delivered either insulin, insulin-like growth factor (IGF-1), or phosphate-bufferred saline (PBS) via continuous intraventricular infusion, and performed quantitative neuropathology one week after transient cerebral ischemia. In fed Wistar rats, cortical ($p<0.05$) and striatal ($p=0.01$) damage were significantly reduced by 23 IU/rat/day insulin ($n=8$), and hippocampal necrosis was less severe at all coronal levels ($p<0.05$). This intraventricular dose of insulin reduced the blood sugar significantly ($p<0.01$), probably by leakage of insulin into the periphery. However, low dose insulin (7 IU/rat/day; $n=10$), which did not reduce the blood sugar, also reduced selective necrosis in striatum ($p<0.05$) and one level of the hippocampus ($p<0.05$). IGF-1 (50 μg/rat/day) also caused no alterations in blood glucose, but significantly ameliorated hippocampal damage in four of the six hippocampal levels in fed rats ($p<0.05$; $n=8$). We conclude that insulin has a dose-dependent direct neuroprotective effect on CNS tissue, and that this direct effect may be mediated partly by IGF receptors. The results also confirm the more powerful, previously described neuroprotective effect of insulin-induced hypoglycemia in transient ischemia.

435.10

MR IMAGING OF BLOOD OXYGENATION AND WATER DIFFUSION CHANGES IN HYPERACUTE BRAIN ISCHEMIA

C Pierpaoli, A Righini, J Mattiello, R Miletich*, J Alger, G di Chiro
Neuroimaging Branch, NINDS, NIH, Bethesda MD 20892

Fast echo planar magnetic resonance imaging EPI-MRI techniques, permit the investigation of evolving brain ischemia in-vivo with high temporal resolution. Pathophysiological events occurring during ischemia can be evaluated by measuring two parameters: the tissue water Apparent Diffusion Coefficient (ADC), which is sensitive to ischemic injury, and the Blood Oxygenation Level Dependent signal (BOLD), which depends upon tissue perfusion and oxygen consumption. We used an EPI sequence that monitors these parameters simultaneously to study a cat model of global ischemia. We investigated both incomplete ischemia (induced by inflating vascular occluders positioned around the innonimate and left subclavian arteries) and complete ischemia (cardiac arrest following KCl i.v.). ADC and BOLD were calculated with a 43 s time resolution from EPI images acquired with a 2 tesla magnet.

Following cardiac arrest, BOLD decreases abruptly, reaching a plateau at 94% of the control value after only 90 s, consistent with rapid oxygen desaturation of the stagnant blood. The ADC decreases more slowly reaching a plateau at 60% of the control value in 8-10 minutes. During incomplete ischemia (12 minutes long) the ADC reduction is small (5-15%) and there is a complete return to normal values after reperfusion. The BOLD signal drop can be as large as in complete ischemia, although the change is delayed (4-6 minutes to reach the plateau), consistent with a less rapid oxygen desaturation due to residual perfusion. After reperfusion the BOLD signal overshoots above the preischemic values (postischemic hyperperfusion). EPI-MRI permits one to grade the degree of severity of the ischemic insult in vivo, with high temporal and spatial resolution.

435.11

EVALUATION OF PATHOLOGICAL STAGE AND TEMPORAL EVOLUTION OF ISCHEMIC BRAIN INJURY BY COMBINED MRI (DWI, T2WI AND T1WI)

K.Matsumoto, E.H.Lo*, A.Pierce and Y.Pan. Center for Imaging and Pharmaceutical Research, Massachusetts General Hospital and Harvard Medical School, Charlestown, MA 02129.

MRI may provide the ability to nonivasively differentiate pathophysiological conditions related to the amount and biophysical state of water in brain tissue. We evaluated the temporal evolution of ischemic brain damage using diffusion weighted imaging(DWI),T2WI and T1WI from the acute (30-90 min) to chronic(6 day) stage following permanent middle cerebral artery occlusion (MCAO) in Sprague-Dawley rats(250-350g). Infarction area was confirmed by TTC and cresyl-violet staining. MCAO was induced under halothane anesthesia using Tamura's subtemporal approach. Immediately after surgery the rat was placed in a GE-2T-CSI with gradient coils. 30-90min after occlusion, signal intensity was increased in the occluded hemisphere on DWI but no apparent changes were seen on T2WI or T1WI. On the 2nd day after occlusion, increased signal intensity areas were seen on both DWI and T2WI. However, on the 6 day after occlusion, DWI intensity decreased dramatically in the occluded hemisphere and in some cases the lesion showed a well demarcated low signal area with a high intensity rim. High or mixed intensity areas were seen on T2WI and low signal areas appeared on T1WI. TTC and cresyl-violet staining showed infarction in the basal ganglia and cortex. The basal ganglia tended to demonstrate a cystic necrosis which corresponded to the circumscribed low signal lesion on DWI. DWI combined with T2WI may be valuable not only for detecting early ischemic changes but also for evaluation of the pathological stage or condition of infarction in the chronic phase that may be related to ischemic density.

Support: Sterling-Winthrop Pharmaceuticals

435.12

MRI AND IMMUNOHISTOCHEMICAL STUDY OF ISCHEMIC EDEMA AND GLIAL REACTION IN RABBIT FOCAL CEREBRAL ISCHEMIA.

Y. Pan*, E. H. Lo, K. Matsumoto and N. W. Kowall. Center for Imaging and Pharmaceutical Research, Massachusetts General Hospital and Harvard Medical School, Charlestown, MA 02129.

Although MRI is a sensitive means of detecting ischemic cerebral injury, the correlation between in vivo scans and the underlying pathophysiology is not well understood. A rabbit model of focal ischemia was used to compare in vivo MRI with immunohistochemical techniques for demonstration of neuronal damage, BBB disruption and astrocytic reaction. Four hrs transorbital occlusion of the left internal carotid, middle cerebral and anterior cerebral arteries were performed in 6 halothane anesthetized rabbits. After 6 hrs reperfusion, T2W, T1W, Gd-enhanced and dynamic MRI were performed in a 1.5 T magnet. T2W scans showed increased signal intensity in the cerebral cortex and basal ganglia of the ischemic hemisphere. T1W images showed decreased intensity in corresponding areas. Dynamic MRI demonstrated delayed hypoperfusion in the ischemic hemisphere compared to the contralateral side. Gd-enhanced scans showed signal enhancement in the center of the lesions in 4 rabbits and peripheral lesion enhancement in the other 2. In all 6 rabbits, pyknotic, shrunken neurons and IgG leakage were noted in areas similar to the T2W lesions. Astrocyte hypertrophy and increased GFAP staining were observed in the ischemic hemisphere, especially near the edge of lesions that showed peripheral Gd-enhancement. The difference between Gd leakage and IgG leakage may be due to the biphasic opening of BBB during reperfusion. IgG leakage may be caused by transient reactive hyperemia after unocclusion, whereas Gd leakage may be due to BBB damage during infarction. *Support: Sterling-Winthrop Pharmaceuticals.*

CYTOSKELETON TRANSPORT AND MEMBRANE TARGETING II

436.1

THE N-TERMINAL DOMAIN OF GAD 65 IS SUFFICIENT TO TARGET A SOLUBLE PROTEIN TO THE PERINUCLEAR REGION OF TRANSFECTED CELLS

Michele Solimena*, Ronald Dirkx , Adrian Hayday and Pietro De Camilli.
Howard Hughes Med Inst, Dept of Cell Biology, Yale Univ School of Medicine and Dept of Biology, Yale Univ, New Haven, USA.

Glutamic acid decarboxylase (GAD), the enzyme responsible for the synthesis of GABA in neurons and pancreatic ß-cells, is represented by two isoforms of 67 kD (GAD 67) and 65 kD (GAD 65), respectively. GAD 65 is a dominant autoantigen in Stiff-Man syndrome and insulin-dependent diabetes mellitus. GAD 65 and GAD 67 are 65 % similar to each other and differ primarily at their N-terminal region. In neurons and ß-cells a pool of GAD is concentrated at the cytoplasmic surface of synaptic vesicles and synaptic-like microvesicles, respectively, as well as in a perinuclear area corresponding to the Golgi complex. The mechanism responsible for the targeting of GAD to these organelles is not known, but is thought to involve palmitoylation of cystein residues. The targeting of the two GAD isoforms was analyzed by immunofluorescence in CHO cells transfected with rat GAD 65 or GAD 67. GAD 67 was homogeneously diffuse in the cytoplasm. Conversely, both GAD 65 and a chimeric GAD 65-GAD 67 protein in which a. a. 1-88 of GAD 67 were replaced by a. a. 1-83 of GAD 65 were concentrated at the area of the Golgi complex. Addition of the N-terminal fragment of GAD 65 (a.a. 1-83) to the N-terminal of ß-galactosidase (which otherwise would have a diffuse distribution), also resulted in a perinuclear distribution of ß-galactosidase, demonstrating that the signal(s) required for the targeting of GAD 65 to membranes resides within the first 1-83 amino acids. Individual point mutations of each of the six cysteins contained within this region of GAD 65 are insufficient to determine a redistribution of GAD 65 similar to that observed for GAD 67. Further studies of the N-terminal domain of GAD 65 will establish the role of palmitoylation and of primary sequence motifs in the targeting of GAD 65.

436.2

HERPES SIMPLEX VIRUS (TYPE 1) INFECTIONS OF HUMAN RETINAL PIGMENT EPITHELIAL CELLS *IN VITRO*.

K. S. Topp*, N. D. Saks, K. Bisla and J. H. LaVail. Dept. of Anatomy and Neuroscience Program, UCSF, San Francisco, CA 94143.

We have cultured human retinal pigment epithelial cells (RPE) derived from perinatal donors on permeable filters to study transport of Herpes simplex virus (HSV) in an intact polarized epithelial cell *in vitro*. After 2 months, the cells are tightly coupled by junctional complexes, as determined by electron microscopy, immunofluorescent staining of tight junctions and measurements of transepithelial resistance > 350 ohms cm^2. Indirect immunofluorescence and confocal microscopy were used to visualize microtubule orientation. In subconfluent RPE cells, the microtubules were dispersed and often arranged parallel to the filter surface. In contrast, the microtubules in confluent, polarized RPE cells were arranged in bundles, oriented perpendicular to the filter surface. Furthermore, the microtubules were polarized with plus ends directed toward the basal surface of the cells. We infected RPE cells at the apical surface with HSV and assayed the uptake and transport of virus from the apical surface to the nucleus by quantitative immunoblot and immunocytochemical staining for the HSV immediate early gene product, ICP4. ICP4 antigen first appeared in RPE cell nuclei at 2 hrs after infection. Treatment of the cells with 33 μm nocodazole, a microtubule-destabilizing drug, delayed the appearance of nuclear ICP4 staining by 1 hr. These data indicate that human RPE cells are infectable with HSV at the apical surface and that the centripetal transport is dependent on intact microtubules. The data are consistent with the hypothesis that the transport of HSV from the apical surface is mediated by a plus-end directed motor molecule, e.g. kinesin.

436.3

MICROTUBULE POLARITY IN THE PERIPHERAL PROCESSES OF TRIGEMINAL GANGLION CELLS: RELEVANCE FOR THE RETROGRADE TRANSPORT OF HERPES SIMPLEX VIRUS. J.H. LaVail,* K. S. Topp and L.B. Meade. Dept. of Anatomy and Neuroscience Program, UCSF, San Francisco, CA 94143.

The directional movement of many organelles in neurons is dependent on polarized microtubules and direction-specific motor molecules. Microtubules also mediate the retrograde transport of Herpes simplex virus (HSV) in sensory neurons. We have investigated the polarity of microtubules in the peripheral axons of trigeminal ganglion neurons. The long ciliary nerves of rabbits were prepared for a standard "hook assay" of microtubule polarity. The axons contained microtubules with the fast growing, plus ends located distal to the cell body and the slow growing, minus ends directed centrally. To determine the role of microtubules in the retrograde transport of HSV in these axons, we injected the retrobulbar space of mice with the microtubule-inhibiting drugs, colchicine, vinblastine or nocodazole, or with the microfilament-inhibitor, cytochalasin D, and 1 day later inoculated the cornea with HSV. We found that colchicine, vinblastine or nocodazole reduced by 52% to 87% the amount of virus recovered from the ganglion 3 days postinoculation, compared to vehicle-treated animals. In contrast, cytochalasin D or β-lumicolchicine did not significantly reduce the amount of HSV recovered from the ganglion. We conclude that the retrograde axonal transport of HSV from axon endings in the cornea to the trigeminal ganglion cell bodies requires intact microtubules and occurs in a plus to minus direction on the microtubules. Our data are consistent with the hypothesis that the retrograde axonal transport of HSV is mediated by a minus end-directed motor molecule, e.g., cytoplasmic dynein.

436.4

PROTEIN–RNA INTERACTIONS AT THE SYNAPSE. M.E. Chicurel,* C. DeFranco, @D.M. Terrian, and H. Potter. Program in Neuroscience, Dept. Neurobiology, Harvard Medical School, Boston, MA 02115; @Dept. Anatomy and Cell Biology, East Carolina School of Medicine, Greenville, NC 27858-4354.

Using a preparation of synaptosomes from the MF-CA3 region of the hippocampus, we have identified a series of RNA molecules that are specifically localized to the synapse. Some appear to be localized in the dendritic spines, and some in the astrocytic processes. Other mRNAs are excluded from the synaptosome preparation. Our studies indicate that at least two of the synaptosomal RNAs, GAP-43 and RC3, form complexes with protein in synaptosomal extracts. We have delineated two protein binding sites on both the GAP-43 mRNA and RC3 mRNA. These binding sites lie within the 3' untranslated regions of the mRNAs. It appears that at least some synaptosomal proteins that bind to GAP-43 mRNA also bind to RC3 mRNA. In addition, a preparation of microtubule-associated proteins, like some synaptosomal proteins, interact with the 3' UTRs of GAP-43 and RC3 mRNAs. Specifically, we have identified one of the proteins that interact with both GAP-43 and RC3 mRNAs as the microtubule- associated protein, MAP1. Possible functions of these cytoskeletal interactions include transport and anchoring of RNAs to the region of the synapse and regulation of local translation.

436.5

GENETIC INTERACTIONS BETWEEN OSM-3, UNC-104, AND UNC-116, THREE LOCI ENCODING DIFFERENT MEMBERS OF THE KINESIN SUPER FAMILY IN CAENORHABDITIS ELEGANS. Shahid S. Siddiqui*, Lab. of Molecular Biol. Toyohashi Univ. of Technology, Tempaku, Toyohashi, 441, JAPAN.

We report here a genetic analysis of interactions in mutants of three genes that encode kinesin like proteins in the nematode C. elegans. Previous work has shown that unc-104 encodes a large member of kinesin family (6.0 kb transcript), mediates axonal transport of synaptic vesicles and locomotory behavior (Otsuka et al., 1991; Hall and Hedgecock, 1991). Recently we have cloned the osm-3 gene, which encodes another kinesin like protein (3.0 kb mRNA), and that is 44.5% homologous to the unc-104 kinesin (M. A. Shakir et al. unpublished). Mutants in osm-3 fail to avoid high concentrations of salts and sugars, and fail to take up fluorescein dyes in chemosensory neurons due to a short cilium structure(Culotti & Russell, 1978; Perkins et al., 1986). Finally, unc-116 encodes another member of the kinesin superfamily (3.0 kb mRNA), mediates locomotion, and affects axonal outhrowth and guidance (Patel et al. 1991, J. Mancillas; S. Siddiqui, unpublished). We have constructed double mutants between different alleles of osm-3 gene, and various alleles of unc-104 gene, to study the epistatic relations between these loci. Similarly, we plan to construct various doubles with the unc-116 alleles to learn how these three kinesins interact genetically in the structure and function of the C. elegans nervous system.

436.6

SUBCELLULAR TARGETING OF TWO DEVELOPMENTALLY REGULATED ISOFORMS AND MUTATED VARIANTS OF THE MEMBRANE ASSOCIATED PROTEIN SNAP-25. *L. C. Bark* P. P. Mehta & M. C. Wilson. Dept. of Neuropharmacology, The Scripps Research Institute, La Jolla, CA 92037.*

The nerve terminal protein SNAP-25 has been recently implicated as one of several neuron-specific receptors of a 20S protein complex that may help target vesicle fusion at the presynaptic plasma membrane *(Söllner et al., 1993, Nature 362, 318-324)*. SNAP-25 is a 206 amino acid polypeptide that contains two domains which may play important roles for biological function and targeting. The amino terminus can form an amphipathic helix and at least one out of four clustered cysteine residues, present in a central domain in the protein, is substrate for posttranslational palmitoylation. There exist two alternatively spliced isoforms of SNAP-25, which show distinct and regulated expression during development *(Bark & Wilson, Neurosci. Abs. 38.19, 1992)*. The differences are only 9 amino acids between the two forms but results in altered organization and sequence context of the cysteine quartet, suggesting that the substrate for palmitoylation is changed.

To evaluate the potential function of the alternative fatty acylation domains of SNAP-25, the two isoforms and mutated variants with the different cysteine residues changed have been expressed in CHO fibroblasts. Our studies have shown that the cysteine residues are crucial for localizing the protein to the periphery, apparently to the plasma membrane in CHO cells, suggesting palmitoylation as a requirement for targeting the protein to membrane compartments. To determine the importance of the 9 amino acid differences in targeting and localizing the alternative isoforms in neuron-like cells, tagged cDNA constructs were transfected into NGF differentiated PC12 cells. In induced PC12 cells, both isoforms of SNAP-25 are translocated into developing neurites, although with different efficiency and subcellular targeting preferences. These data show that the two forms exhibit distinct subcellular targeting and biochemical properties that might correlate with different membrane fusion events. *Supported by Swedish NSRC B-PD9714-303 (I.C.B.) and NSF IBN9121121 and PHS MH 48989 (M.C.W.).*

SEROTONIN: ANATOMY, REGULATION, AND CLINICAL STUDIES

437.1

SEROTONERGIC NERVE TERMINALS IN RAT CEREBRAL CORTEX AND HIPPOCAMPUS: NEUROVASCULAR RELATIONSHIPS. Z. Cohen* and E. Hamel. Montreal Neurological Institute, McGill University, Montréal, QC, Canada, H3A 2B4.

Physiological evidence suggest that serotonin (5-HT) is a potent intracerebral vasoconstrictor which can regulate cerebral blood flow (CBF). We studied the relationships between 5-HT nerve terminals and microvessels (MVs) in the rat cerebral cortex and hippocampus (Hi) by electron microscopic immunocytochemistry. Vibratome-cut, transverse sections of the fronto-parietal cortex and Hi were immunolabelled for tryptophan-5-hydroxylase (TPH), the synthesizing enzyme for 5-HT. All perivascular (located within a 3μm perimeter from vessel walls) TPH-immunostained terminals were systematically photographed and analysed in single thin sections. In the cortex, 2.8% of all perivascular terminals (n=250) appeared to be directly apposed to the basal lamina, 11.6% were separated from it by an astrocytic leaflet with an additional 12% located further away up to 0.25μm from the MVs. In the Hi, none of 108 perivascular TPH terminals was in direct contact with the MVs, 9.3% exhibited an intervening glial process and another 3.7% were within 0.25μm from MVs. Perivascular terminals in the Hi were more distant (1.31 ± 0.08 μm) from MVs than those in the cortex (0.98 ± 0.05 μm) but were of similar size (0.23 ± 0.02 μm^2,Hi, and 0.22 ± 0.01 μm^2,cortex). In both areas, perivascular TPH terminals never exhibited membrane specializations at the neurovascular or neuroglial interface. Analysis of cortical (n=1321) and Hi (n=1024) TPH terminals in similarly vascularized regions showed that 11% and 4%, respectively, were within 3μm from MV walls. The results show that 5-HT terminals are more closely associated with cortical MVs than with those of the Hi, a finding consistent with the reported changes in cortical, but not Hi CBF following raphe stimulation. Overall, the data suggest an important role for perivascular astroglia in the neurogenic control of brain microcirculation by serotonin. Supported by grant MA-9967 of the Medical Research Council of Canada.

437.2

ASCENDING PROJECTIONS FROM THE DORSAL (DR) AND MEDIAN RAPHE (MR) NUCLEI: POSSIBLE FUNCTIONAL IMPLICATIONS WITH RESPECT TO ETHANOL TOLERANCE. A.J. Lança, W.J. Irving, P.H. Wu, H. Kalant and J.A. Saint-Cyr*. Dept. of Pharmacology, Univ. of Toronto, and Addiction Research Foundation, Toronto, Ont., Canada.

Vasopressin (AVP) can maintain ethanol tolerance, but only in the presence of intact ascending projections from the brainstem, including both the serotonergic (5HTergic) and catecholaminergic components. As part of a study of the neuroanatomical circuitry underlying these interactions, a combined retrograde tracing and immunocytochemistry (ICC) approach was used to demonstrate the origin and the neurochemical nature of the ascending raphe projections to the medial septum (MS) and the hypothalamic paraventricular nucleus (PVN). Adult male Sprague-Dawley rats (n=27) were stereotaxically injected with true blue (TB) in the MS, and with diamidino yellow (DY) in the left PVN. After a 3-8 day survival time the animals were sacrificed, and serial brain sections were processed for ICC with antibodies against 5-HT, tyrosine-hydroxylase (TH), and substance P (SP). Tracer studies revealed that both the MS and PVN receive ascending projections originating from both the DR and MR nuclei. ICC showed that most of these projections originate in 5HT-immunoreactive (IR) perikarya located in the DR and MR alike. However, a substantial part of these projections also originated in TH-IR (dopaminergic) neurons of the DR and MR. No SP-IR cell bodies were seen in the DR and MR nuclei. SP-IR fibers were seen in the periaqueductal gray (including the DR), and in the lateral part of the interpeduncular nucleus. These results suggest that the MS, as well as the PVN, are possible loci of functional interactions between the 5HTergic and AVPergic systems in the regulation of tolerance to alcohol. Supported by NIAAA grant #1 R01-AA08212-03.

437.3

TOPOGRAPHY OF DUAL PROJECTIONS FROM THE DORSAL RAPHE NUCLEUS (DRN) TO THE PREFRONTAL CORTEX AND NUCLEUS ACCUMBENS. A. Biswas, E. J. Van Bockstaele, V.M. Pickel and J. Chan*, Dept. of Neurol. & Neurosci., Cornell Univ. Med. Coll., New York, N.Y. 10021.

Diverse physiological actions have been reported for 5-hydroxytryptamine (5-HT) in the medial prefrontal cortex (MPFC) and the nucleus accumbens (Acb) suggesting that the 5-HT innervation of these forebrain areas may be derived from different neurons. We examined this possibility by mapping the distribution of 5-HT-immunoreactive (ir) and non-5-HT-ir neurons containing retrograde labeling following injections of different tracers into both these target regions. The analysis was focused in the DRN of the midbrain since 5-HT pathways to the MPFC and Acb primarily originate from this area. Microinjections of Fluoro Gold (FG) and a protein gold complex (CT-Au) were placed into the MPFC and Acb, respectively, of the same rat. Sections through the DRN were further processed for immunofluorescent localization of 5-HT using a rhodamine marker. Neurons retrogradely labeled from the Acb were greater in number than those projecting to the MPFC. In addition, Acb-projecting neurons extended into the lateral wings of the DRN, whereas MPFC-projecting neurons were more restricted to the midline. Both groups of labeled neurons, however, were more numerous in the caudal aspect of the DRN and were scattered amongst 5-HT immunoreactive perikarya. Of 783 ± 69 CT-Au cells, 15% also contained the FG label and 11% contained FG and 5-HT immunoreactivity. Of 613 ± 48 FG cells, 24% also contained the CT-Au label and 21% were also immunoreactive to 5-HT. The results suggest a more prominent input to the Acb from neurons in the caudal aspect of the DRN and further indicate that while most 5-HT-ir and non-5-HT-ir neurons project differentially to both forebrain regions, a few cells also show collateralization to the MPFC and Acb. Such collateralization to divergent targets may integrate cognitive and motor activities in response to pharmacological manipulations of ascending 5-HT pathways. Supp. by NS09100-01, MH40342, 00078.

437.4

CELLULAR ELECTROPHYSIOLOGY OF 5-HT$_{1A}$-MEDIATED RESPONSES IN CA1 AND CA3 HIPPOCAMPAL SUBFIELDS WITH AGING. M.I. ARENTSEN and J.M. LAKOSKI*. Dept. HBC&G, University Texas Medical Branch, Galveston, TX 77555-0498.

We have investigated age-related alterations of serotonergic responses in the hippocampus. Changes in neuronal sensitivity to selective 5-HT$_{1A}$ compounds were assessed in both CA1 and CA3 hippocampal subfields using extracellular microiontophoretic recording techniques in chloral hydrate anesthetized female Fischer 344 rats (Young, ovariectomized [OVX] 2-6 mo; Middle-aged, OVX, 14-16 mo; and Old, 22-26 mo).

Average spontaneous firing rates in anterior CA1 and CA3 pyramidal cells peaked at Middle age and then significantly declined in Old age; CA3 cell firing rates were consistently less than CA1 across all ages. The 5-HT$_{1A}$ agonist 8-OH-DPAT more potently inhibited cell firing in CA1 than CA3. An age-related *increase* in pyramidal cell response to DPAT was observed in both CA1 (Young, 39.0±5.5; Middle, 44.6±4.9; Old 54.8 %inhibition at 60nA) and CA3 (Young, 23.3±13.6; Middle, 48.7; Old, 68.2 at 60nA). The results identify significant age-related changes in hippocampal physiology and serotonin function.

Publication No. 5A supported by USPHS Grant PO1 AG10514 and AG00450, awarded by the National Institute on Aging.

437.5

IN VIVO MODULATION OF SEROTONIN RELEASE IN FRONTAL NEOCORTEX. F. Petty*, G.L. Kramer, M. Moeller, S. Jordan, VA Med. Ctr., Univ. Tex. Southwestern Med. Schl., 4500 S. Lancaster Rd., Dallas, TX 75216.

The hypothetical neurochemical map we have proposed to account for the development and maintenance of learned helplessness (LH) in the rat involves a central role for serotonin (5-HT) in prefrontal cortex (PFC). LH develops with increased 5-HT release leading to depletion of intracellular 5-HT. Microinjection of 5-HT into PFC reverses LH, and reversal by chronic antidepressant drug treatment is accompanied by restoration of intracellular 5-HT stores. Other data suggest glutamate, GABA, and norepinephrine to also influence LH. Therefore in vivo pharmacological interactions influencing PFC 5-HT release were studied with brain microdialysis. Drugs were administered both IP and through the microdialysis probe (IC). 5-HT measured was K+ stimulated, Ca^{++} dependent, and decreased by tetrodotoxin. The 5-HT releasing agent fenfluramine, the uptake blockers, fluoxetine and fluvoxamine and the tricyclic antidepressants imipramine and desipramine increased 5-HT release both IP and IC, and the 5-HT precursors did so IP. Buspirone increased IC but decreased IP, suggesting systemic administration to differentially influence impulse flow at the raphe level. Propranolol, with both beta-adrenergic and 5-HT1a antagonism, increased IC. GABA agonists muscimol and baclofen had no effect IP, but gamma-vinyl GABA increased 5-HT IP, while bicuculine increased IC. Alpha-2 agent yohimbine decreased but clonidine had no effect IC. Haloperidol increased IC, but apomorphine had no effect. Atropine also had no effect. Overall these results suggest a complex regulation of PFC 5-HT release, with 5-HT agents having expected results, D2 and GABA-A blockade releasing 5-HT, and GABA releasing 5-HT though a mechanism independent of GABA-A or GABA-B modulation. Of the agents tested IC, only yohimbine decreased 5-HT release.

437.6

BRAIN SEROTONERGIC NEURONS DEMONSTRATE NORMAL AXONAL TRANSPORT FOLLOWING SHORT- AND LONG-TERM TREATMENT WITH DEXFENFLURAMINE. M. Kalia* and N. P. O'Malley. Dept of Neurosurgery, Jefferson Medical College, Thomas Jefferson University, Philadelphia PA 19107

There is now convincing evidence that serotonergic perikarya in the raphe nuclei are unaffected by fenfluramine *(Appel et al, '89,* and *Molliver and Molliver, '90).* There is however an observed biochemical and immunocytochemical (IC) reduction in 5-HT in regions of the brain where 5-HT terminal fields are located. This latter finding (which is consistent with the pharmacological effect of fenfluramine and is rapidly reversible - *Kalia, '91*) is frequently interpreted to represent neuropathology of the 5-HT terminals. We examined the viability of 5-HT terminals following treatment with the more potent fenfluramine isomer, dexfenfluramine (DFF) by testing the ability of 5-HT terminals to take up the retrograde label (cholera toxin horseradish peroxidase-CTHRP) and transport it to the cell bodies of origin. DFF was administered orally (8 & 16 mg/kg/day) for 4 or 21 days. The animals survived 18 hrs to 3 weeks post-treatment following which selected areas of the parietal cortex *(Mamounas and Molliver, '88)* were injected with 2 nl of CTHRP under chloral hydrate anesthesia. Following a 3 day survival period, the animals were perfused and the brain stem was processed using single and double labeling techniques for HRP-histochemistry and 5-HT-IC *(Rye et al, '85).* A positive control was done with para-chloramphetamine (6 mg/day for 2 days). Analysis of variance revealed no significant treatment effect. In the PCA treated animals the number of retrogradely labeled neurons in the dorsal raphe was markedly reduced 61% (*Mamounas and Molliver '88* had found a 66% decrease with their retrograde label) as compared with controls. These results confirm earlier observations that had established the functional viability of serotonergic nerve terminals. This viability is not influenced by dose, duration of treatment and post-treatment survival. (Supported by Interneuron Pharmaceuticals Incorporated & IRIS).

437.7

EFFECTS OF ACUTE AND CHRONIC FLUOXETINE ON 5-HT OVERFLOW IN THE FRONTAL CORTEX AND RAPHE REGIONS. K.M. Wozniak*, A Pert and M.Linnoila, LCS/NIAAA and BPB/NIMH, Bethesda, MD 20892.

This study investigates further the effects of the antidepressant fluoxetine (Flu) on serotonin (5-HT) in two different brain areas of the rat, monitored simultaneously. Male Sprague-Dawley rats (300-350g) were anesthetised with chloral hydrate and stereotaxically implanted with microdialysis probes into both the terminal region of the frontal cortex and the cell body region of the raphe nuclei. In one set of experiments, Flu was applied focally (100µM) to either of these areas or given systemically (15 mg/kg i.p.) to naive rats. Another group of rats were treated chronically with Flu (15 mg/kg/day for 14 days) and were similarly challenged about 24 hours after their last injection. Basal levels of 5-HT in the cortex and raphe regions of naive rats were similar (averaging about 9 fmoles/5µl), and both areas displayed equal sensitivities to focal Flu, with peak local increases in 5-HT of about 400 %. Rats receiving chronic Flu pretreatment had markedly elevated 5-HT as compared to naive rats, only in the raphe region. Although both the cortex and raphe regions still displayed similar sensitivities to focal Flu, these were significantly reduced compared to naive rats, with maximum increases in local 5-HT of less than 200%. Systemic challenge with Flu significantly increased 5-HT (by about 300%) only in the raphe region of naive rats. In contrast, there was no significant effect of systemic Flu in either region of chronically pretreated rats. The results indicate that systemic fluoxetine has differential effects in the two brain areas. The increase of 5-HT in the raphe presumably activates somato-dendritic autoreceptors, which tends to inhibit firing of raphe neurones projecting to the cortex. The apparent subsensitivity of both regions to focal or systemic Flu following a chronic pretreatment regimen, suggests the development of an adaptational reduction in 5-HT transporter sensitivity, amongst other possible mechanisms. We are further investigating the exact nature of this effect.

437.8

NEUROENDOCRINE PROFILE OF A POTENTIAL ANXIOLYTIC DRUG, S-20499: COMPARISONS WITH BUSPIRONE.

L.D. Van de Kar*, A.D. Levy, Q. Li, & M. Gustafsson, Dept. Pharmacol., Loyola Univ. Chicago, 2160 S. First Av, Maywood, IL 60153.

The endocrine profile of a 5-HT$_{1A}$/D$_2$ agonist and potential anxiolytic drug S-20499 was compared with that of a structurally related compound, buspirone. In male Sprague-Dawley rats, S-20499 (0.01 - 20 mg/kg, i.p.) dose-dependently elevated plasma ACTH and corticosterone concentrations, with maximal effects observed at 30 and 60 minutes respectively. Buspirone (0.05 - 10 mg/kg, i.p.) produced similar effects. In contrast, S-20499 reduced, while buspirone increased plasma prolactin concentration, while only buspirone reduced plasma renin activity. The minimal doses of S-20499 and buspirone that increased plasma ACTH and corticosterone concentration were 5 mg/kg, while a dose of 1 mg/kg of S-20499 reduced plasma prolactin concentration. Injection of 1 mg/kg (i.p.) of S-20499 also reduced blood pressure and heart rate within 10 min post-injection, suggesting reduced sympathetic output. Pretreatment with the 5-HT$_{1A}$/β antagonist (-)-pindolol (0.3 mg/kg i.p.) significantly attenuated the stimulatory effects of S-20499 on plasma ACTH and corticosterone concentrations. (-)-Pindolol alone also reduced plasma prolactin concentration, and this effect was not altered after injection of doses of S-20499. Taken together, the data suggest that S-20499 stimulates the hypothalamic-pituitary adrenal axis by activating 5-HT$_{1A}$ receptors. Additionally, it reduces prolactin secretion, presumably by activating dopamine D$_2$ receptors in the pituitary. Supported in part by NIMH MH45812 and by IRIS (France).

437.9

HIGH SEROTONIN ACTIVITY OF NON-EMIGRATING MALE RHESUS MONKEYS. J.R. Kaplan*, M.B. Botchin, J.J. Mann, S.B. Manuck, J. Berard. Dept. of Comp. Med., Bowman Gray Sch. of Med., Winston-Salem, NC, 27157-1040.

Male rhesus monkeys typically leave their groups of birth to become social transients when they are between three and five years of age. Some males, however, do not emigrate, and instead retain membership in their birth group. In the current study we evaluated concentrations of 5-hydroxyindoleacetic acid (5-HIAA), 3-methoxy-4-hydroxyphenylglycol (MHPG), and homovanillic acid (HVA) in the cerebrospinal fluid (CSF) of 60 randomly selected adolescent and adult male rhesus monkeys captured on Cayo Santiago during annual trapping. There was a significant inverse correlation between age and CSF concentrations of both 5-HIAA ($p < 0.01$) and HVA ($p < 0.001$); thus, all further analyses used age as a covariate. Comparisons among animals less than five years of age indicated no significant differences between emigrating and non-emigrating monkeys in CSF monoaminergic metabolites. However, among the 39 animals five years of age or greater, the six monkeys remaining in their birth groups had significantly higher concentrations of CSF 5-HIAA than did animals that had emigrated ($p < 0.03$). No significant differences were observed in HVA or MHPG. These data indicate that the tendency of some males to remain in their birth groups instead of becoming socially transient may reflect, in part, elevated central serotonergic activity and associated behavioral characteristics (including reduced impulsivity and increased sociability).

437.10

IN VIVO IMAGING OF THE 5-HYDROXYTRYPTAMINE REUPTAKE SITE IN PRIMATE BRAIN USING SPECT AND [^{123}I]5-IODO-6-NITROQUIPAZINE. W.J. Jagust*, J.L. Eberling, J.A. Roberts, K.M. Brennan, S.M. Hanrahan, H.F. VanBrocklin, A.Biegon, C.A. Mathis Center for Functional Imaging, Lawrence Berkeley Laboratory, Berkeley, CA 94720.

Previous experiments in rats using in vitro binding and ex vivo autoradiography have demonstrated that 5-iodo-6-nitro-2-piperazinylquinoline (5-I-6-NQP) is a potent and selective ligand for studying brain 5-HT reuptake sites. We performed in vivo imaging in non-human primates using single photon emission computed tomography (SPECT) and [^{123}I]5-I-6-NQP. 8 experiments were performed in 4 adult male macaca mulatta using a SPECT tomograph with 6 mm in-plane resolution. Animals were injected with 4 - 8 mCi of tracer i.v., and images were acquired for either 0 - 6 h in one set of experiments or from 5 - 9 h in another set of experiments. Each type of experiment was performed in quadruplicate; for each set 2 experiments were performed with paroxetine pretreatment (2 mg/kg i.v.).

These studies showed rapid brain uptake of the tracer, with slow egress from the brainstem, a region rich in 5-HT reuptake sites. Loss of the tracer from regions with a lower density of these sites, such as cerebellum, was relatively more rapid. Pretreatment with paroxetine increased the washout of tracer from the brainstem to rates similar to that seen in cerebellum. Brainstem to cerebellar ratios of tracer accumulation were >2 by 8 hours after injection, and in paroxetine pretreated animals remained close to 1. These results indicate that the radiotracer has characteristics suitable for use as a SPECT imaging agent of serotonin reuptake sites.

POTASSIUM CHANNEL STRUCTURE, FUNCTION, AND EXPRESSION II

438.1

CLONING OF A NOVEL HUMAN BRAIN ION CHANNEL HIGHLY SIMILAR TO K_{ATP} CHANNEL IN RAT KIDNEY Gabriel G. Haddad[1]*, Trushna Desai[2], and Santosh N. Krishnan[1] [1]Department of Pediatrics (Section of Respiratory Medicine), and [2]Department of Genetics, Yale University School of Medicine, New Haven, Connecticut 06510.

Previous work from this laboratory has demonstrated the important role of ATP-regulated potassium channels in anoxia-induced depolarization and neuronal survival during anoxia. A number of questions regarding the structure and regulation of this channel, however, remain to be answered. For example, it is not clear what the relationship is between glibenclamide binding site and the channel. In order to further understand the function of this protein, we decided to clone it from the brain.

Starting from the sequence of an ATP-regulated potassium channel cloned from rat kidney (Nature **362:** 31), we used the PCR to clone the brain isoform. We isolated a PCR product from human cerebral cortex mRNA that is highly similar to the rat ATP-regulated potassium channel. This product has an M0, M1, and H5 (pore-forming region) domains as described for the rat kidney channel. With this PCR product, we will screen for cDNA clones from a human fetal brain cDNA library in λZAP and genomic clones from a human genomic library in λEMBL3. Using fluorescent in situ hybridization (FISH), we will determine the chromosomal location of this gene. Genomic organization and functional expression studies are also in progress.

438.2

mSlo, A COMPLEX MOUSE GENE ENCODING HIGH CONDUCTANCE CALCIUM-ACTIVATED K^+ CHANNELS. D. McCobb[1]*, A. Wei[1], S. Tsunoda[1], A. Butler[1], N. Fowler[1], M. Schreiber[1], J. Krause[1], M. Saito[3], C. Solaro[3], C. Lingle[3], and L. Salkoff[1,2]. [1]Dept. of Anatomy and Neurobiology, [2]Dept. of Genetics, [3]Dept. of Anaesthesiology, Washington University School of Medicine, Box 8108, 660 S. Euclid Avenue, St. Louis, MO 63110.

cDNAs from mSlo, a gene encoding mouse brain and skeletal muscle calcium-activated K^+ channels, were isolated, sequenced, and expressed in the Xenopus oocyte expression system. mSlo channels had properties of high conductance "maxi" or "BK" channel types; single channel conductance was 272 pS with symmetrical potassium concentrations, and whole cell and single channel currents were blocked by charybdotoxin, iberiotoxin, and TEA. cDNAs encoding many alternative forms of mSlo channels were isolated. The large number of variant channel forms may reflect the need for regulatory diversity, as well as a diversity of biophysical properties. mSlo channel peptides are conserved with their Drosophila homologue, slo, over a large region encompassing ten hydrophobic domains. Conservation of the S1 through S6 region suggests that mSlo belongs to a vast gene family that includes voltage-gated K^+ channels. The mSlo protein, however, is more complex than that of the voltage-gated K^+ channels, having areas of highest conservation with Drosophila slo outside of those conserved with the voltage-gated channels. Perhaps over the course of evolution, the calcium gating mechanism and/or regulatory domains have been appended to the basic structure of a voltage gated K^+ channel.

438.3

PRIMARY STRUCTURE AND FUNCTIONAL EXPRESSION OF A POSSIBLE G-PROTEIN COUPLED INWARD RECTIFIER E. Reuveny*, Y. Kubo, P.A. Slesinger, Y.N. Jan and L.Y. Jan, Howard Hughes Medical Institute, Univ. of Calif. Med. School. San Francisco, CA 94143.

Two novel inward rectifier potassium channels have been recently cloned, IRK1 (Kubo et al., Nature, **362:**127, 1993) and ROMK1 (Ho et al., Nature, **362:**31, 1993). Both of these channels differ from voltage-gated potassium channels in that they have two putative membrane spanning regions, rather than six, and may comprise a new superfamily of potassium channels. Based on sequence homology, we have searched for other members of this superfamily, such as a G-protein coupled inward rectifier from heart. We designed degenerative primers to the core region (M1-M2) of the channel and amplified a fragment by PCR. Using this fragment as a probe, we have isolated a cDNA from rat heart library that has ~40% homology with IRK1 and ROMK1. The cRNA expressed in Xenopus oocytes produces a channel that is inwardly rectifying and is active in patches which are excised into a solution containing 100 μM GTPγS, 2-4 mM MgATP and 90 mM K^+. The channel has a single-channel conductance of ~35 pS (in symmetrical 90 mM K^+) which is similar to the heart G-protein activated channel. We are currently investigating whether this channel is indeed a G-protein coupled inward rectifier.

438.4

EXPRESSION AND CLONING OF AN ATRIAL G-PROTEIN-ACTIVATED POTASSIUM (KGA) CHANNEL IN *XENOPUS* OOCYTES. Nathan Dascal*, Nancy F. Lim, Wolfgang Schreibmayer†, Weizhen Wang, Norman Davidson, Henry A. Lester. Division of Biology, California Institute of Technology, Pasadena, CA 91125. (Permanent addresses: * School of Medicine, Tel Aviv University, Ramat Aviv 69978, Israel; † Institut für Medizinische Physik und Biophysik, Universität Graz, A-8010 Graz, Austria).

Injection of rat atrial RNA into *Xenopus* oocytes resulted in the expression of a G protein-activated K^+ (KGA) channel. Current through the channel could be activated by acetylcholine (ACh) or, if RNA encoding a neuronal 5HT1A receptor was co-injected with atrial RNA, by serotonin (5HT). A 5HT-evoked current (I_{5HT}) was observed in oocytes injected with ventricle RNA fractions (of 2.5-5.5 kb size) and 5HT1A receptor RNA. I_{5HT} displayed strong inward rectification, showing very little conductance above the K^+ equilibrium potential; was highly selective for K^+ over Na^+; and was blocked by 5-300 μM Ba^{2+}. I_{5HT} was suppressed by intracellular injection of the non-hydrolysable analog of GDP, GDP-β-S, but not by treatment with pertussis toxin (PTX), suggesting coupling of the receptor to the KGA channel via a PTX-insensitive G protein possibly endogenously present in the oocyte. Co-expression of the α subunit of a PTX-sensitive G-protein, G_{i2}, rendered I_{5HT} sensitive to PTX inhibition. Native oocytes displayed a constitutively active inward rectifying K^+ current with a lower sensitivity to Ba^{2+} block; expression of a similar current was also directed by atrial or ventricle RNA of 1.5-3 kb size. A directional cDNA library from atrial poly(A) RNA was constructed in pBluescript II (Stratagene), and *Xenopus* oocytes were injected with RNA transcribed *in vitro* from cDNA pools, together with 5HT1A receptor RNA. Further progress in cloning of the KGA channel will be reported at the meeting. Supported by: Israel-US BSF, GM-29836, MH-49176, NRSA, Austrian Research Foundation.

438.5

FUNCTIONAL EXPRESSION OF DELTA OPIOID RECEPTOR K56 IN XENOPUS OOCYTES. C Chavkin[1], D Henry[1]*, N Dascal[2], N. Lim[2], W Schreibmayer[2], BL Kieffer[3], C Gaveriaux-Ruff[3], N Davidson[2] and H Lester[2]. Dept of Pharmacol[1], Univ of Wash, Seattle WA, Div of Biol[2], Caltech, Pasadena, CA, and Ecole Superieure de Biotechnologie, Strasbourg, FR[3].

When cRNA prepared from the delta opioid receptor cloned from NG108-15 cells was injected into Xenopus oocytes, delta opioid specific binding sites were expressed. Peak expression of 11 pmoles receptor per mg of oocyte membrane protein was obtained at 4 days after injection of 18 ng cRNA. Binding site density was defined using the delta selective antagonist [^{3}H]-naltrindole, and uninjected oocytes do not express specific binding sites. Co-injection of the delta receptor cRNA with cRNA derived from a rat atrial library conferred the ability of delta opioid agonists to activate an inwardly-rectifying potassium conductance similar to that controlled by delta receptors in neurons in several brain regions. The potassium current induced by the delta agonist DPDPE was dependent on the co-injection of atrial cRNA, was blocked by the antagonists naloxone (1 μM) or naltrindole (1 μM), and was also blocked by 100 μM Ba^{2+}. DPDPE activated the inward rectifier with an EC50 of 1.3 nM which closely agrees with its measured binding affinity of 1.6 nM. Although delta receptor coupling to potassium channels in brain is known to be sensitive to pertussis toxin, pertussis toxin treatment of the oocytes (1 μg/ml, 24 hrs), did not affect the response to DPDPE. These results demonstrate that the cloned delta receptor expressed by oocytes is functional and that the oocyte expression system will be useful in the analysis of opioid receptor effector coupling. Supported by DA04123, GM 29836, MH 49176, Israel-US BSF.

438.6

DETECTION OF A HETEROMULTIMERIC POTASSIUM CHANNEL IN RAT BRAIN: POTENTIAL MOLECULAR BASIS OF A PRESYNAPTIC A-CURRENT. Morgan Sheng, T.J. Baldwin, Y. Joyce Liao, Yuh Nung Jan, and Lily Yeh Jan*. Howard Hughes Medical Institute and Dept. of Physiology, UCSF, San Francisco, CA 94143.

The heterogeneity of K^+ channels arises in part from the large number of genes encoding different K^+ channel subunits. In addition, heterologous expression studies suggest that assembly of distinct subunits into heteromultimeric channels may contribute further to K^+ channel diversity. A question has been whether heteromeric K^+ channels actually form in vivo, and if so, whether specific combinations of subunits could account for major K^+ currents identified in neurons. Based on biochemical copurification, and co-immunoprecipitation with subunit specific antibodies, we show that Kv1.4 and Kv1.2, two K^+ channel subunits of the Shaker subfamily, coassemble in rat brain. The Kv1.4/Kv1.2 heteromultimer combines features of both parents, resulting in a dendrotoxin-sensitive A-type K^+ conductance. Based on immunocytochemical localization in axons and nerve terminals, we hypothesize that Kv1.4/Kv1.2 heteromultimers may form the molecular basis of a pre-synaptic A-type K^+ channel involved in the regulation of neurotransmitter release.

438.7

INACTIVATION BALL PEPTIDE FROM THE SKAKER K^+ CHANNEL BLOCKS OPEN CYCLIC NUCLEOTIDE-GATED (CNG) CHANNELS BY BINDING TO THEIR PORE DOMAINS R.H. Kramer*, E. Goulding, and S.A. Siegelbaum Ctr. for Neuro. and Behav., Columbia U., NY, NY 10032, and Dept. of Pharm., Univ. of Miami, Miami, FL 33101.

CNG channels are crucial for olfactory and photosensory transduction. While these channels are ligand-gated (cAMP and/or cGMP) and voltage-insensitive, their primary amino acid sequences resemble that of voltage-gated K^+ channels. Here we show that a 20 amino acid peptide derived from the NH_2-terminus of the Shaker K^+ channel ("ball" peptide) blocks olfactory (OLF) and photoreceptor (RET) CNG channels with a K_i of 22 and 175 μM, respectively. Application of ball peptide followed by cGMP reveals a transient CNG current, indicating that channels must be open before they can be blocked. If cGMP is washed away from the blocked channels, the channels re-open after the block is relieved, indicating that the peptide retards channel closing. We conclude that cGMP binding opens an internal gate that allows the peptide to block, and bound peptide prevents the gate from closing. Wild-type and mutant peptides with different affinities for K^+ channels have similar relative potencies for CNG channels, suggesting that CNG and K^+ channels have conserved peptide binding sites. However, block of CNG channels is 4-fold more voltage-sensitive than block of K^+ channels, suggesting that the peptide inserts deeper into CNG channels. Mutational studies suggest that the peptide directly contacts the pore-lining H5 region of CNG channels. When the H5 of the RET channel is replaced with that of the OLF channel, block of the chimeric channel shows the high affinity (K_i=21 μM) characteristic of the OLF channel. Supported by HHMI and NIH grant NS30685.

438.8

NONE OF THE CYSTEINES IN THE SHAKER POTASSIUM CHANNEL IS ESSENTIAL FOR CHANNEL ACTIVITY OR FOR ZINC MODULATION. L.M. Boland*, M.E. Jurman, G.Yellen, Neuroscience Research Center and Dept. Neurobiology, Mass. Gen. Hosp. and Harvard Med. Sch, Charlestown, MA 02129

All voltage-gated ion channels described to date have several endogenous cysteine residues, many of which are highly conserved. In other proteins, cysteines are essential for functions such as protein folding, enzyme activity, and coordination of metal ions. We investigated whether the cysteine residues in potassium (K) channels are essential for activation and inactivation gating or for modulation of activation gating by external zinc and cadmium.

Shaker H4 K channels were expressed in Xenopus oocytes or a mammalian cell line (HEK293) and macroscopic currents were studied by two-electrode voltage-clamp or excised membrane patch recordings. We used oligonucleotide-directed mutagenesis to replace the seven endogenous cysteine residues at positions 96, 245, 286, 301, 308, 462, and 505 with serine, alanine, or valine. Substitutions were made in a mutant (ShIR, Δ6-46) lacking N-type ("fast") inactivation. Replacement of all cysteines did not alter several properties of the macroscopic currents. There was little or no effect on the activation kinetics or the voltage-dependence of activation gating. Both wild-type channels and those lacking cysteines showed a positive shift and slowing of activation gating upon perfusion with external zinc or cadmium (0.1 - 1 mM). Replacement of the cysteine at position 462 alone or 462 plus other cysteines led to progressive rundown of the current with successive pulses, suggesting a faster rate of entry and slower rate of recovery from the C-type ("slow") inactivated state.

These results indicate that the seven cysteine residues in the Shaker K channel subunit are not essential for channel activity or the modulation of activation gating by external divalents. Not surprisingly, replacement of cysteine 462 alters C-type inactivation, consistent with previous reports for nearby residues in the S6 region.

438.9

MUTATIONS OF A CONSERVED CYSTEINE RESIDUE IN S6 OF Kv2.1 (DRK1) ALTER INACTIVATION AND DEACTIVATION KINETICS. H.-j. Zhang, R.D. Zühlke and R.H. Joho*. Department of Cell Biology and Neuroscience, The University of Texas Southwestern Medical Center, Dallas, TX 75235-9111.

Two cysteines in the putative transmembrane segments S2 and S6 are absolutely conserved among cloned and functionally expressed voltage-gated K^+ channels. We have investigated functional changes in Kv2.1 (DRK1) resulting from site-directed mutagenesis of these two conserved cysteine residues. In S2 (C232), twelve out of thirteen amino acid substitutions maintained channel function in *Xenopus* oocytes after microinjection with cRNA transcripts. In contrast, only seven out of fifteen cysteine replacements in S6 (C393) resulted in functional channels, and the expressed S6 mutants showed alterations of channel properties. Substitutions by glycine, asparagine, and valine shifted the midpoint of activation by 10-20 mV in the depolarizing direction, and C-type inactivation was two to tenfold faster. In addition, asparagine and valine substitutions displayed extremely fast deactivation kinetics. Serine and threonine substitutions, however, slowed C-type inactivation and deactivation. Also, the serine substitution increased the Rb^+/K^+ conductance ratio twofold.

Although our results suggest that the conserved cysteines in S2 and S6 are not essential for channel expression in *Xenopus* oocytes, the cysteine residue in S6 appears to play an important role in channel function; it may participate in controlling the transition from the open to the closed state of the channel. (Supported by NS28407 to R.H.J.)

438.10

POTASSIUM CHANNELS SHOW DIFFERENTIAL EXPRESSION AND SUBCELLULAR LOCALIZATION WITHIN THE MOUSE RETINA. D.J. Klumpp, E.-J. Song, M. Shengt, L.Y. Jant, and L.H. Pinto*. Dept. of BMBCB and Dept. of Neurobiol. and Physiol., Northwestern University, Evanston, IL 60208, and †HHMI, University of California, San Franciso, CA 94143.

Many voltage-gated potassium channels have now been cloned, and their physiological properties have been characterized in vitro. For some channels, gross distribution within the CNS is also known. However, the pattern of expression is not yet understood for a variety of channels within a well-defined tissue. The mouse retina provides an attractive system for studying potassium channels because of its well-characterized anatomy and connectivity, and because retinal neurons exhibit a wide range of physiological properties.

We have studied the expression of K channels within intact mouse retinas and within enzymatically isolated retinal neurons using RNA PCR, in situ hybridization and immunohistochemistry. Using RNA PCR, we found the retina expresses members of the Kv1.x, Kv2.x, Kv3.x and Kv4.x families. In situ hybridization revealed Kv1.2 message in the inner nuclear layer (INL) and in the ganglion cell layer. We detected Kv1.3 message in rods, the entire INL and the ganglion cell layer. Employing immunohistochemistry, we found Kv1.3 expression in rods, punctate labelling in the outer plexiform layer, and light staining of the inner plexiform layer. Staining for Kv1.4 revealed light staining of cell bodies within the INL and heavy, banded labelling within the innerplexiform layer. Kv4.2 protein expression was limited to a population of cell bodies within the ganglion cell layer which project processes that form two bands in the middle of the the inner plexiform layer. Thus, within the mouse retina, potassium channels are differentially expressed, a given neuron may express multiple channels, and channels show subcellular distribution.

438.11

ANALYSIS OF THE SPATIAL DISTRIBUTION AND CO-EXPRESSION OF SHAW RELATED K^+ CHANNELS IN THE RAT CENTRAL NERVOUS SYSTEM. M. Weiser*, E. Vega-Saenz de Miera, C. Kentros, H. Moreno, H. Baker, and B. Rudy. Dept. of Physiology and Biophysics, NYU Medical Center, NY 10016 and Burke Med. Res. Institute, White Plains, NY 10605

Transcripts of the Shaw related or ShIII gene subfamily are thought to encode subunits of tetrameric voltage gated K^+ channels. Recent *in vitro* experiments have suggested that K^+ channels with a large functional diversity may arise from heteromultimeric aggregation of subfamily specific subunits. In order to identify the potential for heteromultimer formation *in vivo*, the current experiments examined the spatial distribution and co-expression of the mRNAs for native ShIII channels. Northern blot analysis demonstated that three of the four K^+ channel genes (KV3.1, KV3.2 and KV3.3) are expressed primarily in the CNS. KV3.4 transcripts also were present in the CNS but were more abundant in skeletal muscle. *In situ* hybridization in the CNS revealed expression of ShIII mRNAs in discrete and cell type specific neuronal populations. Although each ShIII gene exhibits a unique hybridization pattern many neuronal populations expressing KV3.1 also contained KV3.3 mRNA. In addition, KV3.4 transcripts were present, although at lower levels, in several neuronal populations which also expressed KV3.1 and/or KV3.3 providing evidence for possible heteromultimer formation *in vivo*. To further establish the functional role of heteromultimer formation in this subfamily, specific ShIII cRNAs were co-injected into *Xenopus* oocytes. Small amounts of KV3.4 cRNA, which expressed small, fast inactivation currents when injected alone, produced fast inactivating currents that were several fold larger when co-injected with an excess of KV3.1 or KV3.3 cRNA. Together these data suggest the significance of the apparently limited, low level expression of KV3.4. Based upon these findings we propose the existence of a ShIII channel system whose functional repertoire results from variable subunit composition. Supported by NS30989 to B.R. and AG09686 to H.B.

438.12

A RE-EXAMINATION OF THE STRUCTURE OF THE PORE OF VOLTAGE-SENSITIVE CATION CHANNELS EXCLUDES THE ANTI-PARALLEL β-SHEET MODEL. John A. Schetz* and P. A. V. Anderson, Dept. of Neuroscience and the Whitney Laboratory of the Univ. of Florida, Gainesville, Florida 32610.

Functional differences between different members of the voltage-sensitive cation channel superfamily reside primarily in their pore regions where ion selectivity and many specific aspects of the their pharmacology are manifested. The structure of the pore of VSCCs has, therefore, been the subject of considerable attention. Current models envisage the loop between the fifth (S5) and sixth (S6) trans-membrane spanning helices of each domain being formed of two short β sheets (SS1 and SS2) linked by a β-hairpin turn, and that the resulting anti-parallel β-sheet inserts part way into the membrane to form the lining of the pore. However, close examination of the residues designated as forming the turn regions of many channels reveals certain inconsistencies in the assignment of turns at those locations. To better evaluate this, we determined the turn probability (P_{bend}) for all tetrapeptide sequences within the putative pore region of 26 different isoforms from the VSCC superfamily, a total of 80 domains. Tetrapeptide sequences with significant P_{bend} were identified in the S5-S6 loops of all 80 domains . However, these turns were shifted sufficiently far towards the C-terminus of each S5-S6 loop, as to lengthen the SS1 regions and altogether eliminate the SS2 β-sheet. This suggests that the pore of the channel cannot be formed by an anti-parallel β-sheet, as currently envisaged, and that other models of the pore region must be considered. (Supported by NSF grant BNS 9109155 to PAVA.)

CATECHOLAMINE RECEPTORS: DOPAMINE II

439.1

DOPAMINE RECEPTOR-MEDIATED INOSITOL PHOSPHATE ACCUMULATION IN CHICK RETINAL CELL CULTURES. P.M. Iuvone* and J. Gan Dept. of Pharmacology, Emory Univ. Sch. of Medicine, Atlanta, GA.

This study examined the effects of dopamine receptor activation on phospholipase C in retinal cells, using accumulation of inositol phosphates as an index of phospholipase C activity. Monolayer cultures of neurons and photoreceptors were prepared from embryonic day 8 chick retinas, and were cultured for 6 days. The cells were prelabeled with [^{3}H]adenine or [^{3}H]myo-inositol for measurement of cyclic AMP formation or inositol phosphate accumulation, respectively. Accumulation of inositol phosphates was measured in the presence of 10 mM lithium to inhibit their metabolism to inositol. Dopamine stimulated inositol phosphate accumulation by retinal cells with an EC_{50} of approximately 10μM. Apomorphine and SKF82958, a D1 receptor agonist, also stimulated inositol phosphate accumulation. The maximal accumulation of inositol phosphates in response to SKF82958 was greater than that elicited by dopamine. Stimulation of inositol phosphate accumulation by dopamine and SKF82958 was antagonized by SCH23390, a D1 dopamine receptor antagonist, or by haloperidol, a nonselective dopamine antagonist. Both dopamine and SKF82958 produced smaller responses than did carbachol, an acetylcholine receptor agonist, suggesting that a small number of putative dopamine receptors are coupled to phospholipase C or that the coupling efficiency is low. Stimulation of inositol phosphates by dopamine receptor agonists was not a consequence of stimulation of cyclic AMP formation, as the two responses showed different concentration-response relationships and forskolin, an activator of adenylate cyclase, had no effect on either basal or dopamine-stimulated inositol phosphate accumulation. Dopamine-stimulated inositol phosphate accumulation was only slightly reduced by pertussis toxin pretreatment or by reducing extracellular Ca^{2+}. The data suggest that a population of D1-like dopamine receptors on retinal cells is coupled to phospholipase C.

439.2

ADENYLYL CYCLASE ACTIVATION BY THE D1B DOPAMINE RECEPTOR MIMICKS PROPERTIES OF CONSTITUTIVELY ACTIVE MUTATED G PROTEIN-COUPLED RECEPTORS. M. Tiberi*, K. R. Jarvie, S. Cotecchia and M. G. Caron. HHMI Labs, Dept. of Cell Biology, Duke Univ. Med. Ctr., Durham, NC 27710.

Dopamine D1A and D1B receptor subtypes belong to the superfamily of G-protein coupled receptors. These two D1 receptor subtypes are coupled to activation of adenylyl cyclase and exhibit a distinct anatomical localization. The primary structure of the two receptors is highly conserved within the transmembrane domains but differs within the third intracellular loops and the carboxy tails. To identify functional differences, binding and stimulation of adenylyl cyclase were assessed in 293 and COS-7 cells expressing either D1A or D1B receptor. In general, membranes expressing D1B receptors displayed higher affinities for agonists than those expressing D1A receptors, whereas antagonists had generally a lower affinity at the D1B than at the D1A receptor. Basal activity of adenylyl cyclase in 293 cells expressing various levels of D1B receptors was markedly higher than the basal activity measured in cells expressing D1A receptors. Thus, the fold and maximal stimulation of adenylyl cyclase resulting from activation of the D1B receptor was significantly less than that obtained following agonist activation of the D1A receptor. In cells expressing D1B receptors, the increased agonist affinity appeared to translate in an increased potency of agonists in stimulating adenylyl cyclase in comparison to the potencies determined at the D1A receptor. These properties are reminescent of those of constitutively active G protein-coupled receptors obtained by site-directed mutations. Experiments with chimeric D1A/D1B receptors reveal that part of the constitutive activity and increased affinity for agonists might be explained by sequences of the third intracellular loop. The different anatomical distribution and biochemical properties of these D1 receptors strengthen the functional distinctions between the two subtypes.

439.3

DOPAMINE AND D_1 DOPAMINE RECEPTOR AGONISTS: DOPAMINE RECEPTOR SELECTIVITY AND RESPONSE TO GUANINE NUCLEOTIDES IN NONHUMAN PRIMATE BRAIN. B.K. Madras*. Harvard Medical School, New England Reg. Primate Res. Ctr., Southborough, MA, 01722.

D_1 dopamine receptor agonists has been proposed as therapeutic agents for Parkinsonism (Madras et al., 1992) and have been considered for the pharmacological management of cocaine abuse. The behavioral effects of D_1 receptor agonists differ, however, and these divergent effects may reflect differing efficacies of these drugs for stimulating adenylate cyclase or other systems. In order to characterize D_1 dopamine receptor agonists in primate brain, D_1 receptor affinity, D_1/D_2 receptor selectivity and response to guanine nucleotides was determined for a series of dopamine agonists in cynomolgus monkey caudate-putamen. Of the drugs tested, SKF 81297 was the most selective for the D_1 dopamine receptor (300-fold). The drugs responded differently to the nonhydrolyzable analog of GTP, GpNHpp (100 μM). GpNHpp decreased the affinity of dopamine for the D_1 receptor by 12-fold and the affinities of SKF 81297, SKF 83189, SKF 82958, CY 208243 by 3-fold. SKF 38393 affinity was reduced 1.5-fold whereas the affinities of SKF 75670, SKF 77434, and SCH 39166 were unaffected by GpNHpp. Dopamine and D_1 dopamine receptor agonists differ in their responses to guanine nucleotides and these differences may contribute to differing behavioral effects. DA06303, DA00499, RR00168.

439.4

IN VIVO KNOCKOUT OF BRAIN DOPAMINE D_2 RECEPTORS BY AN ANTISENSE OLIGODEOXY-NUCLEOTIDE. Ian Creese* and M. Zhang. Center for Molecular and Behavioral Neuroscience, Rutgers, The State University of New Jersey, Newark, NJ 07102.

A 3 day intraventricular infusion of an antisense oligodeoxy-nucleotide corresponding to the NH_2-terminus of the dopamine D_2 receptor mRNA resulted in a 48% decrease in the number of rat striatal dopamine D_2 receptors, as measured by homogenate binding assays. D_2-receptor autoradiography also indicated a homogeneous knockout of about 50% throughout the striatum and over 70% in the nucleus accumbens. In contrast, the antisense treatment failed to affect the receptor densities of striatal D_1, M_1, or 5-HT_2 receptors, indicating that the knockout was specific for D_2 receptors. The specificity of the antisense treatment was further confirmed by the failure of a random oligodeoxynucleotide to induce changes in D_2 receptor number. Behavioral observations indicated that the antisense knockout of D_2 receptors reliably induced catalepsy, a behavior previously associated with decreased D_2 receptor activation. The catalepsy was maintained for at least 6 days after the offset of antisense oligo delivery. Spontaneous locomotor activity was also significantly inhibited by the antisense treatment. These results suggest that antisense treatment has the ability to affect CNS receptors with specificity and efficacy, and hence may have general value in determining the functional significance of CNS receptors.

Supported by the Stanley Foundation and NIMH Neuroscience Center for Research in Schizophrenia.

439.5

FUNCTIONAL CHARACTERIZATION OF HUMAN D_2 AND D_3 DOPAMINE RECEPTORS. M. N. Potenza*§#, G. F. Graminski#, C. Schmauss^, and M. R. Lemer#¶. Depts. of Cell Biology§, Internal Medicine#, & Pharmacology¶ & Howard Hughes Medical Institute #, Yale Univ. Sch. of Med., New Haven, CT 06510 & Dept. of Psychiatry^ & Brookdale Center for Molecular Biology^, Mount Sinai Sch. of Med., NY, NY 10029

Functional characteristics of human D_2 and D_3 receptors (DRs) were examined using a recently developed bioassay suited for the study of G_i-protein coupled receptors (G_iRs). The bioassay utilizes pigment granule aggregation within cultured *Xenopus laevis* melanophores for the evaluation of ligands as agonists or antagonists upon particular G_iRs. A microtiter plate reader is employed to quantitate pigment aggregation and, as cell sacrifice is not necessary for measurement, time course analyses as well as dose-responses can be readily generated. Melanophores were transiently transfected with cDNAs coding for the human D_{2B}R (short form) or D_3R. Expression of either receptor conferred upon the cells the ability to aggregate their melanosomes in response to selective dopaminergic agonists. The same ligands also inhibited cAMP accumulation within the transfected melanophores, and the agonist-induced melanosome aggregation was shown to be sensitive to pertussis toxin. Although the magnitude of ligand-induced pigment aggregation was after greater in the D_2R-expressing melanophores than in the D_3R-expressing cells, EC_{50} value determinations revealed that agonists activated the D_2R and D_3R at similar concentrations. IC_{50} value measurements showed that antagonists revealed the same rank order potency upon the two dopamine receptors. However, each of the antagonists displaying an effect was more potent upon the D_2R. The results reveal functional similarities and differences between the D_{2B}R and D_3R.

439.6

THREE COMPARTMENT MODELING OF [^{123}I]IBF BINDING TO DOPAMINE D2 RECEPTORS IN HUMAN BRAIN. C. H. Van Dyck*, M. Laruelle, A. Abi-Dargham, S. S. Zoghbi, Y. Zea-Ponce, R. M. Baldwin, D. S. Charney, P. B. Hoffer, R. B. Innis. Yale Univ and West Haven VA Medical Center, West Haven, Connecticut, 06516

The iodinated benzamide analog, [^{123}I]IBF (iodobenzofuran), a high affinity dopamine D2 antagonist (KD = 0.11 nM, Kung et al, J Nuc. Med., 30:88-92, 1989), was used as a SPECT radiotracer to measure D2 receptor binding potential (BP = Bmax/KD) in healthy humans (n=4, age = 29 ± 5 years, ±SEM). Data were acquired with the brain dedicated, high resolution, CERASPECT camera for 160 min after bolus injection of the tracer (6.0 ± 0.1 mCi). Arterial plasma activity was measured and corrected for metabolites by HPLC. Plasma clearance of the parent compound was 682 ± 380 l/h. Striatal activity equilibrated at 40-80 min. Occipital and striatal time activity curves were fit to a two and three compartment model, respectively, to estimate transfer rate constants, K1 to k4. Occipital equilibrium distribution volume (DV) was calculated as K1/k2*f1, where f1 is the measured free fraction in the arterial blood. Striatal BP was calculated as K1*k3/k2*k4*f1 with K1/k2 ratio constrained to the value derived in the occipital region. Occipital DV was 63 ± 13 and striatal BP was 197 ± 49. The striatal BP to occipital DV ratio was 3.0 ± 0.2. Assuming an *in vivo* KD of 0.11 nM, these values indicate a Bmax of 22 ± 4 nM, witch is in accordance with the literature. These studies demonstrated the feasibility of measuring D2 receptor BP with SPECT using three compartment kinetic modeling. As this method corrects for between subjects differences in plasma clearance, it is more accurate than simple striatal to occipital ratio methods for measurement of receptor density.

439.7

BINDING OF [^{125}I](R)*trans*-7-HYDROXY-2-(N-n-PROPYL-N-3'-IODO-2'-PROPENYL)AMINOTETRALIN ([^{125}I](R)*trans*-7-OH-PIPAT) TO DOPAMINE D3 RECEPTORS IN RAT OLFACTORY TUBERCLE. K. D. Burris*, T. M. Filtz, M-P. Kung, C. Foulon, S. Chumpradit, H. F. Kung and P. B. Molinoff. Depts. of Pharmacology and Radiology, Univ. of Penn., Sch. of Med., Philadelphia, PA 19104-6084

The lack of selective ligands for members of the D2-like family of receptors has made it difficult to study D2, D3 and D4 receptors in tissues expressing multiple subtypes. [^{125}I](R)*trans*-7-OH-PIPAT binds with high affinity to D3 receptors expressed in Sf9 cells. No specific binding is seen in Sf9 cells expressing a high density of D2 receptors. However, [^{125}I](R)*trans*-7-OH-PIPAT bound with high affinity to membranes from HEK-293 cells expressing transfected D2 or D3 receptors and to membranes from CHO cells expressing 5-HT_{1A} receptors. Binding of [^{125}I](R)*trans*-7-OH-PIPAT to 5-HT_{1A} and D2 receptors was decreased or eliminated in the presence of GTP or guanylyl-imidodiphosphate (Gpp(NH)p), however binding of [^{125}I](R)*trans*-7-OH-PIPAT to D3 receptors was not affected by Gpp(NH)p. Therefore, coupling of receptors to G-proteins appears to be a requirement for the binding of [^{125}I](R)*trans*-7-OH-PIPAT to D2 and 5-HT_{1A}, but not D3, receptors. Saturation binding of [^{125}I](R)*trans*-7-OH-PIPAT to membranes from rat olfactory tubercle resulted in markedly curvilinear Scatchard plots suggesting that the radioligand was binding to more than one class of receptors. In the presence of Gpp(NH)p, linear Scatchard plots with K_d values of 0.1-0.3 nM and densities of 100-200 fmol/mg of protein were obtained. Agonists and antagonists inhibited binding of [^{125}I](R)*trans*-7-OH-PIPAT with K_i values which are in good agreement with values obtained using membranes from HEK-293 cells expressing D3 receptors. These results suggest that, in the presence of Gpp(NH)p to eliminate binding to D2 and 5-HT_{1A} receptors, [^{125}I](R)*trans*-7-OH-PIPAT labels D3 receptors in rat olfactory tubercle. (USPHS NS18591, NS09245, NS24538)

439.8

***IN VIVO* AGONIST PROPERTIES OF 7-OH-DPAT, A DOPAMINE D_3-SELECTIVE RECEPTOR LIGAND.** **J.F. McElroy*, K.A. Amy, K.A. Ward, K.L. Zeller, J.F. Cawley and A.L. Mazzola. CNS Diseases Research, The Du Pont Merck Pharmaceutical Co., Experimental Station, P.O. Box 80400, Wilmington, DE 19880-0400**

7-OH-DPAT (7-hydroxy-N,N-di-n-propyl-2-aminotetralin), recently identified as a high affinity and selective ligand at dopamine D_3 receptors (Levesque et al., *Proc. Natl. Acad. Sci.*, 89: 8155-8159, 1992), was evaluated in a battery of *in vivo* tests highly predictive of dopamine agonist or antagonist activity. In contrast to well established dopamine receptor antagonists such as haloperidol and eticlopride, 7-OH-DPAT was inactive in the rat conditioned avoidance and catalepsy tests and did not antagonize apomorphine-induced stereotypy or climbing behavior in mouse (all ED_{50} values > 30 mg/kg, s.c.). However, similar to the dopamine agonist apomorphine, 7-OH-DPAT produced stereotypical sniffing in mouse and rat (ED_{50}'s < 3.0 mg/kg, s.c.), increased locomotor activity in rat (ED_{50} < 10 mg/kg, s.c.), and caused 6-OHDA-lesioned animals to rotate in a contralateral direction (ED_{50} < 1.0 mg/kg, s.c.). Taken together, these results indicate that 7-OH-DPAT is an agonist at dopamine receptors, *in vivo*. Antagonism studies using antagonists at dopamine D_1 (SCH23390), D_2/D_3 (haloperidol, eticlopride), and D_4 (clozapine) receptors are in progress.

439.9

THE EFFECT OF CHRONIC NICOTINE ON DOPAMINE RECEPTOR - EFFECTOR MECHANISMS IN THE NUCLEUS ACCUMBENS AND STRIATUM. L. L. Holt* and T.C. Westfall. Dept. of Pharm. Phys. Sci. St. Louis Univ. Med. Ctr. St. Louis, MO 63104.

Previous studies from this laboratory have demonstrated development of tolerance to the effects of nicotine on dopamine (DA) synthesis in the nucleus accumbens (NAc) without a similar development of tolerance in the striatum (Str) (Holt, I.L. and Westfall, T.C.; Soc. Nsci. Abstr. 517.5, 1989). Using a similar experimental paradigm for chronic treatment of nicotine, pellets implanted subcutaneously designed to administer 1 mg/ kg/ day for 14 days, we explored potential mechanisms for tolerance development. We examined the effects of chronic nicotine on dopamine D1 and D2 receptors in the NAc and Str and found no change in either the Bmax or Kd of either D1 or D2 receptors in either area after chronic treatment. We further examined the effects of chronic nicotine on D1- or D2- mediated effects on cAMP efflux in both dopaminergic target areas and found that D1 agonist SKF-38393-induced enhancement of cAMP efflux in the NAc was attenuated after chronic nicotine but was not affected in the striatum. We did not observe any effects of nicotine on D2 agonist quinpirole-induced inhibition of cAMP. We additionally examined the effects of chronic NIC on autoreceptor activity in both tissues by treating animals with GBL to block nerve activity and DA release (and hence remove autoreceptor activation) in DAergic neurons and observed the ability of quinpirole to reactivate autoreceptors. This was measured by the ability of quinpirole to inhibit the enhanced DA synthesis stimulated by GBL-induced removal of autoreceptor activity. Although NIC was able to moderately decrease the ability of GBL to enhance DA synthesis, it did not affect autoreceptor induced inhibition of synthesis. These results suggest that chronic nicotine may effect a functional uncoupling of D1 receptors to adenylate cyclase in the NAc, not Str, and that chronic nicotine does not appear to modify D2 or autoreceptor function in either tissue.

439.10

5,7-DIHYDROXYTRYPTAMINE DESTRUCTION OF SEROTONIN FIBERS ELIMINATES THE ENHANCED ORAL ACTIVITY RESPONSE TO SKF 38393 IN NEONATAL 6-HYDROXYDOPAMINE-LESIONED RATS. R.M. Kostrzewa[1]*, R. Brus[2], K.W. Perry[3] and R.W. Fuller[3]. [1]Department of Pharmacology, East Tenn St Univ, Johnson City, TN 37614; [2]Silesian Academy of Medicine, Zabrze, Poland and [3]Lilly Research Labs, Indianapolis, IN.

Previous experiments show that neonatal 6-hydroxydopamine (6-OHDA) lesioned rats exhibit an enhanced oral activity response to the dopamine (DA) D_1 agonist SKF 38393 and that this response is mediated by serotonin 5-HT_{1C} receptors. Therefore, we tested whether the 5-HT neurotoxin, 5,7-dihydroxytryptamine (5,7-DHT), would eliminate SKF 38393 effects. At 3 days after birth rats were treated with desipramine (20 mg/kg IP) 1 hr before vehicle, 6-OHDA (134 μg, ICV) and/or 5,7-DHT (50 μg). At 2-4 months rats treated with 6-OHDA alone showed an enhanced response to SKF 38393 and the 5-HT_{1C} agonist *m*-chlorophenylpiperazine (*m*-CPP). However, there was no change in the dose response curve for these drugs in rats treated neonatally with vehicle or 5,7-DHT alone. In rats treated neonatally with both 6-OHDA and 5,7-DHT the *m*-CPP response was still present, while the SKF 38393 response was fully attenuated. Neonatal 6-OHDA and 5,7-DHT treatments reduced striatal DA and 5-HT contents by >98% and >90%, respectively. The findings demonstrate that the enhanced DA D_1 agonist-induced oral activity response of DA lesioned rats is dependent on 5-HT fibers. Supported by NS 29505 and the Fogarty Int'l Center.

439.11

TYPICAL AND ATYPICAL NEUROLEPTICS DIFFERENTIALLY AFFECT STRIATAL DOPAMINE RELEASE AND METABOLISM STUDIED BY MICRODIALYSIS IN FREELY MOVING RATS. K. S. Rayevsky, R. R. Gainetdinov, T. V. Grekhova, T. D. Sotnikova, and A. Kharlamov*. Institute of Pharmacology, Russian Academy of Medical Sciences, Moscow 125315, Russia.

The dopamine (DA) D3 receptor is a novel D2-like receptor yet its pharmacological profile differs from that of D2. Both receptors are considered as the target for the antipsychotic effect of neuroleptics and it has been suggested that they both might be functioning as presynaptic autoreceptors responsible for the regulation of DA release and metabolism in DAergic neurons. In the present study we examined the effects of a number of typical and atypical neuroleptic drugs on the extracellular level of DA, homovanilic (HVA) and dihydroxyacetic (DOPAC) acids in the striatum of freely moving rats using a transcranial microdialysis probe and HPLC/ED technique. Classical neuroleptics such as haloperidol (0.2 mg/kg), thioproperazine (0.2 mg/kg) and spiperone (0.07 mg/kg, all injected i.p.) produced a gradual increase in the level of extracellular DA (up to 50%) and much greater elevation of the DOPAC (up to 75%) and HVA (up to 200%), while the atypical neuroleptic clozapine (20 mg/kg, i.p.), unlike haloperidol, produced a sharp increment in DA release (up to 100%) followed by delayed increase of the metabolite level. The D3 antagonist (+) AJ-76 (14 mg/kg, i.p.) resulted in a large increase of DA release with less pronounced elevation of HVA and DOPAC. In conclusion, classical and atypical neuroleptics might be clearly distinguished by their different effect upon extracellular levels of DA and related metabolites.

Supported by a grant from the Scottish Rite Foundation.

439.12

Irreversible monoaminergic receptor antagonism partially blocks the striatal neurotensin mRNA response to haloperidol in young but not senescent rats. D.J.Dobie*, M.R.Adams, K.M. Merchant, E.R. Peskind, and D.M. Dorsa. Depts. of Psychiatry and Pharmacology, Univ. of WA, Seattle WA 98195 and GRECC, VAMC, Seattle WA 98108.

To assess further the impact of the age-related decline in dopaminergic function on the induction of neurotensin/neuromedin (NT/N) mRNA by haloperidol (H), we gave the irreversible monoaminergic receptor antagonist N-ethoxycarbonyl-2-ethoxy-1,2 hydroquinolone (EEDQ) to 3 month and 24 month old Fischer 344 rats. Rats received an acute (7 hour) challenge of either H (1 mg/kg i.p.), EEDQ (10 mg/kg i.p.), or vehicle. Separate groups of rats were pretreated with EEDQ either 3 days or 7 days prior to an acute challenge with H or saline. *In situ* hybridization was performed for NT/N mRNA in the dorsolateral striatum (DLSt).

Acute administration of EEDQ or H each induced a significant NT/N mRNA response in the DLSt in young and old animals compared to controls. The magnitude of this response was greater in the H-treated than in the EEDQ-treated animals in both age groups. In young rats, pretreatment with EEDQ resulted in a significant blunting (although not total elimination) of the NT/N mRNA response to H at both 3 and 7 days. In aged rats, the NT/N mRNA response to acute H was significantly diminished when compared to the response in acutely H-treated young rats. Pretreatment with EEDQ did not affect further the blunted response to acute H in aged rats. These data suggest that, while dopamine receptors may play an important role in regulating the NT/N mRNA response to H in the striatum, receptors insensitive to EEDQ inactivation may also be involved. (Supported by NIA, NARSAD, NIH, and Dept. of Veterans Affairs).

INVERTEBRATE LEARNING AND BEHAVIOR IV

440.1

A POSSIBLE ROLE FOR PKC IN THE EXPRESSION OF ENHANCED EXCITABILITY IN LATERAL TYPE A PHOTORECEPTORS OF CONDITIONED *HERMISSENDA*. R.J. Frysztak* & T. Crow. Department of Neurobiology & Anatomy, Univ. Texas Medical School, Houston, TX 77225

Identified type A photoreceptors of *Hermissenda* express differential effects of excitability following conditioning. Lateral type A photoreceptors of conditioned animals exhibit decreased spike frequency accommodation and an increase in excitability to both the conditioned stimulus (CS; light) and extrinsic current (Frysztak & Crow, 1993). In contrast, medial type A photoreceptors do not express enhanced excitability, although they do show an increase in the amplitude of spike-elicited IPSPs following conditioning. Protein kinase C (PKC) contributes to the induction of enhanced excitability in identified type B photoreceptors produced by one-trial conditioning. The broad spectrum kinase inhibitors H-7 and sphingosine, applied before conditioning, can block the induction of enhancement in type B photoreceptors. We report here preliminary evidence for the effects of various PKC inhibitors on the expression of excitability in identified lateral type A photoreceptors of conditioned animals. Bath application of H-7 (Seikagaku; final concentration 100 μM) resulted in a decrease in the frequency of current-elicited action potentials in lateral type A photoreceptors of conditioned animals ($\bar{x}$=61.3%; n=3), as compared to pseudorandom controls ($\bar{x}$=40.2%; n=3). The intracellular application of PKC inhibitor 19-36 (Gibco BRL) into identified lateral type A photoreceptors of conditioned animals resulted in a decrease in the frequency of action potentials elicited by extrinsic current 30 minutes after cell penetration ($\bar{x}$=70.8%; n=5). Pseudorandom controls treated with PKC 19-36 did not show a decrease (n=3), nor did conditioned animals treated with the non-inhibitory PKC analog (n=2). These results indicate that the enhanced excitability expressed in the lateral type A photoreceptor following conditioning may be mediated in part by protein kinase C.

440.2

G-PROTEIN MEDIATED UP- AND DOWN-MODULATION OF POTASSIUM CURRENTS AS A FUNCTION OF INTRACELLULAR CALCIUM CONCENTRATION L.D. Matzel*, R.F. Rogers, and A.C. Talk
Department of Psychology, Rutgers University, New Brunswick, NJ 08903

Numerous receptors are linked to second messenger systems that require the interaction of regulatory G proteins. For instance, stimulation of hair cells in the vestibular system of *Hermissenda* results in a K^+ efflux and resultant hyperpolarization of ipsilateral B photoreceptors which is dependent on a receptor stimulated, pertussis toxin-insensitive G protein. Here we report that in isolated B photoreceptors from *Hermissenda*, hydrolysis-resistant stimulation of G proteins by bath application of fluoride ions (AlF_4^-; 5 mM NaF + 5 μM Al) selectively increases (≈25%) a slow outward, TEA-sensitive K^+ current (I_K), while the magnitude and steady-state inactivation of a fast, voltage-dependent K^+ current (I_A) was unaffected. If the application of AlF_4^- was accompanied by a depolarizing train, both I_A and a Ca^{2+}-dependent K^+ current ($I_{K\text{-}Ca}$) were markedly reduced (≈50-60%). The direct enhancement of I_K by AlF_4^- was unaffected by intracellular injection of the Ca^{2+} chelator EGTA (25 mM), but the pairing-specific reduction of I_A was eliminated, indicating that depolarization modified the response to AlF_4^- by increasing intracellular Ca^{2+} concentrations. Preincubation (24 hr) of the nervous system in the G_i protein inhibitor pertussis toxin blocked the reduction of currents induced by AlF_4^- paired with depolarization. These results suggest that a G protein-mediated, Ca^{2+}-dependent regulation of a second messenger system may contribute to the modulation of membrane excitability when presynaptic activity is paired with postsynaptic depolarization. This process may serve a critical role in the induction of short-term storage of associative memory in *Hermissenda*.

440.3

PERSISTENT ACTIVATION OF THE CA^{2+}-ACTIVATED PKC IN *APLYSIA* REQUIRES PROTEIN SYNTHESIS. W. S. Sossin* and J. H. Schwartz. Center for Neurobiology and Behavior, Columbia University College of Phys. and Surg., New York, NY

Aplysia neurons contain only two isoforms of protein kinase C, the Ca^{2+}-activated Apl I and the Ca^{2+}-independent Apl II. The pattern of activation of the two isoforms suggests that they play distinct roles in inducing long-term synaptic plasticity. Both isoforms are activated 30 min after a 90-min application of 20 µM 5-HT, a protocol that produces long-term presynaptic facilitation of sensory-to-motor synapses. But, only Apl I remains active 2 h after the treatment. This persistence of Apl I requires protein synthesis: it is prevented by anisomycin during the 5-HT application. Anisomycin alone has no effect on activity of Apl I nor does it block its short-term activation. In contrast , Apl II is not stimulated by a short-term 5-HT protocol, and is only transiently stimulated by the long-term protocol. It is difficult to determine whether the transient increase in Apl II is due to changes in gene expression as the inhibitor of protein synthesis alone activates Apl II also. The stimulation of Apl II by anisomycin suggests that Apl II is continually under regulation by proteins that turnover quickly.

Pharmacological agents that distinguish between physiological actions of 5-HT also have differential effects on short-term activation of Apl I. The activation of Apl I by applying 5-HT for 30-min is not blocked by 10 mM THFA, an inhibitor of adenylyl cyclase, but is blocked by 200 µM cyproheptidine (CYP), a serotonin-receptor antagonist. This provides further evidence that the stimulation of PKC by 5-HT is independent of the cAMP pathway and that CYP antagonizes the 5-HT-receptor coupled to PKC activation.

440.4

TEMPORAL ASPECTS OF ACTIVATION OF THE Ca^{2+}/CaM-SENSITIVE ADENYLYL CYCLASE: POSSIBLE CONTRIBUTION TO DETECTION OF CS-US PAIRING DURING CONDITIONING. T.W. Abrams* and J. Galun. Inst. of Neurological Sciences, Univ. of Penn., Phila., PA 19104.

During classical conditioning in *Aplysia*, Ca^{2+}/CaM-sensitive cyclase serves as a molecular site of convergence within the siphon sensory neurons for Ca^{2+} and 5HT, the cellular representations of the conditioned and unconditioned stimuli (CS and US). Using a perfused membrane cyclase assay, we previously found that a prepulse of Ca^{2+} resulted in both a faster rate of cyclase activation and greater peak activation by a brief pulse of 5HT than did a backwards pulse of Ca^{2+}. This suggested that Ca^{2+}/CaM binding to the cyclase might enhance the rate of activation by receptor and G_S. This interaction appeared to be specific for G_S because there was no similar interaction between a Ca^{2+} pulse and a pulse of forskolin. To more directly test the hypothesis that Ca^{2+} modulation of G_S interactions with cyclase underlies the pairing requirements of conditioning, we have begun to study interactions between recombinant type I cyclase and preactivated $G_S\alpha$. Preliminary experiments in which a Ca^{2+} pulse was paired with a brief pulse of preactivated $G_S\alpha$ suggested that pairing results in more powerful cyclase stimulation than the sum of the separate stimulations. We further observed that the CaM-mediated stimulation of cyclase by Ca^{2+} has a relatively delayed and prolonged time course; this delay may contribute to the requirement that the CS (and Ca^{2+} influx) begin before the US (and 5HT binding). In contrast, the direct, inhibitory effect of Ca^{2+} occurs rapidly and is relatively brief.

440.5

MOLECULAR CHARACTERIZATION OF *DROSOPHILA* CALCIUM/CALMODULIN-DEPENDENT PROTEIN KINASE II: A REQUIRED ELEMENT IN BEHAVIORAL PLASTICITY L.C. Griffith* and B. GuptaRoy Dept. of Biology and Center for Complex Systems, Brandeis University, Waltham, MA 02254

Inhibition of calcium/calmodulin-dependent protein kinase II (CaM kinase) activity in transgenic *Drosophila* carrying a gene for a specific peptide inhibitor produces defects in both associative and nonassociative plastic behaviors [Griffith, et al. (1993) *Neuron* **10**:501-9]. To understand the molecular basis of this phenomenon, we are have cloned and characterized several CaM kinase isoforms. Unlike mammals, which have several highly related genes for CaM kinases, *Drosophila* uses a single gene to produce many CaM kinase isoforms. These isoforms differ in primary amino acid sequence only at a point immediately distal to the regulatory domain of the kinase. CaM kinase isoforms are expressed throughout development in *Drosophila* and in the adult nervous system, gut and ovaries. The functional significance of isoform variability is being assessed in terms of catalytic properties and tissue distribution of the different forms. Expression of full length cDNAs in COS cells produces immunoreactive protein for every isoform tested. Each isoform also has calcium/calmodulin-dependent activity when assayed with a specific peptide substrate under saturating activator conditions. At subsaturating conditions, however, amino acid insertions at the variable region change the affinity of the enzyme for calmodulin and may have effects on autophosphorylation and regulation of enzyme activity.

440.6

STOMACH DISTENTION INHIBITS BOTH FEEDING BEHAVIOR AND THE SWALLOWING MOTOR PROGRAM IN THE NUDIBRANCH, *MELIBE LEONINA*. W. H. Watson III* and M. J. Iannucci, Zoology Dept., Univ. of New Hampshire, Durham, NH 03824 & Friday Harbor Labs, Friday Harbor, WA 98250

Melibe respond to prey by increasing their feeding activity from 0.2 hood closures/min to 1-3 closures/min. After feeding for several hours prey capture rate returns to resting levels. This decrease is closely associated with expansion of the stomach and it is likely that stomach distention inhibits feeding behavior.

We tested this hypothesis by: (1) injecting inert material into the stomachs of hungry animals to induce satiation and; (2) cutting the posterior nerves which lead from the stomach to the buccal ganglia and brain. Animals with artificially distended stomachs responded like fed animals, and those with posterior nerve lesions failed to become satiated after feeding for 24 hours. These data indicate that stomach distention activates receptors which feedback to the CNS and depress the neural circuits underlying feeding and possibly swallowing behavior.

This hypothesis was tested using several semi-intact preparations. First, during stomach distention, we recorded a large increase in the firing rate of several units in the posterior nerve. Second, we discovered that stomach inflation suppressed both spontaneous and stimulus-induced expression of the swallowing motor program by the buccal ganglia. Suppression was accompanied by: (1) inhibition of constriction motorneurons and; (2) excitation of dilation neurons and the large SCPb-containing neuron B1. Distention was mimicked, somewhat, by application of either SCPb or serotonin. The influence of stomach distension on the feeding motor program in the brain is presently being investigated and our long-term goal is to identify the site(s) where the feeding system is being modulated, and then determine the neural mechanisms that are responsible.

We would like to thank the staff of the Friday Harbor Laboratories for their help. This work was supported by a NIH grant (NS29555) to WHW.

440.7

ASSOCIATIVE LEARNING IN *C. ELEGANS*: CONTEXT CONDITIONING OF HABITUATION. C.H. Rankin*, Department of Psychology, University of British Columbia, Vancouver, B.C. V6T 1Z4

Many studies of associative learning have shown the importance of context in the retention of the learned association. Context is often composed of the environmental stimuli that were present when the original association was made. In this experiment the context in which worms were given habituation training was varied by the presence or absence of a chemical ion during training. The protocol involved habituating worms to tap in the presence or absence of the ion, and then, following a 1 hour recovery period, retesting them in either the presence or absence of the ion. Thus worms were rehabituated in either the same context as the original training, or a different context (counterbalanced: half of the worms received original training in the presence of the ion, half in the absence of the ion). Using this protocol and habituating at either a 10 s ISI or a 60 s ISI, worms appeared to retain habituation training to a greater extent if the context of rehabituation was the same as the context of the original habituation than if the context changed between original training and rehabituation. Worms rehabituated significantly more rapidly in the same context than in the different context (60 s ISI slopes: $t(38)=2.01$, $p<.05$). In addition, worms had significantly smaller response magnitudes on the first response of rehabituation in the same context than in a different context (60 s ISI response magnitude: $t(68)=2.01$, $p=.05$).

440.8

INDEPENDENT MEMORIES IN *DROSOPHILA* AFTER PAVLOVIAN CONDITIONING. T. Tully*[1,2], M. DelVecchio[1], S. Boynton[2] and T. Preat[2,3]. [1]Center for Learning & Memory, Cold Spring Harbor Laboratory, Cold Spring Harbor NY 11724 U.S.A., [2]Department of Biology, Brandeis University, Waltham MA 02254 U.S.A., [3]Theodor-Boveri-Institut (Biozentrum), Lehrstuhl für Genetik, Am Hubland, D-8700 Würzburg FEDERAL REPUBLIC OF GERMANY

With extended training using a Pavlovian odor avoidance procedure, we have produced long-lasting memory retention in wild-type *Drosophila*. Massed training produces moderate 24-hr retention, which then decays to zero within four days; spaced training produces strong 24-hr retention, which still is 30% of initial learning levels 7 days later. When flies are fed the protein synthesis inhibitor cycloheximide, 24-hr retention after massed training is normal -- indicating that such memory is protein synthesis independent. Cycloheximide affects memory after spaced training, however, producing 24-hr retention scores similar to those after massed training (with or without drug). These results indicate that spaced training induces a protein synthesis-dependent form of LTM above and beyond the protein synthesis-independent form.

Interestingly, 24-hr retention after massed training is zero in *radish* flies, suggesting that the protein synthesis-independent form of memory is blocked by this single-gene mutation. Nevertheless, spaced training of *radish* flies produces memory lasting several days, suggesting that the protein synthesis-dependent form of memory is intact in these mutants.

Taken together, these behavior-genetic experiments reveal the existence of (at least) two phases of memory after extended Pavlovian conditioning. Massed training induces a shorter-lived form, the expression of which is not dependent on protein synthesis. Spaced training induces the protein synthesis independent memory along with a longer-lived, protein synthesis dependent form -- which most likely represents a bona fide long-term memory (LTM). Finally, since LTM can form in *radish* mutants in the absence of the protein synthesis-independent phase of memory, these two types of memory must be genetically distinct and must function independently.

440.9

INTERACTIONS BETWEEN NEURONS IN THE ABDOMINAL GANGLION OF APLYSIA. Y.Tsau, J-Y.Wu, L.B.Cohen*, D.Schiminovich, H-P.Hopp, and C.X.Falk, Department of Physiology, Yale University School of Medicine, New Haven, CT 06510

It is important to record as many neurons as possible and know how these neurons interact in order to understand the mechanism by which a nervous system works. This is especially true when many neurons are thought to be involved in generating a behavior. We employed optical recording to simultaneously monitor the spike activity of about half of the neurons in the abdominal ganglion of Aplysia, and cross-correlation analysis to investigate the interactions between the neurons. We used two stimuli, current steps from an intracellular microelectrode in a cell body (probably L10) or a light touch to the siphon skin. The data were stored and analyzed on a Motorola VME bus computer and a Silicon Graphics workstation. One cross-correlation, the normalized cross-covariance function (NCCVF) was calculated to estimate the interactions between neurons. Approximately 100-200 out of $2\text{-}10\times10^3$ pairs had larger NCCVF values. Most of these had a wide peak half-width (about 1000 msec), implying that most of the synaptic interactions between these neurons are slow. Because there were many simultaneously active neurons it was difficult to determine the synaptic interactions responsible for these slow time-scale correlations. A few pairs (about 20) were found with a short time delay (<100ms) and narrow peak half-widths (<50ms), implying that only a few synaptic interactions are large and fast. It was easier to interpret this kind of cross-correlation in term of synaptic interactions. The interaction pattern changed from animal to animal, implying that the same behavior might be executed by different neuronal circuitry. Supported by NIH grant NS08437.

440.10

NEURONAL ACTIVITY DURING THE APLYSIA GILL WITHDRAWAL REFLEX AND SPONTANEOUS GILL CONTRACTIONS SUGGESTS A DISTRIBUTED NEURONAL ORGANIZATION. Jian-young Wu*, Lawrence B. Cohen, and Chun Xiao Falk, Dept. of Physiol., Yale Univ. Sch. of Med., New Haven, CT 06510

In a distributed neuronal organization behaviors are generated by different configurations of a large integrated neuronal network rather than dedicated circuits. Neuronal activity in the Aplysia abdominal ganglion suggests a distributed neuronal organization. A large fraction of neurons in the abdominal ganglion was optically monitored during three forms of spontaneous or evoked behavioral events (respiratory pumping, the gill withdrawal reflex, and a small spontaneous gill contraction). About 300 of neurons were activated during all three of the behaviors. More than 90 per cent of the neurons activated during the gill withdrawal reflex were also being activated during both small and large spontaneous gill contractions. While the active neuronal populations and the total numbers of spikes during three behaviors were similar, the activity patterns were substantially different during the three events. When an evoked gill withdrawal was elicited a few seconds after a large spontaneous gill contraction, substantial changes in the evoked neuronal activities were observed. These results imply that a distributed neuronal network is reconfigured to generate these different forms of gill contractions. It was known that the gill withdrawal reflex and the respiratory pumping share many motor neurons and interneurons. Our data suggest that a much larger integrated circuit, containing a large fraction of the neurons in the ganglion, is shared by those forms of gill withdrawal behaviors. *Supported by NIH grant NS08437.*

440.11

RECORDING OF MEMBRANE POTENTIAL CHANGES FROM NEURONAL PROCESSES BY FLUORESCENCE MEASUREMENTS. S. Antic and D. Zecevic*, Univ. of Belgrade Sch. of Biology, Belgrade, Yugoslavia

We are investigating optical signals from neurons injected with voltage-sensitive dyes: our aim is to analyze signal integration in neuronal processes. Experiments were done on ventral cerebral giant cells of Helix pomatia that project large axons into the commissures and peripheral nerves. The anatomy of giant cells is particularly suitable for epi-fluorescence measurements. Multi-site optical recording was done using 124 or 464 element rectangular photodiode array supported by Motorola VME bus computer. Best results were obtained with positively charged styryl dyes. Using dye designated JPW1114 we found that signals from soma and distant processes can clearly be distinguished. Signal-to-noise ratio in recording did not require extensive averaging. The temporal resolution was sufficient to enable us to follow the spread of the signal along the axon and determine the site of spike initiation. To further improve signal-to-noise ratio we are currently investigating the effect of size of the neuron, and the effect of the amount of time allowed for the dye to spread into the processes on signal size. We are also testing newly synthesized close analogues of dye JPW1114 with different degrees of hydrophobicity. Supported by NIH grant NS28443.

PEPTIDES: POSTTRANSLATIONAL PROCESSING

441.1

HYPOTHALAMIC TRH STIMULATES NEUROPEPTIDE Y SYNTHESIS IN THE ANTERIOR PITUITARY GLAND. M. Michalkiewicz*. Department of Physiology, West Virginia Univ. Morgantown, WV 26506.

We have shown that neuropeptide Y (NPY) expression in the anterior pituitary gland (AP) is increased in hypothyroid rats and that this effect is mediated by some hypothalamic factor. In the present study we examined the hypothesis that stimulating effect of low thyroid hormone on NPY synthesis in the AP is mediated by the hypothalamic TRH. Male Sprague Dawley rats (7-9 per group) were anesthetized and one of the surgery was performed: 1) Sham thyroidectomy (STx); 2) Thyroidectomy (Tx) + radiofrequency lesion of the paraventricular nucleus (PVNx); 3) Tx+Sham PVNx; 4) Tx+PVNx+TRH(100 μg/day, continuous infusion using osmotic pumps); 5) Tx+PVNx+Placebo; 6) Tx + anterolateral hypothalamic deafferentation (ALHx); 7) Tx+ALHx+TRH; 8) Tx+ALHx+Placebo. 12 days after surgery animals were killed and tissue samples collected for measurement of the AP NPY, plasma TSH, T_3, and T_4 (by RIA). In addition, NPY was measured in blood, superior cervical ganglion, adrenal gland, and prefrontal cerebral cortex. Identity of radioimmunoassayable NPY extracted from the AP and other organs was confirmed by HPLC. Hypothyroidism was verified by low plasma T_3 and T_4 and high TSH. Only animals with complete hypothalamic lesions (confirmed histologically) were included for analysis. In the hypothyroid rats the AP contents of NPY were significantly increased. These changes were completely prevented by PVNx or by ALHx. Treatment with TRH significantly increased AP contents of NPY in the PVNx and ALHx rats. Effect of TRH was specific to the AP because no changes in NPY levels in other organs were observed. These data suggest that the hypothalamic TRH of the PVN origin stimulates the synthesis of NPY in the AP gland. Supp.: WVU BRS #2S07RR05433-31.

441.2

MICRODIALYSIS SAMPLING FROM THE PARAVENTRICULAR NUCLEUS OF THE HYPOTHALAMUS REVEALS INCREASED NEUROPEPTIDE Y IN RESPONSE TO DIABETES AND FOOD DEPRIVATION. P. D. Lambert, J .P. H. Wilding, S. G. Gilbey, M. A. Ghatei and S. R. Bloom. (SPON: Brain Research Association) Department of Endocrinology, Royal Postgraduate Medical School, Hammersmith Hospital. London.W12 0NN.

Microdialysis is widely used for monitoring changes in the release of various substances in areas of the brain. However, consistent recovery of some neuropeptides has been impossible due to their inherent physical properties. We have developed a microdialysis method to sample neuropeptide Y (NPY) and determined the effect of diabetes and food deprivation on NPY release. Microdialysis probes (CMA/12, 2mm) were stereotactically implanted into the paraventricular nucleus of the hypothalamus (PVN) of anaesthetized male Wistar rats and perfused (3μl min^{-1}) with phosphate buffer containing a polyclonal antibody to NPY (YN10, 1:2000). The experiment was carried out in satiated and fasted animals. After one hour, six 30 minute samples were collected and NPY measured using radioimmunoassay. We also dialysed from the PVN of freely-moving normoglycaemic and streptozotocin-diabetic rats. The concentration of NPY sampled from anaesthetized rats was $<$ 1fmol/50μl and increased to 2.4$\pm$0.3fmol/50μl ($p<0.01$) after a 48hr fast. In freely-moving rats, NPY release was significantly greater in streptozotocin-diabetic rats (15$\pm$2fmol/50μl, n=5) compared to controls (8$\pm$1fmol/50μl, n=5, $p<0.01$). In conclusion, NPY release is reduced by anaesthesia and diabetes leads to an increase in NPY release which may induce hyperphagia.

441.3

DIRECT ROLE OF *FURIN* IN MEDIATING ENDOPROTEOLYTIC CLEAVAGE OF PROSOMATOSTATIN (PSS) IN COS-7 CELLS. A. Galanopoulou and Y.C. Patel*. Fraser Labs, McGill University, Montreal.

We have previously reported that mammalian PSS significantly cleaves at monobasic Arg^{64} and Lys^{13} sites to yield respectively SS-28 and $PSS_{[1-10]}$ in COS-7 cells (JBC 268:6041, 1993). In addition, SS-14 is produced in low quantities via cleavage at a third Arg-Glu-Arg-Lys^{78} site. We suggested that *furin*, the only known converting enzyme endogenously expressed in COS-7 cells, may be responsible for these cleavages. Here we have directly assessed the role of *furin* in PSS processing. COS-7 cells were infected either with wild type vaccinia virus (VV:wt) at different concentrations or VV:*furin* and subsequently transfected with the vector p601:rPSS expressing rat prePSS cDNA under a VV promoter. Cell extracts and media were analysed by HPLC and region-specific RIAs.

	VV:wt	VV:Furin (0.8 pfu/cell)	VV:Furin (2.4 pfu/cell)	VV:*Furin* (12 pfu/cell)
SS-14 (% SS-14 LI):	10	13	10	24
SS-28 (%):	27	38	38	51
unprocessed (%)	63	49	52	25

Furin induced dose-dependent conversion of PSS to SS-28. It also cleaved SS-14 weakly, but had no additional effect on $PSS_{[1-10]}$ conversion, beyond that observed with control vaccinia infections. CONCLUSIONS: (1) *Furin* is capable of monobasic cleavage and qualifies as a SS-28 converting enzyme. (2) Another endoprotease, not *furin*, appears responsible for $PSS_{[1-10]}$ conversion in the constitutive pathway. (3) The low affinity cleavage to SS-14 at the R-X-R-K site, and the inability to cleave at monoLys^{13} site compared to monoArg^{64} site suggests preference for Arg versus Lys at P-1 site in *furin* mediated cleavage.

441.4

IN VIVO REGULATION OF PEPTIDE α-AMIDATING ACTIVITY IN THE HEART: EVIDENCE FOR TWO SEPARATE MECHANISMS. M. Altarac, D.B. Newman* and G.P. Mueller. Departments of Physiology and Anatomy, Uniformed Services University of the Health Sciences, Bethesda, MD 20814

Peptidylglycine hydroxylating monooxygenase (PHM) catalyzes the rate limiting step in the conversion of glycine-extended peptides to active α-amidated products. PHM is concentrated in cardiac atrium where its expression *in vitro* is induced by glucocorticoids. Recently we have determined that *in vivo* treatment with disulfiram (DIS) increases the V_{max} of PHM (assayed *in vitro*) without altering its biosynthesis. The present investigation sought to determine if these separate mechanisms interact in the control of PHM *in vivo*. Male rats were treated daily with the glucocorticoid, dexamethasone (DEX), DIS, or the 2 agents in combination for seven days; samples were collected and assayed for PHM activity under optimal conditions *in vitro*. Both DEX and DIS treatments increased soluble PHM activity in the atrium by approximately 2-fold; their combined effects were greater (3-fold) than either alone. This pattern of response was tissue specific; PHM activity in the anterior and neurointermediate pituitary was increased by DIS, but not affected by DEX, and activity in blood and brain (hypothalamus & cortex) was unaltered by the treatments. These findings indicate that the expression and activity of PHM in atrium may be regulated by two separate mechanisms *in vivo*; synthesis of new PHM protein (DEX) and modification of PHM protein to increase its V_{max} (DIS).

441.5

IN VIVO INHIBITION OF PEPTIDE α-AMIDATING ACTIVITY BY 4-PHENYL-3-BUTENOIC ACID (4P3B). G.P. Mueller*, and M. Altarac. Department of Physiology, Uniformed Services University of the Health Sciences, Bethesda, MD 20814,

Peptidylglycine α-amidating monooxygenase (PAM) catalyzes the sequential hydroxylation and lyase steps in the bioactivation of α-amidated peptides. Peptidylglycine hydroxylating monooxygenase (PHM) is rate limiting, whereas the peptidylhydroxyglycine α-amidating lyase (PAL) step occurs more rapidly. 4P3B has been shown to inhibit PHM in vitro irreversibly. This study evaluated 4P3B as a tool for investigating α-amidation in vivo. 4P3B caused dose and time related reductions in PHM activity in serum, and extracts of atrium, anterior and neurointermediate pituitary and brain of rats. Levels of serum PHM activity were reduced to less than 5% of control values 3h after sc injection of 500 mg/kg 4P3B; levels were restored to normal by 24h. Similar, though less dramatic responses were observed for PHM activity present in tissue extracts. Interestingly, the activity of PAL was unaffected by 4P3B. The effects of 4P3B were not cumulative; animals receiving daily injections of 500 mg/kg for up to 10 days had nearly normal levels of serum and tissue PHM activity 24 h after the last treatment. Inhibition of extracted PHM by 4P3B was not reversed by dialysis or solvent extraction. Thus, inhibition of PHM by 4P3B in vivo may be overcome by either synthesis and secretion of new PHM protein or via a mechanism for PHM regeneration that is not evident in vitro.

441.6

IN VITRO PROCESSING OF POMC BY PURIFIED RECOMBINANT PC1. T.C. Friedman, K.T. Kalogeras*, Y.P.Loh and N.P. Birch. LDN, NICHD and CNE, NIMH, NIH, Bethesda, MD 20892 and School of Biological Sciences, Univ. of Auckland, Private Bag 92019, New Zealand.

The prohormone convertases, PC1 (also called PC3) and PC2, are subtilisin-like serine proteases capable of processing neuropeptide precursors. In cotransfection experiments, other investigators have found that PC1 and PC2 can process pro-opiomelanocortin (POMC) to peptide products found in the pituitary. In this study, recombinant rat PC1 was stably expressed in a mouse L cell line and partially purified. Mouse POMC was cleaved by recombinant PC1 to generate N-POMC-ACTH intermediates, ACTH, 16 kDa N-POMC and β-LPH. Purified β-LPH was cleaved to a very small extent to β-endorphin, while purified bovine N-POMC$^{1-77}$ was not processed to γ^3-MSH. The pH optima for these cleavages were 6.0. Media from L-cells not transfected with PC1 did not process POMC. We conclude that purified recombinant PC1 is capable of processing POMC *in vitro* at paired basic residues to peptides similar to those found in the anterior pituitary. The specificity of PC1 is consistent with a role for this enzyme in the biosynthesis of biologically active peptides from POMC.

441.7

UNIQUE SUBSTRATE SPECIFICITY OF 'PROHORMONE THIOL PROTEASE' RELATED TO PROENKEPHALIN PROCESSING. A.V. Azaryan* and V.Y.H. Hook. Dept. of Biochemistry, Uniformed Services University of the Health Sciences, Bethesda, MD. 20814.

'Prohormone thiol protease' (PTP) that cleaves at dibasic and monobasic sites has been found to be the major enkephalin precursor processing enzyme in bovine adrenal medullary chromaffin granules. In this study, further characterization of PTP's cleavage site specificity has been assessed with a series of peptide-MCA substrates containing paired and single basic residues: Z-Arg-Arg-MCA, Boc-Gln-Arg-Arg-MCA, Boc-Gly-Arg-Arg-MCA, Z-Arg-Val-Arg-Arg-MCA, Boc-Gly-Lys-Arg-MCA, Boc-Glu-Lys-Lys-MCA, Z-Phe-Arg-MCA, Bz-Arg-MCA, Boc-Gln-Gly-Arg-MCA, Bz-Val-Leu-Lys-MCA, and Ac-Lys-MCA. After incubating these substrates with PTP, measurement of the generated AMC fluorescent leaving group with and without aminopeptidase M treatment provided information concerning PTP cleavage at the COOH- or NH_2-terminal side of basic residues. PTP demonstrated: (a) cleavage at dibasic- and monobasic sites; (b) preference for cleaving paired basic residues on the NH_2-terminal side of the pair and between the dibasic residues, compared to the COOH-terminal side; (c) cleavage at both the COOH- and NH_2-terminal sides of a single basic residue with high affinity for Gly-Arg bond. These results are consistent with PTP's cleavage pattern with proenkephalin-derived intermediates -- peptides E and F, and BAM-12P. PTP's unique cleavage specificity, compared to other prohormone convertases, provides further support for this protease as a novel prohormone processing enzyme.

441.8

α_1-ANTICHYMOTRYSIN-LIKE PROTEASE INHIBITOR REGULATION OF A PROENKEPHALIN PROCESSING ENZYME. V.Y.H. Hook*, A.V. Azaryan, and T. Purviance. Dept. of Biochemistry, Uniformed Services University of the Health Sciences, Bethesda, MD.

Evidence is presented showing that α_1-antichymotrypsin (ACT)-like protein inhibits and is colocalized with the novel 'prohormone thiol protease' (PTP) involved in processing the enkephalin precursor. These results are the first demonstration that neuropeptide precursor processing may be regulated by an endogenous protease inhibitor. ACT immunoreactivity was colocalized with PTP within secretory vesicles of bovine adrenal medulla and posterior pituitary. The purified 60 kDa bovine pituitary ACT-like protein was a potent inhibitor of both PTP and chymotrypsin in the nanomolar range. Typical of serpin protease inhibitors, the bovine pituitary ACT-like protein formed SDS-stable complexes with chymotrypsin, and PTP formed SDS-stable complexes with human liver ACT. PTP cleavage of enkephalin-containing peptides at the NH_2-terminal side of paired basic residues (Lys-Arg, Arg-Arg, Lys-Lys) that flank the COOH-terminus of (Met)enkephalin (Tyr-Gly-Gly-Phe-Met), indicates methionine at the P_1 position of the cleavage site. These results showing PTP processing at a Met residue resemble the allowable P_1 specificity of ACT and are compatible with inhibition of PTP by ACT. The estimated molar ratio of PTP/ACT-like protein of 2-3 within secretory vesicles of adrenal medulla suggests that PTP in vivo may be partially inhibited; this is consistent with the presence in vivo of a high level of incompletely processed proenkephalin.

441.9

SECOND MESSENGER EFFECTS ON CCK, PC1, AND PC2 mRNA IN THE HUMAN NEUROBLASTOMA CELL LINE SK-N-MCIXC. **B. L. Mania-Farnell*, B. J. Merrill, R. Day, N.G. Seidah, and T. P. Davis. Department of Pharmacology, College of Medicine, University of Arizona, Tucson, AZ 85724.**

Regulation of cholecystokinin (CCK), pro-hormone convertase 1 (PC1) and pro-hormone convertase 2 (PC2) mRNA expression was studied in SK-N-MCIXC cells. These cells express the human CCK gene at high levels and perform postranslational processing of CCK (Verbeeck and Burbach, FEBS 268, 1990). SK-N-MCIXC cells also express mRNA for PC1 and PC2 (Konings et. al.., Neuropeptides, 1993), which are neuroendocrine precursor processing enzymes. To examine the effect of the cyclic AMP (cAMP) second messenger pathway on mRNA levels, SK-N-MCIXC cells were treated with the phosphodiesterase inhibitor isobutyl-methylxanthine (IBMX, 0.5mM). Phorbol-12-myristate-13 acetate (PMA, 0.5 μM) was used to determine if the protein kinase C (PKC) pathway regulated mRNA levels. Messenger RNA levels were quantitated using Northern blot analysis in combination with cRNA hybridization probes, human CCK, human PC1 and rat PC2. After 12 hour treatments CCK mRNA levels were raised 1.8 -fold (IBMX), 2.5 fold (PMA) and 2.7 -fold (PMA in combination with IBMX). Similar results were seen at 6 hours. PC1 mRNA levels were raised 1.3 -fold (IBMX), 1.5 -fold (PMA) and 1.7 -fold (PMA in combination with IBMX) with 3 hour treatments. Similar results were seen at 6 hours. Six hour treatments with second messengers did not affect PC2 mRNA levels. Our results show that the cAMP and PKC pathways regulate CCK and PC1 mRNA levels in SK-N-MCIXC cells and raise the question that PC1 may play a role in processing CCK. Supported by N.I.H. Grant DK 36289 and MH42600 and MRC Grants MT11268 and PG2.

441.10

EFFECT OF DEVELOPMENT ON CARBOXYPEPTIDASE H AND LEVELS OF CHOLECYSTOKININ IN THE RAT HYPOTHALAMUS AND CORTEX. M. G. Oakes*, M. C. Beinfeld and T. P. Davis. Dept. of Pharmacology, University of Arizona College of Medicine, Tucson, AZ 85724.

Bioactive cholecystokinin (CCK-8) is produced by sequential cleavage of Pro-CCK by a series of peptidases. Specific enzyme assays were run to measure the activity of the processing enzyme, Carboxypeptidase H (EC 3.4. 17.10, CPH) responsible for the cleavage of the glycine extended form of CCK-8 prior to amidation, and two metabolic enzymes: Neutral Endopeptidase (EC 3.4.24.11, NEP) and Metallo Endopeptidase (EC 3.4.24.15, MEP). In addition, four immunoreactive forms of CCK present at birth and post-natal ages 4, 7, 30, and 90 days were measured. In hypothalamus, Pro-CCK-like forms remain constant, whereas CCK-33-like forms predominate at birth and increase from day 7 to 3 fold higher at adult age. The glycine extended forms and CCK-8 show a significant increase at ages 30 (2-fold) and 90 days (10 fold). Total CPH activity increased two fold from birth to adult reflecting the increase of bioactive CCK-8. NEP activity increased two-fold from birth to day 7 and remained constant to adult, whereas MEP activity decreased from day 30 to 90. In cortex, CCK-33-like forms predominate at birth and Pro-CCK forms remain constant. Interestingly, CCK-33 decreased throughout the life of the rat suggesting a more rapid conversion of Pro-CCK forms to bioactive forms than that found in the hypothalamus. CCK-8 increased significantly over birth levels on days 7, 30 and 90 whereas glycine-extended forms showed no effect. The constant level of Pro-CCK-like peptides in the hypothalamus and cortex as compared to significant shifts in the levels of CCK-33 and CCK-8 suggests that Pro-CCK may not be fully regulated by transcription alone but could be affected by peptidases. Supported by N.I.H. Grant HD26013 and MH42600.

441.11

PEPTIDASES AT THE BLOOD BRAIN BARRIER: EFFECT ON PERMEABILITY. E.A. Brownson*, T.J.Abbruscato, T.P.Davis. Dept. Pharmacology, University of Arizona College of Medicine, Tucson, AZ.

The present study characterized the presence of specific membrane associated peptidases at the blood brain barrier (BBB). An *in vitro* model of the BBB using bovine microvessel endothelial cells (BMEC) was employed to measure the effect of aminopeptidase and enkephalinase activity on the permeability of two opiate compounds, [Met^5]-Enkephalin and an enzymatically protected analogue of Met-Enk, DPDPE. The measured enzyme activity of BMEC grown to confluency was 1215, 447 and 224 pmol/mg protein/min for total aminopeptidase, aminopeptidase M (3.4.11.2) and enkephalinase (3.4.24.11), respectively. Permeability coefficients (PC) were calculated based on the diffusion of Met-Enk and DPDPE across the BMEC with and without peptidase inhibitors. We have found that in the presence of specific inhibitors of aminopeptidase (bestatin, puromycin) or enkephalinase (phosphoramidon, thiorphan) the PC of Met-Enk was increased three-fold ($6x10^{-4}$ cm/min) compared to Met-Enk alone ($2x10^{-4}$ cm/min). However there was no difference in the PC of DPDPE alone ($10x10^{-4}$ cm/min) compared to DPDPE with inhibitors ($9x10^{-4}$ cm/min). The data from this *in vitro* model of the BBB provide strong evidence for the presence of peptidases at the BBB that prevent blood to brain passage of peptides. Further, the potential for the delivery of drugs across the BBB can be enhanced by the development of enzymatically protected and biologically active peptide analogues. Supported by USPHS grants DA06284 and MH42600.

441.12

EVIDENCE FOR A TWO-STEP MECHANISM OF GnRH METABOLISM BY PROLYL ENDOPEPTIDASE AND METALLOENDOPEPTIDASE EC 3.4.24.15. R. A. Lew,[1] T. Tetaz,[1] M. J. Glucksman,[2] K.E. Sheppard,[1]* J.L. Roberts,[2] and A.I. Smith.[1] [1]Baker Med. Res. Inst., Prahran, Victoria, Australia 3181; [2]Fishberg Res. Ctr. Neurobiol., Mount Sinai Sch. Med., NY, NY, USA 10029.

The soluble metalloendopeptidase EC 3.4.24.15 has been implicated in both the central and peripheral clearance of gonadotropin-releasing hormone (GnRH), and in crude preparations cleaves GnRH at the Tyr^5-Gly^6 bond. Although 24.15 activity is high in rat brain, to date the enzyme has only been sequenced and cloned from the testes (Pierotti et al., *Biochemistry* 29: 10323, 1990). In the present study, we determined the substrate specificity of both brain and recombinant testicular 24.15 for several GnRH-related peptides. When crude recombinant 24.15 (5 µg protein) was incubated with GnRH (25 µM) for 1 hr, only 10% of the peptide was degraded to $GnRH_{1-5}$ as determined by HPLC, compared with 83% of GnRH free acid and 97% of des-[Gly-NH_2]-GnRH, suggesting that C-terminal amidation hinders enzyme-substrate interaction. Interestingly, a very similar enzyme (EC 3.4.24.19) will not cleave GnRH unless the C-terminal Gly-NH_2 residue is removed by a prolyl endopeptidase (Camargo et al., *J. Biol. Chem.* 257:9265, 1982). To test the hypothesis that crude brain extracts contain an activity which cleaves between the Pro^9 and Gly^{10}-NH_2 residues, thus generating a much more favorable substrate for 24.15, we examined the effects of a specific inhibitor of 24.15, CPP-AAY-PAB, and an inhibitor of prolyl endopeptidase, bacitracin, on degradation of GnRH by soluble extracts of sheep hypothalamus. In the absence of inhibitors, most GnRH was degraded to $GnRH_{1-5}$, with some production of $GnRH_{1-9}$. Addition of CPP-AAY-PAB (5 µM) reduced $GnRH_{1-5}$ production, with a concomitant increase in $GnRH_{1-9}$; in contrast, bacitracin (0.1 mg/ml) reduced both $GnRH_{1-5}$ and $GnRH_{1-9}$ generation. These results suggest that the degradation of GnRH may occur via a two-step process involving first removal of Gly-NH_2 by prolyl endopeptidase, followed by cleavage by 24.15 at the Tyr^5-Gly^6 bond.

NEUROENDOCRINE REGULATION: GENE EXPRESSION AND CO-LOCALIZATION

442.1

COLOCALIZATION OF MULTIPLE NEUROPEPTIDES (ENKEPHALIN, NEUROTENSIN AND DYNORPHIN) AND THEIR MESSAGES WITHIN TUBEROINFUNDIBULAR (TIDA) NEURONS OF LACTATING RATS. I. Merchenthaler*, D.E. Lennard and D.M. Bronstein. Functional Morphology Section, LMIN (I.M. and D.E.L.) and Laboratory of Integrated Biology (D.M.B.), NIEHS/NIH, Research Triangle Park, NC 27709.

TIDA neurons in the dorsomedial subdivision of the arcuate nucleus (dmAN) project to the median eminence (ME) where dopamine (DA) is released into the hypophysial portal circulation. Prolactin secretion from the pituitary is under the tonic inhibitory control of DA produced by TIDA neurons. We report here that, in contrast to cycling female or male rats, TIDA neurons of lactating rats coexpress enkephalin, dynorphin and neurotensin. mRNA of enkephalin and dynorphin were demonstrated with in situ hybridization histochemistry using ^{35}S- or digoxigenin-labeled cRNA probes or ^{35}S-labeled deoxynucleotide probes. The peptides were detected with single- or double-labeling immunocytochemistry (ICC). When retrograde labeling from the ME with Fluoro-Gold was combined with double-labeling ICC, we found that every DA neuron (demonstrated by the presence of tyrosin hydroxylase) coexpressing these peptides was connected to the portal circulation. These findings indicate that the multihormonal TIDA neurons in the dmAN may possess a hypophysiotropic function. Since enkephalin, dynorphin and neurotensin exhibit prolactin-releasing activity, these results suggest that in lactating rats a cocktail of these peptides released by TIDA neurons may be responsible for the elevated prolactin and consequent milk secretion. The activity of TIDA neurons in synthesizing and releasing DA is inhibited by the suckling stimulus. Therefore, the action of the peptides cosynthesized with DA during lactation may be particularly important during the non-suckling periods of lactation.

442.2

COLOCALIZATION OF ANDROGEN RECEPTOR AND THYROTROPIN RELEASING HORMONE PROHORMONE IN RAT BRAIN. H.Xu, E.M. Wilson, R.A. King*, and M. Sar. Dept. of Cell Biol. & Anat., Labs for Reprod. Biol., Univ of N.C., Chapel Hill, NC 27599.

Evidence from our laboratory and others has indicated a direct action of androgen on thyrotropes in rat pituitary while androgen action on TRH neurons is not known. This study was conducted to determine whether thyrotropin releasing hormone prohormone (pro-TRH) neurons contain androgen receptors (AR). Adult male rats treated with testosterone propionate sc also received colchicine. Twenty hr later the rats were perfused with Zamboni's fixative. Brains were frozen and 10µm serial sections were processed for dual immunostaining. Sections were immunostained with antipeptide antibody AR32 using DAB and with anti pro-TRH antibody using 4-chloro-1-naphthol. AR was localized in nuclei of neurons in several nuclear groups of the brain where immunoreactive TRH or pro-TRH cells exist. These include the preoptic region, bed nucleus of the stria terminalis, periventricular nucleus, parvocellular portion of the paraventricular nucleus, dorsomedial nucleus and basolateral hypothalamus. Nuclear localization of AR was observed in a subpopulation of immunoreactive pro-TRH cells in preoptic nucleus, bed nucleus of the stria terminalis, periventricular hypothalamic nucleus and basolateral hypothalamus. The results suggest that androgen may have direct effects on certain TRH neurons. (Supported by NIH Grant NS17479).

442.3

ESTROGEN RECEPTOR IMMUNOSTAINING IN THE PREOPTIC AREA AND HYPOTHALAMUS OF FEMALE GUINEA PIGS AFTER ESTROGEN AND PRAZOSIN TREATMENT. K.F. Malik*, H.H. Feder, and J.I. Morrell, Inst. of Animal Behav., Rutgers Universiy, Newark, NJ 07102.

Systemic administration of the alpha-1-adrenergic antagonist prazosin decreases binding of estrogen to hypothalamic lysates of estradiol benzoate (EB) treated females (Brain Res., 330:197-199). We investigated if prazosin treatment alters estrogen receptor (ER) protein content as reflected by changes in ER-immunoreactivity. We used the rat monoclonal antibody H222 directed against ER to detect ER-immunoreactive (ER-ir) cells in eight preoptic and hypothalamic neuronal groups in ovariectomized guinea pigs treated with 10 µg EB and prazosin (1.0 mg/kg, i.p.; N=8) or EB and vehicle (N=8). Neuronal groups studied included the medial preoptic area, the central nucleus of the hypothalamus, the ventrolateral nucleus of the hypothalamus, and the infundibular nucleus. Immunocytochemical parameters that provided optimum conditions for detection of even modest changes in ER-immunoreactivity were first established. Using these optimum conditions we detected no changes in 1) mean number of ER-ir profiles, 2) mean density of ER-ir staining, or distribution of ER-ir staining density readings 6 h after prazosin treatment in any of the eight brain regions investigated. These data suggest that mechanisms other than alterations in ER protein should be considered when interpreting effects of prazosin on retention of estradiol by hypothalamic lysates. Supported by HD 04467 to H.H.F. and J.I.M., and a Sigma XI Grant-In-Aid of Research to K.F.M..

442.4

INDUCTION OF CFOS EXPRESSION IN BRAINSTEM AREAS IN RESPONSE TO NMA AND KAINATE: DIFFERENTIAL PATTERNS OF ACTIVATION IN CYCLING AND LACTATING RATS R. Abbud*, G.E. Hoffman and M.S. Smith. Department of Neurobiology, University of Pittsburgh, Pittsburgh, PA 15261.

During lactation, there is an inhibition of cortical and hippocampal activation in response to NMA, but not to kainate. The lack of response to NMA is revealed as the absence of both behavioral excitation and the induction of cFos expression. This lack of responsiveness to NMA could be due to suckling-induced inhibition of pathways that provide excitatory afferent input to the cortex and hippocampus. To examine this idea, we used the expression of cFos to identify brainstem areas activated in response to either NMA (40 mg/kg iv, 4 injections, one every 10 min) or kainate (2 .5 mg/kg iv, 4 injections, one every 10 min) injected into cycling (diestrus-1) or lactating (day 10 suckling 8 pups) rats. NMA or kainate administration to cycling animals induced cFos expression in many areas of the brainstem, including the locus coeruleus (LC), the dorsal raphe (DR), the nucleus tractus solitarius (NTS), the parabrachial nucleus (PBN) and the A1 noradrenergic cell bodies. During lactation, NMA induced cFos expression in similar areas of the brainstem as during the cycle, except in the LC and DR, where there was little evidence for cFos expression. In contrast, in response to kainate, there were no differences in the patterns of cFos expression between cycling and lactating animals. In summary, these data reveal significant differences between cycling and lactating animals with respect to the brainstem areas activated in response to NMA, but not to kainate. The lack of NMA-induced activation of the LC and DR, areas which send major afferent projections to the cortex and hippocampus, may contribute to the lack of cortical activation during lactation.

442.5

EXPRESSION OF CYTOCHROME P-450 SIDE-CHAIN CLEAVAGE ENZYME AND 3-β HYDROXYSTEROID DEHYDROGENASE IN THE RAT CENTRAL NERVOUS SYSTEM. J.-L. Sanne and K.E. Krueger.* Fidia-Georgetown Institute for the Neurosciences, Georgetown University School of Medicine, Washington, D.C. 20007

Recent developments regarding the possibility of steroid synthesis in the brain prompted the present studies to study the expression of two enzymes involved in the initial steps of steroid synthesis. The mitochondrial cytochrome P-450 side-chain cleavage enzyme ($P\text{-}450_{scc}$) which converts cholesterol to pregnenolone, and 3β-hydroxysteroid dehydrogenase (3β-HSD) which catalyzes the oxidation of pregnenolone to progesterone were examined. Using polymerase chain reaction the presence of the mRNAs for $P\text{-}450_{scc}$ and 3β-HSD are found in the adult rat brain, although there levels are considerably lower than those found in rat adrenals and testes. Proper control experiments verified that the amplified products derived from total RNA of rat brain were not due to genomic DNA or other contaminants having these sequences. Cerebral cortex, cerebellum, and spinal cord all contain comparable levels of mRNA for both enzymes. Furthermore, the two known isozymes of 3β-HSD were identified as determined using descriminative restriction enzymes and sequencing analysis of the amplified brain products. Primary glial and cerebellar granule neuronal cultures prepared from neonatal rat brains were used to examine cell type expression. Both cultures were found to express $P\text{-}450_{scc}$ at comparable levels, however, glial cultures express much higher levels of mDRC which is known to participate in mitochondrial steroidogenic activity. In contrast, 3β-HSD was clearly identified in granule neuronal cultures but only barely detectable in glial cell cultures. These findings support the proposal that the central nervous system may have the capacity to synthesize steroids and suggest that in addition to glial cells certain neuronal cells may play an important part in there expression.

442.6

FUNCTIONAL POSTNATAL RAT HYPOTHALAMIC NEURONS IN CULTURE. S.Hellbach, A.H.S.Hassan & O.F.X.Almeida*. Max Planck Institute of Psychiatry, Kraepelinstrasse 2, W-8000 Munich 40, Germany.

A new method for culturing selected neuron types from postnatal (up to 4 week-old) rat hypothalami is described, with initial data on the morphological and functional characteristics of the cultures. Slices of the arcuate (ARC) or paraventricular (PVN) nuclei are dispersed with papain and plated on PDL-coated cover-glasses in a custom-designed serum- and steroid-free medium. Immunostaining (ICC) for neurofilament proteins revealed only a minor content of glial cells. *In situ* hybridisation and ICC demonstrated that cells from the PVN and ARC synthesize and store corticotropin-releasing hormone (CRH) and ß-endorphin, respectively, as would be expected from *in vivo* observations. Acute treatments with either veratridine (1 μM) or tumor necrosis factor-α (10^{-5}M; a known secretagogue of CRH) stimulated the release of CRH-like immunoreactive material, whereas colchicine (10^{-6}M) significantly inhibited CRH release. Chronic exposure of cultures to either 17ß-estradiol (5 pM) or progesterone (10^{-7}M), markedly reduced basal CRH release; this trend was reversed following withdrawal of both steroids. Contrary to expectations based on *in vivo* results, chronic dexamethasone treatment (10^{-9}M) caused increased release of CRH; subsequent withdrawal of the glucocorticoid resulted in a tendancy towards basal levels of CRH release. The fact that neurons *in vitro* lack their normal afferents may account for these last findings. Our cultures appear to offer a promising tool for the study of long-term adaptational processes in differentiated neurons. Preliminary studies involving co-culture of neurons originating from different hypothalamic nuclei, suggest that we may better our understanding of neuronal interactions by first, teasing the system apart, before putting it back together again. Supported by DFG (SFB 220/C8)

442.7

GONADOTROPIN-RELEASING HORMONE NEURONS (GTI-7) EXPRESS IGF-I AND IGF-II RECEPTORS. BR Olson*, DC Scott, LK Nieman, Developmental Endocrinology Branch, NICHD, NIH, Bethesda, MD, 20892.

Insulin growth factor I (IGF-I) and insulin regulate metabolism and modulate the expression of differentiated cellular functions in some systems. Changes in insulin and IGF-I systemically parallel changes in hypothalamic gonadotropin releasing hormone (GnRH) and pituitary gonadotropin release. It is not known whether systemic or central IGF's and insulin play any role in the activity of the reproductive axis and in particular the GnRH neuron. Studies on explant cultures of rat hypothalamic fragments have suggested that IGF-I stimulates GnRH release. We used a well-characterized, immortalized, GnRH-secreting mouse cell line (GTI-7) to investigate whether these neurons express IGF-I, IGF-II and insulin receptors and to study the effects of the IGF's and insulin on GnRH secretion. All experiments were done on confluent static cultures in serum-free media. Competitive binding studies with I^{125} IGF-I, I^{125} IGF-II detected specific IGF-I (Kd = 3.22 x 10^{-9}) and IGF-II (Kd = 1.95 x 10^{-9}) receptors. The specific binding for I^{125} insulin was too low to allow characterization of an insulin receptor on intact cells. GnRH peptide secretion was measured by RIA in media harvested at 2 and 8 hours after incubation with IGF-I or IGF-II at dose of 1-100 ng/ml, and after 2 hours with insulin at doses 1-1000 ng/ml. Insulin did not affect GnRH secretion. GnRH secretion was decreased from basal values by IGF-I and IGF-II at the physiologic dose of 1 ng/ml, though at different time points. We found no dose response effect at the doses tested, thus the physiologic significance of these findings awaits studies using lower doses of these growth factors. Nonetheless, the presence of these receptors on th GTI-7 cell line suggests a possible role for these growth factors in the regulation of the GnRH neuron, and represents a new system in which to investigate further the functions and mechanisms of action of these growth factors in vitro.

442.8

DEVELOPMENTAL EXPRESSION OF THE ENDOTHELIN-1 (ET-1) AND OXYTOCIN (OT) GENES IN THE HYPOTHALAMUS AND PLACENTA OF THE PREGNANT RAT. M.J. Horwitz, N.B. Kim, J.A. Amico. Dept. of Medicine, Univ. of Pittsburgh and VA Medical Center, Pittsburgh, PA 15261.

ET-1, an endothelial-derived peptide with potent vasoconstrictive properties, has been found in the rat placenta and hypothalamus, suggesting a role for this peptide in reproductive-related events. Within the hypothalamus, ET-1 IR and mRNA occur in the same distribution as OT within the supraoptic (SON) and paraventricular (PVN) nuclei. Synthesis of OT also occurs within the placenta. The co-existence of OT and ET in these organs suggests that these hormones may work in concert during gestation. We investigated the developmental pattern of ET and OT gene expression within the placenta and hypothalamus of the gravid rat. Blot hybridizations of placental and hypothalamic mRNA with a ^{32}P labeled cDNA probe specific for ET-1 revealed a single ET-1 transcript, ≈ 2.3 kb. Placental ET-1 mRNA increased 3.5 fold from day 14 to 18 and 7 fold from day 14 to 21 gestation ($p = 0.01$, Kruskal Wallis test). ET-1 mRNA also increased significantly in the SON ($p = 0.01$, ANOVA) from early (day 4) to late (day 21) gestation. Blots were reprobed with ^{32}P-labeled cDNA probe specific for Exon C of the OT gene. Placental OT mRNA abundance did not change significantly during gestation but showed a downward trend. In contrast, OT gene expression increased significantly in the SON from early (day 4) to late (day 21) gestation ($p = 0.01$, ANOVA). OT transcript size within the SON remained stable throughout gestation. Placental and hypothalamic ET-1 transcripts display stage-specific increases during gestation. OT gene expression, on the other hand, displays both tissue-specific and stage-specific regulation. ET-1 may exert paracrine effects upon the placenta or uterus and may be a neuroendocrine regulator of pregnancy-related events in the rat, where it may act in concert with OT.

442.9

IDENTIFICATION AND CHARACTERIZATION OF GROWTH HORMONE RELEASING FACTOR (GRF) BINDING SITES IN THE HYPOTHALAMUS OF THE RAT. K.J. McDonnell, J.W. Bordeaux, J.I. Koenig*, K.J. Kellar, and M.D. Lumpkin. Depts. of Physiology and Pharmacology, Georgetown Univ. Med. Sch., Wash., D.C. 20007.

We previously reported that administration of small doses of GRF into the third ventricle results in attenuation of growth hormone (GH) secretion from the pituitary. An ultrashort-loop feedback model was proposed to explain this finding. In this model, GRF released by the hypothalamus may modulate hypothalamic GRF receptors (Endo Soc #1372, p.394, 1992) resulting in an autoinhibition of GRF release and subsequent decrease in GH secretion. The present study sought to identify these GRF binding sites in the hypothalamus of the rat. Tissue homogenates were prepared from freshly harvested hypothalamic fragments dissected to a depth of 2 mm with the following landmarks: anteriorly, the optic chiasm; posteriorly, the mamillary bodies; and laterally, the hypothalmic sulci. We observed binding of human (3-[^{125}I] iodotyrosol) GRF 1-44 amide to hypothalmic homogenates. Binding was displaceable by both 1uM human GRF and 1uM GRF antagonist [N-acetyl-Tyr, D-Arg- GRF (1-29)]. No displaceable binding of ligand could be obtained in any previously frozen tissues. At a concentration of 100 pM, 0.84 fmol/mg hypothalamus was specifically labeled by ^{125}I hGRF, and specific binding represented 28-40% of total binding. Specific binding was also present in anterior pituitary homogenate (2.64 fmol/mg pituitary) but absent in the homogenate of brain cortex. These data represent the first demonstration of GRF binding sites in neural tissue and provide support for the proposal that hypothalamic GRF receptors mediate GRF ultrashort-loop feedback inhibition. (Supported by NIH RO1 NS 23036 to MDL)

442.10

EFFECT OF HYPOPHYSECTOMY ON PITUITARY ADENYLATE CYCLASE ACTIVATING POLYPEPTIDE (PACAP) GENE EXPRESSION IN RAT. Y. Shuto, J. T. Weber*, H. Onda, and A. Arimura, US-Japan Biomed. Res. Labs, Tulane U. Hebert Ctr, Belle Chasse, La, 70037; Dept. of Anatomy, Tulane U. Sch. of Med., New Orleans, LA, 70112; and Discovery Res. Div. Takeda Chem. Ind., Tsukuba, Japan.

Pituitary adenylate cyclase activating polypeptide (PACAP) may be a novel hypothalamic hypophysiotropic hormone. It stimulates adenylate cyclase of cultured rat pituitary cells, and its specific receptors are present in the pituitary cells. Our previous immunohistochemical study indicated that the PACAP-like immunostaining in the paraventricular nucleus and supraoptic nucleus, and in the median eminence considerably increased after hypophysectomy suggesting a negative feedback regulation between hypothalamic PACAP synthesis and the pituitary function. In the present study, we determined whether the transcriptional rate of PACAP gene in the rat hypothalamus would change after hypophysectomy. The probe used was PACAP cDNA plasmid PRB38P1 which contained the entire coding region of rat prepro-PACAP and used to remove selectively the labeled PACAP mRNA from the pool of entire transcripts. On the contrary to our expectation, the transcriptional rate of PACAP gene decreased one and two weeks after hypophysectomy as compared with the control animals. The discrepancy between the immunohistochemical findings and the present observation could be explained by that the transcriptional rate of PACAP gene secondarily decreased as an autofeedback mechanism following accumulation of PACAP in the PACAP-synthesizing neurons in the hypothalamus which might be resulted from the prevention of the release of the peptide after stalk section associated with hypophysectomy. However, a possible feedback between the level of pituitary hormone(s) in the blood and the synthesis of PACAP in the hypothalamic neurons cannot be excluded. (Supported in part by NIH grant DK09094 and a research aid from Takeda Chemical Ind.)

442.11

COOPERATIVE GLUTAMATE- AND GABA-EVOKED CALCIUM SIGNALING IN GnRH NEURONS. D.J. Spergel*, L.Z. Krsmanovic, S.S. Stojilkovic and K.J. Catt. ERRB, NICHD, NIH, Bethesda, MD 20892.

Glutamate and GABA receptors in GnRH neurons have been proposed to regulate Ca^{2+}-dependent GnRH secretion. To determine which GABA receptor subtypes are expressed in GnRH neurons, and how glutamate and GABA act together, we measured $[Ca^{2+}]_i$ responses to glutamate and to GABAergic agonists and antagonists, alone and in combinations, in the GT1-7 cell line of GnRH-secreting hypothalamic neurons. GT1-7 cells were cultured on poly-L-lysine coated glass coverslips (5×10^5 cells/cm^2) for 2-3 days, during which they formed monolayer networks, and were then loaded with the acetoxymethyl ester of fura-2 for $[Ca^{2+}]_i$ measurements. Glutamate and GABA each induced a moderate, concentration-dependent increase in $[Ca^{2+}]_i$, with EC_{50}'s of 10 μM and 2 mM, respectively, which was markedly attenuated in Ca^{2+}-free solution and by 5 μM nifedipine. The action of GABA was mimicked by the $GABA_A$ agonist, muscimol (EC_{50} = 4 μM), but not by the $GABA_B$ agonist, baclofen (100 μM). The $GABA_A$ antagonists (all 10 μM), bicuculline, (+)-hydrastine and tert-butyl-bicyclo[2,2,2]phosphorothionate (TBPS) decreased basal $[Ca^{2+}]_i$. Bicuculline (100 μM) decreased basal $[Ca_{2+}]_i$ and the response to GABA (1 mM), but also decreased the response to glutamate (100 μM). The response to a combination of 100 μM glutamate and 1 mM GABA (or 100 μM muscimol) was nearly equal to the sum of the individual responses to these two agonists, but was smaller than the sum when glutamate and GABA were applied sequentially. In contrast, the response to 100 μM glutamate was unaffected by simultaneous or prior addition of 100 μM baclofen. The results indicate that GnRH neurons express both glutamate and $GABA_A$ receptors. Activation of $GABA_A$ receptors is followed by Ca^{2+} influx through L-type voltage-sensitive Ca^{2+} channels, which is enhanced by simultaneous activation of glutamate receptors. (D.J.S. is a National Research Council-ADAMHA/NIH Associate. L.Z.K. is supported by a grant from Sigma-Tau, Rome, Italy.)

442.12

DEMONSTRATION OF MEMBRANE-ASSOCIATED TUBULIN IN THE RAT ANTERIOR PITUITARY PLASMA MEMBRANES. R. Ravindra*, R. G. Nagele, and S. A. Patel. Departments of Cell and Molecular Biology, UMDNJ-School of Osteopathic Medicine, Stratford, NJ 08084.

We have recently observed that colchicine and taxol, drugs that interact with tubulin, inhibited GnRH and TRH-stimulated G protein GTPase activity in plasma membranes from the rat anterior pituitary lobe, suggesting the presence of tubulin in association with these membranes (J. Reprod. Fertil. 97:27-33, 1993). In the present study, we have further tested for the presence of membrane-associated tubulin. Plasma membranes were prepared from anterior pituitary lobes of adult male Sprague-Dawley rats using discontinuous sucrose gradient centrifugation. Ouabain-sensitive Na^+-K^+ ATPase activity, a plasma membrane marker, in the pituitary homogenates and plasma membranes was 2.68 ± 0.14 and 20.84 ± 3.9 μmol/min/mg protein, respectively (n = 3). For detection of tubulin using immunofluorescence, membranes (1-2 mg/ml) were fixed briefly in 2% paraformaldehyde, exposed to monoclonal anti-β tubulin antibodies (1:500 dilution) or non immune IgG, and treated with FITC-labeled anti-mouse IgG. Specimens were mounted in glycine-glycerol buffer (pH 8.6) and visualized with Nikon Optiphot microscope equipped with epifluorescence optics, a SIT-66 video camera, and a DAGE digital image processor. Tubulin-specific fluorescence was very prominent in the membranes incubated with tubulin antibody and was negligible in membranes incubated with non immune IgG or FITC-labeled anti-mouse IgG alone. These results suggest that tubulin is a component of plasma membranes of the rat anterior pituitary and are consistent with the notion of tubulin involvement in signal transduction.

NEURO-ONCOLOGY I

443.1

PHOSPHOROTHIOATE ANTISENSE OLIGONUCLEOTIDES TO PROTEIN KINASE C (PKC) α INHIBIT HUMAN GLIOMA GROWTH *IN VITRO*. N.P. Dooley*, G.H. Baltuch, J.-G. Villemure, and V.W.Yong. Neuroimmunology Unit, MNI, McGill University, Montreal, CANADA, H3A 2B4.

The PKC family of serine/threonine kinases consists of both Ca^{2+}-dependent (α, β_1, β_2, and γ) and -independent isoforms (δ, ε, ζ, and η). The various isoforms of PKC have been shown to play a central role in both the differentiation and proliferation of transformed cells. We have previously demonstrated using reverse trancriptase - polymerase chain reaction (RT-PCR) that of the Ca^{2+}-dependent PKCs, glioma cell lines (A172, U251, and U563) express only the α isoform. To determine whether PKC α was essential in glioma growth, phosphorothioate antisense oligonucleotides (20-mers) specific for the translation initiation site of PKC α were applied to cell cultures (10μM) every 36 hours over a 4 day period. The effect of the antisense treatment was monitored using ^{3}H-thymidine incorporation and I^{125} Western blotting. In serum-free media the proliferation rate of the glioma lines treated with antisense oligonucleotides was decreased by more than 40% over sense controls. A lactate dehydrogenase assay (Sigma) confirmed that this effect was not a result of cytotoxicity. These preliminary findings are consistent with the postulate that protein kinase C α is critical in glioma proliferation.

443.2

COLLAGENASES EXPRESSED IN HUMAN MALIGNANT GLIAL TUMORS AND MICROVASCULAR ENDOTHELIUM; IMPLICATIONS FOR NEOVASCULARIZATION. P.C. Costello*, and R.F. Del Maestro, Brain Research Laboratory, University of Western Ontario, London, Ontario. N6A 4G5

During solid tumor angiogenesis, endothelial cells must bridge the basement membrane. Either the tumor is degrading the basement membrane to acquire a new blood supply using proteases and/or the endothelium are responding to angiogenic stimuli and breaking down the surrounding matrix themselves. Using Northern hybridization analysis, only collagenase IV (gelatinase A) mRNA was detected in human malignant glial tissues in significantly higher levels than in non-tumor brain tissue. To determine the role of the endothelium from human brain capillaries, expression of collagenase IV mRNA was determined using *in situ* hybridization on cell cultures. mRNA levels were highest in sub-confluent cultures. The collagenase IV and general protease activity levels secreted by endothelial cultures were assessed and consistently found to be 20 to 30 % higher in sub-confluent cultures than confluent cultures. Primary glial cell conditioning generated an overall decrease in the endothelium's general protease activity and in collagenase IV activity. Malignant glial tumor conditioning caused an increase in collagenase IV and general protease activity, but only in sub-confluent endothelium. Immunohistochemistry revealed gelatinase A antigenicity in glial tumor cells and microvessels of sections from human surgical specimens. Detection of type IV collagenase (gelatinase A) mRNA, protein, as well as activity in human brain tumors and blood-brain barrier endothelium strongly implicates a role played in the neovascularization cascade of malignant glial tumors.

443.3

GENE THERAPY FOR EXPERIMENTAL GLIOMAS USING VIRUS VECTORS E. Antonio Chiocca, M.X. Wei, E. Boviatsis, M. Chase, P. Pechan, T. Tamiya, N. Kowall, D. Kennedy, P. Filipek and X. O. Breakefield*, Neuroscience Center, Massachusetts General Hospital, Charlestown, MA 02129

Virus vectors provide an efficient means of delivering foreign genes into tumor cells for therapeutic purposes. We have focused our work on gliomas as they are resistant to current modes of therapy and should be selectively vulnerable in the adult brain by virtue of being essentially the only dividing cell population there. Both recombinant herpes simplex type 1 (HSV1) and Moloney murine leukemia-based retrovirus vectors have been used for selective killing of glioma cells in adult rat brains. Changes in tumor size over time have been evaluated by computerized volumetric analysis of gadolinium-enhanced magnetic resonance images. HSV1 vectors, bearing the lacZ marker gene and deleted in genes for thymidine kinase or ribonucleotide reductase, are able to replicate in and kill 9L tumor cells, gradually spreading throughout the tumor with only localized necrotic effects on surrounding normal tissue. Herpes vectors can infect tumor cells that have migrated some distance from the tumor mass. Additional features are being introduced into these "backbone" vectors to increase their selective toxicity for tumor cells. Grafting of a packaging cell line which releases a replication-defective retrovirus vector bearing the HSV1 thymidine kinase (TK) gene into 9L glioma tumors, combined with ganciclovir therapy, has also been tested with and without co-infection with wild type retrovirus. Variable regression of tumors has been observed in this latter model. Combinatorial retrovirus therapy using other conditionally toxic genes, such as IL4 (see abstract by Wei et al), is currently being evaluated to increase the extent of tumor regression. These studies represent a new mode of therapy for brain tumors which is highly versatile and can be combined with traditional therapies.

443.4

GENETIC TREATMENT OF INTRACEREBRAL RODENT BRAIN TUMORS WITH AN INTERLEUKIN-4 RETROVIRUS VECTOR M. X. Wei, R. K. Hurford Jr., R. I. Tepper, X. O. Breakefield, E. A. Chiocca*. Neurosci. Ctr. and Cancer Ctr., Mass. Gen. Hosp., Charlestown, MA 02129, Harvard Med. Sch., Boston, MA 02115.

There are no effective therapies resulting in long-term survival for patients suffering from glioblastoma multiforme, the most common adult primary central nervous system neoplasm. One reason for the lack of tumor regression may be a poor host response against the tumor due to its poor antigenicity and to the blood-brain barrier. Retrovirus vectors can be used to deliver genes to tumor cells, rendering them sensitive to drugs or to other oncolytic mechanisms. Interleukin-4 has been previously shown by one of us (R.I.T.) to provoke an eosinophilic response against subcutaneous sarcomas and to prolong the life of nude mice harboring human U87 intracranial gliomas. Rat C6 glioma cells were infected in culture with a retrovirus vector bearing an interleukin-4 (IL4) gene (or as a control, a lacZ gene) to demonstrate efficient gene transfer. We then tested the ability of IL4 retrovirus-producer cells (CREIL4) or, as a control, lacZ retrovirus-producer cells (CRElacZ) to inhibit the subcutaneous growth of rat C6 glioma cells in nude mice (the ratio of tumor cells to retrovirus producer cells was 1:10). The CREIL4-treated C6 gliomas exhibited only a 3.9-fold increase in tumor volume over a 21 day period, while the CRE-, CRElacZ-, and untreated-C6 gliomas exhibited 69.2-, 53.3-, and 86-fold tumor volume increases respectively. As an additional control, plain CRE cells were not tumorigenic in these mice. To further test the ability of IL4 retroviruses to prolong survival of rodents harboring brain tumors, we inoculated 10,000 rat C6 glioma cells into the frontal lobes of nude mice. Three days later we stereotactically delivered either CREIL4 or CRElacZ cells (1.5×10^5) into the tumor. The average survival for the CRElacZ-treated mice (n=9) was 24.66 days, while that for the CREIL4-treated mice (n=9) was 33.22 days.

443.5

ALTERATIONS OF CHROMOSOMES 9q, 11p, AND 17p IN MEDULLOBLASTOMA. S. Albrecht, A. von Deimling, T. Pietsch, S. Brandner, P. Kleihues, O.D. Wiestler*. Depts. of Neuropathology, University of Bonn, D-5300 Bonn, Germany, and University of Zürich, CH-8091 Zürich, Switzerland.

Little is known about genetic alterations involved in the pathogenesis of cerebellar medulloblastoma (MB). There is evidence for MB loci on 11p & 17p. An additional candidate locus is the locus for Gorlin syndrome (GS), an autosomal dominant syndrome consisting of multiple basal cell carcinomas, epithelial jaw cysts, & skeletal anomalies. Since GS can be associated with MB, we examined sporadic cases of MB for loss of heterozygosity (LOH) on chromosome 9 where a putative GS locus has been localized to band q31. Nineteen paired blood & tumor DNA samples from 16 patients (11 primary tumors, 2 primary with recurrent tumors, 1 primary tumor and cell line, 2 cell lines) were studied by PCR analysis of microsatellites with > 70% heterozygosity at D9S55 (9p12), D9S15 (9q13-q21.1), D9S109 (9q31), D9S127 (9q13-q31), D9S12 (9q22.3), D9S58 (9q22.3-q31), GSN (9q33), ASS (9q34.1), D9S67 (9q34.1-q34.3), TH (11p15.5), D17S5 (17p13.3), TP53 (17p13.1) & by RFLP analysis at WT-1 (11p13). Only 2 of 19 tumors had LOH on 9q. One was non-informative at D9S15 & GSN but had LOH at D9S109, D9S127, D9S12, D9S58, ASS, & D9S67. The other was non-informative at D9S15, D9S58, & D9S67 but had LOH at D9S12, D9S109, D9S127, GSN, & ASS. This is compatible with loss of most or all of one copy of 9q. Both cases were informative for D9S55 without LOH. No case had LOH on 11p. Four cases had LOH at both D17S5 & TP53 & another case was non-informative at D17S5 but had LOH at TP53. None of the cases with LOH on 9q had LOH on 17p & vice-versa. These data indicate that LOH on 9q occurs rarely in sporadic MB. This does not rule out a role for the GS locus in sporadic MB but makes it less likely.

443.6

IN VIVO HIGH-RESOLUTION OPTICAL IMAGING OF NEURONAL TUMOR TISSUE IN RATS AND HUMANS. D.W. Hochman*, M.S. Berger, A.M. Spence, and M.M. Haglund. Department of Neurological Surgery, University of Washington, Seattle, WA 98195.

Currently, intraoperative methods for identifying and grading brain tumors depend primarily on 1) ultrasound and 2) histological criteria. Both techniques suffer drawbacks - ultrasound cannot be used to reliably identify tumor margins once the resections has begun, and the acquisition of the intraoperative biopsy suffers from sampling error.

We have developed a new optical imaging technique that can be used to provide a high-resolution intraoperative map of tumor vs. non-tumor tissue in rat and human brain. Furthermore, our technique can provide online information regarding the tumor grade and presence of residual tumor in resection margins.

A CCD camera is attached to the operating microscope. Either the cranium or exposed brain is illuminated with light (>690 nm). Images are digitized every 1-4 sec and combined to produce percentage difference maps by subtracting images acquired prior to indocyanine green (ICG) injection with images acquired at various times following ICG injection.

The % optical changes imaged in the rat glioma model at various times after ICG injection were as follows:

	EXPOSED BRAIN			INTACT SKULL	
Time	Tumor	Tumor Surround	Normal	Tumor	Normal
2 sec	40.5±9.6	16.4±6.8	9.7±4.7	13.9±3.9	8.2±2.3
60 sec	27.9±5.7	8.3±2.0	4.4±2.1	5.8±1.6	3.1±0

In eight patients undergoing surgical resection for primary gliomas, graded optical changes were observed - the largest in high-grade gliomas, followed by low-grade and then normal brain. Optical changes in high-grade gliomas persisted for more than 10 min. after ICG injection, while normal brain returned to baseline within 1 min.

Optical imaging has the potential to provide real-time intraoperative information with near cellular resolution (50 µm - 1.5 mm/pixel) of margin biopsy sites.

443.7

SHORT-TERM EFFECTS OF PROTON MICROBEAM IRRADIATION ON FELINE LGN. D.C. Lau, C.S. Reder, S.J. Ashwal, and M.A. Kirby*. Depts. of Pediatrics and Anatomy, and the Division of Perinatal Biology, Loma Linda University School of Medicine, Loma Linda, CA 92350.

Little is known of the short-term effects of proton irradiation on neural tissue. We used a 1.0mm microbeam on the cat lateral geniculate nucleus (LGN) to quantify the short-term effects of radiation damage. The LGN is a large structure with well defined afferent, efferent, and receptive field properties.

Electrophysiological and histological techniques were used to examine the effects of the microbeam at 20, 50, and 75 gray, administered as a single bolus. Recordings of light evoked responses in the LGN were obtained using microelectrodes in nine animals within 12 weeks of irradiation. Receptive fields were mapped onto a tangent screen using standard receptive field techniques. Histological measures included afferent termination, myelination, and soma/nuclear area.

Physiological and histological abnormalities were found in the 75 and 50 gray, but not the 20 gray animals. In contrast to controls, areas unresponsive to visual stimuli were found in the irradiated LGN that were surrounded by normally responsive regions. A disruption in the progression of the size of the visual fields in the irradiated LGN was found. Histological analyses correlated with the physiological data by the absence of afferent termination to regions of the LGN, and having significantly larger soma areas ($p<.05$) on the irradiated vs. the control LGN. No necrosis was observed. All effects were isolated to the irradiated LGN.

These results suggest that the proton beam is capable of disruption of function in the absence of acute cellular necrosis. Moreover, this disruption was confined to a single target structure with no observable disruption to surrounding regions.

PAIN: PATHWAYS I

444.1

MECHANOSENSITIVE CHANNELS IN ADULT HUMAN DRG NEURONS T.K. Baumann[1,2*], K.J. Burchiel[1] and M.E. Martenson[1], Division of Neurosurgery[1] and Department of Pharmacology[2], Oregon Health Sciences University, Portland, OR 97201

In recent years, many different cell types have been shown to possess mechanosensitive (MS) ion channels. Surprisingly, with the exception of one abstract,[1] there appear to have been no studies of MS channels in mammalian primary afferent somatosensory neurons. Such channels are likely to play a role not only in the normal functioning of such neurons, e.g., mechanotransduction or volume regulation, but also in pathological states following nerve injury when the mechanosensitivity of primary afferent neurons is known to be markedly increased. We have used patch clamp recording techniques to search for MS channels in the somata of neurons cultured from dorsal root ganglia excised from patients treated surgically for chronic pain. In a large proportion of cell-attached recordings (using electrodes filled with 160 mM KCl, 10 mM HEPES and 8.13 mM EGTA), the application of tension to the patch caused bursts of large amplitude unitary currents (-20 pA, electrode held at 0 mV). Increased tension (negative pressure between 0 and -3 kPa) resulted in an increased probability of channel openings.

[1] X.C. Yang, F. Guharay and F. Sachs, Biophys. J. 49:373a, 1986.

444.2

CLUSTERING SKIN AFFERENTS OF IDENTICAL MODALITY IN HUMAN NERVES HAVE OVERLAPPING RECEPTIVE FIELDS. **R.Ekedahl, R.G.Hallin*, G.Wu, Dept of Clinical Neurophysiology, Huddinge University Hospital, 141 86 Huddinge, Sweden.**

The human palm and finger tips are densely innervated. Thus, even restricted skin stimuli might occasionally excite a few cutaneous afferents of the same modality. Since, in contrast to previous reports (1,2), we recently demonstrated how peripheral myelinated fibres are orderly organized by both modality and somatotopy (3,4) the above phenomenon might be more common than previously realized.

To elucidate the issue we used concentric needle electrodes which image both functional and structural aspects of human nerves (3,4). The studied median nerve units sometimes had separate receptive fields. However, neighbouring myelinated fibres regularly had the same modality and innervated adjacent or even overlapping receptive fields. A combination of natural and/or electrical stimuli applied simultaneously to the area where these fibres ended facilitated the otherwise difficult unit identification. At certain stimulus intensities and repetition frequencies latency changes or blockings were elicited in the myelinated afferents. Reminiscent but more marked refractory phenomena were previously demonstrated and used to characterize cutaneous C units in man.

Thus, subtle recording and stimulation techniques facilitate the identification of neighbouring myelinated afferents in human nerves with overlapping receptive fields in the skin.

References: 1) Sunderland S. Brain 1945, 68, 243-299; 2) Schady WJL, et al. Brain Res 1983, 277, 249-261; 3) Hallin RG. J Neurol Neurosurg Psychiat 1990, 53, 736-744; 4) Hallin RG, et al. Muscle and Nerve 1991. 14. 157-165.

444.3

CORTICOTROPIN RELEASING FACTOR (CRF) AND INTERLEUKIN-1ß (IL-1ß) PRODUCE ANTINOCICEPTION IN INFLAMMATION MEDIATED BY PERIPHERAL OPIOID RECEPTORS.

M. Schäfer, L. Carter, S.R. Goldberg and C. Stein*, NIDA Addiction Research Center and Dept. of Anesthesiology, Johns Hopkins Hospital, Baltimore, MD 21224

In vitro studies have shown that opioid peptides can be released from immune cells by different agents. In our animal model of subcutaneous inflammation we demonstrated such peptides within inflammatory cells and opioid receptors on sensory nerves in situ. The present experiments examined whether CRF or IL-1ß induce endogenous opioid release in inflamed tissue and result in antinociception.

Male Wistar rats received an injection of Freund's complete adjuvant into one hindpaw and developed unilateral localized inflammation. After four days, when injected into the inflamed paw, CRF and IL-1ß produced potent antinociception. This effect was dose dependently antagonized by the specific antagonists alpha-helical-CRF and IL-1-receptor antagonist, respectively, as well as by naloxone and other opioid antagonists. To demonstrate the involvement of immune cells we abolished this effect by immunosuppression using intraperitoneal cyclosporine A.

Our results suggest that CRF and IL-1ß, by activation of their receptors on immune cells, cause a release of opioids which subsequently occupy their receptors on sensory nerves resulting in inhibition of pain.

444.4

Aβ MECHANO-ALLODYNIA AND COLD HYPERALGESIA FOLLOWING LARGE DOSES OF INTRADERMAL CAPSAICIN.

R.H. Gracely, S.A. Barcellos, S.J. Saltzman, M.G. Byas-Smith, M.B. Max and G.J. Bennett*. Neurobiology and Anesthesiology Branch, NIDR, NIH Bethesda MD 20892.

Intradermal injection of 1000 µg capsaicin into the volar forearm in 8 subjects evoked an immediate intense spontaneous pain sensation that rapidly decreased to "weak" or less at 45 min in all but one subject. An area of mechano-allodynia developed rapidly, peaked at 11.4 min, and persisted to the end of all sessions (75-312 min).

In these experiments and in those using lesser doses (500 µg n=5, 250 µg n=4, 100 µg n=3), a 1-s train of constant current electrical stimuli (1-msec cathodal pulses at 100 Hz) was painful at the threshold for detection in each of the 12 cases evaluated, indicating that the mechano-allodynia was mediated by large diameter Aβ low-threshold mechano-receptive (Aβ-LTM) afferents. Aβ allodynia was supported further by reaction times to painful detection-level stimuli in one subject, and the concurrent abolition of allodynia and touch sensation during tourniquet cuff blocks (n=4). Local anesthetization of the injection site in 2 subjects (25 and 82 min post capsaicin injection) did not abolish allodynia. Stimuli (0-23°C) perceived as cold before the injection evoked a distinct pain sensation following 11/19 injections.

The presence of Aβ-LTM allodynia without spontaneous pain, and during local anesthesia of the injection site, suggest that the central processes mediating allodynia can persist in the absence of ongoing nociceptive input. Intradermal capsaicin may provide a model of the initiation of neuropathic pain syndromes.

444.5

SPINOTHALAMIC NEURONS CONTAIN N-ACETYL-ASPARTYL-GLUTAMATE (NAAG) IMMUNOREACTIVITY IN THE RAT.

K.E. Miller*, D.L. O'Donoghue, C.R.D. Poff. Dept. Anatomical Sciences, Oklahoma Center for NeuroSciences, Univ. Oklahoma Health Sciences Center, Oklahoma City, OK 73190.

NAAG is a dipeptide found in abundance in the mammalian CNS. It has been suggested that NAAG has a role as an excitatory neurotransmitter or as a source for excitatory amino acid neurotransmitters. The distribution of NAAG immunoreactive neurons in the spinal cord is similar to the organization of spinothalamic (STT) neurons. The purpose of this study was to examine STT neurons for NAAG immunoreactivity (IR). The thalamic VPL nucleus was injected with colloidal gold labelled, apo-HRP (Au-HRP). After 48-72 hrs, animals were perfused transcardially with ethylcarbodiimide. Cryoprotected spinal cord segments (C1,C7,T9, L4,L6) were sectioned on a cryostat. Sections were processed for silver intensification of Au-HRP and NAAG immunohistochemistry. Most silver labelled STT neurons also contained NAAG-IR. Silver labelled STT neurons containing NAAG-IR were found in the marginal zone, nucleus proprius, lateral cervical and spinal nuclei, and central gray region (lamina X) from cervical to lumbosacral segments. We conclude from these results that NAAG may be used as a neurotransmitter by STT neurons.

Supported by NS27213 (KEM).

444.6

SPINAL CORD LAMINA I TERMINATIONS IN BRAINSTEM CATECHOLAMINE CELL GROUPS.

K.N. Westlund* and A.D. (Bud) Craig. Marine Biomedical Institute, UTMB, Galveston, TX 77555-0843, and Div. of Neurobiol. and Neurosurg., Barrow Neurol. Inst., Phoenix, AZ 85013.

Ascending lamina I projections were identified with *Phaseolus vulgarus* leuco-agglutinin (PHAL) and the relationship of the lamina I termination in the brainstem to catecholamine (CA) cell groups examined with immunofluorescent double-labeling techniques.

The lamina I terminations were labeled with Texas Red and the CA cells were labeled with FITC using a primary antibody to tyrosine hydroxylase. The double DAB process (for black terminals and brown somata) was also employed. In both cats and monkeys ascending lamina I fibers course through the contralateral A1, C1, A5, and A7 cell groups that form a column through the ventrolateral medulla extending into the pons. Lamina I terminations were observed in each of these groups on both the ipsilateral and contralateral sides, and also in A2 and A6 more dorsally. Many of the terminations were located very near and not in apparent contact with CA processes, but others were found that were in close apposition to CA cells and their dendrites. Such putative synaptic contacts were most common in A1 and A7.

These anatomical findings provide a sound basis for thermoreceptive and nociceptive inputs to the brainstem CA system, a major system that coordinates somatic and visceral motor activity and influences sensory processing. These inputs may modulate nociceptive information by direct or multisynaptic descending pathways. These projections may include the substrates for spino-bulbo-spinal somatosympathetic reflex activity and the relay of specific thermoregulatory afferent information to the hypothalamus. (Supported by NIH grant NS25616; NS11255; and NS01445, RCDA to KNW).

444.7

LOCALIZATION OF α_2-ADRENERGIC RECEPTOR SUBTYPES IN NOCICEPTIVE PATHWAYS IN RAT CNS.

E.L. Gustafson*, M.M. Durkin, J. Bard, C. Forray, Y. She, A. Illy, R.L. Weinshank, and T.A. Branchek, Synaptic Pharmaceutical Corp., Paramus, NJ 07652.

The involvement of adrenergic receptors in nociceptive processes takes place at multiple sites in the CNS. We have used receptor autoradiography and *in situ* hybridization histochemistry to define the distribution of the α_{2A} and α_{2C} receptors and their mRNAs in rat CNS. Radioligand binding studies were carried out using [^{3}H]MK-912. The distribution of the α_{2A} and α_{2B} + α_{2C} adrenoceptors were defined by their sensitivity to oxymetazoline and prazosin, respectively. Nonspecific binding was determined using the radiolabelled ligand in the presence of phentolamine. Alpha$_2$ mRNA localization was performed using ^{35}S-labelled antisense and sense oligonucleotide probes (45mers) to the rat RG-20 (α_{2A}) and RG-10 (α_{2C}) adrenoceptor mRNAs. In rat, radioligand binding studies indicate that oxymetazoline-sensitive α_{2A} receptors are the most prominent α_2 subtype in nociception-related structures, including the periaquaductal gray, locus coeruleus, nucleus of the solitary tract, lateral reticular nucleus, and dorsal horn of the spinal cord. *In situ* hybridization studies suggest that α_{2A} mRNA predominates in the locus coeruleus, nucleus of the solitary tract, and lateral reticular nucleus, while α_{2C} is the most prevalent subtype mRNA in the spinal cord dorsal horn, in sensory afferent neurons of the dorsal root ganglia, and in some midline thalamic nuclei. The localization of α_{2A} and α_{2C} adrenoceptor subtypes, and their respective mRNAs, in nociceptive pathways indicates that multiple α_2 adrenocepter subtypes are involved in the regulation of pain. The development of subtype-specific α_2-adrenergic drugs may help to alleviate some of the deleterious side-effects associated with traditional analgesics and anesthetics.

444.8

NOCICEPTIVE- AND THERMORECEPTIVE-SPECIFIC NEURONS IN A DISCRETE REGION OF THE MONKEY LATERAL THALAMUS.

M.C. Bushnell and A.D. (Bud) Craig*. Univ. Montreal, Quebec, Canada and Barrow Neurological Inst., Phoenix AZ.

In 1911 Head and Holmes hypothesized a specific substrate for pain and temperature sensation in the ventral caudal part of lateral thalamus. This hypothesis is supported by the recent identification of a dense, topographic lamina I spinothalamic and trigeminothalamic projection to the posterior part of the ventral medial thalamic nucleus (VMpo). We now demonstrate that this region contains a concentration of nociceptive and thermoreceptive cells.

In 7 barbiturate-anesthetized cynomolgus monkeys, tungsten microelectrodes were used to record from 87 single somatosensory units located histologically in the cytoarchitectonic region of VMpo. Receptive fields (RFs) found on the face, head, hand, and arm were usually small (1-3 cm^2, 48%), but were sometimes large (whole head and/or forelimb, 15%) or bilateral (11%). Cells with trigeminal RFs were located anterior to cells with cervical RFs. Of the 87 units, 71 were nociceptive, 8 were cold-specific, 3 were low-threshold, and 5 were inhibited by noxious or cold stimuli. Neurons responding to noxious stimuli were NS (90%) or WDR (10%) cells; a few neurons responded to cold as well as to noxious heat and pinch. Quantitatively tested NS and WDR cells produced graded responses to noxious heat (46-58°C) or pinch (300-1200 g on 3 mm^2). Notably, small clusters of nociceptive cells with overlapping RFs were recorded in the middle of this region and large clusters of "cold" cells with RFs on the tongue were found at the rostrodorsal limit of this region.

We conclude that VMpo processes specific temperature and pain information. The recent proposal that lamina I conveys the "feelings" of the body (Gemeingefühl) is congruent with the conjoint processing of temperature and pain in VMpo and with VMpo's location relative to VMb and VP. This also implies that VMpo may process not only pain and temperature but also other body sensations such as itch and tickle. (Supported by NIH grant NS 25616)

444.9

THALAMIC OSCILLATIONS GENERATED BY THE INTERACTIONS BETWEEN THALAMIC VENTROBASAL AND RETICULAR NUCLEI IN VITRO. R.A. Warren* and E.G. Jones. Dept. of Anatomy and Neurobiology, Univ. of California, Irvine, CA 92717.

Thalamic neurons display oscillatory activity during periods of drowsiness and slow wave sleep. The oscillations are associated with the synchronization of the cortical EEG during these behavioral states. The cortical feedback acting upon the reticular nucleus of the thalamus (RTN) is thought to play an important role in the generation and maintenance of thalamic oscillations. We found that the excitatory projections from thalamic relay neurons to RTN neurons can also induced oscillations.

Whole cell recording from ventrobasal (VB) and RTN neurons was obtained in a mouse thalamocortical slice preparation (Agmon and Connors, Neuroscience 41:365, 1991) from animals aged 12 to 21 days. A single electrical shock to the internal capsule routinely induced recurrent IPSPs at a frequency of 1-4Hz for 2 to 8 seconds in VB neurons. These IPSPs often generated typical bursting of VB neurons and were blocked by the application of bicuculline. The same stimulus evoked oscillatory bursting in RTN neurons that was sustained by a rhythmic sequence of EPSPs at a frequency similar to the IPSPs observed in VB neurons. The addition of bicuculline to the perfusing medium abolished the rhythmic EPSPs but spared the initial short latency EPSPs in RTN neurons, suggesting that the recurrent EPSPs in RTN were evoked by input from bursting VB relay neurons.

We conclude that the synaptic connectivity between RTN and VB is sufficient to generate thalamic oscillations and that the excitatory projections from thalamic relay neurons to the RTN may be important in the generation of thalamic oscillations in behaving animals.

444.10

DIFFERENCES IN CORTICAL AND SUBCORTICAL RESPONSES TO NOXIOUS HEAT AND COLD STIMULI IN HUMANS. K.L. Casey*[1,2], S. Minoshima[3], R.A. Koeppe[3], T.J. Morrow[1,2], and K.A. Frey[1,3] Depts of Neurology[1], and Physiology[2], and Div. of Nuclear Medicine, Dept of Internal Medicine[3], Univ. of Michigan, and Neurology Research Lab., VAMC, Ann Arbor, MI 48105

We investigated regional increases in cerebral blood flow (CBF) responding to three types of noxious thermal stimuli using positron emission tomography (PET). In each subject, stimulation and baseline studies were performed using the following stimuli: 1) phasic noxious heat stimuli (50°C, 5 sec pulse) and phasic warm baseline stimuli (40°C) delivered using a contact thermode (n=9), 2) tonic noxious cold stimuli (6°C, 60 sec) and cold but not noxious baseline stimuli (24°C) delivered using ice and cold water (n=9), 3) phasic noxious cold stimuli (1°C, 10 sec pulse) and phasic cold but not noxious baseline stimuli (15°C) delivered using ice and cold water (n=7). Each stimulus was delivered to subjects' left forearm 1) and hand 2), 3) during 60 sec PET data acquisition following an intravenous bolus injection of 66 mCi [O-15] water. Stereotactic subtraction and summation analyses were performed to localize increased CBF areas due to noxious stimuli in comparison with baseline stimuli for each group. Different patterns of increased forebrain CBF involving several cortical and subcortical structures were produced by each type of noxious stimulation. Contralateral thalamic activation was seen only with phasic stimuli, but the contralateral anterior cingulate gyrus was most active during tonic cold pain. These results suggest that a reciprocal relationship between thalamic and cortical pain processing.

444.11

POSITRON EMISSION TOMOGRAPHY (PET) STUDIES OF PAIN AN ALLODYNIA IN NORMALS AND PATIENTS WITH CHRONIC NEUROPATHIC PAIN. M. J. Iadarola*, K. F. Berman[a], M. Byas-Smith, R. H. Gracely, M. Max, T. Zeffiro[b], G. J. Bennett. Neurobiology & Anesthesiology Branch, NIDR; [a]Clinical Brain Disorders Branch, NIMH; [b]Medical Neurology Branch, NINDS; NIH, Bethesda MD, 20892.

The PET oxygen-15 water bolus method was used to image regional brain activity during a) capsaicin-induced pain and allodynia in normals and b) 3 patients with spontaneous pain in one limb. Sixty sec tissue count data were acquired for normal subjects (7M,4F) during 6 experimental conditions: 1) rest, 2) brushing, 3) intradermal injection of capsaicin (250ug, volar forearm) to produce pain, 4) and 5) during the waning period of capsaicin, and 6) allodynia produced by brushing adjacent to the capsaicin injection. Fifteen slices with resolution of 6-6.5 mm were acquired for each scan and analyzed with statistical parametric mapping. Capsaicin activated contralateral somatosensory cortex, thalamus, anterior cingulate cortex, cerebellar vermis and insular cortex and putamen bilaterally. A distinctly different regional profile of activation was seen during allodynia which was characterized by prominent activation of orbito-frontal cortex but comparatively little in anterior cingulate cortex or cerebellar vermis. PET scans of the 3 patients revealed that chronic spontaneous neuropathic pain is associated with a decrease in thalamic activity contralateral to the affected limb. The decrease is similar to that reported for cancer patients with chronic lateralized pain (Di Piero et al, Pain, 46:9, 1991). The capsaicin/allodynia data suggest that pain driven by C-fiber activation involves a different regional network compared to allodynia produced by light brushing. The initial observations with the patients suggest that functional alterations in thalamic processing may be an important component of chronic pain.

444.12

IMAGING PAIN IN HUMANS WITH HIGH RESOLUTION FUNCTIONAL MAGNETIC RESONANCE IMAGING (MRI) AT 4 TESLA (4T). H. Wen, S. Wolff, R. Balaban, R. Turner, D. Kenshalo[b]* K.F. Berman[a] and M.J. Iadarola[b]. Laboratory of Cardiac Energetics, NHLBI, [a]Clinical Brain Disorders Branch, NIMH; and [b]Neurobiology & Anesthesiology Branch, NIDR; National Institutes of Health, Bethesda MD, 20892.

This study focuses on mapping changes in neural activity induced by pain in primary somatosensory cortex. Increases in brain activity produce increases in blood flow which can be assessed with recently developed MRI methods sensitive to hemodynamic parameters. These parameters include blood oxygenation level and blood flow and are manifested as magnitude and phase changes in the MR signal. A 4T MRI system, volume head coil and spoiled gradient recalled echo sequence, were used to image brain activity evoked by placing a finger on a metal plate maintained at 49-50°C in multiple alternations with a room temperature stimulus. Image acquisition was 8.5 or 12 sec and voxel size of transaxial or coronal slices was 1 x 2 x 8 mm. Activation produced by thermal stimuli was also compared to that obtained by moving the digit. Noxious stimuli produced magnitude increases and phase changes that were (a) spatially contiguous (b) observed in postcentral gyrus and vessels in postcentral sulcus, (c) coincident with periods of stimulation, (d) up to 20% above the basal condition and (e) contralateral to the stimulus. Dorsoventrally, the active region was highly localized: in one subject only one of 3 sequential 8 mm thick transaxial slices contained the pain signal. Motor activity was mainly localized in precentral gyrus and vessels of precentral and central sulcus and this profile is readily distinguishable from that evoked by thermal pain. The high anatomical resolution of the functional scans reveals the focal nature of the hemodynamic changes. This resolution, coupled with the capacity for multiple stimulus presentations, yields a new approach to investigating neural processing of pain in conscious man.

444.13

SOMATOSENSORY CORTICAL NEURONS in MONONEUROPATHY MODEL. M. Backonja*, G. Miletich. University of Wisconsin, Madison, WI 53792.

Cortical neurons are thought to have only minimal involvement in processing sensory information related to pain. Somatosensory cortical neurons probably participate in encoding sensory-discriminative properties of painful stimuli. It is proposed that cortical neurons undergo plasticity changes, as well as do neurons at lower levels of neuraxes, in the case of neuropathic pain.

Somatosensory cortical electrophysiological recordings with 1M NaCl filled glass electrodes were made in anesthetized ventilated male rats during chronic phase of mononeuropathy 2-3 weeks after surgery for loose ligation of sciatic nerve (Bennett and Xie, 1988). Control animals had sham surgery. A battery of mechanical and thermal stimuli were used for characterization of receptive fields.

Forty neurons were recorded in the study group and thirty six in the control group. In each group a largest subpopulation of neurons had characteristics of wide dynamic range responses. Neurons of rats with painful mononeuropathy had larger receptive fields and more neurons in this group had prolonged afterdischarges.

Data from this study demonstrates that cortical neurons respond to painful stimulation and that the response is altered (increased receptive fields and prolonged afterdischarges) in the case of mononeuropathy.

445.1

STRUCTURE OF THE ISOLATED BRAIN PREPARATION. JE Moreira, PM Reuss, EC Ribeiro, TS Reese and RR Llinás*. NINDS, Bethesda MD, 20892 and Physiol. & Biophys. NYU, NY, NY 10016.

A new technique for perfusion of isolated brains has proven useful for a variety of physiological studies (Llinás et al., *J Physiol.*, 4114:16P, 1989). We examined the structural features of isolated brains from 30 day-old mice and compared the results with those obtained by freeze-substitution. The ultrastructure of surface samples of isolated brains rapidly excised, quick frozen, and freeze substituted served as a benchmark for a variety of fixation experiments designed to determine whether deeper cortical regions of perfused brains were structurally intact. Three perfusion fixative solutions were compared: 1) buffered 2% glutaraldehyde+2% paraformaldehyde; 2) buffered 3% glut. containing 22 mM H_2O_2 and 4% PVP; 3) 3% glut.+1% paraform. in an 3% dextran 70 "artificial blood" (de Curtis et al., *Hippocampus*, 1:4 341, 1991) modified by adding 10 mM H_2O_2. Results closest to those from freeze substitution were obtained by method 2 and, especially, method 3. Areas CA1 to 4 and dentate gyrus in the hippocampus as well as cerebellar folia showed neuropil arrangements with precise rounded shapes and straight plasma membranes, well defined cytosolic density, and synapses and vesicles resembling freeze substituted samples. Method 1, in contrast, produced sinuous membrane profiles which often appeared empty. Microtubule structure also showed marked improvement with H_2O_2-containing fixatives. The morphology of synaptic structures in the isolated brain throughout the two hours of perfusion remained equivalent to the control brain perfused *in situ*. . These results provide an excellent method for structural work on the isolated brain preparation and explain the persistence of many electrophysiological functions in this preparation. Supported by NINCDS 13742

445.2

CABLE ANALYSIS OF FOUR INSECT MUSCLES SUGGESTS THAT PROPERTIES OF THE INDIVIDUAL SYNAPTIC UNITS UNDERLY DIFFERENCES IN NEUROMUSCULAR TRANSMISSION. M.B. Rheuben* and S.M. Baer. Dept. of Anatomy, Michigan State Univ., East Lansing, MI 48824 and Dept. of Mathematics, Arizona State Univ., Tempe, AZ 85827.

The amplitudes of the excitatory junction potentials (EJPs) of four flight muscles of Manduca are consistently different when examined under subthreshold conditions produced by low Ca^{++} saline. The fibers, however, of each muscle differ in diameter and perimeter, and their neuromuscular junctions have characteristic lengths and shapes. To see if the EJP amplitudes could simply be determined by differences in the total number and distribution of synapses relative to the dimensions of the particular fiber type, we examined the morphometric data using a new cable model derived from continuum cable theory (Baer and Rinzel, 1990, J. Neurophysiol., 65: 874). This model allows us to simulate the amplitude and timecourse of EJPs generated by multiterminal innervation from a single motor axon. In simulations where properties of individual synaptic units were held constant, the number and distribution of synapses relative to the muscle fiber sizes predicts subthreshold EJPs of quite similar amplitudes, suggesting a close matching of number and length of junctions to muscle fiber dimensions. The actual differences in function must then rely on physiological differences in membrane properties of the muscle fibers or on pre- and postsynaptic characteristics of the synaptic units. Ultrastructural differences in the active zones and postsynaptic specializations support this latter hypothesis. Supported by NIH Grant NS17132 (to MBR) and NSF DMS-9107538 (to SMB).

445.3

ACETYLCHOLINESTERASE SITE DENSITY AT FROG NEUROMUSCULAR JUNCTIONS. L. Anglister*, J. R. Stiles[1], and M. M. Salpeter[1]. Dept. Anatomy, Hebrew Univ. Med. Sch., Jerusalem 91010, Israel. [1]Section of Neurobiology & Behavior, Cornell Univ., Ithaca, NY 14853.

Muscle acetylcholinesterase (AChE) is concentrated at neuromuscular junctions, where it exists largely in association with the synaptic basal lamina. Positioned between the presynaptic terminal and the postsynaptic acetylcholine receptors (AChRs), it terminates synaptic transmission by hydrolyzing acetylcholine. Knowledge of the junctional AChE and AChR concentrations is crucial for understanding the function of this synapse. Although AChR density has been measured in endplates of several species and shows little variability, AChE concentration has been determined only in mouse endplates. The aim of this study was to measure the concentration and catalytic activity of AChE at the neuromuscular junction of frog, and to investigate the physiological importance of our findings using computer modeling. Frog *cutaneous pectoris* muscles were first incubated with diisopropylfluorophosphate (DFP) to inactivate covalently all serine-hydrolases, including AChE. Subsequently, true AChE sites were reactivated by pyridine-2-aldoxime methiodide (2-PAM) and labelled by ^{3}H-DFP. Quantitative EM-autoradiography showed approximately 600 AChE-sites/μm^2 of postsynaptic surface, a value which is about 4-fold lower than the AChE site density for mouse endplates. Biochemical studies supported the frog AChE site density value. After correction for extra-junctional activity, solubilized extracellular frog muscle AChE hydrolyzed 12.7±1.3 pmol ACh/sec/muscle (mean ± s.e.m, n=27). The catalytic turnover number per active site (Kcat) was 6500-11000 mol/sec, more than 3-fold greater than that for rat or chicken AChE, but significantly less than that for eel enzyme. The total junctional activity divided by Kcat and by the total junctional surface area yielded an AChE density similar to the value measured by autoradiography. Further analysis indicates that the major extracellular molecular form of AChE at the neuromuscular junction was a small globular form (4-6S), although substantial amounts of asymmetric collagen-tailed forms were also present. The physiological implications of the AChE concentration and catalytic turnover number were examined by Monte Carlo simulations of miniature endplate currents. Surprisingly, such modeling showed that normal neuromuscular transmission is possible over a wide range of values for AChE site density and Kcat. (Supported by Israel-US BSF and Bruno-Goldberg Foundation (LA), and NIH F32NS09126 (JRS) and NS09315 (MMS).

445.4

A novel epitope of entactin is found at the neuromuscular junction. *A.Y. Chiu* and J. Ko, Division of Neurosciences, Beckman Research Institute of the City of Hope, Duarte, CA 91010*

The extracellular matrix (ECM) at the neuromuscular junction (NMJ) is biochemically and functionally specialized, and bears molecules that can regulate both the development and activity of this peripheral synapse. We have previously purified one synaptic component of the muscle ECM - a unique laminin isoform named s-laminin - from a schwannoma cell line (*Chiu et al.,J. Neurochem. 59:10*). To develop new probes for the ECM, monoclonal antibodies were generated against other components produced by this cell line. One of these antibodies, 9H6, binds very selectively at the synaptic cleft of NMJs in adult rats, but not at extrasynaptic sites on the muscle surface. On Western blots, 9H6 recognizes a 150 kD band that co-localizes with the laminin-binding, ECM glycoprotein, entactin, under both reducing and non-reducing conditions. N-terminal sequence analysis also indicates that the 9H6 antigen is an entactin-related molecule. However, polyclonal antibodies to entactin stain both synaptic and extrasynaptic sites on rat muscles. Thus, 9H6 appears to recognize an entactin epitope that has a very restricted distribution. Recent studies have shown that novel isoforms of laminin, acetylcholinesterase, agrin and collagen IV are selectively sequestered at the NMJ. Our results suggest that the entactin present at the synaptic cleft also differs from entactin present outside the synapse. The synaptic form of entactin may contribute to the unique functions of the ECM at the neuromuscular synapse.

445.5

MEMBRANE TRAFFICKING AT THE NERVE TERMINAL: THE SYNAPSE-SPECIFIC PHOSPHOPROTEIN F1-20 IS IDENTICAL TO THE CLATHRIN ASSEMBLY PROTEIN AP-3. E.M. Lafer*, S. Zhou, N. Tannery and W. Ye. Dept. of Biological Sciences, University of Pittsburgh, Pittsburgh, PA 15260.

F1-20 was cloned and characterized because of its synapse-specificity. AP-3 was purified and studied biochemically because of its function as a clathrin assembly protein. Monoclonal antibodies against F1-20 and AP-3 both specifically recognize a single protein from mouse brain with an apparent molecular weight of 190 kD on SDS-PAGE. These monoclonal antibodies also specifically recognize bacterially expressed F1-20, and purified bovine AP-3. The anti-F1-20 and the anti-AP-3 Mabs specifically recognize the same spot on a 2-D gel run on a bovine brain clathrin coated vesicle extract. Purified preparations of bovine AP-3 and bacterially expressed F1-20 give identical patterns of protease digestion with bromelain and subtilisin. Bacterially expressed F1-20, like AP-3, specifically binds clathrin.

By having connected the synapse-specific phosphoprotein F1-20 with the clathrin assembly protein AP-3, we are now able to integrate our knowledge of these two molecules into a single model for the role of F1-20/AP-3 in synaptic function. During synaptic vesicle exocytosis, the synaptic vesicle membrane fuses with the pre-synaptic plasma membrane. The synaptic vesicle membrane proteins are clustered in a multimeric complex within the presynaptic plasma membrane. The soluble form of F1-20/AP-3 is able to recognize a feature of this complex and bind to it. The N-terminal domain of F1-20/AP-3 then initiates the assembly of clathrin coated pits, which drive the re-internalization of the synaptic vesicle membrane proteins in CCVs. It is possible that phosphorylation and/or alternative splicing of F1-20/AP-3 serve to regulate this process. Our model further predicts that the developmental expression of the F1-20/AP-3 protein is required for the maturation of the synaptic vesicle recycling mechanism, and as such represents an important event in synapse development. Future work is aimed at testing the predictions of this model.

445.6

VAT-1, AN ABUNDANT COMPONENT OF TORPEDO SYNAPTIC VESICLE MEMBRANE IS A CALCIUM BINDING PROTEIN. M. Linial* and O. Levius. Dept. of Biological Chemistry, Institute for Life Sciences, The Hebrew University of Jerusalem, 91904, Israel.

VAT-1, is an abundant 41 kDa membrane protein expressed in the cholinergic synaptic vesicles of *Torpedo*. To elucidate its biological function we produced *Torpedo* VAT-1 in bacterial expression systems, purified it and examined its Ca^{2+}-binding properties in solution and in solid phase interactions. An overlay assay using $^{45}Ca^{2+}$ as a tracer, demonstrated the ability of a recombinant VAT-1 to bind calcium. High yields of recombinant VAT-1 was obtained from the glutathione S-transferase (GST) expression system. A direct Ca^{2+}-binding study was performed with purified VAT-1 by a quick spin column technique. A quantitative analysis revealed a 1:1 molar stoichiometry for binding of Ca^{2+} to VAT-1, with a dissociation constant of 130μ M. A GST-linked truncated protein consisting of 13 kDa from VAT-1 carboxy-terminal domain was found to retain the capacity to bind Ca^{2+}. Our findings demonstrate that VAT-1 is a low affinity Ca^{2+}-binding protein and attribute a Ca^{2+}-sensory function to this vesicular protein within the nerve terminal. (Supported in part by the Israel-USA Binational Science Foundation).

445.7

IMAGING OF CALCIUM VARIATIONS IN LIVING DENDRITIC SPINES OF CULTURED HIPPOCAMPAL NEURONS. M. Segal*
Dept. Neurobiology, The Weizmann Institute, Rehovot 76100 Israel.

Dissociated rat hippocampal neurons were grown in tissue culture plates for 1-6 weeks. Cells were loaded with the calcium indicator Fura-2 using microelectrodes, and were visuallized with 100x oil objective in an inverted microscope, equipped with a cooled CCD camera. Segments of dendrites containing spines were selected for analysis. $[Ca]_i$ variations in spines ($[Ca]_S$) and dendrites ($[Ca]_D$) resulting from exposure to NMDA, synaptic stimulation and drugs which regulate $[Ca]_i$ were measured. A brief exposure to NMDA caused a rapid rise of $[Ca]_S$ and a parallel, but smaller (by up to 40%) rise of $[Ca]_D$. The recovery of $[Ca]_i$ to pre-NMDA occured in the spine and the parent dendrite simultaneously, and was delayed in sodium-free medium. Synaptic potentials were activated using latrotoxin. In these cases $[Ca]_S$ was more elevated than the adjacent $[Ca]_D$. Conversely, release of Ca^{2+} from mitochondria, using proton uncouplers, elevated $[Ca]_D$ more than $[Ca]_S$. It is suggested that regulation of $[Ca]_S$ and $[Ca]_D$ are independent, but closely coupled.

445.8

CALCIUM CURRENT MAGNITUDE CORRELATES WITH PRESYNAPTIC FUNCTION IN CHICK COCHLEAR HAIR CELLS. P.A. Fuchs* and K.A. Fagan.
Dept. Physiol. and Neurosci. Prog., U. Colo. Sch. Med., Denver, CO, 80262.

It has been suggested that voltage-gated calcium channels are found only at sites of transmitter release in frog saccular hair cells (Roberts *et al., J. Neurosci.* **10**:3664-3684, 1990). If a similar rule applies in the chick's cochlea then calcium currents should be larger in tall (inner) hair cells that make many afferent contacts, and smaller in short (outer) hair cells that make few afferent synapses (Fischer, *Hear. Res.* **61**:167-178, 1992).

We have used whole-cell, tight-seal recording to measure voltage-gated inward current carried by barium (20 mM) in hair cells isolated 1 mm from the apical pole of the chick's cochlea. We observed rapidly-activating, non-inactivating barium currents positive to -50 mV and peaking near -10 mV in 26 tall, intermediate and short hair cells. While the kinetics and voltage-dependence of the barium currents were similar in all cells, the peak currents were usually larger in taller hair cells than in shorter hair cells. The 11 tallest isolated cells had average peak barium currents of 209 pA (± 79 S.D.). Based on morphological studies performed in this laboratory (B.W. Murrow Ph.D. thesis, U. Colo.), these originated from within the neural (innermost) 30% of the cochlear cross section. The 6 shortest cells originated from the abneural (outermost) 30% of the cochlear width and had smaller peak currents, 51 pA (± 24 S.D.). Nine intermediate cells had an average peak current of 71 pA (± 26 S.D.).

Preliminary electron microscopic studies showed that the frequency of appearance of release sites declines several-fold from the tallest cells to the shortest cells at this cochlear location. The co-variance of barium (calcium) current magnitude with number of release sites supports the hypothesis that voltage-gated calcium channels in hair cells are found only at these presynaptic membrane specializations. Supported by NIDCD DC00276.

445.9

A NEW METHOD TO QUANTIFY SYNAPTIC VESICLE EXOCYTOSIS: UPTAKE OF ANTIBODIES TO THE LUMENAL DOMAIN OF SYNAPTOTAGMIN I. K. Kraszewski[1,2], O. Mundigl[1,2], C. Verderio[4], R. Jahn[1,3], P. De Camilli*[1,2] and M. Matteoli[4]. [1]Howard Hughes Med Inst, [2]Dept Cell Biol. and [3]Pharm. Yale Univ. Med School, New Haven, USA and [4]CNR Center of Cytopharm, Univ. of Milan, Italy

The elucidation of molecular mechanisms of neurotransmitter release is crucially dependent on the availability of assays to quantify synaptic vesicle (SV) exocytosis. Until now SV exocytosis has been primarily studied by recording postsynaptic currents or by detecting neurotransmitter release into extracellular media. It will be very useful to have available an assay which measures SV exocytosis independently of neurotransmitter release. We have recently reported the possibility to monitor SV exocytosis by detecting lumenal epitopes of synaptotagmin which are exposed at the surface of a neuron as a result of SV fusion with the plasmamembrane. We have used this approach to study exo-endocytosis of SV in isolated processes of hippocampal neurons developing in primary culture (Matteoli et al.JCB.117:849-861]. We report here that this method can be used to quantitate SV exocytosis. Hippocampal neuronal cultures were incubated at 37°C with rabbit antibodies (Abs) directed against the lumenal domain of synaptotagmin I (Syt_{lum} Ab) for 5 - 20 min in media with different ionic concentrations or including neurotoxins known to affect SV exocytosis. At the end of the incubation cells were fixed, permeabilized, stained with CY3-conjugated secondary Abs and counterstained for the cytoplasmic domain of synaptotagmin I with FITC-conjugated Abs. Images were collected by a chilled CCD camera and Metamorph image-processing-system. Levels of SV exocytosis were inferred from the ratio between CY3 (Syt_{lum} Ab uptake) and FITC (total synaptotagmin I pool) label. Incubation with high K^+ (55 mM) of cultures rich in synaptic contacts evoked significant increase in this ratio over control levels. The increase was dependent on extracellular Ca^{++}. An even higher stimulation was produced by α-latrotoxin (5 nM). Tetanus toxin (50 nM) caused a massive decrease in Syt_{lum} Ab uptake. A Ca^{++} and depolarization dependent increase in SV exocytosis was already observed in isolated axons before the onset of synaptogenesis.

445.10

The kinetics of synaptic vesicle recycling measured at single presynaptic boutons. T. A. Ryan, H. Reuter§, B. Wendland, F. E. Schweizer, R. W. Tsien, S. J Smith*. Department of Molecular and Cellular Physiology, Stanford University, Stanford CA , 94305.§Department of Pharmacology, University of Bern, CH-3010 Bern, Switzerland.

We used the fluorescent membrane probe FM 1-43 to label recycling synaptic vesicles within the presynaptic boutons of dissociated hippocampal neurons in culture. By applying quantitative timelapse fluorescence imaging as well as rapid superfusion techniques we directly measured exocytosis , the kinetics of endocytosis as well as the recycling time of synaptic vesicles within single CNS synaptic boutons at room temperature (~ 24° C). These measurements indicate that endocytosis persists much longer than exocytosis with a $t_{1/2}$ ~ 60 sec and that once internalized, vesicles are available for exocytosis in ~ 30 sec. Furthermore we have shown that endocytosis is not dependent on membrane potential and unlike exocytosis, that it is independent of extracellular Ca^{2+}.

CARDIOVASCULAR REGULATION: BRAINSTEM INTEGRATION

446.1

STEREOSELECTIVE ACTION OF S-NITROSO-L-CYSTEINE (L-SNC) IN NUCLEUS TRACTUS SOLITARII (NTS) OF RATS. H. Ohta*, W.T. Talman, J.N. Bates and S.J. Lewis. Departments of Neurology, Pharmacology and Anesthesiology, University of Iowa and VAMC, Iowa City, IA 52242

Possible roles for nitric oxide (NO) in neurotransmission in the central nervous system have received much attention. Free NO may diffuse through membranes to activate soluble guanylate cyclase and then increase cyclic GMP in the intracellular space. However, some have suggested that NO may react with thiols and may exist as S-nitrosothiols before activating soluble guanylate cyclase in target cells. The present study was designed to investigate involvement of an S-nitrosothiol in signal transduction. Male Sprague Dawley rats anesthetized with chloralose were instrumented for recording arterial pressure and heart rate and injecting agents into NTS. Injection of L-SNC (50 - 1250 pmol) into NTS produced dose-related depressor and bradycardiac responses. Maximum depressor (-38 ± 4 mmHg) and bradycardiac (-66 ± 9 bpm) responses were obtained at 250 pmol. Responses produced by similar doses of other NO moieties, D-SNC and sodium nitroprusside, injected into NTS were one third of those produced by L-SNC. Both L- and D-SNC when injected intravenously produced equivalent depressor responses. Furthermore, both isomers released the same amount of NO in a brain homogenate. Responses to L-SNC (150 pmol) injected into NTS were blocked by prior administration of methylene blue (500 pmol) but not by oxyhemoglobin (5 pmol). Injection of L-cystine, a metabolic product of L-SNC, did not produce significant effects. These results indicate that actions of exogenous nitrosothiols are not due to release of NO extracellularly. Rather, L-nitrosothiols may be efficient NO transporters in the extracellular space and may act at a specific recognition site on the membrane to release NO into the intracellular space. Support: VA Merit Review and Clin. Investigator (WTT), and NIH HL32205 and HL14388.

446.2

NMDA RECEPTORS IN THE NUCLEUS TRACTUS SOLITARII (NTS) ARE INVOLVED IN THE BRADYCARDIC RESPONSE TO CAROTID CHEMORECEPTORS (CC) STIMULATION. B.H. Machado*, E. Colombari, D.A. Chianca Jr., L.G.H. Bonagamba and A.S. Haibara. Dept. Physiol., School of Medicine of Ribeirão Preto, USP, 14049, Ribeirão Preto, SP, Brazil.

Stimulation of CC with potassium cyanide (KCN, 80 µg/kg, i.v.) in conscious rats produces pressor and bradycardic responses, which are not observed in rats with ligature of carotid body artery. Activation of CC was performed by KCN and the chemoreflex neurotransmission into the NTS was studied using AP-5, a selective NMDA receptor antagonist. KCN was injected before and after bilateral microinjection of AP-5 (2 nMoles/100 nl) into the NTS of conscious rats. The results (n=6) show that the pressor response to CC stimulation (+55±3 vs +51±3 mmHg) was not changed, while the bradycardia (-193±15 vs -83±22 bpm) was significantly reduced from 5 to 30 min after AP-5 microinjection. These data suggest that bradycardic response to chemoreceptor activation is mediated by NMDA receptors while the pressor response seems to be mediated by non-NMDA receptors. (Supported by FAPESP and CNPq)

446.3

PHASE-RELATED ACTIVITY IS ALTERED IN BAROSENSITIVE NEURONS IN THE RODENT NUCLEUS OF THE SOLITARY TRACT BY SYSTEMIC ARTERIAL PRESSURE CHANGES. R. F. Rogers, J. F. R. Paton, B. Brown*, and J. S. Schwaber. *Institute of Neurological Sciences, Univ. of Pennsylvania, Philadelphia, PA 19104-6074 and Neural Computation Group, E. I. Du Pont de Nemours & Co., Wilmington, DE 19880-0323.*

We have analyzed baroreceptor inputs onto single nucleus tractus solitarius (NTS) neurons (n=71) which responded with short (<5 ms) and consistent (<±0.5 ms) latency evoked action potentials to ipsilateral aortic nerve stimulation (3-15 V, 0.1 ms, 1 Hz) in anesthetized, paralyzed, artificially ventilated rats. The vast majority of these neurons (61/71) also received a synaptic input following ipsilateral vagus nerve stimulation. Ten of these neurons were analyzed for cardiac phase-related and tracheal pressure-related activity, interspike interval distribution, power spectra of action potential times, and activity during induced pressor responses (phenylephrine, 0.5-3.0 mg i.v.). It was found that 9/10 neurons showed clear peaks in their R-wave triggered histograms (PRWTHs), while 4/6 showed clear peaks in their tracheal pressure triggered histograms. Furthermore, 3/9 neurons revealed novel peaks in their PRWTHs during elevated arterial pressures which were not present at resting pressures. These results suggest that individual baroreceptors, once they reach threshold, may directly shape cardiac phase-related activity in NTS neurons at the first stage of afferent signal processing. These results also imply that at least some NTS neurons are active in controlling both cardiovascular and respiratory function, as indicated by pulmonary afferent influence on barosensitive NTS neuronal activity patterns.

446.4

KYNURENATE ATTENUATES VAGUS NERVE (VN) AND CAROTID SINUS NERVE (CSN) EVOKED DISCHARGE IN NTS NEURONS. S.W. Mifflin and W. Zhang. Spon: R.D. Huffman*. Dept. of Pharmacology, University of Texas Health Science Center, San Antonio, TX. 78284

Microinjection studies have shown that excitatory amino acid receptors (EAAs) within NTS play an important role in arterial baro- and chemo-reflexes. To examine the role of EAAs at the single cell level, the effects of iontophoretic application of kynurenate (KYN) on VN and CSN evoked discharge of NTS neurons were examined in pentobarbital anesthetized rats. Barrels of a multi-barrelled pipette were filled with: KYN (100mM, pH 8.1); xanthurenate (XAN, 100mM, pH 8.1); glutamate (20mM, pH 8.6), substance P (1.5 mM, pH 4.5); 4M NaCl (current balancing); 2% Chicago Sky Blue for marking recording sites. A single electrode, filled with 2M NaCl and used for extracellular recording of VN (n=7) or CSN (n=5) evoked discharge, was positioned so that its tip extended 20-30μm beyond the tip of the multi-barrelled electrode and glued in place. Discharge evoked by 25 stimuli was compared under control conditions, during iontophoresis of either KYN or XAN, and following cessation of iontophoresis. Application of KYN with currents of 30-75nA attenuated VN and CSN evoked discharge (45±7 spikes/25 stimuli during control periods vs. 13±3 spikes/25 stimuli during KYN iontophoresis; p<.01) while XAN had no effect. The percent reduction of VN and CSN evoked discharge during KYN iontophoresis was the same whether the input was monosynaptic (82±9; n = 2 CSN, 1 VN) or polysynaptic (67±8; n = 3 CSN, 6 VN) (p>.2). KYN ejecting currents >100nA were associated with local anesthetic effects. The results suggest EAAs play a role in VN and CSN excitation of NTS neurons at the level of both first order and higher order neurons. (Supported by HL-41894)

446.5

IMPORTANCE OF THE VENTROLATERAL MEDULLA FOR THE CENTRAL CARDIOVASCULAR EFFECTS OF ENDOTHELIN. R. Mosqueda-Garcia*, K. Yates, M. Appalsamy, T. Inagami. Departments of Medicine and Biochemistry, Vanderbilt University, Nashville, TN 37232.

Intracisternal administration of endothelin 1 (ET-1) evokes a hypotensive and bradycardic phase that is followed by hypertension. With increasing doses there is a subsequent impairment of respiratory function that results in cardio-respiratory collapse and death. In the present studies we investigated whether the effects of intracisternal ET-1 are mediated by neuronal cell groups within the ventrolateral medulla oblongata. An intracisternal catheter was placed in urethane anesthetized rats for ET-1 administration. The animals were placed in groups that received bilateral microinjections of saline (group 1, 60 nl, n=4) or the ET-receptor antagonist, BQ-123 (2 pmol/per site, group 2, n=4) in both the rostral (RVLM) and caudal (CVLM) ventrolateral medulla. Two other groups of rats received microinjections of BQ-123 in either the RVLM (group 3, n=6) or into the CVLM (group 4, n=4).

In group 1, the intracisternal administration of ET-1 (20 pmol) decreased mean BP from 90±7 to 71±5 mmHg and HR from 373±14 to 335±23 bpm within 5 min. This phase was followed by sustained hypertension (+50±15 mmHg) and bradycardia. Mortality was 25%, 75% and 100% at 5, 10, and 15 min after ET-1 injection. In group 2, pretreatment with BQ-123 completely inhibited the hypotensive phase and reduced by 83% the subsequent rise in BP evoked by ET. Importantly, cardio-respiratory arrest was prevented in all the animals in this group. In group 3, the initial ET-1-hypotensive phase was left intact, the hypertensive period was attenuated by 39%, with an overall mortality of 67%. Bilateral microinjection of BQ-123 into the CVLM did not prevent the hypertensive phase and cardiorespiratory arrest was present in 75% of the animals in this group. These results suggest that the ventrolateral medulla is an important site for the mediation of effects of circulating CSF-ET. (Supported in part by the Smokeless Tobacco Research Council)

446.6

EFFECT OF PHARMACOLOGIC INDUCED BLOOD PRESSURE CHANGES ON CAT ROSTRAL VENTRAL MEDULLARY SURFACE ACTIVITY ASSESSED WITH HIGH TEMPORAL RESOLUTION OPTICAL IMAGING. R.M. Harper*, D.M. Rector, X-W. Dong and D. Gozal. Dept. of Anatomy & Cell Biology, UCLA Sch. of Med.;Childrens Hosp., USC Sch. of Med., Los Angeles, CA.

The rostral ventral medullary surface (RVMS) plays a significant role in cardiovascular regulation. We examined temporal properties of activity in RVMS regions at high sampling rates to determine patterns associated with pharmacologic manipulation of blood pressure. The VMS was surgically exposed in 5 adult anesthetized, spontaneously breathing cats. A 3.2 mm coherent optical fiber bundle, attached to a charge-coupled device (CCD) camera, was positioned over the RVMS. Neural tissue was illuminated with 660 nm light-emitting diodes, and images were digitized at 60 fields/sec before, and during intravenous injections of phenylephrine (5, 10, 15 and-20 μg/kg). Significant bradypnea and increases in blood pressure (BP) followed all injections except after 5 μg/kg, where only a mild increase in BP was noted. Spectral analysis of the overall reflectance changes revealed consistent decreases in spectral power within the 8-13 Hz range at all phenylephrine doses except with 5 μg/kg, where no effect on spectral peaks was observed. Since increases in 8-13 Hz spectral power accompany ventilatory stimulation, we speculate that phenylephrine induces significant decreases in activity of respiratory neuronal pools within the RVMS.

(Supported by HL-22418 & NIDR DE 07212)

446.7

EXCITATION OF SYMPATHOEXCITATORY RETICULOSPINAL NEURONS OF ROSTRAL MEDULLA BY HYPOXIA OR CYANIDE IS DIRECT AND NOT MEDIATED BY ACTIVATION OF NITRIC OXIDE SYNTHASE OR PROTEIN KINASE C NOR BY GLUTAMATE. M.-K. Sun* and D.J. Reis. Div. of Neurobiol., Cornell Univ. Med. Coll., New York, NY 10021.

In rats, sympathoexcitatory reticulospinal neurons of the rostral ventrolateral medulla (RVL) are rapidly and reversibly excited by hypoxia and by locally applied cyanide *in vivo* and *in vitro* (Sun *et al., Am. J. Physiol.* 262:R182-R189, 1992; Sun and Reis, *Soc. Neurosci. Abstr.* 18:1269, 1992), resulting from a rapid activation of membrane calcium channel conductance. We investigated whether excitation of these RVL neurons by hypoxia or cyanide could be due to a rapid activation of nitric oxide synthase (NOS), protein kinase C (PKC), and/or release of L-glutamate via a calcium-independent mechanism. *In vivo*, in anesthetized chemo-denervated rats, the rapid increase in sympathetic nerve activity (by 120%) elicited by hypoxia (PaO_2: 27 mmHg) was not attenuated by intracisternal administration of the specific NOS inhibitor N^{ω}nitro-L-arginine (NO_2-Arg, 2 μmol), the broad spectrum excitatory amino acid receptor antagonist kynurenate (2 μmol), or 1-(5-isoquinolinesulphonyl)-2-methyl-piperazine (H-7, 1 μmol), a specific inhibitor of protein kinase C. *In vitro*, cyanide (300 μM, 40 s) applied to RVL pacemaker neurons recorded in slices under voltage-clamp (V_H=-70 mV) and in the presence of tetrodotoxin (10 μM) evoked a rapid inward current (peak: 0.5-0.6 nA), which was not attenuated by NO_2-Arg (500 μM), kynurenate (500 μM), or H-7 (100 μM). We conclude (a) the rapid excitation of RVL sympathoexcitatory neurons by hypoxia or cyanide cannot be attributed to generation of nitric oxide, activation of PKC, or release of excitatory amino acids; (b) hypoxia excites RVL sympathoexcitatory neurons directly and non-synaptically; (c) sympathoexcitatory neurons of RVL may be central oxygen detectors.

446.8

Impairment of Baroreceptor Reflexes Following Kainic Acid Lesions of the Lateral Tegmental Field M.E. Clement* and R.B. McCall, Cardiovascular Diseases Research, The Upjohn Company, Kalamazoo, MI 49001. The lateral tegmental field (LTF) has been proposed as a site for the generation of basal sympathetic nerve activity. Although LTF contains both baroreflex modulated sympathoexcitatory and sympathoinhibitory neurons, there is little evidence to suggest that the LTF is an important relay in the baroreflex arc. We examined the effects of kainic acid lesions of the LTF on baroreceptor function in the anesthetized cat. Baroreflex function was determined by evaluating the reflex sympathoinhibition during pressor responses, the temporal locking of sympathetic slow waves to the cardiac cycle and the power spectra of sympathetic slow waves. We found that kainic acid lesions of the LTF mimicked baroreceptor denervation in that it abolished the baroreceptor-mediated inhibition of sympathetic nerve activity in response to increased blood pressure. Baroreceptor denervation has also been demonstrated to temporally uncouple sympathetic slow waves and the cardiac cycle. Following kainic acid microinjection, sympathetic activity was no longer temporally related to the cardiac cycle. We used power spectral analysis routines to determine the frequency components contained within sympathetic nerve activity. As previously shown with baroreceptor denervation, the power spectra of sympathetic activity shifted to higher frequencies following LTF lesioning. In conclusion, our data indicates that the LTF is an integral part of the baroreflex and lesions of this area produce an impairment of the baroreceptor effects on sympathetic activity.

446.9

PARTIAL COHERENCE ANALYSIS REVEALS SELECTIVE RELATIONSHIPS AMONG THE 10-HZ RHYTHMIC DISCHARGES OF DIFFERENT SYMPATHETIC NERVES. G.L. Gebber*, S. Zhong, Y. Paitel, and S.M. Barman. Dept. Pharmacol. & Toxicol., Michigan State Univ., East Lansing, MI 48824.

The 10-Hz rhythms in the discharges of postganglionic sympathetic nerves with different targets are strongly correlated as evidenced by coherence values near one. In the current study on baroreceptor-denervated, decerebrate cats, partial coherence analysis was used to determine whether the 10-Hz rhythmic discharges of the cardiac (C), lumbar (L), renal (R), and vertebral (V) sympathetic nerves are differentially related. This analytical technique mathematically removes the portion of the ordinary coherence between two signals attributable to a third signal. Specifically, the coherence functions relating the 10-Hz discharges of two sympathetic nerves were compared before and after removal of the influences of the central sources of the activity of a third nerve (as reflected by its discharges). We observed nonuniform changes in coherence at 10 Hz for different nerve pairs after partialization. For example, removal of R discharges sometimes eliminated the coherence at 10-Hz for C and L discharges with minimal effect on the coherence for C and V discharges. We also found cases when the coherence value relating the 10-Hz discharges of two nerves (e.g., C and V) was reduced to a greater extent after removal of the influences of R discharges than L discharges. Importantly, the pattern of differential relationships could change within and between experiments. The results might be explained by 1) differential gating of the outputs of a single 10-Hz generator to its targets, 2) differential crosstalk among central circuits receiving inputs from the same generator, or 3) differential coupling of multiple 10-Hz oscillators. (Supported by NIH grants HL-13187 and HL-33266.)

446.10

MEDULLARY LATERAL TEGMENTAL FIELD NEURONS PLAY A PERMISSIVE ROLE IN GOVERNING THE 10-HZ RHYTHM IN SYMPATHETIC NERVE DISCHARGE. S.M. Barman* and G.L. Gebber. Dept. Pharmacol. & Toxicol., Michigan State Univ., East Lansing, MI 48824.

Recordings from sympathetic nerves in decerebrate cats show a variable mixture of 10-Hz and 2- to 6-Hz discharges. Although medullary lateral tegmental field (LTF) neurons are considered to be a source of the 2- to 6-Hz oscillation in sympathetic nerve discharge (SND), their role in the control of the 10-Hz rhythm has not been critically evaluated. This issue served as the focus of the current study. In the first series of experiments, spike-triggered averaging of inferior cardiac SND was used in an attempt to identify LTF neurons with activity correlated to the 10-Hz rhythm in SND. The discharges of only one of 120 LTF neurons studied were correlated to this component of SND. In two of these experiments, we were able to identify neurons (4 of 17) in nucleus raphe pallidus with activity correlated to the 10-Hz rhythm in SND. In experiments in which a 2- to 6-Hz oscillation was prominent, we identified LTF neurons (17 of 79) with activity correlated to this component of SND. These data indicate that LTF neurons neither receive input from nor are components of the 10-Hz rhythm generator. In a second series of experiments, muscimol (1 nmol in 100 nl) was microinjected slowly (10-20 s) into each of four LTF sites on both sides of the medulla. Chemical inactivation of the LTF with muscimol either eliminated the 10-Hz rhythm or reduced the power and peak frequency in this band of SND in five of six cats. Partial recovery of the 10-Hz rhythm occurred within 90 min after completing the injections. These data support the view that LTF neurons have a permissive role in governing the 10-Hz rhythm in SND, likely by acting on elements of the rhythm generator located elsewhere. (Supported by NIH grants HL-33266 and HL-13187.)

446.11

CHRONIC CAFFEINE TREATMENT ATTENUATE THE CARDIOVASCULAR EFFECTS OF ADENOSINE IN RAT BRAINSTEM. H.C. Lin, C.S. Tung, and C.J. Tseng*. Dept. of Pharmacology and Physiology, National Defense Medical Center, Taipei, Taiwan, R.O.C.

We reported previously that microinjection of adenosine into the nucleus tractus solitarii (NTS) decreased mean blood pressure (MBP) and heart rate (HR) in normotensive rats through the putative adenosine receptors. The purpose of this study is to investigate the effect of chronic caffeine treatment on the cardiovascular effects of adenosine in the NTS. Male Sprague-Dawley rats were pretreated with either caffeine-containing water (0.1 %) or water for different period of time. Microinjection (60 nl) of adenosine in the NTS produced a dose-related decrease in HR and MBP in water pretreated rats. However, a gradual attenuation of the hypotensive and bradycardic effects of adenosine were noted as the caffeine pretreatment maintained for more than 3 days. After one week of caffeine treatment, the cardiovascular effects of adenosine in the NTS were completely abolished. These effects recovered completely after the rats were abstained from the caffeine-containing water for more than one week. We concluded that the cardiovascular effects of adenosine in the NTS were attenuated by the pretreatment of caffeine, which might be due to a development of tolerance to caffeine in rats.

446.12

NITRIC OXIDE SYNTHASE IN RAT SYMPATHETIC PREGANGLIONIC NEURONS: AN IMMUNOHISTOCHEMICAL AND ELECTROPHYSIOLOGICAL STUDY. N. J. Dun,* S. Y. Wu, S. L. Dun and U. Förstermann. Dept. of Anatomy, Medical College of Ohio, Toledo, OH 43614 and Abbott Laboratories, Abbott Park, IL 60064.

The presence of nitric oxide synthase-immunoreactivity (NOS-IR) was detected in the rat spinal cord using an antibody against rat cerebellar NOS. NOS-IR was found to be concentrated in rat sympathetic preganglionic neurons (SPNs) but not in ventral horn motoneurons. NOS-IR was also detected in neurons of the dorsal horn, particularly laminae I and II and around the central canal. To evaluate a possible role of NO in SPNs, whole-cell patch clamp recordings were made from these neurons in transverse (500 μm) thoracolumbar spinal cord slices removed from immature (12-16 day) rats. Superfusion of L-arginine (L-Arg; 0.1-0.5 mM), the precursor of NO, reversibly increased the inward current induced by NMDA and AMPA applied to the SPNs by pressure. This effect was stereospecific, as D-Arg in comparable concentrations was ineffective. Superfusion of N^G-Methyl-L-arginine (NMA; 100 μM), a potent inhibitor of NOS, slightly decreased the NMDA- and AMPA-induced currents. Pretreatment of the spinal cord slices with NMA nullified the potentiating effect of subsequent application of L-Arg. Superfusing the slices with 8-bromo-cGMP and 3-isobutyl-l-methylxanthine increased the NMDA/AMPA-induced currents in a reversible manner. In addition, L-Arg reversibly increased the outward current induced by pressure application of glycine. Our results show that agents that stimulate NO formation increase the responses induced by NMDA/AMPA and glycine in rat SPNs, and agents that inhibit NO formation exert the opposite effect. As cGMP mimics these effects, the intracellular mechanism may involve phosphorylation of membrane proteins by cGMP-dependent protein kinase.

LEARNING AND MEMORY: SYSTEMS AND FUNCTIONS VIII

447.1

REGIONAL CEREBRAL BLOOD FLOW CHANGES DURING CLASSICAL EYEBLINK CONDITIONING IN MAN. T.A.Zeffiro*, T.Blaxton, J.Gabrieli, S.Y.Bookheimer, M.Carrillo, E.Binion, J.Disterhoft, and W.Theodore MNB and ERB, NINDS, National Institutes of Health, Bethesda, MD; Stanford University, Palo Alto, CA; Northwestern University, Chicago, IL.

Classical conditioning studies of the rabbit nictitating membrane reflex have implicated the cerebellum and hippocampus as putative sites for the storage or formation of the association between the unconditioned and conditioned stimuli. In this study we explored the neuroanatomical localization of this type of learning.

rCBF changes were recorded with positron emission tomography in 15 subjects using bolus injection of $H_2{}^{15}O$ in 3 conditions: (1) central fixation, (2) uncorrelated tone and airpuff (pseudoconditioning), and (3) correlated 400 msec tone and 150 msec airpuff (short-delay conditioning). Trials were administered in 2 blocks of pseudoconditioning and 4 blocks of conditioning, with each block lasting 2 minutes. Scans were spatially standardized to a common coordinate system. Planned comparisons made between the task and control conditions yielding statistical parametric maps of rCBF change.

Contrasting conditioning and pseudoconditioning blocks revealed conditioning-related rCBF changes including: (1) increases in ipsilateral cerebellar nuclei, ipsilateral hippocampus, contralateral putamen and bilateral prefrontal cortex ($p<.01$); and (2) decreases in ipsilateral cerebellar cortex ($p<.01$).

These results support a role for the cerebellum and hippocampus in associative learning as suggested by previous animal studies. The prefrontal cortex and putamen may play a more prominent role in short-delay eyeblink conditioning in man.

447.2

NEUROPHYSIOLOGICAL SUBSTRATES OF HUMAN MEMORY IMPAIRMENTS: ALTERED REGIONAL CEREBRAL GLUCOSE UTILIZATION IN ALCOHOLIC KORSAKOFF'S SYNDROME AND ALZHEIMER'S DISEASE, AS MEASURED BY POSITRON EMISSION TOMOGRAPHY (PET). K.A. Paller*, B.C. Richardson, A.P. Shimamura, O. Plaisant, B.R. Reed, and W.J. Jagust. Center for Functional Imaging, Lawrence Berkeley Laboratory (MS 55-121), Berkeley, CA 94720, and Department of Neurology, University of California, Davis.

Although structural neuroimaging indicates that Korsakoff's syndrome is associated with damage to the medial thalamus and the mammillary bodies, additional brain areas (such as the hippocampus) may also be dysfunctional and thus responsible for the amnesic symptoms in these patients. We investigated this possibility by measuring regional cerebral metabolic rates for glucose using the radiotracer [^{18}F]-fluorodeoxyglucose and PET imaging with high spatial resolution (2.6 mm in-plane, FWHM). Comparisons were made between patients with Korsakoff's Syndrome, alcoholic control subjects, nonalcoholic control subjects, and patients with Alzheimer's Disease. The procedure included a continuous recognition test as a behavioral challenge during the radiotracer uptake period, and performance measures were obtained assessing recognition (as a function of retention delay) as well as stem-completion priming. Dissociations between recognition and priming were found, replicating prior studies. PET results showed that Alzheimer's disease was associated with abnormally low metabolic rates in temporal and parietal neocortical areas, whereas Korsakoff's syndrome was associated with a more widespread decline in function in both neocortical and subcortical regions. Further, there was no evidence that hippocampal metabolism was disproportionately affected in Korsakoff's syndrome.

447.3

FUNCTIONAL NEUROANATOMY OF VERBAL MEMORY AS REVEALED BY WORD LIST RECALL DURING PET SCANNING. J.T. Becker*, M.A. Mintun, D. Diehl, S.T. DeKosky, J. Dobkin, Depts. of Psychiatry & Radiology, University of Pittsburgh Medical Center, Pittsburgh, PA.

Data obtained from normal individuals using functional neuroimaging provides the opportunity for testing existing neuropsychological models. In the present study, 12 healthy, young individuals performed word recall tasks while undergoing $H_2{}^{15}O_2$ Positron Emission Tomography (PET) scanning.

When the subjects repeated lists of words exceeding their memory span, areas activated included the dorso-lateral prefrontal cortex (Areas 9&10) and the anterior cingulate cortex. No activation was seen in the temporal lobes in addition to that observed during word repetition. By contrast, when comparing 1-word or 3-word repetition to resting state, cortical areas of activation included the superior temporal cortex and the mesial temporal regions in the vicinity of the hippocampus.

These within-subject analyses are consistent with those between-subject studies reported by Grasby and colleagues and suggest that mesial temporal activation occurs during any memory task, even when those brain regions are not critical for the performance of the task. Further, the pattern of activation and of free recall performance suggests that the prefrontal activation is secondary to current concepts of normal memory functions, and to the brain structures which support them.

447.4

DOUBLE DISSOCIATION OF SPATIAL AND OBJECT WORKING MEMORY IN NORMAL HUMANS USING PET. J. Jonides*, E.E. Smith, R. Koeppe, S. Minoshima, and E. Awh. Department of Psychology (Jonides, Smith, and Awh) and Department of Nuclear Medicine (Koeppe and Minoshima), University of Michigan, Ann Arbor, MI 48109.

Behavioral experiments on brain-injured humans as well as electrophysiological experiments on monkeys suggest that working memory for spatial and object information may be mediated by different processing pathways. The present experiments show that the processes of spatial and object working memory can be doubly dissociated using PET in normal humans.

Subjects were given one of two tasks while PET measurements were taken. In a spatial task, visual targets were presented at three locations, and after a 3-sec delay subjects were given a probe and had to indicate whether it appeared at one of the previously occupied locations. In an object task, two target objects were presented, and after a 3-sec delay subjects had to indicate whether a probe object was identical to one of the previously presented targets. The spatial task engaged processes of right hemisphere occipital, parietal, prefrontal and premotor areas while the object task engaged processes of left hemisphere temporal, parietal, prefrontal, and cingulate areas. These results identify different pathways for the two working memory tasks. One difference is in the hemisphere predominantly responsible for processing in the two tasks. Another difference is that the spatial task recruits processes of a dorsal processing system while the object task recruits processes of a ventral system. Yet another difference is in the locus of processes presumably involved in retention of the respective stimuli in prefrontal cortex.

447.5

INTACT CONCEPTUAL PRIMING BUT IMPAIRED CUED RECALL IN PATIENTS WITH FRONTAL LOBE LESIONS. F. B. Gershberg* and A. P. Shimamura. Dept. of Psychology, University of California, Berkeley, CA 94720.

Studies of patients with damage to dorsolateral prefrontal cortex have revealed deficits in free recall and use of organizational strategies but normal recognition memory and perceptual priming. The present study examined performance of frontal patients on implicit tests of conceptual priming and explicit tests of cued recall and free recall. In Experiment 1, 7 frontal patients and 12 controls studied a 15-word, categorized list (5 exemplars from each of 3 categories). They were then given an implicit test in which they were asked to produce 8 exemplars for each of 6 categories (3 studied and 3 new). Priming occurs when the production of studied exemplars is greater than the baseline guessing rate for new categories. After a break, subjects studied a new categorized list and then were given an explicit test using category names as cues for recall. Frontal patients showed normal priming on the implicit test but showed impaired performance on the explicit test. Baseline guessing rate, however, was lower for patients than for controls. Experiment 2 was conducted to address this problem by using an easier test task involving free association. Six frontal patients and 12 controls studied lists made up of 5 clusters of 5 words linked to a common associate (e.g., circus, hunter, tamer, mane, king). The procedures were identical to those of Experiment 1 except that the test cues consisted of 6 single words, 3 of which were associates of studied words (e.g., lion). For the implicit test, subjects were asked to produce 8 words related to each cue word, and for the explicit test, they were told to use the cue words to help them recall list words related to the cues. Frontal patients again showed normal priming, this time with a normal baseline. The cued recall impairment was also replicated. Taken with previous findings, these results suggest that frontal patients are impaired on tests which tap the encoding of list organization, as do explicit tests of category- and associate-cued recall, but normal on tests which tap primarily the conceptual processing of individual list items, as do implicit tests of category and associate generation. (Supported by NIH Grant AG09055 to APS and an NSF Graduate Research Fellowship to FBG.)

447.6

AMNESIC PATIENTS SHOW NORMAL PRIMING AND A NORMAL DEPTH-OF-PROCESSING EFFECT IN A CONCEPTUALLY DRIVEN IMPLICIT MEMORY TASK. M.M. Keane*, J.D.E. Gabrieli, L.A. Monti, J.M. Cantor, and J.S. Noland. Memory Disorders Research Center, Boston University School of Medicine and DVAMC, Boston, MA 02130; and Rush Alzheimer's Disease Center, Chicago, IL 60612.

Although patients with global amnesia show impaired performance on recall and recognition memory tests (explicit measures of memory), they can show intact performance on repetition priming tasks (implicit measures of memory), which measure the influence of prior exposure to test stimuli on subsequent performance. Some investigators have argued that implicit/explicit memory tasks typically confound data-driven/conceptually driven processes, and have suggested that the latter distinction may provide a better account of spared and impaired memory function in amnesia. By this view, data-driven processes (invoked in tasks that require analysis of surface features of stimuli) are spared in amnesia, and conceptually driven processes (invoked in tasks that require analysis of stimulus meaning) are impaired in amnesia. We evaluated these competing theories by comparing the performance of 13 amnesic patients (AMN) and 16 control subjects (CS) on a conceptually driven, implicit memory task: category exemplar production. In a study phase, subjects answered questions that induced deep ("Natural or man-made?") or shallow ("Uppercase or lowercase letters?") processing of category exemplars (e.g., "mango".) In the test phase, subjects generated examples of given categories (e.g., "fruit".) The measure of priming was the degree to which subjects were biased above baseline to generate exemplars from the study list. The results showed that priming was 1) equivalent in the AMN and CS groups; 2) greater following deep than shallow processing; and 3) similarly affected in both groups by the depth-of-processing manipulation. Amnesic patients were impaired in an explicit measure of category exemplar cued recall. Like prior findings, these results demonstrate that memory processes spared in amnesia can support normal performance on conceptually driven memory tasks, provided that those tasks do not depend upon deliberate reference to a prior study episode. Supported by NINDS grant NS 26985 and grant IIRG-92-063 from the Alzheimer Association.

447.7

INTACT CONCEPTUAL TRANSFER ON AN IMPLICIT PERCEPTUAL TASK IN GLOBAL AMNESIA AND ALZHEIMER'S DISEASE. D.A. Grosse*, J.D.E. Gabrieli, S.L. Reminger, L.A. Monti, J.A.Weiner, and R.S. Wilson. Rush Medical College, Chicago, IL, 60612 and Dept. of Psychology, Stanford University, Stanford, CA. 94305.

A profound explicit memory deficit is a defining feature of global amnesia (GA) and of Alzheimer's disease (AD). In contrast, GA and AD patients have shown intact implicit memory on a number of perceptual repetition priming tasks, such as the perceptual identification (PI) of briefly presented words. Normal PI priming is thought to be primarily perceptual (data-driven) since study-phase reading yields greater priming than study-phase generating of the unseen word. PI priming that is evident after study-phase generation can be considered conceptual transfer in a perceptual task. PI priming in memory-disordered patients has been examined only after perceptual encoding. It is unknown whether these patients would show intact conceptual transfer on this perceptual task. Our subjects were 16 patients with mild AD, 16 normal elderly controls (NC), and 5 patients with GA. For perceptual encoding, subjects saw and *read* single words aloud. For conceptual encoding, subjects *generated* a word when given a short definition and the first letter of the word. For the implicit PI test, subjects identified briefly presented and masked words, and priming was measured as the advantage in identifying repeated vs. baseline words. For the explicit yes/no recognition task, subjects made recognition judgments about singly presented studied and unstudied words. The NC subjects were more accurate in their recognition judgments for generated than for read words, and were more accurate in both conditions than the two patient groups. Both GA and AD patients showed intact PI priming magnitudes for the perceptual and conceptual encoding conditions. All subjects performed best for words read, second best for words generated, and least well for baseline (new) words. The patients' intact priming in the conceptual generate condition indicates that they had intact conceptual transfer occur on an implicit perceptual task. These results suggest that the same memory system retains both perceptual and conceptual information on a perceptually-driven task, and that this neural system probably does not include structures damaged in GA and early AD. Supported by the Alzheimer's Association, grant # IIRG-92-063.

447.8

RECOGNITION MEMORY IN PARKINSON'S DISEASE AND ALZHEIMER'S DISEASE: EVIDENCE FOR INTACT FLUENCY. G.T. Stebbins*, J.D.E. Gabrieli, D.A. Grosse, J.A. Weiner, C.G. Goetz. Dept. of Neurological Sciences, Rush Medical College, Chicago, IL 60612

Two components to explicit memory have been proposed: recollection (controlled/conscious processes) and fluency (automatic/unconscious processes). Little is known about the neural bases for these putatively separable explicit memory processes. We examined the status of fluency in patients with known damage to limbic and neocortical structures (Alzheimer's disease (AD)) and to the basal ganglia (Parkinson's disease (PD)). 16 mildly demented AD patients with 16 matched control subjects (NC), and 16 non-demented PD patients with 10 matched NC participated in the study. Jacoby's Fame Judgement task was employed to assess fluency. Subjects first read aloud a list of 20 moderately famous and 20 non-famous names. Subjects next viewed a list of 80 mixed old and new famous and non-famous names and were asked to judge whether or not a name was famous. Fluency was measured by the probability of calling an old name famous minus the probability of calling a new name famous. Subjects next read a new list of 40 famous and non-famous names, and then made yes-no recognition judgements on a list of those names combined with 40 foils. PDs showed similar recognition memory to their NCs, while ADs were significantly impaired in comparison to their NCs. Fluency was comparable across all groups. These findings suggest that: 1) despite impaired recognition memory in AD, fluency is intact in comparison to age and education matched NCs; 2) automatic, fluent memory is not impaired by either the limbic or the neocortical damage found in AD, nor by damage to the basal ganglia found in PD. Supported by the Office of Naval Research and the Illinois Department of Public Health.

447.9

TEXT-SPECIFIC IMPLICIT MEMORY IN PATIENTS WITH ALZHEIMER'S DISEASE AND GLOBAL AMNESIA: EVIDENCE FOR IMPLICIT MEMORY FOR NEW ASSOCIATIONS. L. A. Monti*, J. D. E. Gabrieli, R. S. Wilson, N. J. Cohen, S. L. Reminger, C. Vaidya, and A. Ortony. Rush Alzheimer's Disease Center, Chicago, IL 60612.

Patients with Alzheimer's disease (AD) and global amnesia are impaired on tests of explicit memory (recall and recognition), but intact on some implicit memory tasks. Most of these implicit tasks involve the processing of individual words or pictures and, therefore, do not permit the construction of new associations between items. Two experiments were designed to investigate whether memory-impaired patients can implicitly learn and retain new associations between words. In the first, ten mildly demented AD patients and ten age-matched normal control (NC) subjects read two passages three times, as quickly as possible, and answered recognition memory questions after the third reading of each passage. The AD patients had poor explicit memory for the passages, as evidenced by impaired recognition memory performance, but had intact implicit memory for the passages, as evidenced by normal decreases in the times for successive readings. The baseline reading times for AD patients and NC subjects were comparable. Even though one third of the words were shared by both passages, there was no cross-passage facilitation, indicating that the rereading effect was a text-specific rather than a word-level effect. In a second experiment, amnesic patients read: (1) a normal passage three times, (2) a randomized version of a passage three times, and (3) three different random versions of a passage. Results showed significant priming except in the last of these conditions. These findings suggest that the rereading effect is mediated by rereading words in their original contexts, in which new between-word associations are learned, rather than by individual word repetition or general reading skills. Supported by grants from the NIA #F32-AG05568-01A1 and the Alzheimer's Association #IIRG-92-063.

447.10

CONTRIBUTION OF THE STRIATUM AND CEREBELLUM IN VISUOMOTOR SKILL LEARNING. J. Doyon*, R. Laforce, Jr., D. Gaudreau, M. Castonguay, P.J. Bédard, J-P. Bouchard, & J. Roy, Centre de Recherche en Neurobiologie, Faculté de Médecine, Université Laval, Québec, Canada, G1J 1Z4.

There is now considerable evidence from both animal (e.g., Wang et al., 1990; McDonald & White, 1993) and human studies (e.g., Heindel et al., 1989) indicating that the implicit learning of a variety of skills may depend upon the integrity of a complex cortico-striatal neural pathway. Some investigators have recently shown, however, that the cerebellum may also play a role in motor and non-motor skill learning. The goal of the present study was thus to investigate further the contribution of these structures in the incremental learning of a visuomotor skill. The performance of patients with idiopathic Parkinson's Disease (PD) or with a circumscribed lesion of the cerebellum (Ce), was compared to that of matched normal-control subjects (NC) on an adapted version of the Repeated Sequence Test (Nissen & Bullemer, 1987). This test consist of a choice reaction-time task in which the stimuli follows a set sequence of ten items that repeats itself 10 times in a block of trials. Subjects received 4 blocks of trials on each of the six training days, which took place once a week over a period of 6 weeks. Following the last experimental block of trials on Day 6, subjects were required to respond to a short questionnaire and to complete two blocks of trials in a self-generated sequence task in order to evaluate their declarative knowledge of the sequence and to examine directly the possible dissociation between the two memory systems. The results demonstrate that both PD and Ce patients were impaired on the learning of the repeated sequence over training trials, whereas their performance did not differ from that of the NC subjects on the declarative memory tests. Our findings suggest that the incremental learning of a visuomotor skill (but not declarative memory) may depend, along with the complex cortico-striatal system, upon a parallel cortico-cerebellar circuit.

447.11

DEFICITS IN WORKING MEMORY APPEAR EARLY IN HUNTINGTON'S DISEASE. A. W. Deckel*. Division of Neuropsychology, Dept. of Psychiatry, UCONN Medical School, Farmington, CT 06030.

Huntington's Disease (HD) is an autosomal dominant, slowly progressive neurodegenerative disorder that leads to impaired motor functioning and results in a progressive dementia. Although memory deficits are a frequent finding, deficits in so-called "working memory" have not been systematically evaluated early in the course of the disease.

Three groups of patients, including those with "early" HD, detoxifying substance abusers, and normal controls were asked to memorize a 12 square "grid" filled either with visual designs (visual-spatial) or 3 letter words (verbal-spatial). After assessing immediate recall, they were given 4 "learning" trials during which they were asked to learn the "order" of the designs/words in space, and then were assessed for their immediate and delayed "post -learning" memory. They were also given a task which required them to place in temporal "order" a story they were read.

HD patients had difficulty in learning the "template" during the learning trials, regardless if the trial employed a word or design. Even patients who were "early" in the course of the disease and generally appeared intact cognitively demonstrated difficulty with the task. HD patients also had impaired delayed recall of the grid compared to controls. Similarly, their ability to "order" a story was impaired.

These results suggest that working memory is impaired early in the course of HD, possibly because of the contribution of frontal-lobe dysfunctioning or, alternatively, because of disturbances in the ability of the basal ganglia to "integrate" frontal lobe functioning.

447.12

HIPPOCAMPAL SPECIFIC ATROPHY IS ASSOCIATED WITH DELAYED SECONDARY MEMORY IMPAIRMENT IN NORMAL HUMAN AGING. J. Golomb, A. Kluger, M.J. de Leon, A. Convit, H.Rusinek, A.E. George, S. de Santi, S.H. Ferris*. New York Univ. Sch. of Med. New York, NY 10016.

The hippocampal formation (HF) is an important mediator of declarative secondary memory; atrophy of this structure has been described in Alzheimer's disease and other amnestic disorders. HF dysfunction may also explain milder secondary memory deficits seen in normal aging. We used magnetic resonance imaging (MRI) to test the hypothesis that in normal human aging, after controlling for generalized cortical atrophy, volume reductions of the HF but not of the superior temporal gyrus (ST) are specifically associated with relatively impaired delayed secondary memory performance.

54 healthy elderly persons (ages 55-87, $\bar{x}$=69.0 ± 7.9) participating in a study of normal aging were consecutively selected on the basis of a Global Deterioration Scale score ≤ 2 and a Mini Mental State Exam score ≥ 28. Subjects were administered tests of primary memory (digit span), and tests of secondary memory with immediate and delayed recall components (paragraph, paired associate, list recall; facial recognition). Separate composite scores for the immediate and delayed components were created by combining, with equal weighting, the subtests of each category. The WAIS vocabulary subtest was used as a control measure for language and intelligence. Coronal T1 weighted MRI scans were used to derive head size normalized measurements of the HF, ST and extraventricular CSF (to estimate generalized cerebral atrophy).

After partialing out the effects of age, sex, WAIS vocabulary and generalized cerebral atrophy, normalized HF size was found to correlate significantly with delayed secondary memory performance ($r=.32$, $p < .05$), but not with immediate secondary memory or primary memory. Parallel analyses revealed no significant partial correlations for normalized ST size.

Our results suggest that HF size may be an anatomically specific correlate of delayed secondary memory function in normal human aging.

447.13

ANATOMIC AND COGNITIVE SUBGROUPS AFTER ACoA ANEURYSM. **L.M. Grattan[*1], P.J. Eslinger[3], D. Rigamonti[2], S.M. August[1], & A. Depatri[2]. Depts. of Neurology[1] & Neurosurgery[2], Univ. of MD Med. Sch. Baltimore, MD 21201 & [3]Penn State Univ. College of Med., Hershey, PA 17033.**

Based upon the study of a consecutive series of anterior communicating artery aneurysm (ACoA) patients we reported considerable heterogeneity in the nature of their memory disturbance and lesion sites. This investigation examines in detail patterns of memory disorders and their neuroanatomic correlates after rupture and surgical correction of ACoA aneurysms. We report the neuroanatomic and cognitive findings in a series of 8 patients studied during acute recovery. Lesion data were obtained from CT/MRI studies and intraoperative reports. Standard and experimental cognitive measures examined specific components of learning and memory. These included measures of attention, learning levels, rate of forgetting, subjective organization, spatial and temporal judgment and serial position effect. Findings indicated 5 distinct neuroanatomic subgroups: 1) isolated involvement of the basal forebrain, 2) involvement of basal forebrain together with the vascular territory of the recurrent artery of Heubner (raH), 3) involvement of the basal forebrain with raH and mesial frontal cortex, 4) involvement of the basal forebrain with mesial frontal cortex and 5) involvement of the basal forebrain, raH and lateral frontal cortex. Each subgroup presented with a different pattern of performance on standard and experimental measures of learning and memory. It is conceivable, that like its counterpart in neuroanatomy, patterns of memory impairment after ACoA aneurysm may reflect disruption of different components of memory.

447.14

DISTINCTIVE FORMS OF PARTIAL RETROGRADE AMNESIA. **P.J. Eslinger[*1], L.M. Grattan[2] & A. Easton[1]. Depts. of Neurology, [1]Penn State Univ. Coll. of Med., Hershey, PA 17033, & [2]Univ. of Maryland Med. Sch., Baltimore, MD 21201.**

In contrast to extensive retrograde amnesia (RA), partial forms of RA can elucidate component characteristics of memory disorder associated with select neural lesions. We describe 2 cases (EK, DR) of partial RA from asymmetric temporal lobe lesions after herpes encephalitis. Despite impaired scores on standardized RA measures, experimental probes indicated contrasting findings in the subjects. EK could not name famous faces from the past, nor complete famous name stems, or spontaneously match names and faces. On forced choice testing, her selection of famous names was barely above chance level but selection of famous faces and temporal ordering of faces were normal. These findings contrast sharply with DR who demonstrated normal name completion and name selection. However, DR was poor in famous face selection compared to name selection and when presented facial stimuli compared to name stimuli in name-face matching tasks. These cases show striking lesion localization differences with EK's lesion predominantly in the left medial (amygdala, hippocampus, parahippocampal gyrus) and lateral temporal regions, while DR's lesion was predominantly in the right homotopic areas. The findings indicate dissociated forms of partial RA with distinct left and right temporal cortex contributions to RA.

448.1

SCIATIC NERVE AXOTOMY INCREASES EXPRESSION OF BETA-ACTIN mRNA. L.M. Lund, C.H. Block and I.G. McQuarrie*. Neural Regen. Ctr., VA Med. Ctr., Cleveland, OH 44106.

During development, mRNAs for neuronal actin and tubulin are made in high quantities. After axons connect to appropriate targets, these mRNAs are downregulated. We are interested in axonal regeneration in the peripheral nervous system as a recapitulation of development. We looked at changes in mRNA levels for beta-actin, a protein which (with tubulin) is upregulated after axotomy. Bilateral sciatic nerve crush (or sham) was done at the level of the hip joint. Using an appropriate riboprobe, in combination with Northern blot analysis of mRNA extracted from spinal motor neurons, we found that beta-actin mRNA increases 3-5 fold at Day 4 postaxotomy (PA), whereas, levels are approximately normal at 28 hours and below control at Day 14. Transcription factors are known to be regulators of mRNA synthesis. We are interested in determining which transcription factors are responsive to axotomy. To study this, we used Northern blot analysis with riboprobes of several candidate factors. The Jun family is promising, and we found that Jun D expression is induced 3-fold by 28 hours PA, remains elevated at Day 4 PA, and returns to normal by Day 14.

448.2

THE MOLECULAR GENETICS OF NEURONAL GROWTH: CHARACTERIZATION OF THE Tα1 α-TUBULIN PROMOTER IN TRANSGENIC MICE. A. Gloster*, A. Speelman, J. Toma, E. Chan, and F.D. Miller, Dept. Anatomy. & Cell Biol., University of Alberta, Edmonton, Alberta, T6G 2H7.

The Tα1 α-tubulin gene is one member of the α-tubulin multigene family that is regulated as a function of neuronal growth in both developing and mature mammalian neurons. We have generated transgenic mice carrying a fusion gene comprised of 1100 nucleotides of the upstream, putative Tα1 promoter region linked to a nuclear β-galactosidase reporter gene. At embryonic days 14 and 16, a developmental period when expression of the endogenous gene is maximal, the transgene is expressed in the nervous system of 5 out of 8 founder lines, with expression varying independently of copy number. Although expression in one of these lines is only partial, expression in the remaining three occurs throughout the embryonic nervous system, in a pattern that is indistinguishable from that of the endogenous gene. Developmentally, expression of the transgene appears early during embryogenesis and appears to be coincident with neurogenesis. Expression of the transgene remains high during neuronal morphogenesis, at birth, and is subsequently downregulated following neural maturation, as indicated both by X-gal staining and by Western blot analysis. At all timepoints the transgene is specific to the nervous system. Thus, the sequences responsible for coupling gene expression to neuronal growth exist within this promoter region. We are currently analyzing the expression of this transgene in specific neuronal populations during development and following injury in mature neurons.

448.3

STABILIZED MICROTUBULES ARE ENRICHED IN THE PROXIMAL REGION OF AXONAL NEURITES DURING EARLY OUTGROWTH IN NB2a/d1 NEUROBLASTOMA TB Shea*, PA Paskevich, SL Vincent and ML Beermann McLean Hospital, Harvard Medical School, Belmont MA

Acetylation and detyrosination of α-tubulin are correlated with microtubule (MT) stabilization. We examined the appearance and distribution of stablized MTs during early (day 1-7) dbcAMP-mediated axonal neurite outgrowth in NB2a/d1 cells with antibodies specific for acetylated (acet), detyrosinated (detyr), tyrosinated (tyr), or all forms (total) of α-tubulin. Day 1 neurites contained tyr MTs along their length, but detyr MTs extended only into the proximal neurite region. Acet MTs were not visible at day 1, suggesting that this modification is delayed relative to detyrosination. We quantitated the relative distribution of stablized MTs in day 3 and day 7 neurites as follows. The neurite shaft of 50–100 cells in multiple fields was divided into approximate halves according to the phase-contrast image. Neurites were scored according to the relative intensity of immunoreactivity in the proximal (P) versus the distal (D) neurite half. The majority of day 3 and 7 neurites displayed roughly equivalent total and tyr MT reactivity in both P and D halves; the remaining percentages for each antibody were equally distributed between neurites displaying more staining in the P or D halves. By contrast, the majority of day 3 and 7 neurites displayed more intense acet and detyr reactivity in the P neurite half, with the remainder of neurites displaying roughly equivalent staining in P and D. Differential distribution of acet MTs was highlighted in one or more longitudinally-oriented planes of focus obtained in a Z-series during confocal microscopic analyses, suggesting that that acet MTs may also be differentially distributed through the neurite shaft radius. That the distribution of acet and detyr MTs differs from the total MT distribution indicates that these modified MTs represent a small percentage of total axonal MTs. This small subset of selectively stabilized MTs may reflect the existence of an older or more slowly transported MT population within the P half of the developing neurite.

-Supported by MH47349 and BNS-9010981

448.4

MANGANESE-INDUCED NEURITE OUTGROWTH IS ACCOMPANIED BY ELEVATED LEVELS OF MAP1B. J.M. Aletta*, M. Pacheco, J. Roth and K. Larsen Dept. Pharmacol. SUNY@Buffalo School of Medicine, Buffalo, N.Y.

Manganese chloride (Mn^{2+}) stimulates neurite outgrowth from a variant line of PC12 cells (Lin et al., *J. Neurosci. Res. 34:* 546-561, 1993). Mn^{2+}-treatment also induces peripherin and GAP-43 in these cells. The present study focuses on the microtubule-associated protein, MAP1B based upon its putative role in neurite initiation. We have examined the effects of Mn^{2+} (100 μM in RPMI+1%serum) on its steady state protein level and on its phosphorylation (P). Additional experiments using the wild type PC12 (WT) cells treated with Mn^{2+} were also carried out. Results from the variant line indicate that MAP1B levels rise as early as 12h after treatment. After 2d of treatment, MAP1B measured by western blot is elevated 4-fold (±0.8 SEM, n=4) relative to total cellular protein. Metabolic radiolabeling of MAP1B with 32P-orthophosphate followed by gel scanning densitometry showed that phosphate incorporation at this time is elevated 1.7-fold (±0.1, n=7). These results imply that a significant proportion of the elevated amount of MAP1B is not phosphorylated. In WT cells treated with NGF, process outgrowth is accompanied by increases in both the amount of MAP1B and its P. When treated with Mn^{2+}, WT cells flatten, but do not extend neurites of significant length (<20μm). Mn^{2+} induction of peripherin levels is also absent in WT. Regeneration of neurites from WT, however, can be stimulated transiently by 100μM Mn^{2+} (38±3.6% of the NGF response @12-20h n=6). Mn^{2+} can also potentiate the NGF response in WT as shown previously for the variant line, but these limited neuritogenic effects of Mn^{2+} are not accompanied by changes in P of MAP1B. We conclude that large increases in the P of MAP1B are not necessary for initiation of neurite formation. Variant PC12 cell lines that exhibit different phenotypes regarding neuritogenesis should facilitate further inquiry into the mechanisms of nerve process formation.

448.5

EXPRESSION OF MICROTUBULE-ASSOCIATED PROTEIN, MAP-1A, IN DORSAL ROOT GANGLIA (DRG). R.K. Yip*, L. Boyne, and I. Fischer. Dept. of Anatomy and Neurobiology, Medical College of Pennsylvania, 3200 Henry Ave., Philadelphia, PA 19129.

MAP-1A is a microtubule-associated protein expressed late in development of the nervous system. This protein has been proposed to play a role in the stabilization of the adult neuronal cytoskeleton, but no primary neuronal cultures had been available to study its expression. We prepared specific antibodies against purified MAP-1A and examined the distribution of the protein in DRGs in vivo and in purified neuronal cultures. The staining of MAP-1A in the DRGs was present in cell bodies and axons. However, not all the neurons stained to the same degree. Cultured DRG neurons from neonates did not exhibit MAP-1A staining. In contrast, the MAP-1A antibodies intensely stained the cell bodies of cultured adult DRG neurons, while the neurites stained mostly in their proximal regions. Our results demonstrate that adult DRG cultures can provide a unique neuronal system to study the expression and role of MAP-1A. Supported by NIH NS24707 and NS24725.

448.6

HIGH IMMUNOREACTIVITY FOR A PHOSPHORYLATED MAP1B ISOFORM CORRELATES WITH ADULT CNS AREAS THAT RETAIN CAPACITY FOR STRUCTURAL PLASTICITY. F. Nothias*, I. Fischer[1], P. Vernier and J.-D. Vincent., Inst. A. Fessard, CNRS, 91198 Gif/Yvette, France; [1]Dept. Anat. and Neurobiol., Med. College of PA, Philadelphia, PA 19129.

Microtubule-associated proteins (MAPs) are major structural components of the neuronal cytoskeleton and their expression is regulated during brain development. MAP1B is highly expressed in newborn rat brain and decrease dramatically in adult brain, particularly its phosphorylated isoforms. The present study was undertaken to determine whether MAP1B is specifically expressed in areas of adult rat brain which undergo morphological reorganization under particular physiological and experimental conditions. High immunostaining, using a monoclonal antibody 1B-P that recognizes only the phosphorylated isoform of MAP1B, was observed in restricted areas such as hypothalamic system, olfactory nuclei as well as other regions that also retain the expression of embryonic neural cell adhesion molecule (PSA-NCAM). At the cellular level, immunostaining of phosphorylated MAP1B was localized only in axons and not in perikarya. In contrast, immunostaining of total MAP1B, revealed by antibodies that recognize all its isoforms, was more widespread and not as restricted. MAP1B mRNA was also ubiquitous, present in most of the neurons. Since MAP1B, in particular its phosphorylated isoforms, is present predominantly in growing axons, our observations support the hypothesis of its involvement in morphological plasticity in adult CNS.

448.7

Expression and distribution of phosphorylated MAP1B in growing axons of cultured hippocampal neurons. L. Boyne, E. Paige, J. Deitch*, I. Fischer. Dept. of Anatomy and Neurobiology, Medical College of Pennsylvania, Philadelphia, PA 19129

Microtubule associated protein, MAP1B, is a major structural component of growing axons. It is expressed at high levels early in development including a phosphorylated isoform. In previous studies (Mol. Cell. Neurosci. 2:39, 1991) we have shown that in cultured primary hippocampal neurons MAP1B is sorted into growing axons and is concentrated in the distal tip of axons. In the present study a specific monoclonal antibody, 1B-P, against phosphorylated MAP1B was used to study the distribution of the phosphorylated isoform in hippocampal cultures compared to total MAP1B. During the early stages of minor process extension (1 day in culture) phosphorylated MAP1B was co-localized with total MAP1B and MAP2 in cell bodies and processes, but 1B-P labeled the growth cones more extensively. When neurons acquired axonal and dendritic polarity (2-4 days in culture), MAP2 was segregated to the dendrites, whereas MAP1B was concentrated in the axons. However, phosphorylated MAP1B became gradually restricted only to axons, and was not present in dendrites and cell bodies. By the end of the first week in culture, levels of phosphorylated MAP1B decreased, but that of total MAP1B decreased only after 3 weeks. Treatment of the hippocampal cultures with MAP1B antisense oligonucleotides resulted in a significant decrease in neurite outgrowth. These results suggest that MAP1B plays a role in axonal development and that it is phosphorylated in the growing axons. Supported by NIH grants NS24725 and T32NS07287.

448.8

THE LOCALIZATION OF PHOSPHORYLATED CYTOSKELETAL ELEMENTS DURING THE ESTABLISHMENT OF POLARITY BY CULTURED HIPPOCAMPAL NEURONS D.L. Benson*, F.H. Watkins, J. W. Mandell, and G. Banker Department of Neuroscience, University of Virginia School of Medicine, Charlottesville, Virginia 22908

The differential distribution of phosphorylated and non-phosphorylated neurofilaments is commonly used as a marker of neuronal polarity. SMI31, an antibody directed against a phosphorylated epitope of neurofilament H, is commonly used as a marker for axons. We investigated the development and distribution of SMI31 immunoreactivity in hippocampal cultures. Western blots revealed that at E18 (when hippocampal neurons are taken for culture) virtually all SMI31 immunoreactivity was concentrated in a band that comigrated with MAP1B rather than neurofilament H, confirming an earlier report by Fischer and Romano-Clarke (J. Neurochem. 55:328, 1990). When the blots were treated with alkaline phosphatase, all immunoreactivity for SMI31 was eliminated. In the first few hours after plating hippocampal neurons, SMI31 immunostaining was faint and uniformly distributed throughout neuronal cell somata and minor processes. When a single process exceeded the others in length, becoming the axon, SMI31 immunoreactivity became concentrated in that process. By 3 days, SMI31 immunoreactivity was entirely confined to axonal processes. Within the axon, immunostaining was distributed in a gradient which became more intense distally. SMI31 immunoreactivity was frequently excluded from the most proximal portion of the axon. We conclude that early in culture, the majority of SMI31 immunoreactivity represents MAP1B. The phosphorylated form of MAP1B can stabilize microtubules and is thought to play a role in neurite outgrowth by PC12 cells. In hippocampal neurons, as in cortical neurons (Mansfield et al., J. Neurocytol. 21:1007, 1991), MAP1B may contribute to the selective outgrowth of the axon that is an early event in the establishment of neuronal polarity. *Supported by NIH grant NS23094, NS09491 and postdoctoral fellowships MH10301 and NS09491.*

448.9

A SPATIAL GRADIENT OF TAU PROTEIN PHOSPHORYLATION IN NASCENT AXONS. J.W. Mandell*, D.L. Benson and G.A. Banker, Dept. of Neuroscience, University of Virginia School of Medicine, Charlottesville, VA 22908.

Tau, a major microtubule-associated protein, has been implicated as a critical molecule for axonogenesis (Caceres and Kosik, 1990; Nature, 343:461). Because the ability of tau to promote microtubule assembly is regulated by phosphorylation, we have measured tau phosphorylation at the subcellular level in cultured rat hippocampal neurons undergoing axonogenesis. Semi-quantitative analysis of tau phosphorylation was achieved by fluorescence ratio measurements on cells double-labeled with a combination of two anti-tau antibodies: tau-1, a monoclonal antibody recognizing a tau epitope only in its dephosphorylated state, and 7A5, a polyclonal antibody recognizing amino terminal epitopes on tau independent of phosphorylation state. Measurements on neurons cultured for 24 hr revealed three consistent findings: 1) the distribution of total tau protein was relatively unpolarized; it was found not only in axons but also in somata and minor processes (dendrite precursors). 2) the distribution of dephospho-tau was markedly polarized to the nascent axon. 3) the polarized axonal distribution of dephospho-tau was not discontinuous but took the form of a spatial gradient: the ratio of dephospho-tau to total tau increased smoothly in a proximo-distal fashion. Minor processes, whether from cells with axons or without, lacked a detectable phosphorylation gradient. Enzymatic dephosphorylation of fixed cells with alkaline phosphatase dramatically increased the tau-1 signal in all cellular compartments without affecting 7A5 immunofluorescence. The magnitude of the alkaline phosphatase effect was greater in somata and minor processes than in axons, consistent with the presence of more highly phosphorylated tau in these compartments. The generation of subcellular phosphorylation gradients may be an early step in neuronal polarization. Current studies are aimed at understanding the genesis of the tau phosphorylation gradient and the relationship between axonal growth and local control of tau phosphorylation. Supported by grants NS17112, NS07199, NS09491, MH10301.

448.10

PHOSPHORYLATION OF HUMAN TAU PROTEIN. S. Green, E. M. Clark, R. Carlsen*, and R. Vulliet, Dept. of Veterinary Pharmacol. and Tox., Dept. of Human Physiol., Univ. of Calif., Davis, CA 95616

Human tau protein was observed to be multiply phosphorylated when expressed in SF-9 cells. Infection of SF-9 cells with tau containing baculovirus resulted in the multiple bands of tau protein. Exposure of the infected cells with okadaic acid, a phosphatase inhibitor, decreased the rate of migration of tau protein when analysed by SDS PAGE. Treatment of the BCV expressed-tau with either alkaline phosphatase or hydrofluoric acid increased the rate of tau migration. This rate of migration was identical rate to tau protein that was expressed in *E. Coli.* Using ^{32}P-labelled inorganic phosphate to label tau *in situ*, the rate of migration of the tau protein was inversely related to its degree of phosphorylation. Tryptic analysis of the phosphorylation sites revealed the presence of sites in addition to those known to be phosphorylated by the proline-directed protein kinase (cyclin A/p34 kinase).

448.11

THE LOCALIZATION OF CALCINEURIN IN CULTURED NEURONS AND THE FUNCTIONAL CONSEQUENCES OF ITS INHIBITION. A. Ferreira, R.L. Kincaid and K.S.Kosik*, Harvard Med. Sch. Boston, MA 02115 and NIAAA, Bethesda, MD 20852.

In vitro studies have shown that calcineurin (CN), a Ca^{2+}/calmodulin dependent protein phosphatase highly enriched in neurons, is involved in the dephosphorylation of tau, MAP-2 and tubulin among other substrates. Here we show that CN is localized at the growing tip of neuritic processes in cultured cerebellar macroneurons. This localization depends upon intact actin filaments and microtubules. These observations suggest that CN is well positioned to mediate the interaction among different cytoskeletal elements during neurite outgrowth. To test this hypothesis, E15 cerebellar macroneurons were treated with the CN inhibitors, cyclosporin A and the autoinhibitory peptide C, for 24 hs. The results show that : 1) although treated cells normally extend minor neurites they failed to elongate one as an axon; 2) tau proteins no longer remain attached to microtubules; 3) there is a lack of immunoreactivity for Tau-1, an antibody directed to a tau dephosphorylated epitope, both by immunocytochemistry and immunoblot analyses; 4) acetylated microtubules are present in a single neurite, suggesting that microtubule stabilization, an early step in polarity acquisition has taken place. Taken collectively, these results suggest that CN may be involved in axonal elongation by regulating the state of phosphorylation of tau proteins. This phosphorylation event may regulate tau binding to microtubules and the rapid elongation phase of neurite outgrowth.

448.12

DEVELOPMENTAL EXPRESSION OF A NOVEL SET OF MICROTUBULE-ASSOCIATED PROTEINS DURING CELL DIFFERENTIATION IN THE RAT BRAIN. T.A. Comery*, N. Hawrylak, T.L. Karr and W.T. Greenough. Depts. of Psych., Biochem., Cell and Structural Biol., Neuroscience Program, and Beckman Institute, Univ. of Illinois, Urbana, IL 61801.

We have been investigating immunoreactivity to three novel proteins identified in Drosophila that co-purify with the microtubule fraction (Alcantara et al., *Soc. Neur. Abst.*, 1992; Srinivasan et al., *Soc. Neur. Abst.*, 1992). Immunoblot analysis indicates that these proteins are developmentally regulated in the rat cerebellum (see adjacent poster). Here we describe the spatiotemporal patterns of expression of immunoreactivity to the three antibodies (termed FLAT-MAPs 1, 2 and 3) on postnatal days 1, 5, 7, 10, 14, 21 and 35.

Expression of immunoreactivity to all three antibodies within the cerebellum displays no consistent pattern prior to P7. Expression of FLAT-MAPs 1 and 3 is diffuse throughout the cerebellar cortex at P5 with punctate labeling of Purkinje cell somata and dendrites at P7 and P10. Diffuse labeling in molecular, Purkinje cell and granule cell layers diminishes gradually following P10. FLAT-MAP2 is consistently observed in parallel fibres at P7 but not at earlier or later developmental ages. All three cerebellar cortical layers express diffuse FLAT-MAP2 labeling prior to P7, with expression declining to background levels at later developmental ages. Similar developmental regulation of the expression of these proteins is observed in other brain regions. The expression of these proteins corresponds to periods of process extension, cell differentiation and synapse formation.
Supported by MH40631 and NSF BNS 88 21219.

448.13

CHARACTERIZATION OF THREE NOVEL MICROTUBULE-ASSOCIATED PROTEINS EXPRESSED IN *DROSOPHILA MELANOGASTER* EMBRYOS AND RAT BRAIN. S. Srinivasan, C.Q. Doe* and T.L. Karr Dept. of Biochem., and Cell & Structural Biology, Beckman Institute, Univ. of Illinois, Urbana, IL 61801

Microtubule-Associated Proteins (MAPs) are a group of heterogeneous proteins that co-purify with tubulin during microtubule purification. The functional roles of MAPs in the cell are poorly understood. We are interested in using the *Drosophila* embryo to characterize neural MAPs. We have used purified MAP fractions from 16 hour *Drosophila* embryos as antigens and obtained three monoclonal antibodies of which two recognize proteins expressed in the *Drosophila* ventral nerve cord. These three antibodies recognize proteins in rat brain and hence we have named these cross reactive antibodies FLy-rAT or FLAT-MAPs. Two dimensional immunoblots with microtubule proteins from 6 and 16 hour *Drosophila* embryos with FLAT-MAPs 1,2 & 3 indicate that these proteins are expressed in different isoforms during embryonic development. Western blots with cerebellar cell extracts obtained from rats at post natal days 1,5,7,10,14,21,35 & 90 indicate that FLAT-MAPs 1,2, & 3 increase and subsequently decrease during development of the rat cerebellum. Furthermore, all three FLAT-MAPs are present in the purified (Microtubule Proteins) MTP fraction from rat brain. Immunoblots with FLAT-MAPs 1,2, & 3 were performed on *Drosophila* MTP taken through four cycles of assembly and disassembly. FLAT-MAPs 2 & 3 were determined to be Quantitative MAPs or Q-MAPs since the ratio of MAP/tubulin remains constant through successive assembly/disassembly cycles.

448.14

ANALYSIS OF mRNA AND PROTEIN DISTRIBUTION OF THE MICROTUBULE-ASSOCIATED PROTEIN TAU DURING NEURONAL DIFFERENTIATION. P. LoPresti*, Y. Wang, R.P. Zinkowski and L. I. Binder. Dept. of Cell Biology, Univ. of Alabama at Birmingham, Birmingham, Alabama 35294-0005.

The microtubule associated protein tau is concentrated in the axons of neurons. However, tau proteins are also found in the somatodendritic compartment as well. Recently, our laboratory reported the presence of nuclear tau in cultured human cells concomitant with the expression of a novel 2 Kb tau transcript (Wang et al. J. Cell Biol. 121:257-267, 1993). We have utilized fluorescence microscopy to analyze tau protein distribution by immunofluorescence and tau mRNA distribution by Fluorescence In Situ Hybridization (FISH) during differentiation of the human neuroblastoma cell line SHSY5Y induced by aphidicolin and nerve growth factor. Immunostaining of undifferentiated SHSY5Y cells with the Tau-1 antibody demonstrated the expression of both nuclear and cytoplasmic tau. Detection of tau mRNA by FISH using a labeled 3-repeat tau cDNA revealed that the majority of tau message displayed perinuclear localization. In differentiated SHSY5Y we observed reduced nuclear tau immunostaining coupled with a dramatic increase in tau immunostaining along numerous neuronal processes. As well, FISH revealed a redistribution of tau mRNA during differentiation. Tau mRNA appeared to be distributed uniformly throughout the cell body and many of the processes formed during differentiation exhibited patches of fluorescence suggesting that pools of the message are targeted to specific regions of neurites. As expected, some extensions did not have any staining, suggesting that they represent axon-like neurites. Currently, we are attempting to identify tau mRNA positive extensions as dendritic- or axon-like neurites by differential immunostaining and FISH. We are also attempting to correlate the changes in nuclear versus cytoplasmic tau immunostaining with alternative targeting of tau mRNA in undifferentiated versus differentiated cells. (Supported by NIH grant AG06969 to L.I.B.)

448.15

DYNAMICS OF PHOSPHORYLATION AND ASSEMBLY OF THE HIGH MOLECULAR WEIGHT NEUROFILAMENT PROTEIN IN MOUSE CNS I Fischer, ML Beermann, RA Nixon, MA Edwards* and TB Shea
McLean Hospital, Shriver Center, Harvard Med. School, Med. Coll. Penn.

Phosphorylation of the high molecular weight neurofilament subunit (NF-H) results in multiple isoforms that migrate on SDS-gels at approximately 200kDa (Hphos). Hphos was detectable in immunoblots of cytoskeletal fractions at embryonic day 17 (E17), increased from postnatal day 14 (P14)-P60, and remained high from P60-P120. A transient appearance of Hphos was detected in the Triton-soluble fraction from P14-60 by 3 different antisera directed against Hphos and a monoclonal antibody (SMI-31) directed against phosphorylated NF-H and NF-M epitopes. Certain polyclonal antisera demonstrated that over 50% of the H-200 was Triton-soluble between P14-60. However, soluble Hphos was not reactive with RT97 or SMI-34, although cytoskeletal Hphos was strongly reactive with these antibodies, indicating that certain phosphorylation events are only characteristic of cytoskeletal Hphos. Alkaline phosphatase treatment diminished Triton-soluble Hphos immunoreactivity. Unlike NF-H, NF-M and NF-L were at all times present only in the cytoskeletal fraction.

Retinas and proximal axons of adult mouse retinal ganglion cells contain Triton-soluble Hphos as determined by immunocytochemical and immunoblot analyses with SMI-31 and polyclonal anti-Hphos antibodies. Pulse-chase radiolabeling analyses followed by immunoprecipitation demonstrated that a percentage of newly-synthesized NF-H subunits were converted to Hphos forms within retinas before they were axonally transported and incorporated into Triton-insoluble structures. These findings raise the possibility that NF-H assembly behavior may differ from that of NF-M and NF-L during postnatal development, and that the interaction of NF-H with neurofilaments may be more dynamic than is generally recognized.

–Supported by MH47349, BNS-9010981, AG05604, NS24725

448.16

NEUROFILAMENT PROTEINS AS MARKERS FOR NEURITE MATURATION IN DISSOCIATED EMBRYONIC SPINAL CORD CULTURES OF *XENOPUS LAEVIS*. W. Lin* and B.G. Szaro. Dept. of Biol. Sci., SUNY-Albany, Albany, NY 12222.

Dissociated embryonic spinal cord cultures of *X. laevis* have been used to study channel activities, membrane properties, synaptogenesis and tubulin dynamics of developing neurons. A major question in any dissociated culture system is how closely it matches what happens in situ. During development, newly differentiated neurons express neurofilament (NF) proteins in a progression indicative of axonal maturation. We used a panel of NF antibodies, previously characterized on developing spinal cord neurons in situ, to assess neuritic development in *Xenopus* cultures started at stage 22. These antibodies react with two low molecular weight neurofilament proteins [NF-L and *Xenopus* neuronal intermediate filament protein (XNIF)], non-phosphorylated NF-M and NF-H, and two distinct phosphorylated epitopes of NF-M. As determined by immunofluorescence, both XNIF and non-phosphorylated NF-M began to appear in neurites within the first 3 hours. Gradually over the next two days, NF-M in neuritic processes became progressively more phosphorylated, and NF-L was also expressed. These early events followed a similar timecourse in culture as in situ. However, few cultured neurons survived long enough to have mature axons expressing NF-H. On the third day of culture, less than 10% of surviving neurons expressed NF-H. Nevertheless for the first two days in culture, neurites closely resembled axons differentiating in situ. NIH R29-NS30682.

448.17

A PHOSPHORYLATION-DEPENDENT EPITOPE OF THE NEUROFILAMENT PROTEIN NF-M EVOKED IN CHICK SENSORY AXONS BY SECOND MESSENGERS AND TURNING. M. Szente and L. Landmesser*, Dept. of Physiology & Neurobiology, University of Connecticut, Storrs, CT 06269-3042.

In vivo, Mab 5E10 recognizes a phosphorylation dependent epitope of NF-M that is expressed by chick lumbosacral sensory and motor axons during initial outgrowth beginning at specific points where subsets of growth cones diverge to grow toward their own targets, apparently upon detection of target-derived guidance cues. In explant cultures of E6-7 day DRG neurons two types of 5E10 expression were evoked. First, a variety of external stimuli including depolarization, calcium ionophore and phorbol ester treatment caused a transient increase in expression of 5E10 that returned after several hours to the low background level characteristic of DRG neurons. Expression was reduced below background by treatments that inhibit PKC, such as long term-TPA, staurosporine, and sphingosine. Second, a much more intense and stable expression was evoked in the growth cones and distal neurites of cells as they encountered a boundary, and expression was maintained in that portion of the neurite that turned and continued to grow along the boundary. Associated with this was a cytoskeletal rearrangement in which more of the 5E10 antigen was incorporated into the triton-insoluble cytoskeleton, and in which neurites became more resistant to agents that cause axon retraction, including those that depolymerize microtubules. Axons treated with staurosporine did not increase 5E10 expression at the boundary and did not turn. It is suggested that 5E10 expression and/or some associated cytoskeletal rearrangement is part of a mechanism by which growth cones respond to certain extrinsic guidance cues and alter their trajectories.

448.18

CYTOSKELETAL MECHANISMS AND SUBSTRATE INTERACTIONS THAT INITIATE NEURITE OUTGROWTH. C.L. Smith*. Lab. of Neural Control, NINDS, NIH, Bethesda, MD 20892.

Neurite formation by chick peripheral ganglion neurons grown *in vitro* involves invasion of filopodia by cytoplasm from the perinuclear region. In cultures grown on polyornithine, invasion can be initiated by contact of the tip of a filopodium with a large polyornithine-coated polystyrene bead. After the tip of the filopodium attaches to the bead, the filopodium detaches from the substrate and straightens, suggesting that it is under tension. Cytoplasm begins to invade the filopodium just as it begins to straighten or shortly thereafter. Concurrently, the entire cell body moves toward the base of the filopodium. For this sequence of events to occur, the bead must be attached to the substrate and, if it detaches, the cytoplasm retracts back into the cell body. A model of the cytoskeletal interactions that produce these movements is proposed, based on the observation that filopodia retrogradely transport small beads that attach to their surfaces and the hypothesis that the beads move because they are coupled to actin filaments that are pulled retrogradely by molecular motors (Sheetz, et al., Cell 61: 231, 1990). The present model suggests that when a large, immobile bead becomes coupled to actin filaments, the retrograde movement of the filaments is impeded. The stress produced by the molecular motors pulling actin filaments toward the cell body straightens the filopodium, causing it to detach from the substrate. The same motors might pull perinuclear cytoplasm into the filopodium. The prediction that actin filaments in filopodia cease moving retrogradely when they become distally anchored is currently being tested by monitoring the movements of small beads on the surfaces of filopodia while their tips attach to large beads.

448.19

TIME SERIES ANALYSIS OF GROWTH CONE MOTILITY AND SIMULATED MICROTUBULE DYNAMICS. D.J. Odde* and H.M. Buettner. Deptartment of Chemical and Biochemical Engineering, Rutgers University, Piscataway, NJ 08855.

Cytoskeletal dynamics are an important feature of neurite outgrowth. An outstanding problem is the relationship between microtubule assembly and growth cone motility. Here we apply time series analysis to provide an objective and quantitative framework for characterizing the net displacements of rat superior cervical ganglion (SCG) growth cones and simulated microtubules. Microtubule length life histories were simulated assuming constant growth and shrinkage rates coupled with random selection of growth and shrinkage times from gamma probability distributions, a formulation based on the dynamic instability model of microtubules. A basic parameter set was chosen, 10 μm/min growth and shrinkage rates and 60 sec mean growth and shrinkage times, and then varied over several orders of magnitude. Net microtubule displacements were calculated over discrete time intervals and characterized using the power spectrum as well as the autocorrelation and partial autocorrelation functions. Simulated microtubules showed significant autocorrelation and periodicity depending on the sampling rate and the dynamic parameters. The time series analysis was sensitive to variations in the growth and shrinkage time probability distributions and the mean time spent in one phase relative to the other but not to the growth and shrinkage rates. Since growing axons contain parallel arrays of microtubules, we simulated and characterized noninteracting microtubule populations. The time series behavior was not significantly different than that for a single microtubule with the same parameter set. For comparison, we characterized experimental measurements of rat SCG growth cone centroid displacements in the directions parallel and perpendicular to the axon. Five growth cones had uncorrelated, nonperiodic displacements while 1 was correlated and periodic. Our results indicate that growth cones and simulated microtubules possess a variety of potential dynamic behaviors, in keeping with the complex and highly adaptable nature of the nerve growth cone.

Supported by NSF Grant BCS 92-10540.

448.20

Cytoskeletal changes and retarded neurite outgrowth accompany the inhibition of AChE in non-cholinergic neurons. J.L. Dupree and J.W. Bigbee*. Dept. of Anatomy, Med. Coll. Va., Richmond, VA 23298

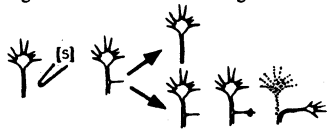
While various non-cholinergic roles for AChE have been suggested, a consistent finding is its transient expression during neurite outgrowth. Our previous results have shown that pharmacological inhibition of AChE retards neurite outgrowth from non-cholinergic dorsal root ganglion neurons (DRGN) grown on collagen. In the same system, neurite outgrowth and AChE activity co-vary with the permissiveness of the extracellular matrix (Gupta and Bigbee, J.Neurosci.Res., 31:454,1992). In the present study, dissociated DRGN from E15 rat embryos were grown on Type I collagen or the reconstituted basal lamina, Matrigel™ in the presence of BW 284c51 (BW), a specific AChE inhibitor, at 10^{-4}M to 10^{-7}M. After 14 days, morphometric analyses of outgrowth and electron microscopy were performed while additional cultures were allowed to recover from the BW treatment. BW treatment resulted in a dose dependent reduction in outgrowth on both substrata with more pronounced inhibition on the less permissive collagen. Analysis of cell number revealed no difference in cell survival between groups and removal of BW resulted in normal recovery of outgrowth. Interestingly, the cell bodies of BW-treated neurons contained large spiralling masses of 8-10 nm filaments which resembled Pick's bodies or inclusions seen in ganglioneuromas and were often associated with large Golgi bodies. These data suggest that AChE may play a role in axonal outgrowth and that the induction of cytoskeletal accumulations may provide a model for reversible neuropathies.

PROCESS OUTGROWTH, GROWTH CONES, AND SPROUTING VII

449.1

IN VITRO INDUCTION OF BACKBRANCHES: IMPLICATIONS FOR AN ALTERNATE MECHANISM OF TARGET LOCATION. C.V. Williams*, R.W. Davenport, P. Dou, S.B. Kater. Anatomy and Neurobiology, Colorado State University, Ft. Collins, CO 80523.

Backbranches may play a critical role in the formation of neuronal connections during development. One model suggests that filopodial-like outgrowths can be induced along the neurite shaft in response to environmental stimuli [S]. If appropriate cues are detected, the outgrowth could be maintained, transformed to a backbranch and possibly become the primary axon.



We have begun to study the hypothesis that filopodial-like outgrowths can become neurites, which eventually form synaptic connections. We have studied the *in vitro* induction of backbranches in response to locally applied stimuli known to induce growth cone filopodia in cultured Helisoma neurons. Outgrowths can be consistently induced along relatively smooth neurites in response to a locally applied electric field (>90%, n>50). These outgrowths rapidly elongate toward the electric field source, often reaching lengths of 30-40um. These dimensions are comparable to those of induced filopodia. Also like filopodia, induced outgrowths orient toward the cathode side of the field. This phenomenon is a developmentally constrained process, since these outgrowths can not be induced along neurites of neurons at stable state. This is the first *in vitro* evidence demonstrating the initial steps in the induction of backbranches and supports the first step in the proposed model.

449.2

DIFFERENTIATION OF *DROSOPHILA* PHOTORECEPTOR CELLS IN PRIMARY CULTURES. Chinglu Li* and I.A. Meinertzhagen. Life Sciences Centre, Dalhousie University, Halifax, Nova Scotia, Canada B3H 4J1.

In order to procure synaptogenesis and explore possible trophic interactions in *Drosophila melanogaster* between cultures of photoreceptor and optic lobe cells we have developed a primary culture system and optimized the conditions for co-culturing the two cell types. Eye imaginal disc fragments and optic lobe cells from wild-type 3-hr white pupae were cultured in several media using supplements of fetal bovine serum (FBS). Eye discs were more exacting in their culture requirements than optic lobe cells. While optic lobe cells survive and differentiate well in nearly all conditions tested, the best survival of, and neurite outgrowth from, eye disc cultures was obtained with MM3 medium supplemented with 2% FBS or from 27% Liebovitz L-15 medium with 5% FBS. Greater FBS concentrations delayed neurite outgrowth, while with lower FBS concentrations both survival and neurite outgrowth were poorer. Both eye disc fragments and optic lobe cells underwent mitoses in both culture conditions;-optic lobe BrdU incorporations were most frequent after 2 hrs *in vitro*, declining about 50% by 10hrs, mirroring development *in vivo*. Vigorous neurite outgrowth was observed from both cultured cell types. Ommatidial clusters recognizable in some eye disc fragments persisted during the culture period. By 4d *in vitro*, a transparent cornea-like sheet covered some eye disc fragments. Both optic lobe and eye disc fragment cells were immunoreactive to anti-HRP, an insect neuronal marker. In addition, eye disc cultures were immunoreactive to photoreceptor-specific monoclonal antibodies 22C10 and 24B10. We also examined histamine immunoreactivity, which is found in the mature photoreceptor. Cultured eye disc fragments and their neurites were histamine-immunoreactive, and double staining revealed that histamine positive fibers were also immunoreactive to 24B10. Absence of histamine immunoreactivity in some 24B10 positive cells could reflect earlier onset of immunoreactivity to 24B10 than histamine.

Supported by grants from the Killam Fund (C.L.) and NIH EY-03592 (to I.A.M.).

449.3

***IN SITU* HYBRIDIZATION REVEALS CHANGING DISTRIBUTION OF LAMININ EXPRESSION DURING NEURONAL DEVELOPMENT AND AFTER INJURY IN THE LEECH CNS.** A.E. Luebke*, I.M. Dickerson, and K.J. Muller. Dept. of Physiol. & Biophysics and GRECC, Univ. of Miami Med Sch, Miami, FL 33101

A laminin-like molecule extracted from the leech nerve cord promotes neurite outgrowth *in vitro* (Masuda-Nakagawa *et al.*, 1988). Laminin-like immunoreactivity, which in the adult CNS is confined to the perineurial sheath, reappears within the CNS after injury. Yet, the source of laminin is not known. To determine which cells in the leech CNS are synthesizing laminin, a probe was generated for leech laminin by degenerate PCR based on conserved regions of vertebrate and *Drosophila* B2-laminins.

Messenger RNA was isolated from juvenile leeches, reverse-transcribed, and subjected to PCR. The resulting probe was 1.3 kB in length, and showed striking sequence homology to other laminins. A digoxigenin-labeled riboprobe was synthesized using RNA polymerase and visualized using an alkaline phosphatase-conjugated antibody.

In 10-14 day embryos, laminin is expressed in cells throughout the perineurial sheath and in the vicinity of axon-bundles. In embryos older than 17 days and adults, laminin expression is confined to the perineurial sheath despite probe access deeper within the ganglia and connectives. If a crush is made to the connectives in either 17 day old embryos or adults, laminin expression markedly increases within the nerve cord, matching published increases in appearance of laminin immunoreactivity. Preliminary results indicate that microglia can synthesize laminin at the crush site. (Supported by NIH and AHA grants).

449.4

DYNAMIC ANALYSIS OF ACTIN FILAMENTS IN GROWTH CONES. C.S. Cohan* and P. Torreano. Depts. of Anatomical Sciences and Biophysics, SUNY at Buffalo, Buffalo, N.Y. 14214.

The motility of growth cones is closely associated with their morphological properties. For example, stimuli that inhibit growth cone movements cause a loss of filopodia and retraction of the lamellipodium. The morphological properties of growth cones are derived from their cytoskeleton, which consists of microtubules and actin filaments. The distribution of these components differs such that microtubules are restricted to the central mound whereas actin filaments are present throughout the lamellipodium as a meshwork and as bundles in filopodia. The focus of this work was to determine whether changes in growth cone motility and structure result from changes in the organization of actin filaments.

Actin filaments in growth cones were directly visualized using a fluorescent precursor, g-Actin. Neurons of the pond snail Helisoma were pressure injected with rhodamine-labeled g-Actin. Pictures were acquired using a CCD camera at exposures of 400 msec.

Three to four hr after injection of g-Actin into cell bodies, actin filaments were brightly stained in growth cones. Actin bundles in filopodia as well as actin filament meshwork in the lamellipodium could be visualized. Dynamic movements of actin filaments were studied in isolated neurons by time-lapse images acquired at 1-2 min intervals. These movies demonstrated the retrograde movement of actin filaments in both the lamellipodial meshwork and filopodial bundles. Application of KCl, as reported previously, caused retraction of the lamellipodium and loss of filopodia. Actin filaments were still visible in these growth cones, and actin bundles extended through filopodia that lengthened as growth cone retraction ensued. These experiments make it possible to correlate changes in actin filaments with alterations in growth cone function.

(Supported by NIH grant # NS25789)

449.5

DIFFERENCES IN REGENERATIVE GROWTH FROM CRAYFISH TONIC AND PHASIC MOTOR AXONS. K. Egid* & G.A. Lnenicka Dept. of Biol. Sci., State Univ. of New York, Albany, New York 12222

Terminals of tonically and phasically active motoneurons of crayfish have characteristic differences in physiology and morphology (Lnenicka, N.Y. Aca. Sci. 627: 197-211, 1991). To examine the development of these differences, we used an explant of the crayfish abdominal nerve cord in which tonic and phasic axons grow from separate branches of the third roots onto a polylysine substrate. The *in vitro* axonal growth differed between tonic and phasic neurons. The rate of neurite outgrowth from phasic axons (X=1.72 mm/day) was significantly greater than for tonic axons (X=0.36 mm/day). Neuritic arbors of the phasic axons had a denser appearance due to the fact that they produced twice as many branches within the same area as did tonic axons (67.4 vs 33.4). Growth cones of phasic axons were larger than those of tonic axons. Our results suggest that some of the morphological differences between the tonic and phasic motor terminals are related to differences in the growth programs of the motoneurons. For identified motoneurons, we are further quantifying the relationship between the neurite branching pattern and diameter observed *in vitro* and the morphology of motor terminals examined *in situ*. In addition, we are investigating the role of impulse activity in these differences in axonal growth. (Supported by NSF grant IBN-9121757)

449.6

CYPROHEPTADINE BLOCKS 5-HT INHIBITION OF LESION-INDUCED SPROUTING IN THE SNAIL CNS. M. W. Baker* & R. P. Croll. Dept. of Physiol. & Biophys., Dalhousie Univ. Halifax N.S. Canada.

We have previously documented (Baker & Croll, Soc. Neurosci. Abstr, 18) that, following a cut to the cerebral-buccal connective in the snail *Achatina fulica*, serotonin content in the ipsilateral buccal ganglia is depleted as a result of distal degeneration of fibers from the ipsilateral, serotonergic, metacerebral giant (MCG) cell. Dye-injections, electrophysiological measures and HPLC quantification all suggest that the uninjured, contralateral MCG normally sprouts into the denervated buccal ganglion, but this sprouting can be suppressed by injections of 5-HT (every second day) following the lesion. These findings suggest that neuritic sprouting by the MCG in the buccal ganglia may normally be tonically inhibited by the serotonergic innervation of that ganglion.

In this report we use selected serotonergic antagonists to investigate the receptor(s) involved in this inhibition. Intracellular recordings from the MCG soma reveal that focally applied 5-HT results in a slow depolarization, which is characterized by an increase in R_m and which reverses near E_{K^+}, suggesting that one action of 5-HT may be a decreased gK^+-dependent depolarization. This depolarization is effectively blocked *in vitro* by the vertebrate 5-HT_2 blocker cyproheptadine (CYP). CYP (0.5 mg in saline) when administered 1 hour prior to injection of 5-HT (0.5 mg in saline every second day) also blocks the inhibitory influence of 5-HT on MCG sprouting following the lesion; sprouting by the uninjured MCG 4 weeks following the lesion is within the range expected of untreated lesioned animals and lesioned animals treated by CYP alone.

These results provide *in vivo* evidence that a neurotransmitter can exert receptor-mediated influences upon neuronal outgrowth and provide a model system in which to examine how 5-HT might govern normal innervation in an intact nervous system.

Supported by a grant from NSERC (Canada) to R.P.C.

449.7

THE DEVELOPMENT OF THE PERIPHERAL TERMINAL FIELDS OF AP AND DORSAL T NEURONS DEPENDS ON PIONEER DORSAL P NEURONS W.B. Gan, L.R. Wolszon* and E.R. Macagno Dept. of Biological Sciences, Columbia University, New York, N.Y. 10027

Pioneer neurons are necessary for the initial outgrowth of late-developing neurons in some systems. This study investigates their function in the formation of the terminal fields of some late-developing neurons in the leech *Hirudo medicinalis*. The dorsal pressure-sensitive neurons (P_D) are among the first neurons to send axons along the dorsal-posterior nerve towards the dorsal body wall. By staining with two different fluorescent dyes (DiI and DiO), the peripheral branches of the AP and T_D neurons are found to follow precisely the processes of the P_D cells, up to their fourth-order branches. Four to five days after killing the P_D cell at embryonic day 8, the AP and T_D cells showed significantly reduced axonal outgrowth and dramatically abnormal branching patterns in the dorsal body wall. This result suggests that the P_D cell is a pioneer neuron that is critical in promoting axonal outgrowth of the AP and T_D neurons, and in guiding the normal formation of their terminal fields. Interestingly, the P_D cells have large numbers of filopodia along their axons and at their growth cones, most of which disappear gradually, whereas the AP and T_D cells have few or no filopodia. Currently, we are investigating the function of these filopodia in pathfinding by these neurons.

449.8

APLYSIA HEMOLYMPH ENHANCES NEURITE OUTGROWTH FROM IDENTIFIED HELIX NEURONS IN CELL CULTURE . Santarelli L. Ghirardi M. Casadio A. Montarolo P.G. Dept. of Human Anatomy & Physiology, C.so Raffaello 30 - 10125 Torino. Italy(Spon:ENA)

Isolated cells grown in culture provide an excellent system for examining synapse formation and plasticity. We have obtained a robust growth of identified neurons from the cerebral ganglion of Helix Pomatia in dissociated cell culture. The two large cerebral neurons C1 and C3 from small adult animals were isolated by enzymatic and mechanical dissociation and plated onto polylysine-coated dishes that contained either conditioned medium or that were pretreated with Aplysia hemolymph. The conditioned medium was obtained by exposing Helix cerebral and circumoesophageal ganglia in L15, adjusted to the osmolarity to the Helix hemolymph, for 72 hrs. The Aplysia hemolymph was added to cover the bottom of the polylysine-treated dishes and left for at least 24 hrs, being removed just before the cells were added..

We have compared the growth effect of the two conditioning factors by measuring neurite elongation and the extent of branching. We have found that: 1) with both conditioning factors cells isolated with their axons remain essentially polar, whereas cells without their original axons sprout numerous processes from their cell bodies. 2)The Aplysia hemolymph remarkably enhances the extent and rate of linear neurite outgrowth and the degree of branching. Hemolymph also decreases the diameter of the outgrowing neurite fascicle. Intracellular recording made in Helix saline showed that the resting and action potentials of cultured cells were comparable to those of cells in vivo.

449.9

CILIARY NEUROTROPHIC FACTOR INDUCES NEURITE OUTGROWTH IN CULTURED HELISOMA NEURONS. D.-B. Wang, A. G. M. Bulloch* and K. Lukowiak. Dept. Med. Physiol. and Neurosci. Res. Group, Univ. of Calgary, Calgary, Alberta, Canada T2N 4N1

The response of identified neurons from the fresh water snail, Helisoma trivolvis, to different neurotrophic conditions was investigated in vitro. Individually isolated motoneurons or interneurons from adult Helisoma central nervous system were cultured in defined medium (DM), DM supplemented with recombinant rat ciliary neurotrophic factor (CNTF), or brain-conditioned medium (CM). These neurons did not exhibit significant neurite outgrowth in DM alone. However, the cells showed extensive neurite outgrowth after two to three days in either CM or CNTF-supplemented DM. This observation, together with previous experiments on the related mollusc Lymnaea (Bulloch et al., Soc. Neurosci. Abstr, 1992), supports that CNTF-like factors may exist in more than one mollusc species. This result also indicates that the effect of CNTF on neurite outgrowth is not mediated indirectly via non-neuronal cells or other factors but directly on the neurons themselves, suggesting the existence of CNTF receptors on these cells. Supported by MRC (Canada).

449.10

NEURITE OUTGROWTH MAY BE INHIBITED BY A RETROGRADELY TRANSPORTED FACTOR IN A *HELISOMA* NEURON. P. J. Kruk* and A. G. M. Bulloch. Department of Medical Physiology and Neuroscience Research Group, University of Calgary, Calgary, Alberta, Canada, T2N 4N1.

This report continues our study of the mysterious failure of axonal regeneration that can occur in buccal neuron 4 (B4) of the mollusc *Helisoma trivolvis*. This neuron has two axons which innervate the two salivary glands. Both axons can regenerate; however, the ipsilateral axon fails to regenerate when axotomized close to the soma if the contralateral axon is left intact (*J. Neurosci. Res.* 31: 401-412, 1992). This failure occurs both *in vivo* and in organ culture. To test the possible involvement of target-derived factors in this phenomenon, we attempted to block retrograde axonal transport in the intact contralateral axon by local exposure of its distal segment to the antimitotic drug colchicine. Two series of experiments were performed on cultured buccal ganglia with the salivary glands attached. First, the effectiveness of 1 μM colchicine to block retrograde transport in neuron B4 was demonstrated using Fluoro Ruby. Second, contralateral block of transport after proximal ipsilateral axotomy resulted in an increased success of axonal regeneration of the ipsilateral axon (blind assessment, $p < 0.05$, Mann-Whitney test). This result suggests that the failure of regeneration may be attributed to a factor transported retrogradely from the terminals of the intact contralateral axon. **Supported by M.R.C. (Canada) and Alberta Heritage Foundation for Medical Research.**

450.1

FIRST INGROWTH OF RETINAL AXONS IN THE RHESUS MONKEY EMBRYO
C.H. Meissirel* and L.M. Chalupa, Dept of Psychology and the Center for Neuroscience, University of California, Davis, CA 95616.

In the developing rhesus monkey, the retinal decussation pattern is highly specified at early stages unlike other mammals (Chalupa and Lia, 1991). Thus, this species may provide a unique model to understand the cellular interactions that play a role in sorting out crossed and uncrossed retinal axons at the chiasm. In order to examine the first ingrowth of retinal axons in the monkey we implanted pairs of embryonic retinae in fixed specimen using two carbocyanide dyes DiI and DiA. After brains were stored at 37°C for 5 to 11 weeks, 100-200 µm thick sections were cut with a vibratome and labelled retinal axons were examined using conventional and confocal microscopy. Five fetal rhesus monkey ranging in age from embryonic (E) days 30 to E50 (gestation 165 ± 2 days) have been studied.

Optic axons reach the presumptive chiasm by E35-36. Only a few fibers are just crossing the midline at that age, with the great majority of the retinal axons taking an ipsilateral route. Crossed fibers are intermingled with uncrossed axons before turning abruptly toward the midline. At this age, some cells in the vicinity of the developing chiasmatic region were also labelled, suggesting that closed contacts are formed between ingrowing axons and midline cells. By E40 and E42 numerous labelled axons are located within the chiasm and the most advanced crossed fibers have reached the optic tract. At this stage, there is a clear partial segregation of the crossed and the more numerous uncrossed retinal fibers. Uncrossed axons are mainly located in the deep part of the tract, whereas crossed axons are more superficially placed. By E50 the vicinity of the visual targets is reached.

These results indicate that the leading retinal projections are initially uncrossed in the optic tract of the monkey. Thus, it appears that binocular interactions do not play a role in the choice made by axons when they first enter the optic tract. *(Supported by NIH EY08763 and RR00169. CM is supported by a postdoctoral fellowship from "Ministere de l'Enseignement Superieur et de la Recherche").*

450.2

CHANGES IN THE ORGANIZATION OF GLIAL PROCESSES AND OPTIC AXONS IN THE DEVELOPING CHIASMATIC REGION OF THE FERRET
B.E. Reese*, T.M. Maynard, D.R. Hocking and S.F. Geller Neuroscience Research Institute and Deparment of Psychology, University of California at Santa Barbara

The present study has compared the optic fiber reorderings of the chiasmatic region to changing features in the developing glial environment. Three groups of fetuses, sampled at three day intervals beginning on E-24, were used: 1) immunocytochemistry was used to study the distribution of glial processes containing the intermediate filament proteins, vimentin and glial fibrillary acidic protein (GFAP); 2) crystals of DiI were used to label the optic axons as they course through the chiasmatic region, or to label the processes of the radial glial cells; and 3) thin sections of the chiasmatic region were examined with the electron microscope.

Optic axons have invaded the developing chiasmatic region on E-24, with few fibers reaching the optic tract. Vimentin staining is weak within the optic stalk, while staining within the ventral diencephalon is intense and primarily radial. At all later ages, the pattern of vimentin-staining is distinct within the stalk, chiasmatic region, and optic tracts. The stalk continues to be characterized by weakly immunoreactive processes of varying orientation; the chiasmatic region is composed of primarily radial processes that course in a concave direction at the lateral margins; and the optic tract contains both radial as well as parallel vimentin-positive processes, the latter being particularly dense at the deep border of the tract. Those latter processes arise from radial glial cells, as they can be labelled from the ventricular surface. GFAP-positive processes, by contrast, are not observed until E-36, being restricted to the chiasmatic midline, oriented mainly in the radial axis.

The change in the distribution of vimentin-immunoreactive processes between the stalk and chiasmatic region is coincident with the onset of the chronotopic reordering of optic axons, although the distribution of growth cones occupies a greater proportion of the depth of the chiasmatic midline than in the tract. Hence, the chronotopic reordering is established only gradually as fibers traverse the chiasmatic region. The change in the organization of vimentin processes between the chiasmatic midline and the tract is coincident with the sudden segregation of dorsal from ventral optic axons that occurs in this region. The spatial distribution of GFAP-positive profiles is coincident with the locus where temporal optic axons become directed ipsilaterally. However, at least at the stages when GFAP is expressed, optic axons arriving at the chiasmatic midline all take a crossed course.

450.3

PROTEOGLYCAN EXPRESSION BY TECTAL MIDLINE GLIA IN RELATION TO THE GROWTH OF RETINOTECTAL AND INTERTECTAL AXONS IN DEVELOPING HAMSTERS .
Sonal Jhaveri*,Dept. Brain & Cognitive Sci., M.I.T., Cambridge, MA 02139.

Glial cells along the tectal midline form a barrier for developing retinal axons and contribute to maintaining the laterality of the retinotectal projection (Wu et al., SN Abstr., 1990). Chondroitin sulfate Proteoglycan (CSPG), deposited along the tectal midline in postnatal animals, may provide a molecular substrate for this barrier function (Wu et al., 1991; Snow et al., 1990). Yet, intertectally projecting axons succeed in crossing through this same glial boundary. Using immunohistochemistry, the developmental expression of CSPG is compared with the times of retinotectal axon ingrowth and of intertectal axon crossing.

CSPG is first detected rostrally along the midbrain raphe around E14, when retinal axons are just beginning to enter the tectum. As retinal axons extend into the caudal tectum, the expression of CSPG proceeds from rostral to caudal along the midline. Some intertectal axons (visualized with TuJ1, an antibody to neuron-specific B–Tubulin, courtesy A. Frankfurter) have crossed the tectal midline by E14, prior to the expression of CSPG along the raphe glia. However, increasing numbers of axons continue to be recruited across the midline over the next few days.

Thus, the early axons projecting across the tectal midline may provide a substrate for later-growing intertectal fibers. Alternatively, retinotectal and intertectal axons may be differentially responsive to CSPG.

Support: NIH Grant EY 05504

450.4

TWO CHONDROITIN SULFATE PROTEOGLYCANS EXPRESSED DURING CHICK EMBRYO VISUAL SYSTEM DEVELOPMENT.
C. Ring*, W. Halfter, and V. Lemmon. Dept. of Neurobiology, Univ. of Pittsburgh, 15261.

Chondroitin sulfate proteoglycans (CSPGs) purified from cartilage have been shown to be inhibitory to neurite outgrowth *in vitro*. Based on these findings, it has been hypothesized that CSPGs function as barrier molecules to neurite outgrowth *in vivo*. We wanted to examine if CSPGs derived from embryonic tissues would have similar effects. We have isolated two monoclonal antibodies (mAbs), 2B9 and 9BA12, which recognize two CSPGs that are developmentally expressed in the chick embryo. The 2B9 mAb recognizes a 1000 kD CSPG with a core protein of approximately 270 kD. Its staining pattern in polyacrylamide gels under reducing conditions, its appearance in rotary shadowed preparations, and its developmental expression in chondrogenic tissues and the vitreous body, suggest that it is a collagen type IX PG. Neurite outgrowth assays reveal that the 2B9 antigen is not a favorable substrate for retinal axon outgrowth alone, however, substrate mixtures of the 2B9 antigen with retinal basal lamina extract, support axonal outgrowth. Neurites growing on these substrates appear fasciculated, with numerous side branches along their lengths.

The 9BA12 mAb recognizes an as yet unidentified CSPG expressed in the retinal optic fiber layer and brain during early embryonic development. The brain-derived 9BA12 antigen does not support retinal fiber outgrowth alone, but is supportive in combination with basal lamina extract. In contrast to the 2B9 antigen, retinal fibers in these cultures appear longer, less fasciculated, and have fewer side branches, indicating that the brain-derived 9BA12 antigen is more supportive for neurite outgrowth than the 2B9 antigen. Our study demonstrates the existence of two species of CSPGs in the developing chick visual system. Their neurite outgrowth supporting activity in combination with basal lamina proteins in *in vitro* culture systems would seem to contradict the hypothesis that chondroitin sulfate proteoglycans are a class of molecules which are inhibitory to neurite outgrowth.

450.5

MEROSIN IS FOUND WITHIN THE PATHWAY OF AVIAN RETINAL PROJECTIONS DURING DEVELOPMENT.
N. Morissette* and S. Carbonetto. Center for Research in Neuroscience, Montreal General Hospital Research Institute and McGill University, Montreal (Quebec) CANADA H3G 1A4.

The developmental regulation of integrins in avian retinal ganglion cells (RGCs) correlates temporally with the growth of these cells to their targets in the tectum. Merosin, an A-chain laminin isoform, is a potent ligand for integrin-mediated neurite outgrowth by embryonic chick retinal ganglion cells in vitro (Cohen et al. (1991) *J. Cell Science* 15, 1-7). In the present study, we provide evidence that merosin is present during development of avian RGC projection pathways.

During the formation of chick retinal projections (embryonic days 7 to 12), merosin immunoreactivity was detected within the optic nerves and chiasm, and within the retinal ganglion cell and nerve fiber layers where ganglion cell bodies and axons are located respectively. In addition, merosin expression was detected along the optic tracts and along the outer surface of the optic lobes. Within the optic tectum, merosin-positive tracts were found associated with the tectobulbar fibers that converge at the base of the tectum to form the tectobulbar tract. The distribution of merosin in the chick retina and along the optic pathway suggests that it serves as a ligand for integrin-mediated outgrowth of RGCs to the tectum.

450.6

DEAFFERENTED TARGET REGIONS REEXPRESS SPECIFIC GUIDANCE INFORMATION FOR REGENERATING RETINAL AXONS.
A. Wizenmann+§, F. Bonhoeffer+, S. Klostermann+ and M. Bähr+*. *Neurologische Universitätsklinik, Hoppe-Seyler-Str. 3, and +Max-Planck-Institut für Entwicklungsbiologie, Spemannstr. 35/I, W-7400 Tübingen

During CNS development a topographic projection of ganglion cells from the retina to the superior colliculus is established. In higher vertebrates, putative guidance molecules for retinal axons seem to be expressed only during the time when the retino-collicular projection is being formed.

We have used the stripe assay to compare the growth of rat and chick retinal axons on membranes from (a) normal adult (control, not deafferented) and (b) deafferented adult rat SC. Deafferentation was achieved by cutting the contralateral optic nerve about 2 weeks before preparation of the SC membranes. Our results show that putative guidance molecules, which are not detectable in the normal adult SC, are reexpressed after optic nerve transection in adult rats. Guidance of both, nasal and temporal retinal axons could be observed in vitro as indicated by a preferential growth of retinal axons on membranes from appropiate target regions of the deafferented SC. Furthermore, we found that regenerating retinal axons of adult rats were able to elongate on these membrane preparations and also showed a clear preference for membranes from their appropiate target region.

These findings show that target specific guidance information is reexpressed after deafferentation of the SC in vivo and that regenerating adult retinal axons respond to such guidance cues. These results support the hope that topographically ordered projections could possibly be reestablished after lesions in the adult mammalian CNS.

Supported by the Kuratorium ZNS

450.7

MAPPING CUES THAT DETERMINE THE LAMINA-SPECIFIC BEHAVIOR OF RETINAL AXONS IN CHICK OPTIC TECTUM
M. Yamagata and J.R. Sanes*. Dept. Anatomy and Neurobiology, Washington University Sch. Med., St. Louis, MO 63110.

Retinal axons enter the chick optic tectum through a superficial lamina, the *stratum opticum* (SO); they then extend branches into distinct deeper laminae, arborize, and form synapses (1). To identify cues that guide these behaviors, we developed an organotypic culture system in which a 200 μm-thick transverse slice of tectum is mounted on a supporting membrane and overlaid by a retinal explant large enough to have direct access to all tectal laminae. When E7 retinal explants were combined with E13-16 tectal slices, retinal axons grew selectively along the SO. Some axons sent branches into the retinal receptive laminae; few extended beyond these laminae. Similar patterns of outgrowth were observed on tecta from embryos that had been enucleated at E3, suggesting that retinal axons were recognizing tectal cues, not merely previously-arriving retinal axons. To test whether these cues are specific for retinal axons, or generally permissive for growth, we prepared cultures in which E6 tectal explants (2) confronted tectal slices. Tectobulbar axons that emerged from the explants did not choose to grow into the SO, but instead grew into the deep laminae that they normally traverse *in vivo*. When tectal slices were methanol-fixed before retinal explants were added, the retinal axons still grew along the SO and branched into retinal target laminae, suggesting that non-diffusible cues guide these behaviors. The experimental accessibility of this culture system may facilitate the cellular localization and molecular identification of these cues. (Refs: 1. Mey & Thanos (1992) J. Hirnforsch. 33:673; 2. Kröger & Walter (1991) Neuron 6:291. Supported by NIH.)

450.8

HEPARIN BLOCKS TARGET RECOGNITION IN THE DEVELOPING VISUAL SYSTEM OF *XENOPUS* .
S. McFarlane*, A. Walz and C.E. Holt, Dept. of Biology, Univ. of California San Diego, La Jolla, CA 92093-0322.

The axons of developing retinal ganglion cells (RGC) navigate along a defined route and, on reaching the optic tectum, they stop and begin to arborize. Target recognition is mediated by specific interactions between the axon tips and the surfaces of their local environment. Since growth factors and their receptors are expressed in specific regions of the developing brain, we have begun to investigate the possibility that these molecules play a role in recognition events, using heparin and other reagents known to block the action of growth factors.

Exposed brain preparations were used to bath apply reagents to the developing optic tract during the period when RGCs axons grow from the base of the optic tract to the tectum (stages 33/34 to 40), and axons were visualized with HRP at stage 40. Heparin (0.1 mg/ml) had little effect on the trajectories of axons in the optic tract, but had striking effects on retinotectal targeting. Axons typically ignored the tectum, scouting around its edges, and continued to grow beyond it. Rostrally, axons frequently crossed over the dorsal midline, while ventrally, axons extended into the medulla and the spinal cord. Axons that entered the tectum either grew back out, dived deep into the neuroepithelium, or continued on caudally and did not appear to arborize. To test for specificity of action, compounds which act similarly to heparin were also used. Pentosan polysulfate (0.1 mg/ml) showed similar effects to heparin, interfering with normal target recognition, whereas suramin (0.03 mg/ml) retarded growth. Immunohistochemistry showed that basic fibroblast growth factor (bFGF), a heparin-binding growth factor, and heparan sulfate, a heparin-like glycosaminoglycan, appear to colocalize in the developing optic tract. Since heparan sulfate is required for the function of bFGF, it is possible that the effects of heparin and pentosan polysulfate reflect their binding to bFGF. These results show that heparin blocks target recognition without interfering with axonal extension or guidance and suggest that heparin-binding growth factors may be involved in target recognition.

(Supported by NIH #NS23780 and PEW Scholars Award).

450.9

REGENERATION OF RETINOTECTAL FIBERS IN THE PRESENCE OR ABSENCE OF IPSILATERALLY PROJECTING OPTIC FIBERS IN THE LEOPARD FROG.
B.C. Weber and E.R. Gruberg*. Biology Dept., Temple University, Philadelphia, PA 19122.

We previously showed that after midsagittal sectioning of the optic chiasm most retinotectal fibers regenerate to the ipsilateral tectal lobe. These frogs recover vision, but respond to prey and looming stimuli as if a stimulus was not in its actual position but at the symmetrical location in the contralateral field. Are regenerating retinotectal fibers misdirected by mechanical disruption or by cues in the optic chiasm? We made selective cuts in the region of the chiasm: After extracranial, bilateral sectioning of the optic nerves, animals recover with accurate responses. After intracranial, bilateral sectioning of the optic nerves animals respond to the actual stimulus position approximately 80% of the time and to mirror image position 20% of the time (80%A:20%M). After transverse sectioning of the chiasm animals respond with a ratio 80%A:20%M. When transverse and midsagittal cuts are produced together, animals respond with a ratio of 60%A:40%M. After paired, symmetrical, parasagittal cuts of the lateral chiasm, animals respond quite differently from each other (range - 0%A:100M to 70%A:30% M). After unilateral lesion to the optic tract individual animals either show normal behavior (100%A:0%M) or mirror image behavior (0%A:100%M). Using HRP and DiI histochemistry we find that in normal animals ipsilateral projecting fibers have trajectories restricted to the lateral part of the chiasm. Our results suggest that if ipsilaterally projecting optic fibers are spared when retinotectal fibers are cut, mirror image responses are likely. Mechanical disruption plays an additional role in misdirecting regenerating fibers. Supported by NIH EY04366.

450.10

ALTERNATE PROJECTION PATHWAYS FOR XENOPUS RETINAL FIBERS AFTER TECTAL ABLATION.
K.M.MacLeod,S.Cohen-Cory, V.Goss, and S.E.Fraser*, Division of Biology, California Institute of Technology, Pasadena, CA 91125.

We have investigated the possible guidance cues for retinal ganglion cell (RGC) axons as they project to the optic tectum during development. After disruption of their local target environment we observed the paths taken by fluorescently labelled fibers over several successive days *in vivo* . Ablations of the left tectum or the adjacent diencephalon were performed on stage 37-45 *Xenopus laevis* embryos, and RGC axons were labelled by pressure injection of contralateral temporal or nasal retina with DiI. The live embryos were viewed by video microscopy over a 24-72 hour period. In embryos in which the diencephalon only or diencephalon and rostral tectum were removed, a significant number of both temporal and nasal axons showed a tendency to reverse direction after passing the optic chiasm but before reaching the tectum and course toward the ipsilateral side, in most cases reaching and arborizing in the ipsilateral tectum. Other path fates included passing the chiasm and continuing down the contralateral optic nerve toward the eye or projecting rostrally into the forebrain. If the diencephalon were left intact but rostral tectum removed, the axons stalled and remained at the diencephalic-tectal border. Often multiple axons in a single embryo displayed two or more path choices. If only the caudal portion of the tectum were removed, most axons projected contralaterally, but one or two nasal axons projected ipsilaterally. These results suggest that both local cues and longer ranged interactions are important for the guidance of optic axons and formation of a normal retinotectal projection.

450.11

THE EARLY DEVELOPMENT OF DESCENDING TECTAL PROJECTIONS IN CHICK.
I.T.Shepherd and J.S.H.Taylor. SPON: Brain Research Association Dept.Human Anatomy, University of Oxford, OX1 3QX, UK

The development of two descending projections from the chick tectum has been studied. An initial cohort of axons arises between stages 14-16 and grows dorso-ventrally to the ventral margin of the tectum, where they turn 90^0 to form a caudally directed tract. This tract descends into the hindbrain past the region of the trigeminal nerve and is assumed to be the tectobulbar tract. A second population of axons emerges at stage 17, initially in the rostral part of the tectum, which also grows dorso-ventrally.These axons fail to turn at the tectal margin, continuing to the ventral midline where the axons decussate and turn caudal, joining the contralateral ventro lateral tract (VLT). These form the tectospinal projection.

Embryos which had either the rostral or caudal tectum ablated at stage 12 and allowed to develop to stage 16/17, show that rostral tectobulbar axons are not dependent on caudal tectal axons for guidance into the hindbrain. It appears that the guidance cues are within the undifferentiated neuroepithelium.

Immunocytochemistry, using an antibody to chondroitin sulphate (CS), shows an increase in staining which delineates the border at which the tectobulbar axons turn caudally. The CS staining at later stages also delineates the VLT. To test whether CS is inhibitory for tectal axons, dissociated cells from E6 tecta have been grown on laminin substrate(100μg/ml) dotted with spots of CS. Axon outgrowth is inhibited on the CS spots, an effect that can be reversed by treatment with Chondroitinase ABC. Interestingly on low concentrations of substrate laminin (10μg/ml) CS forms a poor yet permissive substrate for tectal axon growth.

To investigate adhesion molecules which might be involved in the guidance of the tectospinal axons, an immunocytochemical study has been carried out. An antibody to chick TAG-1 (a gift from J.Dodd) has been found not to stain the tectobulbar axons, but does stain later developing axons. The expression is along the whole length of these axons and does not show a down regulation at the ventral midline.

450.12

INTERSTITIAL BRANCHING OF CORTICAL AXONS RELATED TO THE LOCAL ENVIRONMENT OF THEIR PATHWAY.
M. Bastmeyer*, M.M. Daston, H. Possel, D.D.M. O'Leary., The Salk Institute, La Jolla, CA 92037.

Previous studies have shown that the corticopontine projection is established by interstitial axonal budding from corticospinal axons. Corticospinal axons growing in the cerebral peduncle, pass by a major target, the basilar pons, and collateral branches arise from the axon shaft well behind the advancing growth cone. Evidence suggests that a diffusible chemoattractant, released by the pons, acts at a distance to promote the formation of collaterals and to direct their growth. On the other hand, specialized glia and extracellular matrix (ECM) molecules have been implicated in directing axon growth in other brain areas. The goal of this study is to determine whether the spatiotemporal patterning of cellular or molecular components in the cerebral peduncle correlates with the initiation and/or direction of axon collateral growth.

We have performed an immunohistochemical analysis of rat cerebral peduncles in the region overlying the basilar pons. The distribution of glia, dendrites, and ECM components was examined over the time that collaterals form. We have found that glial processes (identified by RC-1 and anti-GFAP) surround, but do not invade the cerebral peduncle at sites where interstitial branching occurs. We also found that the spatiotemporal distribution of cytotactin and CSPG does not correlate with branch formation. However, dendritic processes (identified by anti-MAP-2) are present in the cerebral peduncle and are oriented parallel to the direction of collateral elongation.

Our findings show that radial glia are not involved in the initiation and directed growth of interstitial branches, but the MAP-2 stain suggests that, in addition to the chemoattractant, dendrites may participate in this process. An ultrastructural study to confirm these results is in progress.

Supported by the DFG (Ba 1034/4-1) and the American Paralysis Association.

450.13

DESCENDING BRAINSTEM-SPINAL AXONS MAKE CORRECT ROSTRO-CAUDAL POLARITY CHOICES IN CULTURE. S.Liu* and R.H. Nordlander, Depts. of Oral Biol., Anat., Ohio State Univ., Columbus OH 43210

Many brainstem neurons normally innervate targets in the spinal cord. To investigate whether their axons recognize polarity cues along the rostrocaudal neural axis, brainstem and spinal cord explants from Xenopus larvae (st 40-45) were cocultured up to 7 days in collagen matrices. Outgrowth from brainstem explants toward cord segments of differing axial orientations were compared. Pairs of segments were placed at about 40° to, but at opposite sides of the brainstem axis and at a distance of 300-400 um from the brainstem.

If presented with equidistant cord segments of opposite orientations, most neurites extended toward the segment whose rostral end faced the brainstem. DiI introduced in these segments marked axons and cells of the raphe and reticular formation, both of which normally send axons into the spinal cord. If both cord segments have the same orientation, equal numbers of neurites go to each segment, with more fibers entering the segments if rostral ends face the brainstem. If presented with the choice of one or several rostrally oriented pieces, a dose effect is exhibited. Our results suggest that descending brainstem axons can recognize and respond to polarity cues inherent in the spinal cord and perhaps diffusing from it.

Supported by NS 18773 to RHN.

450.14

GROWTH PROMOTING MOLECULES IN THE DEVELOPING CORTEX GUIDE THALAMIC AXONS. M. Hübener*, M. Götz, and J. Bolz, Friedrich-Miescher-Labor der Max-Planck-Gesellschaft, 7400 Tübingen, Germany.

Thalamic axons do not grow into an embryonic cortex, but they do grow into an early postnatal rat cortex. We have previously shown that substrate bound molecules in the cortex control thalamic fiber ingrowth (Götz et al., Development **116**, 507, 1992). Here we present evidence that growth promoting molecules in the postnatal cortex, rather than growth inhibiting molecules in the embryonic cortex, are responsible for this behavior. Time-lapse video-microscopy revealed that thalamic fibers grow slower and collaps more often on embryonic day 16 (E16) than on postnatal day 7 (P7) cortical membranes. On a laminin substrate growth-rate and frequency of growth-cone collapses were about equal as on E16 membranes, indicating that the differences in growth behavior on both membrane substrates are due to growth promoting factors present on P7 cortical membranes. Heat-inactivation of embryonic cortical membranes at different temperatures never enhanced their growth promoting properties. Moreover, when thalamic fibers were given a choice between heat-inactivated E16 membranes and P7 membranes in a stripe-assay, the fibers always preferred P7 membranes, again giving no indication of inhibitory molecules on E16 membranes. Most fibers encountering a border between E16 and P7 cortical membranes either stopped their growth or changed their direction of growth. The majority of the fibers that performed turns upon reaching a border did so without a growth-cone collapse, also arguing against inhibitory molecules on E16 membranes. During development *in vivo* thalamic fibers are always confined to regions which can be stained intensely with the lectin peanut agglutinin (PNA; Götz et al., Development **116**, 507, 1992). In the membrane stripe-assay addition of PNA to the culture medium significantly decreased the preference of thalamic fibers for the P7 membranes. Taken together these results suggest that during cortical development thalamic axons are guided by the differential distribution of growth promoting, PNA-binding molecules.

Supported in part by DFG.

450.15

Decrease of astrocytes during development of rat cortex affects the projection of thalamic axons to cortex. M. F. Barbe* and P. Levitt, Dept. of Physical Therapy, Temple Univ., Philadelphia, PA 19140 and Dept. of Anatomy and Neurobiology, Med. Coll. of Pennsylvania, Philadelphia, PA 19129.

Developing thalamic axons projecting to the cortex may wait in the subplate with the most significant growth occurring postnatally. Gliogenesis begins late prenatally and extends into the postnatal animal. In order to investigate whether astrocytes play a role in the ingrowth and collateralization of thalamic axons into the cortex, we injected an antimitotic agent, methylazoxy methano acetate (MAM), into pregnant rat dams to specifically destroy glial precursors. One injection of either 20 or 25 mg/kg body weight was made into dams on E19, E20 or E21. The brains were collected on postnatal (P) day 7, midsagittally sectioned and DiI inserted into midthalamus. 12 weeks later, the brains were sectioned and the DiI-labeled axons examined in the internal and external capsules, subplate and cortical plate. Brains of siblings were cut on a cryostat and immunostained with anti-glial fibrillary acidic protein (GFAP). The thalamocortical axons of pups that had received 20 mg/kg MAM at E19 appeared similar to controls, showing substantial growth into the cortex. Most of the labeled thalamocortical axons of pups injected with 20 mg/kg MAM at E20 did not extend past the lateral border of the internal capsule, suggesting a retraction of axons. Brains of pups that received 20mg/kg MAM on E21 had a few axons that were present in the external capsule, but the numbers extending into the cortex were significantly decreased from control rats or those injected at E19. The number of GFAP-immunoreactive astrocytes present in P7 brains was greatly reduced in pups injected with MAM on E20 or E21 when compared to controls or those injected on E19. Brains of pups injected with 25 mg/kg MAM appeared to have undergone reactive gliosis. DiI-labeled thalamic axons in brains of sibling pups of these latter groups stopped abruptly at the border between the internal and external capsule, with pronounced endings that appeared growth-cone-like. The data indicate that astrocytes are important for regulating thalamic ingrowth into the cortical plate during development. (Supported by Temple Univ. and NIMH Grant MH45507).

450.16

3-D CONFOCAL MICROSCOPIC STUDY ON THE EARLY DEVELOPMENT OF THALAMOCORTICAL PROJECTIONS IN THE RAT

Zoltán Molnár[1]*, Richard Adams[2] and Colin Blakemore[1,2] University Laboratory of Physiology[1] and MRC Research Centre in Brain and Behaviour[2], Parks Road, Oxford OX1 3PT, UK.

We are interested in the topography and interrelationship of descending axons from the cortical subplate and the early thalamocortical projection. A confocal microscope (Leica) was used to image fibres revealed with carbocyanine dyes (DiO, DiI) placed into different parts of the thalamus and cortex in fixed tissue from rat embryos aged E14 to E21. Sections were counter-stained with the chromatin stain, acridine orange. To obtain high-resolution reconstructions of the entire fibre pathways we used both serial sections and fine optical sections to construct 3-D datasets. Discrete placement of small crystals into putative sensory thalamic nuclei appeared to label thalamic axons alone and did **not** back-label cells in the cortex . Neighbouring thalamocortical axons show three distinct patterns of organization in different parts of the pathway. Adjacent axons remain parallel and closely associated throughout the internal capsule. Upon entering the striatum the tight, homogeneous bundle breaks up and the fibres reorganize into small fascicles. While the gross topography of the projection is maintained there is some crossing of axons between neighbouring fascicles. At the lateral edge of the striatum the fibres defasciculate and individual axons course towards the cortex along parallel paths in the white matter. Between E14 and 15, early corticothalamic and thalamocortical axons labelled with different dyes placed in corresponding regions of cortex and thalamus are intimately intermingled and share the same fascicles in the region of the striatum.

3D-reconstruction of the entire fibre pathway was performed after multiple restricted dye placements into the cortex at E16 to E19 (labelling both thalamic and subplate axons). This showed that contiguity of ascending and descending axons and gross topographic order were maintained at all points along the pathway.

450.17

MATURATIONAL CHANGES IN MEMBRANE ASSOCIATED MOLECULES IN THE CORTICAL PLATE MAY CONTROL DEVELOPMENT OF THALAMOCORTICAL AND INTRACORTICAL CONNECTIONS. R. Tuttle*, B.L. Schlaggar, and D.D.M. O'Leary, The Salk Institute, La Jolla, CA 92037.

Thalamocortical afferents (TCAs), the first to grow into the neocortex, follow an intracortical path that is centered on the subplate layer and avoids the overlying cortical plate (CP). TCAs reach occipital (visual) cortex on E17 and extend into the CP around E19. This pattern of growth may be controlled, in part, by the CP, which may initially be too immature to attract or permit the ingrowth of TCAs.

To study this, explants of rat CNS were placed onto a substrate composed of alternating stripes of E18 vs. P0 or E19 vs. P1 rat CP membranes dissected from the occipital neocortex. E18 lateral geniculate (LG) neurites displayed a clear preference for the neonatal CP membranes. E18 cortical neurites also grew well on CP membranes and preferred neonatal CP. However, these CP membranes were generally a poor substrate for neurite growth from explants of E18 retina and P7 cerebellum, neither of which project to cortex.

These findings suggest that membrane associated molecules in CP undergo maturation-dependent changes that thalamocortical axons are responsive to and, therefore, might promote their invasion into the CP. The behavior of the cortical neurites suggests that these molecules may also regulate the onset of the development of intracortical connections.

450.18

CORTICAL GROWTH CONE BEHAVIORS IN EXPLANT CO-CULTURES REFLECT PERMISSIVE OR INHIBITORY TARGET CUES. M.Merline,* R.Z.Kuang, K.Kalil. Neuroscience Training Program and Dept. of Anatomy, University of Wisconsin, Madison, WI 53706.

During development *in vivo* corticospinal axons are highly specific in their innervation of spinal targets. Similarly, *in vitro*, cortical axons invade spinal targets but are repelled from cerebellar explants, as shown by DiI labeling of fixed co-cultures (Kuang et al. Soc. Neurosci. Abs. 1992). In order to characterize growth cone behaviors that mediate target selection by growing cortical axons, we carried out video microscopy on living cortical explants co-cultured with either spinal cord or cerebellum. Explants of P0-P1 hamster sensorimotor cortex were co-cultured in collagen gels with explants of P0-P1 cervical spinal cord or cerebellum. After 1-2 days video microscopy was carried out under phase optics. Control cortical growth cones growing in the collagen gel in the absence of target tissue were only occasionally interrupted in their forward advance by collapse of the growth cone lamellipodium and contraction of the motile tip into a static tapered or club shaped ending. Collapsing behaviors by control growth cones were most often temporary, and growth cones rapidly resumed forward extension. In co-cultures with cerebellar targets, cortical growth cones contacting cerebellar explants showed rapid and dramatic collapsing behaviors, and often went through repeated cycles of collapse and withdrawal. In contrast, cortical growth cones contacting spinal cord targets extended smoothly into the explants. They sometimes paused or slightly retracted but did not go through large withdrawals or repeated collapsing behaviors. These results show that contact mediated growth cone interactions with target cells form the basis for appropriate target selection by cortical axons. Growth cone collapse and withdrawal inhibit axon extension into inappropriate targets whereas growth cones entering appropriate targets rarely show inhibitory behaviors. Supported by NIH Grant NS14428 (K.K.) and Training Grant T32GM07507 (M.M.).

450.19

ANALYSIS OF PARALLEL FIBER ELONGATION IN THE WILD TYPE AND MUTANT MOUSE CEREBELLUM. M.W. Vogel*, J. Soha, J. Prittie, and J.E. Crandall. MPRC, Baltimore, MD 21228., Dept. of Surgery, Yale Medical School, New Haven, CT 06511, and Shriver Center, Waltham, MA 02254.

Granule cell parallel fibers extend for long distances in the cerebellum in an ordered parallel array. While parallel fibers begin to grow soon after granule cells start to differentiate in the external granule cell layer, little is known about the regulation of their development. We have begun an investigation of parallel fiber growth in wild type, *lurcher*, and *staggerer* mice using DiI labeling in fixed cerebellar tissue.

Wild type and mutant mice (P2 to P41) were killed by cardiac perfusion with 4% phosphate buffered paraformaldehyde. A DiI coated micropipette was inserted into the vermis of the fixed cerebella (pyramis or declive). After incubation in fixative for 24 hours to 1 week in the dark (RT or 37° C), the cerebella were sectioned coronally on a vibratome, and the sections analyzed with an epifluorescence microscope.

In whole mounts of wild type cerebella, the DiI micropipette labels a thin band of fibers stretching across the vermis. In coronal sections, DiI labeled parallel fibers and granule cells extend on either side of the injection site. Surprisingly, the apparent maximum length of parallel fibers did not increase greatly from P3 to P41. This is consistent with the hypothesis that parallel fibers attain their maximum length before granule cells migrate into the inner granule cell layer. Despite Purkinje and granule cell loss in *+/Lc* , parallel fibers appear to reach normal lengths in *+/Lc* pups. We are in the process of examining parallel fiber labeling in *staggerer* pups and verifying DiI parallel fiber labeling by examining the ultrastructure of photoconverted DiI labeled fibers.

Research support provided by NIH grants NS29277, NS32163, and NS24386.

450.20

NEW EVIDENCE FOR A CORRELATION BETWEEN THE EARLY NEURONAL ORGANIZATION AND THE PATTERN OF AXONAL TRACTS. M.-C. Bélanger,*F. Auclair, N. Valdés, L. Bertrand and R. Marchand. Centre de Recherche en Neurobiologie, Hôp. de l'Enfant-Jésus, 1401, 18e rue, Québec (Qc), Canada, G1J 1Z4.

The primitive columnar organization of early generated neurons predicts the pattern of several longitudinal axonal tracts (Bélanger et al. '93). To improve our knowledge of the correlation that appears to exist between the position of early neurons and the pattern of axonal bundles, one or two injections of bromodeoxyuridine (BrdU, 60 µg/g) were made to gestating rats between days 11.5 and 12.5 in order to visualize early generated neurons that will appear as *unlabeled* for BrdU. One to three days later, fixed embryos originating from these litters received crystals of carbocyanines along different points of the columns of early generated neurons as in the chiasmatic ridge, the prospective accessory olfactory bulb and the amygdaloid complex among other sites which are known to contain populations of early generated neurons. Fluorescent bundles of axons and cell bodies were photographed from cryostat sections which were then processed for anti-BrdU immunostaining. Comparison of both labeling patterns confirmed that axons travel along columns of unlabeled neurons. These axons represent recognizable axonal tracts of the adult brain like the medial forebrain bundle, the accessory olfactory tract and the mesencephalic root of the trigeminal nerve for example. These results support the hypothesis that the primitive organization of early neurons might be involved in the elaboration of a terrain favorable to axonal growth. Supported by the MRC (Canada), FRSQ and FCAR (Québec).

450.21

AXON PATHFINDING IN INITIAL TRACT FORMATION IN THE EMBRYONIC MOUSE MIDBRAIN. GS Mastick and SS Easter, Jr.*, Dept. of Biology, Univ. of Michigan, Ann Arbor, MI 48109-1048.

The embryonic mouse CNS has a stereotyped pattern of axon outgrowth starting on embryonic day 8.75 (E8.75) as revealed by labelling of fixed embryos with an antibody to neuron-specific class III ß-tubulin (Easter, *et al.*, J. Neurosci. 13:285-299). The first axons arise from near the dorsal midline of the mesencephalon, course caudally as a loose group, and turn laterally around the cerebellar plate, to form a tight, longitudinal tract (dtmesV) (*ibid*).

More detailed examination of small groups of axons near the mesencephalon/metencephalon border (E9.25) revealed pathfinding errors, suggesting a reduced precision in guidance cues at this point. At E10.0, local injections of DiI into the wall of the rostral mesencephalon labelled the caudal projections of dtmesV axons and a second ventrally-projecting population of axons, superficial to dtmesV. They projected across the ventral midline just caudal to the oculomotor nerve.

As a first step toward examining growth cone dynamics of the initial CNS axons *in situ*, embryos were cultured *in vitro* from E8.75 to E9.5. Both anti-ß-tubulin and DiI labelling demonstrated that these cultured embryos had normal timing and pattern of early axon pathfinding.

AXON GUIDANCE MECHANISMS AND PATHWAYS V

451.1

INFLUENCE OF CHONDROITIN SULFATE ON RETINAL AXON GROWTH IN VITRO. B.D. McAdams and S.C. McLoon*. Dept. of Cell Biology & Neuroanatomy, Univ. of Minnesota, Minneapolis, MN 55455.

An inhibitory or impedance effect on neurite extension has been attributed to chondroitin sulfate-bearing proteoglycans. These proteoglycans have been suggested to be components of barriers to axon growth during development. However, chondroitin sulfate immunoreactivity has previously been observed in the developing retinofugal pathway of chick. Furthermore, the distribution of immunoreactivity suggests that chondroitin sulfate is produced by retinal ganglion cells or adjacent cells. The present study utilized immunocytochemistry to determine whether neurites from chick retinal explants are immunoreactive for chondroitin sulfate and to examine their behavior in the presence of chondroitin sulfate. Chondroitin sulfate immunoreactivity was observed in association with retinal neurites grown on laminin. The immunoreactivity was present both along the neurite, on the growth cone, and immediately adjacent to some neurites and explants. Therefore, the chondroitin sulfate immunoreactivity observed in vivo may be of retinal ganglion cell origin. To determine the effect of chondroitin sulfate on neurite extension, retinal neurites were grown on vitreous or with chondroitin sulfate-supplemented medium. Retinal explants extended neurites in intact or cryostat-sectioned chondroitin sulfate-containing embryonic chick vitreous. When medium supplemented with chondroitin sulfate was added to cultured retinal explants, growth cones transiently collapsed and new filopodia extended from the neurites and growth cones within minutes. These results suggest that, while chondroitin sulfate may influence growth cone behavior, it does not prevent growth cone extension. (Supported by EY05371.)

451.2

EXPRESSION OF MUTANT ß1 INTEGRIN AND N-CADHERIN IN THE DEVELOPING RETINA. A. Lilienbaum, R. H. Riehl, A. A. Reszka, A. F. Horwitz and C. E. Holt*. Dept. of Biology, UC San Diego, La Jolla, CA 92093-0322.

Integrins and N-cadherin are substrate and cell adhesion molecules that act synergistically to promote neurite outgrowth from retinal cells *in vitro*. These molecules are expressed in the optic pathway of *Xenopus laevis* embryos at a time when retinal ganglion cells (RGCs) are sending their axons to the tectum. To determine the role that these molecules play in axonal growth and guidance *in vivo* we have expressed mutant forms of ß1 integrin and N-cadherin in embryonic retinal cells with the aim of blocking their function in developing RGCs.

cDNAs were introduced into the retina by multiple focal injections of lipofectin/cDNA made directly into the proliferative neuroepithelium of the eye primordium. We used a *myc*-tagged dominant-negative form of *Xenopus* N-cadherin with a large extracellular deletion and three different chicken ß1 integrin mutants (cytoplasmic deletions and amino acid substitutions) with severely reduced adhesion plaque-forming activity. A non-functional N-cadherin mutant and luciferase were used as controls. Transfected cells expressing mutant N-cadherin and ß1 integrin proteins were detected immunohistochemically using *c-myc* and chicken specific antibodies respectively. Transfections were done at stage 19-20 and strongly expressing cells were assayed 48 hours later at stages 40 and 41. Entire transfected RGCs were visualized from soma to axon tip in 50µm DAB stained vibratome sections.

Retinal cells expressing high levels of mutant N-cadherin or ß1 integrin exhibited normal patterns of laminar migration and were present in all layers of the differentiated retina in numerous cell types. The number of processes (dendrites and axons) that ß1 integrin and N-cadherin expressing RGCs elaborated, however, was significantly reduced from those transfected with the luciferase reporter (13-50% vs 80%). RGC axons expressing mutant adhesion molecules followed the correct pathway and terminated in the tectum. These results suggest that loss of or reduced adhesion molecule function interfers with the initiation and/or the rate of axonogenesis in RGCs *in vivo* but not with axonal guidance. Supported by NIH #NS23780, PEW Scholars Award, March of Dimes and CNRS.

451.3

AXONIN I IS SELECTIVELY EXPRESSED IN DEVELOPING PRIMARY AFFERENT PATHWAYS IN THE AVIAN BRAIN. Y.P.L. Yip*, L. Felder[1], C.D. Balaban[1], W. Halfter and J.W. Yip. Departments of Neurobiology and Otolaryngology[1], University of Pittsburgh, Pittsburgh, PA 15261

Axonin I (Ax-I), the avian homolog of TAG-1, is a membrane adhesion molecule that may facilitate axon fasciculation and neurite growth. Our previous studies demonstrated that Ax-1 is expressed selectively in cutaneous and visceral sensory afferents and in the retinotectal pathway during axon outgrowth. This study examines the time course and regional distribution of Ax-1 expression in developing brain. Immunocytochemical staining with a specific monoclonal antibody (1A12) shows that Ax-1 is expressed by primary visual, auditory, somatosensory and visceral sensory pathways. Immunoreactive axons are observed on E4 and could be traced to the dorsal funiculi, trigeminal root and spinal trigeminal tract, retino-pretectal, retino-thalamic and accessory optic visual pathways, olfactory nerve, vestibular and cochlear nuclear afferent pathways and the tractus solitarius. However, expression is absent in the mesencephalic trigeminal tract and nucleus, components of a proprioceptive primary afferent pathway. A striking down-regulation of Ax-1 immunoreactivity is observed in the optic tectum and vestibular and cochlear nerves by E8. By day 19, weak expression remains only in the dorsal funiculi. This study confirms that non-proprioceptive somatic and visceral sensory primary afferents selectively express Ax-1 during development. This suggests that Ax-1 plays an essential role in the development of primary sensory pathways.

451.4

Differential Effects of Glycosaminoglycans on Neurite Extension on L1 and Laminin Substrates. Chang-Lin Dou* and Joel M.Levine. Department of Neurobiology and Behavior, SUNY at Stony Brook, Stony Brook, NY 11794

The glycosaminoglycans (GAGs) keratan-sulfate (KS) and chondroitin-sulfate (CS) are thought to be negative regulators of axon growth (Exp.Neurol.,109:111). This hypothesis remains controversial since 1) soluble GAGs may promote neurite growth (Development,114:17) and 2) some proteoglycans inhibt neurite extension after treatment with GAG hydrolases (J.Neurosci.,11:822). To examine the role of GAGs as modulators of neurite growth, we tested the ability of these negatively charged carbohydrates to inhibit neurite extension on L1 and laminin substrates.

Tissue culture surfaces were coated with poly-L-lysine followed by either L1 or laminin alone (both at 2μg/ml), or a mixture of laminin (or L1) and various amounts of CS or KS. Cerebellar granule neurons were isolated from postnatal day 5 and 6 rats, seeded onto the surfaces in media supplemented with bFGF, and after 24hr, the extent of cell attachment and neurite outgrowth was quantitated. The neurons attached equally well to all substrates tested. On laminin alone, 50% of the cells extended neurites and the mean neurite length was 85μ. When laminin was mixed with KS (10-1000μg/ml), only 25% of the cells grew neurites and the mean neurite length was 50μ (40% inhibition). CS inhibited neurite growth on laminin in a dose-dependent manner, reaching a maximum of 45% inhibition at 50μg/ml or higher concentrations. On L1 surfaces, 42% of the cells extended neurites with a mean length of 98μ. KS (10-1000μg/ml) did not inhibit neurite growth on L1 coated surfaces and 1mg/ml of CS was needed to achieve a 50% inhibition of neurite growth. Digestion of CS with chondroitinase ABC reversed its inhibition completely on both laminin and L1 substrates.

These results suggest that CS and KS may not exert strong negative influences over axonal elongation in the developing cerebellum and other CNS regions where L1 is abundant. GAGs may play a role in modulating the growth of axons of the PNS which extend through an environment rich in laminin.

451.5

L-14, AN ENDOGENOUSLY SYNTHESIZED LECTIN OF THE RAT OLFACTORY SYSTEM: IS IT A BIFUNCTIONAL PROTEIN INVOLVED IN AXON GUIDANCE? N. K. Mahanthappa*, D. N. W. Cooper, S. H. Barondes and G. A. Schwarting. Dept. of Biomed. Sci., The Shriver Center, Waltham, MA 02254; Dept. of Psychiatry, UCSF, San Francisco, CA 94143; and the Prgrm. in Neurosci., Harvard Med. Sch., Boston, MA 02115.

L-14, a divalent lectin found in a variety of vertebrate tissues, shows specific binding to terminally lactosaminylated glycans and polylactosamine. The protein is expressed at high levels in a subset of rat dorsal root ganglion neurons where it is co-expressed with a lactosamine-immunoreactive epitope and thus thought to potentially mediate specific adhesive interactions (Regan et al., *PNAS (1986) 83:2248*). In the rat olfactory system, we have demonstrated that L-14 is expressed at high levels in the olfactory nerve, and co-localizes with two ligands: a lactosaminylated glycolipid on the surface of nascent axons, and a laminin-related protein in the extracellular matrix. Both ligands bind L-14 in a carbohydrate-dependent manner. We have thus hypothesized that L-14 may crosslink the axonal surface to the extracellular matrix and thus play a role in the guidance of olfactory axons to the olfactory bulb.

In vitro we have found that L-14 appears to promote two distinct adhesive interactions among primary olfactory neurons. The first occurs at high L-14 concentrations (~10mM), is carbohydrate-dependent, and promotes intercellular adhesion. The second interaction occurs at low L-14 concentrations (~10μM), is carbohydrate-independent, and promotes substrate adhesion. Neither interaction is inhibited by RGDS peptides or anti-integrin antibodies. We are presently investigating whether a novel, high affinity, carbohydrate-independent, L-14 binding site (similar to that reported on fibroblasts by Wells and Mallucci, *Cell (1991) 64:91*) is present on olfactory neurons. We are also performing in situ hybridzation studies to determine the site of L-14 synthesis in the olfactory system.

451.6

EXPRESSION OF E587 ANTIGEN IN EMBRYONIC ZEBRAFISH CNS AND *IN VITRO* FUNCTIONAL ANALYSIS. S. Giordano*, M.Bastmeyer, A.Y. Loos and C.A.O. Stuermer. Faculty for Biology, University of Konstanz, Germany

The E587 antigen (Ag), which was previously identified as a likely cell adhesion molecule in the goldfish visual system , is associated with growing retinal axons in embryos and regenerating retinal axons in adults but is absent from mature axons (Vielmetter et al., 1991).

The expression of E587 Ag in zebrafish embryos was examined using new mono- and polyclonal antibodies (Ab) against the purified protein. Staining was observed on retinal ganglion cell axons in the retina, optic nerve/tract and in the tectum of two day old embryos. Staining was also present in the spinal cord.

To determine if E587 Ag can influence the behavior of growing axons, growth cones from goldfish retinal explants were observed advancing on polylysine stripes. In this assay 80% of growth cones fasciculate. In the presence of E587 monoclonal Ab, however,only 60% of growth cones fasciculate. In addition the increase in velocity growth cones exhibit when in fascicles, as compared to growth on polylysine, was abolished by this Ab.

In vitro growth cones also show a preference for growth on goldfish oligodendrocytes, which express E587 Ag (Bastmeyer et al., 1993). In contrast, in media containing E587 Ab, there is a signifigant reduction in the number of contacts growth cones have with oligodendrocytes and they appear to lose their preference for these cells.

These results demonstrate that E587 Ag contributes to axon fasciculation and axon-glial cell interactions in vitro and suggest this may be a function for this molecule in vivo. This view is strengthened by the expression of E587 Ag on outgrowing axons in development and its re-expression during retinal axonal regeneration.

451.7

MOLECULAR CLONING OF NEUROLIN AND ITS EXPRESSION IN GOLDFISH AND EMBRYONIC ZEBRAFISH CNS. U. Laessing*, S. Giordano, F.Lottspeich+ and C.A.O. Stuermer. Faculty of Biology, University of Konstanz, and +MPI Biochemie, München, Germany

Neurolin, a ≈90Kd cell surface glycoprotein is found on all retinal ganglion cells (RGC) and axons in the goldfish embryo but is restricted to new RGC axons in adults and re-expressed on all regenerating RGC axons following optic nerve transection (Paschke et al.,1992).

We report here a 500 bp cDNA coding for the C-terminus of Neurolin which was obtained using PCR and specific primers determined from amino acid sequencing of the purified protein. There is a striking homology between the predicted amino acid sequence of Neurolin and DM-Grasp protein (Burns et al., 1991) extending 60 amino acids from the carboxy terminus. This similarity, however, is not extended throughout the entire PCR fragment and the overall amino acid homology is about 50%. A cDNA library from regenerating goldfish retina is now being screened for a full length Neurolin clone to determine if Neurolin is the fish homolog to DM-Grasp or a related protein.

In addition, two new monoclonal antibodies against purified Neurolin were used to examine Neurolin expression in zebrafish embryos. Staining in whole mounts was on retinal axons, spinal cord neurons, DRGs and in the hindbrain of 2 day old embryos.

Neurolin expression in the developing and regenerating CNS and its homology to DM-Grasp indicate that Neurolin may be important for the formation of CNS fiber tracts in fish.

451.8

CLONING AND CHARACTERIZATION OF A ZEBRAFISH cDNA SIMILAR TO CHICK DM-GRASP, A NEURAL CELL SURFACE PROTEIN IN THE IMMUNOGLOBULIN SUPERFAMILY. J.P. Kanki * and J.Y.Kuwada, Dept. of Biology, University of Michigan, Ann Arbor, MI 48109.

A number of neural cell surface molecules have been characterized that localize to restricted subsets of axons and are members of the immunoglobulin superfamily of cell adhesion molecules. These molecules are likely to be involved in axon guidance during early neural development. One such molecule that has been characterized in the chick is DM-GRASP, also known as SC1 or BEN. We have recently cloned and begun to characterize a zebrafish cDNA that is similar to DM-GRASP. $6x10^5$ zebrafish clones were screened with a 1.6kb restriction enzyme fragment of the Chick DM-GRASP cDNA (Burns, et al. 1991). One of these clones contains a 2.1kb insert which shares 54.6%identity with the nucleotide sequence of Chick DM-GRASP (Pearson and Lipman, 1988). Northern analysis indicates that this zebrafish transcript is expressed during the period of early axonogenesis. This clone contains a single open reading frame of 610 amino acids which shares 35.1%identity as well as structural homology with the Chick DM-GRASP protein . A portion of the Zebrafish DM-GRASP coding region was inserted into the pGEX-KN vector (Hakes and Dixon, 1992) and over-expressed in *E. Coli.* This fusion protein was purified and injected into rabbits for the production of antisera. Initial immunohistological results indicate that the Zebrafish DM-GRASP protein is expressed on the axons of a subset of neurons during early zebrafish development. These results indicate that we have cloned either the zebrafish homolog of DM-GRASP, or a very closely related member in the immunoglobulin superfamily. (Supported by NIH)

451.9

Purified Chick DM-GRASP Supports Neurite Extension From A Subpopulation Of Neurons In Vitro A.P. DeBernardo and S. Chang*
Department of Neuroscience, U. of Penn., Philadelphia, PA 19104

DM-GRASP (DM) is an integral membrane glycoprotein expressed on a restricted set of axons in the developing chick nervous system. Currently, three lines of evidence suggest that DM may be involved in axonal pathfinding: (1) Its expression pattern is temporally dynamic and roughly coincides with axonogenesis. (2) Antibodies to DM can block the extension of sympathetic neurites on sympathetic axons. (3) Purified DM can support the extension of dorsal root ganglion (DRG) neurites. We have extended these findings by demonstrating that purified DM supports neurite extension in a distinct subpopulation of neurons. This subpopulation is defined by DM expression. Both DRG and spinal motor neurons (SMN) express DM in vivo. Dissociated and explant cultures of DRG and SMN extend neurites on laminin, G4/L1, and DM substrates that are significantly longer than on a control BSA substrate. Preincubation of substrates with 100 ug/ml monoclonal antibody against DM blocks extension on DM, but does not affect extension on other substrates. In contrast, retinal ganglion cells do not express DM. Dissociated or explant cultures of retina do not extend neurites on DM that are longer than on BSA. Retinal neurons are able to extend neurites on laminin or G4/L1 under the same culture conditions. This data suggests that purified DM selectively supports neurite extension from DM bearing subclasses of neurons. The selective fasciculation model of pathfinding predicts that particular axonal tracts support axonal extension from subclasses of neurons. Known substrate molecules support extension from a wide variety of neurons. The finding that DM can selectively support neurite extension suggests it may be involved in selective fasciculation.

451.10

GROWTH CONES OF COMMISSURAL AXONS IN THE ZEBRAFISH SPINAL CORD CHANGE THEIR SUBSTRATE AT THE VENTRAL MIDLINE. **R.R. Bernhardt*, E. Tongiorgi, and M. Schachner. Neurobiology, ETH-Hönggerberg, 8093 Zürich, Switzerland.**

Commissural (Co) axons initially extend ventrally from dorsally located cell bodies. After crossing the ventral (ve) midline they grow dorsally to ultimately extend anteriorly in a dorsal longitudinal pathway. Why do Co growth cones first extend to, but then away from, the ve midline? A switch in the adhesive properties might be triggered when the axon passes the ve midline. EM analysis of labeled Co growth cones has now revealed morphological evidence consistent with such a switch. Growth cones which have arrived in the ventrolateral cord, but not yet crossed the ve midline, have many filopodia which make extensive contacts with neuroepithelial cells, and possibly with ventral neurons, but show very little apposition to the basement membrane (BM). In striking contrast, growth cones which occupy equivalent positions after having crossed the ve midline have few filopodia and show extensive contact with the BM. We are now exploring molecular mechanisms which may underlie this change. Candidates are recognition molecules at the cellular surface and in the extracellular matrix. One among them is L1, the spatially and temporally regulated expression of which has been implicated in influencing Co axonal pathfinding in mammals. In situ hybridisation demonstrates that Co neurons in zebrafish express an L1 homologue. The regulation of L1 expression by Co neurons is currently under investigation.

451.11

CLONING AND ANALYSIS OF DEVELOPMENTAL EXPRESSION PATTERN OF L1 AND TENASCIN HOMOLOGUES IN ZEBRAFISH.
E. Tongiorgi*, R.R. Bernhardt and M. Schachner
Neurobiology, ETH-Hönggerberg,8039 Zürich, Switzerland

Zebrafish homologues of chicken tenascin and mouse L1 have been isolated from a 33-36h embryonic zebrafish cDNA library. Their expression patterns during embryonic development have been analysed by *in situ* hybridisation.

High levels of tenascin message are expressed in the hypochord, a single row of cells located ventral to the notochord from 16h, the earliest stage tested. At 16h, tenascin-specific message is detectable also in somites. The signal is strongest in the rostral half of each somite and restricted to cells occupying medial positions in the central third of each somite, including muscle pioneers. From 18h, in more mature somites, the message is present over the entire width of the somitic anterior-posterior axis and transiently over a larger region along the dorso-ventral axis. At 19h, tenascin positive cells are detectable in dorsal regions of the trunk. The time of appearance, positition and shape suggest that they are neural crest cells. Onset of L1 expression appears correlated with axonogenesis. At 18h, groups of neurons with early axonogenesis such as the early clusters of brain neurons, hindbrain neurons and head sensory ganglia are conspicuously L1 positive. Nuc PC neurons with later axonogenesis are first positive at 21h. In the spinal cord the first L1 positive cells are the Rohon-Beard cells. Other spinal neurons are labelled slightly later. In the eye retinal ganglion cells (RGC) also start to be L1 positive by the time of their axonogenesis (about 36h). During post-larval development L1 expression in the RGC layer becomes restricted to the retinal margin. In general higher levels of L1 message are seen during embryonic development than at early post-larval and adult stages.

451.12

HNK-1 AND TENASCIN DISTRIBUTION IN THE DEVELOPING NEUROMUSCULAR SYSTEM OF XENOPUS. T. Somasekhar and R.H. Nordlander*. Depts. Oral Biol. and Anat., Ohio State Univ., Columbus, OH 43210.

The antibody HNK-1 marks developing neurons in Xenopus, including outgrowing motor axons (Devel. Br. Res., 50:147-153, '89). HNK-1 recognizes an epitope common to many cell adhesion molecules, including tenascin (TN). Both the HNK-1 antigen and TN are involved in neurodevelopment. This study examines staining patterns of HNK-1 and anti-TN at developing myotomes of Xenopus in order to: 1) compare their distributions, and 2) determine whether TN may account for HNK1-immunoreactivity (HNK1-IR) at the developing myotomal neuromuscular junction.

HNK1-IR occurs on the surfaces of all peripheral axons in a graded rostrocaudal pattern. HNK-1 labeling also outlines the presynaptic nerve terminals at the intermyotomal furrows, the sites of myotendinous and neuromuscular junctions. On muscle, HNK1-IR is restricted to synaptic sites, first labeling the motor axon and later the postsynaptic membrane. TN-IR also appears at the furrows in a graded pattern. It precedes the arrival of motor axons. Unlike HNK1-IR, TN-IR is not closely associated with axons and neuromuscular junctions. These mutually exclusive distributions suggest that TN does not carry the HNK-1 epitope during innervation and maturation of the axial musculature in Xenopus.

Supported by NS-18773 to RHN.

451.13

STUDIES OF S-LAMININ FUNCTION *IN VITRO* AND *IN VIVO*. B.E. Porter, P.G. Noakes*, M. Gautam#, J.P. Merlie# and J.R. Sanes.
Departments of Anatomy and Neurobiology, and of Molecular Biology and Pharmacology#, Washington University School of Medicine, St. Louis, MO 63110

Axons preferentially reinnervate original synaptic sites on muscle fibers. We are testing the idea that s-laminin, a laminin-like chain concentrated in the basal lamina of the neuromuscular junction, is one of the molecules that influences motor axons at synaptic sites. S-laminin fusion proteins contain a binding site, LRE, to which motoneuron-like cells from chick ciliary ganglion (CG) or a spinal-cord derived cell line (NSC-34) selectively adhere (CSHSQB 55:419, 1990). Although adhesive, these proteins do not promote neurite outgrowth. We now show that s-laminin fusion proteins inhibit neurite outgrowth from both CG and NSC-34 cells. Fusion proteins with LRE mutated to QRE or LRA do not inhibit outgrowth. Outgrowth is not inhibited from PC12 or sensory cells, which do not adhere to LRE. These results suggest that LRE might serve as a stop signal for motor axons, an idea we tested with patterned substrates. CG cells growing on laminin stop at borders of s-laminin-LRE but cross borders onto s-laminin-QRE. We are now determining if growth cones differentiate into nerve terminals at s-laminin-LRE borders. In addition, we will assess the function of s-laminin *in vivo* using a gene knockout approach in embryonic stem (ES) cells. We have isolated s-laminin genomic clones, constructed targeting vectors, and generated ES cells with an inactivated s-laminin gene. We are currently injecting these cells into blastocysts to eventually generate s-laminin-null mutants. These two approaches will test the role of s-laminin in the formation and stabilization of neuromuscular synapses. (Support: NIH and MDA).

452.1

DEVELOPMENTAL EXPRESSION OF NEURONAL NICOTINIC RECEPTOR SUBUNITS IN THE CENTRAL NERVOUS SYSTEM.

R. Krauss*, K.M. Rosen and G.D. Fischbach. Department of Neurobiology, Harvard Medical School, Boston, MA 02115.

Neuronal Nicotinic Acetylcholine Receptor (NnAChR) subunits belong to a family of closely related genes. They display discrete (sometimes overlapping) patterns of gene expression in CNS. The NnAChRs are multimers containing a mixture of α and β subunits which determine their physiological and pharmacological properties. The α7 subunit forms channels highly permeable to Calcium and may participate in regulatory processes including synaptogenesis. The steady-state expression of the α7 NnAChR subunit mRNA was studied by Northern blots in different regions of rat CNS and compared to the expression of α3 and α4. Total RNA samples were analyzed at E19, P0, P3, P6, P9, P12, P18 and P21.

In most regions the α7 mRNA is low around birth and then increases. In the cerebral cortex (Cx) α7 increases at P3, then maintains steady levels until P21. This pattern is also followed by α3 and α4. In the Hippocampus (Hip), α7 increases at P6 and, as in Cx, is maintained at steady levels until P21. Similarly to Cx this increase at P3 is followed by α3 and α4 but, as a difference α3 declines after 3 days. In the Brainstem (Bs) α7 appears at P3, remains constant through P12, then undergo a transient decrease at P15. The same pattern is followed by α4, but α3, which is similar from E19 to P12, declines after P15. In the Diencephalon α7 has two transient peaks, one at P3 and a later one at P9-P12. By contrast, α4 increases at P9 and this level is subsequently maintained at steady levels until P21, whereas α3 maintains constant levels throughout development. A different situation occurs in the Cerebellum (Cb) where α7 has an increase at birth, then declines and increases again at P9 to be maintained steady through P21. By contrast α3 is present at late embryonic stages (E19-P0) then decreases sharply at P3 and finally disappears.

It is concluded that the expression of the α7 subunit is regulated differently in different brain regions. Moreover, in some areas (i.e. Cx, Hip, Bs) α7 is regulated coordinately with one or more α subunits, whereas in others (i.e. Di, Cb) the regulation of α7, α3 and α4 is independent from each other.

452.2

MAINTENANCE OF PHENOTYPE OF MATURE SWEAT GLAND NEURONS AFTER DISRUPTION OF CONTACT WITH TARGET.

S. Herculano and S.C Landis*. Dept. of Neurosci., CWRU, Cleveland OH 44106.

Downregulation of noradrenergic properties in and acquisition of the mature cholinergic and peptidergic phenotype by rat sweat gland neurons depends on interaction with their target. It is not clear, however, whether maintenance of the neurotransmitter phenotype of the mature sweat gland neurons requires continued interaction with the target. To examine this question we identified sweat gland neurons in the lower lumbar sympathetic ganglia of adult rats by retrograde labeling from hind footpads with fast blue, and then analyzed their neurotransmitter properties at various times after axotomy. Sweat gland neurons constitute a minor population of sympathetic neurons. They either have faint or no tyrosine hydroxylase immunoreactivity (TH-IR), lack neuropeptide Y immunoreactivity (NPY-IR) and contain vasoactive intestinal peptide immunoreactivity (VIP-IR). When retrogradely labeled neurons were examined in sham-operated animals, 81.8±9.1% had faint or no TH-IR, 84.6±11.9% were negative for NPY-IR, and 81.3±8.3% were positive for VIP-IR. The expression of TH-IR, NPY-IR and VIP-IR within retrogradely labeled neurons did not change significantly after axotomy. Fourteen days after surgery, 89.5±4.7% of retrogradely labeled neurons on the axotomized side had faint or no TH-IR, 86±0% were negative for NPY-IR, and 86±0% were positive for VIP-IR. The continued absence of TH-IR and presence of VIP-IR after axotomy suggests that axotomy does not alter the neurotransmitter phenotype induced in sweat gland neurons by interactions with their target tissue during development. Further, our results provide evidence that maintenance of the transmitter phenotype of mature sweat gland neurons does not require continued contact with the target.

452.3

SYNTHESIS OF HISTAMINE IN DEVELOPING RAT BRAIN.

A. Vanhala, A. Yamatodani and P. Panula*. Dept. Anatomy, Univ. Helsinki, 00170 Helsinki, Finland, Dept. Molec. Physiol. Chem., Osaka Univ., Osaka, Japan and Dept. Biology, Abo Akademi University, Biocity, 20520 Turku, Finland.

High histamine (HA) concentration is found in embryonal rat brain in transiently HA-ir neurons. We detected low HA concentrations in prosencephalon/telencephalon, mesencephalon/diencephalon, and pons/medulla on day E12 using HPLC fluorometry. A clear increase in all areas occurred on day E13, and by day E18 the concentration declined in all areas. The only HA-ir neuron population during that period was in the developing raphe area. In this area, a subpopulation of cells were immunoreactive for both serotonin and HA. A specific antiserum against histidine decarboxylase (HDC) revealed immunoreactivity in these cells. After day E18, these cells were still intensely immunoreactive for serotonin but no HA-ir was detected. Oligonucleotide probes complementary to rat HDC detected a small population of cells in the raphe area on day E16. The results suggest that histamine is synthesized in developing rat brain in a subpopulation of developing raphe neurons, which sends projections to other areas.

452.4

REGULATION OF GALANIN EXPRESSION IN THE ADULT SUPERIOR CERVICAL GANGLION (SCG) AFTER AXOTOMY.

R. C. Schreiber*, T. A. Bennett, H. Hyatt-Sachs, and R.E. Zigmond. Department of Neurosciences, Case Western Reserve University, School of Medicine, Cleveland, OH 44106.

Postganglionic axotomy of sympathetic neurons results in an increased expression of substance P and vasoactive intestinal peptide-like immunoreactivity (IR) and their mRNAs (Hyatt-Sachs et al, J Neurosci, 1993; Rao et al, J Neurobiol, 1993). Another neuropeptide, galanin, has been shown to increase after axotomy of sensory neurons (Hokfelt et al, Neurosci Lett, 1987) and cranial motor neurons (Saika et al, Brain Res, 1989; Moore, J Comp Neurol, 1989).

We have examined the regulation of galanin-IR in the rat SCG after cutting the cervical sympathetic trunk (CST), internal and external carotid nerves (ICN, ECN), and after explant culture. Forty-eight hours after cutting the ICN and ECN or placing the SCG in culture, galanin-IR increased 238- and 438-fold, respectively, as measured by radioimmunoassay (RIA). Immunohistochemical analysis indicated galanin labeled neurons and fibers distributed throughout the SCG. When only the ECN was cut, a 127-fold increase in galanin-IR resulted, and the immunostained neurons were highly concentrated in the caudal portion of the ganglion. Cutting the ICN also resulted in a 127-fold increase in galanin-IR and galanin labeling of neurons and fibers in the rostral portion of the SCG. Lastly, cutting the CST resulted in only a 2.6-fold increase in galanin-IR and a few immunostained neurons near the caudal pole of the SCG. Based on previous retrograde labeling studies (Bowers & Zigmond, J Comp Neurol, 1979) this distribution pattern is consistent with the view that changes in galanin-IR occur primarily, if not solely, in axotomized neurons. The content of galanin mRNA was shown to increase by Northern blot analysis after the ECN and ICN are transected, after explantation and, to a much smaller extent, after the CST was cut. Thus, these data, together with previous studies, indicate that galanin expression increases in sensory, motor, and sympathetic neurons after peripheral nerve transection.

452.5

INCREASED GALANIN AND VASOACTIVE INTESTINAL PEPTIDE MESSENGER RNA EXPRESSION IN SYMPATHETIC NEURONS OF THE ADULT RAT SUPERIOR CERVICAL GANGLION AFTER AXOTOMY.

R.P. Mohney*, R.E. Siegel, and R.E. Zigmond. Department of Neurosciences, Case Western Reserve University, School of Medicine, Cleveland, OH 44106.

In the sympathetic nervous system, galanin-like immunoreactivity (IR) is absent in neurons of the adult rat superior cervical ganglion (SCG; Schreiber et al., preceding abstract) and vasoactive intestinal peptide (VIP)-IR is present at a low level (Hokfelt et al., Neurosci., 1977). Immunoreactivity for both neuropeptides increases *in vivo* after postganglionic axotomy of sympathetic neurons, as measured by immunohistochemistry and radioimmunoassay (Schreiber et al., preceding abstract; Hyatt-Sachs et al., J. Neurosci., 1993).

We have examined changes in messenger RNA for galanin and VIP by *in situ* hybridization using radiolabeled oligonucleotide probes. The level of galanin mRNA in the SCG increased after 48 hr in culture, and this increase is seen in neurons throughout the SCG. *In vivo* axotomy of both postganglionic trunks of the SCG, the internal and external carotid nerves (ICN, ECN), resulted in an increase in galanin mRNA qualitatively similar to that seen in culture. The number of neurons containing detectable galanin mRNA after axotomy was higher than the number containing detectable galanin-IR. Lesioning the ICN resulted in an increase in neuronal cell bodies expressing galanin mRNA located primarily in the rostral half of the SCG. Conversely, cutting the ECN produced an increase in galanin mRNA in neurons residing primarily in the caudal portion of the SCG. Decentralization of the SCG, produced by lesioning the cervical sympathetic trunk, increased galanin mRNA expression in a small population of neurons near the caudal end of the SCG. These data are consistent with the hypothesis that galanin mRNA increases predominantly, if not solely, in neurons that have been axotomized. *In vivo* and *in vitro* experiments produced similar increases in VIP mRNA, but a smaller population of neurons was affected than in the case of galanin mRNA. No evidence for an increase in either peptide in non-neuronal cells in the SCG was obtained.

452.6

REGULATION OF GALANIN IN AXOTOMIZED SYMPATHETIC NEURONS IN THE MIDDLE AND INFERIOR CERVICAL GANGLIA.

A. M. Shadiack*, S. A. Vaccariello, and R. E. Zigmond. Dept. of Neurosciences, Case Western Reserve Univ, School of Med, Cleveland, OH 44106.

Sympathetic neurons in the rat superior cervical ganglion (SCG) respond to axotomy by increasing levels of certain neuropeptides. Our laboratory has previously shown that vasoactive intestinal peptide and galanin increase 22-fold and 238-fold, respectively, 48 hrs after axotomy.

These observations raised certain questions: 1) Is this a phenomenon restricted to the SCG or do other sympathetic neurons in other ganglia respond similarly? 2) Since axotomy is performed within millimeters of the SCG, is local tissue damage the trigger of this response? 3) Is the peptide regulation occurring in the same neurons that are being axotomized? In order to answer these questions we turned to the middle and inferior cervical ganglia (MICG) which is caudal to the SCG in the sympathetic chain and contains a population ofneurons which project rostrally through the cervical sympathetic trunk (CST) (Bowers & Zigmond, Neurosci 1981).

Animals were anesthetized and their CST transected nearly 2 cm away from the MICG so as to leave the ganglion undisturbed. The number of neurons exhibiting galanin-immunoreactivity (IR) increased by 7 days after surgery. We then applied the retrograde label fast blue to the transected CST which resulted in the labeling of a modest portion of the total neurons in the MICG. This tissue was then examined for galanin-IR by immunohistochemistry. Galanin-positive cells followed a similar distribution to the axotomized cells with approximately 75% of the immunostained neurons containing fast blue. As controls, sham-operated animals showed very few galanin-positive cells and, in retrograde labeling experiments, the contralateral MICG showed only a handful of fast blue labeled cells.

452.7

ALTERATION IN ENKEPHALIN EXPRESSION BY SYMPATHETIC NEURONS FOLLOWING INTERRUPTION OF TARGET CONTACT. S. Tyrrell* and S. C. Landis. Dept. of Neurosci., Case Western Reserve Univ., Cleveland, OH 44106.

Sympathetic neurons are plastic with respect to their transmitter phenotype. The factors which regulate this plasticity *in vivo* are poorly defined. Previous studies have implicated target tissues in the development of neuropeptide phenotypes. We examined the effect of target contact on enkephalin (Enk) expression by disrupting interactions between the superior cervical ganglion (SCG) and its targets with one dose of the neurotoxin, 6-hydroxydopamine (6-OHDA) on postnatal day (P)1. During normal development, leucine (L)-Enk and methionine (M)-Enk immunoreactive (-IR) SCG neurons were first detected by immunocytochemistry (ICC) on P2. The number of Enk-IR cells and the concentration of Enk-IR assayed by radioimmunoassay (RIA) increased during the first 2 postnatal weeks but decreased dramatically after 3 weeks. *In situ* hybridization studies with a probe complementary to proenkephalin mRNA showed that the percentage of cells with grain densities above background increased until P21. In addition, the mean density of grains over neurons increased until P14 while after P21 neither the proportion of cells with grain densities above background nor the mean grain density changed. Two days after treatment of neonates with 6-OHDA, the density of tyrosine hydroxylase (TH)-IR fibers within the submandibular gland, a target of SCG Enk-IR neurons, was dramatically reduced but by P14 the density of TH-IR fibers was equivalent to that in control animals. Enk-IR detected by ICC and RIA was substantially reduced in the SCG of 6-OHDA treated rats until after P21, while NPY-IR assayed with ICC was unchanged. Moreover, *in situ* hybridization studies showed decreased proenkephalin mRNA in adult SCG. These data indicate that expression of Enk during development and in the adult is altered by neonatal 6-OHDA treatment which disrupts neuron-target interactions. Since we were unable to detect any peak in Enk-IR, and less proenkephalin mRNA was detected in the adult, we conclude that the normal increase in Enk expression was not just delayed, but prevented.

452.8

BEHAVIORAL EFFECTS OF PRENATAL EXPOSURE TO AN ALPHA-1 AGONIST AND ANTAGONIST. W. Briner*, B. Miller, and E. Martin. Dept. of Psychology, Univ. of Nebraska at Kearney, Kearney, NE 68849.

It has been suggested that many of the traditional neurotransmitters may modulate neuronal development. For instance, 5-HT has been shown to exert a trophic effect on embryonic CNS neurons and norepinephrine (NE) innervation is required for normal development of the cerebellum. We have examine the role of the α-1 receptor in early neural development by administering the α-1 agonist phenylephrine (PH) and the α-1 antagonist terazosin (TZ) to rats during early gestation. Female rats were given injections of PH (20.5mg/kg), TZ (171.1mg/kg), injected with saline from days 6-10 of gestation, or left untreated. After birth the pups were examined for the emergence of developmentally significant behaviors. Pups that received the α-1 agonist PH were delayed in the emergence of the surface righting reflex, but advanced for walking. In open-field testing these animals engaged in more rearing behavior than did the other groups. The α-1 antagonist (TZ) group did not differ significantly from the controls. These findings reinforce the concept that the NE system is involved in cerebellar development, but the α-1 receptor may not modulate development in the same direction or manner for all behavioral systems.

452.9

DEVELOPMENT OF NEUROPEPTIDES AND NADPH-DIAPHORASE IN THE ENTERO-PANCREATIC INNERVATION OF THE RAT. T. Tharakan, A. Kirchgessner, L. V. Baxi, T. Rothman* and M D. Gershon. Columbia Univ. P&S New York, NY 10032

Pancreatic ganglia are formed by neural crest-derived precursors that migrate first to the bowel and then to the pancreas. These cells are innervated by enteric neurons, which evoke amylase and insulin secretion. Most of the neuropeptides found in the bowel are also present in the pancreas. In addition, the enzyme NADPH-diaphorase (d) is located in a subset of enteric and pancreatic neurons, where it is coexpressed with neuropeptide Y (NPY), vasoactive intestinal peptide (VIP) gastrin releasing peptide (GRP) and galanin (GAL). The expression of neuropeptides and NADPH-d was studied in the fetal and neonatal rat gut and pancreas (E12-P28) *in situ* and in organotypic cultures of pancreatic rudiments and foregut. Neural markers included the immunoreactivities of GAP-43 and NC-1 (which is expressed by crest-derived cells). Neurotransmitter-related markers included dopamine β-hydroxylase (DBH), NPY, VIP, GAL, GRP. At day E12, NC-1 and GAP-43-immunoreactive cells were found in the primordial stomach. These cells were also DBH-immunoreactive. Cells that expressed these markers were visualized in the pancreas one day later, on day E13, along with NPY, which appeared to be present in endocrine cells. Pancreatic NPY-immunoreative neurons were detected by day E18. GRP immunoreactivity (IR) was first detected in the stomach at day E18 and in the pancreas by birth. VIP-IR could not be detected in the bowel until day E20 and not in the pancreas until birth. GAL-IR appeared in the gut at day E18 and in the pancreas at E20. NADPH-d activity was first found in neurons in the presumptive stomach on day E13. Scattered neurons that expressed NADPH-d activity were visualized in the pancreas on day E14. NADPH-d activity was found in neurons in pancreatic rudiments explanted on day E13; therefore, pancreatic neural precursors must have been present in the pancreatic rudiments when they were explanted. These observations support the idea that pancreatic ganglia develop from transiently catecholaminergic precursors that migrate to the pancreas from the fetal foregut. Supported by grants NS27645, NS01582, and NS15547.

452.10

INNERVATION OF POSTNATAL RAT HEART BY NEUROPEPTIDE Y (NPY)- AND TYROSINE HYDROXYLASE (TH)- IMMUNOREACTIVE (IR) NERVES C. Nyquist-Battie*, S. Sands, P. Cochran and B.M. Chronwall. School of Biological Sciences, University of Missouri - Kansas City, Kansas City, MO 64108

Postnatal development of cardiac NPY-IR nerves was documented and compared to that of TH-IR nerves to determine if these two putatative co-localized markers of cardiac sympathetic nerves exhibited the same temporal and spatial developmental pattern. On postnatal day (PN) 2, the NPY-IR sympathetic trunk was easily recognized as it entered the heart. Intense NPY-IR nerve plexuses were present in nodal areas at birth but their density declined with age. Additionally, in newborn hearts individual NPY-IR fibers were present in the myocardium of the atrial septum, being quite noticeable at the atrio-ventricular junction. In all areas, the epicardium contained a NPY-IR nerve plexus on PN2. A rapid NPY-IR innervation of the myocardium occurred between PN2 and PN21 with atrial innervation proceedings ventricular innervation. Perivascular innervation was present before myocyte innervation in most instances. The density of NPY-IR fibers in ventricular myocardium decreased between the third postnatal week and adulthood. TH-IR innervation developed similarly to that of NPY, confirming the localization of NPY in sympathetic fibers and suggesting a co-regulation of NPY and TH expression in developing cardiac sympathetic nerves.

452.11

DEVELOPMENT OF THE DOPAMINE TRANSPORTER (COCAINE BINDING SITE) IN THE RAT MIDBRAIN: AUTORADIOGRAPHY WITH [^{3}H]WIN 35,428. C. L. Coulter, H. K. Happe, L. C. Murrin*. Dept. of Neurology, Creighton Univ. Sch. Med., Omaha NE 68131 and Dept. of Pharmacology, Univ. Neb. Med. Sch., Omaha, NE 68198.

Information on the development of dopaminergic mesencephalic nuclear groups is important in understanding the growth of the mesolimbic and nigrostriatal systems. Radioligands that bind to the dopamine transporter (cocaine binding site) allow visualization of these dopaminergic neurons and their projections. We used [^{3}H]WIN 35,428 to study the developmental distribution of the dopamine transporter in rat midbrain from birth to adult. Rat brain was sectioned at 16 μ and thaw-mounted onto subbed slides. Sections were incubated in 10 mM sodium phosphate buffer, 0.32 M sucrose, 120 mM NaCl, pH 7.4 at 4°C for 120 min, then rinsed 2 x 2 min in ice-cold buffer. Sections were apposed to Hyperfilm for 90 days (adult rats) to 180 days (day 0 rats). Quantitative analysis of autoradiograms showed early maturation of dopamine transporter sites in substantia nigra and ventral tegmental area. Substantia nigra, zona compacta reached adult levels of binding by day 5, as did the posterior portion of the substantia nigra reticulata. The more anterior portion of the substantia nigra reticulata reached adult levels by day 10. In adults there was a caudal (higher) to rostral (lower) gradient of binding in the substantia nigra reticulata that was not appreciable at earlier ages. The ventral tegmental area appeared to develop rapidly since binding to transporter peaked at day 5 and regressed to adult levels by day 15. The subthalamic nucleus showed moderately low levels of binding until day 10, then steadily increasing binding through days 15 and 21. Normative data on development of the dopamine transporter will allow investigation of the effects of perinatal insults from anoxia, toxins (drugs), or infection on the dopaminergic system. Supported by NS 23975.

452.12

FETAL MOTOR BEHAVIOR AND THE DOPAMINE SYSTEM: EFFECTS OF CENTRAL ADMINISTRATION OF D1 RECEPTOR AGONIST AND ANTAGONIST. E. S. Petrov, E. I. Varlinskaya, Institute for Experimental Medicine, Russian Academy of Medical Sciences, St. Petersburg,; S. R. Robinson, and W. P. Smotherman*Laboratory of Perinatal Neuroethology, Center for Developmental Psychobiology, Binghamton University, Binghamton, NY 13902-6000.

Intraperitoneal (IP) administration of D1 receptor agonists and antagonists has been shown to produce behavioral effects in rats during the prenatal and early postnatal periods. Understanding the functional role of developing dopaminergic systems may be advanced by central administration of agonist and antagonist drugs and in vivo measurement of fetal motor behavior. The D1 agonist SKF-38393 and antagonist SCH-23390 were administered to rat fetuses on E21 by intracisternal (IC) or intrahemispheric (IH) injection. Both routes of agonist administration resulted in increased motor activity. IC injection of SKF-38393 in various dosages (0.2-10.0 μg) elicited a pronounced increase in movements of the head, limbs and body trunk, which comprise the main categories of movement during spontaneous activity. IC injection of the D1 antagonist did not change overall activity in the dosage range of 5.0-20.0 μg, but fetuses treated with SCH-23390 showed an increase in specific behavioral categories seldom observed during spontaneous activity (facial wiping, mouthing and licking movements), and a decrease in common movement categories (head, forelimb and rearlimb). Unilateral IH injection resulted in similar changes in fetal behavior, suggesting that the right and left hemispheres of the fetal brain are sensitive to D1 receptor manipulation. Differences in behavioral effects of left and right IH injections suggest lateral asymmetry in sensitivity to a D1 agonist, but only in females. These data confirm that central dopamine systems play an important role in the regulation of motor behavior during the prenatal period.

Supported by Grants HD 16102 to WPS and HD 28231 to WPS & SRR.

452.13

DEVELOPMENT OF SEROTONIN CONTENT AND TURNOVER AFTER NEONATAL 5,7-DIHYDROXYTRYPTAMINE TREATMENT IN RATS.

M. Mercugliano*, H.Q. Nguyen, S. Djali, and I. Lucki. Children's Seashore House and Departments of Pediatrics, Pharmacology, and Psychiatry, University of Pennsylvania, Philadelphia, PA. 19104.

The neurochemical effects of treatment with the serotonin (5-HT) neurotoxin, 5,7-dihydroxytryptamine (5,7-DHT) were examined by HPLC 4, 8, and 12 weeks after neonatal i.c.v. administration (100 ug free base). Region-specific patterns in the development of 5-HT and 5-hydroxyindoleacetic acid (5-HIAA) content were found in control rats. In the hippocampus, 5-HT was unchanged while 5-HIAA increased from 4 to 12 weeks. In the striatum, 5-HT increased while 5-HIAA was unchanged. In the cortex, both 5-HT and 5-HIAA increased. In treated rats, 5-HT was reduced by 82-94 % without evidence of recovery or further decrease in these regions from 4 to 12 weeks. 5-HIAA was also significantly reduced, but declined further from 4 to 12 weeks. The ratio of 5-HIAA to 5-HT (turnover) differed in each region. In the cortex it was consistently decreased , in the striatum it was transiently increased, and in the hippocampus, it declined from 4 to 12 weeks. In the brainstem, 5-HT and 5-HIAA content were similar to controls until 12 weeks when both were significantly decreased. Normal 5-HT turnover was maintained. These results indicate differences between the forebrain and brainstem response to neonatal 5,7-DHT treatment, as well as differences between individual forebrain regions in subsequent 5-HT turnover. Supported by USPHS grants MH 36262, HD 26979 and K08 NS01594-01.

452.14

SEROTONIN-IMMUNOREACTIVE NEURONS DEVELOP IN CULTURES OF THE EMBRYONIC CHICK HYPOTHALAMUS.

A.M. Gabaldon, V.A. Roe, Y.M. Bellis, A.Y. Gonzales and J.A. Wallace*. Dept. of Anatomy, Univ. of New Mexico Sch. of Med., Albuquerque, NM 87131.

We have reported previously on the presence of tryptophan hydroxylase (TPOH) immunoreactive neurons in the hypothalamus of the chick embryo near hatching (SON Abs. 18:1471,'92). However, while TPOH is the first enzyme in the synthesis of serotonin (5-HT), no 5-HT immunoreactive cells are found in hypothalamic regions where TPOH+ neurons occur. To investigate whether hypothalamic TPOH+ cells have the potential to synthesize 5-HT during development, primary cultures of the embryonic hypothalamus were examined for the presence of 5-HT-containing neurons. Dissociated cells from the diencephalon of chick embryos at 8 days of incubation were plated on collagen-coated Petri dishes and grown for 5-7 days. Cultures grown in media (DMEM-F12) containing either 10% horse serum or 10% Serum Plus (Hazelton) produced numerous, intensely stained 5-HT immunoreactive neurons. The occurrence of these cells may indicate plasticity in the ability of TPOH+ neurons to synthesize 5-HT, or the induction of a novel population of 5-HT synthesizing or accumulating cells. Supported by grants DHHS GM08222 and RR08139.

452.15

DEVELOPMENTAL REGULATION OF OCTOPAMINE BIOSYNTHESIS IN THE CNS OF *MANDUCA SEXTA*.

H.K. Lehman*, C. Murgiuc, and J.G. Hildebrand. ARL Div. of Neurobiology, Univ. of Arizona, Tucson, AZ 85721.

Octopamine is a widespread neurotransmitter in *M. sexta*, and the enzyme tyramine ß-hydroxylase (TßH) is essential for the biosynthesis of octopamine. To study the developmental regulation of the octopaminergic phenotype, we have analysed the biochemical and morphological development of octopaminergic neurons in the insect nervous system.

In *M. sexta*, two octopaminergic neurons in each abdominal ganglion develop during embryogenesis. TßH activity remains unchanged in each ganglion from early to mid stages of adult development, but in later stages (beginning at pupal day 10), TßH activity increases 6-fold specifically in the terminal abdominal ganglion (TAG). The rise in TßH activity is coincident with the appearance of putative octopaminergic neurons in the TAG. A copper histochemical technique was used to visualize TßH containing cells during metamorphosis; 4 neurons appear in the male and 3 in the female. In addition, increased TßH levels are temporally correlated with changes in steroid hormone levels during development. The transformation from a larva to an adult is controlled by three pulses of ecdysteroids during metamorphosis: a small commitment peak, a prepupal peak, and a large pupal peak. Results of ligation experiments indicate that the largest pulse of 20-hydroxyecdysone (20-HE), the pupal peak, is required for normal development of TßH. Infusions of 20-HE to mimic the pupal peak are being used to confirm that the steroid is necessary and sufficient for the development of TßH in the TAG. [Supported by NSF IBN-92309780 (HKL).]

452.16

EFFECTS OF SEROTONIN (5HT) ON SOMATOSENSORY CORTEX OF NEONATAL RATS *IN VITRO*.

M.-Y. Shi*, R.D. Mooney, C.A. Bennett-Clarke, and R.W. Rhoades. Medical College of Ohio, Toledo OH 43699.

Recent data from our lab indicate that $5HT_{1B}$ receptors in S1 cortex are found on terminals of thalamocortical afferents during the first two postnatal weeks. We have examined the function of these receptors by intracellular and whole-cell recording using brain slices cut obliquely in order to preserve the thalamocortical projection. A stimulating electrode was placed in ventrobasal thalamus. To date we have tested 29 cells in S1 in animals 7-12 days of age. Using bath application, 5HT invariably reduced the amplitude of evoked EPSPs but also produced changes in membrane resistance and resting potential. To evaluate the role of the $5HT_{1B}$ receptors, we have used selective $5HT_{1B}$ agonists, TFMPP and CGS12066B, and have also applied 5HT after pretreatment of slices with ligands selective for $5HT_{1A}$ and $5HT_2$ receptors. In 90% (n=10) of cells tested with $5HT_{1B}$ agonists, the evoked EPSPs were reduced by at least 50% in amplitude without any corresponding change in membrane potential. In the presence of $5HT_2$ antagonist ketanserine plus $5HT_{1A}$ agonist 8-OH-DPAT, 5HT produced no additional change in membrane potential but reduced EPSP amplitude by >50% for all 7 cells tested. After pretreatment with less selective antagonists, methiothepin, cyproheptidine and spiperone, 5HT in each case (n=6) reduced EPSP amplitude and either hyperpolarized or depolarized the neurons according to whether $5HT_2$ or $5HT_{1A}$ receptors were antagonized, respectively. In summary, 5HT produces a number of effects upon S1 cortical neurons consistent with the presence of several subtypes of 5HT receptors. Moreover, the $5HT_{1B}$ receptor appears to mediate presynaptic inhibition of thalamocortical transmission in neonates.

Supported by EY 04170, EY 08015, EY 08661 and IBN-9208211.

452.17

CYCLIC AMP ACCUMULATION DUE TO DOPAMINERGIC DRUGS IN HUMAN SECOND TRIMESTER FOETAL BRAIN

M.H.M. Bakker[1], J.F. Dong[2], E.R. Hitchcock[2], D.A. Kendall[1], G.W. Bennett[1], C.A. Marsden[1](SPON: Brain Research Association). [1]Dept of Physiology and Pharmacology, Medical School, University of Nottingham, Nottingham NG7 2UH, UK. [2]Dept of Neurosurgery, University of Birmingham, Birmingham B67 7JX, UK.

With the use of foetal neural tissue as donor tissue for transplantation in the treatment of Parkinson's disease, it has become necessary to expand our understanding of the functional development of dopaminergic (DA) neurones in the brain. In this study we have investigated the development of DA function by measuring cyclic AMP accumulation in the presence of DA and the selective partial D1 receptor agonist SKF38393 in the ventral mesencephalon which contains the substantia nigra and the striatum of 17 to 20 week old human foetal brain. cAMP accumulation was measured in cross-chopped slices by a prelabelling assay using 3H-adenine in the presence of the phosphodiesterase inhibitor rolipram.

In the ventral mesencephalon, cyclic AMP production was increased by DA and to a lesser extend by SKF38393. In the striatum however, no increase was observed with DA receptor activation. The direct adenylyl cyclase activator forskolin increased cyclic AMP levels in both brain areas.

These results indicate the presence of the functional enzyme in both the ventral mesencephalon and in the striatum. A functional link between the DA receptor and the second messenger system appears only to be established in the ventral mesencephalon at this stage of development, indicating that the functional linkage of the DA receptors in the striatum occurs at a later stage.

452.18

NEUROTRANSMITTER STIMULATION OF G PROTEIN GTPASE ACTIVITY IN DEVELOPING CHICK HEART.

R.S. Aronstam* and T.L. Creazzo, Guthrie Research Institute, Sayre, PA 18840 and Dept. Cellular Biology & Anatomy, Medical College of Georgia, Augusta, GA 30912.

The ability of muscarinic and β-adrenergic agonists to activate the G protein cycle during development of chick heart was determined by monitoring agonist stimulation of G protein GTPase activity. GTPase activity was determined by the method of Cassel and Selinger. The reaction was carried out for 10 min at 37°C, and then quenched by the addition of 5% activated charcoal in 20 mM phosphoric acid (pH 2.5). Low K_M GTPase activity was calculated by subtracting activity measured in the presence of 100 μM GTP. Acetylcholine stimulated low Km GTPase activity by up to 120% (incubation day 12) with an EC50 of 1 μM. This stimulation was completely blocked by atropine with an IC50 of 100 nM. Isoproterenol stimulated GTPase activity by 40% with an EC50 of 3 μM; this stimulation was blocked by propranolol. Basal GTPase activity measured in the absence of receptor agonist increased steadily from incubation day 4 to incubation day 17 (from 8.8 to 17.6 pmol/mg protein/min). The amount of activity stimulated by both acetylcholine and isoproterenol was constant from incubation day 4 to incubation day 12, then rose 25% by day 17. G protein activation as indicated by fractional stimulation of basal activity declined steadily throughout development until day 12, after which it remained constant. These findings are consistent with the presence of G protein-coupled muscarinic and β-adrenergic receptors early in the development of chick heart, prior to sympathetic and parasympathetic innervation. (Supported by GM-46408 and the Medical Research Service of the Veterans Administration).

453.1

MORPHOLOGY AND CALCIUM CURRENT IN PC12D CELLS AFTER TREATMENT OF DOPAMINE(DA)-DEPLETED STRIATAL TISSUE EXTRACT. H. Hida, K. Nakajima, T. Hashitani, A. Fukuda, Robert E. Petroski[2], M. Inase*[1] and H. Nishino. Dept. Physiol., Nagoya City Univ. Med. Sch., Nagoya, 467. [1] Nat,l Inst. Physiol. Sci., Okazaki, 444, Japan. [2] Robert Wood Johonson Med. Sch., Piscataway, NJ 08854.

Development in morphology and Ca-current in PC12D cells were investigated after treatment of tissue extract from DA-depleted striatum. In Wistar rat, 6-OHDA was microinjected in the substantia nigra(SN) unilaterally to make chemical lesions in the nigrostriatal DAergic pathway. Two weeks after the lesion, DA-depleted striata were dissected out from rats that exhibited significant metamphetamine-induced rotations. These DA-depleted striatal and intact striatal tissues were homogenized with leupeptin and mercaptoethanol, centrifuged (35000 rpm,1h) and the supernatant was collected. PC12D cells were plated on poly-l-lysine or collagen coated dishes with 2% horse serum and 1% fetal culf serum. Extracts were added to the culture every three day. The DA-depleted striatal extract promoted neurite outgrowth in PC12D cells in concentration dependent manner. The effect was stronger than the intact striatal extracts. The extract induced a rather straight neurite extension with fewer arborizations compared to the treatment of NGF. Under whole cell patch clamp, with standard bath solution containing TTX and cesium-containing pipette bigger long-lasting inward current were observed in cells treated with DA-depleted striatal extract than in control cells. Data suggest the increase in Ca current may partly be the basis of differentiation of PC12D cells after treatment of DA-depleted striatal tissue extract.

453.2

IN VITRO DEVELOPMENT OF IMMORTALIZED MURINE HIPPOCAMPAL PROGENITOR CELLS. R. Rozental*[1,2], M.F. Mehler[2,3], M. Morales[2], A.F. Andrade-Rozental[1,2], J.A. Kessler[2,3] and D.C. Spray[2]. [(1)]Institute of Biophysics "Carlos Chagas Filho", UFRJ, 21941, Brazil; and Departments of [(2)]Neuroscience and [(3)]Neurology, A. Einstein College of Medicine, Bronx, New York, 10461, USA.

We examined the temporal interrelationship among gap junctions, voltage- and ligand-gated responses during neuronal maturation. In immortalized murine embryonic hippocampal progenitor cells (Mehler *et al., Nature*, 1993), *in vitro* differentiation was induced by either interleukin (IL)-7 alone or concurrently with TGFα and bFGF and evaluated under patch clamp, dye-coupling and Ca^{2+} imaging techniques. Electrotonic coupling between untreated neuroblasts was observed within 24 hours of plating in serum-free media. However, as neuroblasts differentiated into neurons both the junctional conductance and the extent of dye coupling progressively decreased. Voltage-dependent inward currents, that in time became TTX-sensitive, were observed under whole-cell patch clamp technique within two to six days of neuroblast treatment with cytokines. At this stage, however, these cells did not respond to glutamate and glycine (≤ 100 μM) or to acetylcholine (≤ 300 μM) (outside-out patch configuration). Contrary to expectation, the neuronal steady-state $[Ca^{2+}]_i$ did not change during ontogeny. Together, these findings indicate that certain cytokines may be involved in the development of excitability in neurons. Furthermore, the onset of voltage-dependent responses and the disappearance of intercellular coupling in these hippocampal cells seem to precede the expression of chemosensitivity to a variety of neurotransmitters.

453.3

CHARACTERIZATION OF THE ACUTE SYNAPTIC EFFECTS OF THE NEUROTROPHINS NT-3 AND BDNF. A.M. Lohof*, N.Y. Ip[+], and M.-m. Poo, Department of Biological Sciences, Columbia University, New York, NY 10027 and [+]Regeneron Pharmaceuticals, Tarrytown, NY 10591

We have previously shown that acute exposure to either neurotrophin-3 (NT-3) or brain-derived neurotrophic factor (BDNF) can produce rapid potentiation of the spontaneous and impulse-evoked synaptic activity at developing *Xenopus* neuromuscular junctions in culture; similar treatment with nerve growth factor (NGF) has no effect. The effect of NT-3 or BDNF on the frequency of spontaneous synaptic currents (SSCs) appears to reflect an increase in the frequency of spontaneous acetylcholine (ACh) secretion rather than an increase in post-synaptic ACh receptor sensitivity. Neither the mean SSC amplitude nor the distribution of SSC amplitudes changed following application of the neurotrophins. Further characterization of the synaptic potentiation produced by NT-3 indicates that the continuous presence of the factor is required: the frequency of SSCs returns to control levels after NT-3 is removed from the recording bath, whether the time of exposure to NT-3 is 15 min or 1 hr. This result suggests that exposure to the neurotrophin does not produce a lasting modulation of the synapse. Rather a rapid and reversible alteration in the secretion mechanism probably produces the change in SSC frequency. Finally, the effect of NT-3 appears to be mediated by Trk receptors, since pretreatment of cultures with 200 nM K252a prevented any change in SSC frequency when NT-3 was applied. Pretreatment with K252b, which does not inhibit Trk receptors at this concentration, did not prevent the NT-3 effect. Together, our results suggest that neurotrophins may play a role in regulating the function of developing synapses.

453.4

SODIUM CURRENT PROPERTIES OF POSTNATAL RAT CHROMAFIN CELLS EXPOSED TO NGF *IN VITRO* GRADUALLY RESEMBLE THOSE OF DIFFERENTIATED RAT SYMPATHETIC NEURONS. Islas-Suárez I., Gómez-Chavarín M., Verdugo-Díaz, L., Méndez M., Drucker-Colín, R., and Hernández-Cruz, A*. Instituto de Fisiología Celular, UNAM; A.P. 04510, México city, México.

Postnatal adrenal chromaffin cells undergo neuron-like morphologic diferentiation in response to NGF. An increased excitability has been documented in their neoplastic correlate (PC12 cells), were NGF markedly increases sodium current (INa) density. Although PC12 cell differentiation is widely used as a model for the development and maturation of cells from the simpatoadrenal lineage, it fails to reproduce some key observations. For instance, peak current potential, voltage dependence of activation and inactivation, and reversal potential of INa differ markedly between chromaffin cells and mature sympathetic neurons, however, the properties of INa in PC12 cells remain unchanged after NGF treatment. This work describes changes both in the current density and in the functional properties of INa during the NGF-induced neuronal differentiation of postnatal chromaffin cells. Rat chromaffin cells from postnatal day 7 were grown either in control media (DMEM, 10% FCS and antibiotics) or control media supplemented with 50-100 ng/ml NGF for periods up to 18 days. Freshly dissociated chromaffin cells and sympathetic SCG neurons from 18 day old rats were also used for comparison. Whole-cell patch-clamp experiments were conducted at different stages of diferentiation. (Pipette solution (mM): CsCl 110, HEPES 10, EGTA 11, $MgCl_2$ 1, pH 7.3; External solution (mM): NaCl 120, Hepes 10, $MgCl_2$ 2, KCl 2.8, $CaCl_2$ 1.8, pH 7.4). As a result of NGF treatment for 18 days, cell capacitance increased from 6.5 to 24 pF reflecting soma growth and neurite extension. At the same time, the average peak INa increased from 240 to 3000 pA, and the current density from 52 to 142 uA/cm^2. This compares with an average of 170 uA/cm^2. for mature sympathetic neurons. The peak current potential, half-activation, half-inactivation and reversal potential of INa in mature chromaffin cells were, respectively, +10, -10.9, -37.6 and +60 mV. In contrast, mature simpathetic neurons had -15, -35, -43 and +30. In cultured chromaffin cells these values started at +9, -9.7, -41.4, and +60 on day 0 and reached -2, -25.4. -43.6 and +43 after 18 days of NGF treatment. Thus, during NGF-induced neuronal differentiation, the properties of INa which are characteristic of the chromaffin phenotype change to resemble those of the sympathetic phenotype. Current investigation is aimed to find out if complete transformation requires more prolonged NGF treatment or if a limit to the complete transdifferentiation exists. Supported by grants CONACyT F052 and DGAPA 200992.

453.5

REGULATION OF VOLTAGE-GATED ION CHANNELS BY NEUROTROPHIC FACTORS IN NEURAL CELL LINES. S. S. Lesser and D. C. Lo*. Department of Neurobiology, Box 3209, Duke University Medical Center, Durham NC 27710.

Although the development and maintenance of neuronal excitability is thought to be strongly influenced by neurotrophic factors, their regulation of ion channel and neurotransmitter receptor expression remains largely uncharacterized. We have conducted a preliminary study of the regulation of voltage-gated sodium, potassium, and calcium channel expression by neurotrophic factors in "neural" cells such as the human neuroblastoma line SK-N-SH, the parent line of SH-SY5Y cells. Because the SK-N-SH cell line has been used extensively in signal transduction studies of neurotrophic factors and also as a model for neuronal differentiation, it is particularly attractive for examining neurotrophic control over the development of excitability.

We found that NGF treatment caused an increase in sodium channel expression (2-fold in peak current; N=40), potassium channel expression (2-fold; N=8) and calcium channel expression (5-fold; N=52) as measured by whole-cell, perforated-patch, and single-channel recording. Induction was seen within 2-3 days after NGF addition, but became most pronounced by 5-6 days of treatment. Although it has previously been shown that SK-N-SH cells and SH-SY5Y cells respond to CNTF by increased *c-fos* expression, CNTF addition did not significantly alter sodium or calcium channel expression either alone (N=20) or in combination with NGF (N=18). Similarly, BDNF addition had no effect on sodium or calcium channel induction in SK-N-SH cells (N=17), even though BDNF has been shown to induce *c-fos* in SH-SY5Y cells. Differentiation by TPA (20 nM), led to an increase in sodium and calcium channel expression smaller than that induced by NGF (N=14), while retinoic acid (10 μM) affected neither sodium nor calcium channel expression (N=13). These and other experiments demonstrate that neural cell lines will provide a good system for the study of neurotrophic regulation of neuronal excitability through a combined use of molecular, imaging, and electrophysiological approaches.

453.6

NGF INCREASES VOLTAGE-DEPENDENT SODIUM CURRENT IN PANCREATIC β-CELLS. T.Rosenbaum, D.Sánchez-Herrera, R.Vidal-Tamayo, M.C.Sánchez-Soto, M.Hiriart*. Inst.Fisiología Celular Dept. Bioenergética, UNAM, México D.F., 04510, MEXICO.

We have previously shown the induction of neurite outgrowth by NGF in pancreatic β-cells (Soc. Neurosci., 1992, Abst.542.4). Following the study of the phenotypical NGF-induced changes in pancreatic β-cells, we have also assessed the cytoskeleton role in neurite outgrowth, and found that 92 % of the neurites are positive to tubulin (IF), suggesting that the β-cell cytoskeleton is actively participating in the process of neurogenization.

On the other hand, it has also been observed in other cell types that respond to NGF, like PC12 cell- line, that neurogenization is accompanied by changes in membrane electrical properties, which include a significant increase in sodium channel density. Normal pancreatic β-cells have voltage-dependent sodium channels which are important for membrane depolarization. In this study, the effect of 2.5S NGF on voltage-dependent sodium current of isolated β-cells was investigated. Islet cells were cultured for one week in the presence of 2.5S NGF (50 ng/ml), and inward ionic currents were recorded with a WCR variant of the patch clamp technique. The results indicate that NGF induced a 43% increase in macroscopic TTX-sensitive Na current with respect to control cells, from -37.89 ± 4.5 pA to -54.09 ± 5.3 pA (n=32 for each one, $p<0.05$). Our data suggest that one of the mechanisms of action of NGF on pancreatic β-cells is similar to that observed in other systems that respond to this factor with an increase in sodium channels expression.

Supported by DGPA grant IN206291, UNAM, MEXICO.

453.7

A SENSITIVE AND QUANTITATIVE BIOASSAY FOR NEUROTROPHINS USING SERUM-FREE MEDIUM.

S.A. Scott, X. Tian and K.A. Crutcher. Department of Neurosurgery, University of Cincinnati School of Medicine, Cincinnati OH 45267.

Assays employing embryonic chick sympathetic and dorsal root ganglia are routinely used to demonstrate the biological activity of NGF and related neurotrophins. These assays vary in sensitivity for several reasons, notably the type of dissection (explants versus dissociated cells) and the components of the tissue culture medium. When measuring neurotrophin-like bioactivity in tissue extracts, the ability to reliably measure very low levels (i.e., less than 10 pg/ml) is often necessary. We have developed a quantitative and serum-free bioassay for such purposes, using explants from sympathetic and dorsal root ganglia of E9 chick embryos. Medium consisted of Ham's F12 supplemented with selenium, putrescine, progesterone, human transferrin, and antibiotics, along with recombinant human proteins NGF (Genentech), BDNF or NT-3 (both from Amgen) ranging in final concentration from less than 1 pg/ml to over 100 ng/ml. Cultures were grown overnight in polyornithine-treated petri dishes and subsequently fixed and stained with silver nitrate. Neurite outgrowth was assessed using both image analytic and semiquantitative approaches.

The sympathetic ganglion assay consistently detected NGF in the range of 1-3 pg/ml, considerably below the limit of detection reported in previous studies. NT-3 was detectable below 10 pg/ml. BDNF was ineffective in the sympathetic assay but did stimulate neurite growth from sensory ganglia (as did both NGF and NT-3) at concentrations as low as 10 pg/ml. Areal measurements of neurite outgrowth alone correlated very highly with NGF levels up to 200 pg/ml. At higher concentrations, the density of neurites continued to increase whereas areal outgrowth (i.e., neurite length) did not. The maximally effective concentration of NGF was ~ 500 pg/ml, in accordance with a previous report (Greene, 1974), suggesting that biologically relevant levels of NGF are detected with this assay.

453.8

HIGH-LEVEL PRODUCTION AND CHARACTERIZATION OF RECOMBINANT BRAIN-DERIVED NEUROTROPHIC FACTOR AND NEUROTROPHIN-3 EXPRESSED IN INSECT CELLS.

D. M. Lang, S. L. Meyer, M. E. Forbes, E. Knight, Jr., J. D. Hirsch, S. P. Trusko, J. C. Kauer*, and R. W. Scott. Cephalon, Inc., West Chester, PA 19380.

Bioactive brain-derived neurotrophic factor (BDNF) and neurotrophin-3 (NT-3) were produced at high levels using the baculovirus expression system and purified to homogeneity using ion-exchange and reversed-phase chromatography. Yields of purified neurotrophin-3 (300-500 μg/L) were similar to levels reported for baculovirus-expressed nerve growth factor (NGF), whereas initial yields of BDNF were significantly lower (20-50 μg/L). Improved production of BDNF (150-200 μg/L) was achieved by expressing BDNF from a chimeric preproNGF/mature BDNF construct using the *Trichoplusia ni* insect cell line, Tn-5B1-4. Examination of the distribution of BDNF protein from both the chimeric preproBDNF and the chimeric preproNGF/mature BDNF viruses in Sf-21 and Tn-5B1-4 infected cells suggests a specific deficiency in the Tn-5B1-4 cells in processing the nonchimeric precursor. In addition, the vast majority of the BDNF protein at was intracellular and insoluble. Reversed-phase HPLC separated both the BDNF and NT-3 into two species, having similar bioactivity and identical N-terminal amino acid sequences. SDS-PAGE analysis under non-reducing conditions revealed that one HPLC species is the neurotrophin monomer and the other species is the dimer. Bioactivity was characterized *in vitro* on primary neuronal cultures from the CNS and PNS.

453.9

STRUCTURE/ACTIVITY RELATIONSHIPS OF NGF/BDNF CHIMERIC PROTEINS.

D. Dawbarn*, S.M. Colebrook, R. Feeney, S.H. MacGowan, S.J. Allen and M. Ashcroft. Molecular Neurobiology Group, Dept. Med. (Care of the Elderly), Bristol Univ., Bristol, BS2 8HW.

We have identified four regions of variability between the otherwise highly homologous protein sequences of the human neurotrophins (NGF, BDNF, NT-3 & NT-5). PCR homologue mutagenesis was used to generate a series of mutant genes, each encoding the human NGF gene with one or more of these four variable domains replaced with the equivalent domain from BDNF. The chimeric mutant proteins were expressed in Sf21 cells using the baculovirus system and production quantified using a two site ELISA. The neurotrophic biological activity of the mutants was assessed using a PC12 cell neurite outgrowth assay for NGF and a chick nodose ganglia assay for BDNF. The receptor binding and phosphorylation ability of each mutant was studied using p140^{trkA} and p145^{trkB} receptors expressed in COS-7 cells. These studies have identified regions within the neurotrophins that are required for activation of specific trk receptors.

453.10

SYNERGISTIC TROPHIC ACTIONS REVEALED BY NGF/BDNF CHIMERIC MOLECULES.

I.B.Black*, W.J. Friedman, H. Persson[1], and C.F. Ibáñez[1] Department of Neuroscience and Cell Biology, UMDNJ-Robert Wood Johnson Medical School, 675 Hoes Lane, Piscataway, N.J. 08854, USA, and [1]Lab of Molecular Neurobiology, Karolinska Institute, Box 60400, S-10401 Stockholm, Sweden

Members of the neurotrophin family of trophic factors act on specific neuronal populations to influence survival and/or function. We have previously shown that different neurotrophins have distinct influences on basal forebrain cholinergic neurons: NGF increased ChAT activity but had no effect on cholinergic cell survival, while BDNF, NT-3 and NT-4 all increased survival.

We have now employed a purified chimeric trophic molecule combining active domains of NGF and BDNF (Ibáñez et al., EMBO J. 10, 2105-2110, 1991) to examine the combined effects of the two trophic activities. We compared the influence of this chimera on substantia nigra (SN) dopaminergic (DA) neurons (which are influenced by BDNF but not NGF) with the basal forebrain (BF) cholinergic neurons (which are differentially influenced by the two factors). In pure neuronal cultures from the SN, the chimera reproduced the effects of purified BDNF, eliciting an approximate 15% increase in DA neurons. In contrast, in BF neuronal cultures the chimera was 100x more potent than BDNF in promoting cholinergic cell survival. Identical results were obtained when NGF and BDNF were added together to the cultures. These data suggest that although NGF itself does not influence cholinergic cell survival, this factor may potentiate the survival effects of BDNF in the BF. Supported by NIH grants HD 23315 and NS 10259.

453.11

NEUROTROPHIC ACTIVITY OF RAT HIPPOCAMPAL MOSSY FIBER SYNAPTOSOMAL FRACTIONS

Ph. Taupin*, Y. Ben-Ari, M.P. Roisin and G. Barbin.
INSERM U29, 123 Bd Port-Royal, 75014 Paris, France.

In the hippocampus, the axons of the dentate granule cells, the Mossy Fibers, project to CA3 pyramidal neurons.

We have developed a procedure for the isolation from Wistar adult rat hippocampal Mossy Fiber synaptosomes on 3-steps Percoll gradients from the post-nuclear pellet. The PII and PIII fractions are enriched in zinc, in dynorphin and in typical Mossy Fiber synaptosomes (EM). All these features are absent in synaptosomes prepared from neonatally irradiated hippocampus to destroy the granule cells and Mossy Fibers (Ph. Taupin et al, submitted 1993).

We now report that Mossy Fiber synaptosomal fractions promote mitotic reinitiation on 3T3 fibroblasts and neurite outgrowth on PC12 cells. These activities are heat-inactivated, trypsin-sensitive and retained with molecular weight cutt off device of 10 kDa (centricon). These results suggest that Mossy Fiber endings contain proteic factor(s) exhibiting neurotrophic activities. These factor(s) may be acting as paracrine or autocrine factors; the modification of expression of these factor(s) might be involved in Mossy Fiber sprouting. The granule cells synthetize a variety of trophic factors including NGF, BDNF, NT3, FGF. We are now pursuing the characterization of this factor(s) and testing these fractions on primary cultures of hippocampal neurons. (supported by INSERM and Servier)

453.12

PROTO-ONCOGENE JUN-B AS A TARGET FOR ACTIVIN ACTION IN NEURONAL CELLS

Makoto Hashimoto, V.J. Roberts* and W.W. Vale, Clayton Foundation Laboratories for Peptide Biology, The Salk Institute for Biological Studies, La Jolla, CA 92037

Activins are implicated in the regulation of growth, development and differentiation of a variety of biological systems; for example, they are involved in the control of pituitary hormone secretion, neuronal cell survival, retrograde regulation of somatostatin expression in ciliary ganglion cells and the induction of embryonic mesoderm. We have sought to identify immediate early genes whose altered expression may provide a common nuclear event which mediates a myriad of activin-regulated phenotypic changes in neuronal and other cell types. We utilized PC12 pheochromocytoma cells, in which activin was found to suppress cell proliferation and reduce tyrosine hydroxylase expression, but did not cause neurite formation. We analyzed the expression of mRNAs for the proto-oncogenes junB, c-jun, and c-fos at various times following activin stimulation. Activin treatment selectively increased expression of junB mRNA within 1hr, whereas neither c-jun nor c-fos mRNA were observed in treated or untreated cells. The transient induction of junB expression was transcription-dependent and protein synthesis-independent. Similar results were obtained using human K562 myelogenous leukemia cells, indicating that JunB induction may be a common step in the activin signal transduction pathway in a diverse set of tissues, including the central nervous system.

453.13

A NOVEL NEURITE-PROMOTING FACTOR FROM HUMAN T-CELL HYBRIDOMA: IDENTIFICATION AS EOSINOPHIL MAJOR BASIC PROTEIN PRECURSOR WITH CHONDROITIN SULFATE GLYCOSAMINOGLYCAN. Y. Arakawa*, K. Isahara, Y. Shikata and S. Tachibana. Tsukuba Res. Labs., Eisai Co., Tsukuba 300-26, Japan.

There are extensive reports on crosstalk between nervous systems and immune systems as exemplified by cytokines and neurotrophic factors. Here we found neurite-promoting activity (NPF act) in serum-free medium conditioned by human T-cell hybridoma (C-108) using serum-free culture of fetal rat septal neurons. NPF act was purified by batchwise and gradient elutions on DEAE-Sepharose columns and by gel-filtration chromatography on Sepharose CL-4B. Since the factor, designated as CII, contains many free cysteine residues, it was reduced and carboxymethylated for the stabilization of the activity (designated as CII-CM). Both CII and CII-CM showed potent NPF act on septal neurons with half-maximal activity at 130ng/ml and 71ng/ml, respectively. A broad band of CII-CM on SDS-PAGE at 88-110 kDa, shifted to 35kDa after chondroitinase ABC digestion (CII-CM core), and after further digestion with glycopeptidase A to 32kDa, indicating that CII-CM has glycosaminoglycans and N-oligosaccharides. CII-CM core still showed a significant activity (1/6 of CII-CM), suggesting that the core protein plays a key role in NPF act. The amino acid and sequence analysis revealed that the protein part of CII is identical to eosinophil major basic protein precursor. Secretion of NPF act from an immune cell line suggests the involvement of immune cells in the regeneration of CNS after damage.

453.14

REVERSAL BY NGF OF CYTOSTATIC DRUG-INDUCED REDUCTION OF NEURITE OUTGROWTH IN RAT DORSAL ROOT GANGLIA IN VITRO P.N.M. Konings*, W.K. Makkink, H.L.A. Philipsen, P.R. Bär[1], G.S.F. Ruigt. Organon International BV, PO 20, 5340 BH Oss, The Netherlands, [1]Dept. Neurology, Univ. Utrecht, Heidelberglaan 100, 3584 CX Utrecht, The Netherlands.

Cytostatic drugs, e.g. cisplatin, vincristine, and taxol, damage the sensory neurones present in dorsal root ganglia (DRG). We, therefore, use intact DRG from rats (E15) in vitro as a model to study the neuropathy caused by cytostatic agents and the potential neuroprotective effect of nerve growth factor (NGF). Neuritogenesis from DRG was measured with an image analysis system. Several doses of cytostatic drugs were tested on intact DRG (1 DRG/well, n=10 per dose) in the presence of 3 ng NGF/mL and Ara-C (10^{-6} M). Relative neurite outgrowth in intact DRG in culture was reduced dose-dependently a) by vincristine starting at a dose of 400 pg/mL for 2 days (-33% as compared to control; $P<0.001$, Student's t-test); b) by taxol 10 ng/mL (-60%; $P<0.001$), and c) by cisplatin 4 µg/mL (-56%, $P<0.001$). Cisplatin also prevented the migration of fibroblasts/glia cells from the intact DRG into the well in contrast to control, vincristine, or taxol. To evaluate the neuroprotective potential of NGF in this in vitro cytostatic neuropathy model, we incubated intact DRG with cytostatic agents in combination with increasing amounts of NGF. The neurite outgrowth from DRG treated with vincristine (400 pg/mL) + NGF (6 ng/mL) for 2 days was significantly higher, (+72%) than vincristine alone (400 pg/mL; $P<0.01$). The neurite outgrowth from DRG treated with taxol (10 ng/mL) + NGF (6 ng/mL) for 2 days was significantly higher (+49%) than taxol alone (10 ng/mL; $P<0.01$). Neuritogenesis from DRG treated with cisplatin (4 µg/mL) + NGF (6 ng/mL) for 2 days was not significantly higher (+14%) than cisplatin alone (4 µg/mL). NGF, therefore, has a clear neuroprotective effect in vincristine- and taxol- induced neuropathy as demonstated in this in vitro model.

453.15

STABLE TRANSFECTION OF C6 ASTROCYTOMA CELLS WITH BASIC FIBROBLAST GROWTH FACTOR SENSE AND ANTISENSE cDNA. G. J. Redekop and C. C. G. Naus*. Departments of Anatomy and Clinical Neurological Sciences, The University of Western Ontario, London, Canada, N6A 5C1.

Peptide growth factors play a major role in the control of cell division and differentiation. Aberrant autocrine control by such growth factors is postulated as a mechanism by which disordered regulation of cell proliferation may occur. We have used the C6 astrocytoma cell line as a model to study the role of basic fibroblast growth factor (bFGF) in tumor growth. C6 cells produce bFGF messenger RNA and at least two bFGF protein species, with molecular weights of 18 and 22.5 kDa. In addition, they have high affinity FGF receptors through which exogenous bFGF acts as a mitogen, confirming its function as an autocrine growth factor. To further investigate the role of bFGF we have transfected C6 cells with bFGF cDNA in both the sense and antisense orientation using a stably incorporated plasmid vector (pLTR/gpt). Clones with high mRNA expression of the sense construct exhibit increased rates of proliferation in cell culture when compared to the parent cell line and show increased levels of intracellular and extracellular bFGF by immunocytochemistry. Clones with high expression of the antisense construct show a diminished rate of proliferation in culture and reduced levels of immunologically detectable bFGF. These C6 bFGF transfectants will be useful for further in vivo and in vitro study of the role of bFGF in tumor growth. Supported by the Medical Research Council of Canada and the Brain Research Fund.

453.16

NEU DIFFERENTIATION FACTOR ISOFORMS HAVE DIFFERENTIAL EFFECTS ON SCHWANN CELL PROLIFERATION AND DIFFERENTIATION. T.J. Neuberger,*A. Welcher, N. Liu, R. Koski, D. Wen, and G.H. De Vries Dept of Biochemistry, Med. Coll. Va., Richmond Va. and Amgen Inc. Thousand Oaks Ca.

Neu-differentiation factor (NDF) is a 44kD polypeptide which exists in an alpha(NDF-α) and beta (NDF-ß) form. While the addition of NDF-α had no effect on Schwann cell (Sc) proliferation, addition of NDF-ß resulted in a dose dependent increase in proliferation. Pretreatment of primary Schwann cells (Sc) with NDF-α resulted in a diminished mitogenic response when the Sc were subsequently challenged with a number of known Sc mitogens including fibroblast growth factor (FGF), transforming growth factor ß (TGFß) and the axolemma enriched fraction (AEF). In contrast, simultaneous addition of NDF-α to either FGF, TGFß or AEF had no effect on the mitogen induced Sc proliferation. Western blotting of AEF using an anti-NDF antisera demonstrated a major 25kD band and a minor 44kD band. Pre-incubation of the AEF with this same antisera resulted in enhanced Sc proliferation. NDF-ß, when added with AEF, had a synergistic effect on Sc proliferation while NDF-ß plus FGF was additive. In contrast, the response Sc to NDF-ß was diminished by the addition of TGFß. While treatment with NDF-α had no effect on the normal morphology of the Sc, addition of NDF-ß resulted in a dramatic alteration in morphology. Within 24 hours, cell aggregates composed of 5-10 Sc with short processes and thick cell bodies dissociated into individual Sc with long processes and thin cell bodies. We conclude that AEF contains a NDF related molecule (probably the NDF-α form) and that NDF-ß and NDF-α have opposite effects on the proliferation of cultured Sc. NDF-α inhibits proliferation while NDF-ß acts like a potent mitogen (Supported by NS10821, NS15408, Neurotech and Amgen).

453.17

NEONATAL OLIGODENDROCYTES EXPRESS NEU AND RESPOND TO NEU DIFFERENTIATION FACTOR

S. Suy, T. Neuberger, A. Welcher*, N. Liu, R. Koski, D. Wen, and G.H. De Vries. Dept. of Biochem.& Biophysics. MCV/VCU, Richmond,VA 23298-0614 Amgen, Thousand Oaks, CA 91320

Neu differentiation factor(NDF) is a glycoprotein with a molecular weight of 44 kda which is believed to be a ligand for Neu-receptor. Addition of either of the two isoforms of NDF- (α or ß) to primary neonatal oligodendroglia(OLG) had no effect on OLG proliferation as measured by ^{3}H-thymidine uptake. However, pretreatment of OLG with either NDF-α or NDF-ß for 3 days followed by the addition of basic fibroblast growth factor (bFGF) caused a decrease in the mitogenic response of OLG relative to non-pretreated OLG. Pretreatment of OLG with NDF-α followed by the addition of axolemma enriched fraction(AEF) had no effect on AEF mitogenicity, whereas pretreatment with NDF-ß followed by the addition of AEF resulted in an increase of OLG proliferation. Addition of anti-NDF antibodies to AEF resulted in a decrease in ^{3}H-thymidine uptake relative to AEF treated OLG. OLG showed a strong positive immunoreactivity to both the intracellular and extracellular domain of the Neu-receptor. We conclude that under these conditions neither NDF isoform by itself acts as a mitogen for OLG, AEF contains NDF-related molecules that potentiate the AEF mitogenicity for the OLG and that neonatal OLG express Neu-receptors. (Supported by NS 15408, Neurotech and Amgen)

453.18

CNTF MODIFIES THE RESPONSE OF CULTURED OLIGODENDROCYTES TO GLUTAMATE AND TNF Y. Lev-Ram*[1], J-C. Louis[2], R.Y. Tsien[3] and M.H. Ellisman[4] Depts. Pharmacology[1]; Biology[2]; Pharmacology & Chemistry and Howard Hughes Medical Institute[3]; Neurosciences[4]; University of California San Diego, La Jolla, CA 92093

Developing oligodendrocytes undergo spontaneous death *in vitro* and *in vivo*, unless sufficient amounts of trophic factors are available. We found that ciliary neurotrophic factor (CNTF), a cytokine produced by astrocytes in the CNS, promotes the survival and maturation of cultured oligodendrocytes. CNTF also protects oligodendrocytes from tumor necrosis factor (TNFα) and glutamate cytotoxicity. To investigate the protective effect of CNTF we studied the response of cultured oligodendrocytes to glutamate and TNFα by monitoring their intracellular calcium levels ($[Ca^{++}]_i$). The fractional change in fluorescence was determined using the non-ratiometric calcium indicator fluo3 and quantitative measurements were made using fura2. Both dyes were loaded into the cells via their AM-esters. Cells were itreated with CNTF for 3 and 4 days, after which their responses to glutamate and TNFα were tested. Control cells responded to glutamate with a large and prolonged increase in $[Ca^{++}]_i$. $[Ca^{++}]_i$ levels declined to baseline levels within 10 minutes following removal of glutamate. Repetitive application of glutamate, after recovery, induced similar transients. Challenging CNTF treated cells with glutamate resulted in more rapid transients and faster recovery. However, subsequent exposures, after the first glutamate challenge, did not yield $[Ca^{++}]_i$ transients. $[Ca^{++}]_i$ transients in response to TNFα were detected in some CNTF treated cells whereas in control conditions most of the cells had a large and fast recovering $[Ca^{++}]_i$ transient (60-120 sec.). In addition, the CNTF-treated cells differed from the control cells in their response to thapsigargin, with control cells exhibiting a large sustained $[Ca^{++}]_i$ increase and CNTF treated cells responding with a $[Ca^{++}]_i$ transient. These findings suggest that the ability of CNTF to modify changes in $[Ca^{++}]_i$ responses of cells to injurious signals may underlie its survival-promoting activity.

453.19

NERVE GROWTH FACTOR EFFECTS ON PRIMARY GLIAL CULTURES. L.A. Hutton*, C.P. Turner & J.R. Perez-Polo. Dept. of HBC&G, Univ. of Tx. Med. Branch, Galveston, TX 77555.

Cultures of rat pup cortical type I astrocytes respond to NGF treatment by increasing levels of p75 mRNA (J.Neurosc.Res.32:375-383,1992). They also express trkA and trkB mRNAs which encode the NGF and BDNF receptors. Both p75 and trkA are involved in high affinity NGF binding and in the biological response to NGF. Since primary cultures of type I astrocytes express p75 and trk A, we examined the effects of NGF on primary astrocytes. GFAP+ cells assumed a fibrous morphology when cultured for five days in serum free medium with 50ng/ml NGF. In control cultures GFAP+ cells remained polygonal after 5 days of serum deprivation. NGF had no effect on the morphology of GFAP+ cells in the presence of fetal calf serum. ^{125}I-NGF was used to measure NGF internalization in purified astrocyte cultures. Pretreatment with 50ng/ml NGF for 72h prior to labeling with ^{125}I-NGF decreased the amount of internalized ^{125}I-NGF compared to untreated cultures. Together these data support the notion that astrocytes respond to NGF treatment in vitro. Publication No.8A supported by USPHS grant PO1 AG10514 awarded by the National Institute on Aging and a grant from the American Paralysis Association.

453.20

A PERIPHERAL NERVE-DERIVED NEUROTROPHIC FACTOR. G.M. Villegas*, Y.R. Olivares, A.T. Haustein, J.C. Bournat and R. Villegas. Instituto Internacional de Estudios Avanzados (IDEA), Apartado 17606, Caracas 1015-A, Venezuela.

A new factor was found in a conditioned medium (CM) prepared with adult rat sciatic nerves cultured in DMEM containing fetal bovine and horse sera during nine days. This factor caused (1) sympathetic-like neuron differentiation of PC12 cells, and (2) survival and neurite outgrowth of the cells of dorsal root (DRG) and ciliary ganglia (CG) of eight-day old chicken embryos (E8). The effect on the E8 ganglia was identical to those described for the Neuronal Growth Factor (NGF) on the DRG and the Ciliary Neurotrophic Factor (CNTF) on the CG. The PC12 differentiation caused by the CM was similar to that produced by the NGF. The changes caused by the CM were not inhibited by antibodies against NGF, b-Fibroblast Growth Factor, laminin and fibronectin. No morphological changes of the PC12 cells were produced by CNTF. Trypsin (0.2 mg/ml) abolished the CM effect. Heating at 95°C, 20 min, decreased by 50% the CM activity with complete inhibition after 1 h. The CM effect on the levels of Na^+ channel type II mRNA of PC12 cells was similar to that of NGF. MC ultrafiltration followed by gel exclusion chromatography (Sephadex G100 & Sephacryl S300) produced an active fraction enriched in a 65-75 kD peptide observed by SDS-PAGE electrophoresis.

Supported by grants S1-1485 (G.M.V.) and S1-2180 (R.V.), CONICIT, Venezuela; and donations from the Vollmer Research Fund of CEC-Caracas, and Prof. Betty G. Uzman.

453.21

BDNF INCREASES GFAP+ PROCESS-BEARING CELLS IN A SUBPOPULATION OF ASTROCYTES. L.M. Buono, L.S.Aibel, I.B. Black and C.F. Dreyfus*. Dept. Neuroscience & Cell Biology, Robert Wood Johnson Medical School, UMDNJ, Piscataway, NJ 08854 and Cook College, Rutgers University, New Brunswick, NJ 08903.

Previous studies from our laboratory suggested that neurotrophins may affect glial function. For example, the p75 neurotrophin receptor, as well as low-affinity binding sites for NGF were localized to non-neuronal cells in mixed neuron-glial cultures derived from embryonic day 17 rat basal forebrain (bf). Moreover, NGF (1µg/ml.) elicited a 9-fold increase in the number of glial fibrillary acidic protein (GFAP+) cells (Yokoyama et al., 1991). GFAP is a marker for astrocytes . In the present study we investigated the putative role of related neurotrophins.

Cultures of enriched bf astrocytes were established from postnatal day 1 rats and found to contain > 90% GFAP+ cells. To determine whether neurotrophins influence these populations, cultures were stained for the receptor, trk, using a pan-trk antibody, as well as for trkB and p75 receptors. Both trk and trkB were localized to a subpopulation of astrocytes. Similarly, p75 was co-localized to a GFAP+ subpopulation, suggesting that astrocytes are responsive to neurotrophins. Cultures were treated with NGF, BDNF, NT-3 or NT-4 produced by cos cells transfected with appropriate plasmids. (Cos cell medium was generously provided by W. Friedman.) BDNF significantly increased GFAP+ process-bearing cells. Concomittantly, GFAP negative cells decreased. Other neurotrophins did not elicit these effects. Our observations suggest that BDNF elicits differentiation of a subpopulation of astrocytes, possibly working through the trkB and/or p75 receptors.

Supported by NIH HD23315 and NINDS NS10259, The Foundation of UMDNJ and the George H. Cook Scholar's Program, Cook College.

453.22

PHENOTYPIC RESPONSES OF SCHWANN CELLS TO NEUREGULIN (GLIAL GROWTH FACTOR) TREATMENT O. Bermingham-McDonogh, S. Scherer[1], C. Nolan, R. Toms, C. Kirk, D. Gwynne* and M. Marchionni. Cambridge NeuroScience, Cambridge MA 02139 and [1]Department of Neurology, University of Pennsylvania, Philadelphia, PA 19104.

Proliferation of Schwann cells appears to be an important step in nerve regeneration following injury. Glial Growth Factor (GGF), a mitogen for Schwann cells has been recently cloned and found to be a member of a large superfamily including the heregulins and ARIA, which are all likely to be alternative spliced products of the same gene. With the availability of recombinant GGF material we have undertaken a study of the changes in gene expression after GGF treatment. We will present data showing levels of expression of growth factor genes and their receptors, and also differentiation markers such as myelin genes.

453.23

RECOMBINANT CNTF ENHANCES ADRENAL MEDULLA CELL SURVIVAL *IN VITRO* M.A. Tokiwa* and L.C. Doering, Division of Anatomy, McMaster University, Hamilton, Ontario, Canada, L8N 3Z5.

Previous experiments showed the long term survival of adrenal medulla (AM) cells when transplanted into the rat peripheral nerve environment (Doering and Tokiwa, 1991; Brain Res. 551:267). We are currently assessing the effects of recombinant trophic factors and non-neuronal cells (fibroblasts, Schwann cells) from peripheral nerve on AM cell survival in culture.

Recombinant rat ciliary neurotrophic factor (rr CNTF), supplied by Genentech, was tested on enriched neonatal AM cell cultures at concentrations of 25-500 ng/ml in serum-free media. Viable chromaffin cells were quantitated after 4 days *in vitro* by tyrosine hydroxylase (TH) immunohistochemistry. The addition of CNTF to the cultures enhanced AM cell survival ($p<0.001$ for 500 ng/ml).

AM cells were also co-cultured with neonatal rat peripheral nerve fibroblast monolayers. Increased survival of TH-positive cells occurred when co-cultured with the fibroblasts ($p<0.001$ compared to controls). Experiments are in progress to determine if Schwann cells can similarly elevate the survival of chromaffin cells.

The results indicate that recombinant growth factors in addition to NGF, as well as non-neuronal cells of the peripheral nerve (fibroblasts) can support the survival of chromaffin cells *in vitro*. **(Supported by The Parkinson Foundation of Canada)**

453.24

NERVE GROWTH FACTOR CONVERTS GH-3 CELLS INTO LACTOTROPH-LIKE CELLS. F. Boroni, S. Sigala*, C. Missale and P.F. Spano. Div. of Pharmacology Dept. of Biomed. Sci. and Biotech, University of Brescia, Italy.

Nerve growth factor (NGF) promotes the growth and differentiation of sensory and sympathetic cells and of cells of the immune system. In the present study we first report that NGF dictates the differentiation of a cell line of endocrine origin. An established cell line (GH-3), which differs from pituitary lactotrophs in the production of both prolactin (PRL) and growth hormone (GH) and in the lack of D-2 receptors for dopamine (DA) was employed. GH-3 cells possess both the gp75 and the gp140trk components of the NGF receptor as shown by both Western and immunofluorescence experiments. Following exposure to NGF, GH-3 cells markedly decreased their proliferation rate. This effect was maximal 3 days after beginning of treatment and was maintained during the following days of exposure. This was paralleled by a change in the hormone production. The PRL secretion was indeed increased 6 fold, while that of GH was remarkably inhibited. GH-3 cells also re-expressed the lactotroph-specific D-2 receptor protein in response to NGF, as evidentiated with the D-2 receptor ligand N-(p-aminophenetyl)spiperone coupled to fluorescein. An intense fluorescence was in fact evident in NGF-treated cells, but not in untreated cultures. The pharmacological characterization of the receptor labelled with [^{125}I]iodosulpride was consistent with its D-2 nature. The expressed D-2 receptors were functionally active in inhibiting PRL secretion. GH-3 cells thus respond to NGF by acquiring a phenotype similar to that of pituitary lactotrophs.

454.1

RESPONSIVENESS OF MOTOR NEURONS TO NEUROTROPHIC FACTORS BECOMES RESTRICTED IN THE ADULT Young W. Kwon*[1], Qiao Yan[2], and Mark E. Gurney[1]. [1]Dept. of Cell, Molecular and Structural Biology, Northwestern Univ., 303 E. Chicago Ave., Chicago, IL, 60611 and [2]Neurobiology Program, Amgen Inc., Amgen Center, Thousand Oaks, CA. 91320.

Local administration of ciliary neurotrophic factor (CNTF) causes sprouting by healthy, undamaged, adult motor neurons. As a corollary to that study, we addressed the three following issues. One, can CNTF cause motor neuron sprouting after systemic administration? Two, are the sprouts induced by CNTF qualitatively similar to those caused by muscle paralysis? Three, can any other neurotrophic factors, to which motor neurons respond either in culture or during development *in vivo*, induce sprouting by adult motor neurons? We found that high dose injections of CNTF at 0.4 mg/kg/day and 1.2 mg/kg/day for 7 days acted systemically to cause sprouting from 33±4% (SEM) and 27±6% respectively of the terminals examined. The sprouts induced by CNTF, from either systemic or local injections, differed little in type or quality from the sprouts caused by muscle paralysis following injection of botulinum toxin. Muscle paralysis did, however, induce more terminal sprouts per endplate (1.32±0.12 vs. 1.13±0.05) as well as more nodal sprouts (10±1.2 vs. 3.3±0.5 per 100 preterminal axons). Finally, none of the other neurotrophic factors that we tested were able to cause sprouting by adult motor neurons. Leukemia inhibitory factor (LIF), oncostatin M (OSM), acidic and basic fibroblast growth factors (aFGF, bFGF), insulin-like growth factor type 1 (IGF1) and type 2 (IGF2), brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3), and nerve growth factor (NGF) all failed to induce sprouting by healthy, undamaged, adult motor neurons. Since CNTF is the only factor that induces sprouting by adult motor neurons, our results imply that responsiveness of motor neurons to neurotrophic factors becomes restricted in the adult.

454.2

NEUROTROPHIC FACTORS RESET THE TIMING OF PROGRAMMED SYNAPSE ELIMINATION IN NEONATAL MUSCLES ME Gurney* and YW Kwon Dept. Cell, Molec. Struct. Biol., Northwestern Univ. Med. Schl. Chicago IL 60611

Neonatal muscle fibers are innervated by multiple motor axons at a single endplate. Elimination of excess synaptic inputs occurs after birth and is due in part to competition between motor inputs for maintenance of the synaptic contact. Although it has been proposed that motor inputs may compete for a neurotrophic factor that is in limiting supply, we find no evidence for that hypothesis. Instead, the related factors, leukemia inhibitory factor (LIF), ciliary neurotrophic factor (CNTF) and oncostatin M (OSM), delay synapse elimination in neonatal mouse muscles without affecting the rate of the underlying process that is driving withdrawal of synapses. This occurs independently of the effects of such factors upon neuronal survival. The effect of LIF was dose- and time-dependent. Maximum delay of synapse elimination in the mouse tensor facia latae muscle was obtained with 100-500 ng LIF injected per day. A maximum delay of synapse elimination of 5 days was obtained with LIF exposure beginning at 3 days after birth. Acidic and basic fibroblast growth factors had no effect.

454.3

IS THERE A SENSITIVE PERIOD FOR THE EFFECT OF CILIARY NEUROTROPHIC FACTOR ON NEUROMUSCULAR SYNAPSE ELIMINATION? A.W. English* and G. Schwartz. Dept. of Anatomy and Cell Biology, Emory Univ. Sch. of Med., Atlanta, GA 30322

Daily intramuscular injections of ciliary neurotrophic factor (CNTF) for one week in neonatal rats results in a retention of multiple innervation of muscle fibers. However, these animals gain weight less rapidly than their untreated litter mates. To examine whether this systemic effect influenced the outcome of neuromuscular synapse elimination, we made single injections of recombinant rat CNTF into the lateral gastrocnemius (LG) muscle and then assayed the amount of multiple innervation present during the second postnatal week using intracellular recording. For animals injected at postnatal age 2 days (P2), a dose-dependent retention of multiple innervation was found. At 100 μg/kg, we observed a transient but significant increase in the amount of multiple innervation. At higher doses, we found a more sustained elevation, nearly doubling the normal amount. In animals injected at age P6, LG contained the normal amount of multiply innervated fibers at all doses examined. In animals injected at P0 or as E18 fetuses, we found nearly three the times normal proportion of multiply innervated fibers as late as P14. Normal weight gain was unaffected by these treatments in any animals, suggesting that CNTF-induced retention of multiple innervation was not due to an effect on normal postnatal growth. These results suggest that a sensitive period does exist during which developing neuromuscular synapses are susceptible to treatment with CNTF. Supported by Regeneron Pharmaceuticals, Inc., the BRSG of Emory University, and NS20545 from the USPHS.

454.4

CILIARY NEUROTROPHIC FACTOR DELAYS SYNAPSE ELIMINATION IN THE RAT LEVATOR ANI MUSCLE. C. L. Jordan*. Dept. Psych., Univ. California, Berkeley, CA 94720.

During developmental synapse elimination, synapses may compete for target-supplied trophic substances. Because exogenous ciliary neurotrophic factor (CNTF) prevents the normal loss of synapses during synapse elimination in the rat gastrocnemius muscle (English & Schwartz, Soc. Neurosci. Abs. 18: 618), CNTF may be the object of competition. I have extended these findings to the rat levator ani (LA) muscle in which synapse elimination is regulated by gonadal androgens.

Gonadally intact male rat pups received daily sc perineal injections of human recombinant CNTF [a gift of Regeneron (0.5 or 1.0 μg/g BW)] or vehicle during the normal period (14-28 day) of synapse elimination in the LA. At 28 days, LA muscles were stained with tetranitroblue tetrazolium and the number of stained motor axons contacting individual muscle fibers counted. **The percentage of multiply innervated fibers was much higher in CNTF-treated than in vehicle-treated LA muscles.**

DOSE	CNTF	VEHICLE
HIGH	81 ± 2%	24 ± 6% (fibers multiply innervated)
LOW	68 ± 3%	25 ± 1%

Thus, androgen may prevent synapse elimination in the LA muscle by increasing the amount of CNTF available to nerve terminals. Supported by HD15021 and IBN-9210229.

454.5

NEUROTROPHIC FACTORS RESCUE MOUSE MOTONEURONS FROM NATURALLY OCCURRING AND AXOTOMY-INDUCED CELL DEATH. L. Li*, R. W. Oppenheim, M. Lei and L. J. Houenou Dept. of Neurobiology & Anatomy, Bowman Gray Sch. of Med., Wake Forest Univ., Winston-Salem, NC 27157.

We have examined the ability of different neurotrophic agents to prevent naturally occurring and axotomy-induced cell death in the developing mouse spinal cord. In utero treatment with 10μg BDNF or CNTF on embryonic day 14 (E14) rescued 50-60% motoneurons (MNs) from naturally occurring cell death when examined on E18. Although in utero treatment with NGF did not affect MN survival, it rescued the sensory neurons in dorsal root ganglia. After postnatal unilateral section of the sciatic nerve, most MN loss occurs in the fourth lumbar segment (L4). Axotomy-induced cell death occurred after surgery performed on or before postnatal day 5 (P5). In contrast, no significant cell loss was found when axotomy was performed after P10. Axotomy on P5 resulted in a 41% loss of MNs in L4 on P12. Implantation of gelfoam (2mm³) presoaked with neurotrophic factors in the lesion site significantly rescued axotomized MNs. CNTF rescued 20-30% of MNs whereas BDNF and NT-3 rescued virtually all axotomized MNs. In summary, the in utero studies reported here are the first to demonstrate an effect of neurotrophic agents on naturally occurring MN death in mammals in vivo. The data from the postnatal studies support previous investigations in showing that CNTF, BDNF and NT-3 can rescue mammalian MNs from injury-induced cell death. Supported by NIH, MDA and IRIP.

454.6

OVEREXPRESSION OF NGF IN SKIN OF TRANSGENIC MICE CAUSES HYPERTROHY OF THE SENSORY AND SYMPATHETIC NERVOUS SYSTEM. K.M. Albers*, D.E. Wright, K.B. Seroogy, H. Traurig, and B.M. Davis. Departments of Pathology* and Anatomy & Neurobiology, University of Kentucky Sch. of Med., Lexington KY, 40536.

Previously we described alterations in the sensory nervous system of transgenic mice expressing a fusion gene that contained the mouse NGF cDNA linked to the keratin 14 (K 14) gene promotor. The K14 promotor is expressed in the epidermis at ca. E14 during the period of naturally occuring neuronal death. K14-NGF mice exhibited overexpression of NGF mRNA in the skin and consequently a hypertrophy of the sensory ganglia and peripheral sensory nerves. We now report on the effect of NGF overexpression on the sympathetic nervous system and extend our observations on the sensory nervous system. Calcitonin gene related peptide (CGRP) immunoreactivity and tyrosine hydroxylase (TH) immunoreactivity was used as a marker of sensory and sympathetic processes, respectively. A major increase in both CGRP and TH immunoreactive positive fibers was detected in K14-NGF skin. Moreover, an increase in the number of CGRP positive neurons was observed in the trigeminal ganglion. Cell counts of transgenic trigeminal ganglia were two-fold higher than control values. This increase is consistent with the number of cells lost through naturally occurring cell death. In comparison, an increase in the number of cells in the superior cervical ganglion was several fold greater than control values suggesting that increased mitosis occurred as well as blockade of cell death. This difference may reflect the dependence of sympathetic neuron survival on NGF thoughout life versus sensory neuron dependence on NGF that occurs during a brief developmental period. Supported by AR40873 to KMA and NS 31826 to BMD.

454.7

CHANGES IN SENSORY THRESHOLDS IN TRANSGENIC MICE EXPRESSING NGF SENSE AND ANTISENSE mRNA. B.M. Davis*, G.R. Lewin[1], L.M. Mendel[1], M. Jones[2], and K.M. Albers[2]. Depts. of Anatomy & Neurobiology* and Pathology[2], Univ. of Kentucky Sch. of Med., Lexington, KY, 40536; Dept. of Neurobiology & Behavior[1], SUNY at Stony Brook, Stony Brook, NY 11794.

Previous experiments have demonstrated that chronic or acute injections of NGF or anti-NGF antisera can effect nociceptive responses in rats. To examine the role of NGF in sensory perception we have created two lines of transgenic mice that overexpress NGF and two lines that express antisense NGF mRNA in the skin. Both sense and antisense mRNA expression is driven by the keratin 14 (K14) promotor that is expressed in skin at ca. E14. These mice provide noninvasive models in which either an increased level of NGF peptide is produced by a target of NGF dependent sensory neurons (the NGF sense mice) or a decreased level of NGF peptide is produced (the antisense mice). In mice expressing increased amounts of K14-NGF sense mRNA, a dramatic increase in the size of the trigeminal ganglion was observed whereas mice expressing the K14-NGF antisense mRNA showed no discernable change in trigeminal size. These results likely reflect the temporal overlap of expression of the K14 promotor with the period of naturally occuring cell death that begins at ca. E15. In K14-NGF sense mice elevation of NGF levels blocks cell death. Production of antisense NGF may not increase cell death since no significant atrophy of the trigeminal occurred. Analysis of the threshold for mechanical stimulation using Von Frey hairs indicates that mechanical thresholds are decreased 80% in K14-NGF sense mice (compared to controls) whereas in K14-NGF antisense mice an increase of 144% occurred. Supported by AR40873 to KMA and NS31826 to BMD.

454.8

PERTURBATION OF CARDIAC DEVELOPMENT BY INCREASED EXPRESSION OF NGF IN TRANSGENIC MICE. A. Hassankhani*, C. A. Altar[†], and H. J. Federoff. Departments of Neuroscience and Medicine, Albert Einstein Coll. of Med. Bronx, N.Y. 10461, and [†]Regeneron Pharm., Tarrytown, N.Y. 10591.

We have previously reported the production and analysis of transgenic mice that over express a nerve growth factor (NGF) mini-gene driven by the cardiac specific α-myosin heavy chain (α-MHC) promoter (Hassankhani *et al., Soc. Neurosci. Abstr.*, Vol. 18, part 2, p. 1289, 1992). In these mice the α-MHC promoter turns-on the expression of the NGF transgene in the heart as early as E13.5 and expression persists throughout the animal's life. Transgenic mice develop cardiomegaly, which is produced by two components: an increase in ventricular myocardial mass and a hyperplasia of presumptive neural crest derivatives within the base of the heart, particularly in the atrial appendages. Our data suggest that over-expression of NGF leads to increased survival of the innervating cardiac sympathetic neurons, thus creating a hyperinnervated heart. To determine the mechanisms that could induce ventricular growth we have begun to examine the role of adrenergic neurotransmission in this process, since catecholamines have been implicated in the development of myocardial hypertrophy. Immunohistochemical analysis for the catecholamine biosynthetic enzyme tyrosine hydroxylase reveals abundant expression within nerve fibers and ectopic neural crest cells. Increased nerve fiber bundle density was also demonstrated by immunolabeling with an antibody against neurofilament M, which identifies neuritic processes. Measurements of catecholamines and their metabolites were made by HPLC and the data indicate that transgenic heart has significant elevation of norepinephrine (NE), dopamine and several catecholamine metabolites. Catecholamine measurements were performed at several developmental time points. We have observed a 30-fold increase in the NE content in one week old transgenic atria and ventricles compared with non-transgenic mice. This difference is less marked at later developmental times, including adults. Similarly, NE content was elevated 7-fold in the atrial appendage of transgenic mice at one week of age. Whereas, in the atrial appendages of adult transgenic mice only 2.7 fold increase in NE content was observed. These data suggest either that the transgenic heart is innervated prematurely or that the increased catecholamines derive from the ectopic cells found largely within the atrial appendages. Supported by PHS grant HD27226.

454.9

CHARACTERIZATION OF THE DORSAL ROOT AFFERENT PROJECTION TO SPINAL CORD IN THE *trk*B "KNOCKOUT" MOUSE. [1]L. Silos-Santiago, [1]L. Zhang*, [1]W.D. Snider, [2]R. Klein, [2]M. Barbacid, [2]R.J. Smeyne. [1]Dept. Neurology, Washington Univ., Med. Sch., St. Louis, MO. [2]Dept. Molecular Biology, Bristol-Myers Squibb Pharmaceutical Research Institute, Princeton, NJ.

An ideal tool to address the function of neurotrophin receptors is the analysis of mice carrying targeted deletions of the gene(s) encoding these proteins. Recently, using homologous recombination in ES cells, a mouse carrying a "knockout" of the *trk*B gene, *trk*B^{TK}(-/-), was generated. This animal demonstrated a loss of 40-60% of the neurons in the dorsal root and trigeminal ganglia, and cranial and spinal motor pools. To characterize the populations of DRG cells lost, we compared the dorsal root afferent projection to the spinal cord of PN0 wild-type and *trk*B^{TK}(-/-) mice. In the *trk*B^{TK}(-/-) mice, no gross abnormalities in the laminar architecture of the spinal cord were detected. The most clearcut finding was normal-appearing Ia afferents projecting to the motor pool and branching to Clarke's column. In the dorsal horn, we observed some fibers characteristic of hair follicle afferents entering medially, projecting to lamina IV, turning upwards and terminating in lamina III. This suggests that some members of this class of low-threshold mechanoreceptors (LTMs) are present. Laminae I and II also contained large number of fibers. Although the distribution of spinal cord afferents appears normal, the overall reduction of DRG neurons in the *trk*B^{TK}(-/-) suggests that some classes of these cells may not have a full complement of fibers. It is also possible that some classes of LTMs or fine cutaneous afferents are eliminated. We conclude that at least some DRG neurons innervating muscle-spindles and hair follicles can differentiate, extend axons to appropriate targets and survive without expressing *trk*B. Whether these neurons do not require BDNF or NT4/5 during normal development or whether compensatory mechanisms occur in the *trk*B^{TK}(-/-) mice remains to be determined.

454.10

FGF, BDNF, AND NT3 GENE EXPRESSION DIRECTED TO NORADRENERGIC CELLS IN TRANSGENIC MICE. Stephen Skirboll, Brigid Stirling, Phyllis Harbor, Mark Bothwell*. Dept of Physiol. & Biophysics, Univ of Washington, Seattle, WA 98195.

To further discern the role of various neurotophic factors in the development of specific neuronal systems, we are examining the gene expression of basic fibroblast growth factor (FGF), brain-derived neurotrophic factor (BDNF) and neurotrophin-3 (NT3) directed to noradrenegic cells in transgenic mice using the 5' flanking region from the human dopamine-beta-hydroxylase (DBH) gene. A number of mouse lines have been generated which stably carry the DBH-FGF, DBH-BDNF and DBH-NT3 gene constructs. Expression of the transgene was characterized by *in situ* hybrdization using a probe to a mouse protamine-1 DNA tag included in each construct. Initial studies demonstrated expression of the DBH-FGF transgene in adrenal chromaffin cells, the enteric nervous system, and in the locus coeruleus and other brainstem nuclei. Preliminary studies show similar expressions patterns for DBH-BDNF and DBH-NT3. Other possible sites of expression are being examined. A variety of phenotypic abnormalities have been observed in these transgenic mice. The possible role of transgene expression in generation of these phenotypes is under investigation. This work was supported by NIH grant NS30305 and a grant from the Research Foundation of the American Association of Neurological Surgeons.

454.11

GENDER AND REGIONAL DIFFERENCES IN [^{3}H]GBR 12935 BINDING IN THE BRAINS OF TRANSGENIC MICE. R. Goldberg, L. Hilakivi-Clarke, A. Hitri, S.I. Deutsch*. Psychiatry Dept. Georgetown University, Lombardi Cancer Res.Ctr., NIDA Research/Psychiatry Serv., DVA Med. Ctr. Washington DC.20422

Recently, transforming growth factor alpha ($TGF2_{alpha}$) mRNA, protein and receptors in rodent brain have been demonstrated; however, $TGF2_{alpha}$'s role has not been elucidated. Transgenic CD-1 mice overexpressing $TGF2_{alpha}$ exhibit gender specific alterations in locomotor activity and aggressive behavior. Furthermore, $TGF2_{alpha}$ is known to stimulate dopamine (DA) uptake in the rat fetal dopaminergic neurons. The present study examined [^{3}H]GBR 12935 labeled DA transporter alterations in the male and female transgenic $TGF2_{alpha}$ mice. The results indicate a 26% decrease in [^{3}H]GBR 12935 binding in the cerebellum of both male and female $TGF2_{alpha}$ mice, compared to normal control mice. In the frontal cortex, while there are no gender differences in the control mice, the gender differences are clearly present in $TGF2_{alpha}$ mice: [^{3}H]GBR 12935 binding in the frontal cortex of the male $TGF2_{alpha}$ mice is 31% ($p<0.05$) lower than in $TGF2_{alpha}$ female mice. The data suggest regional differences in $TGF2_{alpha}$ modulation of [^{3}H]GBR 12935 binding and are consistent with the role of $TGF2_{alpha}$ in DA uptake and dopaminergic transmission.

454.12

TARGET TISSUE REGULATION OF p65 (SYNAPTOTAGMIN) IN SYMPATHETIC GANGLIA IN VITRO K.F. Greif*, H. Fahl, D. Frederick , F. Kim and S. Yang. Dept. of Biology, Bryn Mawr College, Bryn Mawr, PA 19010.

The expression of the synaptic vesicle protein, p65 (synaptotagmin), in rat superior cervical ganglion (SCG) can be influenced by anterograde transsynaptic factors both in vivo and in vitro. Axotomy of the SCG in vivo reduces the levels of p65, but the factors responsible for this reduction cannot be determined directly. The present study investigates whether interactions with target tissues contribute to the regulation of expression p65 in vitro. SCGs from 2-day old rats were grown in explant culture in defined medium (Linderman and Greif, 1990), either in isolation or co-cultured with explants of either salivary gland or skin. Protein levels were determined by RIA. p65 levels significantly increased after seven days co-culture with salivary gland, a normal target of SCG neurons. Co-culture with skin, a non-target tissue, did not significantly increase p65 levels. Histological analysis confirmed that target tissues survived in the defined medium. To rule out the possibility that the salivary gland effect was due to elevated levels of NGF, SCGs were cultured alone with a ten-fold excess of NGF added to basal medium. Levels of p65 were not altered in cultures grown in elevated NGF.

These results indicate that interactions with target tissue play a significant role in regulation of expression of p65 in sympathetic neurons. Further experiments are required to determine the biochemical nature of the factor produced by salivary gland.

Supported by NSF Grant IBN 88-19763.

454.13

BDNF SUPPORTS NEURITE OUTGROWTH FROM OCULOMOTOR NEURONS IN VITRO AND IS RETROGRADELY TRANSPORTED IN VIVO. Y. Kinoshita*, M. Bothwell, and C.S. von Bartheld. Department of Physiology & Biophysics (SJ-40), University of Washington, Seattle, WA 98195.

BDNF supports the survival of many populations of motoneurons in vivo. We have tested if BDNF promotes neurite outgrowth of oculomotor nucleus explants in vitro. To identify the oculomotor nucleus, the fluorescent tracer DiI was injected periocularly in 11-13 day-old chick embryos in ovo. Following retrograde transport of the dye for 16-20 hours, the labeled motor nucleus was identified in brain slices, dissected and explanted in Matrigel. Neurite outgrowth was considerably enhanced with treatment of BDNF (4 ng/ml) for 5-14 days when compared with controls. To determine if BDNF is retrogradely transported from the eye muscles in vivo, radio-iodinated BDNF was injected periocularly and/or intraocularly in 14-day old chick embryos in ovo. The animals were sacrificed after 16-18 hours, and sections through the oculomotor nucleus were processed for autoradiography. Following exposure for 5 weeks, radioactive BDNF was apparent in many oculomotor neurons and in particular in the Edinger Westphal component of the oculomotor complex. These findings show that BDNF supports not only the survival but also neurite outgrowth from oculomotor neurons in vitro and that BDNF is retrogradely transported by developing oculomotor neurons in vivo.

Supported by NIH grants HD 29177 and NS 30305.

454.14

RETROGRADE AND ANTEROGRADE TRANSPORT OF NEUROTROPHINS FROM THE EYE TO THE BRAIN IN CHICK EMBRYOS. C.S. von Bartheld, L.C. Schecterson*, and M. Bothwell. Department of Physiology & Biophysics (SJ-40), University of Washington, Seattle, WA 98195.

BDNF and NT-3 are expressed in the retina and in the tectum of chick embryos, with substantial levels of BDNF expression by presumptive retinorecipient tectal neurons. Previous studies have shown that BDNF supports the survival of retinal ganglion cells, and that BDNF and NT-3 support the survival of neurons in the isthmo-optic nucleus (ION) in chick embryos. Increased levels of NGF, however, enhance developmental cell death in the ION (von Bartheld et al., 1992, Soc. Neurosci. Abstr. 18:1291). Negative effects of NGF may be due to inhibition of BDNF or NT-3 binding to the 75 kD (low-affinity) neurotrophin receptor (p75), thereby blocking retrograde signaling. To test this hypothesis, radio-iodinated BDNF or NT-3 was co-injected into the eye of 14-day old chick embryos with an excess of cold NGF, with cytochrome C, or with antibodies against the p75 receptor. BDNF and NT-3 alone or in combination with cytochrome C were transported heavily to the ION within 8-20 hours. When cold NGF or antibodies against p75 were co-injected, retrograde transport of BDNF was substantially reduced. Transport of I-125 NGF was not observed in the midbrain. Radioactive BDNF and NT-3 were also transported anterogradely, apparently by retinal ganglion cells, to the layer of afferent retinal fibes in the contralateral optic tectum. These results provide first direct evidence that neurons can transport neurotrophins in the anterograde direction. Supported by NIH grants HD 29177, NS 30305, and a grant from the Muscular Dystrophy Association.

454.15

NORADRENERGIC NEURONS IN LOCUS COERULEUS: EFFECTS OF NEUROTROPHINS IN VITRO AND IDENTIFICATION OF TARGETS IN VIVO. M. Bothwell[1], Y. Kinoshita[1], A. Schober[1], S. Borson*[1,2], and C.S. von Bartheld[1]. Departments of [1]Physiology & Biophysics (SJ-40) and [2]Psychiatry (RP-10), University of Washington, Seattle, WA 98195.

Noradrenergic neurons of the locus coeruleus express the low-affinity neurotrophin receptor (p75) in chicken (von Bartheld and Bothwell 1992, J. Comp. Neurol. 320:479-500). To determine which of the neurotrophins may support the noradrenergic subpopulation, coeruleus neurons from 18-20 day-old chick embryos were treated in primary culture with NGF, BDNF or NT-3 (7 days, 1 ng/ml), and noradrenergic neurons were immunolabeled with an antibody against dopamine-beta-hydroxylase (EugeneTech). NT-3 supported the survival of noradrenergic neurons two-threefold when compared with controls or treatment with NGF or BDNF. To identify targets of developing noradrenergic coeruleus neurons, the tracer DiI was injected into various regions of the brain in E15-19 chick embryos in vivo, and frozen sections through the brain were subsequently processed with an antibody against the p75 neurotrophin receptor (gift of Dr. H. Tanaka, Kumamoto University). Following injections of DiI into the telencephalon, about 800 neurons were retrogradely labeled in the locus coeruleus proper and up to 240 in the nucleus subcoeruleus. Some of these neurons could be double-labeled with the p75 antibody. We are currently testing if NT-3 is retrogradely transported to the noradrenergic subpopulation in the locus coeruleus. Supported by NIH grants HD 29177, NS 30305, and a grant from the German Science Foundation.

454.16

NGF, BDNF AND NT-3 PLAY UNIQUE ROLES IN THE PATTERNING OF INNERVATION OF THE INNER EAR H. Staecker, W. Liu, V. Galinovic-Schwartz, P. Lefebvre, G. Moonen, T.R. Van De Water*. Lab of Developmental Otobiology, Albert Einstein College of Medicine, Bronx, N.Y., 10461.

Development and innervation of the sensory epithelia of the inner ear have been shown to depend on a number of factors that include epithelial mesenchymal interactions, the presence of specific substrate molecules as well as the action of numerous soluble and bound growth factors. Among the most important of these is the Nerve Growth Factor (NGF) family of neurotrophins which include NGF, Brain Derived Neurotrophic Factor (BDNF) and Neurotrophin-3 (NT-3). To evaluate their significance in the developing inner ear, we have specifically and individually downregulated the production of NGF, BDNF and NT-3 in organotypic E 10.5 day otocyst-cochleovestibular ganglion cultures using antisense oligonucleotides directed against these three neurotrophins. After three days in vitro, cultures were double stained with anti 66 Kd neurofilament (FITC label) and anti laminin (TRITC label) and subsequently analyzed with confocal microscopy to evaluate neuronal morphology. In these organotypic cultures of the developing inner ear, downregulation of BDNF results in neuronal degeneration, downregulation of NGF causes inhibition of neuritogenisis, whereas downregulation of NT-3 results in a loss of targeting of the neurites to the otic epithelium. Furthermore using in situ reverse transcription PCR (IS RT-PCR) we have shown that not only BDNF and NT-3 but also NGF are actively produced in the neuroepithelial tissues of the developing inner ear.

(Supported by NIH Grant DC00088 to TRV and the Deafness Research Foundation to HS)

454.17

COMPARISON OF THE BIOLOGICAL ACTIVITY OF OTOCYST-DERIVED FACTOR TO NEUROTROPHINS AND FGF. L.M. Bianchi* and C.S. Cohan Dept. of Anatomical Sciences, SUNY at Buffalo, Buffalo, NY 14214

The developing inner ear (otocyst) releases a survival and neurite-promoting factor that influences the associated statoacoustic ganglia (SAG). This unidentified factor is released during the early stages of auditory and vestibular development, at the period preceding target cell innervation (E4-E6 in the chick).

We have found that the ODF is both heat and trypsin sensitive, suggesting that ODF has proteinaceous properties. Previous work from this lab demonstrated that the activity of the ODF was not mimicked by NGF, BDNF or CNTF. Further comparisons of ODF to identified growth factors revealed that NT-3, aFGF and bFGF also failed to mimic the activity of the ODF. In cultures of dissociated E5 SAG neurons, ODF promoted both neuronal survival and outgrowth. However, in the presence of NT-3, aFGF or bFGF, the amount of SAG survival and outgrowth was similar to controls cultured in the absence of any growth factor. Furthermore, combinations of the various identified growth factors did not increase SAG survival or outgrowth above control levels. These results indicate that none of the growth factors tested, alone or in combination, produced effects that were similar to ODF. Thus, ODF may represent an untested factor or combination of growth factors, or a unique target-derived factor.

(Supported by Deafness Research Foundation, March of Dimes #92-0994 and NIH #NS25789)

454.18

BASIC FGF AFFECTS DEVELOPMENT OF CHICK EMBRYO ACOUSTICO-VESTIBULAR NEURONS IN VITRO. D.K. Morest*, X. Zhou, A. Brennan, and C. Baier. Anatomy Dept., Univ. CT Health Ctr., Farmington, CT 06092.

Effects of basic fibroblast growth factor (bFGF) on presumptive auditory and vestibular neurons from the medulla were studied in primary cell cultures. The part of the rhombic lip that forms nuc. magnocellularis (homologue of the mammalian anteroventral cochlear nucleus) was explanted from White Leghorn embryos at Hamburger-Hamilton stage 28, when precursors of the magnocellularis bushy cells migrate and begin to differentiate in situ. In vitro the neuroblasts migrate onto 2-D substrates of purified collagen and differentiate in Ham's F12 base with L-glutamine and defined additives (ITS+, Collaborative). Half of the cultures were supplemented with fetal bovine serum (Nuserum IV, Collaborative); the others, with human recombinant bFGF (UBI), either 10 ng/ml (replaced daily) or 250 ng/ml (replaced every other day) for 5-7 days. Neuroblast migration and neurite outgrowth were measured and averaged for 154 cultures. Compared to serum, bFGF at either concentration produced a 20-fold increase in the area covered by migration and neurite outgrowth during the first 3 days. Migration began sooner in bFGF but was surpassed in serum after the 2nd day. Longer neurites grew in bFGF but usually degenerated after 4-5 days; their growth in serum continued for several weeks. Differentiation of neuronal morphology, including axons and dendrites, began within 2-3 days in bFGF but required at least 5-7 days in serum. Preliminary histochemical observations with an antibody to bFGF receptor (UBI) demonstrated immunopositive patches on many young neurons in the stage 28 medulla. The findings suggest that bFGF stimulates early migration and initial differentiation of neuroblasts in the cochlear nucleus. bFGF may accelerate cell death by overstimulating neuroblasts, but no doubt other factors are needed to sustain them. Supported by NIH grant NS29613.

454.19

BIOLOGICAL EFFECTS OF NEUROTROPHINS ON DEVELOPING DRG CELLS IN VIVO.[1]L.Zhang [1]L.Kutka' [2]D.Privette [2]R.Oppenheim and [1]WD Snider [1]Dept.Neurology, Washington Univ. Sch. of Med St.Louis. MO 63110. [2]Dept. Neurobiol & Anat. Wake Forest Univ. Bowman Gray Med.Sch.Winston-Salem, NC 27157

It is unknown whether newly described neurotrophins exert the same spectrum of biological effects on their responsive neurons as the prototypical factor NGF. The dorsal root ganglion (DRG) is a favorable population of neurons in which to address this question. In order to determine the effects of neurotrophins on DRG development, we have administered mouse NGF and human recombinant BDNF and NT3 to rat embryos *in utero* and chick embryos *in ovo* and assessed neuronal survival, neuronal size and axon growth in spinal cord. Chicks were studied at E10 after injections on E6-E9. Rats were studied at E16 (after injections on E14,15) and E19 (after injections on E15, E17). As expected, NGF had dramatic effects on all parameters studied. DRGs were markedly larger than in controls, size-frequency histograms were shifted strikingly toward larger soma areas and growth and collateral branching of certain classes of dorsal root axons in the spinal cord were enhanced. BDNF had a substantial effect on cell survival and a slight effect on cell size in chick, but, surprisingly, NT3 influenced neither of these parameters. In contrast to the marked effects of NGF on dorsal root axon growth and branching, neither BDNF nor NT3 induced clearcut changes in the shape or laminar distribution of dorsal root axons in gray matter. In fact, NT3 consistently delayed collateral axon branching in gray matter at early developmental stages. Whether BDNF and NT3 have more subtle effects on parameters such as number of axon collaterals or growth cones is currently under investigation.

We conclude that NGF induces a spectrum of effects on DRG cells *in vivo* with striking influences on cell size and axon growth. In contrast, influences of BDNF and NT3 were more subtle, and we were unable to demonstrate major effects on the dorsal root projection to the spinal cord in chick or rat. Whether this represents lack of efficacy of human recombinant factors in these sytems, a greater responsiveness to these factors at developmental stages earlier than those studied here, or differences in the way that different DRG cell types respond to neurotrophins *in vivo* remains to be determined.

454.20

INTERLEUKIN-7 IS A NEURONAL GROWTH FACTOR. M. D. Michaelson*, H. Xu, M. F. Mehler and J. A. Kessler. Departments of Neurology and Neuroscience, Albert Einstein College of Medicine, Bronx, NY 10461.

Numerous parallels exist between mechanisms regulating development of the hematopoietic and neuropoietic systems, including the importance of various cytokines in each. In this study, we have found that IL-7 is present *in vivo* in adult and developing murine brain, and that it has neuronotrophic actions *in vitro*. Studies using reverse transcription-polymerase chain reaction (RT-PCR) confirm the presence of IL-7 mRNA in adult whole brain, and in specific regions including cerebellum and striatum; further, IL-7 may be enriched in neonatal brain relative to adult brain. Embryonic neurons from selective regions including cortex, hippocampus, striatum, and hypothalamus respond *in vitro* to IL-7 treatment in a dose-dependent manner with significantly enhanced survival and increased neurite outgrowth. These lines of evidence point to a role for IL-7 as a neuronal growth factor. Similarly, experimental evidence *in vivo* and *in vitro* suggests a developmental role for IL-5. In contrast, by RT-PCR analysis, the IL-12 p35 subunit is present in adult but not in developing brain. Characterization of the spatial and temporal expression profiles of these and other non-traditional neural growth factors will advance our understanding of neuropoiesis.

454.21

VISUAL FORM DEPRIVATION INDUCES MYOPIA IN THE INFANT RABBIT,WHICH IS REDUCED BY MUSCARINIC ANTAGONISTS APPLIED DIRECTLY TO THE SCLERA. R.W.BEUERMAN*,S.J.CHEW. LSU EYE CENTER,NEW ORLEANS,LA 70112. THE ROCKEFELLER UNIVERSITY NEW YORK, NY 10021

MYOPIA IS A REFRACTIVE ERROR DUE TO EXCESS ELONGATION OF THE EYE. RETINAL NEUROTRANSMITTERS ARE KEY TO THE MODULATION OF ACTIVE SCLERAL GROWTH. ACETYLCHOLINE (ACh) IS IMPLICATED BY THE ATROPINE INHIBITION OF MYOPIA IN CHILDREN AND FORM-DEPRIVATION MYOPIA IN TREE SHREWS AND CHICKS. WE FOUND THAT MUSCARINIC ANTAGONISTS REDUCE PROLIFERATION OF HUMAN NEONATAL SCLERAL FIBROBLASTS IN CULTURE. THIS STUDY WAS PERFORMED TO TEST ITS EFFECTS IN VIVO IN A NEW MAMMALIAN MODEL OF MYOPIA. WE APPLIED 2-MM FILTER PAPER DISKS SOAKED WITH 0.1M NaOH FOR 5SEC TO THE APICAL CORNEAS OF 15 INFANT NZW RABBITS. IN 5 OF THESE, THE OPACIFIED EYE ALSO RECEIVED TWICE-WEEKLY RETROBULBAR INJECTIONS OF ATROPINE 1MM. THIS ROUTE OF ADMINISTRATION MAXIMIZED THE SCLERAL DRUG DOSE. FORM DEPRIVATION CAUSED BY OPACIFIED CORNEAS INDUCED A 0.5MM EXCESS GLOBE ELONGATION AT 2 WEEKS, AND 1.4MM BY 4 WEEKS. ATROPINE-TREATED EYES DID NOT SHOW SIGNIFICANT DIFFERENCES IN LENGTH, AS ASSESSED BY ULTRASONOGRAPHY, FROM CONTROLS. AXIAL LENGTHS AT 2 WEEKS WERE 1 MM LESS THAN UNTREATED FORM-DEPRIVED EYES.WE SUGGEST THAT MUSCARINIC ANTAGONISTS CAN ACT DIRECTLY ON THE SCLERA TO CONTROL FIBROBLAST PROLIFERATION AND COLLAGEN SYNTHESIS.

454.22

INDICATIONS THAT MATERNAL VIP REGULATES PRENATAL GROWTH AND DEVELOPMENT. J.M. Hill*, S.K. McCune, J. Keimowitz and D.E. Brenneman, Lab. of Dev. Neurobiol., NICHD, NIH, Bethesda, MD 20892; Dept. of Neonatol., Children's Natl. Med. Ctr., George Washington Univ., Washington, DC 20010.

VIP has neurotrophic properties (PNAS **83**:1159,1986; Nature **343**:564,1990) and influences neurobehavioral development (Peptides **12**:87,1991). It has recently been shown that VIP has a dramatic effect on *in vitro* embryonic growth and that this growth-enhancing action occurs through VIP receptors localized to the CNS (Nature **362**:155,1993). Additional recent work has shown that while VIP receptors are abundant during both pre- and postnatal development, and express changing patterns of distribution related to ontogenic events, the mRNA for VIP is not expressed prenatally in the CNS. This data suggests that extraembryonic VIP may bind to prenatal VIP receptors and regulate fetal development. *In situ* hybridization revealed no mRNA for VIP in placenta suggesting that maternal serum may be a potential source of ligand. The present study examined maternal serum daily (E9-E21) for levels of VIP and somatostatin. VIP concentrations exhibited oscillatory changes ranging from 20-280 pg/ml. *In vitro* experiments indicated that VIP concentrations in the upper range were maximally efficacious in increasing survival of spinal cord neurons. Somatostatin measurements from the same serum samples showed no significant change. In addition, the pattern of VIP degradation also changed throughout pregnancy. Intravenous injection of radiolabeled VIP into pregnant rats revealed that radiolabeled material reached the fetal brain and that at least some co-eluted on FPLC with intact VIP. Given the potent neurotrophic properties of VIP and its demonstrated effects on fetal growth, these data suggest that maternal VIP plays a role in the regulation of prenatal growth and development.

454.23

NEURAL INFLUENCE ON MUSCLE DYSTROPHIN: PROTEIN LOCALISATION AND GENE EXPRESSION.

G. Poiana*, R. Pierantoni[§] and C. Perrone-Capano. *Dept. Cell Biol.& Development, Rome Univ."La Sapienza", Italy; [§]Dept Hum. Physiol. & Integr. Biol., Naples Univ., Italy; Dept. Gen. Environm. Physiol., Naples Univ.,Italy and Interml. Inst. Genetics Biophys., CNR, Naples, Italy;

We have studied the influence exherted by neurons and embryo extracts on the localization of dystrophin in cultured muscle cells, on its distribution in the subsynaptic area and on dystrophin gene transcription. Immunofluorescence staining shows that dystrophin is expressed at a very low level in 1-day old myoblasts from chick embryo pectoral muscles and in 4-day old myotubes cultured in the absence of extracts. Dystrophin immunopositivity is increased in myotubes co-cultured with chick embryo ciliary ganglion neurons, and it is very high in all myotubes in cultures supplemented with embryo extracts.

A quantitative RT-PCR assay was used to evaluate the expression of dystrophin mRNA in myotubes cultured in the different conditions described above. Preliminary data strongly suggest that dystrophin is up-regulated in myotubes cultured with brain extracts; when cocultured with ciliary ganglion neurons, there was also an increase of dystrophin mRNA, but to a lesser extent. Such findings suggest the presence of a factor in embryo extracts (whole embryo or brain extracts) that specifically increases the production of dystrophin in cultured myotubes, also indipendently from the presence of neurons in the culture.

Experiments aimed at the detection and quantitation of neuronal dystrophin are in progress in order to examine the transcriptional regulation of the neuronal molecule.

The financial support of Telethon-Italy to the project "Molecular events during *in vitro* synaptogenesis" is gratefully acknowledged.

454.24

MULTIPLE COLONY-STIMULATING FACTORS (CSFs) DIFFERENTIALLY REGULATE CNS GROWTH AND DEVELOPMENT. H. Xu*, M. F. Mehler and J. A. Kessler. Depts. of Neurology and Neuroscience, Albert Einstein College of Medicine, Bronx, NY 10461.

Converging lines of evidence suggest that neuropoiesis and hematopoiesis may be mediated by a series of growth factors common to both systems. Four CSFs, granulocyte-macrophage (GM-CSF), multifunctional (IL3), granulocyte (G-CSF) and macrophage (CSF I), are known to regulate early, intermediate and later stages of hematopoiesis. To examine the potential CNS developmental role for these functionally diverse cytokines, we established primary dissociated cultures of five rat embryonic brain regions in serum-free media at different densities to preferentially assess growth factor effects on neuronal survival and differentiation. In hippocampus and hypothalamus, GM-CSF, G-CSF and IL3 exhibited dose-dependent, but qualitatively distinct enhancement of neurite outgrowth, terminal branching and cellular survival. Further, in striatum and cerebellum, IL3 showed, respectively, additional short and intermediate-term effects on process outgrowth and survival. By contrast, in cortex or hypothalamus, CSF I demonstrated significant and dose-dependent potentiation of process-outgrowth, branching and cellular survival. Cytokine effects were apparent by 24 hrs of treatment, peaked by 48-60 hrs and showed phenotypic specificity. These observations establish a differential regional and cellular developmental role for the four known hematopoietic CSFs in CNS growth and differentiation.

454.25

INCREASED TRANSFORMING GROWTH FACTOR-ß1 (TGFß-1) mRNA IN THE BRINDLED MOUSE (MO^{br}) BRAIN IS REVERSED BY COPPER C.Peterson , T.E.Morgan, J.Valverde and N.Nichols Dept. Gerontology, University of Southern California, Los Angeles, CA 90089

The Brindled mouse (MO^{br}) is a murine model of the X-linked inherited disorder Menkes disease which is characterized by a defect in copper transport. Major neuropathological changes include severe neuronal loss, swollen mitochondria, decreased microtubules and cytoskeletal inclusions. Astrocytic alterations accompany neuronal degeneration in the MO^{br} brain. Between post-natal days 12 and 15 there is a progressive increase in GFAP immunoreactivity that is preceded by an increase in GFAP mRNA. In lesion models of neurodegeneration, alterations in GFAP mRNA are preceded by an elevation in the cytokine, transforming growth factor beta-1 (TGFß-1) mRNA; therefore, we studied TGFß-1 in the MO^{br} brain.

An increase in TGFß-1 mRNA is detected by northern blot hybridization by post-natal day 12 in the striatum, hippocampus, cortex and midbrain of MO^{br} male mice. No increases in TGFß-1 mRNA were observed in normal male or heterozygous female littermates. A developmental time course using *in situ* hybridization indicates that the increase in TGFß-1 mRNA appears between postnatal days 10-12. *In situ* hybridization localizes this increase in TGFß-1 mRNA to areas where there are changes in astrocytes and microglia. Systemic treatment of MO^{br} mice with copper on days 7 and 10 prevents the increases in brain TGFß-1 mRNA and decreases the neuropathological alterations. Although the alterations in TGFß-1 mRNA may be an indirect effect of the copper transport defect, the results of these studies will help determine the role that cytokines play in developmental neurodegeneration. Supported by AG07855 and AG05589.

NEUROTROPHIC FACTORS: BIOLOGICAL EFFECTS VIII

455.1

NEUROTROPHIN-LIKE ACTIVITY IN SCIATIC NERVE AFTER TRANSECTION LESION: THE EFFECTS OF ELECTROMAGNETIC FIELDS. BF Sisken*, FM Longo, J Walker, S Hamilton, JF Hyde and R Stach. Center for Biomed Engr & Dept Anat & Neurobiol., Univ. Kentucky, Lexington, KY 40506; Dept. of Neurology, UC San Francisco, CA 94143; Div. Orthop. Surg., Univ. of Kentucky, Lexington, KY 40506; Dir Res, Univ. Mich-Flint, Flint, MI 48502

We are using pulsed electromagnetic fields (PEMF) to stimulate nerve regeneration (Sisken et al, 1989). To understand the underlying mechanisms of this stimulation, we are investigating the role of neurotrophic activity of lesioned nerves. Twelve rats subjected to transection lesions were separated equally into control and treated groups. Control animals were restrained for 4 hours/day and treated animals were restrained and exposed to low levels of PEMF (amplitude 3 gauss, rep rate 2 Hz). Two days post-transection 5 mm lengths taken of unoperated nerve, and of proximal and distal segments of transected nerves were frozen. Neurotrophin-like activity was determined in nerve extracts using a neurite growth assay of dissociated chick dorsal root ganglia neurons. Preliminary findings indicate that neurotrophin-like activity in lesioned stumps was 5-10 fold higher than in unoperated nerve segments. In the distal stumps of the operated nerves there was 79% more activity than in the proximal stumps ($p=0.001$). There was no significant difference between untreated animals and animals treated with PEMF in unoperated nerves, proximal stumps or distal stumps, nor any PEMF effects on ratios of proximal vs distal activities. Although neurotrophin-like activity may play a role in the mechanism of PEMF stimulation of nerve regeneration, we find no compelling evidence that this role is primary. CNTF-like activity is also being investigated. Supported by NIH NS 29621-02, Orthop. Res. Educ. Found., NIH AG 09873, United Cerebral Palsy and the V.A.

455.2

EFFECT OF BASIC FIBROBLAST GROWTH FACTOR (BFGF) ON RETROGRADE DEGENERATION IN THE DORSAL LATERAL GENICULATE NUCLEUS (LGN). S. Agarwala* and R.E. Kalil. Center for Neuroscience and Dept. of Ophthalmology, Univ. of Wisconsin, Madison, WI 53706

In most mammals, a lesion of visual cortex leads to the retrograde degeneration of neurons in the LGN. In many species, the rate and magnitude of retrograde degeneration in the LGN is variable, being influenced, for example, by age at the time of the cortical lesion or the extent of the cortical damage. Recently, it has been demonstrated in several regions of the mammalian CNS that certain neurotrophic factors also may influence the time course and/or severity of neuronal degeneration. We have studied the effect of one of these factors, bFGF, on the retrograde degeneration of LGN neurons after a lesion of visual cortex. A unilateral lesion of visual cortex was made by subpial suction in 7 adult (250-300g) rats. Three rats(RVC) received only a lesion of visual cortex, while 4 rats (RVCF) were given 5 injections (0.5μl) of bFGF (0.25mg/ml in saline or 2% rat serum) directly into visual cortex 2 days prior to making the lesion. In addition, a gelatin sponge soaked in an identical solution of bFGF was placed on the surface of the lesion in the injected animals. After 1 week, the animals were perfused with 4% paraformaldehyde, and serial sections through the brain in the frontal plane were stained with cresyl violet. Material from 3 normal adult rats also was available.

The total number of neurons in each LGN ipsilateral to the cortical lesion was estimated by counting samples of neurons with a 100x objective, and correcting the sample counts for the measured volume of the nucleus. Similar estimates of neuron number were made in the normal rats. After 1 week, the approximate number of neurons surviving in RVC rats is 36% of normal and it is 46% of normal in RVCF rats. Thus bFGF appears to promote about a 28% improvement in LGN neuron survival after a lesion of visual cortex. This suggests that bFGF may be an effective neuroprotectant in the LGN, especially if long-term neuroprotection can be shown.

455.3

FUNCTIONAL AND HISTOLOGICAL EFFECTS OF LOCALLY APPLIED EXTRACELLULAR MATRIX WITH AND WITHOUT bFGF FOLLOWING SPINAL CORD TRANSECTION. B.B.Walters*, E.S.Bachman, A.E.Houston. Div. of Neurosurgery, Univ. of North Carolina at Chapel Hill, NC 27599-7060.

23 Sprague-Dawley rats underwent an open midthoracic procedure under methoxyflurane anesthesia within 36 hours of birth. 5 had laminectomies alone (LAMI), 4 had intradural transection and one segment myelectomy (TRANS), 7 had myelectomies with implantation of approx. 7 µl of Matrigel (ECM), and 7 had implants containing approx. 5 µg of bFGF. The animals were evaluated in blinded fashion by a subset of the combined behavioral score of Wrathall (Exp. Neurol. 88: 123, 1985) 3 times weekly for 6 weeks. The LAMI animals performed significantly better than all other groups at all times. For weeks 1-3, the other animals performed similarly to each other. During weeks 4-6, ECM and bFGF performed the same and were significantly better than TRANS. At 6 weeks, spinal cord tissues spanning the injury sites were harvested, serially sectioned, processed with solochrome-Van Gieson stain, and in blinded fashion semiquantitatively examined by true color image processing to evaluate relative areas of myelin and scar. Preliminary analysis reveals LAMI had mean spinal cord areas of 3.71 mm^2 & mean myelin staining of 83% of that area; for all other groups these values were significantly lower. Mean areas and myelin % of the tissue defined as "cord" were 1.03 mm^2 and 52% for TRANS, 0.77 mm^2 and 36% for bFGF, and 0.59 mm^2 and 33% for ECM. The myelin % is significantly higher for TRANS than bFGF or ECM. Though the areas of scar within the "cord" tissue did not differ among TRANS, ECM, and bFGF, the mean total harvested scar areas were significantly higher with bFGF (0.18 mm^2) than with TRANS (0.11 mm^2) or ECM (0.10 mm^2). Other quantitative comparisons and correlations will be presented.

455.4

SOLUBLE FACTORS FROM SKELETAL MUSCLE PROMOTE SURVIVAL OF NEURONS IN SENSORY GANGLIA AFTER AXOTOMY. W.H.A. Yu*[1], R.M.W. Chau[2] and F. Ren[2]. [1]Dept. of Cell Biol. and Anat. Sci., City Univ. of New York Medical School, New York, NY 10031 and [2]Dept. of Anat., Univ. of Hong Kong, Hong Kong.

Using the protein band-fishing by cells" method, two protein bands migrated at 35kD and 22kD on Phast gel electrophoresis from extracts of the peroneal muscles of young rats were identified to contain soluble factors which enhanced neurite outgrowth and promoted the survival of anterior horn motoneurons in vitro. Putative neurotrophic factors in 35kD and 22kD protein bands were named as MNTF1 and MNTF2, respectively. Application of minced Phast gel containing MFTF1 and MNTF2 to stumps of transected facial and sciatic nerves also promoted the survival of axotomized motoneurons in vivo. In this study, the efficacy of MNTF1 and MNTF2 was tested on the survival of sensory neurons after their peripheral processes were cut in order to further characterize MNTF1 and MNTF2. The infraorbital and sciatic nerves of 10-day-old rats were transected unilaterally under hypothermia. MNTF1 and MNTF2 in Phast gel minced to small pieces were placed between transected nerve stumps. At 2 weeks postaxotomy, rats were perfused intracardially with formaldehyde fixative. The trigeminal ganglia (TG) and dorsal root ganglia (DRG) from L_{4-5} were excised bilaterally and embedded in Paraplast. Neuronal cell numbers in TG (excluding the mandibular portion of the ganglia) and DRG were counted in serial sections stained with cresyl violet to determine the percentage survival. Results indicated that about 50% of the ganglion cells were lost from axotomy. Treatment with either MNTF1 or MNTF2 significantly attenuated neuronal cell loss in TG and DRG, suggesting that MNTF1 and MNTF2 can also protect sensory neurons from axotomy-induced cell death.

455.5

EFFECTS OF CILIARY NEUROTROPHIC FACTOR ON AXOTOMY-INDUCED DEATH IN RETROGRADELY-LABELLED SCIATIC MOTONEURONS IN NEONATAL RATS. R. Vejsada, Y. Sagot and A.C. Kato*, Division Clinical Neurophysiology & Dept. Pharmacology, CMU, Univ. Geneva, Geneva, Switzerland

Death of motoneurons after nerve lesion in immature animals provides a means to study effects of neurotrophic factors on neuronal degeneration. We have developed a quantitative method for examining short and long-term survival of axotomized sciatic motoneurons in neonatal rats in presence or absence of exogenous ciliary neurotrophic factor (CNTF). Under cold anaesthesia, the sciatic nerve was cut either on postnatal day 3 (P3) or 7 (P7). Direct detection and quantitation of the lesioned motoneurons was obtained by their retrograde labelling by fluorescent dye (FluoroGold, FG), applied in a small sealed tube on the central nerve stump. Either CNTF or bovine serum albumin (BSA) was simultaneously added to the tube.

After 1 or 2-week survival, the sectioned spinal cords were examined for fluorescent motoneurons. In rats that had been operated on P3, only 100-200 motoneurons survived in the controls, whereas significantly more motoneurons (700-800) were still present in rats treated with CNTF after 1 week. However, following 2 weeks of survival, the number of surviving motoneurons in control and experimental animals was the same. As in BSA-treated control rats, the FG label appeared in cell debris and in abundant microglia. Similar results were obtained in rats operated on P7; since degeneration rates were significantly lower in the control animals, differences were less important. These findings suggest that rescuing immature axotomized motoneurons from degeneration may require repeated application of neurotrophic factor. (*CNTF was generously provided by Regeneron*)

455.6

DEATH OF AVIAN MOTOR AND SENSORY NEURONS AFTER AXOTOMY AND THEIR RESCUE BY NEUROTROPHIC AGENTS. A. C. Lo*, R. W. Oppenheim and L. J. Houenou. Dept. of Neurobiology & Anatomy, Bowman Gray Sch. of Med., Wake Forest Univ., Winston-Salem, NC 27157.

In neonatal mammals axonal injury (axotomy) results in a substantial loss of motor and sensory neurons. Furthermore treatment with exogenous neurotrophic agents can promote the survival of axotomized rat motoneurons (MNs). The present study was aimed at determining the time-course of motor and sensory neuron death following sciatic nerve axotomy in the chick, In addition, we have examined whether neurotrophic agents, including the neurotrophins and CNTF, can promote the survival of axotomized avian neurons. Results indicate that chick MNs are vulnerable to axotomy up to embryonic day (E) 12, whereas injury-induced sensory neuron death occurred up to E14. Four days after axotomy on E12, 43% of the motor neurons died whereas 62% of the sensory neurons were lost. Treatment of axotomized embryos with NGF rescues significant numbers of both motor (21%) and sensory (22%) neurons from cell death. Daily administration of CNTF completely prevents axotomized MNs from dying and significantly increased DRG neurons survival by 30%. Because of the ineffectiveness of NGF on normal MN death, these data suggest that injured avian MNs may aberrantly express NGF receptors. Alternatively, NGF may affect MN indirectly via the rescue of DRG cells. The effects of other members of the neurotrophin family will also be discussed.

Supported by MDA, IRIP, and Synergen, Inc.

455.7

BDNF PREVENTS ATROPHY OF RAT RUBROSPINAL NEURONS AFTER AXOTOMY. W. Tetzlaff*, N.R. Kobayashi and A.M. Bedard Dept. of Physiology, University of Ottawa, Ottawa, Ontario, Canada, K1H 8M5

Rat rubrospinal neurons undergo massive atrophy between day 7 and 14 after axotomy at the cervical level of the spinal cord (C3). This atrophy is accompanied by a drop in total tubulin and actin mRNA expression (Tetzlaff et al., J. Neurosci. 11:2528, 1991). We have previously demonstrated the presence of trkB and $p75^{NGFR}$ mRNA in normal and axotomized rubrospinal neurons (Soc. Neurosci. Abstr. Vol.18:1294, 1992). This provided the rationale to test whether BDNF, the presumed ligand at trkB receptors, could prevent this neuronal atrophy and sustain regeneration associated gene expression. BDNF was applied at doses of 125 or 500 ng/µl/h via an osmotic minipump (Alzet) between days 7 and 14 after axotomy. In those experiments with BDNF infusion less than 1 mm away (n=3) from the red nucleus a full prevention of neuronal atrophy was observed, i.e. mean soma area was 97.2% of contralateral, compared to 54.6% in saline controls. The effect was only partial with greater infusion needle to red nucleus distances. Axotomized BNDF treated neurons were chromatolytic and expressed high levels of GAP43 mRNA. A detailed analysis of regeneration associated gene expression is currently under way. To the best of our knowledge, this is the first demonstration of a response to neurotrophic factors in rubrospinal neurons. (BDNF was a generous gift of REGENERON PHARMACEUTICALS Inc.. Supported by MRC, Canada and Rotary International).

455.8

NEUROTROPHIN-4 (NT-4) SUPPORTS THE SURVIVAL OF RETINAL GANGLION CELLS AND NEURITE OUTGROWTH FROM ADULT RAT RETINAL EXPLANTS *IN VITRO*. A. Cohen, J. Beer*, G. M. Bray and A. J. Aguayo. Centre for Research in Neuroscience, McGill University and Montréal General Hospital Research Institute, 1650 Cedar Avenue, Montréal, Québec H3G 1A4, Canada.

NT-4, a novel member of the neurotrophin family, has been reported to induce neurite outgrowth from chick dorsal root ganglia explants with almost no effect on sympathetic or nodose ganglia. In this study we compared the effects of NT-4, brain derived neurotrophic factor (BDNF) (both provided by Regeneron Pharmaceuticals Inc.), nerve growth factor (NGF), ciliary neurotrophic factor (CNTF) and basic fibroblast growth factor (bFGF) on retinal ganglion cell (RGC) survival and neurite outgrowth from adult rat retinal explants *in vitro*. The responses to NT-4 were dose dependent. Maximal neurite outgrowth was observed with 5 ng/ml and the half-maximal effect was about 0.5 ng/ml. This NT-4 dose-response was similar to that obtained with BDNF. NT-4 and BDNF also supported the survival of Fluorogold^{R} prelabeled RGCs in the retinal explants. In contrast, we did not observe significant effects of NGF, CNTF and bFGF in these culture conditions. These results demonstrate that among the neurotrophic factors tested, NT-4 and BDNF are the most effective in promoting RGC survival and neurite outgrowth. Combination of saturating concentrations of NT-4 and BDNF did not further augment neurite outgrowth suggesting that NT-4 and BDNF share the same signaling pathway.

455.9

EFFECT OF NEUROTROPHIN-4 ADMINISTRATION ON THE SURVIVAL OF AXOTOMIZED RETINAL GANGLION CELLS IN ADULT RATS. D.B. Clarke, Y.-C. Wang, G.M. Bray, M. Rasminsky*, and A.J. Aguayo. McGill University Centre for Research in Neuroscience, Montreal General Hospital Research Institute, 1650 Cedar Avenue, Montréal, Québec, H3G 1A4.

To determine the survival effect of neurotrophin-4 (NT-4) on axotomized retinal ganglion cells (RGCs), the RGC densities were measured in adult rats after intraorbital optic nerve (ON) cut and a concurrent single intravitreal 5µl injection of NT-4 (0.6µg/µl; Regeneron Pharmaceuticals Inc.) in 0.1M phosphate buffered saline (PBS). Two groups of animals with cut ONs were used as controls: one group had an injection of the vehicle (5µl of PBS) and the other had no further treatment.

Single injection of NT-4 posteriorly through the sclera and retina prevented the loss of more than one quarter of the RGCs by maintaining a normal RGC population at one week. In the NT-4 injected group, 99% of RGCs survived after 7 days, compared with 52% ($p<0.01$) after ON cut alone and 72% ($p<0.01$) after injection of PBS. At 10 days, 62% of RGCs survived after NT-4 injection, compared with 21% after ON cut ($p<0.01$) and 22% after injection of PBS ($p<0.01$). At 14 days, the RGC survival effect of NT-4 was almost completely dissipated. The increased survival of RGCs caused by injection of vehicle without NT-4 was more pronounced with anterior injections into the vitreous chamber through the cornea-sclera junction than with posterior injections through the sclera and retina.

The single intravitreal administration of NT-4 has a potent, though short-lived, survival effect on axotomized RGCs. The RGC survival effect of control injections may be due to synthesis within the eye of trophic factors stimulated by ocular injury.

455.10

STRENGTHENING OF FUNCTIONAL CONNECTIVITY IN THE SPINAL CORD AS A RESULT OF NGF-DEPENDENT SPROUTING OF UNDAMAGED PERIPHERAL NERVES. B.A. Urschel*, K. Mearow, and J. Diamond. Dept. of Biomedical Sciences, McMaster University, Hamilton, Ontario L8N3Z5.

Using induction of the immediate early gene c-fos as a marker of functional connectivity, we have recently demonstrated that collateral sprouting of undamaged peripheral nociceptive axons results in a concommitant increase in the number of c-fos immunoreactive cells in the dorsal horn of the spinal cord. This strengthening of functional connectivity within the cord was not observed if the peripheral sprouting was prevented with daily systemic injections of antibodies to Nerve Growth Factor (NGF). We have undertaken several morphological approaches in order to determine if the increase in the number of c-fos positive cells can be explained by intraspinal sprouting and synaptogenesis, or whether other mechanisms of increasing synaptic effectiveness are involved. Counts of both myelinated and unmyelinated axons in the dorsal roots above and below the root corresponding to the peripheral nerve of interest revealed no difference between chronically and acutely isolated sides; thus if intraspinal sprouting is occurring it is not a result of degeneration of the central processes of the adjacent cut peripheral nerves vacating sites within the spinal cord. Currently, tracing and immunocytochemical approaches are being used to look for morphological evidence of intraspinal sprouting. We expect to present the results at the meeting. We also hope to report the results of associated PCR studies examining NGF, BDNF, Neurotrophin-3, trk, trkB, and $p75^{NGFR}$ expression in the dorsal horn of the affected spinal cords.

Supported by the Canadian Network of Centres of Excellence (NCE) for Neural Regeneration and Functional Recovery, and the MRC (Canada), which also provides a postdoctoral fellowship to B.A.U.

455.11

SPATIAL DISTRIBUTION OF NGF MAY DEFINE REGIONS TO BE INNERVATED BY NGF-SENSITIVE FIBERS J.M. Conner* and S. Varon
Department of Biology, University of California San Diego, La Jolla, CA 92093

Cholinergic deafferentation of the hippocampal formation stimulates fibers from the superior cervical ganglion to innervate this CNS territory. The distribution of sympathetic fibers in the denervated hippocampal formation differs from the preexisting pattern of cholinergic terminals and is restricted primarily to the mossy fiber pathway. While NGF has been suggested to play a role in this sprouting response, the extent to which it is involved and the mechanism by which it may act remain to be elucidated. In the present investigation, correlations were made between the distribution of NGF immunoreactivity (NGF-ir) and the pattern of sympathetic ingrowth into the cholinergically deafferented hippocampal formation of adult rats. Following a fimbria fornix transection, superior cervical ganglia were dissected from neonatal rats and transplanted into the lesion cavity. Following a 16 or 30 day survival interval, sympathetic fibers from the ganglia had innervated the mossy fiber region as reported. This distribution of fibers correlated well with the distribution of NGF-ir seen in adjacent sections. If, at the time of the ganglion transplantation, the animal also received an entorhinal cortex lesion ipsilateral to the transplant, an additional band of NGF-ir appeared in the outer molecular layer of the dentate gyrus. The appearance of this new "patch" of NGF-ir was accompanied by new sprouting of sympathetic fibers into this region. These results support the hypothesis that NGF is involved in the sprouting response of sympathetic fibers following cholinergic deafferentation of the hippocampal formation and further suggests that a topographical distribution of NGF may define the region to be innervated by NGF-sensitive sympathetic fibers. Supported by NINCDS NS-16349 and NS-27047.

455.12

DURATION OF NERVE GROWTH FACTOR-INDUCED CEREBROVASCULAR SPROUTING. L.G. Isaacson*[1], F. Simpson[1], and K.A. Crutcher[2]. [1]Dept Zoology, Miami University, Oxford, OH 45056; [2]Dept Neurosurgery, University of Cincinnati College of Medicine, Cincinnati, OH 45267.

We have previously shown that intraventricular infusion of nerve growth factor (NGF) into the adult rat brain elicits sprouting from mature cerebrovascular axons associated with the intradural internal carotid artery (ICA). A 3-fold increase in the total number of perivascular axons was found at the ultrastructural level (Isaacson et al.1990). Recently we determined that the majority of the sprouted axons are sympathetic in origin (Isaacson et al. 1992) and concluded that mature cerebrovascular innervation can be modulated by NGF infusion. We carried out the present study to determine how long the sprouted axons persist following the two week infusion period. The animals received a 2-week intracerebroventricular infusion of either NGF or cytochrome C. Following the infusion period, two groups of rats (NGF; n=3 and VEH; n=3) were sacrificed immediately for electron microsopy. Other rats were reanesthetized and the pump tubing was clipped to halt the flow of pump infusate. Following either a 1 week (NGF-1; n=4; VEH-1; n = 4) or 3 week (NGF-3; n=4; VEH-3; n = 4) survival, these animals were sacrificed. The total number of perivascular axons associated with the ICA was determined at the ultrastructural level. Similar to previous reports, NGF elicited a 3-fold increase in the number of perivascular axons (x=1771) when compared with the VEH rats (x=607). One week after NGF infusion was halted (NGF-1), the number of axons (x=977) was significantly higher than that observed in NGF-3 (x=575) and all control groups (VEH-1=560; VEH-3=647) but significantly less than NGF rats (x=1771). The number of perivascular axons in NGF-3 rats (x=575) was comparable to that observed in control animals. The results of this study indicate that, even though NGF can elicit sprouting of perivascular axons in the adult, continued infusion of NGF is necessary to maintain the axonal sprouts.

455.13

RECOMBINANT HUMAN IGF-I, Des (1-3)IGF-I and R^3 IGF-I ENHANCE FUNCTIONAL RECOVERY FROM SCIATIC CRUSH IN MICE. Cathy Steffler, David B. Stong, Erya Yu, Kathy Callison, Malini Dasgupta, Jeffry L. Vaught and Patricia C. Contreras*. Cephalon Inc., West Chester, PA 19380.

The purpose of this study was to compare the ability of recombinant human IGF-I (rhIGF-I) to that of des (1-3)IGF-I and R^3 IGF-I, two analogs of IGF-I with reduced affinity for IGF binding proteins, to enhance functional recovery from sciatic nerve crushes. After both sciatic nerves were crushed, mice were injected sc. daily with 0.1 or 1 mg/kg of rhIGF-I, des (1-3)IGF-I or R^3 IGF-I or vehicle until Day 14 post crush. Initially after both sciatic nerves were crushed, mice lost the ability to grip an inverted wirescreen with their hindpaws and parameters of gait, toe spread, internal toe spread and angle between hindfeet, were adversely affected, but the mice slowly regained control of their hindfeet over the next 14 days. However, when mice were dosed with rhIGF-I (0.1 and 1 mg/kg), des (1-3)IGF-I(1 mg/kg) and R^3IGF-I (0.1 and 1 mg/kg), gripping ability returned back to control levels faster than that of vehicle-treated mice. The different parameters of gait consistently returned back to control levels sooner than that of vehicle-treated mice when animals were treated with rhIGF-I (0.1 and 1 mg/kg) or R^3IGF-I (1mg/kg). In contrast, des(1-3)IGF-I only returned toe spread values back to control sooner than that of vehicle-treated mice. The reason that R^3IGF-I and des(1-3)IGF-I were less potent/effective may be due to the finding that rhIGF-I has a longer $t_{1/2}$ in plasma than des(1-3)IGF-I or R^3IGF-I.

455.14

RECOMBINANT HUMAN INSULIN-LIKE GROWTH FACTOR-I (rhIGF-I) PREVENTS DEVELOPMENT OF VINCRISTINE-INDUCED NEUROPATHY IN MICE. Patricia C. Contreras, Cathy Steffler, Shelley Dennis, Joseph C. Arezzo, Michael E. Lewis, Stuart C. Apfel, John A. Kessler, John A. Gruner* and Jeffry L. Vaught. Cephalon, Inc., West Chester, PA 19380.

One of the major dose-limiting side-effects of vincristine is induction of a severe peripheral neuropathy. To assess whether rhIGF-I, which supports the survival of motoneurons and dorsal root ganglionic neurons *in vitro* and enhances neuronal sprouting *in vivo*, could prevent the development of a vincristine-induced neuropathy *in vivo*, mice were treated twice a week with 2 mg/kg of vincristine administered ip for 8 weeks. Mice were also treated concurrently with rhIGF-I (1 or 0.3 mg/kg) or vehicle administered sc. daily for the duration of the study. Vincristine treatment decreased body weight, affected several parameters of gait, reduced ability to grip an incline plane and decreased the response to a noxious stimulus. Vincristine also increased latency and reduced the amplitude of the compound action potential of the caudal nerve and decreased the amplitude of muscle contraction in response to stimulation of the tibial nerve. All of the effects of vincristine, except decreased amplitude of the action potential of the caudal nerve, were prevented by co-treating mice with 1 mg/kg of rhIGF-I. The effect of rhIGF-I was dose-dependent as 0.3 mg/kg of rhIGF-I was not as efficacious as 1 mg/kg of rhIGF-I. These results suggest that rhIGF-I may be useful clinically in preventing the vincristine-induced peripheral neuropathy.

455.15

TROPHIC AND TROPIC INFLUENCE OF EXOGENOUS NT-3 AND BDNF AFTER NEONATAL SPINAL CORD LESIONS. P. Diener-Ostfield*, P.S. DiStefano, M.M. McAtee, and B.S. Bregman. Dept. of Anatomy and Cell Biology, Georgetown University School of Medicine, Washington, DC 20007, and Regeneron Pharmaceuticals, Inc., Tarrytown, NY 10591.

Immature central and peripheral neurons are dependent upon target derived trophic support for survival. Axotomy at birth results in massive retrograde cell loss of the injured neurons. After spinal cord lesion at birth, transplants of fetal spinal cord tissue permanently rescue immature axotomized brainstem-spinal neurons and permit regrowth of their axons into and through the transplant. Nontarget transplants (cortex,CX, hippocampus, HC) rescue these neurons and support axonal growth only transiently. Dorsal root neurons survive central axotomy and maintain axonal projections in both target and nontarget transplants. The current series of experiments tested the capacity of different combinations of neurotrophic support and fetal striatal transplants (which lack a trophic influence) to rescue immature axotomized brainstem-spinal and dorsal root neurons and support regrowth of their axons. Neurotrophic factors (NT-3, BDNF) or fetal striatal transplants with or without exogenous NT-3 or BDNF were inserted directly into the lesion site created by a thoracic spinal cord hemisection in 3 day old rat pups. Neuronal survival and axonal elongation were examined at one and four weeks after injury. NT-3, BDNF and striatal transplants rescue neurons in the red nucleus and locus coeruleus. Unlike fetal HC or CX, however, striatal transplants fail to support the growth of brainstem-spinal or dorsal root axons at either survival period. This suggests that the requirements of immature axotomized neurons for survival differ from their requirements for axonal elongation and that a sustrate, per se, is insufficient for axonal elongation. Exogenous NT-3 or BDNF combined with striatal transplants, however, elicited regeneration of a subpopulation of dorsal root but not brainstem-spinal axons. This observation suggests that there may be pathway specificity in the tropic influences of particular neurotrophic factors. Other guidance cues unique to fetal spinal cord tissue also may be required for regeneration of brainstem-spinal axons. Supported by NIH NS 19259 and RCDA NS01356 to BSB. Purified NT-3, BDNF provided by Amgen/Regeneron.

455.16

RETINOTECTAL REGENERATION: bFGF EFFECTS IN CO-CULTURE PREPARATIONS PARALLEL *IN VIVO* RESULTS. G.E. Schneider* & D.F. Chen. Dept. Brain & Cognitive Sciences, M.I.T., Cambridge, MA

We have shown that retinofugal axons will reinnervate the superior colliculus (SC) of the Syrian hamster with transection of the brachium of the SC on postnatal day 3 (P3) or earlier (So et al., 1980). The regrowth fails after transections on P4, but a gelfoam implant soaked in basic fibroblast growth factor (bFGF) extends the period of regeneration well beyond P3 (Carman & Schneider, submitted). Now we are obtaining comparable results with an organotypic co-culture system in which retinal explants from postnatal hamsters are placed adjacent to the superficial gray layer of living slices of SC, at sites of pial disruption. After 5 days, the co-cultures are fixed in paraformaldehyde and outgrowth of axons is assessed by placing crystals of DiI in the retina. With source and target tissue taken from pups of the same age, retinal axons extend into the tectum in cultures from P0 animals (N=3), but not from P1-P5 animals (N=18). Since axons take 2-3 days to reach and penetrate an SC slice, the age of the tissue in the P0 cases when axons penetrate the tectum is 2-3 days. However, adding bFGF (30 ng/ml) to cultures of tissue taken from P5 animals results in a dramatic increase in retinal axon growth and penetration of the superficial midbrain (N=3).

In heterochronic co-cultures, very limited axonal growth from P19 and adult retinae occurred into E13 SC (see Chen et al., this vol.). However, with bFGF added to similar cultures, large numbers of axons succeed in interconnecting retina and tectum .

The co-culture model should facilitate the specification of optimal conditions for induction of regenerative growth, as well as spur progress towards understanding the mechanisms of growth-promoting and inhibiting molecular factors. (Support: NIH grants EY00126, EY05504, EY02621; bFGF provided by Creative Biomolecules of Hopkinton, Mass.)

456.1

CORRESPONDENCE BETWEEN HRP-LABELLED OLIVOCEREBELLAR FIBERS AND L7/PCP2+ PURKINJE CELLS IN TRANSGENIC MICE DURING EMBRYOGENESIS. M.A. Paradies*§, R.J. Smeyne†, J.D. Oberdick¥, J.I. MorganΩ and L.M. Eisenman§. §Dept. of Anatomy and Devel. Biology, Thos. Jefferson Univ., Philadelphia, PA; †Dept. of Molec. Biology, Pharm. Res. Inst., Bristol Myers-Squibb, Princeton, NJ; ¥Dept. of Anatomy and Neurobiology, Ohio State Univ., Columbus, OH; ΩRoche Inst. of Molec. Biology, Nutley, NJ.

There is great interest in determining the factors responsible for afferent organization in the mammalian cerebellum. Purkinje cells (PC) have been suggested to perform this function as they express biochemical heterogeneities during embryonic development in the rat. By using an in vitro perfusion system, it is possible to selectively label groups of olivocerebellar (OC) fibers to determine how their termination zones relate to biochemically-defined groups of PC. The transgenic mice used carry a ß-gal reporter gene linked to an L7/PCP2 promoter. In the cerebellum L7/PCP2 is expressed exclusively in PC and is upregulated heterogeneously during embryonic development. OC fibers labelled after horseradish peroxidase (HRP) application to the caudal medial accessory olive at E17 terminate primarily among L7/PCP2+ PC located in parasagittal bands in the medial cerebellum. In addition, the boundaries between HRP-labelled and -unlabelled areas appear to correspond to those between L7/PCP2+ and L7/PCP2- PC clusters in many areas. However, not all HRP-labelled fibers are colocalized with L7/PCP2+ cells, questioning the nature of the relationship between L7/PCP2 expression and OC fiber organization.

456.2

IDENTIFICATION OF a cDNA ENCODING A PUTATIVE TRANSCRIPTIONAL REGULATOR OF L7-EXPRESSION IN PURKINJE CELLS. C. Kürschner and J.I. Morgan*, Roche Institute of Molecular Biology, Roche Research Center, Nutley, NJ.

The Purkinje cell-specific gene, L7, is expressed in mouse cerebellum in a dynamic temporal and spatial pattern that resembles segmental gene expression in developing invertebrates, such as Drosophila. To identify transcription factors that regulate L7 expression, a mouse cerebellum cDNA library was generated, that allows for functional screening in yeast strains carrying an L7 promoter-lacZ fusion reporter construct. With this approach, we isolated a cDNA clone that codes for a protein ("LZCP" for leucine zipper containing protein) with a "leucine zipper" motif and a "basic region", the putative DNA binding domain of the protein. We assume that LZCP, like other proteins of this type, is a transcription factor. The target DNA sequence of LZCP in the L7 promoter was identified using gel shift assays and transient expression activation assays in yeast. The effects on the map of L7 expression of mutating this site is analysed in transgenic mice carrying a mutant L7 promoter-lacZ fusion construct as a transgene.

456.3

NITRIC OXIDE SYNTHASE EXPRESSION REVEALS COMPARTMENTS OF CEREBELLAR GRANULE CELLS AND SUGGESTS A ROLE FOR MOSSY FIBERS IN THEIR DEVELOPMENT Karl Schilling* & Stephan L. Baader
Dept. Anatomy & Cell Biology, University of Ulm, D-89069 Ulm, Germany

Nitric oxide (NO) is a pluripotent molecule involved in multiple functions, including neurotransmission. It has been theorized that NO signalling also affects neural development. To address this issue experimentally, we investigated the developmental expression of nitric oxide synthase (NOS) in the murine cerebellum and correlated it to the well established developmental history of the cerebellar cortex. NOS was visualized by NADPH-diaphorase histochemistry. Cerebellar granule cells (GC) expressed NOS only after reaching the internal GC layer. Initially, the nascent internal GC layer stained uniformly for NADPH-diaphorase throughout the cerebellum. Following invasion by mossy fibers, a pattern of differential NOS expression by GCs emerged, such that clusters of heavily stained GCs became separated by areas of unstained GCs. Analysis of cultured GCs indicated that initial induction of NOS is independent of mossy fiber signals and that the level of NOS expression becomes sensitive to electrical activity once GCs establish functional synapses. These findings show that GC precursors are endowed with an intrinsic program which regulates NOS induction and which is executed independently of correct positional cues. The data also suggest that synaptic activity of mossy fibers plays an important role for development of GC compartments. These compartments are defined by differential expression of NOS and may contribute to the functional organization of the cerebellar cortex.

Supported by BMFT grant 0316915A.

456.4

MOLECULAR DISTINCTIONS BETWEEN THALAMIC NUCLEI DURING DEVELOPMENT: DIFFERENTIAL EXPRESSION OF KERATAN SULFATE PROTEOGLYCANS. B. Miller*, A.M. Sheppard and A. L. Pearlman. Depts. of Cell Biol. and Neurology, Washington Univ. Sch. of Med., St. Louis, MO 63110.

Thalamic nuclei are distinct in their connectivity and function, but the molecular signals that act during development to determine these distinctions are not known. Components of the peri- and extracellular matrix, including proteoglycans, have important roles in development, particularly in cell migration, axonal elongation, and response to growth factors. We have analyzed the distribution of keratan sulfate proteoglycans (KSPGs) in the developing thalamus of the rat (E16-P14) by immunohistochemistry with a monoclonal antibody (5D4, generously provided by B. Caterson) to the glycosaminoglycan side chains. We find three striking distinctions between developing thalamic nuclei in the expression of KSPGs. i) Nuclear groups differ appreciably in their level of immunolabeling. For example, lateral nuclei (L, LP, LGd) are intensely immunolabeled throughout the period of expression while anterior nuclei (AV, AM) label less intensely. ii) Selected nuclei (e.g. LGv, AD) within groups express no detectable KSPGs while their neighbors (LGd, AV) are strongly immunolabeled. iii) Expression patterns within a nuclear group are temporally distinct; both VBm and VBl have intense immunolabeling at E18, but a rapid decline in labeling begins in VBm several days before a similar decline occurs in VBl. Immunolabeling in all nuclei is developmentally regulated, beginning at E16-18 and declining in the first or second postnatal week. Thalamic axons express KSPGs as they leave the thalamus and enter the internal capsule. Thus neurons of the thalamus express KSPGs in developmentally regulated, differential patterns that respect the boundaries of thalamic nuclei. Expression along their axons suggests that these distinctions may be involved in processes that determine connectivity such as pathfinding, fasciculation, and target selection.

456.5

EFFECTS OF PRE- AND POSTNATAL SEROTONIN DEPLETION UPON THE ORGANIZATION OF THALAMOCORTICAL AFFERENTS IN THE RAT'S PRIMARY SOMATOSENSORY CORTEX. C.A. Bennett-Clarke*, N.L. Chiaia, M.J. Leslie and R.D. Lane. Dept. of Anatomy, Medical College of Ohio, Toledo, OH 43699

A previous study (Blue, M.E. et al., Cerebral Cortex, 1991;1:380-389) has shown that depletion of serotonin (5-HT) from the developing somatosensory cortex in rats by means of systemic injection of *p*-chloroamphetamine delays, but does not prevent, the formation of a vibrissae-related pattern by thalamocortical afferent axons. This interesting study was limited by the facts that the extent of the 5-HT depletion was not quantified and appeared modest and that all depletions were carried out after birth, an age at which the vibrissae-related pattern can already be discerned. We employed single subcutaneous injections of 5,7-dihydroxytryptamine (5,7-DHT) on either embryonic day (E-) 16 or the day of birth (P-0) to deplete cortical 5-HT and evaluated the organization of thalamocortical afferents on P-5 through P-8. In most of the animals used for the assessment of thalamocortical organization, the amount of 5-HT in one hemisphere was measured with high pressure liquid chromatography. Anatomical and biochemical results from experimental animals were compared with those from age-matched normal animals. Injection of 5,7-DHT on E-16 resulted in an 89% reduction in cortical 5-HT; injections on P-0 produced an average reduction of 91%. Neither pre- nor postnatal depletion of 5-HT resulted in a significant alteration in the vibrissae-related pattern of thalamocortical afferents within the primary somatosensory cortex.

Supported by EY 08861 and DE 07734

456.6

Delayed barrel boundary development in the somatosensory cortex of thyroxine-deficient rodents. E D. Laywell, J. A. Brodkey, L. Van Middlesworth, J. Evans, T. F. O'Brien*, and D. A. Steindler. Depts. Anat. & Neurobiol., Neurosurg., and Physiol., Univ. of Tenn., Memphis, TN 38163

Previous studies have shown that transient cellular (astrocyte) and molecular (e.g. tenascin) boundaries surround individual cortical whisker barrels during the first week of postnatal development. Boundaries are most distinct on postnatal day 6 (P6), and they persist until the second to third postnatal week. Boundary expression is associated with a so-called critical period during which manipulations of the periphery (i.e. vibrissae lesions) lead to changes in central cytoarchitecture. We describe here a 1 week delay in whisker barrel boundary appearance in the pups of female rats and mice maintained during pregnancy and weaning on an iodine-free diet supplemented with propylthiouracil (PTU) to interfere with thyroxine synthesis. PTU pups fail to show barrel boundaries until P13, using conven-tional barrel-boundary labeling methods. This delay does not seem to be due to a general retarded development of the whisker-barrel pathway , since normal boundaries are present at apparently normal developmental times in the brainstem trigeminal complex of PTU pups. Studies are underway to correlate this altered boundary molecule expression with the development of thalamocortical afferent projections, and to establish that this delay in boundary development corresponds to a lengthened critical period. The lengthened development of cortical barrel boundaries provides a useful model for studies of barrel development because of the protraction of the normally short period of postnatal barrel maturation. Cellular and molecular mechanisms behind PTU effects on cortical barrel maturation may reflect a crucial interaction between thyroid hormone and neural cells involved in normal CNS circuit construction and plasticity. Support - NIH, NS 20856.

456.7

AXONAL TRANSPORT BLOCKADE MIMICS EFFECTS OF NEONATAL INFRAORBITAL NERVE TRANSECTION UPON VIBRISSAE-RELATED PATTERNS. N.L. Chiaia*, R.S. Crissman, C.A. Bennett-Clarke and R.W. Rhoades. Dept. of Anatomy, Medical College of Ohio, Toledo, OH 43699

Uninterrupted connections with the periphery are necessary for the normal development and maintenance of central somatotopic patterns. This has been shown to be true for the representation of the mystacial vibrissae in the trigeminal (V) system, but additional studies have demonstrated that neither NGF deprivation nor neuronal activity blockade disrupts vibrissae-related patterns. To determine if a peripheral factor was necessary for the maintenance of vibrissae-related patterns, we applied ELVAX chips impregnated with colchicine to the infraorbital nerve (ION) at birth (P-0) and killed animals between P-4 and P-7. In brainstems stained for cytochrome oxidase, there was a complete loss of the dense patches corresponding to the mystacial vibrissae follicles ipsilateral to the colchicine implants. Labelling of thalamocortical afferents with Di-I or staining of cortices with an antibody directed against serotonin demonstrated a pattern with five rows, but no individual patches. This pattern is also observed after neonatal ION transection. Retrograde labelling of V ganglion cells after Di-I injection into the face in fixed tissue and direct examination of treated nerves demonstrated that the colchicine-impregnated ELVAX did not sever the ION. However, the number of myelinated fibers in the nerve was reduced. None of the effects reported above were observed in animals that received blank ELVAX implants. These results suggest that a peripheral factor other than NGF may be necessary for the maintenance of central vibrissae-related patterns.

Supported in part by DE 07734, DE 08971, and NS 28888

456.8

RETENTION OF VIBRISSAE-RELATED PRIMARY AFFERENT PATTERNS AFTER NEONATAL ION TRANSECTION IN THE RAT. F.A. White*, C.A. Bennett-Clarke, N.L. Chiaia, R.S. Crissman and R.W. Rhoades. Dept. of Anatomy, Medical College of Ohio, Toledo, OH 43699

Immunocytochemistry for galanin (GAL) and anterograde labelling with Di-I were used to evaluate trigeminal (V) brainstem organization in rats that sustained perinatal damage to either the infraorbital nerve (ION) or individual vibrissae follicles. After nerve damage on the day of birth (P-0), dense GAL-immunoreactivity appeared throughout the V brainstem complex and had a patchy distribution similar to that of vibrissae-related V primary afferents in normal rats. Damage to a row of vibrissae follicles or a single follicle at birth produced a single row or patch of GAL-immunoreactive terminals in the ipsilateral V brainstem complex. This immunoreactivity was apparent by P-3 and disappeared at some time after P-14. Upregulation of GAL immunoreactivity occurred in rats that sustained ION damage as late as P-21. Experiments in which nerve damage was followed by destruction of the V ganglion demonstrated that the GAL-immunoreactivity was in primary afferent axons. The combination of immunocytochemistry and electron microscopy showed further that many myelinated primary afferent fibers in the nerve-damaged animals were GAL-immunoreactive. Placement of Di-I in the V ganglion of neonatally nerve damaged rats demonstrated that labelled primary afferent axons filled central ION territory and that their terminal distribution, while less dense than normal, was patchy. These results indicate that the V ganglion cells that survive neonatal peripheral axotomy retain somatotopically organized projections to the V brainstem complex for at least a limited postnatal period. Supported by NS 28888, DE 07734, and DE 08971

456.9

FETAL INFRAORBITAL NERVE TRANSECTION INCREASES THE AREA DEVOTED TO THE PRIMARY SOMATOSENSORY CORTICAL REPRESENTATION OF THE LOWER JAW. S.E. Fish*, H.P. Killackey, N.L. Chiaia, C.A. Bennett-Clarke, and R.W. Rhoades. Dept. of Anatomy, Medical College of Ohio, Toledo, OH 43699, Dept. of Psychobiology, University of California, Irvine, CA 92717 and Dept. of Anatomy, Marshall University Medical School, Huntington WV 25704

Nerve lesions at different stages of development were used to determine the role of the periphery in the establishment of cortical territories devoted to representations of different portions of the body surface. Transection of the infraorbital nerve (ION), the trigeminal branch that supplies the whisker pad, in fetal (embryonic day 15 through embryonic day 18), but not newborn, rats resulted in a significant reduction in the area within the primary somatosensory cortex devoted to the representation of the mystacial vibrissae as demonstrated by staining the cortex with an antibody to serotonin or labelling of thalamocortical afferents with Di-I. Such lesions in fetal, but not neonatal, rats also resulted in significant increases in the cortical area devoted to the representation of the lower lip and jaw. There was a significant positive correlation between the reduction in the vibrissae representation and the expansion of that of the lower jaw. Damage to the ION in either neonatal or fetal rats failed to significantly increase the amount of cortex devoted to the representation of the forepaw. These results indicate that the primary afferent innervation of the periphery does influence the cortical areas devoted to representations of different parts of the body surface and that the representation of one region can expand significantly when that of another body part is reduced. Supported by NS 28888, DE 07734, DE 08971, and BNS 90-22168

456.10

REORGANIZATION OF PRIMARY AFFERENT PROJECTIONS TO THE GRACILE NUCLEUS AFTER FETAL HINDLIMB REMOVAL IN RAT. H.P. Killackey*, N.L. Chiaia, B.F. Hoeflinger and R.W. Rhoades. Dept. of Anatomy, Medical College of Ohio, Toledo, OH 43699 and Dept. of Psychobiology, University of California, Irvine, CA 92717

Previous experiments from our laboratories have shown that sciatic nerve afferents invade the cuneate nucleus when the forelimb is removed prior to birth. Since hindlimb-related primary afferents are among the last to arrive in the dorsal column nuclei, we asked, in the present experiment, whether earlier arriving axons from the forelimb exhibited the same plasticity. The central projections of the axons comprising the brachial plexus were traced in normal adult rats and in animals that sustained removal of the hindlimb on embryonic day 16 using a combination of WGA-HRP, cholera toxin-conjugated HRP, and free HRP. Tracing in normal rats revealed no brachial plexus afferents in the gracile nucleus. Labelled axons were present throughout the cuneate and external cuneate nuclei, and a number of labelled fibers were also visible in the lateral portion of the trigeminal brainstem complex primarily in subnucleus interpolaris. In adult rats that sustained fetal hindlimb removal, labelled brachial plexus axons were present in all of the above-described targets and they could also be seen in the gracile nucleus. The latter labelling consisted of small bundles of axons and isolated fibers whose terminations were mainly restricted to the peripheral portions of the nucleus. This labelling was visible in each (N=8) of the experimental animals. The present results thus demonstrate that forelimb- as well as hindlimb-related primary afferents can alter their central projections as a result of prenatal primary afferent lesions.

Supported by NS 28888, DE 07734, DE 08971, and BNS 90-22168

456.11

AREA-SPECIFIC REGULATION OF THE $GABA_A$ RECEPTOR α1-SUBUNIT IN DEVELOPING RAT NEOCORTEX DEPRIVED OF AFFERENT INNERVATION. J.Paysan[1], A.Kossel[2], R.Thanos[2], J.M.Fritschy[1], H.Mohler[1] and J.Bolz[2]*, [1]Institut für Pharmakologie der Universität Zürich, Switzerland; [2]Friedrich-Miescher Labor der Max-Planck Gesellschaft, Tübingen, Germany.

The mechanisms underlying the specification of functionally and architectonically distinct cortical areas during development are not known. Using an antiserum specific for the α1-subunit of the $GABA_A$-receptor, we found that immunohistochemical staining for this antigen delineates the primary visual (V1) and somatosensory (Sm1) cortex during early postnatal development. Already at the day of birth (P0), staining for the α1-subunit revealed a band restricted to the lower cortical plate and immature layer 4 in V1 and Sm1. This pattern was very prominent at P2 and clearly demarcated these areas from adjacent cortical regions during the first postnatal week. Later in development, staining was gradually detected in all neocortical layers and areas. Since at birth thalamic fibers have not yet reached layer 4, these observations suggest that the boundaries of Sm1 and V1 are already defined at this early age outside the zone of thalamic afferents. To further analyze whether this area-specific distribution of the α1-subunit is regualted by intrinsic cortical mechanisms or by the ingrowth of thalamic axons, the emergence of this pattern was examined in vivo after lesions of the thalamus performed at P0, and in vitro using slice cultures prepared from rats between embryonic days 18-20. The results of both types of experiments indicated that the regional specificity of α1-subunit staining in neocortex was preserved in the absence of afferent innervation. Thus, our results suggest that cortical parcellation is induced prenatally, and that factors intrinsic to the maturing cortex are involved in the specification of cortical areas.

456.12

THY-1 IMMUNOREACTIVITY DISTINGUISHES PATCHES FROM MATRIX IN THE EARLY POSTNATAL STRIATUM OF RAT. C.W. Shults*, VA Med. Ctr. and Dept. of Neurosci., Univ. of Cal., San Diego, San Diego CA 92161

The cell adhesion molecule Thy-1 emerges in specific regions of neurons at certain stages in development and appears to be involved in inhibition of neurite outgrowth and stabilization of synapses. Coronal sections from rat embryos and pups were immunolabelled for both Thy-1 and tyrosine hydroxylase (TH). At P0, patches of increased density of TH-immunoreactive (TH-IR) axons were noted in the striatum. At this age, we also noted patches of increased amounts of Thy-1-immunoreactive (Thy-1-IR) material, which coincided with the patches of dense dopaminergic axons. Patches of increased density of Thy-1-IR material were noted on P2 and P3, but by P5 the distribution of Thy-1-IR material was homogeneous throughout the developing striatum. Patches of dense dopaminergic axons were still noted at P9. The most parsimonious explanation for our finding is that after neurons destined to be incorporated in the patch compartment complete their initial extension of axons to the midbrain, during the late prenatal period, production of Thy-1 and deposition in the developing dendrites is increased. During this period, the neurons in the later developing matrix, which only begin to extend axons to the SN during the early postnatal period, have relatively less Thy-1-IR material. As neurons in the matrix compartment complete the initial elongation of axons to the SN, they begin to accumulate Thy-1, and the patch and matrix compartments are no longer distinctive because of differing amounts of Thy-1 on neurons in each compartment. Thy-1 may serve as a signal to suppress further outgrowth from dopaminergic neurites and stabilize developing synapses with the striatal neurons.

456.13

The Development of Striatal Compartments by 5-Bromo-deoxyUridine Immunocytochemistry. D. D. SONG* AND R. E. HARLAN. Department of Anatomy and Neuroscience Training Program, Tulane University School of Medicine, New Orleans, LA, 70112.

To study birthdates and migration patterns of striatal patch and matrix cells, 5-Bromo-deoxyUridine (BrdU) was injected into pregnant rats at embryonic days 14 (E14) or E19, respectively. Immunocytochemistry for BrdU was performed on coronal cryostat sections through the forebrain at 2hrs post-injection and E16, E19, and postnatal day 0 (P0). At E14 2hrs post-injection, strongly labelled cells could be identified throughout the striatal neuroepithelium, in both ventricular (VZ) and subventricular zones (SVZ). By E16, strongly BrdU labelled cells were only found in more differentiated portions of the neostriatal anlage. Only weakly labelled cells were found in the VZ and SVZ, indicating dilution of BrdU in mitotically active cells. By E19, cells labelled at E14 were distinctly congregated along the ventrolateral border of the CPu. Other E19 embryos injected 2hrs previously with BrdU, had strongly labelled cells throughout the VZ and SVZ of the CPu and nucleus accumbens (Acb). Very few labelled cells were present in the more differentiated CPu. By P0, a dense narrow ventrolateral accumulation of E14-labelled BrdU cells was present along the external capsule. Some labelled cells were dispersed medially in the CPu with clumps or patches of labelled cells being found in the middle of the CPu just caudal to and at the level of the posterior limb of the anterior commissure. Double labelling for the patch marker Substance P (SP) resulted in correspondence of SP and E14-BrdU patches and subcallosal streak. Despite the presence of SP patches, progressively fewer E14-labelled BrdU cells were found at more rostral levels. In contrast, E19-labelled BrdU cells at P0 were quite numerous rostrally, spreading out ventrolaterally into the CPu and Acb. Thus, early developing patch neurons appear to be initially aggregated in the ventrolateral CPu and are dispersed dorsomedially into patches as later matrix neurons migrate around them ventrolaterally. Neurons remaining in the ventrolateral CPu spread out along the external capsule to form the subcallosal streak of the patch compartment. Supported by DA05523 (DDS) and NS24148 (REH).

456.14

DEVELOPMENTAL PLASTICITY IN THE HABENULO-INTERPEDUNCULAR SYSTEM CORRELATES WITH HETEROGENEOUS EXPRESSION OF GAP-43, TENASCIN, HNK-1 AND CHONDROITIN SULFATE PROTEOGLYCAN

Carol A. Murphy and Marion Murray*. Dept. of Anatomy and Neurobiology, Medical College of PA, 3200 Henry Ave., Philadelphia, PA 19129

The medial habenular nuclei (MH) project to discrete subnuclei of the interpeduncular nucleus (IPN) via the fasciculus retroflexus (FR). Substance P (SP) neurons in the dorsal MH innervate the lateral subnuclei of the IPN, while cholinergic cells in the ventral MH project to the central and intermediate subnuclei. SP innervation of the IPN is present at birth and retains plasticity in the adult, whereas cholinergic innervation is not seen until the second postnatal week, and does not show plasticity after this age. The developmental expression of two extracellular matrix molecules, tenascin and chondroitin sulfate proteoglycan (CSPG), of GAP-43, a molecule associated with axonal growth cones, and of HNK-1, an epitope common to a number of cell adhesion and matrix molecules, were evaluated in the habenulo-IPN system of neonatal and adult rats. At P0, CSPG-IR clearly delimited the subnuclei of the IPN, consistent with CSPG's putative role in the formation of boundaries. At P7, CSPG and HNK-1 were expressed almost exclusively in the central subnucleus, coinciding with the onset of the period of cholinergic innervation. In contrast, tenascin was uniformly present in the IPN at P7 and P14, but confined to the SP target subnuclei in adults. GAP-43 was selectively expressed in the lateral subnuclei at P0 and in adults. Within the MH, HNK-1 was present in cells in the ventral region while GAP-43 was selectively expressed by cells in the dorsal region. The differential expression of tenascin and CSPG in the IPN suggests glial heterogeneity among adjacent subnuclei. The non-overlapping patterns of HNK-1 and GAP-43 expression in both MH and IPN suggest differences in molecular expression by cholinergic and SP neurons, respectively. We conclude that these developmental changes in the molecular expression contribute to the differential plasticity of the two systems.

456.15

A THREE-DIMENSIONAL RECONSTRUCTION OF THE HUMAN HYPOTHALAMUS

J.K. Young and G.B. Stanton*, Dept. Anatomy, Howard Univ. Washington, D.C. 20059.

Our previous 3-dimensional reconstruction (3-DR) of the rat hypothalamus (Young and Stanton, Brain Res. Bull. 26:279, 1991) revealed an organization of nuclei into 3 major clusters. This clustering may relate to the presence of 3 hypothalamic anlagen in the embryo and to a restriction of marker proteins to specific regions of the hypothalamus during development (Stainier, et al., Dev. Biol. 147:22, 1991; Mathis, et al., Embo. J. 11:2551, 1992). To see if a similar clustering was present in the human hypothalamus, and to examine volumetric homologies of human nuclei with those of the rat, a 3-DR of the hypothalamus from a male cadaver was prepared. Hypothalamic nuclei from serial, Nissl-stained, frozen sections were projected onto paper, traced and digitized with a graphics tablet.

The 3-DR showed a clustering of human hypothalamic nuclei into 3 anterior-posterior groups. With reference to the well-defined rat and human supraoptic nuclei, the suprachiasmatic, supramammillary and lateral mammillary nuclei were proportionately 10-fold smaller in the human than in the rat. Human and rat anterior nuclei were proportionately equal in size; middle hypothalamic nuclei were proportionately 4-5 fold larger in the rat, and medial- and tubero-mammillary nuclei of the posterior cluster were proportionately larger in the human.

456.16

EXPRESSION PATTERN AND DEVELOPMENTAL CONTROL OF TOP_{AP}: A GRADIENT MOLECULE IN THE CHICK RETINOTECTAL SYSTEM.

J.M. Savitt, D. Trisler*, C.Cargile and D.C. Hilt. Depts. of Biochemistry, Human Genetics and Neurology, Univ. of Maryland Sch. of Med., Baltimore, MD 21201. Laboratory of Biochemical Genetics, NHLBI, NIH, Bethesda, MD 20892.

TOP_{AP} is a 40 kD protein expressed in a position-specific, continuosly graded, fashion in the chick retina and tectum. Antibody binding studies and immunocytochemical staining have shown that TOP_{AP} is preferentially expressed in the more posterior region of the retina and in the more anterior region of the optic tectum. This pattern of expression mirrors that of the retinotectal projections themselves, thus it is possible that TOP_{AP} plays a role in the generation of position-specific neuronal connections. In an attempt to learn more about the structure, function and expression of this protein, a cDNA clone encoding TOP_{AP} was isolated from a posterior quadrant chick retina library. Analysis of the 3.2kb cloned sequence demonstrated a near full length cDNA with an open reading frame coding for 359 amino acids. The translated sequence showed limited similarity to known protein-coding regions found in the SWISS-PROT database. Nucleic acid probes as well as polyclonal antibodies have been generated and used to demonstrate the expression pattern of the TOP_{AP} protein and mRNA on positional, temporal, tissue-specific and subcellular levels. Initial studies have shown that the graded expression of TOP_{AP} is present at the mRNA level and that the protein and mRNA are up-regulated late in embryonic development. The mRNA and protein are differentially expressed in various tissues with the highest expression seen in heart and retina. Finally, biochemical characterization of native and cloned protein, coupled with immunocytochemical staining of primary retinal cells and transfected cells have implied a complex subcellular localization pattern including the presence of TOP_{AP} in membranes.

456.17

EARLY HISTOGENESIS OF THE CENTRAL MOTOR COMPONENTS OF THE FACIAL NERVE IN THE RAT EMBRYO.

F. AUCLAIR, N. VALDES, R. MARCHAND*. Centre de recherche en Neurobiologie, Univ. Laval (Hôpital de l'Enfant-Jésus), Québec, Canada, G1J 1Z4.

The primary goal of this study was to describe the early histogenesis of the motor components of the facial nerve within the hindbrain. Specific labeling of one or the other motor component of the nerve could be achieve by precise dye deposition at peripheral sites. The injections included the main branchiomeric trunk of the facial nerve, the chorda tympani or the greater petrosal nerve. DiI crystals were used on fixed embryos of 13, 14 and 15 gestational days of age (E13, E14, E15). On E13, branchiomeric motoneurons were distributed in a longitudinal column lying close to the midline. They migrated first caudally and then ventrolaterally on E14 and E15. Most of them had reached their "adult" ventrolateral position in the tegmentum by E15. At the same stages of development, visceral motoneurons could also be labeled. On E13, they extended laterally from the medial branchiomeric group to the region of the sulcus limitans, and longitudinally, from a region slightly more caudal than the level of exit of the motor nerve to the level of the caudal end of the medial branchiomeric group. On E14, they continued to slide more laterally and on E15, most of them were lying in the prospective parvocellular reticular formation. Another group of laterally situated visceral motoreurons appeared more rostrally, extending from the level of the exit point of the motor nerve to a level slightly more rostral. It contained less neurons than the first visceral group. Branchiomeric and visceral central components of the facial nerve could further be distinghished by the course of their axons. Branchiomeric axons coursed first rostrally and then turned laterally to exit the hindbrain. Visceral axons coursed first laterally and then turned rostrally to exit the hindbrain. These results describe the evolution in time of the relative position of the branchiomeric and visceral motor components of the facial nerve in a mammalian embryo and suggest that both types of motoneurons are generated in a region close to the midline. Supported by MRC of Canada and FRSQ.

456.18

EFFECTS OF NEONATAL ION DAMAGE UPON THE DISTRIBUTION OF GALANIN RECEPTORS IN THE RAT'S TRIGEMINAL BRAINSTEM COMPLEX.

M.J. Leslie*, C.A. Bennett-Clarke and N.L. Chiaia. Dept. of Anatomy, Medical College of Ohio, Toledo, OH 43699

We have recently demonstrated a marked and transient up-regulation of galanin (GAL)-immunoreactive axons in trigeminal (V) nucleus principalis (PrV) and all of the V spinal subnuclei after perinatal damage to the infraorbital nerve (ION). In this study, radioligand binding with [^{125}I]-GAL was used to determine how this marked up-regulation in GAL immunoreactivity affected the distribution of receptors for this peptide. Receptor binding experiments were carried out in normal rats and in animals that sustained ION damage during the first week of life. In normal adult and perinatal rats, GAL receptors in PrV, subnucleus interpolaris, and the magnocellular portion of subnucleus caudalis form a pattern which is the negative image of vibrissae-related primary afferent terminations. In rats killed 7 days after neonatal ION transection (an age at which there is a clear up-regulation in damaged V primary afferents), there is a dramatic reduction in the density of GAL receptors ipsilateral to the lesion. The decrease occurs in all V subnuclei. In rats killed at longer (>60 day) intervals after neonatal ION damage when the up-regulation of GAL immunoreactivity is no longer present, the density of GAL receptors in the V brainstem complex ipsilateral to the lesion is much closer to normal, but the vibrissae-related pattern can no longer be discerned. These results thus indicate that the dramatic and transient lesion-induced increase in GAL immunoreactivity in the V brainstem complex is associated with a marked and equally transient down-regulation of receptors for this peptide. Supported by NS 28888, DE 08971, DE 07734

457.1

PATTERNS OF *GOTCH* mRNA EXPRESSION IN DEVELOPING GOLDFISH. S.A. Sullivan, B.L. Largent & P.A. Raymond*. Department of Anatomy & Cell Biology, University of Michigan, Ann Arbor, MI 48109-0616.

Using digoxigenin-labeled RNA probes derived from *Gotch*, a goldfish *Notch* homologue cloned previously, we have characterized *Gotch* mRNA expression patterns in the developing goldfish via whole embryo *in situ* hybridization. Some embryos were subsequently frozen and cryosectioned. Robust expression of *Gotch* message was seen as early as the embryonic shield stage [st. 15, Kajishima *Jap. J. Icthyol.* 1960] in the presumptive neural keel and associated structures. Expression remained primarily specific to the CNS through hatching [st. 25], at which time hybridization signals decreased markedly. At all stages *Gotch* expression was localized to regions of cell proliferation and differentiation (*e.g.* periventricular layer in the brain and spinal cord, peripheral germinal zone in the retina), in accord with its postulated role as an enabler of cell fate decisions in *Drosophila*.

Supported by NIH R01EY04318 (P.A.R.)

457.2

ONTOGENY OF CORTICOTROPIN RELEASING HORMONE GENE EXPRESSION IN THE RAT INFERIOR OLIVE. D. Chang, M.C. Citron* and T.Z. Baram. Dept. Neurology Univ. Southern Calif.; Div. Neurology, Childrens Hosp. Los Angeles, Los Angeles, CA 90027.

Corticotropin releasing hormone (CRH) has been shown to be a neurotransmitter in the inferior olive (IO) of both the rat and the human. CRH excites neurons in a variety of neuronal circuits. We have demonstrated age-specific potency and rapidity of the convulsant effects of CRH in the neonatal/infant rat.

We analyzed IO of 25 rats by semi-quantitative in situ hybridization histochemistry using an S^{35}- labelled deoxy-oligonucleotide probe directed against CRH-messenger RNA. We determined the onset and anatomic distribution of CRH-mRNA in rats aged 1,2,3,5,7,10, 14 and 18 days in comparison with adults. CRH-mRNA was first detectible on postnatal day 2, most prominently in the principal and medial accessory subnuclei. CRH-mRNA abundance in IO increased with age, in contradistinction with the ontogenic course of CRH gene expression in the paraventricular nucleus. We conclude that the CRH neurons in IO may be functional during the first postnatal days.

457.3

DISTRIBUTION OF NICOTINIC ACETYLCHOLINE RECEPTOR ALPHA3 SUBUNIT mRNA IN DEVELOPING RAT BRAIN. T.A. Austin* and J.L. Fuchs. Dept. of Biological Sciences, University of North Texas, Denton TX 76203.

The regional distribution of nAChR alpha3 subunit mRNA was examined in rat brains from embryonic day 16 through adult. Cryostat sections were hybridized with [^{35}S]mRNA, and film autoradiographs were prepared. Most brain regions that express high levels of alpha3 mRNA in adults were also prominently labeled in prenatal or early postnatal development. These brain regions typically showed a peak of intense labeling during postnatal weeks 1 or 2, with a subsequent decline to adulthood. The time of peak labeling varied across brain regions. Labeling in the habenula and retina was strong as early as E16. Regions that were prominently labeled in adult and also in perinatal brains included the pineal, certain thalamic nuclei, substantia nigra pars compacta, and patches in the caudate-putamen.

In neocortex, dense labeling distinguished layer IV beginning in area 17 on postnatal day 2 (P2). Labeling in layer IV reached a peak around P14 before declining, but remained prominent in layer IV of all areas of adult neocortex, especially visual cortex.

The hippocampal formation was somewhat unusual in being well labeled only in immature brains. Levels in str. pyramidale of CA3-CA4 were moderate in neonates, highest around P4, and then declined in subsequent weeks. Labeling in the dentate str. granulare was moderately low beginning on P4 and peaked around P6-14 before declining to low levels in adults.

These results suggest that neurons that express high levels of alpha3 nAChR mRNA generally acquire this identity early in ontogeny. Transiently high levels of receptor mRNA in immature brain regions might serve to stabilize or increase the effectiveness of developing synapses.

Supported by NIMH MH41865. We thank J. Patrick for the cDNA.

457.4

A NOVEL RECEPTOR-TYPE PROTEIN TYROSINE PHOSPHATASE EXPRESSED DURING NEUROGENESIS OF OLFACTORY NEURONS. B. L. Largent*, K. J. Martell, J. E. Dixon and K. M. Walton. Biol. Chem. and Anat. & Cell Biol., Univ. of Michigan, Ann Arbor MI 48109-0616.

Tyrosine phosphorylation is a principal mechanism for regulating biological processes. Protein–tyrosine phosphatases (PTPs) have emerged as an important component in the regulation of tyrosine phosphorylation. We postulated that the olfactory system, with its capacity of neural regeneration, might express specific PTPs serving a regulatory role in regenerative processes such as neural proliferation or axon extension. To identify both known and novel PTPs expressed in olfactory epithelium, we amplified putative PTP sequences by PCR using degenerate oligomers which hybridize to highly conserved sequences in PTPs. The amplified products were cloned and analyzed by DNA sequencing. Fourteen unique clones were identified: 8 clones were previously reported PTPs (or the rat homolog); and 6 clones represented novel PTPs. The RNA expression pattern of these novel clones was predominantly neural. For one clone, PTP NE3, we have determined the full length cDNA sequence which predicts a transmembrane protein with extracellular domains of Ig and FN III–like repeats and two cytoplasmic phosphatase domains. PTP NE3 appears neural specific with highest expression in the olfactory epithelium and the brain hippocampal formation. In situ hybridization demonstrates PTP NE3 expression by neural precursors and sensory neurons of the olfactory epithelium. The predicted transmembrane structure with extracellular adhesion motifs suggests a role in axon extension and/or neural soma migration.

457.5

CONSTRUCTION OF RETROVIRAL COEXPRESSION VECTORS TO INVESTIGATE MOLECULAR MECHANISMS OF CNS DEVELOPMENT. E.A.Grove[1]*, H.Moreau[2], V.Calaora[2], G. Rougon[2] and J.Price[1], [1]N.I.M.R, London, U.K., and [2]Universite d'Aix-Marseille, France.

Investigation of the molecular mechanisms of CNS development is hampered by the limited means of altering the expression of specific genes in the mammalian embryo. We have constructed several retroviral coexpression vectors that will allow us to alter the expression of selected neurotrophins and cell surface molecules in embryonic cells, and to mark the cells so that their fate can be followed. These vectors, based on murine MuLV vectors provided by J. Majors (Washington University School of Medicine, St. Louis), carry the gene of interest (or an antisense or mutatated form), followed by the picornavirus internal ribosome entry site (IRES), followed in turn by the marker gene *lacZ*. Expression of both genes is thus driven by the endogenous retroviral promotor in the LTR. We have found that a high proportion of COS-7 or C6 cells transfected with constructs of this form express both genes. A construct containing the gene for alkaline phosphatase in the first position will allow us to quantify coexpression. We are testing the levels of gene expression in infected cells by functional assays *in vitro*. Effects of altered gene function on normal cerebral and cerebellar cortical development will be determined by following the fate of cells marked by infecting germinal layer cells in the rat CNS.

457.6

A DEVELOPMENTALLY REGULATED NGF-INDUCED GENE, VGF, IS REGULATED BY ELECTRICAL ACTIVITY IN THE RAT VISUAL SYSTEM. A. Lombardo, F. Cremisi, S. Rabacchi, T. Pizzorusso, M.C. Cenni, G. Barsacchi, L. Maffei, R. Possenti,* Scuola Normale Superiore, Pisa; Universita' di Pisa, Sez Biologia Cellulare e Sviluppo, Pisa; Istituto di Neurofisiologia CNR, Pisa; Istituto di Neurobiologia CNR, Roma; ITALY.

In the pre-natal and post-natal rat brain we studied the expression of VGF, a gene previously found to be induced by NGF and whose protein is released following depolarization in PC12 cells. RNAse protection, in situ hybridization and immunohistochemical techniques have been performed. Both the mRNA and the protein are particularly abundant in the developing thalamic nuclei that project to primary sensory cortical areas, appearing respectively at embrional day 16 and 18. Strong immunoreactivity (but no hybridization signal) has also been found at the cortical subplate, presumably reflecting the presence of the protein in axonal terminals of thalamic neurons. The peak distribution of VGF mRNA in the dorsal geniculate nucleus is reached during the second postnatal week, followed by a gradual decline of expression.

Interestingly, the blockade of afferent electrical activity by intraocular injection of tetrodotoxin strongly reduces the level of VGF mRNA in the dorsal geniculate nucleus. The temporal correlation between the arrival of retinal fibers and the first expression of VGF suggests that retinal afferents switch on this gene either by afferent electrical activity or by anterograde factors. VGF spatio-temporal expression and regulation by electrical activity point to a role of this protein in the process of synaptogenesis and/or synaptic stabilization in the developing geniculo-cortical connections and introduces the possibility that this factor is involved in the communication processes between pre- and post-synaptic partners.

457.7

IDENTIFICATION OF NUCLEAR PROTEINS THAT ARE DEVELOPMENTALLY REGULATED IN EMBRYONIC RAT BRAIN. F.R. Thormodsson*§, L. Redmond and S. Hockfield. Section of Neurobiology, Yale University Sch. of Med., New Haven, CT 06510 and §Dept. of Anatomy, University of Iceland, Med. Sch., 101 Reykjavik, Iceland.

To identify nuclear proteins that might play a role in the acquisition of neuronal phenotype, 2D-PAGE was used to analyze proteins differentially expressed over the course of embryonic rat brain development. Metabolically labeled rat brain nuclear proteins from embryonic day 14 (E14) were compared to proteins from embryonic day 19-20 (E19-20). Over this period, the rat brain develops from a collection of relatively homogeneous precursor cells into a complex structure containing many different classes of neurons.

Analysis of 2D-PAGE fluorograms, using a computerized gel scanner, permitted the identification of 11 proteins that show increases in their rate of synthesis between E14 and E19-20. Twenty proteins that consistently appear at E19-20 are not detectable on fluorograms of E14 nuclear proteins, even after long exposures, and thus may be consider to appear *de novo*. Fifty-eight proteins show consistent down-regulation between E14 and E19-20, and of these, 19 were not detectable on fluorograms of E19-20 nuclear proteins. The electrophoretic properties of many of these proteins suggest that they are previously unreported, developmentally-regulated nuclear proteins. Comparisons of gels of brain nuclear protein to gels of liver nuclear proteins indicate that several of these proteins may be enriched in brain relative to non-neural tissues. Some of the developmentally regulated, brain enriched nuclear proteins identified here may play a role in regulating the expression of neural genes important for cellular differentiation in the mammalian CNS. [Supported by NS22807.]

457.8

STRUCTURE, EXPRESSION, AND FUNCTION OF A NOVEL RECEPTOR PROTEIN-TYROSINE KINASE, TYRO-3. Martin J. Gore[1,2], Cary Lai[3], Paul Shilling[2], and Greg Lemke*.[1] [1]Molecular Neurobiology Laboratory, The Salk Institute, [2]UCSD Department of Neurosciences, and [3]Department of Neuropharmacology, The Scripps Research Institute, La Jolla, CA 92037.

We have previously described the isolation and characterisation of cDNA clones encoding a series of novel protein-tyrosine kinases (PTKs) including the neural Tyro-3 [Neuron 6 691-704]. The deduced amino acid sequence of Tyro-3 suggests a transmembrane receptor PTK with an extracellular region similar to neural adhesion molecules such as N-CAM, L1, TAG-1, and fasciclin II. We have now studied Tyro-3 expression in the embryonic and adult CNS. We have also generated fibroblast cell lines that stably express this neural receptor, in order to (a) assess its functional properties, and (b) identify its ligand. We have in addition isolated Tyro-3 genomic clones, which are currently being adapted for use in elucidating the developmental role of Tyro-3 *in vivo*.

457.9

A TYROSINE PHOSPHATASE EXPRESSED IN DEVELOPING AND ADULT RAT BRAIN. L.M. Boulanger*[1], G. Apicelli[1], P. Kim[1], G. Matthews[1], P.J. Lombroso[2], and J.R. Naegele[1,2]. [1]Department of Biology, Wesleyan University, Middletown, CT 06459, and [2]Child Study Center, Yale University School of Medicine, New Haven, CT 06473.

Protein phosphorylation is one of the key mechanisms underlying neuronal growth, proliferation, and function. We have investigated the patterns of protein and mRNA expression of a tyrosine phosphatase designated STEP (Lombroso et al, 1993) in developing and adult rat brain. Monoclonal antibodies against an 18 amino acid sequence from the non-phosphatase domain recognized several forms of STEP on Western blots, including a cytosolic triplet of 46, 37, and 33 kDa and a membrane band of about 65 kDa. The membrane band appeared by birth, preceding the cytosolic bands, and both forms were present at adult-like levels by postnatal day 15 (PND 15). In striatum, STEP immunoreactivity shifted from small clusters of cells and neuropil to a continuous pattern between PND 0 and PND 15. STEP protein and/or mRNA was also detected in the retina, olfactory bulb, septum, hippocampus, choroid plexus, cerebellum, brainstem, and cerebral cortex, including the cortical subplate. Comparison of tyrosine hydroxylase, DARPP, and STEP immunoreactivity demonstrated that most of these cells are also dopaminoceptive, suggesting STEP is localized to a functionally distinct neuronal subset.

457.10

MOLECULAR CHARACTERIZATION OF A DEVELOPMENTALLY REGULATED PROTEIN FROM THE EMBRYONIC RAT CORTEX. J.E. Minturn*, H.J.L. Fryer, and S.Hockfield. Section of Neurobiology, Yale Univ. School of Medicine, New Haven, CT 06510

Using 2-dimensional PAGE we previously identified a 64 kD protein (protein 310) that is abundant in the embryonic rat cerebral cortex and is down regulated postnatally (J.Neurosci.9:4304). Microsequencing of gel-purified protein identified 5 peptides that show no significant homology to previously characterized proteins. Polyclonal antisera raised to 2 synthetic peptide fragments demonstrate that protein 310 is expressed by early post-mitotic neurons and is down-regulated during the second post-natal week.

Degenerate oligonucleotide primers to these two peptide sequences were used to amplify cDNA from post-natal day 5 rat neocortex. PCR products were subcloned and sequenced. The predicted amino acid sequence of one of the products contains protein 310 peptide sequence that extends into the clone beyond the primer region. Developmental northern analysis demonstrates a 4.8 kb mRNA species in rat cortex that is down regulated in the second post-natal week, and is absent from both liver and kidney. *In situ* hybridization at embryonic day 16 reveals that the mRNA is present throughout the developing brain and spinal cord as well as in the dorsal root ganglia. The message is especially abundant in the developing cortical plate and is far less abundant in the ventricular and intermediate zones, consistent with expression by post-mitotic neurons.

BESTFIT analysis of the translated clone reveals modest homology (20% identity, 44% similarity) to the C. elegans protein unc-33 which has been suggested to be important in axonal outgrowth and guidance in the developing nematode. Further sequence information may provide additional indications of the function of protein 310. (Supported by NS22807 and the Howard Hughes Medical Institute).

457.11

CORTEXIN mRNA EXPRESSION IN FETAL AND POSTNATAL RODENT BRAIN. P.M. Coulter II, C.F Landry, J.E. Margulies, A.T. Campagnoni*, and J.B. Watson. Mental Retardation Research Center, Department of Psychiatry and Biobehavioral Sciences, UCLA School of Medicine, Los Angeles, CA 90024.

Cortexin is a novel, brain-specific protein of 82 residues that was first identified as a cDNA clone of cortex-enriched mRNA in rat brain by subtractive hybridization (Watson et al, 1992, Brain Dysfunct. 5:94-105; P.M. Coulter II et al, 1993, J. Neurochem., in press). The amino acid sequence of rat cortexin and its mouse homologue are nearly identical (98% similarity) and both contain a putative single-membrane-spanning region in the middle of each sequence. Northern blot analysis shows that cortexin mRNA is brain-specific, cortex-enriched, and present at significant levels in fetal rodent brain with peak expression occurring in postnatal brain. *In situ* hybridization with ^{35}S-labeled probes detects cortexin mRNA mostly in pyramidal neurons of neocortex laminae II and VI, CA1, CA3, CA4 of hippocampus, and piriform cortex, and in granule cells of dentate gyrus of adult brain. There is little or no expression in midbrain, hindbrain, or white matter regions. Digoxigenin-labeled probes visualized cortexin mRNA in cortical neurons of E14-E18 fetal mouse brain. We postulate that cortexin may play a unique signalling role for neurons in both the developing and adult cerebral cortex. Supported by NIH grant HD 25831(A.T.C., J.B.W.).

457.12

ONTOGENY AND EFFECT OF ACTIVITY ON PREPROTACHYKININ mRNA AND SUBSTANCE P IN THE CHICK: EXPRESSION IN NOTOCHORD, FLOOR PLATE AND SPINAL CORD NEURONS. L.K. Garner*, B. M. Mendelson[2], K. L. Cox, and B.M. Davis. Dept. of Anatomy and Neurobiology, Univ. of Kentucky Med. Cntr., Lexington KY 40536; Dept of Anatomy[2], Univ. of Arkansas Med. Sch., Little Rock, AR 72205.

Activity is included in a list of possible epigentic factors that alter gene expression during development. For these studies, ontogeny of preprotachykinin (PPT) mRNA was determined in chick spinal cord during normal development and in embryos treated with d-tubocurarine during embryonic days 5-13. A chick specific PPT cRNA probe was used, for *in situ* hybridization along with immunocytochemistry for the substance P (SP) peptide. Both PPT mRNA and the SP -like immunoreactivity (SPLI) can be detected in the developing embryo as early as stage 23 (E4) in the notochord and presumptive dorsal and ventral horns. At stage 28 (E6) PPT mRNA is expressed in developing ventral floor plate and PPT mRNA and SPLI are found in cells in the dorsal and ventral horns of the spinal cord. By stage 37, cells in the ventral floor plate no longer express PPT mRNA , however, motoneurons in the ventral horn and cells in the dorsal horn are positive for PPT mRNA and SPLI. At stage 39 (E14), the adult pattern is seen for the PPT mRNA localized in the dorsal horn, intermediate lamina and ventral horn motoneurons. Following blockade of activity using dTc, a downregulation of the mRNA was seen in areas of the dorsal horn and intermediate lamina. Ventral horn motoneurons appear to be unaffected. Supported by AA-09205 to BMM.

457.13

LOCALIZATION OF HUMAN D_3 RECEPTOR mRNA IN MIDGESTATIONAL HUMAN FETAL CORTEX BY *IN SITU* HYBRIDIZATION. A.S. Unis* and D.M. Dorsa. Dept. of Psychiatry and Behavioral Sciences, Univ. of Washington, Seattle, WA 98195.

Although [^{3}H]-YM09151-2 specific binding is distributed in a stratified fashion in the cortical plate and subplate regions of midgestational human forebrain (Unis, 1993), it does not discriminate D_2-like receptor subtypes. In order to determine whether some component of this D_2-like radioligand binding might be due to D_3 receptors expressed in human fetal cortex at the twentieth gestational week, we performed *in situ* hybridization using ^{35}S-labeled cRNA probe transcribed from the human D_3 receptor cDNA (Sokolov et al., 1992).

^{35}S-labeled D_3 riboprobe hybridized in a discrete distribution corresponding to the cortical plate and the ventricular zone in sections obtained from the parietal-occipital cortex. Hybridization signal was likewise detected in human caudate control sections. These data suggest that a component of [^{3}H]-YM09151-2 specific binding, which is localized to the subplate zone at this gestational age, may be to the D_3 receptor. The distribution of hybridization signal suggests that these sites are produced by cells in the cortical plate and the ventricular zone. Further characterization of these D_3 mRNA-expressing cells is in progress using emulsion-dipped sections, counter-stained for histological identification.

457.14

DEVELOPMENTAL REGULATION OF NITRIC OXIDE SYNTHASE IN THE CHICK RETINA. R. Paes-de-Carvalho*, J.L.M. do Nascimento and M.H. de Faria. Dep. Neurobiologia, Univ. Fed. Fluminense, Niterói, RJ, Brazil

NADPH-diaphorase histochemistry, reported to label Nitric oxide synthase (NOS) positive cells, reveals the presence of groups of neurons synthesizing the intercellular messenger Nitric oxide in the CNS. Here we show the presence of NOS and its regulation by Ca^{2+} in the developing chick retina. Homogenates from retinas at several developmental stages were incubated at 37°C in 50mM Tris Ph 8.1 containing 1mM β-NADPH and 0.5mM Nitro Blue Tetrazolium and the absorbance was measured at 585nm to detect NADPH-diaphorase activity. L-N^G-Nitro-Arginine (L-NARG) and L-N^G-Monomethyl-Arginine (NMMA), inhibitors of NOS, blocked 40 to 60% of NADPH-diaphorase activity at all ages studied. Ca^{2+} ions were able to stimulate enzyme activity by 30% and 60% in homogenates of E8 and E14 retinas, respectively. However, Ca^{2+}-dependence could no longer be demonstrated in post-hatching retinas. Inhibition curves for L-NARG indicated the presence of two distinct NOS, one stimulated by Ca^{2+}, present only in embryonic stages of development and inhibited by low concentrations of L-NARG and another independent on Ca^{2+} ions and inhibited by high concentrations of L-NARG. The results indicate the existence of different populations of NOS in the chick retina that could be differentially regulated during development. (Supported by CNPq and PROPP/UFF).

457.15

EMERGENCE OF 13 $GABA_A$ RECEPTOR SUBUNIT AND GAD TRANSCRIPTS IN EMBRYONIC RAT SPINAL CORD. R. Somogyi*, W. Ma, V. Smallwood and J.L. Barker. Laboratory of Neurophysiology, NIH, Bethesda, MD 20892

Intercellular communication by chemical messengers plays a critical role in the concerted differentiation of phenotypes during development. A simple chemical messenger, γ-aminobutyric acid (GABA), and its synthesizing enzyme, glutamic acid decarboxylase (GAD), have been found in the spinal cord at embryonic day 13 (E13) and persisted into adulthood. To elucidate the role of GABA during cord development, we determined when and where the receptors for this amino acid are expressed. We analyzed the expression of mRNA for 13 subunits (α1–6, β1–3, γ1–3, δ) of the $GABA_A$ receptor. Using the most sensitive method for the detection of mRNA, PCR (polymerase chain reaction), we clearly identified transcripts for the α1, α2, α3, α5, β3 and γ3 subunits at E12, while α4, β1 and δ subunits were just above the limit of detectability. At E13, the γ2 subunit appeared, followed by the emergence of the β2 and γ1 subunits at E17. From E12 to P0, the full complement of subunit transcripts emerged and steadily increased in signal intensity, except for the α6 subunit, which could never be detected in spinal cord, and the δ subunit, which fluctuated around the limit of sensitivity. From P0 to adult, a general decrease of expression intensities was found. Using in situ hybridization for the same $GABA_A$ receptor subunits, we found that expression begins at E12-13 in motoneurons and continues to emerge along a ventral-dorsal gradient into other regions of neurogenesis to reach maximum abundance at P0. A region- and subunit-specific depletion of transcripts occurs during the transition from P0 to adult. Although PCR enabled us to identify several subunit transcripts before they were detectable by in situ hybridization, the results obtained using both methods were in general agreement.

457.16

P19 MOUSE EC CELLS PROVIDE MODEL FOR NEURONAL REGULATION OF *Ache* GENE EXPRESSION. B. A. COLEMAN* AND P. TAYLOR. Dept. of Pharmacology, UCSD School of Med., La Jolla, CA 92093.

Acetylcholinesterase (Ache) has commonly been used as a marker for terminal differentiation of neurons and has been proposed to play a role in development of the vertebrate nervous system. Mouse P19 embryonic carcinoma cells are uncommitted, pluripotent cells which can by induced to terminally differentiate along the neuroectodermal lineage. Neurons, glia and astrocytes develop after aggregates, previously cultured with retinoic acid (RA), are plated onto tissue culture dishes. Neuronal cultures up to 90% purity can be obtained by treatment with mitotic inhibitors. We have begun using this cell line to examine the developmental and tissue-specific transcriptional regulation of *Ache*. Ache activity is not expressed in undifferentiated cells but can be detected within 24 hr of plating neuronally-induced cultures and increases to approximately 20 nmols/min/mg protein. This correlates with the increase in cells exhibiting neuronal morphology. Histochemical and immunohistochemical results indicate that Ache activity is associated with neuron-like cells containing processes, but not with flattened, glia-like cells. The presence of *Ache* mRNA, as detected by message protection, parallels expression of the enzyme. Splicing of the *Ache* mRNA appears consistent with that observed in mouse brain. The 5' flanking region (3 kb) of *Ache* was linked to the luciferase gene for transient transfection assays. Levels of the reporter gene rose 3.5-fold in neuronally-induced cultures over that observed in untreated controls suggesting that this upstream region of the *Ache* gene contains elements capable of enhancing transcription in neuronal cultures and that increased transcription occurs after neuronal differentiation. Thus the P19 cell line appears to provide a suitable model for further studies of *Ache* gene regulation during neuronal development. (Supported by USPHS GM18360 and 24437)

457.17

VOLTAGE-GATED CA^{2+} CHANNELS AND DTBHQ-SENSITIVITY ARE UPREGULATED PRIOR TO THE ONSET OF SPONTANEOUS CA^{2+} SPIKING AND NEURITE OUTGROWTH. E. C. Olson* and X. Gu. Department of Biology, UCSD. La Jolla, CA 92093.

Calcium influx through voltage-gated calcium channels (VOCs) regulates aspects of differentiation including neurotransmitter expression and K^+-channel modulation in Xenopus spinal neurons. Agents such as Ni^{2+}, which block VOCs and alter differentiation, eliminate a specific class of spontaneous transient $[Ca^{2+}]_i$ elevations in these neurons, termed spikes. Furthermore DTBHQ, which depletes intracellular calcium stores, reduces the amplitude of elicited spikes and suppresses neurotransmitter expression. In this study the developmental appearance of spikes has been investigated with respect to the appearance of VOCs and DTBHQ-sensitivity.

The appearance of spikes was examined in neural plate cultures on laminin polylysine from 2-7 hr, this period encompasses neural tube formation *in vivo*. Spontaneous spike-like elevations of $[Ca^{2+}]_i$-induced fluo-3 fluorescence are first observed at 4-5 hr in neurons with processes (mean incidence 7/hr; n=5). Depolarization with KCl increases fluorescence in all of these cells. Prior to process extension, most spontaneous elevations of $[Ca^{2+}]_i$ are substantially slower. The percentage of morphologically undifferentiated cells which respond to KCl depolarization increases from 3-7 hr in culture: 11% of cells (n=9) respond at 2-3 hr, 75% (n=4) by 6 hr. These observations imply that upregulation of functional VOCs occurs during this period, prior to neurite outgrowth. The KCl response is an effective predictor of cells that will morphologically differentiate as neurons. Sensitivity to DTBHQ precedes the response to KCl (n=6) in differentiating neurons, implying that changes in $[Ca^{2+}]_i$ homeostasis also occur in this period prior to neurite outgrowth. Supported by the NSF (ECO) and the NIH (XG, NCS).

457.18

SPONTANEOUS CA^{2+} SPIKES AND WAVES IN EMBRYONIC *XENOPUS* SPINAL NEURONS *IN VITRO* AND *IN VIVO*. X. Gu* and N. C. Spitzer. Department of Biology & Center for Molecular Genetics, UCSD, La Jolla CA 92093.

Calcium plays an important role in the normal differentiation of embryonic amphibian spinal neurons during an early period of development. Previous studies showed that cultured neurons exhibit spontaneous rapid Ca^{2+} spikes and slow Ca^{2+} waves during this Ca^{2+}-sensitive period. We have now extended the study over much of this period by semi-continuous, confocal imaging of $[Ca^{2+}]_i$-induced fluo-3 fluorescence at 10 second intervals for 5 hours (7-12 hr *in vitro*). We find that 88% of neurons exhibit spontaneous elevations of $[Ca^{2+}]_i$ in culture (n=25). Among them, 17 show spikes and 19 show waves. Interestingly, most spikes (104 of 177) occur during the first 2 hours, while waves are relatively uniformly distributed throughout this period. The incidence of waves is higher in growth cones (8-9/hr) than in the cell body (1-2/hr), although their temporal distribution is similar.

Spontaneous spikes and waves are also observed in neurons in the intact embryonic spinal cord. At early neural tube stages (corresponding to 2-6 hr *in vitro*), 50% of cells in spinal cord exhibit spikes, at a frequency of 10/hr. The incidence and frequency of spiking decline to 20% and 2/hr at tailbud stages (9-15 hr *in vitro*). The appearance of synchronously active cells characterizes the spinal cord at these later stages, when the neurons are electrically coupled. Groups of 2-4 cells in a field of 100 generate synchronous spikes repeatedly, 2-5 times. Remarkably, these cells are not always contiguous, consistent with neurite extension that begins at these stages. The mechanisms by which these calcium elevations encode these aspects of differentiation remain to be elucidated.

Supported by NIH NS15918.

458.1

IN VIVO CONFOCAL MICROSCOPY OF NERVE STRUCTURES IN THE HUMAN CORNEA. B.R. MASTERS*, A.A. THAER, Institute for Medical Vision Aid, Bahnhofstrasse 19a, 6330 Wetzlar, Germany; Uniformed Services University of the Health Sciences, Department of Anatomy and Cell Biology, 4301 Jones Bridge Road, Bethesda, MD 20814.

The cornea richly supplied with sensory nerves and is unique in that its innervation can be noninvasively imaged in the living human eye without staining. A new scanning slit in vivo confocal microscope was developed for imaging the living human eye. The light source is a halogen lamp. Two sets of adjustable confocal slits provide the optical sectioning capability. A double sided mirror provides both scanning and descanning. An intensified video camera detects the images. The optical sections are 1-2 microns thick. No frame averaging or digital image enhancement is required. The studies we described in this paper were made with a Leitz 50X, NA 1.0 water immersion objective. Other studies which required a larger field of view used a Leitz 25X, NA 0.6, water immersion objective. The confocal microscope operated in the back scattered light mode produced single video frames of corneal nerves. The nerves in the anterior stroma penetrate Bowman's membrane and form a plexus under the basal epithelium. The deep fiber bundles in the stroma and the subepithelial fibers are imaged with high contrast without any staining. We observed long nerve fibers, beaded fibers and nerves bridged by short branches. The ability to noninvasively image the innervation in the anterior cornea of the live human eye provides a new approach to monitor in real-time changes in pattern and structure. This work was supported by a grant from NIH, National Eye Institute, EY-06958 (BRM). The authors acknowledge the cooperation from The Institute for Medical Vision Aid, and Helmut Hund GmbH.

458.2

3-D VISUALIZATION AND MORPHOMETRICS OF MYELIN IN INTACT NERVE SEGMENTS OF NORMAL AND TREMBLER MUTANT MICE BY CONFOCAL MICROSCOPY. B. Kosaras*, M. Tang and R.L. Sidman, Division of Neurogenetics, New England Regional Primate Research Center, Harvard Medical School, Southborough, MA 01772-9102.

We have examined segments of whole nerve many mm in length by confocal microscopy. Intact segments of one-year-old wild-type (+/+) and trembler (Tr/+) mouse nerve from lumbar root levels to the plantaris nerve in the foot were fixed in single or mixed aldehydes, washed in buffer, and dehydrated in 50%, 70%, 90%, and 100% DMSO. Nerves were stained for 0.5 hr at room temperature by immersion in 0.01% DiI (Molecular Probes, Eugene, OR) in DMSO, rinsed in 1-2 changes of DMSO, and then were embedded in glycol methacrylate at 4° C. The transparent blocks were oriented on microscope slides with gum and examined with a Sarastro laser scanning confocal microscope (Molecular Dynamics, Sunnyvale, CA). Nerves were viewed transversely and longitudinally, but were optically clearer in transverse view. The red fluorescent stain was intense and selective for myelin. With 20X-60X oil immersion objectives, optical sections of the full transverse face or of selected fascicles were collected at 0.3 or 0.5 μm intervals for depths >100 μm into the plastic block. By alternate confocal scanning and thick sectioning, the length of nerve that can be imaged is limited only by available computer memory. Morphometric measurements in 2-D and 3-D were made with VoxelView software (Vital Images, Fairfield, IA) on a Silicon Graphics (Mountain View, CA) Indigo computer. Myelin occupied 63-83% of the nerve volume at the various +/+ nerve levels, with the highest value in the dorsal L3 root, but occupied only 2-3% of the nerve volume in Tr/+, except in the dorsal root, where the value was 8%. Myelin concentration was 2.5-4.2% of normal at all levels in Tr/+ except for 9.6% in the L3 dorsal root. In the +/+ controls, myelin was continuous along a given axon except at the nodes of Ranvier, as expected, but in Tr/+ the segments of myelin appeared at random; almost no nodes were identified—confirming the Schwann cell as the site of Tr gene action. (Supported by NIH grant NS20820.)

458.3

AN EASY METHOD FOR ULTRASTRUCTURAL ANALYSIS OF IDENTIFIED GROWING NEURONS WITH THE HELP OF THE CONFOCAL LASER SCANNING MICROSCOPE. H.A. Vischer * and M. Dürrenberger, Dept. Pharmacology and Interdepartemental Electronmicroscopy Facility, Biocenter, Univ. of Basel, 4056 Basel, Switzerland.

A problem in ultrastructural analysis of identified neurons is to localize them with exact precision in the tissue block used for thin sectioning. We have labeled with DiI fibers growing in an immature mammalian spinal cord. After labeling with DiI for 2 days at room temperature, the cord was cut on a vibratome into 100μm thick horizontal sections. After investigation under rhodamine fluorescence, sections exhibiting labeled neurons were soaked in 0.03 ‰ DAB and photoconverted under the same illumination for 1 hour. Sections were placed into folding grids, dehydrated, infiltrated and embedded in Quetol 651 (Pelco Inc.) which allowed immunocytochemistry on thin sections. After trimming, the Quetol blocks were fixed in block holders and mounted on an inverted confocal laser scanning microscope (CLSM; Tracor Odyssey). The block was optically sliced at 1μm intervals with a 40x (NA=0.75) Zeiss Planapo lens in fluorescent light mode (FITC). The 3D position of the labeled fibers, relative to the block surface, could thus be determined with an accuracy of ±0.5μm. With imaging techniques, it was possible to localize not only the exact position of the growth cones of the DiI labeled fibers but also the light microscopic 3D distribution of their axons before sectioning for electron microscopy.
Supported by Swiss National Found (31.362692) to J.G. Nicholls.

458.4

MEASUREMENTS OF PHRENIC MOTONEURON SOMAL VOLUMES USING LASER SCANNING CONFOCAL MICROSCOPY: COMPARISONS WITH ESTIMATES USING THE CAVALIERI PRINCIPLE AND THE NUCLEATOR. Y.S. Prakash*, K.G. Smithson and G.C. Sieck Departments of Anesthesiology, Physiology and Biophysics, Mayo Clinic and Foundation, Rochester, MN 55905

In neuromorphometry, spatial information about neural elements is obtained essentially from two-dimensional (2D) images while the objects themselves are three-dimensional (3D). Until recently, parameters (e.g., cell volume) have been estimated from 2D images using stereological techniques which require assumptions about the size, shape and orientation of the 3D object. This approach can lead to considerable errors in estimating parameters of interest since neuronal shape or orientation can deviate significantly from these assumptions. Recently two new unbiased methods which make no shape assumptions, the Cavalieri principle (Gundersen, H.J. et al. *J Microsc.* 147:229, 1987), and the nucleator (Gundersen, H.J. *J Microsc.* 151:3,1988) have been introduced to estimate cell volume. Alternatively it is possible to make direct measurements of cell volume using confocal microscopy (Prakash, Y.S. et al. *Soc. Neurosci. Abstr.* 18:966,1992). In the present study these three volume estimation methods were compared: 1) confocal microscopy, 2) nucleator, and 3) Cavalieri principle. Optical sections of forty-nine rhodamine-labeled phrenic motoneurons were obtained by confocal microscopy; here neuronal volumes were determined by voxel counting. Nucleator and Cavalieri methods were simulated by computer manipulation of these optical sections. Individual motoneuron volumes were similar for confocal and Cavalieri methods, but were significantly different from volumes estimated by the nucleator. Furthermore, the distributions of neuronal volumes showed a similar relationship. These results suggest that the nucleator was not an accurate method for determining individual cell volume, or the distribution of cell volumes, for phrenic motoneurons. Estimates obtained by both "direct" confocal measurements and by the Cavalieri method were in good agreement suggesting that less stringent optical sectioning parameters may suffice for cell volume measurements when using confocal microscopy. (Supported by NIH grants HL37680, HL34817 and GM08288)

458.5

CHARACTERIZATION AND LOCALIZATION OF ORBICULARIS OCCULI MOTONEURONS FROM 3D RECONSTRUCTIONS. B. F. Lofthus*[1] and T. H. Brown[2,3]. Program in Neuroscience[1] and Depts. of Psychology[2], and Cellular & Molec.ular Physiology[3], Yale Univ., New Haven, CT 06520

The rat eye blink reflex is useful for neurophysiological studies of associative learning and adaptive gain control (Donegan et. al, *Neuro. Abstr. (18), 146.6.* In the present study, we determined the number, location and morphological characteristics of the motoneurons that produce the downward force generating the eye blink. These neurons, which innervate the orbicularis occuli (oo) muscle, are located in the facial motor nucleus (FMN).

Until now, studies of the motoneuron pool innervating a particular facial muscle have typically involved HRP or one of its conjugates in thin sections that have been previously dehydrated. Tissue shrinkage and incorrect cell counts are two common problems with this technique. Our method of retrograde labeling from the oo muscle, combined with confocal microscopy and 3D reconstruction of thick tissue, eliminates these problems. *In vivo* injections of tetramethylrhodamine were made into the oo muscle. Fixed tissue containing the FMN was transversely sliced at 200 μm and optically sectioned using a laser scanning confocal microscope. Cells were then reconstructed using the Voxel View 3D reconstruction system.

Statistical analysis of the number of oo motorneurons revealed a mean (±SE) = 21 ± 1 cells per animal per FMN. The oo motoneurons span 1,400 μm rostrocaudally and form a cap around the posterior edge of the FMN. Soma sizes ranged from 450 - 1,487 μ^2, with larger cells being distributed more rostrally and smaller cells more caudally. In addition to providing quantitative data on dendritic length, width, and arborization, the 3D reconstruction system allowed visualization of novel cell-to-cell interactions. *Supported by NIH (THB), NSF(BFL) and CTAN.*

458.6

CONFOCAL "GRID-MAPPED" FREEZE FRACTURE OF PRESELECTED NEURONS IN SPINAL CORD AND BRAIN

J. E. Rash*, L. R. Whalen, P. B. Guthrie, M. Morita, R. K. Dillman, T. Yasumura and D. Fay-Guthrie. Dept. of Anatomy and Neurobiology, Colorado State University, Fort Collins, CO 80523

Interpretation of freeze-fracture images from complex nervous tissues is subject to many uncertainties, including those introduced by tissue dissection, sample orientation during freezing, the near random nature of the fracture plane, and fragmentation of replicas during cleaning. We have developed the "grid-mapped" freeze-fracture technique to address many of these limitations. Histological slices were mapped in three-dimensions by confocal microscopy, allowing selected neurons to be cleaved using the precision microtome (±0.5 μm) in a JEOL JFD-9000 freeze-fracture machine. After replication, each frozen sample was stabilized in a Lexan film on a gold "Finder" grid. To confirm the location of the fracture plane, samples were thawed and re-mapped by confocal microscopy. A second carbon film applied before removal of the Lexan support film yielded unfragmented, undisplaced replicas as large as 2 x 2 mm. Spinal cord neurons linked by gap junctions were mapped to Rexed Laminae VI, VII, VIII, and IX (motor neurons and interneurons). Similarly, histological stains applied after freeze fracturing allowed us to identify replicated neurons in the hippocampus and suprachiasmatic nucleus. The "grid-mapped" freeze-fracture technique provides for unambiguous identification and mapping of replicated neurons and their major neuritic processes, and is allowing us to develop criteria for identifying neuronal subtypes based on a growing "library" of IMP markers.

Supported by NIH Grant # NS-39503.

458.7

MAPPING OF THE HUMAN BRAIN. THE SINGLE CELL LEVEL, EMPLOYING LUCIFER YELLOY MICROINJECTION, IMMUNOHISTOCHEMISTRY, AND CONFOCAL LASER SCANNING MICROSCOPY.

P.V. Belichenko and A. Dahlström*. Brain Res. Inst., Russian Acad. Med. Sci., Moscow, Russia; and Inst. Neurobiol.*, Dept. of Anat. and Cell Biol., Univ. of Göteborg, Medicinaregatan 5, S-413 90 Göteborg, Sweden.

Modern neuroscience is looking for technologies suitable for mapping of the human brain. Neurons in the CNS integrate more than 95% of the information from other neurons on their dendrites and alterations in the dentritic receptive capacity may play a crucial role in some neuropathological contions. A compete picture of single neurons, with dendrites and axons, the fundamental functional units of organization, must be included in brain mapping. Our strategy for contributing to the "Mapping of the Human Brain" uses intracellular injections of Lucifer Yellow (LY), neurotransmitter immunohistochemistry and confocal laser scanning microscopy (CLSM). Under microscopic control, LY was injected into a great number of single neurons and glial cells in 200µm thick vibratome serial sections from brain specimens removed during neurosurgery. We successfully combined LY injections with immunohistochemistry, using a variety of antisera, e.g. anti-synapsin I, anti-P38, anti-MAO, anti-β-adrenoceptor, etc. Using CLSM, the fluorescence microscope pictures of single cells and neurotransmitter distribution are stored in file format and transferred to a computer, where 3-D structures of the injected cells are created. With this approach we have started to collect a confocal 3-D image library. This approach was tested for frontal, temporal, parietal, and occipital cortices, and of hippocampus in normal and pathology human brains. The clinical application has been the study of neuronal dysplasia in cortical areas of epileptic patients.

458.8

THREE-DIMENSIONAL MORPHOLOGICAL ANALYSIS OF MOSSY-FIBER SYNAPSES IN RAT HIPPOCAMPAL BRAIN SLICES.

T.-P. Yu*[1] and T.H. Brown[1,2] Depts. of Psych.[1] and Cell. and Mol. Physiol.[2], Yale University, New Haven, CT 06520.

The hippocampal mossy-fiber (mf) synapse is attractive for studies of neurophysiology and use-dependent plasticity because of its large size and electrotonic proximity to the soma. We previously reported (Yu and Brown, *Soc. Neurosci. Abstr. 18*, 1992) the visualization and identification of presynaptic mf boutons (expansions) using confocal laser scanning microscopy (CLSM) applied to living brain slices. Here we perform a morphological analysis of the boutons using a volume rendering system.

The methods used for visualization of living mf synaptic expansions were as described previously (*op. cit.*). Living rat brain slices were sectioned at 400 - 450 µm, the mf boutons were stained with dil or diA, and the labelled boutons were then imaged using CLSM. The rendering system enabled three-dimensional (3D) visualization of the boutons at different angles and permitted analysis of area and volume.

The mf boutons were highly variable with respect to both size and shape. In most cases, they were irregular oval structures, occasionally having rough surfaces. About 70% of the boutons were bipolar, being more or less fusiform enlargements on the axis of the mf axon. Sometimes we could see thin protuberances emanating from the expansion. The volumes of the boutons varied from 25 to 510 μm^3 [mean (±SE) = 161 ± 23 μm^3]. The 3D surface areas ranged from 32 to 416 μm^2 [mean (±SE) = 174 ± 18 μm^2].

Unexpectedly, the analysis suggested a linear relationship between mf volume and 3D surface area, suggesting that as the volume increases there is a change in the shape or an increase in the surface irregularity. We are currently examining these possibilities. *Supported by NIMH and CTAN.*

458.9

THE LIVING AND THE DEAD: INTERACTIVE VISUALIZATION OF NERVE CELLS

B.J. Burbach*[1], R.S. Avila[1], L.M. Sobierajski[2], A.E. Kaufman[2], and P.R. Adams[1]
[1]Howard Hughes Medical Institute and [2]Dept. Computer Science, SUNY at Stony Brook, Stony Brook, NY 11794-5230

The electrical properties of neurons are strongly influenced by geometric factors such as membrane capacity, channel localization and diffusion pathways. Laser scanning confocal fluorescence microscopy together with volume rendering are powerful techniques for exploring and quantifying these aspects of three dimensional morphology. We have investigated a variety of preparations including tracer-injected neurons in fixed brain slices (e.g., hippocampus, LGN), immunofluorescently labeled cells (e.g., PC 12), and live vitally stained bullfrog sympathetic ganglion cells in culture. Particular attention was paid to optical abberations, calibration, sampling and thresholding. A volume visualization system, VolVis, was developed and utilized to reconstruct, visualize, and measure serial optical sections. The VolVis system provides the ability to load multiple volumetric and geometric data sets, navigate in real time through a reduced resolution polygonal representation of the data sets, render the scene using several projection algorithms, and perform volume and surface area measurements. Rendering capabilities include a fast ray casting algorithm called PARC (Polygon Assisted Ray Casting) and a volumetric ray tracer, both of which can render a scene with volumetric surfaces or transparency and may perform parallel or perspective projections. The position of the view is interactively manipulated using the navigation component. The VolVis system is running on Silicon Graphics workstations using X/Motif/GL, Sun workstations using X/OpenLook, and Hewlett-Packard workstations using X/Motif/Starbase. VolVis is available free of charge to educational and research institutions. Contact R. Avila at avila@cs.sunysb.edu.

458.10

EVIDENCE AGAINST PERSISTENT NUCLEAR/CYTOSOLIC CALCIUM GRADIENTS IN BULLFROG SYMPATHETIC NEURONS.

DM O'Malley*, SP Yu, BJ Burbach, & PR Adams. Dept of Neurobiology & Behavior and Howard Hughes Medical Institute, SUNY, Stony Brook, NY, 11794.

Cultured neurons were loaded with the calcium indicator fluo 3 via a whole-cell patch electrode and imaged using the Biorad MRC 600 Laser Scanning Confocal Microscope. Cells were held at -70 mV and depolarized to 0 mV for 5 to 200 msec to activate calcium currents. The influx of calcium was visualized in images acquired at 200 msec intervals. A ring of calcium influx was evident under the plasma membrane immediately after the pulse. The calcium then diffused through the cytoplasm and into the nucleus. The absolute fluorescence increase was largest in the nucleus. However, after correcting for autofluorescence, the relative increase in nuclear fluorescence was roughly equal to that in a region of cytoplasm the same distance from the plasma membrane as the nucleus. The relative fluorescence change in these regions was much smaller than the maximal early increase seen under the plasma membrane. Rapid imaging with 2-msec linescans revealed calcium transients entering the nucleus within 10-20 msec after arriving at the nuclear envelope.

Although the nuclear envelope appeared freely permeable to calcium transients, the fluorescence of fluo 3 in the nucleus was about 2.3-fold brighter than in the cytoplasm. To evaluate whether the increased nuclear fluorescence was due to a persistent nuclear/cytosolic calcium gradient, a calibration curve was generated by patching onto cells with pipettes containing different levels of free calcium and 10 mM BAPTA. Using confocal volumes, independent calibration curves were made for the nucleus and cytoplasm. The nucleus was 2- to 2.5-fold brighter than the cytosol at all calcium levels. Perfusion of the patch pipette with different concentrations of calcium or manganese allowed generation of complete calibration curves in individual cells. These calibrations argue against persistent calcium gradients and suggest that nuclear fluorescence exceeds cytoplasmic fluorescence because of the exclusion of fluo 3 by cytoplasmic organelles. [DMO supported by NIH Fellowship NS09113-02].

STAINING, TRACING, AND IMAGING TECHNIQUES I

459.1

ULTRASTRUCTURAL DISTRIBUTION OF CAM KINASE II ISOFORM mRNAs IN RAT HIPPOCAMPUS: ELECTRON MICROSCOPIC IN SITU HYBRIDIZATION USING THICK CRYOSECTIONS.

M.E. Martone, J. A. Pollock[1], Y. Zhang and M. H. Ellisman*, Microscopy and Imaging Resource, Univ. Calif., San Diego, 92093-0608, [1]Biology Dept., Carnegie Mellon University, Pittsburgh, PA.

We investigated the ultrastructural distribution of mRNA for Ca^{2+} calmodulin-dependent protein kinase II (CAMKII) isoforms in the rat hippocampus using an EM in situ hybridization protocol utilizing thick cryosections viewed with intermediate high voltage electron microscopy (IVEM). IVEM offers several advantages over standard electron microscopy for EM in situ hybridization. Thicker sections survive the harsh pretreatments necessary to render the mRNAs accessible better than do thin sections and also include greater numbers of target mRNA molecules per section. Semi-thick and thick (0.5-1um) ultracryosections were obtained from the rat hippocampus and collected on gold EM grids. Grids were floated in 0.1N HCl followed by 2XSSC, rapid heating to 70°, 4% paraformaldehyde, dehydration-rehydration in either ethanol or glycerol with PBS rinses between steps. Grids were incubated overnight at 37° with biotin- or digoxygenin- labeled probes which included a 600 bp cDNA generated from a restriction fragment of CAMKII (provided by Dr. P. Kelly, Univ. Texas, Houston) and synthetic oligonucleotides specific for either the alpha or beta CAMKII isoforms (Burgin et al. J. Neurosci. 1990, 10, 1788). Following a series of high stringency washes, labeled probes were detected using silver intensified 1nm colloidal gold. Various controls were included to test probe and method specificiy. Sections embedded in LR White were viewed at 400 keV using a JEOL 4000EX IVEM.

Labeling for both alpha and beta isoforms was localized to granule and pyramidal cell bodies. Labeling for beta extended into proximal pyramidal cell dendrites but abruptly stopped between 5-10 um from the cell body. In contrast, labeling for alpha was followed up to 30 um into apical dendrites where it gradually diminished. These results confirm and extend light microscopic observations of Burgin et al. (1990) and suggest that the use of semi-thick cryosections provides a sensitive method for EM level detection of mRNAs.

459.2

ANATOMICAL CHARACTERIZATION OF ZEBRAFISH RETINAL ANTIGENS WITH ALKALINE PHOSPHATASE-MEDIATED METHODS USING A NEW FLUOROGENIC SUBSTRATE

†K.D. Larison, ‡R. BreMiller, †V. Paragas, †V. Singer, †J.J. Naleway and †R.P. Haugland. †Molecular Probes, Inc., Eugene, OR 97402; ‡Institute of Neuroscience, University of Oregon, Eugene, OR 97403.

Molecular Probes' researchers have synthesized a new alkaline phosphatase substrate, 2-(2'-phosphoryloxyphenyl)-4(3*H*)-quinazolinone, which we used with standard immunohistochemical techniques to characterize monoclonal antibodies against fish retina (FRet monoclonals). The tissue was first probed with a FRet antibody, followed by sequential application of biotinylated goat anti-mouse, streptavidin, biotinylated alkaline phosphatase, and then finally the substrate. With the methods we have developed, we were able to localize FRets that bind to pigment epithelial cells (FRet 45), to double-cone cells (FRet 43), to nuclear membranes of a subset of retinal cells (FRet 34), to retinal synaptic layers (FRets 8 and 19) and to cell surfaces just scleral to the outer limiting membrane (FRet 6). The substrate's pattern of staining for each of these antibodies was identical to that seen with HRP/DAB (horseradish peroxidase-diaminobenzidine) methods. The advantage of this newly developed method over standard HRP/DAB techniques is that it can be used in conjunction with any number of fluorescent probes, including fluorophore-conjugated immunoreagents, to facilitate multicolor labeling. The substrate produces an extremely bright and photostable fluorescent precipitate (excitation, ~360–420 nm; fluorescence, ~500–540 nm). We have also used the substrate for Western blotting to further characterize these antibodies. Supported by NIH grant R43GM48306-01 to R.P. Haugland.

459.3

VISUALIZATION OF ASPARTATE AND GLUTAMATE DISTRIBUTIONS IN HIPPOCAMPUS USING UNIQUE NEW NETWELL REACTION TRAY SYSTEM. W. K. Newell, M. W. Fleck, and G. Barrionuevo*. *Departments of Behavioral Neuroscience and Psychiatry; University of Pittsburgh, Pittsburgh, PA 15260.*

We evaluated the utility of a new disposable reaction tray system for use in immunohistochemical and general staining of sliced tissues. A new Netwell reaction tray system (Costar) was tested which allows for pre treatment of live tissue slices by superfusion followed by immediate fixation and reaction in disposable trays. Compared with the custom-built apparatus used in previous experiments, the disposable Netwell reaction tray system reduced experimental time and damage artifact while significantly increasing slice yield.

Rat hippocampal slices (100 μm thick) were obtained by Vibratome slicing following rapid cardiac perfusion with oxygenated sucrose solution. Slices were incubated in the reaction trays by superfusion of oxygenated artificial CSF of varying ionic compositions. Superfusion source was then changed to medium containing cacodylate and sodium metabisulfite for 10 min, and then to the same medium containing glutaraldehyde. Slices were separated into two groups and reacted with antibodies raised in rabbit against the glutaraldehyde fixation products of either ASP or GLU (Chemicon). Slices were then incubated with a secondary anti-rabbit antibody raised in goat (Vector). Antigens were revealed with ABC-DAB reactions.

Only weak staining was observed in the three principal cell-body regions, CA1, CA3, and dentate gyrus. Diffuse ASP-like immunoreactivity was apparent in all dendritic regions but especially in area CA1 and dentate. Diffuse GLU-like immunoreactivity was equally robust in all dentritic regions. A thin dense band in the middle third of the dentate molecular layer showed both ASP-like and GLU-like immunoreactivities. In CA3, a thin GLU-like immunoreactive band was observed in stratum lucidum. These results support a neurotransmitter role for GLU and ASP in hippocampus. Supported by NS26288 and an NIMH predoctoral fellowship.

459.4

NEURAL ANTIGEN DETECTION IS NOT IMPAIRED BY DECALCIFICATION. D.B. Henken* & J.R. Martin, NIH, NINDS, LENP, Bethesda, MD, USA, 20892.

Decalcification using EDTA (ethylenediaminetetraacetic acid) has been used by pathologists to examine bone and neural tissues encased by it. Whether the decalcification process reduces the sensitivity of antigen detection in neural tissues has not been addressed. Here, in dissected sensory ganglia and undissected, decalcified preparations that include spinal ganglia and spinal cord, quantitative and semiquantitative comparisons are made of numbers and staining intensities of neurons immunocytochemically labelled with antibodies that include the neuropeptides, substance P (SP) and calcitonin gene-related peptide (CGRP) as well as two other neural markers, neuron specific enolase (NSE) and glial fibrillary acidic protein (GFAP). Adult female BALB/c mice (n=9) were cardiac perfused with 10% formalin. The vertebral columns of 5 mice were removed and decalcified. The sensory ganglia from the remaining 4 mice were dissected. All tissues were embedded in paraffin, sectioned (7 μm), and processed for immunocytochemical detection of the appropriate antigen. In undissected decalcified tissues, the immunoreactivities of these neural antigens were compared with those in dissected sensory ganglia. Decalcification does not reduce either the numbers or staining intensities of antigen-positive neural cells. Tissues are well preserved and complex anatomical relationships are maintained.

459.5

A QUANTITATIVE ANALYSIS OF THE RELATIONSHIP BETWEEN NEURON COUNTS AND OPTICAL DENSITY OF NADPH-DIAPHORASE HISTOCHEMISTRY IN THE RAT STRIATUM. H. Kuo*, J. Hengemihle, W. DeLoriers, S. Grant, and D. K. Ingram, GRC, NIA, NIH, Baltimore, MD 21224 and Dept. of Psychology, Univ. of Delaware, Newark, DE 19716.

NADPH diaphorase histochemistry is a useful technique for examining select neuronal populations in both experimental studies and in human neuropathology and also provides a simple method to localize nitric oxide synthase in the CNS (Hope, *PNAS*, 88:2811, 1991). However, no established method exists for detecting quantitative changes of NADPH diaphorase histochemistry under different experimental conditions. To develop a quantitative procedure, we investigated the correlation between the number of NADPH diaphorase positive cells and the optical density of NADPH diaphorase histochemistry in the rat striatum. Fourteen male F-344 rats (3 mo old) were used. Brains were sectioned parasagittally into 30 μm serial sections on a cryostat. NADPH diaphorase histochemistry (Vincent, *Neuroscience*, 46:755, 1992) was performed at a series of different incubation times (0.5-8 hr). The calibrated optical density of the whole striatum at a specific plan (lateral 2.5 mm) was measured using automated densitometry (RAS system, Amersham) and the number of positive cells per unit in this plan was counted by hand. Our results showed that during the reaction, the cell body was stained first and then the major processes and neuropil. Up to 5 hr incubation, an increase in incubation time increased the optical density of NADPH diaphorase staining. In contrast, the number of NADPH diaphorase positive cells counted was relatively consistent across incubation times. Therefore, little correlation existed between the optical density and cell number. This results indicate that when using NADPH diaphorase histochemistry, the number of NADPH positive neurons is independent of the optical density of the staining, and these two parameters should be considered and treated separately when conducting quantitative analysis related to an experimental treatment.

459.6

ENHANCED IMMEDIATE EARLY GENE EXPRESSION WITH BIOTIN AMPLIFICATION. K.A. BERGHORN, K.D. RYAN*, and G.E. HOFFMAN. Departments of Neurobiology, and Cell Biology and Physiology. University of Pittsburgh, Pittsburgh, PA, 15261.

Identification of activated neurons using c-Fos and/or Fos related antigens (FRAs) is an important technique with which to study functional pathways following a specific stimulus. Expression of Fos, revealed by immunoreactivity, has been used extensively in this laboratory to study the regulation of several autonomic and neuroendocrine systems Increased sensitivity and enhancement of Fos immunocytochemical localization would be of great benefit. Recently, Adams (J Histochem Cytochem 40:1457-1463, 1992) described a biotin amplification procedure (BAP) which increased sensitivity in histochemical stains. Through modification of his protocol, we applied BAP for enhancement of ABC "elite" immunoperoxidase and developed a BAP procedure for immunofluorescent staining of immediate early gene proteins. Using BAP, Fos and FRA staining with immunoperoxidase protocols was enhanced variably over the "elite" ABC peroxidase protocol. Antibody titers could be reduced 2-15X, depending on the antibody. For certain N-terminal-directed Fos antisera, BAP increased background staining. However, in general Fos and FRA staining intensity was increased and sensitivity improved. The greatest advantage came with immunofluorescence staining. There, BAP rmarkedly increased the signal to noise ratio, reduced background staining and permitted decreases in titers In total, the application of BAP to localization has the promise of aiding the scientist in detecting these immediate early gene projects. *Supported by NIH HD 07481 and NS 28730.*

459.7

NON-ISOTOPIC *IN SITU* HYBRIDIZATION IN BRAIN WHOLEMOUNTS OF THE SEA LAMPREY *PETROMYZON MARINUS*. G.P. Swain* and M.E. Selzer Dept. of Neurology & David Mahoney Institute of Neuroscience, Univ. of PA, Phila., PA 19104.

Non-isotopic *in situ* hybridization (NISH) using a variety of colorometric reporter molecules is rapidly replacing autoradiographic techniques because of its more rapid processing time, safety and improved localization of signal. Until now NISH has been limited largely to sectioned material or cultured cells. We have developed a hybridization protocol using digoxigenin-labeled cRNA probes suitable for whole mount *in situ* preparations of the lamprey CNS. The ability to do ISH in wholemounts permits the rapid examination of large sample sizes and thus facilitates investigations of gene expression during CNS development (*e.g.*, metamorphosis) and regeneration. These preparations can then be compared to wholemounts labeled by other methods such as retrograde transport of HRP, immunohistochemistry, etc. The technique is illustrated in this poster using probes transcribed from lamprey neurofilament (NF 180) cDNA. Neurofilament mRNA is expressed in most of the cranial nerve nuclei and in the reticulospinal cells in the larval and adult lamprey. Expression of NF180 in the cells of the oculomotor and trochlear nuclei begins upon metamorphosis (*i.e.*, coincidental with the functional development of the eyes). The ability to do NISH in wholemounted brain, even in adult specimens, is an additional advantage of the lamprey as an experimental model for vertebrate neurobiology. (Supported by grant #NS 14837)

459.8

SEMI-AUTOMATIC IMAGE ANALYSIS FOR IN SITU HYBRIDIZATION EXPERIMENTS. F. T. Banfro[1], C. L. Thouron[1], L. R. Lucas[2], R. E. Harlan[2], and R. R. Mize[1], [1]Dept. of Anatomy and the Neuroscience Center, Louisiana State Univ. Med. Ctr. and [2]Dept. of Anatomy and Neuroscience Training Program, Tulane University Med. School, New Orleans, LA 70112.

We have developed a procedure for semi-automatic computer-based image analysis of in situ hybridization experiments. The procedure is designed to count autoradiographic grains over cells, to measure the number of cells and their size, and to calculate the grain density per cell. The procedure is used with a Joyce-Loebl MD image analyzer running a specially written macro task list. In order to distinguish autoradiographic grains from thionin stained neurons, the task list makes two passes, one to recognize cells, the other to recognize grains. In the first pass, a green filter is used to enhance the cells. A parabola spatial filter and a heighten contrast gray level operator are applied to enhance cell contour. We then set the grey level threshold so that cells are captured in binary memory. Separation and size filters are applied to separate apposed cells and eliminate artifacts. The size and number of cells are then measured. A second pass is used to extract the grains. A blue wratten filter is used to mask the thionin stained cells. Non-linear density and Laplacian filters are applied to enhance grain edges. The grains are then thresholded and separation and size filters applied to extract the grains and store them in a second binary memory. Boolean operators are applied to both binary images in order to selectively count the grains overlying each cell. Grain density is then computed using the cell size and grain measurements. The procedure has been used to quantify levels of mRNA expression in the rat caudate putamen. Comparison of manual and automatic grain counts revealed a high correlation between the two methods, although the computer slightly underestimated the counts obtained manually. Advantages of the procedure include speed and the ability to accurately separate grains from cells. Supported by EY-02973, RR-02800 (RRM) and, NS 24148, DA06194 (REH).

459.9

FLUORESCEIN AS A LABEL FOR NON-RADIOACTIVE IN SITU HYBRIDIZATION IMAGING TECHNIQUES, I. Durrant*, S. Brunning, P. Chadwick and M. Cunningham.

Amersham International R&D Lab., Amersham, Bucks, HP7 9LL, UK.

The current studies were performed to investigate the properties of fluorescein as a label for in situ hybridization probes. This label should not have any endogenous background and high affinity antibody was available. Fluorescein labelled nucleotides have been incorporated into RNA, DNA and oligonucleotide probes. The efficiency of probe labelling is checked in a rapid fluorescence-based assay. Hybridizations, in a new buffer system, can be performed in only 4 hours and the entire process can be completed in 1 working day, with results available the following morning. The probes have been tested on a variety of systems, including the detection of messenger RNA for peptide hormones, including POMC in rat pituitary. Signal is high, resolution is high and background is low.

459.10

VISUALIZATION OF ACTIVITY-DEPENDENT EXPRESSION AND DISTRIBUTION OF M_1 MUSCARINIC ACH RECEPTORS IN LIVING VISUAL CORTEX NEURONAL CULTURES. Y.Wang*, Q.Gu, F.Mao[1], R.P.Haugland[1] and M.S.Cynader. Dept. of Ophthalmology, Univ. of British Columbia, Vancouver, B.C. Canada V5Z 3N9 and [1]Molecular Probes Inc., Eugene, OR 97402.

We have employed the fluorescently labeled muscarinic M_1 receptor selective antagonist BODIPY-pirenzepine to study the activity-dependent distribution and expression of muscarinic M_1 ACh receptors (M_1AChRs) in cultured neurons derived from rat visual cortex. Binding of BODIPY-pirenzepine retained the specificity of pirenzepine for M_1 receptors, with a similar K_i and displacement profile. Using confocal microscopy, the receptors were predominantly localized to cell bodies early in development in the culture environment. After two weeks in culture, the receptors were labeled not only in cell bodies but also in neuritic processes, especially on the initial segments. Chronic membrane depolarization with 40 mM potassium chloride caused a dramatic increase in M_1 receptor expression in these cultured neurons. Conversely chronic blockade of neural activity with 0.1 μM TTX decreased expression of the receptors. Receptor expression increased after cells were treated chronically with 50 nM pirenzepine, whereas it decreased after exposure to 10 μM carbachol. The results demonstrate for the first time the precise location of muscarinic receptors in living cultured neurons and also illustrate activity-dependent expression of M_1 receptors. The results also show that both chronic membrane depolarization and application of antagonists can up-regulate receptor expression, whereas blocking bioelectrical activity and applying agonist down-regulate receptor expression in living cells.

459.11

A NOVEL METHOD TO RELATE OPTICAL DENSITY OF PKC-ISOFORM ANTIBODY STAINING OF HIPPOCAMPAL SECTIONS TO KNOWN PKC EPITOPE CONCENTRATIONS USING AVIDIN-BIOTIN-PEROXIDASE (ABP) IMMUNOHISTOCHEMISTRY ON NITROCELLULOSE FILM SLIDES. M. Rossi*, L. deToledo-Morrell, F. Morrell. Dept. Neurol. Sci., Rush Med. Coll., Chgo, IL 60612.

An innovative technique is introduced to quantify protein kinase C (PKC) epitope-antibody binding in brain tissue sections using ABP immunohistochemistry against known concentrations of purified PKC proteins or peptides of interest. A nitrocellulose (NC) film formulation was developed by Grace Bio-Oncology, Inc. After some modification to the formulation, exceptional histocoherent transfers of unfixed frozen 10 μm thick brain sections were achieved. To prepare calibrated standards, a dilution series for immunodot staining was performed using known quantities of purified PKC-α and γ proteins, as well as synthetic peptide sequences of PKC-α, βI, βI/βII, and γ. All epitopes effectively adhered to the NC film coated glass microscope slides. Double gelatinized glass slides did not significantly bind the proteins or peptides. In addition, using quantitative image analysis, antibody staining of brain sections with serial dilutions of all antibodies were compared on glass and NC film slides. The latter slides showed staining of PKC isoforms with densitometric anatomical resolution superior to that of gelatinized glass slides. Application of this immunohistochemical method where anatomic specificity is preserved will greatly augment the ability to quantify PKC-mediated changes implicated in neural plasticity.

Supported by grants AG08794 from the NIA and IBN-8912372 from NSF.

459.12

A SIMPLE METHOD TO IMPROVE THE RELIABILITY OF IONTOPHORETIC ADMINISTRATION OF TRACER SUBSTANCES. D. Yu* and F.J. Gordon. Dept. of Pharmacology, Emory Univ. School of Medicine, Atlanta, GA 30322.

While conducting experiments using iontophoretic application of wheat germ agglutinin conjugated to horseradish peroxidase (WGA-HRP) we encountered many instances where WGA-HRP was not deposited into brain tissue. Microscopic examination indicated that extracellular fluid, drawn into the pipette tip by capillary action, may have diluted the tracer and reduced the concentration of WGA-HRP available for iontophoretic deposition. Coating the lumen of the capillary tube with silicone to reduce its surface tension, reduced the incidence of failures from 40% to 0%. The lumen of glass capillary tubes (WPI Kwik-fil, 1.2 mm o.d.) was coated with silicone (Sigmacote) by slowly drawing the fluid into the tube and expelling it 3 times. After drying, pipettes were pulled and tips cut back to 20-30 μm o.d. Silicone treated and untreated pipettes were filled with 2% WGA-HRP (Vector or Sigma). In 10 rats, iontophoretic depositions (5 μA anodal current, 5 sec on/5 sec off for 10 min) were made using silicone coated and uncoated pipettes positioned in the basal ganglia. 24 hr later, HRP was developed with TMB-tungstate. For uncoated pipettes, injection size was highly variable and in 4 of 10 cases the injection site could not be detected. Using silicone-treated pipettes, injections were invariably larger, more consistent in size, no failures were observed and the number of retrogradely labelled cells in the substantia nigra was increased. Similar results were found with Fluorogold. These results indicate that coating the lumen of glass micropipettes with silicone can significantly improve the reliability of iontophoretic deposition.

Supported by grants NIH-HL36907 and Amer.Heart Assoc. to FJG.

459.13

SIMULTANEOUS ANTEROGRADE LABELING OF DIFFERENT AFFERENT PATHWAYS USING BIOTINYLATED DEXTRAN AMINE AND CHOLERA TOXIN SUBUNIT B. J.M. Alisky* and D.L. Tolbert. Dept. of Anat. and Neurobiol. and Surgery (Neurosurg.) St. Louis Univ., St. Louis MO 63104

To orthogradely label two cerebellar afferent pathways in the same animal, biotinylated dextran amine (BDA) and cholera toxin subunit B (CTb) were pressure injected into the brainstem or spinal cord of rats. Survival periods varied from 1 to 3 weeks. Sections of cerebellum were mounted onto slides and reacted first for BDA and then for CTb. BDA label was visualized as a black reaction product using cobalt-enhanced diaminobenzidine as the substrate for an avidin-biotinylated peroxidase reaction. CTb was visualized as a brown reaction product by using diaminobenzidine without cobalt in an immunoperoxidase reaction. Terminals labeled by these two tracers could always be differentiated based on their color under brightfield illumination. Cobalt-intensified BDA mossy fiber terminals were always dark black to purple, whereas CTb labeled terminals were always reddish-brown. Under darkfield illumination CTb labeled mossy fiber terminals appeared bright orange while BDA mossy fiber terminals were barely visible or not seen at all. BDA and CTb labeled mossy fiber terminals also differed in their degree of resolution of labeling terminal morphology. BDA labeled terminals were Golgi-like in appearance and preterminal axons were usually labeled. CTb labeled mossy fiber terminals had a granular appearance and preterminal axons were generally not labeled. Controls showed that the sensitivity of BDA and CTb was unaffected by their combined use. BDA and CTb should have a wide range of applications for double anterograde labeling studies. (Supported by NIH grant NS20227)

459.14

EMBRYONIC TRACT TRACING WITH 3 K DEXTRAN AMINES. B. Fritzsch*, D.H. Nichols and M. A. Christensen, Creighton Univ., Dept. Biomed. Sci., Omaha, NE 68104.

Higher molecular weight dextran amines have been used successfully in studies of neuronal morphogenesis. The use of smaller 3K dextran amines gives excellent results where long survival times are a problem due to their rapid rate of spread; up to 2 mm/hr. at room temperature. 3 K Dextran amines spread bi-directionally over identical distances whether or not microtubules have been disrupted using colchicine or nocodazole. Diffusion thus appears the most likely mechanism of spread; at least over short distances. 3 K Dextran amines diffuse little in the extracellular space thus allowing detailed examination near injection sites as well as the specific analysis of outgrowing fibers after focal applications. Applications of a few seconds will fill cut or damaged neuronal processes enough to allow Golgi-like fillings at several millimeters distance. Dextran amines can be combined with other techniques such as immunolocalization or histochemistry to allow both detailed topographical analysis of gene expression and the morphogenesis of neurons potentially affected by those genes. Biotinylated dextrans can be used to obtain permanent records for the detailed analysis of whole mounted embryonic brains.

Supported by the NIH.

459.15

CULTURED NEURONAL CELLS FLUORESCE WHEN INCUBATED WITH 2-ME-HARMALINIUM OR DIHYDROISOQUINOLINIUM COMPOUNDS.
E.J. Neafsey* and M.A. Collins. Departments of Cell Biology, Neurobiology & Anatomy and Molecular and Cellular Biochemistry, Loyola University Medical Center, Maywood, IL 60153.

Primary cultures of fetal (E14) rat mesencephalon were prepared using trituration and enzymatic digestion. After washing and resuspension, a trypan blue viability count was done, and cells were then plated at a density of 7.5×10^5 cells/well in Costar 6 well poly-D-lysine coated plates in DMEM F12 N2-supplemented (Bottenstein) medium. No serum was used. Fluoxydeoxyuridine was added as an anti-mitotic. Cultures were incubated at 37 degrees in 5% CO_2 for 7 days, and the medium was changed every other day. On day 7 either the beta-carboline 2-methyl-harmalinium iodide (2MeHL) or the 2-carboxymethyl-6,7-dhydroxy-3,4-dihydroisoquinolinium (DIQ) product of dopamine and glyoxylic acid was added to individual wells so that their final concentration was 200 μM (2MeHL) or 300 μM (DIQ); the two compounds were synthesized as part of our laboratory's investigation of various putative endogenous neurotoxins. After incubation for 30 minutes (and subsequent replacement of the brightly fluorescent DIQ medium with Hanks buffer), the cells were observed using both phase contrast and epi-fluorescence illumination (50 W Hg) on an Leica DMIL inverted fluorescence microscope using filter cube A2 (BP270-380, BP410-580) or D (BP355-425, LP460). Most cell bodies were fluorescent when viewed with either filter cube; some were very bright while others were barely visible. DIQ also caused many of the fine neurites to fluoresce brightly, suggesting it may be useful in visualizing detailed neuronal cell morphology in culture. (Supported by NIH grant NS 23891; thanks to Dr. Mary Druse-Manteuffel and Ms. Roberta Gillespie for providing the cell cultures.)

NEUROGLIA AND MYELIN V

460.1

TRANSFERRIN AND FERRITIN IMMUNOHISTOCHEMISTRY AND IRON STAINING IN NORMAL AND HYPOTRANSFERRINEMIC (HP) MICE.
T.K. Dickinson* and J.R. Connor. Dept of Neurosci & Anatomy, PSU Sch of Med, Hershey, PA 17033.

The importance of iron regulation in the brain is rapidly evolving as knowledge of the effects and extent of oxidative damage in the brain become better appreciated. In this study, the cellular distribution of transferrin(Tf;iron mobilization protein), ferritin(Frt;iron sequestration) and iron was determined in normal (adult CD1) and Hp mice. Normally, iron is found predominantly in oligodendrocytes. Tf is also found thoughout the mouse brain mainly in oligodendrocytes. Ferritin immunostaining in the mouse was found in predominantly microglial. Astrocytic immunostaining was also observed particularly in white matter tracts. Neuronal staining was observed in select subcortical nuclei most prominently in the nucleus accumbens. Similar to the iron staining, tanycytes also label for ferritin. The Hp mice were investigated because these animals have a mutation in the Tf gene. The resulting phenotype has <1% of the normal circulating plasma level of Tf. The animals require weekly i.p. injections of Tf for survival. The overall pattern of staining for iron, Tf and Frt in the Hp mouse brain was similar to normal animals, although the number of Tf-positive cells was greatly reduced. The normal cellular distribution of iron and the iron regulatory proteins in Hp mice suggests that the exogenously introduced Tf enters the brain from the plasma (carrying iron) and enters appropriate cell types. Alternatively, these data may indicate that endogenous brain Tf is not critical for iron transport to cells in the brain. These observations are critical to the understanding of iron transport within the brain.

460.2

EFFECT OF SODIUM BUTYRATE ON ACYLTRANSFERASE ACTIVITIES OF A CLONAL OLIGODENDROCYTE CELL LINE, CB-II.
S. H. Sun*, Y. W. Chen, K. C. Chen, and A. C. Chang, Neurosciences Institute, National Yang Ming Medical College, Taipei, Taiwan, R. O. C.

Sodium butyrate was supplemented in the culture medium of CB-II, a clonal oligodendrocyte cell line, for 48 hrs, a 40% increase in the [^{3}H]-arachidonic acid incorporation into phosphatidylcholine (PC) and a concomitant 75% decrease in phosphatidylethanolamine (PE) was observed. Only a slight, 2 %, increase in PC was observed when the cells were labeled with [^{14}C]-myristic acid. In this study we examine the effect of sodium butyrate on the activities of [^{3}H]-arachidonic acid-lysophospholipid acyltransferases directly by an *in vitro* assay system. In the absence of sodium butyrate and exogenous lysophospholipids, [^{3}H]-arachidonic acid incorporated into all major phospholipids in 10 min. PC was maximally labeled, and phosphatidylserine (PS) minimally labeled. Phosphatidylinositol (PI), phosphatidylethanolamine (PE) and plasmalogen phosphatidylethanolamine (PLPE) were also labeled effectively. Additional sodium butyrate in the incubation system significantly stimulated the activities of acyltransferases and an increase in the labelling of PC was observed. In the presence of exogenous lysophosphatidylcholine (LPC), lysophosphatidylinositol (LPI), and lysophosphatidylethanolamine (LPE), increased the radioactivities of PC, PI, and PE respectively. Additional sodium butyrate with exogenous LPC, LPI, and LPE, resulted in a further 70%, 60% and 160% increases in the labelling of PC PI, and PE respectively. We conclude that the mechanism for sodium butyrate to induce mophological transformation and alter membrane-phospholipid metabolism is partially due to stimulating the activities of acyltransferases in CB-II.

460.3

CARBACHOL INDUCTION OF c-fos mRNA IN OLIGODENDROCYTES IS MEDIATED BY PROTEIN KINASE C (PKC)
Almazan, G. * and Cohen, R. I.
McGill Univ. Dept. of Pharmacology, Montreal, PQ H3G 1Y6

We have previously reported that in oligodendrocytes carbachol stimulated phosphoinositide hydrolysis (mobilization of $[Ca^{2+}]_i$) and attenuated b-adrenergic stimulated cyclic AMP formation via M1 and M2 type muscarinic receptors, respectively. We postulated that stimulation of the M1 type muscarinic receptor, which results in the formation of DAG (and IP_3) can lead to the activation of PKC. Furthermore, Bhat has recently demonstrated that TPA, a potent activator of PKC, induces *c-fos* mRNA levels in oligodendrocytes (Bhat, N. R. *J. Neurosci. Res.* **32**, 340-349 1992). Therefore, in line with our previous hypothesis, we investigated the effect of carbachol on the expression of *c-fos* mRNA in oligodendrocytes.

Using Northern blot analysis we determined that carbachol (100 uM) caused a maximal *c-fos* induction 30-60' after addition. This was blocked by a 5 minute pretreatment with 10 uM atropine, the non-selective muscarinic antagonist. In addition, the induction was concentration dependent (10 - 1000 uM), and correlated well with our previous PI hydrolysis results. The induction with carbachol was also blocked with the PKC inhibitor H7 (30 uM). Our results suggest that neuronal cholinergic signals affect the expression of *c-fos* in oligodendrocyte cell culture through the activation of PKC. (Supported by the MRC of Canada).

460.4

DETECTION OF NOVEL RNA SEQUENCES HOMOLOGOUS TO THE PROTEOLIPID PROTEIN GENE.
N.L.Nadon* and E.U.Bagriacik. Biology Dept., University of Tulsa, Tulsa OK 74104.

The proteolipid proteins are the most abundant proteins in CNS myelin. There are currently two known products of the PLP gene produced through alternative splicing in exon 3. PLP, the predominant form in adult myelin has 276 amino acids, while DM20 lacks 35 internal amino acids but is otherwise identical to PLP. We have evidence for up to three new RNAs with homology to PLP. Preliminary experiments utilized Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) to amplify the region from exon 3 to exon 4 from the PLP gene transcripts, using very stringent annealing temperatures (65oC). In addition to bands corresponding to DM20 and PLP mRNAs, three extra bands were observed. These extra bands are observed in RT-PCR from mouse, rat and dog brain RNA. The new bands are not observed in E16 mouse brain, when DM20 but not PLP is expressed, but are detected by P1 when PLP has been turned on. Two of the RT-PCR derived cDNA fragments have been cloned, and sequence analysis will determine if they are new isoforms of PLP produced through alternative splicing or products of a gene closely related to the PLP gene. (Supported by grants from the Oklahoma Center for the Advancement of Science and Technology and the National Institutes of Health [N.L.N.])

460.5

MYELINATION BY CELLS FROM THE CG-4 GLIAL CELL LINE FOLLOWING TRANSPLANTATION INTO THE MYELIN DEFICIENT RAT SPINAL CORD. D.R. Archer*, U. Tontsch#, M. DuBois-Dalcq# and I.D. Duncan. School of Veterinary Medicine, University of Wisconsin, Madison, WI 53706 and # NINDS, NIH, Bethesda, MD 20892.

Cells from the bipotential rat glial cell line CG-4 are capable of differentiating into oligodendrocytes or type II astrocytes (Louis et al. J. Neurosci. Res. 1992 31 193). In this study we examined the ability of these bipotential cells to myelinate axons of the myelin deficient rat spinal cord *in vivo*. CG4 cells were kept proliferating with conditioned medium from the B104 neuroblastoma cell line. The CG4 cells were collected and concentrated to 25,000 cells/µl. At least 85% of the cells were A2B5 positive by immunolabelling, indicative of the O2-A phenotype. Two microliters of the cells were injected into the T13/L1 region of myelin deficient rats. Thirteen days later the rats were euthanized by perfusion fixation, the spinal cords removed and tissue at the transplantation site examined by light and electron microscopy. In each of 12 animals, cells with the appearance of normal oligodendrocytes formed patches of myelination in the dorsal columns of the transplant recipients. These results show that cells from a glial cell line are capable of myelination *in vivo* and that they represent a continued source of cells for experimentation and possible use in future therapies for de/dysmyelinating diseases. (Supported by NS23124 and The Myelin Project)

460.6

EFFICIENT GENE TRANSFER INTO RAT AND DOG OLIGODENDROCYTES IN CULTURE Z. Guo, S.S. Jiao†, L. Cheng§, N.S. Yang§, I.D. Duncan, J.A. Wolff†* Dept. of Medical Science, SVM; Dept. of Pediatrics†, Waisman Center, Univ. of Wisconsin; Agracetus, Inc.§, Madison, WI 53706

In order to determine the optimal method of foreign gene transfer into primary cultures of oligodendrocytes, particle bombardment (Accell Gene Gun), cationic liposome-mediated transfection (Lipofectin, BRL), calcium phosphate precipitation, and retroviral infection were tried. Post-natal rat and dog brain tissue were mechanically and enzymatically dissociated. Highly enriched oligodendrocytes (>95% pure) were obtained by sequential immuno-panning with the O1 antibody. The most efficient expression of the ß-galactosidase reporter gene was obtained with particle bombardment. Two days after bombardment with pCMVLacZ, 20% of the rat oligodendrocytes expressed ß-galactosidase. 10% of the rat cells were positive after lipofection with pCMVLacZ. Less than 0.1% ß-galactosidase-positive cells were observed after exposure of the rat cells to FGF (known to stimulate mitosis in these oligodendrocytes) and an amphotropic ß-galactosidase retroviral vector (LTR-ßGAL-SV40 prom.-NEO-LTR; titre > 10^6/ml). Approximately 2% of the rat cells were positive after exposure of the rat cells to calcium phosphate precipitates of pCMVLacZ for four hours, but many of the cells died. Using the luciferase reporter gene, the highest expression was observed also with particle bombardment. Relatively large amounts of luciferase activity (~ 30 X 10^6 total L.U.) was obtained after pCMVLux was bombarded into rat and dog oligodendrocytes. These levels were approximately 4- and 100-fold greater than that with the Lipofectin and calcium phosphate methods, respectively. Particle bombardment enabled similarly high levels of expression with hGH reporter gene. In conclusion, the ability to efficiently express foreign genes in primary cultures of oligodendrocytes will be useful for studies of oligodendrocyte function, promoter analysis and gene therapy. (Supported by NS23124)

460.7

REQUIREMENT OF POLYSIALIC-ACID FOR THE MIGRATION OF THE O-2A GLIAL PROGENITOR CELL IN VITRO. C. Wang, G. Rougon[1] and J. Z. Kiss*, Department of Morphology, University of Geneva Medical School, Geneva, Switzerland, [1]Laboratoire de Génétique et Physiologie du Développement, Marseille, France

While the capacity of O-2A oligodendrocyte progenitors to migrate in cell culture and during in vivo myelin formation is well documented, little is known about factors that regulate the motility of these cells. Here, we report on an in vitro model that allowed us to evaluate the contribution of α 2-8 linked polysialic acid (PSA) to O-2A cell motility. Using explant cultures of newborn rat neurohypophysis, we observed that individual glial fibrillary acidic protein (GFAP) positive cells rapidly disperse from the explants, and that cells of the O-2A lineage predominate in the migratory cell pool. A monoclonal antibody, which recognizes the α 2-8 linked PSA characteristic of the embryonic form of NCAM, revealed immunoreactivity on the surface of O-2A progenitor cells, whereas mature oligodendrocytes, type 2, type 1 astrocytes as well as flat GFAP negative cells were negative. Treatment of the explants with endoneuraminidase purified from phage K1, that specifically removes PSA from the surface of the cell, resulted in a complete blockade of the dispersion of O-2A lineage population from the explant. The effects of the enzymatic treatment were both selective and reversible: migration of GFAP negative flat cells or type 1 astrocytes that are PSA negative was not influenced, and upon removal of the enzyme, cells of the O-2A lineage were readily detectable in the migrating population. These results provide direct evidence that α 2-8 linked PSA contribute to the motility of O-2A, glial progenitor cells.

460.8

MYELIN BASIC PROTEIN MRNA APPEARS NOT TO TRANSLOCATE IN SHARK OLIGODENDROYCTES. Robert M. Gould*, NYS Inst. for Basic Res., Staten Island, N.Y. 10314

Sharks are the most primitive vertebrates that have a myelinated nervous system. Their sheaths, like those in mammals, contain two major proteins, myelin basic protein (MBP) and protein zero (P_0). CRNA probes, ^{35}S- and digoxygenin-labeled, localize mRNAs to MBP and P_0 to oligodendrocytes in sections of the spinal cords and medulla of adults and myelinating embryos. Both mRNAs were concentrated to the perinuclear regions of myelinating oligodendrocytes. This result is expected for P_0, since it requires posttranslational processing in perinuclear Golgi and because mRNAs for its mammalian counterpart, proteolipid protein is similarly localized to the perinuclear oligodendrocyte cytoplasm. In contrast, mammalian MBP mRNAs which are used in long oligodendrocyte processes, *in situ* hybridization studies have demonstrated a more diffuse distribution of its mRNA in tissue sections (Trapp et al, 1987). In the shark, MBP mRNA colocalizes with P_0 mRNA. These results indicate that MBP mRNA is not translocated to oligodendrocyte processes during shark CNS myelination and, hence, mRNA translocation is an event which appears at an evolutionary stage between sharks and mammals. (Supported by grant NS-13980).

460.9

DEVELOPMENTAL MODULATION OF GLUTAMATE TOXICITY IN OLIGODENDROGLIA IN CULTURE. A. Oka, J.W. Lin, P.A. Rosenberg and J.J.Volpe*, Children's Hospital and Harvard Medical School, Boston, MA 02115.

We have reported marked cytotoxicity of glutamate (GLU) to cultured rat oligodendroglia (OL) (J. Neurosci. 1993, 13:1441-1453). We now have addressed the developmental characteristics of this toxicity and the mechanisms of any such developmental vulnerability. We compared the effect of GLU on cells at day 3 after plating, consisting mainly of immature OL, with the effects on day 6 and day 9 cells, which are more mature (as judged by immunocytochemical criteria). EC_{50}'s for 24-hour exposures to GLU for day 3, 6, and 9 OL were 0.3 ± 0.1mM, 2.7 ± 1.6mM, and 7.0 ± 0.4mM respectively. We next evaluated the mechanisms for this striking developmental vulnerability. Because we have shown previously that GLU-induced toxicity is mediated by GLU uptake and subsequent glutathione (GSH) depletion, we measured the reduction rates of GSH at different stages of OL development. Maximal reduction rate of GSH was observed at day 3, when OL were maximally vulnerable to GLU toxicity. Moreover, buthionine sulfoximine, an inhibitor of GSH synthesis, resulted in toxicity only at day 3 but not at days 6 or 9, suggesting that GSH is the critical antioxidative mechanism in immature OL only and that other antioxidative mechanisms develop during maturation. This notion was supported further by showing that GLU leads to accumulation of H_2O_2, visualized by 2'7'-dichlorofluorescin diacetate, presumably because the GSH depletion inhibits the GSH peroxidase (GSH-Px) reaction. In order to define the requirement for GSH further, we studied the development of GSH-Px and catalase (CAT), both scavengers of H_2O_2. While GSH-Px activity, if anything, decreased over the first nine days in culture, CAT activity increased from 2µmole H_2O_2/mg protein/minute at day 3 to 18µmole H_2O_2/mg protein/minute at day 6. In addition, 3mM 3-amino-1,2,4-triazole (AT), which inhibited catalase activity by 87 ± 3%, enhanced GLU toxicity at day 9 (EC_{50} = 3.9 ± 0.5mM N=4). There was no effect of AT on GLU toxicity at day 3. These results suggest that the development of catalase activity may, in part, underlie the emergence of resistance to glutamate toxicity in OL in culture. (Supported by grants from the NIH and United Cerebral Palsy Foundation.)

460.10

MIGRATION AND DIFFERENTIATION OF TRANSPLANTED OLIGODENDROCYTE LINEAGE, VISUALIZED BY TRANSGENIC MARKER. V. L. Friedrich Jr.*, D.S. Rickman and R.A. Lazzarini. Brookdale Center for Molecular Biology, Mount Sinai Medical Center, New York, NY 10029.

Migration of transplanted oligodendrocytes or their progenitors into host CNS has been demonstrated in recipients experimentally or genetically dysmyelinated; however, the behavior of the oligodendrocyte lineage when transplanted into normal CNS remains largely unknown. To approach this problem, we have transplanted CNS bearing the MbetaP transgenic marker. This transgene, which encodes *E. coli* beta-galactosidase under control of a promoter/enhancer fragment from the gene for myelin basic protein, is expressed strongly and specifically in oligodendrocytes. Histochemical (X-gal), and immunostaining mark oligodendrocyte cell bodes and processes as well as myelin internodes.

Tissue pieces and dispersed cell suspensions transplanted from subependymal plate and olfactory bulb of perinatal MbetaP transgenic mice into brains of perinatal nontransgenic hosts yielded X-gal positive, myelin-bearing oligodendrocytes distributed over territories up to 4 mm across. Preparations placed in cerebral cortex yielded labeled cells in surrounding cortex and subcortical white matter but not in adjacent caudoputamen. Preparations placed in caudoputamen or thalamus myelinated deeper structures but yielded little myelination within subcortical white matter or cerebral cortex. These data suggest that migration of oligodendrocytes or their progenitors may be guided or restricted by mechanisms yet to be defined.

460.11

PROGRESSIVE DEMYELINATION IN THE TWITCHER SPINAL CORD. M.TANIIKE AND K.SUZUKI* Department of Pathology; University of North Carolina, Chapel Hill, NC27599-7525

The twitcher (tw/tw) is an authentic murine model of human metabolic demyelinating disease, globoid cell leukodystrophy. We investigated the time course and distribution of demyelinating lesions in the spinal cord with LFB-PAS stain or immunohistochemistry using antibodies against MBP, MAG (s- and l-) and CNPase. Paraffin sections of tw/tw and age-matched controls were examined at 5-45 PND. At 5 PND, only l-MAG and MBP were expressed in the ventral columns and the fasciculus cuneatus. At 15 PND, immunostaining was dense throughout the white matter except for the corticospinal tract, where cauda-rostral gradient of myelination was observed. The myelin markers were expressed in the following order: l-MAG, MBP, CNPase and then s-MAG. There were no differences in the pattern and density of immunostaining between tw/tw and controls up to 15 PND. Around 20 PND, PAS(+) macrophages and demyelinated patches were first recognized in the ventral column of tw/tw. Generally aggregation of PAS(+) cells were coincided with the demyelinated lesions. MBP(-) lesions were discrete while MAG(-) or CNPase(-) lesions were more diffuse. Demyelination appeared to start in the ventral column, become the most extensive in the lateral column after 25 PND, and seems to be more prominent in the cervical and thoracic level than in the lumbar level. The results of this study suggest 1)early myelination is normal in tw/tw; 2)demyelination progresses in an orderly pattern in tw/tw. (Supported in part by U.S.P.H.S Grants NS24453, HD-03110 and ES-01104.)

460.12

TRACER-COUPLING REVEALS THREE CLASSES OF OLIGODENDROCYTE IN RABBIT RETINA. E.C.G.M. Hampson, S.R. Robinson and D.I. Vaney*. Vision, Touch and Hearing Research Centre, The University of Queensland, Australia 4072.

Oligodendrocytes myelinate CNS axons and form gap junctions with astrocytes, suggesting.communicative and/or metabolic support roles. We examined the morphology and gap junction permeability of oligodendrocytes injected with the biotinylated tracers, biocytin (373 Da) and Neurobiotin (286 Da). Rabbit retinae were incubated for 20 min in Ames medium with the fluorescent dye Hoechst 38317, to label the morphologically distinct nuclei of astrocytes and oligodendrocytes. Individual cells near the edge of the medullary rays were injected (+0.5 nA, 1-2 min) with tracers under direct microscopic control. Oligodendrocytes were identified by their characteristic morphology (Butt & Ransom, *Glia* 2: 470-475, 1989) and the absence of immunoreactivity for anti-GFAP, which selectively labels astrocytes. We distinguished three morphological classes of oligodendrocyte."Standard" oligodendrocytes comprised 38% of the injected population and have processes which run parallel to axons in the nerve fibre layer (NFL). "Radial" oligodendrocytes (16%) have dendrites that radiate in all directions within the NFL; they terminate in spines and varicosities."Bilaminar" oligodendrocytes (46%) have dendrites extending parallel to axons in the NFL, and a second arbor of radial processes in the inner plexiform layer. Radial and bilaminar cells rarely tracer-coupled, whereas 50% of the standard cells were coupled to about 30 nearby oligodendrocytes and astrocytes. Thus, small molecules can pass from standard to radial and bilaminar oligodendrocytes, but not back the other way. These findings suggest that these morphologically distinct classes are also functionally distinct.
Supported by ARC & NHMRC.

460.13

ENSHEATHMENT AND EARLY MYELINATION OF RAT SPINAL AND CALLOSAL FIBERS. C. Bjartmar*, C. Hildebrand and K. Loinder. Dept of Cell Biology, Faculty of Health Sciences, S-581 85 Linköping, Sweden.

Ensheathment and initial myelination of prospective large spinal cord (SC) ventral funiculus axons and prospective small corpus callosum (CC) axons in rat, was examined by qualitative electron microscopy at different ages and serial section analysis of individual glial units at two specific developmental stages. The results show that ensheathment commences 19 days after conception in the SC and 12 days postnatally (P 12) in the CC. Multipolar SC and CC oligodendrocytes provide axons with uncompacted cytoplasmic sheaths through slender processes with various lengths. Other axons (not counted) were contacted but not ensheathed by oligodendroglial processes. The average number of axons ensheathed by each cell was 7 in the SC and 13 in the CC . The mean diameter of ensheathed axons was 0.72 µm in the SC and 0.35 µm in the CC. Myelin sheaths (4-8 lamellae) appear at birth and at P 17 in the SC and CC respectively. The mean number of myelinated axons per oligodendrocyte was 3 in the SC and 15 in the CC. The mean diameter of myelinated axons was 1.04 µm in the SC and 0.54 µm in the CC The average midnuclear-midaxonal distance increased 1.4 times in the SC between ensheatment and myelination, while it remained unchanged in the CC. Our results suggest that ensheathing spinal oligodendrocytes contact fewer axons than ensheathing cerebral cells do and that this difference increases with time. It seems that ensheathing spinal oligodendrocytes reduce the number of axonal contacts when they shift from cytoplasmic ensheathment to formation of compact myelin.

460.14

DISRUPTION OF FAST AXONAL TRANSPORT IN VIVO LEADS TO ALTERATIONS IN SCHWANN CELL GENE EXPRESSION. W. Wu, J.G. Toma, H. Chan, R.S. Smith, and F.D. Miller,*Dept. Anat & Cell Biol., University of Alberta.

Following nerve injury, Schwann cells distal to the site of injury downregulate genes associated with myelination. We hypothesized that at least some of these alterations were due to the loss of ongoing homeostatic signals that were transduced as a function of fast axonal transport. To directly address this hypothesis, we selectively blocked fast axonal transport in vivo by locally-cooling the sciatic nerve to 5-8° C (a cold block). Immunocytochemistry, light and electron microscopy show that macrophages do not invade the cold-blocked nerve, and that the nerve distal to the cold block shows no signs of Wallerian degeneration, with maintenance of normal axon and myelin profiles. Thus, any effects of the cold block treatment are not likely due to nerve injury, inflammatory responses, or loss of physical contact between axons and Schwann cells. To determine whether this treatment affected nonneuronal cells, we examined expression of the major myelin protein Po, and p75 NGF receptor, both of which are regulated as a function of Schwann cell:axon contact. Levels of p75 NGF receptor mRNA and protein were unaffected by the cold block. In contrast, levels of Po mRNA were decreased in the distal nerve in a fashion similar to that observed following axotomy. These data suggest that Po and p75 NGF receptor are regulated as a function of two different aspects of Schwann cell:axon communication, one of which involves maintenance of normal fast axonal transport. Furthermore, these data demonstrate that at least some of the axotomy-induced alterations in the peripheral nerve are not due to either macrophage infiltration or Wallerian degeneration, but to disruption of ongoing homeostatic mechanisms.

460.15

TRANSFORMING GROWTH FACTORS-ß1 AND -ß2 SLIGHTLY INHIBIT SCHWANN CELL PROLIFERATION IN NEURON/SCHWANN CELL CO-CULTURES. V. Guenard* and P.M. Wood. The Miami Project & Depart. Neurol. Surg., Univ. Miami School Med., Miami, FL.

Transforming growth factor-ß1 and -ß2 (TGF-ß1, -ß2) are mitogens for Schwann cells (SCs) in culture. However, SCs are always associated with neurons in vivo. In this study, the influence of TGF-ß1 and -ß2 on neuron-induced SC proliferation and myelination was evaluated. Cultures of purified embryonic rat dorsal root ganglion (DRG) neurons were seeded with sciatic nerve-derived SCs. To assess proliferation, serum-free medium supplemented with TGF-ß1 or -ß2 (0-10.0 ng/ml) were tested on SC, DRG/SC or CCL-64 mink lung (Mv1Lu) cells. After 48 hours, the cultures were exposed to [^{3}H]-thymidine (0.5 uCi/ml) and proliferation assessed by autoradiography. As previously observed, TGF-ß1 enhanced the proliferation of isolated SCs in a dose-dependent fashion. In contrast, both TGF-ß1 and -ß2 slightly inhibited SC proliferation on DRG neurites in a dose-dependent fashion. TGF-ß1 and -ß2 affected Mv1Lu cells as expected, by inhibiting their proliferation. The influence of both TGF-ß1 and -ß2 was partially inhibited by their respective specific antibodies (0.1-10.0 ug/ml). For the myelination assay, the cultures were maintained in serum-free feed containing laminin and TGF-ß1 or -ß2 (0-10.0 ng/ml). In a single experiment carried to date, neither TGF-ß1 nor -ß2 show a significant effect on myelination. This study demonstrates that TGF-ß1 and -ß2 are slight inhibitors of SC proliferation on DRG neurites and do not either promote or inhibit myelination. Our data suggest that the response of SCs to TGF-ß depends on whether neurons are present or not. Although TGF-ß does not seem to induce either SC proliferation or myelination, TGF-ß could function by partially controlling the arrest of SC proliferation, a necessary step for SC differentiation. TGF-ß could also stimulate SC division in response to injury when axonal contact is lost. Supported by NIH grant NS28059, NMSS grant RG 2210-A-2 and The Miami Project.

460.16

PURIFICATION OF P_0 AND ITS INTRACELLULAR COOH-TERMINAL DOMAIN. Y. Ding, S. L. Yates*, K. R. Brunden, Univ. Miss. Med. Ctr., Jackson, MS 39216 and Gliatech, Inc., Cleveland, OH 44122

P_0, the major protein of peripheral myelin, has been proposed to play a role in myelin membrane apposition. Little is known about the role of the cytoplasmic domain of P_0 in the formation of the myelin major dense line. We have developed procedures for the rapid purification of P_0 and subsequent isolation of a proteolytic fragment comprising the intracellular domain of this protein. Rat sciatic nerve endoneuria were homogenized in PBS and centrifuged at 160,000 x g. The resulting membrane pellet was homogenized in PBS containing 3% Triton X-100, and the detergent-solubilized proteins were applied to a cellulose phosphate column. The loosely bound proteins were eluted with PBS containing Triton X-100, and P_0 was subsequently removed with buffer containing Triton X-100 and 0.6 M NaCl. Since the intracellular portion of P_0 has only one tryptophan, cleavage at the carboxyl side of this residue with iodosobenzoic acid should give rise to a fragment containing the COOH-terminal 65 amino acids (C-fragment) of the 69 residue cytoplasmic domain. P_0 was cleaved with iodosobenzoic acid in 4 M guanidine-HCl/80% acetic acid at room temperature for 36 to 48 hour. The cleavage reaction was terminated by adding dithioerythritol, and the digest was dialyzed against 2 M NaCl. The dialysate was passed through SM-2 resin to remove residual detergent, and the detergent-depleted sample was dialyzed against PBS and subsequently applied to a DE 52 column. The C-fragment was specifically eluted from the column using a step NaCl gradient. The availability of a peptide comprising the intracellular domain of P_0 will expedite studies aimed at elucidating the role of P_0 in the formation of the myelin major dense line. (NS27587)

460.17

THE PROLIFERATION OF SCHWANN CELLS BY MBP-RELATED PEPTIDES IS ENHANCED BY FORSKOLIN AND b-FGF

S.F. Tzeng[1], G.E. Deibler[2], G.R. Leichnetz[3]*, and G.H. De Vries[1]. [1]Dept. of Biochem., MCV, Richmond, VA 23298-0614. [2]NIH, Building 36, Room 1A-05, Bethesda, MD 20892. [3]Dept. of Anat., MCV, Richmond, VA 23298-0709.

Macrophages treated with myelin enriched fraction (MEF) produced a conditioned media (MEF-Macro CM) which has been shown to be mitogenic for Schwann cells (SCs) (PNAS, 85, 1701, 1988). Myelin basic protein (MBP) incorporated into liposomes also stimulated SC proliferation in vitro (BBRC, 164, 883, 1989). MBP alone (3μM) was not mitogenic for quiescent SCs. Forskolin (1μM) or bFGF (1ng/ml) was a weak mitogen for SCs. Simultaneous addition of forskolin or b-FGF with MBP markedly increased the mitogenicity of MBP for SCs. In contrast to MEF, the mitogenic effect of MBP was not decreased by the addition of NH_4Cl indicating that the response of SCs to MBP did not require lysosomal processing. Forskolin also potentiated the mitogenic effect of MBP fragment 1-88 and 89-169. Relative to MBP 1-88, MBP 89-169 was a weaker SC mitogen and required a longer time to stimulate SC proliferation. Stimulation of SCs with MBP peptide 1-36 or 1-42 yielded a dose-dependent increase in SC proliferation. Peptide 37-88 or 43-88 was not mitogenic for SCs. Addition of MBP or Macro-MEF CM with varying concentrations of bFGF to SC demonstrated a marked increase in the mitogenicity. The increase in mitogenicity was maximal at 0.1ng/ml b-FGF. Therefore, we postulate that elevated intracellular cAMP in SCs can modulate the subsequent mitogenic response to MBP-related peptides. b-FGF was able to synergistically increase the mitogenic effect of MBP or Macro-MEF CM. One mitogenic region of MBP is contained in the initial 42 residues. b-FGF or forskolin may play a critical role in up-regulating the receptor for MBP. (Supported by NS15408)

460.18

SEROTONIN RECEPTORS ON CULTURED SCHWANN CELLS.

E. Yoder*, V. Lev-Ram[1], and M. H. Ellisman. San Diego Microscopy and Imaging Resource, Departments of Neurosciences and [1]Pharmacology, University of California, San Diego, La Jolla, CA, 92093-0608.

$[Ca^{2+}]_i$ transients were measured on primary cultures of rat Schwann cells loaded with the calcium indicator dye fluo-3 introduced via its AM ester. We have previously reported that some cells transiently increase their $[Ca^{2+}]_i$ in response to cholinergic agonists, and that these transients were antagonized by curare [Yoder *et al.*, NS Abst. pg. 1489, 1992]. We now report that a subpopulation of cells respond to pharmacological manipulation in manners consistent with the expression of 5-HT1C or 5-HT2 receptors. 5-HT elicited a dose-dependent transient increase in $[Ca^{2+}]_i$ in many cells. Transients were induced by exposure to concentrations of 5-HT as low as 10nM. A few of these responding cells began oscillating when exposed to a minimal dose of 1μM 5-HT. The 5-HT-induced transients, which occurred in the absence of $[Ca^{2+}]_o$ (with 0.5 mM EGTA), were blocked by ketanserin. The calcium source of transients occurring in 0 $[Ca^{2+}]_o$ is likely to be from IP_3-sensitive intracellular calcium stores, since thapsigargin, but not caffeine, increased $[Ca^{2+}]_i$ and prevented further 5-HT-induced $[Ca^{2+}]_i$ transients. Current studies attempt to correlate the cells' stage of differentiation with expression of the 5-HT-induced $[Ca^{2+}]_i$ transients.

460.19

SCHWANN CELLS IN CONTACT WITH NEURONS MAINTAIN AN ATP-MEDIATED CALCIUM RESPONSE.

S.A. Lyons[1], P. Morell[1]* and K.D. McCarthy[2] Biochemistry and Biophysics[1] and Pharmacology[2] Depts. University of North Carolina at Chapel Hill, NC 27599

Unique exchanges of information occur between Schwann cells (SC) and axons that affect the development, maintenance and reparation of the peripheral nervous system. We were interested in examining neurotransmitter-activated calcium signaling systems of SC during development. We demonstrated previously that 80-90% of freshly-isolated neonatal rat sciatic nerve SC responded to 10μM ATP with an increase in $[Ca^{2+}]_i$. The ATP-induced calcium response appeared to be mediated by purinergic P_{2Y} receptors. The ATP calcium response disappeared with time *in vitro* but could be recovered by growing SC in medium containing cyclic AMP analogs (i.e., dibutyryl-cAMP and 8Br-cAMP) and 1% fetal bovine serum. Immunostaining results indicated that these SC (S100β+) had differentiated toward the myelinating phenotype (P_0+, 192IgG (NGFR)-, O1+). These results suggested that cAMP analogs were necessary for the reappearance of SC sensitivity to ATP in culture and the development of SC stage-specific markers. Neuronal contact has also been reported to upregulate the same SC markers. Together, these findings led us to hypothesize that contact with neurons would upregulate SC calcium responsiveness to P_{2Y} purinergic stimulation. To test the hypothesis, SC were introduced into cultures of purified neurons prepared from rat E15 DRG. Results of these experiments indicated that SC recovered the ability to respond to ATP with increases in $[Ca^{2+}]_i$ when in direct contact with neurons. In addition, there was no ATP response in cocultured SC not in contact with neurites. SC were tested for ATP responses after 6d in the presence of neurons and again after 35d when myelin was present. These data suggested that contact with neurons (which may raise cAMP levels) was necessary for the expression of the ATP-mediated calcium response. Further studies will address the question of what population(s) of SC are responding to ATP (myelinating, non-myelinating or both). NS11615

460.20

PMP22 MODULATES GROWTH OF SCHWANN CELLS.

G. Zoidl, C. Schmalenbach, D. D'Urso and H.-W. Müller*; Molecular Neurobiology Lab., Dept. of Neurology, University of Düsseldorf, FRG.

The peripheral myelin protein PMP22 has recently been cloned. The expression pattern of PMP22 during development, nerve regeneration and in adult tissues follows that of known myelin proteins like Po and MBP. Mutations within the PMP22 coding region in mice (*trembler, tremblerJ*) or chromosomal rearrangements in man (*Charcot-Marie-Tooth Typ1a, HNPP*) affect myelination in PNS. Sequence homologies to the growth-arrest-specific gene gas3 suggest potential growth regulatory functions for PMP22. In order to study the function(s) of PMP22 in myelination and/or Schwann cell growth we established recombinant Schwann cells with altered PMP22 expression by retroviral mediated gene transfer. A set of vectors with partial PMP22 cDNA sequences in both sense or antisense orientation under control of an internal CMV promotor were constructed. Cell culture supernatants collected from high titer Psi2 producer cell lines were used to infect secondary Schwann cell cultures. Selected G418-resistant cells were characterized for either constitutive overexpression or depletion of PMP22 mRNA and protein expression. Schwann cell proliferation is significantly reduced when PMP22 expression is increased. Conversely when PMP22 is expressed at lower than normal levels the proliferation rate of the recombinant Schwann cells is slightly increased. Our results show that recombinant Schwann cell lines with the described alteration in PMP22 expression are a useful tool to elucidate the mechanisms by which PMP22 can modulate cell growth. Using the same cells in an in vitro myelinating cell culture system will allow us to investigate the role of PMP22 in myelination of peripheral axons.(Supported by BMFT and F. Thyssen-Stiftung)

GENE STRUCTURE AND FUNCTION IV

461.1

CLONING AND EXPRESSION OF BASIC HELIX-LOOP-HELIX PROTEINS FROM THE MAMMALIAN CENTRAL NERVOUS SYSTEM

A. Bartholomä, A. Pühlhofer, M. Rossner, M. Schwab, M. Klugmann, and K.-A. Nave* *Center for Molecular Biology (ZMBH), University of Heidelberg, D-6900 Heidelberg, Germany.*

Transcription factors, structurally related by the helix-loop-helix (HLH) domain and a basic DNA binding site, determine cell lineage and differentiation of drosophila proneural stem cells. The finding of mammalian homologues for the drosophila genes *achaete-scute, hairy,* and *enhancer-of-split* suggests that bHLH proteins are also involved in vertebrate neural determination. To identify bHLH proteins regulating the later stages of neuronal and glial development in mammals, we have used highly degenerate primers to PCR amplify novel bHLH sequences from adult brain cDNA. One such clone encodes NEX, a brain-specific bHLH protein that is highly abundant in adult hippocampal pyramidal neurons and throughout the neocortex. In the embryo, NEX expression is restricted to postmitotic neurons which have migrated out of the ventricular zone and form the cortical plate. NEX expression peaks at the time of maximal synaptogenesis in the first postnatal week. Another novel protein identified in this screen is encoded by a mRNA that is most abundant in the CNS and gradually increases throughout brain development, peaking in the adult. A shorter isoform of its transcript is specifically expressed in glial cells of the O-2A lineage. Finally, we screened a λgt11 expression library to clone the bHLH protein that binds to the single E-box target sequence footprinted in the promoter of the PLP gene. Sequence analysis suggests that it may be the rat homologue of TFE3, a bHLH-leucin zipper protein also expressed in cells of the immune system. (Supported by the BMFT #0316000A)

461.2

KINESIN mRNA IS PRESENT IN THE SQUID GIANT AXON.

A.E. Gioio*, J.T. Chun, J. Panza, M. Crispino, C. Perrone Capano, A. Giuditta and B.B. Kaplan. Dept. Psychiatry, Univ. Pittsburgh School of Medicine, Pittsburgh, PA 15213 and Dept. Gen. Physiology, Univ. Naples, Naples, Italy.

Previously, we reported that the squid giant axon contains a heterogeneous population of mRNAs and polyribosomes (Giuditta et al. 1991 J. Neurosci. Res. 28:18). Two of these mRNAs have been characterized and encode β-actin and β-tubulin (Kaplan et al. 1992 Mol. Cell. Neurosci. 3:133). To further characterize the axonal mRNA population, an axonal cDNA library was screened using a squid kinesin clone as a probe. The sequence analysis of several positive clones manifested >98% sequence identity to squid heavy chain kinesin (Kosik et al. 1990 J. Biol. Chem. 256:3278). Results of in situ hybridization histochemistry using [^{35}S]labeled riboprobes established the axonal location of kinesin mRNA. To explore the biological significance of this finding, polysomes were isolated from the giant axon, and kinesin sequences amplified from the RNA using PCR methodology. The results of this experiment clearly demonstrate the presence of kinesin RNA sequences in the polyribosome fraction. Taken together, these findings demonstrate that kinesin mRNA is present in the squid giant axon, and raises the possibility that key elements of the axonal transport system are locally synthesized in the squid giant axon.

461.3

HIPPOCAMPAL EXPRESSION AND ALTERNATIVE SPLICING OF HZF-2, A NOVEL MEMBER OF THE THYROID HORMONE RECEPTOR FAMILY. G.A. Jamieson Jr. and S. Peña de Ortiz* Toxicology Program, Department of Environmental Health, University of Cincinnati College of Medicine, Cincinnati, Ohio 45267-0056.

Signal-transcription coupling is hypothesized to be a major mechanism governing long-term hippocampal responses to stimulation or injury. In the current study, our goal was to isolate rat hippocampal cDNAs which encode novel members of the superfamily of ligand-dependent transcription factors. Our cloning strategy took advantage of the high degree of homology which exists between the zinc finger regions of these nuclear receptors. This approach resulted in the isolation of twelve cDNA clones encoding the DNA binding domain of nuclear hormone receptors, including two novel isolates, HZF-2 and HZF-3. Full length HZF-2 and HZF-3 cDNA clones were isolated from a rat hippocampal library. Sequence analysis revealed HZF-2 to be highly homologous to EAR-1, an ERB-A related orphan receptor. The DNA binding domain of HZF-2 is 90% homologous to that of EAR-1. Reverse transcription-PCR (RT-PCR) analysis was used to study the brain distribution of HZF-2 and determined HZF-2 to be preferentially expressed in the rat hippocampus (hippocampus >> cerebellum >>> cortex). Interestingly, we obtained several clones for HZF-2, varying in size from 2.0 to 1.0 kb, suggesting this gene may be alternatively spliced. Northern blot analyses support this hypothesis. RT-PCR is being used to further examine the expression of multiple HZF-2 isoforms in the brain. *In situ* hybridization analysis will compliment these studies and enhance our understanding of the spatial distribution of HZF-2 isoforms. These experiments will assist our assignment of potential functional roles to HZF-2 mediated regulation of gene expression in the hippocampus. *Supported by ONR N00014-90-J-1898, NIMH Minority Fellowship, Ford Foundation Minority Dissertation Fellowship.*

461.4

SOMATIC DNA CHANGES IN IMMORTALIZED NEURAL CELL LINES. A.L. Barth[1], M.E. Lim[1], B.H. Morimoto[1]*, F.W. Hopf[1], E.M. Eves[2], M.R. Rosner[2], B.H. Wainer[2], and H. Sakano[1]. [1]Dept. Mol. and Cell Biol., Univ. of Calif. - Berkeley, Berkeley, CA 94720 and [2]Dept. Pharm.-Phys., Univ.of Chicago, Chicago, IL 60637.

Transgenic mice were made carrying a recombination reporter gene consisting of recombination signal sequences from immunoglobulin genes flanking lacZ, where inversion of the transgene through recombination can activate lacZ transcription. LacZ expression was detected in lymphoid tissues as well as discrete regions of the brain, and DNA rearrangement was verified by PCR amplification of the recombination breakpoints. Stable transfection of immortalized CNS cell lines with the same recombination substrate showed activation of the reporter gene and the presence of PCR-amplifiable recombination breakpoints. A transfected glial line showed a significantly lower percentage of β-gal positive cells. In both the transgenic and tissue culture systems, the neural-specific recombination event appears different from V(D)J-type recombination in its site-specificity. In addition, RAG-1 expression in at least one transfected neural line showing reporter gene activation was undetectable by northern and RT-PCR, suggesting that RAG-1 is not required for this type of DNA rearrangement. Detection of a recombination activity in neural cell lines allows their use as a tool to analyze the specificity and regulation of this reaction.

461.5

MOLECULAR CHARACTERIZATION OF NeuN, A NOVEL NEURONAL NUCLEAR PROTEIN. D.L. Somers, M.J. Ghosh and C.R. Buck*, Dept. of Cell Biology and Anatomy, Medical Univ. of SC, Charleston SC 29425.

NeuN is a nuclear protein, identified with monoclonal antibody (MAb) A60, that is specifically expressed by vertebrate neurons. The expression of the protein is developmentally regulated, appearing in most mouse neurons immediately following withdrawal from the cell cycle and maintained thereafter. Biochemical analysis of NeuN indicates that it is a soluble nuclear phosphoprotein capable of binding to DNA in a specific manner that is similar to that of known transcription factors. In order to obtain a full length cDNA clone which will allow direct assessment of the functional role of this protein, MAb A60 was used to isolate a cDNA clone from a gt11 expression library. This initial cDNA (1Kb) has been sequenced, confirmed with independent clones from different libraries, and represents about 2/3 of a 1.6Kb mRNA identified by RNA blot analysis. An open reading frame has been identified, but the 5' end of the NeuN cDNA has been refractory to characterization. Genomic Southern blot analysis indicates that this protein is encoded by a single copy gene. Analysis of a mouse NeuN genomic clone and overlapping but divergent cDNA clones indicates that the epitope-encoding sequence may be but one of multiple 5' splicing alternatives. One splice alternative contains two exons not present in our original cDNA. Alternative splicing of NeuN is consistent with immunoblot analysis of mouse brain nuclear proteins indicating three immunoreactive species of 46 to 48Kd, and with primer extension studies suggesting multiple 5' ends for this mRNA. To confirm the authenticity of the putative NeuN cDNA sequence to date, *in situ* hybridization was performed on adult rat brain tissue sections using an antisense oligonucleotide probe that will hybridize to at least two splice alternatives. Film autoradiograms revealed hybridization of this probe over most of the same brain regions identified immunohistochemically with MAb A60. Comparison of the sequence for both splicing alternatives to the sequence databases revealed no significant homologies, supporting the suggestion that NeuN is a novel neuronal nuclear protein.
Supported by NIH NS31011 and MUSC 22040CR25.

461.6

CHARACTERIZATION OF HUMAN BRAIN SEPIAPTERIN REDUCTASE (SR) cDNA. R.A. Levine*[1,2,4], P.Z. Anastasiadis[1], S. K. Demetriou[1], J.C. and States[3*]. [1]William T. Gossett Neurology Labs of Henry Ford Hospital, [2]Cellular and Clinical Neurobiology Pgm, Dept. of Psychiatry, Wayne State University (WSU), [3]Center for Molecular Biology, WSU, Detroit, MI, and [4]Veterans Administration, Allen Park, MI.

Sepiapterin reductase (SR) catalyzes the final reaction in the synthesis of tetrahydrobiopterin (BH_4), the cofactor for tyrosine and tryptophan hydroxylases in dopamine and serotonin synthesis. Cloning of human SR gene will allow the study of altered genetic expression of BH_4 biosynthetic enzymes in neuropsychiatric illnesses caused by altered biogenic amine metabolism. We have recently cloned the cDNA encoding human brain SR (1630 bp) and compared this with the rat liver (1157 bp) and human liver (833 bp) cDNAs. The open reading frames of human brain and liver cDNAs are identical. Human brain SR cDNA contains 260 bp of 5' upstream untranslated region (compared with 22 bp in human liver) and 569 bp of 3' downstream untranslated region (compared with 25 bp in human liver). There are several potential regulatory elements in 5' untranslated region of the human brain cDNA; there is a 78% nucleotide homology and 68% amino acid homology with rat liver SR cDNA. Northern blot analysis of rat and human liver RNA demonstrated that there was weak cross-hybridization of rat and human SR cDNA with mRNA from the opposite species. Human brain SR mRNA was slightly larger than rat SR mRNA. Current studies are focused on comparing human brain and liver mRNA, measuring SR mRNA in neurological diseased postmortem human tissue, and isolating the human SR gene.

461.7

MOLECULAR CLONING OF AN ORPHAN RECEPTOR cDNA FROM OVINE PITUITARY GLAND. M. I. Masana*, R.C. Brown, M.E. Gurney and M.L. Dubocovich. Depts. Pharmacol. and Cell.Mol.Biol., Northwestern Univ. Med. Sch. Chicago, IL 60611

Using degenerate primers designed for the polymerase chain reaction (PCR) based on homologies in the II and VII transmembrane domains between the members of the G-protein receptor (GPR) gene family (Cell 65:175, 1991), we successfully cloned a novel cDNA receptor sequence from the ovine pituitary gland. Template cDNA was obtained by reverse transcription of mRNA from the pituitary stalk (PS) in a block of tissue containing also portions of median eminence and pituitary pars nervosa / pars distalis. The cDNA clone (S856) has a single open reading frame which when translated has a pattern of repeating hydrophobic domain that resemble the membrane spanning domains of GPR sequences and is 56 % homologous over its length to another orphan sequence from human endothelial cells named endothelial differentiation gene: edg-1 (J.Biol.Chem. 265:9308, 1990). Using a random primed ^{32}P-DNA probe of S856 we screened a size-selected cDNA ovine pars tuberalis library prepared in the plasmid expression vector pcDNA I and cloned a sequence of 2500 bp that contains the S856 sequence (edg-2). By Northern analysis of poly(A)+mRNA we found that a transcript of 1.7 kb was present in the ovine PS, pituitary pars nervosa / distalis, retina and cerebral blood vessels. In addition, cDNAs from these tissues and hypothalamus were amplified by PCR using internal oligonucleotide primers and a product of the expected size (415 bp) was observed. Edg1 and edg-2 appears to be distinct genes which may encode protein products that bind the same ligand. Supported by NIH grants: MH42922 (MLD), T32-NS07140 (RCB) and a Glaxo grant

461.8

Cloning and molecular characterization of the mouse astrocyte glycogen synthase

G. Pellegri, C. Rossier, S. de Rham, P.J. Magistretti and J.-L Martin*. Institut de Physiologie, Université de Lausanne, Switzerland

In the brain, glycogen is predominantly localized in astrocytes, although its presence has been detected in certain large neurons, in ependymal and choroid plexus cells. Glycogen is synthesized by the enzyme glycogen synthase (GlyS) from UDP-glucose. Out of the different isozymes, only the muscle and the liver forms of GlyS have been cloned. We have isolated and characterized from a mouse astrocyte λZapII cDNA library, a cDNA clone encoding the mouse cerebral cortical astrocyte GlyS. The 3.5 kb clone isolated contains an open reading frame of 2214 bp encoding a protein of 738 amino acids. The predicted molecular weight of the astrocyte GlyS (83.6 kDa) corresponds to that of the enzyme purified by HPLC from canine brain (88 kDa) (Inoue et al., 1988). The coding region of the mouse astrocyte GlyS cDNA shares 87% and 86% identity with the nucleotide sequences of the human muscle and the rabbit muscle GlyS cDNAs respectively. At the amino acid level the identity is even more striking with 95.5 % and 96.6 %. GlyS activity is regulated by phosphorylation. Out of the nine phosphorylation sites identified *in vivo* for the rabbit muscle GlyS, all are conserved in the mouse astrocyte GlyS.The cellular distribution of the GlyS mRNA studied by Northern blotting shows the presence of a single transcript of 4 kb both in astrocytes and neurons.
We are currently examining by *in situ* hybridization the regional distribution of the astrocyte GlyS mRNA in the mouse brain.
Inoue, N. et al. (1988) J.Neurochem. 50 (2), 400-405.

461.9

CLONING AND EXPRESSION OF RAT ASTROGLIAL INDUCIBLE NITRIC OXIDE SYNTHASE E.Galea*, D.J. Reis, and D.L. Feinstein, Division of Neurobiol., Dept of Neurol. and Neurosci., Cornell University Medical College, New York, NY 10021.

Rat cortical astrocytes in primary cultures express a nitric oxide synthase (aNOS) that is reminiscent of the macrophage enzyme in its biochemical characteristics and inducibility by cytokines and bacterial endotoxin (LPS).

To determine the sequence of the aNOS mRNA, a cDNA library was prepared from astrocytes exposed to LPS for 5 hours, and one million plaques were screened at low stringency (42 °C, 2xSSC) with a fragment of the mouse macrophage NOS cDNA that spanned the two NADPH binding sites. The longest clone isolated and sequenced contained a 2.0 kb-insert corresponding to the carboxyl terminus of the NOS protein, as well as the entire 3' untranslated region. Additional DNA sequence was obtained with polymerase chain reaction (PCR) amplification of upstream regions using degenerate primers directed against highly conserved areas amongst all NOS proteins. The combined DNA sequences reveal that at the coding region the rat aNOS mRNA is 92% identical to the NOS mRNA expressed in mouse macrophages, while the identity between untranslated regions is 81%. The deduced aminoacid sequence shows 93% identity between the NOS's from rat astrocytes and mouse macrophages, 70% of the substitutions being conservative.

A quantitative PCR assay has been developed to evaluate expression of aNOS mRNA *in vivo* and *in vitro*, using primers that flank a 400-bp segment at the end of the translated region. A PCR product of the expected size was detected in cultured astrocytes and in the rat C6 glioma cell line after a 4-hour exposure to LPS and LPS and interferon-γ, respectively, while no product was obtained in untreated cells. This assay is being used to analyze the regulation of aNOS mRNA expression by cytokines and neurotransmitters in brain samples.

461.10

SUBTRACTION CLONING OF CEREBELLAR GENES. Vinodh Narayanan, M.D.*, Departments of Pediatrics and Neurology, The University of Pittsburgh, Pittsburgh, PA.

The granule cells of the cerebellum arise from the external germinal layer (EGL), a structure that persists into the post-natal period. Cells from the pre-migratory zone of the EGL migrate along the surface of Bergmann glial processes to the internal granule cell layer. We have undertaken a molecular biologic approach to isolate genes expressed in differentiated granule cells, and those activated during granule cell differentiation and migration.

Following Altman, et al, we used focal, low-dose X-irradiation of newborn rat pups to ablate the granule cells. Within these agranular cerebella, the Purkinje cells occupied several layers and were mis-oriented. We then extracted mRNA from normal P12 rats (CTRL) and irradiated P12 rats (RADS), constructed directional cDNA libraries in λZAP II (Stratagene Inc.), and used these to generate a subtracted cDNA library (CTRL minus RADS) We have screened this library for clones absent from the RADS mRNA, and have identified differentially expressed clones, by Northern blot analysis. The spatial distribution of the corresponding mRNAs are being analyzed by in situ hybridization. This approach will allow us to study gene expression during neuronal differentiation.

461.11

Characterization of M6 and Identification of a PLP/DM2 gene family

Y. Yan*, C. Lagenaur, and V. Narayanan, Departments of Neurobiology and Pediatrics, University of Pittsburgh, Pittsburgh, PA15261

M6 is a membrane glycoprotein which is expressed on central neurons and certain polarized epithelia from early developmental stage. Antibodies against M6 interfere with cerebellar neurite outgrowth in vitro. Two closely related cDNAs, M6a and M6b, were obtained by expression cloning, both of which showed high homology to the major CNS myelin protein PLP/DM20. Although M6 clones and PLP/DM20 share many molecular characteristics, in situ hybridization revealed nonoverlapping distributions of their mRNAs in mouse CNS, in agreement with previous results that M6 is expressed on neurons in CNS while PLP/DM20 is present on oligodentdrocytes.

Preliminary functional studies of M6 protein showed that M6 antibody affected cerebellar neurite outgrowth when the cells were grown on polylysine and laminin but had less effect on those grown on L1. Antisense oligodeoxynucleotide, supposedly inhibits M6 expression, significantly decreased neuron survival in culture.

The identification of a gene family including neuronal specific M6 and glial specific PLP/DM20 in CNS and the importance of M6 for cell survival revealed by cell culture studies suggest a broader functional role for these molecules than myelination.

461.12

TRANSGENIC ANALYSIS OF THE OMP PROMOTER. E. Walters, M. Grillo*, K. Kudrycki, C. Bocchiaro, A. Phillips, F.L. Margolis. Roche Institute of Molecular Biology, Nutley, N.J., 07110, USA.

This laboratory previously reported the presence of putative novel transcriptional regulatory sequences in the 5' flanking region of the olfactory marker protein (OMP) gene. We proposed that an olfactory-specific trans-acting factor, designated Olf-1 (Kudrycki et al., Molec. Cell. Biol., May, 1993), binds to two upstream sites of the OMP gene. The role of the Olf-1 binding sites in olfactory-specific gene expression was analyzed by studying the expression of E.coli Lac-Z placed under the control of differently truncated regions of the rat OMP promoter. Lac-Z expression in olfactory tissue of transgenic mice was analyzed biochemically and histochemically. Immuno-enzymatic assays using CPRG as a substrate for β-galactosidase generally revealed substantial activity in olfactory tissue from mice carrying a construct with 0.3 kb upstream. In contrast, less activity was measured in olfactory tissue of mice carrying a larger construct (0.8 kb upstream). X-gal staining of olfactory tissue reflected the results of the biochemical assays. X-gal-positive olfactory neurons of mice carrying either construct were apically localized throughout the neuroepithelium. In one line carrying the shorter construct, intensely stained olfactory neurons occurred as clusters primarily in the caudal region of the epithelium. These neurons spanned the entire thickness of the neuroepithelium and correlated with robust staining of some glomeruli in the bulb. Lac-Z expression observed in some regions of the CNS suggests that additional sequences in the OMP gene participate in regulating olfactory neuron-specific gene expression. Overall, these data reveal that as little as 0.3 kb upstream of the OMP translational start site (harboring a single Olf-1 binding motif) is sufficient for expression of OMP and imply the presence of silencer elements in this gene.

461.13

HUMAN OMP GENE: CLONING AND *IN SITU* LOCALIZATION.

O. Buiakova[1], N.S. Rama Krishna[2], H. Baumeister[1], M.L. Getchell[3], T.V. Getchell[2,3], and F.L. Margolis*[1], [1]Roche Inst. of Molecular Biol., Nutley, NJ 07110; [2]Dept. of Physiol. & Biophys., [3]Div. Otolaryngol. Dept. of Surgery, Univ. of Kentucky College of Medicine, Lexington, KY 40503.

We have cloned the genes for olfactory marker protein (OMP) from human, mouse and rat to study conserved sequences that may be associated with gene regulation and the encoding of functional protein domains. OMP is expressed primarily in mature olfactory receptor neurons. The coding regions from the three species were highly conserved. The 162 amino acid OMP protein differs by 3 amino acids in rat vs. mouse and by 17 amino acids in rat vs. human. Almost all the substitutions are conserved. Nucleotide identity was very high in the 5' untranslated region of rat and mouse genes. In human, the first 400 bp upstream to the ATG codon had 75% identity to the rat and mouse, whereas further upstream, the identity was 50%. The 5'-upstream promoter regions of the OMP genes had highly conserved Olf-1 binding motifs (Kudrycki et al., Mol. Cell. Biol., May, 1993). Olf-1 may regulate OMP expression. Another potential regulatory motif, UBE, was identified in rat, mouse and human OMP genes. Olf-1-binding sites and UBEs from the human and mouse genes bound proteins from rat olfactory epithelium, and were competed by the corresponding rat sequences. Initial data indicates UBE contains NF-1 binding activity. Expression was studied in human olfactory epithelium with *in situ* hybridization using human OMP cRNA probes. Hybridization signal was localized specifically in olfactory receptor neurons, but not in other cell types in olfactory or respiratory mucosae. *In situ* localization of OMP mRNA was congruent with the immunocytochemical localization of the protein in human olfactory receptor neurons. Supported by NIH grants DC-00159 (TVG) and DC-001715 (MLG).

461.14

CLONING AND CHARACTERIZATION OF A NOVEL SYNAPTOTAGMIN-RELATED GENE.

B. S. Hilbush* and J. I. Morgan. Dept. of Neurosciences, Roche Institute of Molecular Biology, Roche Research Center, Nutley, NJ 07110.

The discovery of several families of proteins thought to be involved in synaptic vesicle exocytosis has provided the first step in elucidating the molecular events underlying neurotransmitter release. One important family of these molecules is the synaptotagmins, synaptic vesicle proteins which possess properties of a Ca^{2+} sensor and may serve as key regulatory elements in vesicle fusion. Biochemical and molecular genetic approaches were used previously to identify two family members, synaptotagmin I and II. We used a PCR-based strategy to isolate additional family members from the mouse central nervous system. Using this approach, we have cloned a novel synaptotagmin-related gene that contains significant homology to both synaptotagmins. The homology amongst all of these members is greatest in the internal repeats of the C2 domain, a region found in the Ca^{2+}/phospholipid regulatory region of protein kinase C. Northern blot analysis with a probe specific for the new member reveals a single 4.5 kb transcript whose expression is restricted to neural tissues. The identification of a third member of the synaptotagmin family suggests that multiple Ca^{2+} sensitive components of the fusion machinery may be required for regulated exocytosis.

461.15

ISOLATION AND CHARACTERIZATION OF NEUROTRANSMITTER TRANSPORTER-HOMOLOGOUS cDNA CLONES IN *DROSOPHILA*.

W. S. Neckameyer*. Dept. of Pharmacological & Physiological Science, St. Louis University School of Medicine, St. Louis MO 63104.

A *Drosophila melanogaster* adult head cDNA library was screened with an oligomer whose sequence was derived from a highly conserved region of the norepinephrine and γ-aminobutyric acid transporters, and which was used to isolate a dopamine transporter in rat (Shimada *et al.*, Science 254: 576-578, 1991).

Of the three clones initially isolated, a partial sequence of one cDNA clone was found to span 50 amino acid residues encompassing the proposed first and second transmembrane domains. This sequence was remarkably well conserved in *Drosophila*, and therefore this cDNA clone was used in a second screen of the same library. Several cDNA clones have now been isolated, which fall into two major classes by Southern analysis of *Drosophila* genomic DNA. A detailed characterization of these cDNAs encoding putative neurotransmitter transporters will be presented.

461.16

ALTERNATIVE SPLICING OF A HUMAN AMYLOID PRECURSOR PROTEIN MINI-GENE CONSTRUCT IN MOUSE P19 EMBRYONAL CARCINOMA CELLS.

D. Willoughby*, S. A. Johnson, and C. E. Finch. Andrus Gerontology Center and Department of Biological Sciences, University of Southern California, Los Angeles, CA 90089

ß-amyloid is a 42-43 amino acid peptide which is deposited extracellularly in the brains of Alzheimer's disease patients and to a lesser extent in the brains of normal elderly persons. ß-amyloid is a proteolytic fragment of the carboxy-terminal region of the amyloid precursor protein (APP). Unprocessed APP contains 695, 751, or 770 amino acids. These alternative forms of APP are generated by variable inclusion of exon 7 and exon 8 in the mRNA. Exon 7 encodes a 57 amino acid Kunitz protease inhibitor domain (KPI), while exon 8 encodes a 19 amino acid motif with similarity to OX2, a neuron and thymocyte cell surface marker. We and others have found that the ratio of KPI-containing/KPI-lacking-mRNAs is increased in the cortex and hippocampus of Alzheimer's disease patients.

Several groups have shown that APP-695-mRNA, which lacks the KPI exon, is selectively up-regulated during retinoic acid-induced neuronal differentiation of P19 cells. We transfected differentiated P19 cells with a human APP mini-gene splicing construct. The KPI exon was spliced out of most construct-derived-mRNAs in these cells. In contrast, the KPI exon was spliced into most construct derived mRNAs in rat C6 glioma and human U87 astrocytoma. Similarly, in mouse Neuro-2A neuroblastoma and human SK-N-SH neuroblastoma the KPI-exon was contained in most mRNAs derived from the splicing construct. The differences in splicing between P19 cells and other cell lines will be exploited in order to identify cis-acting RNA sequence elements involved in cell type specific alternative processing of the APP pre-mRNA. DW was supported by TG-AG00037-13; this work was supported by AG7909 to C. E. F.

461.17

DIFFERENTIAL RNA SPLICING OF CR16, A NOVEL GENE EXPRESSED IN HIPPOCAMPAL NEURONS.

J.N. Masters*, W.H. Ashman and S.L. Cotman. Department of Cell Biology, Neurobiology and Anatomy and the Ohio State Biotechnology Center, The Ohio State Univ., Columbus, OH 43210

CR16 is a novel gene expressed in subsets of neurons in the brain including hippocampal pyramidal cells CA1-CA3 and the dentate gyrus granule cells. RNA blots identify CR16 as a 3.9 kb mRNA which is reduced 50% in abundance by adrenalectomy (ADX) and elevated 3-4 fold in ADX rats by high levels of exogenous corticosterone (CORT) treatment. Both aldosterone and low levels of CORT raise CR16 to intact endogenous levels in ADX rats suggesting that the gene is regulated primarily by the mineralocorticoid receptor. To further characterize the CR16 gene, we have isolated overlapping cDNA clones from a hippocampal library which span 4.5 kb. No significant homologies to the CR16 DNA sequence were found in GenBank, protein databases or the Procite database indicating that CR16 represents a novel gene. However, the DNA sequence identifies 2 classes of cDNA clones which differ by the inclusion of 102 nt in the carboxyterminal region of the presumed coding region. Six cDNA clones were isolated which span this region and two contain the 102 nt insert while the four others do not. Genomic clones confirm that this difference is due to differential RNA splicing. We have subcloned the differentially spliced exon and are currently using *in situ* hybridization to determine whether this splicing event is specifically regulated in brain areas expressing CR16 and whether steroid treatment effects the relative expression of these sub-types. Supported by NIH AG 09425

461.18

ALTERNATIVE SPLICING OF AN SH2 DOMAIN CONTAINING PROTEIN TYROSINE PHOSPHATASE THAT IS ENRICHED IN THE BRAIN.

L. Mei* and R. L. Huganir. Department of Neuroscience, Howard Hughes Medical Institute, The Johns Hopkins University School of Medicine, Baltimore, MD 21205.

Recent studies demonstrating a high level of expression of protein tyrosine kinases in the central nervous system suggest that tyrosine phosphorylation may be involved in neuronal function. Moreover, abundant expression of protein tyrosine kinases at synapses, both presynaptically and postsynaptically, suggests that tyrosine phosphorylation may be involved in the modulation of synaptic transmission. We are interested in identifying protein tyrosine phosphatases that are important in the regulation of synaptic transmission. We recently isolated cDNAs that encode a protein tyrosine phosphatase with two src homology 2 (SH2) domains from a rat brain cDNA library. Since SH2 domains directly bind to specific phosphotyrosyl proteins, the SH2 domain in a tyrosine phosphatase is believed to play a role in targeting or anchoring the substrates to be dephosphorylated or forming complexes with functionally related proteins. The deduced sequence of this phosphatase shows a high percentage of identity at amino acid level to rat SH-PTP2. However, this phosphatase appears to be a novel form of SH-PTP2 generated by alternative splicing of the same or related gene. Nothern blot analysis revealed its high-level expression in the brain, intermediate-level expression in the lung, skeletal muscle and heart, low level expression in the kidney, intestine and liver. Studies are under way to characterize the substrate specificities and tissue distributions of the alternatively spliced forms of the protein tyrosine phosphatase.

461.19

PROMOTER ACTIVITIES AND UNUSUAL SPLICING PATTERNS ASSOCIATED WITH THE GABRB3 GENE.

E.F. Kirkness* The Institute for Genomic Research, 932 Clopper Road, Gaithersburg, MD 20878.

Transcription of the $GABA_A$ receptor β3 subunit gene (GABRB3) involves the use of alternative exons 1 (exon 1 or 1a) that encode distinct signal sequences (J. Biol. Chem. **268**, 4420-4428). The predominant β3 subunit mRNA species in human brain contains exon 1 sequence and is transcribed from a promoter termed P1. Deletion analysis of CAT-constructs has identified strong P1 promoter activity within a 90bp element of the gene. A region of this element that binds nuclear factors was found to adopt a single-stranded conformation when supercoiled. The binding specificity of nuclear factors has been examined by gel retardation assays. Point mutations that affect binding have been incorporated into CAT-constructs and shown to modulate promoter activity in transfected cells.

A RACE analysis of β3 subunit transcripts has uncovered another alternatively spliced exon that can replace exons 1 or 1a. The DNA sequence of this exon is very highly conserved between species. Primary transcripts that contain the novel sequence can be spliced into at least two forms. In addition to the expected linear RNA, an unusual circular transcript appears to result from the splicing of a downstream exon to the 5' end of the transcript. This unusual splicing pattern may play a novel role in post-transcriptional regulation of β3 subunit expression.

461.20

ALTERNATIVE RNA SPLICING OF THE MOUSE GLUR2 GENE *IN VIVO*.

J. A. Izzo*, C. A. Sherman and J. W. Kusiak. Molecular Neurobiology Unit, GRC, NIA, NIH, Baltimore, MD 21224

Mutually exclusive splicing of the Flop and Flip exons of the glutamate receptor subunit gene GLUR-2, generates functionally distinct protein isoforms. To investigate which sequences are involved in regulating Flop and Flip splicing, we constructed a minigene (MG) containing the Flip and Flop genomic region. We have examined the expression of the endogenous GLUR2 gene and our MG construct in several cell lines by RT-PCR. HeLa cells do not express endogenous GLUR2 mRNA, however RNA from the minigene is spliced accurately with a strong preference for the Flop exon. Splicing together of the flanking constitutive exons is also seen. Undifferentiated PC-12 cells transfected with the minigene express both Flip and Flop containing RNAs in a pattern which mimics the splicing choice of the endogenous gene.

A deletion analysis was conducted to identify important cis-acting sequences. Deletion of a long polypyrimidine tract between Flop and Flip (ΔCT) did not qualitatively change the splicing pattern compared to MG. A 165 bp deletion from -47 to -212 upstream of the Flip 3' acceptor site (ΔEF) also did not change the splicing pattern. Extending the ΔEF deletion to -25 upstream of Flip altered the recognition of the Flop exon donor site. Based on these results, we conclude that our MG minigene construct has the necessary sequences for correct splicing. Deletion of a consensus branch point sequence 31 nt upstream of the Flip exon changes the pattern of splicing suggesting that this sequence is critical for correct recognition of the Flop exon donor site. These results suggest that recognition of the Flop 5' donor site and branchpoint site does not occur independently but is tightly linked.

462.1

EFFECTOR DOMAIN OF Rab3 REGULATES NEUROTRANSMITTER RELEASE IN *APLYSIA* SYNAPTOSOMES. Y. Hu, R. Baston, B.-K. Kaang*, and E.R. Kandel, Center for Neurobiology & Behavior, Columbia University College of Physicians & Surgeons, HHMI, New York, NY 10032.

We have cloned Rab3 cDNA from the central nervous system of *Aplysia* and studied its role in neurotransmitter release from presynaptic terminals. Rab3 is a small GTP-binding protein associated with synaptic vesicles. It is thought to be involved in targeting synaptic vesicles to the active zone and in regulating the fusion of the vesicles with the plasma membrane at release sites. Rab3 is in turn activated by a GTPase activating protein (GAP), which regulates the GTPase activity of Rab3 by interacting with its effector domain. Since the GTP-bound form of Rab3 is active, inhibiting its GTPase activity by blocking GAP with a peptide encoding the Rab3 effector domain might keep Rab3 persistently active and thereby enhance neurotransmitter release.

To test this possibility we have introduced into *Aplysia* synaptosomes the effector domain of Rab3 and studied its effect on neurotransmitter release and on the GTPase activity of Rab3. We found that the effector domain of Rab3 inhibited the GTPase activity of the Rab3 protein and enhanced neurotransmitter release from synaptosomes. We propose that the Rab3 effector domain may compete with the Rab3 protein for the binding of a Rab3-specific GAP in the synaptosome, thereby decreasing the GTPase activity of Rab3 and increasing the concentration of the GTP-bound form of Rab3. Thus, our results suggest that the Rab3 protein is involved in the regulation of neurotransmitter release at the nerve terminal and that the activity of RAB3 depends on its GTPase activity.

462.2

PRESYNAPTIC LOCUS FOR SLOW DEVELOPING POTENTIATION IN THE CEREBRAL GANGLION OF *APLYSIA*. M.V. Storozhuk and S.M. Fredman*. A.A. Bogomoletz Institute of Physiology, Kiev, Ukraine and Dept. of Physiology, Meharry Medical College, Nashville, TN 37208.

The A-B neuron synapse in the cerebral ganglion of *Aplysia* exhibits a novel form of enhanced synaptic transmission, slow developing potentiation (SDP). Brief stimulation of single A neurons (4, 500 msec duration trains of 35 msec pulses @ 20 Hz) causes a long-lasting (~25 min) increase in EPSP amplitudes in B neurons. Previous results suggested that SDP is due in part to the activation of PKC and increases in presynaptic Ca^{2+}. The possibility of a postsynaptic component to SDP was not eliminated. To test this, SDP was induced while recording either pre- or postsynaptic neurons using two electrode voltage clamp. During SDP, the amplitude of EPSCs in voltage clamped postsynaptic B neurons increased in phase with EPSPs in a second simultaneously recorded B neuron. There was a significant correlation ($n=70$, $r=0.81$ $p<0.001$) between EPSC and EPSP amplitudes. With the membrane potential of B neurons clamped between -45 and -60 mV there was no change in holding current during SDP. This eliminates postsynaptic membrane currents and changes in membrane resistance contributing to SDP. Additionally, the reversal potential of baseline and potentiated EPSCs was the same ~-10 to 0 mV. In contrast, the induction of SDP in presynaptic A neurons clamped at -50 mV and stepped to 0 mV, resulted in an increase in total inward current and a decrease in outward current. The ionic basis for these changes is presently being studied. Taken together, the results indicate that SDP has a presynaptic locus and is presumably due to increases in transmitter release by A neurons.

This work was supported by NINDS grant NS28199 and NIGMS (MBRS) grant GM08037 to SMF.

462.3

RECORDINGS FROM CALYX NERVE TERMINALS AND POSTSYNAPTIC NEURONS IN CHICK COCHLEAR NUCLEUS. S. Sivaramakrishnan* and G. Laurent. Division of Biology, California Institute of Technology, Pasadena, CA 91125.

We have developed a preparation to study synaptic transmission in the vertebrate CNS. The synapse is that formed by VIIIth nerve afferents on nucleus magnocellularis (NM) neurons in the chick brainstem. Confocal images of DiI-filled afferents show one or two calyx-type nerve terminals impinging on the soma of NM neurons. 250-300 μm thick brain slices and whole-cell patch-clamp recordings are used to characterize pre- and postsynaptic conductances and postsynaptic currents (PSCs) evoked by stimulation of VIIIth nerve afferents. In NM cells under voltage-clamp conditions, depolarizing steps evoke Na and K currents. The K current activates rapidly and shows no inactivation during 600 ms long voltage steps. It is partially blocked by high concentrations of TEA and 4-AP. Under similar conditions, presynaptic calyces also show inward Na and outward K currents. The K current has two components of decay and is completely blocked by TEA and 4-AP. Calcium currents recorded in the presynaptic calyx in the presence of Na and K channel blockers activate at -55 mV (from a holding potential of -70 mV), reach a peak amplitude of 500-800 pA at +40 mV and slowly inactivate during 500 ms long voltage steps. EPSCs recorded in NM cells by stimulation of VIIIth nerve afferents are 3-5 nA in amplitude at a holding potential of -70 mV and peak currents are reached in about 0.4 ms. Preliminary results indicate that these EPSCs are greatly reduced by omega-conotoxin GVIA.

Supported by NIMH.

462.4

MODULATION OF ACTION POTENTIAL PROPAGATION THROUGH EMBRYONIC DORSAL ROOT GANGLION CELLS BY REPETITIVE STIMULATION. CHR. LÜSCHER*, R.QUADRONI¶, J. STREIT & H.-R. LÜSCHER, DEPT. OF PHYSIOLOGY, UNIVERSITY OF BERN, &¶INSTITUTE OF THEORETICAL PHYSICS, ETHZ, ZÜRICH, SWITZERLAND

A: Impulse conduction in axons is not simply a transmitive process but has integrative functions. The dorsal root ganglion (DRG) of the embryonic rat put into culture forms a monolayer and is thus accessible to conventional microelectrode technique, which allows testing the above hypothesis. We have chosen a dual approach by correlating experimental electrophysiological data from the culture with results obtained from a compartmental computer model. **B:** When a train of stimuli at frequencies of 5-20 Hz is applied to the central or peripheral branch of the axon of a DRG cell the following phenomena can be recorded in the soma: during the first twenty to fifty stimuli action potential (AP) invasion of the soma is reliable, but there is an increase in the latency from time of stimulation to the onset of the AP, a decrease of the upstroke velocity, a decrease of the afterhyperpolarization amplitude and a disappearance of the "calcium-shoulder". For the next stimuli AP invasion intermittently fails, leaving an electrotonic residue (ER) of one discrete amplitude level in bipolar cells and ERs with two discrete amplitude levels in unipolar cells. The smaller ER reflects conduction failure at the branch point and the larger ER conduction failure at the entrance of the axon into the soma. **C:** Our experiments suggest that conduction may fail at sites of subtle impedance mismatch when periaxonal potassium accumulation and Ca-induced Ca channel inactivation occur. A compartmental computer model based on voltage clamp data from the literature reproduced the majority of the experimental findings. (SNF 31-27553.89 to H.-R.L.)

462.5

MODULATION OF GNRH-INDUCED CALCIUM OSCILLATIONS IN PITUITARY GONADOTROPES. A. Tse*, F.W. Tse and B. Hille. Dept. Physiol. & Biophys., U. of Washington, Seattle, WA 98195.

In identified gonadotropes of male rat, gonadotropin-releasing hormone (GnRH) stimulates a rhythmic release of Ca^{2+} from an IP_3-sensitive store, which in turn induces an oscillatory apamin-sensitive $I_{K(Ca)}$ (Tse & Hille, Science 255:462) and bursts of exocytosis (Tse et al., Science 260:82). Since GnRH also activates PKC, we examine the effect of phorbol esters by simultaneously measuring $[Ca^{2+}]_i$ and $I_{K(Ca)}$. Application of PMA (100 nM) has little effect on the basal $[Ca^{2+}]_i$ (n = 3). PMA or PDBu, but not the inactive 4α-PDD (n = 6), reduces the frequency of oscillations by 48 ± 3% and increases peak $I_{K(Ca)}$ by 108 ± 18% (mean ± S.E.; n = 46). However, despite the enhancement of peak $I_{K(Ca)}$, the amplitude of $[Ca^{2+}]_i$ oscillations is not typically increased (n = 15). Both the slowing of oscillations and the $I_{K(Ca)}$ enhancement can be mimicked by 1,2-dioctanoyl-sn-glycerol (50-200 μM; n = 8), and they can be induced during oscillations that have been initiated irreversibly by GnRH in cells loaded with 100 μM GTP-γ-S (n = 15). In contrast, in cells loaded with 10 or 20 μM IP_3, phorbol esters enhance $I_{K(Ca)}$ but have no significant effect on the frequency of oscillations (n = 11). Therefore, the slowing of $[Ca^{2+}]_i$ oscillations by phorbol ester probably involves PKC modulation of steps after G-protein activation but before IP_3 generation. Our results also suggest that phorbol esters can enhance $I_{K(Ca)}$ independently of changes in $[Ca^{2+}]_i$. Thus, caution must be taken when $I_{K(Ca)}$ is used to monitor changes in $[Ca^{2+}]_i$. (Supported by AR-17803, HD-12629 and the Mellon, McKnight and W. M. Keck Foundations.)

462.6

CALCIUM HOMEOSTASIS IN PITUITARY GONADOTROPES F.W.Tse*, A. Tse and B. Hille. Dept. Physiol. & Biophys., U. of Washington, Seattle, WA 98195.

Using whole-cell voltage-clamp and indo-1 fluorometry, we examine cytoplasmic Ca^{2+} dynamics in male rat gonadotropes identified by the reverse hemolytic plaque assay. During depolarizing voltage steps (< 1s) to potentials between -40 and +20 mV, Ca^{2+} entries estimated from the integral of I_{Ca} closely parallel the peak amplitude of $[Ca^{2+}]_i$ rises. For a comparable Ca^{2+} entry, the $[Ca^{2+}]_i$ rise is attenuated 5.4-fold when the total concentration of Ca^{2+} buffers (indo-1 and BAPTA) loaded into cells via the whole-cell pipette is increased from 25 μM to 500 μM. Comparison of depolarization-induced $[Ca^{2+}]_i$ rises and Ca^{2+} entries with different pipette Ca^{2+} buffer concentrations suggests that, in the absence of any pipette Ca^{2+} buffers, the essentially non-diffusible endogenous Ca^{2+} buffers would immediately bind ~99% of the Ca^{2+} entering the cytoplasm. With 25 μM pipette Ca^{2+} buffer, depolarization-induced $[Ca^{2+}]_i$ rises decay with a 2-s time constant. Inhibition of intracellular Ca^{2+}-ATPases by thapsigargin, cyclopiazonic acid or 2,5-di-(tert-butyl)-1,4-benzohydroquinone (BHQ) results in a slow increase of $[Ca^{2+}]_i$. BHQ (10 μM) also slows the decay of depolarization-induced $[Ca^{2+}]_i$ rises by ~3 fold, implicating intracellular Ca^{2+}-ATPases in the slow removal of cytoplasmic Ca^{2+}. These inhibitors do not block the release of intracellular Ca^{2+} induced by gonadotropin-releasing hormone (GnRH), but transform GnRH-induced $[Ca^{2+}]_i$ oscillations into a single, slow monophasic decay. (Supported by AR-17803, HD-12629, and the Mellon, McKnight and W. M. Keck Foundations.)

462.7

REGULATION OF INTRACELLULAR CALCIUM IN ISOLATED SECRETORY NERVE ENDINGS OF THE NEUROHYPOPHYSIS. E.L. Stuenkel* Department of Physiology, Univ. of Michigan, Ann Arbor, MI 48109-0622

Stimulated release of chemical messengers from nerve endings is initiated by a rise in intracellular calcium concentration ($[Ca^{2+}]_i$). The amplitude and duration of impulse evoked Ca^{2+} transients are determined by properties of Ca^{2+} influx and mobilization as well as by intracellular buffering, sequestration and efflux. To characterize these properties $[Ca^{2+}]_i$ and calcium currents were simultaneously measured under whole-cell voltage clamp at isolated secretory nerve endings of the rat neurohypophysis. The peak amplitude of $[Ca^{2+}]_i$ was found to closely parallel calcium currents evoked by depolarizing voltage commands. These results suggest that neither voltage nor Ca^{2+} -induced Ca^{2+} release significantly affect depolarization evoked $[Ca^{2+}]_i$ transients. This is supported by a lack of effect of ryanodine and caffeine on elevated K^+-induced $[Ca^{2+}]_i$ transients. Voltage steps to +10 mV (h.p. -90 mV) led to a rapid equilibration of $[Ca^{2+}]_i$ (< 100 ms) at 10 mM $[Ca^{2+}]_o$. Recovery of $[Ca^{2+}]_i$ to prestimulation levels was gradual (15 - 30 sec) even for brief depolarizations. The rate of $[Ca^{2+}]_i$ recovery was uninfluenced by return to holding potentials over the range of -90 to -50 mV following the step depolarization. The kinetic difference between rapid $[Ca^{2+}]_i$ equilibration and slow recovery allowed estimation of the endogenous Ca^{2+} binding capacity of the nerve endings (method, Neher and Augustine, 1992). The endogenous binding capacity (167 ± 11, $\bar{X}$ ± SEM) remained stable over a period of 5 min following initiation of whole cell recording, suggesting the endogenous Ca^{2+} buffer is of low mobility.

462.8

A MINIATURE SYNAPTIC CURRENT IS GENERATED BY A GROUP OF PERIODIC EXOCYTOTIC BURSTS. **B.R. Maple* and S.M. Wu, Cullen Eye Institute, Baylor College of Medicine, Houston, TX 77030**

Off-center bipolar cells of the salamander retina display spontaneous miniature excitatory postsynaptic currents (MEPSCs), thought to be generated by the vesicular release of glutamate from photoreceptors. These events vary tremendously in amplitude and time course, suggesting that the MEPSCs represent a multivesicular release process. We have performed a two-dimensional analysis which reveals a MEPSC subunit structure.

Bipolar cells in retinal slices were voltage-clamped with patch electrodes, and MEPSCs were observed under conditions where presynaptic calcium influx was suppressed. Bandpass filtered MEPSCs were fit to a filtered difference of exponentials to yield an estimated amplitude and integrated charge for each unfiltered event. A duration (defined as the charge/amplitude ratio) was calculated for each MEPSC, and the events were plotted as a density in a charge vs. duration plane. These plots exhibited a complex structure, with periodicities along both the charge and duration axes.

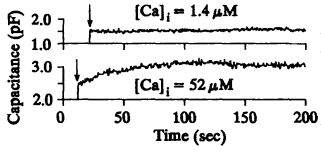
This structure was consistent with a model in which exocytosis occurs as a series of bursts with an interburst interval of about 1.2 msec. In this model the number of vesicles released in the first burst was governed by an exponentially decaying probability density. The number of vesicles released in subsequent bursts was governed by binomial statistics, where the probability of release increased as a function of the number of vesicles released in the previous burst. A further prediction of the model was that the effects of synchronously released vesicles do not sum linearly, but instead display positive cooperativity.

Visual inspection of the MEPSCs showed a tendency for slight inflections in the MEPSC waveforms to occur periodically at the same interval predicted by the model. Thus, it appears that the basic unit of transmitter release from photoreceptors does not correspond to a single glutamate vesicle, but rather to a group of vesicles released in a series of exocytotic bursts. The rate of MEPSC decay, then, does not primarily reflect either channel kinetics or the rate of glutamate uptake, but rather the time course of vesicle mobilization during a synaptic release event.

462.9

CALCIUM-DEPENDENT EXOCYTOSIS IN INDIVIDUAL SYNAPTIC TERMINALS OF A CNS INTERNEURON. **Henrique von Gersdorff and Gary Matthews*. Dept. of Neurobiology, SUNY, Stony Brook, NY 11794.**

Exocytosis was studied in giant synaptic terminals of goldfish retinal bipolar neurons, using combined patch-clamp, fura-2, and capacitance measurements. Dynamics of capacitance changes elicited by Ca influx through Ca channels were described previously (von Gersdorff & Matthews, Biophys. J., 64, A319). With light Ca-buffering (0.5 mM BAPTA), the capacitance jump elicited by a single 250-ms depolarizing pulse averaged 83 ± 20 fF (mean ± s.e.m., N=11);with stronger buffering (10 mM BAPTA), the response to a single pulse was abolished (-9 ± 7 fF; N = 15). After 15 repetitive pulses at 1/sec, the change in capacitance for 10 mM BAPTA (145 ± 54 fF) was little different from 0.5 mM BAPTA (190 ± 31 fF). Thus, BAPTA retarded but did not prevent the increase in capacitance. To examine the Ca-dependence of exocytosis, terminals were dialyzed with solutions containing [Ca] strongly buffered at 1.4 μM (which is similar to the average $[Ca]_i$ reached during sustained activation of Ca current) or 52 μM (see Fig.). With 1.4 μM Ca, no change in capacitance was seen (16 ± 69 fF; N=12). With 52 μM Ca, capacitance increased following break-in (242 ± 83 fF; N=7). This suggests that [Ca] achieved locally during release evoked by Ca current is much higher than the bulk $[Ca]_i$ measured by fura-2 in the whole terminal. *Fig. Capacitance of isolated terminals measured with lock-in amplifier following break-in (at arrows). Ca-buffering (in mM): 1 EGTA/9 CaEGTA (top); 7 NTA/3 CaNTA (bottom). Cs/TEA/ATP/GTP pipette solution. (EY03821)*



462.10

FACILITATION AT CRAYFISH NEUROMUSCULAR JUNCTIONS IN THE PRESENCE OF A CALCIUM IONOPHORE. U.Musser and S.J.Velez*, Department of Biological Sciences, Dartmouth College, Hanover, N. H. 03755.

We examined the facilitation characteristics of synapses made by neurons innervating the superficial flexor muscles of the crayfish Procambarus clarkii while changing the calcium concentration of the Ringers bathing the preparation, in the presence and absence of the calcium ionophore A23187. The nerve was stimulated at 1 Hz and 10 Hz while the junction potentials generated (JP's) were recorded with intracellular microelectrodes impailing the muscle fibers. With the microelectrode in the same muscle fiber, the Ringers solution was changed several times to ones containing 30%, 70%, 100%, 117% and 200% normal calcium concentrations. JP's recorded at 1 Hz were compared to those recorded at 10 Hz and facilitation ratios were calculated (JP at 10 Hz/JP at 1 Hz). These synapses facilitated through all these external calcium changes. Upon addition of a 15 uM solution of the calcium ionophore A23187 in the different calcium Ringers, the amount of facilitation was reduced at the lower calcium concentrations and disappeared in the 200% calcium Ringers, i.e. the JP's generated at 10 Hz were smaller than those generated at 1 Hz. Upon removal of the ionophore, the terminals again facilitated. It appears that too much calcium entry into these terminals affects the mechanism of facilitation.

462.11

WITHDRAWN

462.12

EFFECT OF REPETITIVE STIMULATION ON MINIATURE END-PLATE POTENTIAL FREQUENCY UNDER CONDITIONS OF DEPRESSION OF EVOKED RELEASE. **M.A. Sosa* and J.E. Zengel. Depts. of Neurosci. & Neurosurg., U. of Fla. Coll. of Med. and DVA Med. Ctr., Gainesville FL 32610.**

Repetitive stimulation of the frog neuromuscular junction under conditions of normal or high levels of release results in a depression of endplate potential (EPP) amplitude that has traditionally been attributed to a depletion of available transmitter. The purpose of this study was to determine whether transmitter release in the form of miniature EPPs (MEPPs) is also affected by depression.

MEPPs were recorded from frog sartorius neuromuscular junctions in 3.6 mM Ca Ringer using standard intracellular recording techniques. Muscle action potentials were blocked by 5 μM μ-conotoxin. Nerves were conditioned with trains of 200-4800 impulses (20 imp/sec).

We found that whereas the conditioning stimulation resulted in the expected depression of EPP amplitude, the frequency of MEPPs was dramatically increased. The increase in MEPP frequency following conditioning stimulation could be described by four components with time constants of decay similar to those reported for the components of increased release observed under low quantal conditions when depression is absent.

Our results support the idea that the mechanisms that produce the components of increased release can be present simultaneously with those that produce depression of release. They are not consistent, however, with the hypothesis that depression results from a depletion of available quanta, unless one assumes that evoked release (EPPs) and asynchronous release (MEPPs) arise from different pools of quanta.

462.13

ON THE SIMULTANEOUS MEASUREMENTS OF CALCIUM CURRENTS AND ACETYLCHOLINE RELEASE FROM MOTOR NERVE ENDINGS. Redman, R.S*. and Silinsky, E.M. Dept. Pharmacology, Northwestern U. Med. Schl.,Chicago, IL 60611.

A number of laboratories have been successful in recording Ca currents from motor nerve endings by perineural or 'loose patch' methods. Such measurements are generally made in normal Ca solutions in the presence of high concentrations of the K channel blockers 3,4, diaminopyridine (DAP) and tetraethyammonium (TEA), conditions that cause a rapid depletion of acetylcholine (ACh) quanta. Simultaneous measurements of Ca currents and stable levels of ACh release are thus not possible in such solutions, an unfortunate feature that precludes studies in which effects of an exogenous agent on ACh release may be compared simultaneously to its effect on Ca currents. We have found that Ringer containing 0.9 mM Ca, 10 mM Mg, 100 μM DAP and low TEA concentrations (250 μM) produced sufficient blockade of the Ca activated-K current to allow unobscured measurements of Ca currents and to minimize depletion of ACh release. In this solution, increases or decreases in extracellular Ca produce parallel effects on both the perineural Ca currents and end-plate potentials (EPPs) measured simultaneously. By making simultaneous measurements of presynaptic currents and EPPs in this solution, we have been able to differentiate between agents that affect ACh release by an action on presynaptic ion channels (e.g., increases in release with difluoro-methylornithine or NaF; decreases in release by Co) from those that affect release by other means (decreases in release by adenosine in the frog). (Supported by NIH grants NS12782 and NS30795)

462.14

OPTICAL MEASUREMENTS OF PRESYNAPTIC CALCIUM DYNAMICS IN FROG TECTUM. M. B. Feller , K. R. Delaney*& D. W. Tank, Biological Computation Research Dept., AT&T Bell Laboratories, Murray Hill, NJ 07974.

Visually guided prey-catching in the frog displays a form of behavioral facilitation that decays on a time scale of 1-10 seconds. The biophysical basis of this effect may be short term synaptic enhancement caused by rapid buildup and slow decay of residual calcium in presynaptic terminals. As a first step in testing this hypothesis, we have used optical imaging techniques and the calcium indicator fura-2 to measure the kinetics of presynaptic calcium ion concentration in optic nerve fibers in the optic tectum of *Rana pipien.*

Membrane permeable fura-2AM was bath applied to the optic tectum *in vivo* and to isolated brains. Following labeling, a transient decrease in 380 nm excited fluorescence was observed in response to optic nerve shocks. The transient had a rapid onset (20 msec) and decayed to pre-stimulus levels on a time scale of a few seconds, significantly longer than the decay of extracellular field potentials. Several features of this fluorescence signal suggest that the change corresponds to transient increases in residual calcium in unmyelinated optic nerve fibers. First, bath-applied glutamate antagonists reversibly eliminated post-synaptic components from the field potential but did not affect the measured calcium transients, implying the origin of the signal is pre-synaptic. Second, when the stimulation intensity is lowered so that only the myelinated fibers are activated, the calcium response to the optic nerve shock disappears. Finally, the staining of fura-2 in the tectum is strongest in the outer layers, which is the region which corresponds to the termination zone of the unmyelinated fibers. We have also demonstrated temporal summation of the presynaptic calcium changes during 10-50 Hz stimulus trains.

In summary, we have measured changes in presynaptic calcium in frog optic tectum *in vivo*. Our results suggest that the decay times of presynaptic residual calcium are on the same time scale as the decay of behavioral short term memory effects in frog visually-guided prey catching.

462.15

CHOLINE CHLORIDE CAN INCREASE TRANSMITTER RELEASE AT THE NEUROMUSCULAR JUNCTION OF THE RAT. D. F. Wilson* Zoology Dept. Miami Univ., Oxford, OH, USA 45056.

The presynaptic effect of exogenously applied choline chloride on transmitter release in the rat diaphragm was examined by testing the effects of 0.5 and 0.75 mM choline chloride on evoked transmitter release. Intracellular recording techniques were used to monitor end-plate potentials (EPPs) and miniature end-plate potentials (MEPPs) in the isolated cut-muscle rat diaphragm-phrenic nerve preparation. Quantal release in the presence and absence of choline chloride was examined. In the presence of 0.5 mM choline chloride, no significant changes were observed. In the presence of 0.75 mM choline chloride however the initial output of quantal release was increased by 36% and the MEPP amplitudes were depressed by 42%. It is suggested that the enhancing effect of choline on transmitter release is due to its ability to block nicotinic receptors on the nerve terminal that serves a negative feedback role (Supported by NIH grant NS-27260).

462.16

SIMULTANEOUS BARIUM-INDUCED CATECHOLAMINE QUANTAL RELEASE AND FURA-2 RESPONSE AT SINGLE BOVINE CHROMAFFIN CELLS. J.M. Finnegan, J.A. Jankowski, T.J. Schroeder, and R.M. Wightman*, Dept. of Chemistry, Univ. of North Carolina, Chapel Hill, NC 27599-3290.

Simultaneous measurement of quantal release of catecholamines and intracellular divalent-ion concentration with fura-2 allows the stimulus-secretion coupling at the bovine adrenal chromaffin cell to be probed at the single-cell level. The quantal release of catecholamine is monitored by placing a carbon-fiber microelectrode adjacent to a single cell. Oxidation of released catecholamines by the electrode results in electrochemical current spikes which are due to exocytosis from individual vesicles.

The high conductance of barium through voltage gated Ca^{2+}- channels allows Ba^{2+} to substitute for Ca^{2+} in many intracellular processes, including binding to the divalent-cation probe fura-2. Barium is a potent secretagogue capable of evoking exocytosis with or without extracellular calcium. The intracellular divalent-cation concentration remains at a high plateau for greater than 10 min. with Ba^{2+}, while K^+ depolarizations give a transient response returning $[Ca^{2+}]_i$ to basal levels in less than 40 s. The detected catecholamine packets resulting from Ba^{2+}-induced release are taller, more frequent, and longer lasting than those due to high K^+ depolarizations at the same cell. However, spikes induced by high K^+ applied after Ba^{2+} show characteristics much like those from Ba^{2+} itself. The data indicate that Ba^{2+} causes a measurable alteration of the intravesicular cocktail even after the Ba^{2+}-induced release has subsided.

462.17

PROTON IONOPHORES DELAY ONSET OF BOTULINUM PARALYSIS IN ISOLATED MOUSE SKELETAL MUSCLE. *R. E. Sheridan**. Neurotoxicology Branch, Pathophysiology Division, USAMRICD, APG, MD 21010-5425.

Botulinum and tetanus toxins are thought to enter cells through endocytotic vesicles where acidification is required for release of these toxins into the cytoplasm. The role of vesicular acidification in the development of botulinum intoxication was studied in the isolated mouse phrenic nerve/diaphragm muscle preparation. Isolated hemidiaphragms were incubated at 4°C for 1 hr with or without botulinum toxin. The muscles were then washed with oxygenated Tyrode's solution and transferred to 36°C baths that contained the test drugs. The phrenic nerves were stimulated and the resulting twitches were measured isometrically. Two ionophores, nigericin and monensin, that increase membrane permeability to H^+ and K^+ or H^+, Na^+ and K^+ respectively, block vesicle acidification by acting as H^+ shunts to neutralize pH gradients across vesicular membranes. Both drugs were inherently toxic at concentrations greater than 1 μM, resulting in loss of nerve-elicited contractions. For nigericin at 1 μM, electrical recordings at muscle endplates showed a decrease in MEPP frequency without any change in MEPP amplitude or muscle membrane potential. There was no apparent effect of either ionophore on contractions due to direct electrical stimulation of the muscles. Concentrations of monensin and nigericin $\leq$ 10 nM showed no inherent toxicity over a 4-6 hr period. Either monensin or nigericin treatment (40 nM) of botulinum type A or B (10^{-10} to 10^{-9} M)-exposed diaphragms delayed paralysis 2- to 3-fold compared to muscles in botulinum alone. This delay was roughly equivalent to a 10-fold reduction in the effective concentration of botulinum toxin. Treatment with 1-3 nM nigericin did not significantly delay onset of botulinum type B paralysis. Neutralization of endocytotic vesicles was thus able to slow the onset and reduce the effective concentration for multiple botulinum toxin serotypes.

462.18

ROLE OF N- AND NON-N-TYPE CALCIUM CHANNELS IN TRANSMISSION AT CORTICOSTRIATAL SYNAPSES. D.M. Lovinger*, A. Merritt and D. Reyes. Dept. Molecular Physiology and Biophysics, Vanderbilt Med. School, Nashville, TN 37232-0615.

N-type Ca^{2+} channels are thought to participate in excitation/secretion coupling at presynaptic terminals. However, there is little evidence implicating N channels in transmission in the CNS. We have examined the action of the selective N channel blocker ω-conotoxin GVIA (CgTx) on excitatory synaptic transmission at corticostriatal synapses in rat brain slices. During field potential recording, application of 1μM CgTx depressed the amplitude of the stimulus-evoked, synaptically-driven population spike (PS) by 49±5% (n=27). Depression was irreversible after extensive washing and higher CgTx concentrations did not produce additional depression. The PS produced by direct activation of neurons due to stimulus current was not affected by CgTx. During whole-cell recording, application of 1μM CgTx irreversibly depressed EPSP amplitude by 52± 6% (n=17). CgTx induced inconsistent changes in postsynaptic neuron excitability which did not correlate with synaptic depression. Pretreatment with CgTx did not alter synaptic depression induced by 50μM 1S,3R-ACPD (% decrease in PS amplitude in 6 untreated slices=61±5 in ACPD compared to 63±7 after CgTx, n= 7). CgTx pretreatment also did not prevent potentiaton of transmission by the phorbol ester PDBu (% increase in PS amplitude=33±7 in 8 untreated slices and 55±25 in 5 CgTx-treated slices). Similar results were observed during whole-cell recording. N- and non-N-type Ca^{2+} channels appear to be involved in transmission at corticostriatal synapses; perhaps in glutamate release. Alterations in transmission by presynaptic receptor or protein kinase C activation do not appear to require N channels. Supported by NS 30470.

463.1

GABA$_B$ RECEPTORS INHIBIT CULTURED DORSAL ROOT GANGLION NEURONE CALCIUM CURRENTS VIA G_o: EVIDENCE FROM ANTISENSE OLIGONUCLEOTIDE STUDIES. A.C. Dolphin,* V. Campbell and N. Berrow. Dept. of Pharmacology, Royal Free Hospital School of Medicine, Rowland Hill Street, London, NW3 2PF, UK.

Calcium channel currents (I_{Ba}) were recorded in cultured dorsal root ganglion neurones (DRGs), 24-32 h after microinjection with 20-mer phosphorothioate antisense oligonucleotides complementary either to a $G\alpha_o$ or a $G\alpha_i$ unique sequence, or with a nonsense sequence. The ability of the GABA$_B$ agonist (-)-baclofen (50 μM) to inhibit I_{Ba} was examined. The maximum peak current was inhibited by 35.2±4.0% (n=11) in control noninjected cells, and by 38.1±2.6% (n=11) and 34.8±4.2% (n=5) in nonsense and $G\alpha_i$ oligonucleotide injected cells. Following $G\alpha_o$ oligonucleotide injection, the inhibition of I_{Ba} by (-)-baclofen was significantly reduced ($p<0.05$) to 21.0±3.3% (n=19). Confocal immunocytochemical localisation of $G\alpha_o$ showed prominent staining at the plasma membrane in control DRGs, and this was also present in $G\alpha_i$ and nonsense oligonucleotide injected cells. The $G\alpha_o$ staining at the plasma membrane was much reduced in $G\alpha_o$ oligonucleotide injected cells. In contrast, confocal immunocytochemical localisation of $G\alpha_i$ showed immunostaining in the membrane and cytoplasm of control, $G\alpha_o$ and nonsense injected DRGs, whereas this was greatly depleted in $G\alpha_i$ oligonucleotide injected cells. These results indicate that the GABA$_B$ receptor couples to voltage sensitive calcium channels via the G protein G_o and not G_i, and that antisense oligonucleotides can be used to deplete G proteins in DRGs. Supported by Wellcome Trust.

463.2

SEROTONIN INHIBITS HIGH-THRESHOLD Ca^{2+} CURRENT IN AMYGDALA PYRAMIDAL NEURONS. Reese S. Scroggs* and Robert C. Foehring
Dept. of Anat. and Neurobiol., Univ. of Tennessee, Memphis, TN 38163

The amygdala plays an important role in emotion, motivation, learning, and memory, yet little is known about ion currents and their modulation in amygdala neurons. In this study, high-threshold Ca^{2+} currents were recorded from freshly dissociated amygdala pyramidal neurons using the whole-cell patch-clamp technique. Neurons harvested from the several nuclei in the amygdala were grouped together. In neurons held at -90mV, Ca^{2+} current peaked around -10mV and averaged 1.5nA ± 0.32 SEM. 5μM nifedipine (nifed) inhibited 30% ± 4.1 SEM of peak current, while 5μM Bay K 8466 increased peak current by 55% ± 2.4 SEM. 1μM ω-conotoxin GVIA (ω-CgTx) inhibited 30.4% ± 5.6 SEM of peak current. Thus, both L- and N-type Ca^{2+} channels are important for Ca^{2+} entry in amygdala neurons. 40% ± 6.9 SEM of the current was not blocked by the combination of 5μM nifedipine and 1μM ω-CgTx. About 1/3 of the nifed/ω-CgTx resistant current was blocked by 100nM ω-agatoxin IVA, indicating that these cells also express P-type Ca^{2+} channels. In some neurons, part of the nifed/ω-CgTx resistant Ca^{2+} entry appeared to result from T-type Ca^{2+} channel activity, similar to a report by Kaneda and Akaike, (1989). However, a significant portion of Ca^{2+} entry occurred via uncharacterized Ca^{2+} channels.

2μM serotonin (5HT) was observed to block 25% ± 2.8 SEM of high-threshold Ca^{2+} current in neurons held at -90mV. The effect of 5HT was nearly completely occluded by pretreatment with 1μM ω-CgTx, indicating that most of 5HT's effect was on N-type Ca^{2+} channels. 5HT did not affect the slow component of the tail current in neurons treated with 5μM Bay K 8644, indicating that 5HT receptors were not coupled to L-type Ca^{2+} channels. *Supported by NINDS grant# NS27180 to RCF*

463.3

INTERACTION BETWEEN ENKEPHALIN AND NEFIRACETAM (DM-9384), A NOOTROPIC AGENT, OBSERVED IN CALCIUM CHANNEL CURRENTS AND cAMP IN NG108-15 CELLS. M. Yoshii[1], Y. L. Murashima[1], S. Watabe[2*], T. Shiotani[2] and M. Tanaka[2]. [1]Dept. of Neurophysiol., Tokyo Inst. of Psychiatry, Tokyo 156 and [2]Tokyo R & D Center, Daiichi Pharmaceu. Co. Ltd, Tokyo 134, Japan.

We have previously reported that the nootropic agent, nefiracetam (DM-9384), activates cAMP-dependent processes, resulting in a facilitation of long-lasting (or type II) Ca channel currents in NG108-15 cells (Yoshii et al., Soc. Neurosci. Abstr. 18, 435, 1992). The nefiracetam action reverses an inhibitory effect of Leu-enkephalin on the Ca channels (Watabe et al., Soc. Neurosci. Abstr. 18, 434, 1992). In the present study, we have investigated whether cAMP mediates the interaction between opioid and nootropic by measuring the intracellular level of cAMP (enzymatic method) as well as by recording Ca channel currents (whole cell clamp). When Leu-enkephalin (50 nM) was applied, cAMP levels were enhanced markedly (more than 10-fold) within 5-10 min whereas type II currents were reduced. The opioid-induced increase in cAMP was further enhanced by nefiracetam (1 uM) in an additive manner. Under such conditions, Ca channel currents were recovered from inhibition and even potentiated. The results suggest that cAMP does not mediate the interaction between opioid and nootropic appearing in Ca channel currents. It is also suggested that cAMP-sensitive Ca channels may not be influenced by a group of cAMP enhanced by opioids.

463.4

INVOLVEMENT OF GLUTAMATE RECEPTORS IN LONG-TERM INDUCTION OF Ca^{2+} CURRENTS IN HIPPOCAMPAL NEURONS. D.E. García*[1], P.N. Velázquez[2], A. Cavalié[3] and H.D. Lux[3]. Dept of [1]Physiology and [2]Histology, Fac de Medicina, UNAM. AP 70250, CP 04510, México and [3]Dept of Neurophysiology, MPI für Psychiatrie, Am Klopferspitz 18A, W-8033 Martinsried, FRG.

Voltage-gated Ca^{2+} channels are associated with functional adaptive changes in neurons. We report that periodic exposure of cultured hippocampal neurons to glutamate or potassium depolarization, increased currents from high voltage-activated calcium channels after a latency of a couple of days. This enhancement was not due to change in membrane capacitance but instead indicated that newly functional channels were present in the membrane. The gating was not changed and, therefore, it was presumably due to an increase in channel density. The intracellular calcium transient (Ca_i) was assessed by Fura-2. In the case of glutamate, Ca_i fell to zero during prolonged exposure whereas in the potassium depolarization it overlasted the application. AP5 partially suppressed Ca_i yet the current response was completely blocked suggesting a possible involvement of the glutamate receptors. These observations point out the importance of calcium channels for synaptic transmission in the nervous system.

463.5

ACTIVATION OF METABOTROPIC GLUTAMATE RECEPTORS INHIBITS SPONTANEOUS OSCILLATIONS OF CYTOSOLIC Ca^{2+} IN CULTURED RAT CORTICAL NEURONS. Kenneth A. Stauderman* and Rebecca A. McKinney. Marion Merrell Dow Research Institute, 2110 E. Galbraith Rd., Cincinnati, OH 45215.

Primary cultures of rat cortical neurons develop spontaneous, synchronized, oscillations of cytosolic Ca^{2+} concentration ($[Ca^{2+}]_i$) that depend on glutamatergic neurotransmission (Kuroda et al., Neurosci. Lett. **135**: 255-258, 1992), making them a convenient model to study endogenous synaptic activity. Using fura-2 loaded cells, we found that activation of metabotropic glutamate receptors (mGluRs) with (1S,3R)-1-aminocyclopentane-1,3-dicarboxylic acid (*trans*-ACPD) inhibited spontaneous oscillations of $[Ca^{2+}]_i$ in a dose-dependent manner. Low concentrations of *trans*-ACPD (1-20 μM) inhibited the frequency of oscillations without altering amplitude, whereas higher concentrations (>20 μM) obliterated the oscillations. The effects of 20 μM *trans*-ACPD were not blocked by 300 μM L-AP3, and were often observed without accompanying increases of $[Ca^{2+}]_i$, suggesting that mGluR1 and mGluR5 subtypes may not be involved. Also, L-AP4 was not as effective as *trans*-ACPD at decreasing oscillation frequency, making mGluR4 activation unlikely. We are characterizing in more detail the effects of mGluR activation and the nature of the spontaneous oscillations of $[Ca^{2+}]_i$.

463.6

STIMULATION OF m4 MUSCARINIC RECEPTOR SUBTYPE ENHANCES AN L-TYPE CALCIUM CONDUCTANCE IN AtT20 CELLS. K. E. Pemberton* and S.V.P. Jones. Molecular Neuropharmacology, Department of Psychiatry, University of Vermont College of Medicine, Burlington, VT 05405.

The effects of activation of the m4 muscarinic receptor subtype on calcium conductances were studied in the AtT20 cell line, using the whole cell patch clamp technique. AtT20 cells have been previously shown to express only the m4 receptor subtype. Calcium currents were recorded in extracellular solutions containing (mM) NaCl 155, $MgCl_2$ 1, $BaCl_2$ 5, Hepes 10, Glucose 20. Patch pipettes (5-7 MΩ) contained (mM) NMGCl 100, $MgCl_2$ 4, EGTA 10, Hepes 10, ATP 4, creatine phosphate 5, creatine kinase 50 units/ml. Current voltage (I-V) relationships were constructed from the currents measured at the end of 100-500 ms steps from -90 to 50 mV in 10 mV increments from a holding potential of -90 mV. The I-V relationship indicated that the calcium current activated around -30 mV and peaked around -10 mV. Application of BayK8644 (7 µM) dramatically increased the current amplitude and addition of 2-300µM nifedipine inhibited the current. This is indicative of an L-type calcium conductance. Application of 50 µM acetylcholine (ACh) from a pressure ejection pipette reversibly increased the amplitude of the calcium current 16±3% (n=8). This effect was abolished by pirenzepine (1-3 µM). The ACh-induced increase in calcium current was inhibited in the presence of nifedipine. Preincubation of the AtT20 cells in Pertussis toxin (PTX) overnight also inhibited the ACh effect. It is therefore concluded that stimulation of the m4 muscarinic receptor leads to an enhancement of L-type calcium channel activity mediated by a PTX sensitive G-protein.

463.7

Muscarinic-Cholinergic Stimulated Increases in Cytosolic Calcium in SH-SY5Y Human Neuroblastoma have Two Components. by Vincent J. Andaloro and Fulton T. Crews*. Dept. of Pharmacology and Center for Neurobiology of Aging, University of Florida. 32610.

SHSY-5Y cells were loaded with FURA-II to measure intracellular free calcium ([Ca2+]i) responses. Stimulation by carbachol (CCh), a cholinergic agonist, results in a rapid and transient peak increase in cytosolic calcium which decays within two minutes to a sustained plateau level above baseline. Pretreatment with atropine blocks both peak and plateau responses, whereas treatment during the plateau phase rapidly returns [Ca2+]i to basal levels indicating that both peak and plateau responses require continuous carbachol stimulation. Dose response curves indicate that the ED50 values for peak and plateau are different: 17 ± 4 mM and 0.8 ± 0.2 mM CCh for peak and plateau, respectively. Decreasing extracellular calcium to low levels or the addition of Ni2+ or Mn2+, calcium channel antagonists, abolish the plateau suggesting that the peak primarily represents the release of intracellular calcium stores whereas the plateau requires extracellular calcium. Nifedipine did not change either response. Carbachol dose response, time course, and atropine-antagonism experiments measuring inositol polyphosphate formation are consistent with these second messengers mediating one or both of the [Ca2+]i responses. Pretreatment with CCh results in a rapid loss of the peak [Ca2+]i response within 1 hour which after 24 hours decreases 94% from control. In contrast, the plateau decrease is only 34% after 24 hrs. These data support the hypothesis that the peak and plateau phases are indeed distinct phenomena, and may even have different physiological roles. (Supported by AG10485 and T32 AG00196).

463.8

INHIBITION BY NORADRENALINE AND NPY OF CALCIUM CURRENTS EVOKED BY ACTION POTENTIAL WAVE FORMS IN RAT SYMPATHETIC NEURONS P.T. Toth* and R.J. Miller. Dept. of Pharmacol. and Physiol. Sciences, Univ. of Chicago, Chicago, IL 60637

Which Ca^{2+} channels are activated during physiological stimuli and how are these regulated by neurotransmitters? In order to answer these questions we studied the inhibition of calcium currents by noradrenaline (NA, 10μM) NPY (300nM) and ω-conotoxin GVIA (ω-CgTx, 5μM) evoked by action potential wave forms (APW) and regular square wave forms (SW) in neonatal rat sympathetic neurones within 12-36 hours after isolation. Action potentials were recorded in the current clamp mode of whole cell patch-clamp from sympathetic neurones, and the prerecorded action potential wave form was played back to another sympathetic neuron in a sweep, where a 40 ms square wave depolarization to +10 mV from the same holding potential followed the action potential wave form. The magnitude of APW evoked calcium current was 1.55 times higher than the maximum current evoked by SW (n=24). NA inhibited the APW evoked and the SW evoked calcium currents by 53% and 47% respectively (n=11). NPY inhibition was 40% (APW) and 35% (SW)(n=6). ω-CgTx inhibited the APW evoked and the SW evoked calcium currents by 79% and 70% respectively (n=11). Application of 300 nM nifedipine inhibited the residual calcium current seen after ω-CgTx inhibition by a further 30% (n=5). In another series of experiments the action potential wave form was applied 4 and 8 times (40 and 77 Hz action potential train simulation) in one sweep and the inhibition produced by NA and NPY on the APW evoked calcium currents were compared. Increasing the APW numbers in a sweep gave no relief of the calcium current inhibition by NA and NPY. These results imply that the strength of the presynaptic inhibition produced by NA and NPY at high frequency stimulation would not be decreased. Supported by Fogarty International Fellowship to PTT.

463.9

MODULATION OF HIGH THRESHOLD CALCIUM CURRENTS BY NOREPINEPHRINE IN ACUTELY ISOLATED RAT SENSORIMOTOR CORTICAL NEURONS. Foehring, R. C.*, and Lorenzon, N. M., Dept. of Anatomy and Neurobiology, Univ. of Tenn., Memphis, TN 38163.

We used whole cell recordings of acutely isolated neocortical pyramidal cells to study modulation of Ca^{2+} currents by norepinephrine (NE). NE decreased high-threshold Ca^{2+} currents in a dose-dependent manner. 500 nM NE blocked 12 ± 5 % of the whole cell current and 2 μM NE blocked 42 ± 20 % of the current. This effect was rapid (onset within a few s, maximum within 30 s), repeatable and fully reversible. NE reduced Ca^{2+} currents at all ages examined (P1-adult). In adults, ω-conotoxin blocked 37 ± 17 % of the current and greatly reduced the amount of current blocked by NE, suggesting that the effect is at least partly exerted upon N-type channels. NE had no effect on the large, slow tail currents induced by BayK-8644, suggesting that L- type channels were not involved under these recording conditions (Ba^{2+} as charge carrier, NMG internal,10 mM EGTA in pipette to chelate Ca^{2+}).

Specific agonists (isoproterenol, clonidine, phenylephrine) and antagonists (timolol, yohimbine, prazosin) were used to determine the adrenergic receptor subtype involved. These data suggest the involvement of B and α-2 receptors. The effects of NE or isoproterenol were not reduced by prepulses to very positive potentials (+120 mV). In conclusion, NE reduces high-threshold Ca^{2+} currents in neocortical pyramidal cells by multiple mechanisms, involving different receptors, channel types and signalling pathways. Supported by NINDS grant NS27180plk (R.C.F.) and an NIMH predoctoral fellowship (N.M.L)

463.10

POSTNATAL DEVELOPMENT OF HIGH THRESHOLD CALCIUM CURRENTS IN ACUTELY ISOLATED RAT SENSORIMOTOR CORTICAL NEURONS. Lorenzon, N. M.* and Foehring, R. C. Dept. of Anatomy and Neurobiology, Univ. of Tenn., Memphis, TN 38163.

We studied the postnatal development of voltage-gated calcium currents in acutely isolated rat sensorimotor cortical neurons (P0-adult). In adult cortical neurons, high-voltage activated currents were observed in all cells (average peak current amplitude, 890 ± 378 pA; recording conditions: 5 mM Ba^{2+} as charge carrier, NMG internal with 10 mM EGTA). At a given age, we saw considerable variability in the relative proportions of the various current components. The dihydropyridine antagonist, nifedipine (2-10 μM) blocked 31 ± 13% of the high threshold current, and ω-conotoxin (1-2 μM) blocked 37 ± 17%. The dihydropyridine agonist, BayK 8644 increased the peak current by 14 ± 13%, and produced large slow tail currents. ω-agatoxin reduced the peak current by about 20%. There was a current component that was resistant to all three blockers.

Calcium current density increased during the first postnatal week reaching adult levels at about the second postnatal week. At postnatal days 2-4, high threshold calcium currents were small in amplitude (172 ±85 pA), and were composed of a small nifedipine-sensitive component (6 ± 6%), a larger conotoxin-sensitive component (29 ± 34%), and was dominated by a nifedipine- and conotoxin-resistant component. The relative proportions of these three components to the high threshold current reach adult values at approximately 1 week of age. Supported by NINDS grant NS27180 (R.C.F.) and an NIMH predoctoral fellowship (N.M.L.).

463.11

NOREPINEPHRINE MODULATION OF CALCIUM CURRENT RECORDED IN THE CELL-ATTACHED PATCH CONFIGURATION. Keith S. Elmslie*, Paul J. Kammermeier & Stephen W. Jones. Depts. of Physiology & Biophysics and Neuroscience, Case Western Reserve University, Cleveland, OH 44106.

Norepinephrine (NE) inhibits ~50% of N-type calcium current when recorded in the whole-cell patch configuration. This inhibition is voltage-dependent since it can be reversed by strong depolarization (facilitation). However, Delcour & Tsien (*Science* 259: 980, 1993) found little, if any, voltage-dependence of NE modulation of N-channels recorded in the cell-attached patch configuration. We recorded from cell-attached patches containing many channels (macropatches) to determine if voltage-dependent modulation could be observed.

Nine of 15 patches recorded with 100 μM NE in the pipet showed the facilitation expected from modulation (Fig.). For the 9 responsive patches, the postpulse/prepulse ratio ranged from 1.2-1.6 at peak current, compared to 0.9-1.1 in unresponsive patches. The range for control patches (n=6) was 0.9-1.1. Desensitization may explain the absence of observed modulation in unresponsive patches. Alternatively, it is possible that N-channels in some patches are not coupled to adrenergic receptors.

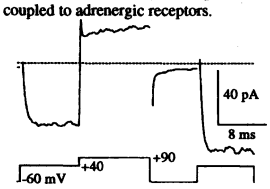


Fig. The trace is an average of 4 runs. The pipet solution contained (mM) 90 $BaCl_2$, 10 TEA-Cl, 10 NMG-HEPES & 100 μM NE.

463.12

DOPAMINE MODULATES SINGLE CHANNEL PROPERTIES OF L-TYPE CALCIUM CHANNELS IN WHITE BASS CONE HORIZONTAL CELLS. C. Pfeiffer-Linn* and E.M. Lasater. Dep't. Ophthalmology, Univ. of Utah, Salt Lake City, UT.

White bass cone horizontal cells contain a voltage-activated calcium current which resembles a sustained L-type current. Recently, using whole cell patch clamp techniques, we have found that dopamine enhanced this L-type current through the D1 type receptor via the cAMP second messenger system (Pfeiffer-Linn and Lasater, J. Gen. Physiol., In Press). Here, we report the effect of dopamine on the single channel properties of the L-type calcium channel using outside-out excised patches obtained from isolated cultured white bass cone horizontal cells. Under pharmacological conditions favoring the sole expression of L-type calcium currents, dopamine significantly effected channel open and close times. Distributions for both the open and close times were best fit with 2 exponentials. Dopamine significantly increased the mean open times by 38% for τ1 and by 35% for τ2 from control conditions. As a result, calcium conductance through channels increased by an average of 138%. Dopamine also increased the frequency of channel openings by an average of 225% from control. Similar results were obtained using specific D1 receptor agonists and a cAMP activator. We conclude that dopamine potentiates the activity of L-type calcium channels in bass cone horizontal cells by increasing the amplitude of calcium current through individual channels and by altering the kinetics of the ion channel to favor the open state.

Supported by NRSA EY06246, NEI EY05972 and grants from Research to Prevent Blindness, Inc.

463.13

EFFECTS OF NERVE GROWTH FACTOR (NGF) ON ACUTE ETHANOL INHIBITION OF CALCIUM (Ca^{+2}) CURRENTS INVOLVE MULTIPLE PROCESSES IN PC12 CELLS D. Mullikin-Kilpatrick* and S. N. Treistman. Department of Pharmacology, University of Massachusetts Medical Center, Worcester, MA. 01655.

Differentiation with NGF requires a period of days before many of the morphological and intracellular changes associated with this treatment occur. NGF treatment (50ng/ml for 7-10 days) of PC12 cells caused a decrease in acute ethanol (25 mM) inhibition of high voltage-activated Ca^{+2} channels from ~45% in undifferentiated (UND) cells to ~17% in treated cells. We examined the time-course of this reduction in ethanol sensitivity, using whole-cell patch clamp techniques. The decrease in sensitivity occurred in two phases. Inhibition of currents in cells treated with NGF for 15 min was comparable to inhibition in UND cells, but fell to ~26% of UND values after a 2 hr exposure to NGF. This spike of insensitivity to ethanol was followed by a return to values indistinguishable from UND values after a 5 hr exposure to NGF. Further exposure (10-240 hrs) led to a second phase which was characterized by a steady decline in inhibition to ~37% of UND values. Ca^{+2} channel sensitivity to ethanol returned to UND cell levels after removal of NGF. We also examined the ability of dbcAMP to affect ethanol inhibition. A 2 hr exposure to 0.5mM dbcAMP caused a sharp drop in inhibition, similar to the transient effect seen after NGF treatment. However, the second phase of reduced sensitivity seen with NGF treatment was absent. These results suggest that the effects of NGF on acute ethanol inhibition of Ca^{+2} currents involve multiple processes with varying time courses. Supported by NIH grant AA05542

463.14

NGF MAY DIRECTLY ACTIVATE VOLTAGE INDEPENDENT CALCIUM CHANNELS IN PC12 CELLS. S.A. Scott* and J.A. Strong, Dept. of Biol. Sci., Purdue University., W. Lafayette, IN 47907.

Voltage independent calcium channels have been described in a variety of cell types. This broad class of Ca channels includes channels that are regulated by either extracellular or intracellular ligands. We reported that PC12 cells contain two novel voltage independent Ca channels that are usually inactive in cell-attached patches which could be activated by patch excision or incubation with pertussis toxin[1]. The question still remains as to what the physiological regulator(s) of these Ca channels are. PC12 cells differentiate when incubated with nerve growth factor (NGF), changing from a proliferating secretory cell to a "sympathetic neuron". Among the many effects of NGF on PC12 cells are very rapid morphological changes which do not require protein synthesis and long term effects such as neurite outgrowth which do require protein synthesis. One of the early effects of NGF is a rapid, transient influx of Ca which occurs at least in part through voltage independent pathways[2,3]. These rapid Ca changes have been measured using Ca sensitive fluorescent dyes and ^{45}Ca influx across the plasma membrane. The single channel basis for this Ca influx is still unknown. Using single channel recording techniques we find that two voltage independent Ca channels are active in cell-attached membrane patches when NGF (50-100 ng/ml) is included in the recording pipette (10 of 17 patches $p<.1$ when compared to 14 control patches). These two NGF-activated channels share several characteristics with the PTX- and excision activated channels: they have the same conductances (3-5 and 16 pS in 20mM Ca), long open times, and lack of voltage dependence. In contrast, when NGF was added to the bath solution no channel activity was seen in cell-attached patches (n=3), suggesting the NGF effect is membrane delimited. These two channels may be subject to both positive and negative regulation, through multiple converging pathways. [1] Soc. Neurosci. Abstr 18:1270. [2]A.P. Alonso *et al*., FEBS Lett 208: 48-51. [3]A. Kozak *et al*., J. Neurosci. Res. 33(1): 30-36.

463.15

EPIDERMAL GROWTH FACTOR (EGF) STIMULATES THE FUNCTIONAL EXPRESSION OF HIGH-THRESHOLD CALCIUM CHANNELS IN CLONAL PITUITARY CELLS. U. Meza, A. Navarrete, A. Marin and G. Cota*. Dept. of Neurosciences, Cinvestav, Mexico, DF 07000.

Chronic treatment of GH_3 cells and related rat pituitary cell lines with EGF stimulates prolactin synthesis. We investigate here whether EGF regulates the functional activity of voltage-dependent Ca channels. GH_3 cells were cultured for 5-6 days under standard conditions or in the presence of 5 nM EGF. Patch-clamp experiments were then carried out to record whole-cell Ca^{2+} currents (5 mM external Ca^{2+}; HP -80 mV). Peak current at +20 mV was -95±12 pA in control cells (mean±SE, n=20) and -208±19 pA in EGF-treated cells (n=18). Cell capacitance was 11.2±0.7 and 13.7±0.6 pF, respectively. Analysis of tail Ca^{2+} currents revealed a selective increase in the activity of high-threshold Ca channels in the EGF-treated cells, with no significant change in channel closing kinetics. Such an effect was long-lasting, and gradually decreased following EGF withdrawal; full recovery took 3-5 days. Regulation of Ca channel expression by growth factors may be a general mechanism for producing long-term changes in the secretory behavior of endocrine cells.

463.16

NEURAL REGULATION OF Ca CHANNEL EXPRESSION IN PITUITARY MELANOTROPES OF NEONATAL RATS. J.C. Gomora*, A. Navarrete, A. Marin and G. Cota. Dept. of Physiology, Biophysics and Neurosciences, Cinvestav, Mexico, DF 07000.

The dopaminergic innervation of the intermediate lobe (IL) of the rat pituitary is first detected on postnatal day 3, and is well developed 9 days later (Gary and Chronwall, *Int. J. Devl. Neurosci.* 10:131, 1992). Here, we show a clear reduction on the activity of high-threshold Ca channels in the endocrine cells of the IL (melanotropes) concomitant with the onset of innervation. IL cell cultures were obtained from 2- or 12-day-old rats (PN2 and PN12 cells, respectively). Whole-cell Ca^{2+} currents were recorded from single melanotropes after 5-22 h in culture by using the patch-clamp technique. In the presence of 10 mM external Ca^{2+}, peak Ca^{2+} current at +20 mV (HP -80 mV) was -80±9 pA in PN2 cells (mean±SE, n=16) and -31±5 pA (n=14) in PN12 cells. Cell capacitance was close to 4.3 pF in both cell groups. The high-threshold Ca^{2+} current was 2.3-fold larger in PN2 cells than in PN12 cells, as determined from tail current measurements. The results suggest that Ca channel expression in melanotropes is drastically inhibited following innervation.

463.17

PREFERENTIAL INHIBITION OF ONE KINETIC COMPONENT OF L-TYPE CALCIUM CHANNEL CURRENT BY TRH IN GH_3 CELLS. D.M. Fass* and E.S. Levitan Depts. of Behavioral Neuroscience and Pharmacology, Univ. of Pittsburgh, Pittsburgh, PA 15260.

Thyrotropin releasing hormone (TRH) inhibits L-type calcium channels in GH_3 cells. To test the voltage dependence of this inhibition, whole cell patch clamp barium tail currents were measured under conditions which isolated L-current. Following a 10 ms depolarization to a maximally activating potential (+50 mV), the tail current measured at -40 mV decayed with a time constant of ~150 μs. Application of the dihydropyridine agonist BAY K 8644 (1 μM) shifted the activation curve to the left by 15 mV and slowed the tail current decay. The BAY K 8644 modified tails measured at -40 mV were fit by two exponentials with time constants of 1.79 ± .29 ms and 7.86 ± 1.56 ms (n = 10). TRH (1 μM) reduced the amplitude of the tail currents by ~25% in a voltage-independent manner both in the presence and absence of BAY K 8644. TRH-inhibited tail currents had altered decay kinetics from controls. Specifically, TRH preferentially reduced the amplitude of the slow component of the decay (by 53%, p = .001), while only slightly reducing the fast component (by 20%, p = .04) The time constants of the tails were not altered by TRH. Preliminary evidence indicates that the two components of the tail may be differentially sensitive to BAY K 8644. One possible conclusion is that two subtypes of L-channels are expressed in GH_3 cells with different sensitivities to TRH and dihydropyridines.

463.18

SUBCELLULAR LOCALIZATION OF NITRIC OXIDE SYNTHASE AND MODULATION OF CALCIUM CHANNELS BY NITRIC OXIDE IN ROD PHOTORECEPTORS. D.E. Kurenny[1], L.L. Moroz[2] and S. Barnes[1]*, Neuroscience Research Group, University of Calgary, Alberta, Canada T2N 4N1[1] and Department of Physiology, Byelorussian State University, Minsk, Belarus[2].

Two subcellular compartments in the outer retina of tiger salamander were identified as likely sites of nitric oxide (NO) production using NADPH-diaphorase histochemistry and NO synthase immunocytochemistry. One, within the photoreceptors, is the ellipsoid region of both rods and cones, the other, surrounding the photoreceptor cell bodies, is confined to the most distal region of Müller (glial) cells. This pattern of staining suggests a role for NO in photoreceptor function, possibly via intra- or trans-cellular modulation of inner segment ion channels.

The actions of NO on the barium conducting L-type Ca channel of rods were investigated via superfusion of NO-generator S-nitrosocysteine (SNC) using the nystatin perforated patch technique. First applications of fresh NO-containing solutions increased barium current by shifting the Ca channel activation curve on the voltage axis. SNC (100-200 μM) shifted activation by -4.1 ± 0.8 mV (n=6, SE) (*e.g.* in the negative direction). Second and third applications of SNC produced shifts of -0.7 ± 0.4 mV and -0.3 ± 0.4 mV, respectively. No change in leak current at the holding potential of -60 mV occurred in barium-containing saline. Degassed solutions were used as controls and had no statistically significant effects (shift of +0.3 ± 0.6 mV). As adjustments in Ca channel activation translate into significant changes in synaptic output, NO could act to alter transmission from photoreceptors. *We thank T. Dawson and S. Snyder for antibodies against rat cerebeller NO synthase. Supported by the Medical Research Council and the Alberta Heritage Foundation for Medical Research.*

463.19

NITRIC OXIDE MODULATES Ca^{2+} CURRENTS OF SUPERIOR CERVICAL GANGLION NEURONS. Chu Chen* and Geoffrey G. Schofield, Department of Physiology, Tulane University School of Medicine, New Orleans, LA 70112.

Increased evidence suggests that nitric oxide (NO) is a central and peripheral neuronal messenger or a new class of neurotransmitter. Recent experiments show that NO is involved in modulation of norepinephrine (NE) release, but the results are quite controversial. It has been reported that NO inhibits NE release from sympathetic nerve terminals, whereas others show that NO synthesis inhibitors decrease the release of NE. We observed recently that NO donors enhance the amplitude of neuronal Ca^{2+} currents which could underly enhanced exocytotic release of NE. In the present study we investigated possible mechanism(s) of NO induced enhancement of Ca^{2+} channel currents in rat sympathetic neurons using the whole-cell patch-clamp technique. 500 μM of the nitric oxide donors, sodium nitroprusside (SNP) and S-nitroso-N-acetylpenicillamine (SNAP) increased Ca^{2+} current amplitudes by either extracellular or intracellular application. Intracellular application of 1 mM cGMP or 100 μM M & B 22948 (a cGMP phosphodiesterase inhibitor) also enhanced the amplitude of Ca^{2+} currents. 100 μM methylene blue decreased the SNP (500 μM) induced enhancement of Ca^{2+} currents. In addition, SNP and SNAP, as well as cGMP prevented NE-induced inhibition of Ca^{2+} currents and reduced NE-induced facilitation of Ca^{2+} current amplitude produced by a depolarizing conditioning pulse. The results suggest that NO may facilitate the release of NE from the sympathetic neurons and that NO induces enhancement of Ca^{2+} currents via stimulation of cGMP formation. (Supported by PHS grant HL-43656)

CHLORIDE AND OTHER ION CHANNELS

464.1

COMPLEMENTARY DNA CLONING OF A CYCLIC NUCLEOTIDE ACTIVATED CATION CHANNEL FROM CHICK BRAIN AND HEART. Leslie C. Timpe* and Katherine A. Logee, Cardiovascular Research Institute, Box 0572, UCSF, San Francisco, California 94143.

Ion channels that select for cations and that are activated by membrane hyperpolarization have been identified in vertebrate photoreceptors, in various central neurons, and in cardiac pacemaker tissue. The cardiac channel is activated directly by cAMP (DiFrancesco and Tortora, Nature 351:145). Sensory transduction channels of vertebrate photoreceptors and olfactory epithelia are also activated by cyclic nucleotides and are cation-selective. We designed degenerate primers for the polymerase chain reaction (PCR) from the sensory transduction channel amino acid sequences, and used them in PCR reactions with cow, Xenopus tadpole and chick heart cDNA as template, in an attempt to clone complementary DNA for a hyperpolarization-activated channel. The PCR reactions yielded 800 base pair products that are approximately 80% identical to the sensory transduction channels in predicted amino acid sequence. Regions of identity include part of a cyclic nucleotide binding domain; there is also limited sequence similarity to a potential pore-lining sequence in Shaker K^+ channels. Northern blots of chick brain, heart, limb and liver A^+ RNA demonstrate a signal of 3.5 to 4 kb in each organ. The PCR fragments are being used for in situ hybridization to identify the tissues that express the message, and for screening cDNA libraries.

464.2

HYPOTONIC STRESS INDUCES A CA^{2+} INDEPENDENT INWARD CURRENT IN HSG-PA CELLS. S. Fatherazi,* K. Izutsu, R. Wellner & C. Belton, Oral Biology, Univ. of Washington, Seattle, WA 98195, & Toxinology Div., USAMRIID, Ft. Detrick, Frederick, MD 21701.

The whole cell patch-clamp technique was used to characterize the inward current activated by hypotonic stress in HSG-PA cells. Cells were perfused with a medium containing (mM): 140 N-methyl-D-glucamine (NMG) Cl, 2 $CaCl_2$, 5 KCl, 1 $MgCl_2$, pH=7.4, and dialyzed with (mM): 135 CsCl, 2 NaATP, 2 $MgCl_2$, 0.1 EGTA, 0.2 GTP, pH=7.4. Cell conductance changes were monitored using a holding potential of -39 mV and 100 msec pulses to 0 mV, the equilibrium potential for Cl ion and -90 mV, the equilibrium potential for K ions. I-V curves were generated using a staircase of 10-20 mV voltage steps of 10 msec duration. Replacement of internal K^+ with Cs^+ and external Na^+ with NMG^+ minimized the K^+ and nonspecific cationic currents. Then, exposing cells to 20% to 50% hypotonic solutions induced an inward current. This current reversed at +3±1 mV (n=5) and shifted towards more positive membrane potential when Cl^- was replaced with the less permeable gluconate ion (45±5 mV, n=5 versus expected reversal potential of +64 mV). Although exposure of Fura-2 loaded cells to 30% hypotonic solutions caused a 3-5 fold increase in intracellular Ca^{2+} concentration, 5-10 mM BAPTA in the pipette did not block the activated current (n=7). Application of 50 μM SITS (4-acetamido-isothiocyanostilbene-2,2'-disulphonic acid), a Cl^- channel blocker, rapidly and reversibly decreased this current by 92.5±2.5% (n=4). These results suggest that hypotonic stress activates a Ca^{2+} independent inward Cl current in HSG-PA cells. Supported by NIDR grant DE09812.

464.3

CALCIUM-ACTIVATED CHLORIDE CURRENTS ELICITED BY FLASH PHOTOLYSIS OF DM-NITROPHEN IN CULTURED RAT SENSORY NEURONES. K.P.M. Currie* and R.H. Scott, Dept. of Physiology, St. George's Hosp. Med. Sch., London SW17 0RE, UK.

We have previously reported that a subpopulation of cultured dorsal root ganglion neurones possess a Ca^{2+}-activated chloride current ($I_{Cl(Ca)}$) which can be activated by release of Ca^{2+} from intracellular stores or by Ca^{2+} influx through voltage-gated Ca^{2+} currents (I_{Ca}). Here we further characterise $I_{Cl(Ca)}$ and report its activation by photorelease of Ca^{2+} from DM-Nitrophen (DM). Cells were voltage clamped using the whole cell recording technique with CsCl based patch pipette solution containing 4mM DM and 2mM Ca^{2+}. Flash photolysis elicited transient inward currents (mean peak amplitude of -417±83pA (n=34)) only in those cells which possessed an inward tail current following I_{Ca}. Flash responses could also be obtained during the decay of the tail currents but were never larger than the peak amplitude of the tail current itself. The flash responses were identified as $I_{Cl(Ca)}$ using the chloride channel blocker niflumic acid (10μM) which inhibited the current by 69±3% (n=5) and by extracellular anion substitution. Our results suggest that under these conditions, at least at negative holding potentials, $I_{Cl(Ca)}$ decay is due to deactivation rather than inactivation, but at depolarised holding potentials only comparatively small flash induced outward currents were ever seen (even at +90mV) suggesting inward rectification of the flash induced current.

464.4

Neurokinin-3 receptors coupled to a chloride conductance in guinea pig myenteric neurons. P. P. Bertrand*, J. J. Galligan. Dept. Pharmacol./Toxicol. Michigan State University, E. Lansing, MI 48824.

In the myenteric plexus, substance P (SP), acting at neurokinin-3 (NK-3) receptors, is a putative mediator of the slow excitatory post-synaptic current (sEPSC). The sEPSC is due to inactivation of resting calcium-activated potassium conductance ($G_{K,Ca}$) and activation of a non-potassium (non-G_K), inward current associated with a conductance increase. SP and the SP analog senktide (a NK-3 selective agonist) mimic the sEPSC. The purpose of this study was to characterize the conductances coupled to NK-3 receptor activation. Conventional single electrode voltage clamp techniques were used to record membrane currents from single AH-cells. The non-G_K was evident in approx. 25% of neurons and reversed at -8±2 mV with a 2M KCl recording electrode. The non-G_K was unmasked by forskolin, which selectively inhibits $G_{K,Ca}$. Niflumic acid (NFA) (3-300μM), a non-specific chloride channel blocker, inhibited the senktide-activated non-G_K (EC_{50} 30μM). In addition, NFA (30-300μM) caused an increase in $G_{K,Ca}$ (rev:-94mV) that was sensitive to calcium channel blockade and available for senktide-dependent closure. Chloride replacement with isethionate, gluconate or glucose (both Na^+ and Cl^- replacement) first attenuated, then blocked the non-G_K. Replacement of NaCl with choline-Cl had no effect. Pressure applied gamma-aminobutyrate (GABA) (1mM) produced a large chloride conductance (G_{Cl}) that was partially blocked by NFA in a use-dependent fashion and was transiently increased, than decreased by chloride replacement. In summary, these data suggest that NK-3 activation inhibits $G_{K,Ca}$ and activates G_{Cl}. NFA blocked the increase in G_{Cl} caused by both senktide and GABA. In addition, NFA opened a population of $G_{K,Ca}$ that was similar to potassium channels closed by NK-3 activation. (Supported by DK 40210)

464.5

SECRETION-ASSOCIATED MODULATION OF CHLORIDE CHANNELS PRESENT IN SECRETORY VESICLES.

H. Tamir*, J. Piscopo, K.P. Liu, M. Adlersberg, E. A. Nunez, and M.D. Gershon, Philips Electronic, Mahwah, NJ; NY State Psych. Inst. and Dept. of Anat. and Cell Biol. Columbia University, New York, NY.

Parafollicular (PF) cells of the thyroid gland are paraneurons; they are derived from the neural crest and extend neurites when exposed to NGF. PF cells exhibit properties, such as biosynthesis and secretion of 5-HT, that are also manifested by serotonergic neurons. 5-HT, is stored in secretory vesicles together with a nerve-specific serotonin binding protein. PF cells secrete 5-HT in response to stimulation by thyroid stimulating hormone (TSH) or ↑ $[Ca^{2+}]_e$. The interior pH in most of the secretory vesicles of unstimulated cells is near neutral; however, the vesicles acidify prior to exocytosis when the cells are treated with a secretogogue. It has been proposed that this acidification is due to the ATP-driven translocation H^+ into vesicles, which is made possible by the secretogogue-evoked opening of Cl^- channels in the vesicular membrane. This channel opening would permit Cl^- to enter the vesicles and dissipate the membrane potential that would otherwise be generated by the movement of H^+ and prevent further H^+ transport. This hypothesis was tested. We now demonstrate that antibodies raised against a 62 kDa Cl^- channel, purified from renal epithelium, specifically react with a 62 kDa protein in ghosts prepared from isolated secretory vesicles of PF cells. X-ray microanalysis, carried out with an electron microscope, revealed that treatment of isolated PF cells with the secretogogues, TSH or ↑ $[Ca^{2+}]_e$ causes the relative concentration of Cl^- (the Cl^-/sulfur ratio) within secretory vesicles to increase 8-10-fold. The possibility the vesicular Cl channel could be gated by phosphorylation was evaluated. Isolated vesicles were found to exhibit endogenous kinase activity and these kinases phosphorylate the Cl- channel in the presence of ^{32}P-ATP. These observations confirm that a Cl^- channel is present in the membranes of the secretory vesicles of PF cells and directly demonstrate that secretion is preceded by the movement of Cl^- into the vesicles. It is suggested that secretion-acidification coupling in the 5-HT-storing vesicles of PF cells is mediated by phosphorylation of the vesicular Cl- channels. This study was supported by grants MH 37575, DK19743, and NS12969.

464.6

VOLUME-SENSITIVE ANION CHANNELS MEDIATE SWELLING-ACTIVATED INOSITOL AND TAURINE EFFLUX FROM C6 GLIOMA CELLS.

K. Strange* and P. S. Jackson, Div. of Nephrology, Dept. of Medicine, and Dept. of Neurosurgery, Children's Hospital, Harvard Medical School, Boston, MA 02115.

C6 glioma cells, like brain cells *in vivo*, accumulate volume regulatory organic osmolytes such as inositol and taurine in response to chronic hypertonic stress. Upon return to isotonic conditions, the cells minimize osmotic swelling by rapid activation of volume-sensitive transport pathways that mediate the efflux of these solutes. The inositol efflux pathway is a Na^+-independent, passive, unsaturable transport system that is blocked 80-100% by a number of anion transport inhibitors, certain lipoxygenase inhibitors and various polyunsaturated fatty acids. The taurine efflux pathway has characteristics that are identical to those of the inositol efflux mechanism including kinetics of activation and inactivation, osmotic sensitivity, pharmacological sensitivity and inhibition by certain Na^+ and Cl^- substitutes. These results suggest strongly that volume-sensitive inositol and taurine efflux are mediated by a common transport mechanism. The inhibition of the transport pathway by anion transport blockers and unsaturated fatty acids suggests indirectly that efflux of these solutes may be mediated by an anion channel. We tested this hypothesis by performing whole cell patch clamp measurements with symmetrical CsCl solutions. Under hypertonic conditions, C6 cells had an extremely small membrane conductance (< 0.02 nS/pF). Following cell swelling induced by return to isotonic medium, however, a large anion conductance (1.5-2 nS/pF) was activated rapidly. This conductance was outwardly rectified, selective for Cl^- and was inhibited 80-100% by blockers of swelling-activated inositol and taurine efflux. The relative taurine permeability (i.e., $P_{taurine}/P_{Cl}$) of the conductance was 0.2. We conclude that a volume-sensitive anion channel mediates that the efflux of structurally diverse organic osmolytes such as taurine and inositol from the cell. (Supported by NIH grants NS30591 and DK45628, and the American Heart Association).

464.7

PURIFICATION OF PHOTOAFFINITY LABELED AVERMECTIN BINDING PROTEINS FROM *CAENORHABDITIS ELEGANS* AND *DROSOPHILA MELANOGASTER*.

S.P. Rohrer*, E. Birzin, E. Hayes, E. Jacobson and J. Schaeffer, Department of Cellular Biochemistry and Physiology, Merck Research Laboratories, Rahway, NJ 07065

The avermectins are a family of macrocyclic lactones isolated as natural fermentation products from the bacterium *Streptomyces avermitilis* and characterized by their potent anthelmintic and insecticidal properties. This class of compounds displays a high degree of selectivity for invertebrate tissues and while they are known to cause an increase in membrane permeability to chloride ions, the precise mechanism by which they exert their paralytic effects in insects and nematodes has yet to be elucidated. Avermectin binding proteins from the free living nematode, *Caenorhabditis elegans* and from the fruit fly, *Drosophila melanogaster* were identified by photoaffinity labeling with a radioactive azido-avermectin analog. Affinity labeled proteins from both *C. elegans* and *Drosophila* were partially purified under denaturing conditions on Sephacryl S-300 gel filtration columns. The second step in the purification scheme involved the use of a monoclonal antibody against avermectin B_{1a} as an immunoaffinity reagent. ^{125}I-Azido-AVM labeled proteins from *C. elegans* as well as the affinity labeled *Drosophila* protein were immunoprecipitated by the anti-AVM MAb coupled covalently to Sepharose beads. 45 pmol of the *Drosophila* AVM binding protein were purified using this approach.

ACETYLCHOLINE RECEPTORS: MUSCARINIC SUBTYPES

465.1

m5 MUSCARINIC RECEPTORS: AUTORADIOGRAPHIC LOCALIZATION IN RAT AND HUMAN BRAIN.

M.T. Vilaró[1], J.M.Palacios[2] and G.Mengod[1]*. 1) Dpt. Neurochemistry, CID/CSIC, 2) Laboratorios Almirall. Barcelona. Spain.

Five genes (m1-m5) encoding muscarinic acetylcholine receptors have been cloned and their mRNAs detected in brain. However, only 4 subtypes (M_1-M_4) have been described pharmacologically. We have combined in situ hybridization histochemistry and in vitro receptor autoradiography to study the possible existence of m5 receptor proteins in brain. m5 mRNA had a restricted distribution in rat brain. Most notably, it was present in hippocampus, substantia nigra compacta (SNC), ventral tegmental area, lateral habenula (LH), hypothalamic nuclei, and parabigeminal nucleus. In previous autoradiographic studies with ^{3}H-4-DAMP, a reported M_1/M_3 selective antagonist, we observed rather high densities of labelled sites in rat SNC. On the other hand, ^{3}H-4-DAMP has high affinities for m1 and m3 cloned receptors but also for m4 and m5 receptors. Thus, it is possible that ^{3}H-4-DAMP sites in SNC correspond to m5 receptors. We studied the pharmacological properties of ^{3}H-4-DAMP sites in SNC and LH in comparisom with sites in regions enriched in M_1, M_3 and M_4 receptors. The compound AQ-RA 741 was used since it has much lower affinities for cloned m5 receptors than for the other 4 subtypes. Brain sections were incubated with ^{3}H-4-DAMP and increasing concentrations of AQ-RA 741. The compound had much lower affinity for ^{3}H-4-DAMP sites in SNC and LH than for sites in M_1-, M_3- and M_4-rich regions, thus suggesting the presence in rat brain of muscarinic receptor proteins encoded by the m5 gene and with pharmacological properties resembling those of cloned m5 receptors. Therefore, the pharmacological classification of muscarinic receptors can tentatively be expanded to include M_5 receptors. Data from similar experiments in human brain will also be presented.
(Part of this work was done at Sandoz Pharma, Basel, Switzerland)

465.2

m1-m5 MUSCARINIC RECEPTOR DISTRIBUTION AND QUANTITATION IN RAT CNS BY RT-PCR AND HPLC.

J. Wei, A. Milici and J.J. Buccafusco*, Department of Pharmacology and Toxicology, Medical College of Georgia, and the DVAMC, Augusta, GA 30912.

Five muscarinic receptor (MR) (m1-m5) genes have been cloned which encode distinct MR subtypes. Because of their structural homology and pharmacological similarity, ligand binding probes currently available do not clearly distinguish among the subtypes. To obtain a clear distribution within the CNS of molecularly-defined MR subtypes, 7 brain regions were examined for the expression of the respective mRNAs. The most sensitive method for detecting mRNA is through amplification of the respective cDNAs. Brain regions were obtained from male Wistar rats and total RNA was isolated using the RNAzol kit. Next, the extracted RNA was extensively treated with RNase-free DNase to remove any residual genomic DNA. 1μg total RNA was reversed transcribed using random primers and reverse transcriptase. The resulting cDNA was amplified using a thermal cycler for 33 cycles at 95°C for 1 min and 62°C for 1min. The PCR-amplified products were analyzed by gel electrophoresis (1.8% agarose gel containing ethidium bromide) and were visualized with fluorescent illumination. PCR-amplified samples were also injected directly onto an HPLC anion exchange column and quantitated by UV detection. At least 4 of the 5 MR subtypes were found in each brain region examined. The m1 subtype was most abundant in cortex and gradually declined in content caudally to the spinal cord. The m2 subtype was most abundant in thalamus and pons-medulla. The m4 subtype was found in greatest amount in the striatum while m3 and m5 were expressed consistently throughout the CNS. Supported by a grant from the DVAMC.

465.3

PREDICTION OF THE THREE-DIMENSIONAL STRUCTURE OF MUSCARINIC ACETYLCHOLINE RECEPTORS BASED ON STUDIES WITH CHIMERIC m2/m5 RECEPTORS. Z. Pittel and J. Wess*. Natl. Inst. of Diabetes and Digestive and Kidney Diseases, Lab. of Bioorganic Chemistry, Bethesda, MD 20892.

Current three-dimensional models of G protein-coupled receptors are based primarily on high-resolution structural data obtained with bacteriorhodopsin, an integral membrane protein whose molecular structure is characterized by the presence of seven α-helical transmembrane domains (TM I-VII). However, since bacteriorhodopsin does not couple to G proteins and shows very little sequence homology with G protein-linked receptors, the general usefulness of such models remains uncertain.

To gain more direct insight into the three-dimensional structure of muscarinic acetylcholine receptors, we have analyzed the pharmacological properties of a series of hybrid human m2/m5 muscarinic receptors. We found that several of the chimeric constructs studied were unable to bind significant amounts of the radioligand [3H]NMS (drastic reduction in Bmax), following their transient expression in COS-7 cells. A common structural feature of these constructs was that TM I was derived from the m5 receptor, whereas TM VII originated from the m2 receptor. When the N-terminal portion (including TM I) of these hybrid receptors was replaced with m2 receptor sequence, the resulting chimeric receptors were expressed at high levels (as determined by [3H]NMS saturation binding) and displayed ligand binding properties similar to the two wild type receptors. These data strongly suggest that the molecular architecture of muscarinic receptors (and, most likely, other G protein-coupled receptors) resembles that of bacteriorhodopsin in that the seven TM helices are arranged in a ring-like fashion such that TM I lies directly adjacent to TM VII.

465.4

DIFFERENTIAL LOSS OF STRIATAL MUSCARINIC AND DOPAMINE RECEPTOR SUBTYPES AND THEIR mRNAS FOLLOWING SPECIFIC LESION OF DOPAMINE OR CHOLINERGIC NEURONS Z. Zang* and Ian Creese. Center for Molecular & Behavioral Neuroscience, Rutgers, The State University of New Jersey, Newark, NJ 07102 USA.

Quantitative solution hybridization/RNase protection assays (SHRPA) were developed to examine the effects of 6-OHDA lesion of the nigrostriatal dopaminergic pathway or AF64A lesion of striatal cholinergic neurons on the levels of rat striatal muscarinic receptor subtype (m1-m4) and D_2 receptor mRNAs. Muscarinic or dopamine (D_1 and D_2) receptor binding was also determined. Three weeks following 6-OHDA lesion, there was a 19% reduction in striatal muscarinic receptor [3H]QNB binding sites. SHRPA showed that D_2 receptor mRNA was increased (+30%) by the lesion. None of the muscarinic receptor mRNAs were altered. Three weeks after intrastriatal AF64A injection non-M_1 receptor binding sites were decreased by 19%, m2 and m4 receptor mRNAs were reduced by −26% and −36% respectively and the striatal D_2 receptor mRNA was also reduced by −27%. However, AF64A lesion did not alter M_1 receptors, m1 or m3 mRNA or D_1 receptor levels. These results suggest that (1) presynaptic muscarinic receptors are found on dopamine neuron terminals, (2) D_2 receptors and a sub-population of muscarinic receptors (m2 and m4) are expressed by striatal cholinergic neurons and (3) D_1, m1 and m3 receptors are expressed by non-cholinergic striatal neurons.

465.5

CHOLINERGIC AND GABAergic NEURONS IN THE RAT MEDIAL SEPTUM EXPRESS MUSCARINIC ACETYLCHOLINE RECEPTORS (mAChRs). E.A. Van der Zee*, T. Matsuyama and P.G.M. Luiten. Dept. of Animal Physiology, University of Groningen, P.O.Box 14, 9750 AA Haren, The Netherlands.

The interaction of the GABAergic and cholinergic systems in the medial septum is crucial for hippocampal functioning, particularly in relation to the generation of theta rhythms. In the present study we determined whether cholinergic and GABAergic neurons of the medial septum express mAChRs employing the monoclonal antibody M35 raised against purified mAChR-protein. Alternating cryosections of 6 young adult male Wistar rats were immunoprocessed for mAChRs, ChAT, or histochemically stained for AChE. Immunofluorescence doublelabeling was performed for mAChRs and the GABA synthesizing enzyme GAD employing FITC and Phycoerythrin-conjugated antisera, respectively. Numerous medial septal neurons were positively stained for mAChRs, GAD, ChAT and AChE. Adjacent alternating sections revealed that the cholinergic cells express mAChRs. Additionally, GAD-positive cells were found to be mAChR-positive. Quantitative immunocytochemistry showed that approximately half of all mAChR-positive neurons (52.3%; 1115 out of 2132 neurons) belonged to the GABAergic cell group. Most single-labeled mAChR-positive neurons were located in the lateral regions of the medial septum. Conversely, nearly all GAD-positive neurons were immunostained for mAChRs (98.6%; 1296 out of 1314 neurons). In general, the GABAergic, muscarinic cholinoceptive neurons were intensely stained for mAChRs, whereas the non-GABAergic, muscarinic cholinoceptive neurons revealed only weak to moderate levels of mAChR immunoreactivity. The current data show that both GABAergic and cholinergic medial septum neurons express mAChRs, adding anatomical evidence for cholinergic neurotransmission upon both groups of cells.

465.6

MUSCARINIC RECEPTOR SUBTYPES IN RAT COCHLEAR NUCLEUS. Kejian Chen[1], Glen E. Green[2], Hardress J. Waller[3]*, Dennis G. Drescher[2], Donald A. Godfrey[1], Depts. of Otolaryngology[1] and Neurological Surgery[3], Medical College of Ohio, Toledo, OH 43699; and Depts. of Otolaryngology and Biochemistry[2], Wayne State University, School of Medicine, Detroit, MI 48201.

Acetylcholine is a neurotransmitter in centrifugal pathways to the cochlea and cochlear nucleus. The cholinergic receptor type was previously found with pharmacological methods in brain stem slice preparations to be predominantly muscarinic in dorsal cochlear nucleus. Cholinergic effects on spontaneous activities of rat dorsal cochlear nucleus neurons were not mediated primarily by M_1, M_2 or M_3 subtypes of muscarinic receptors. Selective drugs are not available for M_4 or M_5 subtypes.

The polymerase chain reaction (PCR) method was used to analyze the expression of muscarinic receptor genes in cochlear nucleus. Total RNA was extracted with guanidine thiocyanate from the cochlear nuclei of 20-60 day old, male and female Sprague Dawley rats, and treated with DNase. Messenger RNA was isolated with oligo-dT cellulose and reverse-transcribed with oligo-dT_{12-18} primer and RNase H-free reverse transcriptase. The resulting cDNA was amplified by PCR. The reverse-transcription step was omitted in each control group. Specific PCR primers corresponded to published nucleotide sequences for rat mAChRs m_1-m_5. Our preliminary data suggest that the muscarinic acetylcholine receptor subtypes expressed in the rat cochlear nucleus are predominantly m_4 and m_5, which is in agreement with the electrophysiological results.

(Supported by NIH grants DC00172 and DC00156)

465.7

FUNCTIONAL EXPRESSION OF THE RAT M5 MUSCARINIC ACETYLCHOLINE RECEPTOR (mAChR) IN FISH CELLS. C.-F. Liao[1]*, Y. G. Lee[1], L.-G. Chen[2], C.-M. Kuo[2] and J.-L. Wu[1] Institute of Zoology[1], Academia Sinica Taipei, Taiwan 11529, R.O.C; Institute of Fisheries Science[2], National Taiwan University, Taipei, Taiwan 10764, R.O.C.

The purpose of this study was to test whether the rat mAChR can interact with a fish G protein. The gene encoding the rat mAChR subtype M5 was subcloned into the expression vector p91023(B). By utilizing calcium phosphate-mediated transfection, the recombinant M5 DNA construct was co-transfected with a neo gene selection marker into the mAChR-negative chinook salmon embryo CHSE-214 cells. Stable transformants were selected with G418 and tested for acquisition of mAChRs by following appearance of specific binding sites for the muscarinic ligand [N-methyl-^{3}H]-scopolamine. Two transfected cell lines, CHSEM5-1 and -2, stably expressing typical mAChRs were obtained. Stimulation of the M5 receptor with carbachol results in time- and dose-dependent increase in phosphoinositide hydrolysis in the CHSEM5 cells. The results suggest that mammalian mAChRs can functionally couple to fish G proteins.

465.8

BINDING OF [^{3}H]-(R)-QUINUCLIDINYL BENZILATE ([^{3}H]-(R)-QNB) AND PIRENZEPINE TO PIGEON BRAIN MEMBRANES E.C.Kohler*, #W.S.Messer, Jr. and V.P.Bingman. Dept. of Psychology, Bowling Green State Univ., Bowling Green, OH 43403 and #Dept. of Medicinal & Biological Chemistry, College of Pharmacy, University of Toledo, Toledo, OH 43606.

There is evidence suggesting the existence of at least five subtypes of muscarinic acetylcholine receptors (mAChRs). Furthermore, there has been autoradiographic demonstration of heterogeneous distribution of mAChR subtypes in the mammalian brain. The data from other studies (Tietje et al., J. Biol. Chem. 265:2828, '90; J. Biol. Chem. 266:17382, '91) suggests that there may also be more than one type of mAChR in the avian brain. Here we examine the possibility of multiple mAChRs by investigating the binding of [^{3}H]-(R)-QNB and the ability of the M_1 receptor antagonist pirenzepine (PZ) to reduce the binding of [^{3}H]-(R)-QNB in membranes from pigeon brain. An excess of atropine was used to assess non-specific binding in each assay. Saturation curves for [^{3}H]-(R)-QNB indicated a B_{max} of 0.296 pmoles/mg protein and an apparent dissociation constant (K_d) of 39.7 pM. An Eadie-Hofstee analysis suggested biphasic binding of PZ to the receptors in the membrane preparation. There appeared to be a small proportion of receptors (<15%) that bind PZ with very high affinity, with a majority of the receptors having a lower affinity. Current investigations include examination of the distribution of putative mAChR subtypes in the pigeon brain. These data will be useful in determining some of the specific roles of the cholinergic system in studies of avian behavior. This work was supported in part by NS 01493.

465.9

THE BLOCKADE OF M-1 MUSCARINIC RECEPTORS IN THE SUBTHALAMIC NUCLEUS ABOLISHES MUSCULAR RIGIDITY IN RESERPINIZED (PARKINSONIAN) RATS. G. Flores, S. Hernández, A. Sierra, J-L. Góngora-Alfaro, M. Rosales and J. Aceves. Dept. of Physiology, Biophysics and Neurosciences. CINVESTAV-IPN. Apartado Postal 14-740. 07000 México, D.F., México.

Here we have tested the possibility that the action of anticholinergic drugs in Parkinson's disease could be mediated by blocking the excitatory cholinergic action in the subthalamic nucleus. The experiments were done in unanesthetized rats in which a cannula was chronically positioned just in the border of the subthalamic nucleus. After 5-6 days of the positioning of the cannula, reserpine (5 mg/Kg i.p.) was injected. After 2 h of the injection the locomotor activity was measured for 1 h and then, the spontaneous EMG activity of the ipsilateral gastrecnemious-soleus muscle was continuously recorded. All reserpinized rats (n=5) presented a drastic reduction in locomotor activity and all presented spontaneous EMG activity (none normal rat presented EMG activity in the same recording conditions). The injection of the selective antagonist of M-1 muscarinic receptors pirenzepine (1 µg/0.25 µl) into the subthalamic nucleus practically abolished (produced more than 90% fall) the EMG activity (the injection of the same volume of saline did not modified the EMG activity). These results suggest that by blocking the activation of M-1 muscarinic receptors in the subthalamic nucleus anticholinergic drugs control muscular rigidity (judged by the appearance of EMG) in experimental parkinsonism.

(Supported by a grant (0586N-9108) from CONACYT of México)

465.10

DIETARY LIPID MODULATION Of LOW Km GTPase ACTIVITY in STRIATA of MATURE F344 RATS. J.F Kelly,* S.A Erat, J.A. Joseph, R.P. Mason and G.S Roth. GRC/NIA/NIH, Baltimore, MD 21224 and U of Conn Health Sciences Ctr, Farmington, Conn.

To investigate the effects of dietary lipid modulation on muscarinic-cholinergic receptor-G protein linked signal transduction in the striatum, we fed 12 mo (mature) male F344 rats purified diets containing 5% soybean oil (Ctrl), 4.5% cholesterol (Hichol), 7.5% coconut oil/2.5% soybean oil (Hisatf) and 0.5% soybean oil (Lofat) for one month. Animals were sacrificed by decapitation and striata frozen and stored at -80 C. Low Km GTPase activity stimulated by oxotremorine(O) 10^{-5} M and carbachol(C) 10^{-3} M was measured using crude striatal membrane incubated with ^{32}P GTP. For O, all groups differed from Ctrl(Duncans tests p< .05). For C, Hichol and Lofat showed significant decreases from Ctrl (Duncans tests p<.05). Preliminary analysis of small angle X-ray diffraction of cortical synaptasomes from Lofat animals showed a decrease in membrane width, suggesting both structural and functional effects of dietary lipid modulation in mature F344 rats.

ACETYLCHOLINE RECEPTORS: NICOTINIC RECEPTOR EXPRESSION

466.1

Neural Crest Cells Express mRNA Encoding Neuronal Nicotinic Acetylcholine Receptor Subunits. Marthe J. Howard* and Michael D. Gershon, Department of Anatomy/Cell Biology, Columbia University, New York, NY 10032.

The neural crest gives rise to neurons that express a variety of different neurotransmitters and neurotransmitter receptors. The kinds of neuron that differentiate in tissue culture are dependent upon the growth medium. A subset of sensory and enteric neurons and all sympathetic and parasympathetic neurons express neuronal nicotinic acetylcholine receptors (nAChRs) *in vivo*. The factors in the embryonic environment that induce the expression of these receptors or regulate the subunit composition are unknown. The current studies were undertaken to examine the expression of nAChRs on quail neural crest cells developing in culture and to examine the coordinate expression of AChR α and β subunits. Catecholaminergic and cholinergic neurons develop under the growth conditions used. The polymerase chain reaction (PCR) was used to assess the time of expression of mRNA encoding nAChR subunits; PCR primers were designed from the published sequence of the chicken nAChR subunits. mRNA encoding the α3 subunit of the nAChR was present as early as two days in culture while the mRNA encoding the β4 subunit was not detected until the cells had been in culture for 4 days. Northern blot analysis of RNA isolated from neural crest cells grown in culture for 8 or 10 days, using a cDNA probe for the α3 subunit, showed 2.0 kb and 3.5 kb mRNA bands; the 2.0 kb mRNA is not present at 8 days in culture but was detectable by 10 days. Cloning and sequencing of α3 PCR products demonstrated that the quail sequence is 94% homologous at the nucleotide level and 100% homologous at the amino acid level to the chicken sequence. Expression of mRNA encoding *c-jun* did not correlate with the expression of nAChR subunits, but did coincide with the appearance of dopamine-β–hydroxylase, a marker relevant to the noradrenergic phenotype. The data suggest that AChR subunit expression is regulated early in neural crest cell development.

This work was supported by NIH grants, HD 28184 and NS 15547.

466.2

POSTNATAL EXPRESSION OF α4 AND β2 SUBUNITS OF THE NEURONAL ACETYLCHOLINE NICOTINIC RECEPTORS IN THE RAT BRAIN. M. Cimino, P. Marini, S. Colombo, F. Cattabeni, N. Brunello*, D. Fornasari and F. Clementi. Inst. of Pharmacol. Univ. of Urbino 61029 Urbino, Dept. of Pharmaceutical Sci. Univ. of Modena, Inst. of Pharmacol. Sci. and CNR Center of Cytopharmacol. Univ. of Milan, Milan, Italy.

Molecular cloning of neuronal acetylcholine receptors (nAChRs) brought to the identification and anatomical localization of mRNAs for several α and β subunits which can generate many subtypes of nAChRs with diverse functional and pharmacological properties. The nAChR formed by the α4β2 subunits is the major brain ACh receptor with high affinity for 3H-L-nicotine. In this study we report the ontogenetic regulation of α4 and β2 mRNAs in selected areas of the rat brain using in situ hybridization. Brains at 1,7,14, 28,56 and 90 postnatal days (PN) were sectioned and hybridized with 35S-labeled riboprobes. At birth high levels of transcripts were already detected although an uneven distribution of α4 and β2 mRNAs was observed. Quantitative analysis revealed that there was a different rate at which the two messages developed in the brain regions. In the hippocampus, a differential ontogenetic expression of the two subunits was observed. Their expresion remained unchanged from birth to aulthood in the Ammon's horn, whereas in the dentate gyrus an age-related increase was shown. In the thalamus, the medial , lateral and ventral nuclei showed a decrease of the β2 signal from birth to PN14 which, then, rise again to reach its plateau atPN56. A similar pattern was observed in the medial habenula for both probes. In contrast, the expression of α4 showed only a modest increase in the medial and lateral nuclei from birth to PN14. The pattern of distribution for α4 and β2, in the thalamic nuclei, was compared with that of the high affinity nicotine binding sites. It is concluded that the expression of both β2 and α4 is differentially regulated during brain maturation and that, at least in the thalamus, it doesn't coincide with the development of the 3H-L-nicotine binding sites.

Partially supported by CNR grant n.91.00124.PF41 (targeted project "Prevention and Controlof Disease Factors"- subproject 2).

466.3

REGULATION OF NICOTINIC ACETYLCHOLINE RECEPTOR TRANSCRIPT LEVELS IN CHICK CILIARY GANGLION NEURONS *IN SITU*. M. Schwartz Levey*, C. Brumwell and M.H. Jacob. Worcester Foundation for Experimental Biology, Shrewsbury, MA 01545.

Nicotinic acetylcholine receptors (AChRs) mediate chemical synaptic transmission in the chick ciliary ganglion (CG) and synaptic AChRs in the CG are composed of α3, α5 and β4 subunits. To gain insights into the events that influence the formation, maintenance and function of synapses in the CG, we have been examining the role of cell-cell interactions in regulating α3, α5 and β4 subunit mRNA levels. We have previously established that the disruption of afferent inputs or efferent connections in the CG of newly hatched chicks results in a 3- to 4-fold decline in α3 mRNA levels. We have now examined α5 and β4 mRNA levels in denervated and axotomized CGs of posthatch chicks using RT-PCR with mutated AChR cRNA internal standards.

Alpha5 and β4 subunit mRNA levels are decreased 3- to 4-fold in denervated ganglia as compared to contralateral control innervated ganglia after 9 days. Five days after axotomy, α5 and β4 mRNA levels are also reduced 3- to 4-fold relative to contralateral control ganglion values. Further, α3 and β4 mRNA levels are decreased 1.5- to 2-fold in embryonic CG neurons that have developed in the absence of these cell-cell interactions following surgical removal of the preganglionic nucleus or the target tissues in the eye prior to synapse formation. These results demonstrate that presynaptic inputs and retrograde signals from the target tissue induce increases in and maintain AChR α3, α5 and β4 subunit mRNA levels in CG neurons *in situ*.

Supported by NIH 21725, the Pfeiffer Fdn and MDA.

466.4

THE EFFECT OF CHOLINERGIC DEAFFERENTATION ON HIPPOCAMPAL NEURON RESPONSIVENESS TO NICOTINE. C.J. Frazier* and G.M Rose. Neuroscience Training Program, UCHSC, and Medical Research, VAMC, Denver, CO 80220

Hippocampal CA1 pyramidal neurons demonstrate an increased responsiveness to nicotine in aged rats (Engstrom et al., *Neurobiol. Aging*, in press). In the periphery, such a change occurs following deafferentation. We therefore decided to test the hypothesis that the loss of cholinergic input to the hippocampus would result in an increase in sensitivity to nicotine. Electrolytic lesions of the medial septal area were made in young male Fischer 344 rats. Approximately thirty days later, the animals were anesthetized with pentobarbital and nicotine was locally applied to electrophysiologically identified CA1 pyramidal neurons using pressure microejection. Neurons recorded from rats with septal lesions required significantly lower doses of nicotine than control animals to demonstrate a similar excitation. This result supports the hypothesis that an age-related loss of cholinergic input could mediate the observed increase in CA1 pyramidal neuron sensitivity to locally applied nicotine. Interestingly, the lesion-induced increase in sensitivity to nicotine was not as great as that previously observed in the aged population. Based on the assumption that the cholinergic denervation was greater in the lesioned than in the aged rats, these results suggest that some factor other than (or in addition to) simple deafferentation must be involved in the age-related increase in nicotine sensitivity. One possibility is an age-related change in nicotinic receptor subunit composition, leading to differences in receptor conductance.

466.5

NERVE GROWTH FACTOR INCREASES THE TRANSCRIPTIONAL ACTIVITY OF THE NEURONAL NICOTINIC ACETYLCHOLINE RECEPTOR β4 SUBUNIT PROMOTER IN PC12 CELLS M. Hu*,[1,2] and P.D. Gardner[1]. [1]Institute of Biotechnology, University of Texas Health Science Center, San Antonio, TX 78245 and [2]Program in Biochemistry, Dartmouth Medical School, Hanover, NH 03755.

Neuronal nicotinic acetylcholine receptors (nAChR) play a critical role in synaptic transmission in the nervous system. However, little information is available concerning the molecular mechanisms regulating expression of nAChR. We have begun studying the transcriptional regulation of the nAChR genes during neuronal differentiation using nerve growth factor (NGF) treatment of the rat PC12 cell line as a model system. PC12 cells respond to NGF treatment with a broad spectrum of changes and the conversion to a sympathetic neuron-like phenotype. Included in this spectrum is an increase in the number of nAChR on the cell surface and increases in the steady state levels of mRNA encoding the α3, α5, α7, β2 and β4 subunits. We investigated the possibility that these increases are a consequence of increased transcription of the receptor genes, focussing on the β4 subunit gene. We isolated a 2.3-Kb 5'-flanking region of the β4 gene and localized the β4 transcription initiation site within this region. We demonstrated that this 2.3-Kb fragment is capable of activating transcription of a reporter gene in transfected neuronal cell lines. The transcriptional activity of this fragment is up-regulated approximately 5-fold following treatment of PC12 cells with NGF. These results are consistent with the observed increase of endogenous β4 mRNA levels in PC12 cells upon NGF treatment.

466.6

NGF INCREASES ACH RECEPTOR GENE EXPRESSION AND CURRENT DENSITY IN WILD TYPE AND PROTEIN KINASE A-DEFICIENT PC12 CELLS L.P. Henderson, M.I. Gdovin, C.L. Liu, P.D. Gardner* and R.A. Maue. Depts of Physiology and Biochemistry, Dartmouth Medical School, Hanover, NH 03755.

Neuronal nicotinic acetylcholine receptors (nAChR) play a key role in synaptic transmission and information transfer in the nervous system. In order to investigate the molecular mechanisms that govern the expression of nAChR and determine their functional properties, we have investigated the nerve growth factor (NGF)-mediated regulation of nAChR expression in rat pheochromocytoma (PC12) cells and protein kinase A (PKA)-deficient PC12 cells (123.7 cells; Ginty et al. J. Biol. Chem. 266:15325). Northern blot analysis of total RNA detected mRNAs encoding the α3, α5, α7, β2, and β4 subunits in PC12 and 123.7 cells. In contrast, mRNAs encoding the α2, α4, α6 and β3 subunits were not detected. NGF treatment increased the steady state levels of the α3, α5, α7, β2, and β4 subunit mRNAs by 2- to 3-fold in both cell types. The NGF-dependent increases in the steady state levels of nAChR mRNAs were correlated with a 2.5-fold increase in the occurrence of ACh-induced single channel activity in excised outside-out patches. Nearly all ACh-induced events in both untreated and NGF-treated PC12 and 123.7 cells could be attributed to a single class, which had a slope conductance of ~46 pS and an average burst duration of ~14 ms at -80 mV. In both PC12 and 123.7 cells, ACh-induced macroscopic current density (at -80 mV) was also increased ~2.7-fold by NGF treatment, from ~16 pA/pF to ~44 pA/pF. There were no significant differences between the kinetic properties of the macroscopic currents elicited in PC12 cells and 123.7 cells, as indicated by the time-to-peak current (~100 ms), time constants of the fast and slow components of the current decay (~0.35 and 5 sec) and the relative contribution of the fast component to the total macroscopic current decay (~75%). The results indicate that the NGF-dependent increase in ACh sensitivity in PC12 cells is correlated with increased expression of specific nAChR subunit mRNAs and that a deficiency in PKA activity does not alter the constitutive expression, NGF-stimulated expression or functional properties of nAChRs in these cells. Supported by NIH NS28668 (LPH), NS30243 (PDG), NS28767 (RAM), AHEI, ALSA (LPH), Alfred P. Sloan Foundation (RAM).

466.7

CALCIUM-DEPENDENT REGULATION OF NICOTINIC ACETYLCHOLINE RECEPTOR GENE EXPRESSION. D. W. Walke and Daniel Goldman.* Dept. of Biol. Chem., Mental Health Res. Inst., Univ. of Michigan, Ann Arbor, MI 48105.

We have previously shown that raising intracellular levels of cAMP will reverse the effects of muscle depolarization on nAChR gene expression (Chahine et al., JBC 268:2893; 1993). In addition, we have found that raising intracellular levels of calcium will decrease the level of nAChR gene expression found in inactive muscle (Soc. for Neurosci. abstract #189.1 p 436, 1992). Therefore, both cAMP and calcium may be mediating the activity-dependent expression of nAChRs in rat skeletal muscle. We now report that the sequences of the nAChR δ-subunit and ε-subunit promoters that mediate calcium regulation map to regions within 102 and 108 nucleotides, respectively, upstream of the start site of transcription. Interestingly these same regions contain sequences conferring activity-dependent regulation on the δ-subunit gene (Chahine et al., Development 115:213; 1992) and synapse-specific expression of the ε-subunit gene (Dulcert et al., PNAS 90:3043; 1993). Internal deletions and site-directed mutagenesis have further localized the calcium-dependent regulatory element in the δ-subunit promoter to a small region containing a MyoD binding site consensus sequence.

466.8

EFFECTS OF DIBUTYRYL CYCLIC AMP OR FORSKOLIN ON MUSCLE- OR GANGLIA-TYPE NICOTINIC RECEPTOR FUNCTION ARE CYCLIC AMP INDEPENDENT. Merouane Bencherif* and Ronald J. Lukas. Division of Neurobiology, Barrow Neurological Institute, 350 West Thomas Road, Phoenix, Arizona 85013, USA.

Dibutyryl cyclic AMP (dbcAMP) and forskolin (FSK) have been widely used as pharmacological tools to implicate cyclic AMP (cAMP)-dependent mechanisms in cellular events. Our studies on muscle- or ganglia-type nicotinic receptor (nAChR) regulation now suggest that effects of dbcAMP or FSK on nAChR expression are unrelated to their putative mimickry of cAMP-dependent signaling. dbcAMP-stimulated downregulation of surface and total nAChR numbers takes hours to develop, reverses with a $T_{1/2}$ of days, is transcriptionally-based, but reflects effects of sodium butyrate alone. At concentrations commonly used to activate adenylyl cyclase (between 5 and 50 uM), FSK acts acutely (within seconds) to non-competitively antagonize nAChR function and to *inhibit* agonist-induced desensitization of nAChR without affecting nAChR numbers. By contrast, studies with 8-(4-chlorophenylthio)-cAMP and modulators of adenylyl cyclase indicate that activation of cAMP-dependent pathways has no effect on agonist-stimulated receptor function and no effect on total nAChR numbers, but induces a 40% increase in cell surface nAChR within 1 day of drug treatment. These results indicate that effects of dbcAMP or FSK are independent of those due to cAMP and are consistent with cAMP-dependent recruitment of intracellular nAChR to the cell surface.

466.9

COOPERATIVITY BETWEEN CYCLIC AMP- AND C-KINASE-DEPENDENT PROCESSES IN THE REGULATION OF NICOTINIC ACETYLCHOLINE RECEPTOR EXPRESSION AND FUNCTION. Ronald J. Lukas* and Merouane Bencherif. Div. Neurobiol., Barrow Neurological Inst., 350 West Thomas Road, Phoenix, Arizona 85013, USA.

We previously have reported that nAChR expression and function in TE671/RD cells can be modulated through stimuli involving transcriptional, translational and/or posttranslational mechanisms (Bencherif and Lukas, Mol. Cell. Neuro., 2: 52-65, 1991; J. Neurochem. in press). Here we report that activation of protein kinase C (PKC) with PMA or mezerein produces (i) immediate enhanced desensitization of nAChR function, (ii) delayed down-regulation of surface nAChR (hours), and (iii) a later increase in total but not surface nAChR numbers (days). By contrast, activation of cAMP-dependent signaling (see Bencherif and Lukas, this volume) enhances recruitment of nAChR from intracellular to cell surface pools. Synchronous activation of PKC and cAMP-dependent pathways produces (i) immediate enhanced desensitization of nAChR function and (ii) an **early** increase in total receptor number (200% at 1 day) followed by (iii) a **marked increase in cell surface receptor** (400% at 2 days). These data and Northern blot analyses suggest that PKC effects are mediated via both transcriptional and posttranslational mechanisms whereas cAMP effects are limited to changes in posttranslational processing of intracellular nAChR and/or nAChR subunits.

466.10

PROTEIN KINASE A MODULATES NICOTINIC RECEPTOR mRNAs IN PC 12 CELLS. TC Madhok*, HS Beyer and BM Sharp, Endocrine-Neuroscience Research Lab., Minneapolis Medical Research Foundation and Dept. of Medicine, Hennepin County Medical Center and University of Minnesota, Minneapolis, MN 55404

To delineate mechanisms regulating the expression of neuronal nicotinic cholinergic receptors (nChRs), we studied cAMP-dependent second messenger systems. We reported previously that specific ^{3}H-nicotine binding was upregulated (up to 4-fold) on PC 12 cells grown in dibutyryl-cAMP, in a dose- and time-dependent manner, for 7 d. On the basis of the following observations that: 1) forskolin (10-100 μM) + isobutyl-methylxanthine (IBMX, 1.0 mM) enhanced ^{3}H-nicotine binding 2-3-fold at 7 d, whereas forskolin and IBMX alone had no effect, and 2) ^{3}H-nicotine binding to PC 12 cell mutants (A126.1B2 and A123.7), which do not have cAMP-responsive protein kinase A (PKA) Types I and II, were unaffected by dbcAMP, we suggest that protein kinase A plays a role in regulating expression of nChRs. To gain insight into mechanism(s), the effect of dbcAMP (0.1 mM) on nChR mRNA levels was studied. Northern gels showed that α-3, α-5, ß-2 and ß-4 mRNAs were present, whereas α-4, α-6, and ß-3 transcripts were not detected. Northern gels also showed that the α-3, α-5, and ß-4 mRNAs were markedly decreased by dbcAMP at 4 hours. However, ß-2 mRNA increased at 4 h, and then returned to baseline by 24 h. These studies indicate that PKA is involved in the regulation of nChRs. These studies also suggest that enhanced ^{3}H-nicotine binding *may not* involve synthesis of new receptor subunit proteins. (Supported by DA04446 and 03977).

467.1

CEREBROCORTICAL NEUROTOXICITY AND CHRONIC MEMORY IMPAIRMENT INDUCED BY MK-801 IN THE MOUSE.

G. Brosnan-Watters, D. F. Wozniak*, M. McEwen, J. W. Olney, Washington University, St. Louis, MO 63110.

Behavioral and histologic experiments were conducted to study the neurotoxic effects of MK-801 in male mice. A modified version of a hole board food search task was used with a repeated measures design and a massed trials protocol to evaluate the acute effects of a low dose of MK-801 (n=10; 0.05 mg/kg) and then the more chronic effects of a high dose of MK-801 (n=8; 10 mg/kg). The 0.05 mg/kg dose (ip) of MK-801 impaired acquisition on one version of the hole board task (learning which hole was baited in a row of 4) relative to saline performance levels but the same dose did not impair acquisition on another version of the task (4-corner stimulus configuration). Mice were then injected (ip) with the 10 mg/kg dose of MK-801 and tested 2 weeks later on the row configuration and 6 weeks later on the 4-corner version of the task. Relative to their earlier saline performances, they were significantly impaired on both the row and 4-corner configurations. Histologic studies were conducted on separate mice to determine if MK-801 produces the same pathomorphological side effects in mouse cerebrocortical neurons that are produced in the rat (cytoplasmic vacuole formation and dissolution of mitochondria in pyramidal neurons from layers III and IV of the posterior cingulate/retrosplenial (PC/RS) cortices). Mice were injected (sc) with either 0.5 or 1.0 mg/kg MK-801 and sacrificed 4 hr later. Brains were embedded in plastic, thin-sectioned and stained with methylene blue/azure II and examined by light microscopy. Fifty percent (3/6) of the mice dosed with 0.5 mg/kg MK-801 showed evidence of vacuoles in the PC/RS cortical area while 100% (6/6) of the mice treated with 1.0 mg/kg had vacuoles. Thus, in mice, a low dose of MK-801 causes transient memory impairment and higher doses cause acute injury of PC/RS neurons and chronic memory impairment. Supported by RSA MH 38894 (JWO) and AG 05681 (DFW/JWO).

467.2

DIFFERENTIAL EFFECTS OF ANTAGONISTS AT THE STRYCHNINE-INSENSITIVE GLYCINE RECEPTOR ON ANTICONVULSANT AND ANXIOLYTIC TESTS. T.C. McCloskey, H.S. White, B.M. Baron, Y. Senyah, A.L. Slone, B.W. Siegel*, B.L. Harrison, F. Salituro, M.G. Palfreyman, and J.H. Kehne. Marion Merrell Dow Research Institute, 2110 E. Galbraith Rd., Cincinnati OH 45215.

The anticonvulsant and anxiolytic potential of antagonists at various sites associated with the NMDA glutamate receptor complex has been demonstrated preclinically, though the relationship between these two activities is not clear. The present study evaluated the anticonvulsant and anxiolytic activities of compounds derived from indole-2-carboxylic acid or quinoline-2-carboxylic acid and containing either an aromatic or a carboxylate-bearing substituent (*J. Med. Chem. 35:* 1835, 1992; *J. Med. Chem. 33:* 3130, 1990; *Bioorg. Med. Chem. Lett. 1:* 455, 1991). Examples of compounds were found which have anxiolytic effects in the rat pup separation-induced vocalization (SIV; Kehne et al., *Eur. J. Pharmacol., 193:* 283, 1991) model and apparent lack of motoric side-effects as measured with the "time on an inclined plane" (TIP) test, relative to competitive NMDA antagonists or ion-channel blockers. Anticonvulsant activity was measured using audiogenic seizure-susceptible DBA/2J mice. Surprisingly, differential effects were seen in that glycine antagonists which were potent anxiolytics tended to have weak anticonvulsant activity, and vice versa. Motoric disruption was better correlated with anticonvulsant than with anxiolytic activity. Anticonvulsant potency of "selective" anxiolytics was not demonstrable upon direct i.c.v. injection, suggesting that simple CNS bioavailability did not explain these differential functional effects. These results might be explained by activity on regionally-specific glycine receptor subtypes, though biochemical characterization to date has failed to reveal apparent differences between the compounds.

467.3

N-METHYL-D-ASPARTATE (NMDA) ACUTELY INCREASES PROENKEPHALIN mRNA IN THE RAT STRIATUM. R.M. Beckstead*. Dept. Cell Biol. & Anat., Med. Univ. of South Carolina, Charleston, SC 29425.

The influence of NMDA receptor activation on proenkephalin (PPE) mRNA levels was examined acutely in the rat striatum and compared to protachykinin (PPT) and α-tubulin mRNA. NMDA (dissolved in 2 µl artificial CSF) was injected into the lateral ventricle at one of 3 doses (1, 10, 25 nmol; 6 rats per dose group). So that any processing effects were common within an experiment, a vehicle injected control group was prepared along with each dose group. Eight hours after the injection, the rats were perfused and processed for *in situ* hybridization with a [^{35}S]dATP end-labeled oligonucleotide probe followed by quantitative film autoradiography. PPE was *increased* in a dose-dependent manner compared to controls, reaching 140% of control level at the highest dose of NMDA. There was no significant change at any dose in the level of PPT or α-tubulin mRNA suggesting that the response was not uniform across striatal neurons nor an unspecific generalized augmentation of transcription. The increase in PPE was completely blocked by pretreatment with the competitive NMDA antagonist CGP 37849 injected systemically (12 mg/kg; ip) 20 minutes prior to the NMDA infusion. The data are consistent with a specific acute effect on PPE regulation by excitatory amino acid transmission mediated by the NMDA receptor. The direction and magnitude of change is consistent with earlier results in which acute depression of PPE was observed after treatment with NMDA antagonists. The data provide further support for the notion that the NMDA receptor mediates a rapid-onset, excitatory amino acid-stimulated up-regulation of met-enkephalin biosynthesis.

Supported by NIH (NINDS) grant NS24971.

467.4

POLYAMINES AND NMDA RECEPTORS IN ELECTRO CONVULSIVE SHOCK INDUCED EPILEPTIC SEIZURES.

Z. Iqbal,* F. Siddiqui, C. Lu and H. Koenig. V.A. Lakeside Medical Center and Northwestern University Medical School & Institute for Neuroscience, Chicago, IL 60611.

We have previously shown that electroconvulsive shock (ECS)- and methionine sulfoximine (MSO)-induced epileptic seizures in rat and mouse are associated with an activation of polyamine (PA) synthesis regulating enzyme ornithine decarboxylase (ODC). We now report that the changes in ODC and PA levels associated with ECS-induced epileptic seizures are linked to N-Methyl-D-Aspartate (NMDA) receptor activation. A single ECS (120 V/1 second) caused a significant (>25%) increase in rat cerebral ODC activity and PA levels within 15 seconds. Pretreatment of animals with PA synthesis inhibitors ∝-difluoromethylornithine (500 mg/kg) and methyl glyoxal bis guanylhydrazone (200 mg/kg) blocked the increases in ODC and PA levels and also prevented the seizures. Furthermore, the ECS-induced increases in cerebral ODC and PA levels associated with epileptic seizures were effectively suppressed when the animals were administered with NMDA receptor antagonist, MK-801. A sharp (<15 seconds) and transient (lasting for 1-2 minutes) increase in ODC and PA levels support the hypothesis that NMDA receptor activation associated epileptic seizures require post translational activation of existing ODC leading to increases in PA levels.

(Supported by VA Merit Review and NIH HL 26835 & NS 1804).

467.5

POLYAMINE NMDA ANTAGONISTS RELATED TO IFENPRODIL REDUCE NMDA-INDUCED, NO-DEPENDENT cGMP SYNTHESIS, INTRACELLULAR CALCIUM LEVELS AND CYTOTOXICITY.

P.M. Beart*, A. Schousboe and A. Frandsen, PharmaBiotec Research Center, Royal Danish School of Pharmacy, DK-2100 Copenhagen, Denmark.

Actions of ifenprodil and related aminoalcohols (e.g. tibalosine), which are polyamine NMDA antagonists (Beart et al., Mol. Neuropharmac. 2: 11, 1992), were investigated in cultured cortical neurones to characterize their NMDA-mediated cytoprotective potential. Mouse cerebral cortical neurones were cultured in the presence of cytosine arabinoside for 7-8 days before assessment of NMDA-mediated functions. NMDA (100µM)-stimulated production of cGMP was blocked by TCP (1µM) and N$^{\omega}$-nitro-L-arginine (100µM). All aminoalcohols dose-dependently inhibited the NO-dependent synthesis of cGMP; ifenprodil (IC_{50} 150 nM) exhibited higher potency than its benzothiazine (IC_{50} 625 nM) and thiochromane (IC_{50} 6µM) analogues. Ifenprodil and analogues also displayed a similar range of potencies as blockers of the NMDA (300µM)-induced increase in $[Ca^{++}]_i$ in mouse cortical neurones; blockade produced by the polyamine NMDA antagonists was complete and dose-dependent. Leakage of lactate dehydrogenase activity from the cultures after exposure to NMDA (100µM, 4h) was used as an index of neuronal death; all aminoalcohols reduced NMDA-induced cytotoxicity with ifenprodil giving almost complete protection at 10µM. Polyamine NMDA antagonists possess potential as cytoprotective agents in ischaemic events - the aforementioned ifenprodil analogues are lipophilic, unlike ifenprodil, and are suitable for systemic administration.

Supported by Danish State Biotechnology Program and NHMRC (Australia).

467.6

NMDA RECEPTOR-DEPENDENT INCREASE IN CYCLIC AMP INDUCED BY HIGH FREQUENCY ELECTRICAL STIMULATION IN THE DENTATE GYRUS OF RAT HIPPOCAMPAL SLICE. M.J. Bonner* and J.M. Sarvey, Department of Pharmacology, Uniformed Services University of the Health Sciences, Bethesda, MD 20814.

Cyclic AMP (cAMP) levels were induced by a high frequency train (HFT; 100Hz, 2 sec) to the perforant path in the dentate gyrus in vitro in hippocampal slices. As previously demonstrated in this lab, the parameters used for the HFT are sufficient to produce long-term potentiation, a form of synaptic plasticity. After HFT the dentate gyrus was cut from the rest of the hippocampal slice, cAMP levels in the dentate were then determined by a protein binding assay. Levels of cAMP were determined at intervals (n=8 rats /interval) during a 1 to 60 min post-HFT time period. Initially post-HFT cAMP levels were greatly increased to 246% of the basal level at the 1 min interval and declined to 147% at the 30 min interval. The NMDA-receptor antagonist CPP, present before and during the HFT, significantly suppressed post-HFT cAMP levels to approx 50% of no-CPP HFT stimulated levels. The suppression of cAMP levels by the presence of CPP (0.1, 1.0, 10.0 µM) was concentration dependent. The presence of 10.0 µM CPP immediately after HFT also resulted in suppression of cAMP levels. Excitatory amino acid receptor antagonists AP3 (100 µM) and CNQX (10µM) did not significantly reduce the levels of cAMP induced by HFT stimulation. These results suggest that stimulation of cAMP by HFT involves a NMDA CPP-sensitive receptor.

467.7

N-ACETYLASPARTYLGLUTAMATE INHIBITS CYCLIC GMP FORMATION IN CEREBELLAR GRANULE CELLS. J. H. Neale* and B. Wroblewska. Department of Biology, Georgetown University, Washington D.C. 20057.

N-acetylaspartylglutamate (NAAG) is a dipeptide present in the brain in very high concentrations. NAAG has been shown to activate NMDA receptors, including homomeric NMDAR1 receptor, with low potency as compared to glutamate. We have reported previously that NAAG inhibits cAMP formation in the cerebellar granule cells, through the activation of metabotropic mGluR receptor(s). These data indicate that NAAG is a candidate for an endogenous agonist at central glutamate receptors. The aim of the present study was to evaluate the role of NAAG in the second messenger responses to glutamatergic stimulation in cultured cerebellar granule neurons. We found that NAAG does not affect phosphatidyl inositol hydrolysis, calcium fluxes, or cGMP formation. However, NAAG inhibits the elevation of cGMP levels which are stimulated by kainate, NMDA, or glutamate. The mechanism of this inhibition is unknown, but NAAG has no effect on nitric oxide synthase activity, nor does it inhibit the direct response of these cells to nitric oxide which activates guanylate cyclase and increases formation of cGMP. NAAG also does not affect directly soluble or particulate guanylate cyclase activity. The release of arachidonic acid which is a stimulator of cGMP formation in granule cells is not affected by NAAG. Inhibition of cGMP formation is specific for NAAG, and not mimicked by N-acetylaspartate or glutamate, indicating that NAAG peptidase is not involved. The inhibitory action of NAAG is not prevented by glutamatergic antagonists of NMDA receptor, MK-801 or Mg^{2+}, but is blocked by non-NMDA antagonist, CNQX.

467.8

POTENTIATION OF NMDA-STIMULATED NITRIC OXIDE SYNTHASE ACTIVITY BY DIBUTYRYL cAMP AND FORSKOLIN. N.J.Toms and P.J. Roberts*. Dept. of Pharmacology, Sch. of Med. Sci., Univ. of Bristol, Bristol, U.K.

In the rat cerebellum, the synthesis of nitric oxide (NO) is coupled to activation of Ca^{2+}-gated NMDA receptors. This occurs primarily within the granule cells and involves the constitutive Ca^{2+}/CaM-dependent enzyme, nitric oxide synthase (NOS). Since glutamate stimulates cyclic AMP (cAMP) formation in cerebellum, possibly by release of other transmitters such as adenosine, we have investigated the ability of forskolin and dibutyryl cAMP to influence NO formation. Exposure of cerebellar slices from 10 day-old rats to NMDA, resulted in robust, dose-dependent increases in NO formation (assayed by conversion of L-[^{3}H]arginine to L-[^{3}H]citrulline.) Addition of either dibutyryl cAMP (1mM) or forskolin (60μM) produced a marked potentiation (203 and 209% over basal) of the response to a submaximal concentration (10μM) of NMDA. These substances both showed negligible direct effects on cAMP formation in the absence of NMDA. Dibutyryl cAMP produced a parallel leftwards shift in the NMDA response curve, without affecting the maximal response and the enhancement was fully antagonised by inclusion of the competitive NMDA antagonist, D-APV (50μM)

These results indicate that a cAMP-dependent process may regulate NMDA receptor/NO coupling in the developing rat cerebellum, probably by direct phosphorylation of the receptor.
NJT is an SERC Research Student.

467.9

SEVERAL IRON LIGANDS, 1,10-PHENANTHROLINE AND 2,2-DIPYRIDYL, AUGMENT THE N-METHYL-D-ASPARTATE-INDUCED RELEASE OF GLUTAMATE AND ELEVATION OF INTRACELLULAR CALCIUM ($[Ca^{2+}]_i$) IN NEURONAL CULTURE. S. Oh* and P. P. McCaslin. Dept. Pharmacol. & Toxicol., Univ. Mississippi Med. Ctr., Jackson, MS 39216.

These studies were designed to examine the effect of the iron ligands, 1,10-phenanthroline and 2,2-dipyridyl, on glutamate neurotransmission. The N-methyl-D-aspartate (NMDA) receptor has several modulatory sites, and it is possible that the receptor is a metalloprotein containing Fe_2S_2 at or near the active site. The glutamate receptor agonists, NMDA and kainic acid, result in the elevation of $[Ca^{2+}]_i$ when measured by fura-2 fluorometry in cerebellar granule neurons. The NMDA- but not the kainate-induced elevation of $[Ca^{2+}]_i$ was potentiated by 1,10-phenanthroline and 2,2-dipyridyl. Similarly results were found for the excitatory amino acid-induced release of glutamate from granule cells. Neither 1,10-phenanthroline or 2,2-dipyridyl had effects on basal levels of glutamate release or $[Ca^{2+}]_i$. The elevation of cyclic GMP is also correlated with elevations of $[Ca^{2+}]_i$, however, 1,10-phenanthroline was without effect on the NMDA- or kainate-induced elevation of cyclic GMP levels. 2,2-Dipyridyl was less potent than 1,10-phenanthroline on the above effects. These observations suggest that a metallic center or a metallic-like center may be an important regulatory site on the NMDA receptor.
Supported by NIDA grant DA 64843.

467.10

Na^+/Ca^{2+} EXCHANGER REGULATES GLUTAMATE-INDUCED Ca^{2+} RESPONSE IN ASTROCYTES. W. T. Kim and A. H. Cornell-Bell*. Dept. of Cell Biology, Yale Univ. Sch. of Med., New Haven, CT 06510.

We have previously demonstrated that Ca^{2+} flux through the plasma membrane is necessary for sustained oscillations and waves in mammalian astrocytes. Several lines of evidence suggest that Na^+ flux is necessary as well. The Na^+/Ca^{2+} exchanger, as a major mediator of both these fluxes, may play a key role in modulating Ca^{2+} oscillations and waves. We used time-lapse confocal laser microscopy and Fluo-3 to monitor $[Ca^{2+}]_{in}$ changes after application of 100 μM glutamate. While being superfused with zero-Na^+ saline, astrocytes exposed to glutamate showed a significant decrease ($p<.01$, $N=30$) in the frequency of oscillations in comparison to Ca^{2+} responses in normal saline. This decrease in oscillation frequency was also seen in a dose-dependent manner when astrocytes were treated with Benzamil (1 μM-1 mM), an amiloride inhibitor of the Na^+/Ca^{2+} exchanger. With 500 μM Benzamil, the oscillation frequency decreased from 6.6±0.6/min to 2.0±0.8/min. Furthermore, no intercellular Ca^{2+} waves were seen. The initial Ca^{2+} peak was unaffected by either zero-$[Na^+]_{out}$ or Benzamil treatments suggesting that the mechanism of release from intracellular stores is independent of oscillation and wave generation. Ionic regulation of the astrocyte through the Na^+/Ca^{2+} exchanger is critical in setting the frequency of Ca^{2+} oscillations and intercellular waves.

467.11

MODIFICATION OF NMDA-EVOKED NEUROTRANSMITTER RELEASE IN THE CEREBRAL CORTEX BY HIGH D-GLUCOSE LEVELS COMPATIBLE WITH HYPEROSMOLAR DIABETIC COMA. M. Göthert*, K. Fink and V. Schmitz. Inst. Pharmacol. Toxicol., Univ. Bonn, Reuterstr. 2b, 5300 Bonn 1, Germany.

The aims of the present study were to investigate whether high D-glucose (GLUC) modifies the [^{3}H]neurotransmitter ([^{3}H]NT) release evoked by NMDA receptor stimulation and whether this property is shared by other hexoses, NaCl and dimethylsulfoxide (DMSO) at equiosmolar concentrations. Rat brain cortical slices preincubated with [^{3}H]GABA and [^{3}H]norepinephrine ([^{3}H]NE) were superfused with Krebs' solution which under control conditions, contained 11 mM GLUC; stimulation was carried out by addition of NMDA (300 μM, unless stated otherwise) for 2 min. Elevation of GLUC by 60 or 100 mM increased the NMDA-evoked [^{3}H]GABA overflow (which could be inhibited by competitive and noncompetitive NMDA receptor antagonists), but decreased the NMDA-evoked [^{3}H]NE overflow. The inhibitory effect on [^{3}H]NE overflow also occurred in the presence of L-glucose, D-fructose, D-galactose, NaCl and sorbitol, but not of the easily membrane-permeable DMSO, at equiosmolar concentrations. When complete concentration-response curves for NMDA were determined, GLUC caused a depression of the maximum response. A decrease in pH from 7.4 to 7.0 did not affect the degree of GLUC-induced inhibition of [^{3}H]NE overflow. The inhibitory effect of 60 mM GLUC was significantly reduced by CGP 35348 (p-(3-aminopropyl)-p-diethoxymethyl-phosphinic acid), a $GABA_B$ receptor antagonist. In conclusion, NMDA receptors mediate a stimulation of [^{3}H]NT release not only from noradrenergic nerve terminals but also from GABAergic interneurones in the cerebral cortex. GLUC modifies the NMDA-evoked [^{3}H]NT release in the same manner as the K^+-evoked release (previous findings; Fink and Göthert, Brain Res., in press). The osmotic gradient extra- versus intracellular plays a crucial role in the mechanism(s) underlying the changes of [^{3}H]NT release. The inhibition of [^{3}H]NE release appears to be partially mediated by an increased stimulation of inhibitory $GABA_B$ receptors, which, in turn, is the consequence of the increased GABA release. The changes in NT release probably play a role in the pathogenesis of hyperosmolar diabetic coma.

467.12

GLUTAMATE RECEPTOR AGONISTS INCREASE INTRACELLULAR CALCIUM AND REGULATE GENE EXPRESSION IN OLIGODENDROCYTE PROGENITORS. V. Gallo*, J. L. Curtis, F. Vaccarino, P. W. Wright and J.T. Russell. Lab. Cell. Mol. Neurophysiol., NICHD, NIH, Bethesda, MD and Yale Child Study Ctr. Yale University, New Haven, CT.

Fura-2 fluorescence measurements performed in a glial progenitor cell line (CG-4) showed that glutamate receptor agonists caused a rapid increase in intracellular Ca^{2+}. Application of kainate, glutamate and quisqualate to both bipolar $LB1^+$ progenitors and multipolar 04^+ pre-oligodendrocytes increased intracellular Ca^{2+}. In progenitor cells, maximal responses to kainate and glutamate were observed after 4 days *in vitro* and were inhibited by CNQX. The kainate-induced Ca^{2+} increase was blocked in the absence of extracellular Ca^{2+}. Addition of nimodipine (10 μM) or Cd^{2+}(100 μM) reduced kainate responses by 75% (n=17) and 57% (n=37), respectively. A rapid and transient elevation of mRNA levels for the primary response gene *zif/268* was observed after the addition of glutamate receptor agonists to CG-4 cells. Under similar conditions, *c-fos* and *c-jun* mRNA levels were unchanged. Induction of *zif/268* by kainate was time-dependent, reaching a 5-fold stimulation after 1 hour and returning to basal levels within 4 hours. Depolarization of CG-4 cells with 40 mM K^+ did not cause any increase in *zif/268* mRNA levels. Our results indicate that excitatory amino acids can induce Ca^{2+} influx in immature glia through voltage-dependent channels and through Ca^{2+}-permeable kainate-activated channels. Activation of these channels selectively regulates the expression of the primary response gene *zif/268*.

467.13

EXCITATORY AMINO ACIDS INHIBIT MEMBRANE-BOUND GUANYLATE CYCLASE IN CEREBELLAR NEURONS. B. Wroblewska*[1], J.H. Neale[1] and J.T. Wroblewski[2]. [1]Department of Biology and [2]Fidia-Georgetown Institute for the Neurosciences, Georgetown University, Washington D.C. 20007.

In primary cultures of cerebellar granule cells excitatory amino acids induce a large accumulation of cGMP by stimulating the nitric oxide-dependent soluble guanylate cyclase. In addition, a smaller accumulation of cGMP is also observed in these cells after application of atrial natriuretic peptide (ANP) which, together with related peptides, is known to activate a family of cell surface receptors expressing the activity of membrane-bound guanylate cyclase. In granule cells ANP potently (EC_{50} = 70 nM) stimulated cGMP accumulation. This effect did not require the presence of extracellular calcium, and was not inhibited by the NO synthase inhibitor nitroarginine. However, the stimulatory effect of ANP was inhibited, in a non-competitive manner, by the excitatatory amino acid receptor agonist quisqualate. The inhibition induced by quisqualate was independent of the presence of the phosphodiesterase inhibitor IBMX. It was, however eliminated by the absence of extracellular calcium and by the ionotropic glutamate receptor antagonist 6-cyano-7-nitroquinoxaline-2,3-dione. A similar inhibitory effect on ANP-induced cGMP accumulation was produced by other agonists of ionotropic glutamate receptors, glutamate, kainate, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid and *N*-methyl-D-aspartate (NMDA), when their actions were tested in the presence of nitroarginine, used to inhibit the stimulation of the soluble guanylate cyclase. In contrast, the agonists of metabotropic glutamate receptors *trans*-1-aminocyclopentane-1,3-dicarboxylic acid and *N*-acetylaspartylglutamate were inactive. The inhibitory effect of glutatamate was partially reduced by the presence of NMDA receptor antagonists Mg^{2+} and MK-801, indicating the involvement of both NMDA and non-NMDA ionotropic glutamate receptors. Our results suggest that alterations of intracellular ionic homeostasis induced by the activation of ionotropic glutamate receptors may play a role in the control of ANP-induced cGMP formation.

467.14

Stimulation of *c-fos* expression by VIP in cortical neurons is mediated by glutamate

J.-L Martin, S. de Rham, D. Gasser and P.J. Magistretti*. Institut de Physiologie, Université de Lausanne, Lausanne, Switzerland

The regulation of *c-fos* expression by VIP was examined in primary cultures of astrocytes or neurons originating from the mouse cerebral cortex. In primary cultures of cortical neurons, VIP increases cAMP levels and stimulates *c-fos* expression. Interestingly however, VIP stimulation is completely blocked by MK-801, a non-competitive NMDA receptor antagonist, but not by CNQX or AP3, which are antagonists of respectively AMPA/kainate and metabotropic receptors. *c-fos* expression stimulated by glutamate also shares the same pharmacology. These results suggest an involvement of glutamate in the stimulation of *c-fos* expression evoked by VIP in primary cultures of cortical neurons.

In cultured astrocytes, VIP also stimulates *c-fos* expression in a concentration-dependent manner with an EC_{50} of 1 nM. VIP stimulation of *c-fos* expression is detectable after 30 min, reaching a maximal value after 4 hours and returning to control values within 9 hours. A comparable profile is also observed, although less pronounced, when VIP is applied as a 10 minute pulse. Noradrenaline (NA) also stimulates *c-fos* expression with a 9.7-fold stimulation already observed at 100 nM. When added together VIP and NA exhibit additive effects on *c-fos* expression, thus suggesting different pathways of activation. Potent activators of either protein kinase A, i.e. dBcAMP, or protein kinase C, i.e. PdBu or mezerein also stimulate *c-fos* expression in primary cultures of astroyctes. When added together dBcAMP and PdBu exhibit additive effects.

467.15

INHIBITION OF POTASSIUM-INDUCED HIPPOCAMPAL RELEASE OF GLUTAMATE AND ASPARTATE BY L-CPPENE IN FREELY MOVING RATS. B.S.Meldrum*, M.H.Millan and A.G.Chapman. Department of Neurology, Institute of Psychiatry, De Crespigny Park, London, UK.

CPPene(3-(2-carboxypiperazin-4-yl)-1-propenyl-1-phosphonic acid) is a potent competitive NMDA antagonist with anticonvulsant activity, where the D-enantiomer is 10-20 fold more potent than the L-enantiomer both as an antagonist and as an anticonvulsant (Aebischer et al. (1989) Helv. Chim. Acta, 72, 1043-1051; Patel et al. (1990) Epi. Res. 7, 3-10).

L-CPPene, on the other hand, is 10 fold more active than D-CPPene in inhibiting K^+-induced release of glutamate (GLU) and aspartate (ASP) in awake rats. When rats implanted with microdialysis probes in the dorsal hippocampus were subjected to high K^+ stimuli (100 mM through probe, 2x 10 min stimuli, 40 min apart) there were rapid 300-500% increases in the dialysate concentrations of ASP, GLU, GABA and TAU in response to both stimuli. The S2/S1 values (ratios between the second and first K^+-responses) were 0.7-0.9 for all the amino acids in the control groups. L-CPPene (25-250 μM in perfusate during second K^+-stimulus) selectively inhibited ASP and GLU release (S2/S1 = 0.2-0.3) without affecting GABA and TAU release.

467.16

D-2-AMINO-5-PHOSPHONOVALEATE BLOCKS INDUCTION OF LONG-TERM DEPRESSION OF THE NMDA RECEPTOR-MEDIATED SYNAPTIC COMPONENT IN RAT HIPPOCAMPUS. P.W. Gean*, J.H. Lin, and C.C. Huang. Dept. of Pharmacology, College of Medicine, National Cheng-Kung University, Tainan, Taiwan, R.O.C.

An in vitro slice preparation of rat hippocampus was used to study the long-term modifications of pharmacologically isolated N-methyl-D-aspartate (NMDA) receptor-mediated excitatory postsynaptic potential ($EPSP_{NMDA}$). Intracellular recordings were made from CA1 pyramidal cells in the presence of 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX, 10μM) and picrotoxin (50 μM) which block non-NMDA and $GABA_A$ receptors respectively. Pairing of low-frequency $EPSP_{NMDA}$ with postsynaptic depolarization induced a long-term depression (LTD) of $EPSP_{NMDA}$. The maximal reduction of $EPSP_{NMDA}$ amplitude amounted to 81.3% of the control 1 min after the pairing. When low-frequency synaptic stimulation was paired with strong postsynaptic depolarization, a long-term potentiation (LTP) of $EPSP_{NMDA}$ could be induced. The LTD of $EPSP_{NMDA}$ was blocked by D-APV. In normal medium, the $EPSP_{NMDA}$ remained depressed (45.2±6.5% of control, n=14) 20 min following the pairing. In the presence of D-APV, the $EPSP_{NMDA}$ returned to control level (101±10.9%, n=6) at 20 min after the pairing. These results suggest that the induction of $EPSP_{NMDA}$ LTD requires an increase in postsynaptic Ca^{++} likely, at least in part, due to synaptic activation of NMDA receptors during concomitant postsynaptic depolarization.

467.17

EFFECT OF MAGNESIUM ON OPEN-CHANNEL BLOCKADE OF NMDA RECEPTORS BY 9-AMINOACRIDINES. M.E. Nelson[1], J.G. Montes[1], and E.X. Albuquerque[1,2]*. [1]Dept. Pharmacol. Exp. Ther., Univ. Maryland Sch. Med., Baltimore, MD, USA 21201; [2]Lab. Mol. Pharmacol., IBCCF, UFRJ, Brazil 21944.

Bis-9-aminoacridines have been found to be potent blockers of NMDA-activated single-channel currents in outside-out patches from cultured rat hippocampal neurons (*Neurosci. Soc. Abs.*, **18**:1510, 1992). The concentration and voltage dependence of the blockade led us to propose an open-channel blocking mechanism to describe the effect. However, analysis of the forward blocking rate constants for 1,2-propylene-bis-9,9′-aminoacridine (1,2-PAA) and 1,4-n-butylene-bis-9,9′-aminoacridine (1,4-BAA), as well as the mono-9-aminoacridine, 9-amino-1,2,3,4-tetrahydroacridine (THA), suggested that a model for open-channel blockade was inappropriate because plots of reciprocal channel open time $(\tau_{open})^{-1}$ versus acridine concentration were not linear, but hyperbolic. This finding was surprising as previous whole-cell studies of NMDA-activated currents in these neurons were consistent with an open-channel blocking mechanism for THA (*Neurosci. Soc. Abs.*, **15**:1166, 1989). It was possible that the 3-5 μM Mg^{2+} present in our "nominally Mg^{2+}-free" solutions might have altered the interaction of the acridines with NMDA-activated channels. Therefore, additional single-channel experiments were performed with the acridines in Mg^{2+}-free solutions (<0.5 μM, by spectrophotometry). The results are consistent with a model of open-channel blockade for describing the interactions of the 9-aminoacridines with the NMDA receptor as evidenced by the linear relationship between $(\tau_{open})^{-1}$ and acridine concentration between -40 and -100 mV and at concentrations up to 10 μM for 1,2-PAA or 1,4-BAA, and up to 50 μM for THA. At -80 mV, τ_{open} was reduced by more than 85% compared to controls at these drug concentrations. These data suggest that the kinetics of interactions of 9-aminoacridines with the ion-channel of the NMDA receptor are significantly altered by the presence of low micromolar concentrations of Mg^{2+}. Supp.: USPHS Grants NS25296, ES05730.

468.1

GABAergic action on identified cholinergic neurones of the nucleus basalis in guinea-pig brain slices. A. Pegna, A. Khateb, P. Fort*, B.E. Jones° and M. Mühlethaler *Dept. of Physiology, CMU, 1211 Geneva 4, Switzerland and °Montreal Neurological Inst., McGill University, Canada H3A 2B4*

Cholinergic neurones within the nucleus basalis are surrounded by GABAergic neurones which may in part provide an innervation to the cholinergic cells as local interneurones. Our goal was thus to determine the action of GABA on identified cholinergic cells, whose properties we have described in the guinea-pig brain. Employing intracellular recordings we found that such neurones were depolarized by GABA which was administered by superfusion in the bath. This effect did not appear to be due to the activation of GABAB receptors since baclofen had no effect. In contrast the effect was strongly mimicked by muscimol, a GABAA selective agonist and blocked by bicuculline, a GABAA antagonist. It appeared that the effect of muscimol was postsynaptic since it persisted in presence of a high Mg^{2+}/low Ca^{2+} solution, which interferes with synaptic transmission. The depolarizing effect of muscimol was accompanied by a drop in membrane resistance and an inhibition of firing in cells which were spontaneously active in control conditions. These results suggest that GABA depolarizes but nonetheless inhibits cholinergic basalis neurons through a mechanism that remains to be elucidated. This action would prevent both the tonic and burst modes of firing by the cholinergic neurones. (Swiss NSF, Canadian MRC, Fondation Fyssen and Lyonnaise des Banques).

468.2

GABA EVOKED WHOLE CELL AND SINGLE-CHANNEL CURRENTS ARE MODULATED BY INTRACELLULAR FREE CALCIUM IN CULTURED CEREBELLAR GRANULE CELLS. M.Martina*, G.Kilic, and E.Cherubini. Biophysics Lab, SISSA, Via Beirut 4, 34013 Trieste, Italy.

The patch clamp technique was used to study the effects of intracellular free calcium $[Ca]_i$ on $GABA_A$ evoked whole cell and single channel currents of cultured cerebellar granule cells.Changes in $[Ca]_i$ were obtained by varying the extracellular calcium concentration $[Ca]_o$ in the presence of the calcium ionophore A23187 (2 μM).The relationship between $[Ca]_o$ and $[Ca]_i$ in the presence (or in the absence) of A23187 was assessed using fluorimetric measurements from Fura 2 AM loaded cells. In a bathing solution containing 10, 100 and 1000 μM calcium, the $[Ca]_i$ was 200, 230 and 230 nM without A23187 and 220, 350 and 1400 nM in the presence of A23187 respectively.In whole cell experiments (symmetrical chloride concentration) in calcium free solution at a holding potential of -50 mV bath application of GABA (0.5 μM for 40-60 s) induced inward currents (14±2 pA) that did not desensitize. The currents were reduced to 7±2 pA in 2 mM $[Ca]_o$. Single channel currents activated by 0.5 μM GABA were also recorded in outside-out configuration. In calcium free medium the open probability was 0.134±0.01. When $[Ca]_o$ was raised to 800 μM the open probability decreased to 0.003±0.002 and no change in single channel conductance or mean open time was observed. It is concluded that $[Ca]_i$ plays an important role in modulating GABA activated whole cell or single channel currents probably by changing the affinity of the amino acid for the $GABA_A$ receptor.

468.3

CONDITIONS NECESSARY FOR $GABA_A$ RECEPTOR-MEDIATED EXCITATION ARE DIFFERENT IN ADULT CA1 *vs* DENTATE GYRUS NEURONS K.J. Staley* Neurology Dept, University of Colorado Health Sciences Center, Denver, CO 80262

$GABA_A$ receptor-mediated excitation has been reported under various experimental conditions in most types of hippocampal neurons. If excitation = action potential generation, then $GABA_A$ receptor-mediated excitation can occur only if 1) the chloride reversal potential (E_{Cl}) is less than action potential (AP) threshold and 2) the resting membrane potential (RMP) is large enough to prevent inactivation of the voltage-dependent Na conductances which underlie the AP.

In dentate gyrus granule cells (DGGC), whole-cell recordings with 131-139 mM KCl electrodes revealed a steady state RMP of -74 mV, an E_{Cl} of 0 mV, and an AP threshold of -55 mV (n=17). Thus in DGGC, chloride loading is sufficient to produce $GABA_A$ receptor-mediated excitation.

In CA1 pyramidal cells, whole-cell recordings with 131 mM KCl electrodes revealed a slow decline of the RMP from -65 to -30 mV, while E_{Cl} reached a steady state of only -22 mV; AP threshold was -55 mV (n=10). Inactivation of Na conductances together with the small driving force (8 mV) for $GABA_A$ receptor-mediated postsynaptic potentials prevented $GABA_A$ receptor-mediated excitation unless the RMP was increased with current injection. Blockade of cation-Cl cotransport with furosemide only enhanced the depolarization of the RMP.

These findings indicate that a large pathway for conductive Cl efflux is present in CA1 neurons but not DGGC. In addition to Cl loading, blocking (or counteracting) this Cl conductance is necessary to produce $GABA_A$ receptor-mediated excitation in adult CA1 pyramidal cells. Supported by NIH NS15173.

468.4

ABILITY OF GABAergic IPSPs TO REDUCE THE EFFICACY OF GLUTAMATE-EVOKED POSTSYNAPTIC POTENTIALS IN DENDRITES OF HIPPOCAMPAL CA1 PYRAMIDAL CELLS *IN VITRO*. D.D. Samulack* and J.-C. Lacaille. Centre de recherche en sciences neurologiques et Département de physiologie, Université de Montréal, Montréal, QC, Canada H3C 3J7.

Populations of inhibitory interneurons which display differences in regional distribution of terminals and that utilize different GABA postsynaptic mechanisms, may show variation in their postsynaptic inhibitory effectiveness. The ability of inhibitory postsynaptic potentials (IPSPs), evoked by microapplication of glutamate (500 μM) in *stratum (str.) pyramidale* (PYR glut-IPSP), or in *str. lacunosum-moleculare* (L-M glut-IPSP), to depress depolarizing postsynaptic potentials (DPs) evoked by glutamate microapplication in the dendrites (mid-*radiatum*), was studied through collision of these IPSPs with the DPs recorded intracellularly from CA1 pyramidal cells in slices (450 μm thickness) of the rat hippocampus (125-250 g, male, Sprague-Dawley). Preliminary data showed that PYR glut-IPSPs ($GABA_A$-mediated; Samulack and Lacaille, Hippocampus, 1993) (n=4) were more effective than L-M glut-IPSPs ($GABA_B$-mediated; Williams and Lacaille, Synapse, 1992) (n=4) in reducing the amplitude of DPs evoked in apical dendrites of the recorded pyramidal cells (28.4% *versus* 14.8% reduction, respectively, with peak-to-peak collision, measured at the apparent equilibrium potential for the IPSP). The ability of the IPSP, to reduce the amplitude of the DP, decremented as the onset of the DP was delayed with respect to the peak of the IPSP. It seems, therefore, that currents from PYR glut-IPSPs ($GABA_A$) which are likely generated by interneurons of the feedback inhibitory loop (e.g. basket cells, vertical cells, etc...) appear more effective than those of L-M glut-IPSPs ($GABA_B$) produced by feedforward inhibitory interneurons (stellate cells) in shunting excitatory inputs to the apical dendrites of pyramidal cells.

(Supported by MRC of Canada, FRSQ, FCAR and Epilepsy Canada/Parke-Davis)

468.5

A PHOTOREACTIVE DERIVATIVE OF 5,7-DICHLOROKYNURENIC ACID THAT DISPLACES [^{3}H] MUSCIMOL BINDING IN MEMBRANE ASSAYS A.C. Nichols* and K.L. Yielding. Department of Pharmacology, University of Texas Medical Branch, Galveston, Tx.

4-Methylamino-5,7-dichloroquinoline-2-acyl azide, a derivative of the glycine antagonist 5,7-dichlorokynurenic acid, was synthesized for testing as a photoaffinity probe for the glycine site. The compound had very low affinity for the glycine receptor (IC_{50} approx. 1mM), but was found to compete for [^{3}H] - muscimol binding in synaptosomal membrane preparations with an IC_{50} of about 30 uM. Gel electrophoresis of synaptosomal membrane fragments photolabeled with a [^{14}C] derivative of the probe had radiolabeled protein which migrated between 80 and 49.5 kDalton standards. Such photosensitive probes may provide a useful means of identifying specific sub-sets of neurotransmitter sites.

468.6

ACTIONS OF NICOTINIC ANTAGONISTS AT THE $GABA_A$ RECEPTOR.

V.E. Wotring* and K.-W. Yoon, Department of Pharmacological and Physiological Science and Division of Neurosurgery, Saint Louis University School of Medicine, St. Louis, MO 63104.

It has previously been noted that d-tubocurarine can act as an antagonist at the $GABA_A$ receptor as well as at the nicotinic acetylcholine receptor. (Labeda et. al., J. Neurophys, 48:3, 1982) We pursued the possibility that other nicotinic antagonists might behave similarly.

Measurements of GABA evoked currents were made using the whole cell patch clamp technique. Recordings were made from rat hippocampal cells grown in culture for 7 to 16 days. Drug applications were made with a U-tube. 30 μM d-tubocurarine reduces GABA current evoked with 10 μM GABA by 52% (n=6). 50 μM trimethaphan reduces the GABA current by 35% (n=5). These blockades were reversible, dose-dependent, and independent of the holding potential. Dihydro-β-erythroidine (100 μM) has no effect on the GABA current (n=7), nor do mM concentrations of open channel blockers at the nicotinic receptor, hexamethonium (n=6) and mecamylamine (n=6). Furthermore, we attempted to elucidate the mechanisms of $GABA_A$ receptor blockades caused by d-tubocurarine and trimethaphan.

468.7

EFFECTS OF $GABA_A$ RECEPTOR ACTIVATION ON CYCLE ALTERNATION IN THE MOTOR RHYTHM OF MOUSE SPINAL CORD, IN VITRO. T.D. Oliver, P. Hernandez* and M.H. Droge. Biology Dept., Texas Woman's Univ., Denton, TX 76204, *Biology Dept., Abilene Christian Univ., Abilene, TX 79601.

The mouse spinal cord-hindlimb (SCHL) preparation exhibits locomotor-like rhythm in gastrocnemius (G) and tibialis anterior (TA) muscle activity (Hernandez et al., Exp. Brain Res. 1991). However, the rhythm typically lacks cycle alternation. The objective of the present study was to determine if $GABA_A$ receptor activation can produce motor rhythm with cycle alternation in the mouse spinal cord, in vitro.

The $GABA_A$ agonist muscimol (5-20 μM) and antagonist bicuculline (5-20 μM) were bath applied to SCHL explants as well as to isolated spinal cords prepared from 1-5 day old Balb/C mice. Recordings of G and TA muscle activity or bilateral ventral root activity were obtained, depending on the preparation. In cases where little spontaneous activity was observed, muscimol typically increased motor activity and could evoke rhythmic EMG or ENG sequences. The muscimol-evoked rhythm included cycle periods ranging from 0.3 to 1.6 s as well as some episodes of cycle alternation. In cases were NMDA (10-15 μM) was applied to evoke motor rhythm, subsequent application of NMDA + muscimol increased the incidence of cycle alternation. However muscimol did not evoke prolonged sequences of cycle alternation. Nevertheless, these data suggest that $GABA_A$ receptor activation contributes to the reciprocal antagonism involved in coordinating motor rhythm

(Supported by NIH #5R29NS25250 and #2S06GM08256).

468.8

ROLE OF GABA (γ-AMINOBUTYRIC ACID) IN ANOXIC-INDUCED ION CHANGES IN THE HIPPOCAMPUS. G.V. Obrocea* and M.E. Morris. Dept. of Pharmacology, University of Ottawa, Ottawa, Canada K1H 8M5.

To determine the role of the inhibitory neurotransmitter, γ-aminobutyric acid (GABA), in the generation of ionic responses of the brain to anoxia, ion-selective microelectrodes were used to measure changes in extracellular $[Na^+]$, $[K^+]$ and $[Cl^-]$ in stratum pyramidale (SP) and stratum radiatum (SR) in CA1 guinea pig hippocampal slices. GABA (5 mM perfusion for 5 min) induced increases (↑s) in $[K^+]_o$ and $[Cl^-]_o$ and a decrease (↓) in $[Na^+]_o$ in SP; in contrast in SR there was an ↑ in $[K^+]_o$, and a ↓ in both $[Na^+]_o$ and $[Cl^-]_o$. Replacement of O_2 by N_2 for 5 min) produced identical changes of the same/greater magnitude. $[K^+]_o$ ↑s evoked in SR by GABA (0.7 mM) and N_2 (3.2 mM) were greater than in SP (0.5 and 2.0 mM, respectively); $[Na^+]_o$ ↓s (-23.5 mM with GABA and -32.8 mM with N_2) in SR were also significantly greater than in SP (-17.0 and -25.2 mM). GABA and N_2 evoked $[Cl^-]_o$ ↑s of +21.3 and +25.1 mM in the SP, and ↓s of -14.8 and -33.8 in the SR. Bicuculline (BMI 100 μM) reversibly blocked or attenuated all ion changes evoked by both GABA and N_2. In SP BMI depressed ↑s in $[K^+]_o/[Cl^-]_o$ with GABA by 90% and with N_2 by 50-60%. In SR K^+ accumulation during GABA was abolished and with anoxia was attenuated by 70%; ↓s in Cl^- were depressed by >50%. $[Na^+]_o$ changes in SP and SR were affected to a lesser extent, being blocked by only 20-30%. The observed similarity of changes supports the hypothesis of a GABA-mediated contribution to the changes in extracellular ion levels which are induced by anoxia.

Supported by the MRC of Canada and NCE Network for Neuronal Regeneration and Functional Recovery

468.9

WITHDRAWN

468.10

INTRACELLULAR CYCLIC GMP MODULATES $GABA_A$ RECEPTOR FUNCTION. D. J. Bradshaw* and M. A. Simmons, Marshall University School of Medicine, Dept. of Pharmacology, Huntington, WV 25755-9310.

Previous research from our lab suggested that GTP is capable of modulating $GABA_A$ receptor ($GABA_AR$) activity in the absence of exogenous ATP. The aim of this study is to determine what mechanism endogenous GTP might employ to enhance $GABA_AR$ function. In all experiments bullfrog dorsal root ganglion cells were voltage-clamped at -70 mV, and the GABA invoked Cl^- current recorded in the presence of tetrodotoxin and cesium (to inhibit Na^+ and K^+ currents, respectively). GABA (100 μM) was applied via superfusion for 5 seconds at 5 minute intervals for 30 minutes postimpalement. We found previously that when the recording electrode contains no ATP or GTP, the 6th responses to GABA decrease to a mean ± s.e.m. of 33 ± 7% (n=5) of the initial response. Modulation by G-protein activation, and cGMP generation were both examined as plausible processes. Intracellular application of 100 μM $AlCl_3$, 10 mM NaF (or KF), and 100 μM GDP did not provide protection against rundown. This suggests direct modulation by a G-protein is unlikely. Intracellular application of varying concentrations of cGMP (500 μM, 0.5 μM, and 0.5 nM) presented 6th responses of 147 ± 18% (n=2), 125 ± 24% (n=3), and 100% (n=1 at 5th response), respectively. The mean initial responses for each cGMP concentration were -10.8 ± 2.0 nA (n=3), -1.9 ± 0.3 nA (n=4), and -0.5 ± 0.1 nA (n=4), respectively. Extracellular application of 20 μM 8-Br-cGMP provided a 6th response of 150 ± 77% (n=3). These preliminary results suggest that an increase in cytosolic cGMP can positively modulate the $GABA_AR$ in the absence of exogenous ATP.

468.11

$GABA_A$ RECEPTOR FUNCTION IS MODULATED BY AGENTS WHICH AFFECT MICROTUBULE ASSEMBLY. V.J. Whatley, S.J. Mihic and R.A. Harris*, Dept. Pharmacology, Univ. Colo. Sch. Med. and Denver VAMC, Denver, CO 80262.

Cytoskeleton-associated clustering of the nicotinic acetylcholine receptor (nACh-R) has been shown to modulate its function, by a mechanism believed to involve microtubules. Since the $GABA_A$ receptor shares significant sequence homology with the n-ACh-R, we questioned whether it would also be affected by clustering. Microtubule-affecting agents were tested for their effects on $GABA_A$ receptor function, by measuring muscimol-stimulated $^{36}Cl^-$ uptake into mouse cerebral cortical microsacs. Colchicine (1-100μM) decreased muscimol-stimulated uptake at 34°C, but had no effect at 0°C, where microtubles have already depolymerized. Microsacs treated with colchicine (1-100μM) for only 3 sec displayed the same inhibition of uptake as membranes treated for 30 min. The inactive analog of colchicine, ß-lumicolchine (1μM) potentiated muscimol-stimulated uptake at 34°C and 0°C. In *Xenopus* oocytes expressing $\alpha_1\beta_2\gamma_{2s}$ GABA receptor constructs, colchicine (1-100μM) inhibited, while ß-lumicolchine (1-100μM) potentiated, GABA-induced currents. Taxol (1μM), an agent promoting microtubule assembly, potentiated muscimol-stimulated chloride uptake. These data suggest that agents which affect microtubule assembly or disassembly can modulate $GABA_A$ receptor function, possibly by a mechanism involving receptor clustering. Supported by the VA and NIH/NIAAA.

468.12

INFLUENCE OF PHOSPHORYLATION EFFECTORS ON $GABA_A$ RECEPTOR FUNCTION AND DESENSITIZATION T.M. DeLorey*, M. Gordey, R. Tyndale, and R.W. Olsen Department of Pharmacology and Brain Research Institute, UCLA School of Medicine, Los Angeles CA 90024

The $GABA_A$ receptor is a macromolecular complex, composed of multiple subunits (α, β, γ, δ). Each subunit contains four membrane spanning regions with a large intracellular loop connecting transmembrane regions 3 and 4. Consensus sequences for phosphorylation by various kinases are located within this loop. Based on the structural homology shared between the nACh receptor and the $GABA_A$ receptor, it has been suggested that phosphorylation may modulate $GABA_A$ receptor desensitization. It has been demonstrated by muscimol-stimulated $^{36}Cl^-$ flux, in rat whole brain microsacs, that drugs such as the adenylate cyclase activator forskolin and the cAMP analog CPTcAMP, drugs which influence the cAMP dependent protein kinase (PKA) pathway, inhibit muscimol-stimulated $^{36}Cl^-$ flux to varying degrees (Leidenheimer *et al. Mol. Pharm.* 38, 823-28, 1990). We have further demonstrated a regional variability in CPTcAMP influence on muscimol-stimulated $^{36}Cl^-$ flux. Chloride flux in the thalamus, inferior colliculus and hippocampus is inhibited by 60% with 1.5 mM CPTcAMP while flux in the caudate putamen and olfactory tubercle is inhibited by 100%. Both the caudate putamen and olfactory tubercle are enriched in the $GABA_A$ receptor β_3 subunit mRNA. The β_3 subunit contains a consensus sequence for PKA phosphorylation. We have further demonstrated using recombinant expression of $\alpha_1\beta_3\gamma_{2s}$ in *Xenopus* oocytes that IBMX, a phosphodiesterase inhibitor, in combination with forskolin leads to an overall reduction in peak height of GABA-mediated chloride conductance. This effect requires the presence of forskolin but was dependent on the dose of IBMX. Furthermore, the forskolin-IBMX combination appears to decrease the overall rate of desensitization. These data suggest that PKA-mediated phosphorylation may influence desensitization of the $GABA_A$ receptor, probably via the β_3 subunit. (Supported by NS 28772)

468.13

GABA-A RECEPTOR REGULATION BY A CHLORIDE-DEPENDENT KINASE AND A SODIUM-DEPENDENT PHOSPHATASE.

R.A. Lanius*, B.A. Pasqualotto & C.A. Shaw, Depts. of Neuroscience, Ophthalmology & Physiology, c/o Dept. of Anatomy, University of British Columbia, Vancouver, B.C., V6T 1Z3, Canada.

We have recently reported that agonist (muscimol) and depolarizing stimulation (veratridine) require kinase or phosphatase activity to regulate GABA-A receptors ([^{3}H]-SR 95531) in slices of adult rat cortex (Pasqualotto et al., NeuroReport 4(4), 447-450, 1993). Muscimol and veratridine both lead to changes in ionic concentrations inside the affected neurons: Muscimol activates Cl^- currents through GABA-A receptors; veratridine blocks Na^+ channel inactivation thus leading to increased intracellular Na^+ concentrations. Based on these data, we predicted that Cl^- and Na^+ serve as triggers for kinase(s) and phosphatase(s) involved in the regulation of the GABA-A receptor. The effects of Cl^- and Na^+ were examined alone and in the presence of protein kinase A (PKA) or alkaline phosphatase (AP). The results showed a decrease in binding after incubation with Cl^- alone; this decrease was further enhanced in the presence of PKA. Both effects could be blocked by a PKA inhibiting peptide. Conversely, an increase in binding was observed after incubation with Na^+ alone; this increase was further enhanced in the presence of AP. In both cases these increases could be blocked by sodium-ortho-vanadate. These results suggest that Cl^- and Na^+ may trigger kinase(s) and phosphatase(s) involved in GABA-A receptor regulation.

468.14

ELECTROPHYSIOLOGICAL EVIDENCE FOR TWO TYPES OF ZOLPIDEM-BINDING RECEPTORS BASED UPON THE ABILITY OF ETHANOL TO ENHANCE GABA-INDUCED INHIBITION.

H.E. Criswell*, G.R. Breese, and A.L. Morrow. U.N.C. Sch. of Med., Chapel Hill NC 27599.

For some time it has been believed that ethanol produces some of its behavioral effects by an action on the $GABA_A$ receptor complex. The specific subunits incorporated into the $GABA_A$ receptors determine their pharmacological properties. Recent electrophysiological studies have shown that ethanol enhances GABA-induced inhibition of some but not all neurons, suggesting that only a subset of the $GABA_A$ isoreceptors are sensitive to ethanol. The localization of ethanol sensitive neurons to brain areas with high levels of zolpidem binding suggested that ethanol acts on a $GABA_A$ isoreceptor which binds zolpidem. We tested this hypothesis by examining the effect of iontophoretically applied ethanol and zolpidem on GABA-induced inhibition in several brain areas. Neurons which responded to ethanol also responded to zolpidem enhancement of GABA-induced inhibition. However, some neurons responded to zolpidem which were unresponsive to ethanol's enhancement of GABA-induced inhibition. These data indicate heterogeneity of $GABA_A$ receptors sensitive to zolpidem and provide further evidence for the selectivity of ethanol's action on $GABA_A$ receptors. This heterogeneity may be due to the presence of differing $GABA_A$ isoreceptors sensitive to zolpidem or differences in post-translational processing of the receptors.
Supported by USPHS grants AA-09122 and AA-08024.

468.15

PROTEIN KINASE C MODULATES ETHANOL ENHANCEMENT OF SYNAPTIC $GABA_A$ CURRENT IN RAT HIPPOCAMPAL CA1 NEURONS.

J.L. Weiner*, L. Zhang, and P.L. Carlen. Playfair Neurosci. Unit, Addiction Research Foundation, Toronto Hosp. Res. Inst., Univ. of Toronto, Dept. of Pharmacol., Canada M5T 2S8.

We have previously demonstrated that clinically relevant concentrations of ethanol and benzodiazepines enhance pharmacologically isolated $GABA_A$ IPSCs recorded in rat hippocampal CA1 neurons in brain slices. Using the whole-cell patch-clamp recording technique, we investigated protein kinase C (PKC) modulation of the sensitivity of synaptic $GABA_A$ currents to ethanol and diazepam.

Pretreatment of slices with selective inhibitors of PKC blocked ethanol (20 mM) enhancement of IPSCs but did not depress the effects of diazepam (100 nM). In addtion, removing ATP from the recording solution or replacing it with AMP-PNP, a non hydrolyzable ATP analog, reduced ethanol-mediated enhancement of $GABA_A$ IPSCs but did not depress the action of diazepam. Furthermore, inclusion of 400 μM GDP-βS in the recording solution also occluded ethanol potentiation of IPSCs. Surprisingly, incubating slices in activators of PKC (3 μM PDBu or 1 μM PMA for 15-60 min.) also significantly reduced ethanol enhancement of IPSCs but did not reduce the potentiation induced by diazepam. Incubation of slices in the inactive phorbol, 4-α PDBu (3 μM) did not interfere with ethanol enhancement of IPSCs.

These results demonstrate, as previously shown for recombinant $GABA_A$ receptors recorded in Xenopus oocytes (Wafford and Whiting, FEBS LET., 313: 113-117, 1992), that the sensitivity of $GABA_A$ receptors mediating inhibitory synaptic transmission in the mammalian CNS may be modulated by PKC. The mechanisms underlying this modulation are currently under investigation.
(Supported by the Medical Research Council of Cananda)

468.16

ALCOHOL-HEIGHTENED AGGRESSION MICE SHOW REDUCED $GABA_A$-BENZODIAZEPINE RECEPTOR BINDING IN VIVO.

K.A. Miczek[1*], E.M. Weerts[1], M. Wiadro[1], H. Barros[1], J.M. Koff[2], L.G. Miller[2] Tufts Univ., Dept. of Psychology[1], Medford, MA 02155 and Dept. of Pharmacology and Experimental Therapeutics[2], Boston, MA 02111, USA

Recent analysis of individual differences in alcohol's effects on aggression have identified subgroups of mice and rats that show qualitatively different effects. While some individuals show alcohol-heightened aggression (AHA), others show only alcohol-suppressed aggression (ASA) or no reliable effects (ANA) in the same low to moderate dose ranges and on repeated occasions. Alcohol-heightened aggressive behavior is antagonized by benzodiazepine receptor antagonists. Do AHA and ASA mice show different binding at the $GABA_A$-benzodiazepine receptor? Male, CFW mice were pair-housed with females for at least 21 days and screened for reliable attack behavior in 5 minute resident-intruder tests. Animals that attacked naive intruder males over four separate test days were administered distilled water or 1.0 g/kg alcohol in alternating sequence so that each animal received alcohol twice. There were no differences in baseline levels of locomotion, aggression, attack latency, body weight gain or reproductive success in AHA, ASA and ANA mice. AHA mice had lower [^{3}H]Ro 15-1788 in vivo binding in cortex when compared to ANA mice. [^{3}H]Flunitrazepam binding in vitro revealed no significant differences between groups. These data indicate that lower binding at the $GABA_A$-benzodiazepine receptor in cortex may be linked to alcohol's pro-aggressive effects in some individuals.

468.17

EFFECTS OF GLUTAMATE AND CALCIUM ON GABA-INDUCED CHLORIDE FLUX.

Tory A. Masonis and Michael P. McCarthy*. CABM/Pharmacology, UMDNJ-RWJMS, Piscataway, NJ, 08854.

Chloride flux through the γ-aminobutyric $acid_A$ receptor ($GABA_A$ receptor) is modulated by a wide number of effectors. We have studied the effects of excitatory amino acids on chloride influx in rat brain membranes using the $^{36}Cl^-$ influx assay of Harris and Allan[1]. Earlier workers found that glutamate enhanced GABA-induced $^{36}Cl^-$ influx in mouse synapto-neurosomes, but did not induce influx in the absence of GABA[2]. We have found that, in the absence of calcium, 100 mM glutamate moderately inhibited GABA-induced $^{36}Cl^-$ influx, but also produced significant influx alone. Aspartate (100 μM) also induced significant $^{36}Cl^-$ influx in the absence of GABA. Glutamate- and aspartate-induced $^{36}Cl^-$ influx was blocked by picrotoxin. However, in the presence of calcium (1 mM), glutamate alone did not induce $^{36}Cl^-$ influx, in agreement with earlier studies[2]. Glutamate may be acting as a partial agonist (in the absence of calcium), as it also inhibited [^{3}H]muscimol binding approximately 50%.

1. R. A. Harris & A. A. Allan (1985). *Science* **228**, 1108-1110.
2. A. Schatzki, M. McMillan, & L. G. Miller (1990) *Brain Res. Bull.* **25**, 239-243.

469.1

$GABA_A$ RECEPTORS IN CELL LINES.

R.F. Tyndale*, A.J. Tobin, R.W. Olsen and T.G. Hales. Depts. of Biology, Pharmacology & Anesthesiology, UCLA, LA, CA 90024.

We have investigated the $GABA_A$ receptor composition using subunit specific oligo-primers and RT-PCR in 14 cell lines (B35, B65, B103, B104, RINm5F, Rat1, PC12, C6, C17, C27, βTC3, NB41A3, AtT-20 & HIT). Thirteen $GABA_A$ receptor subunits have been cloned from mammalian tissue; α1-6, β1-3, γ1-3 and δ. This multiplicity of subunits provides a basis for the diverse pharmacology of the $GABA_A$ receptor. Attempts to understand the regulation and pharmacology of individual subunits and of the hetero-oligomeric receptor combinations have been impeded by a lack of access to pure populations of cells expressing $GABA_A$ receptor subunits. Permanent cell lines provide such a source.

Each of the receptor subunit mRNAs has been identified in at least one cell line and each cell line contained at least one $GABA_A$ subunit mRNA. However, in 6 cell lines examined using the patch-clamp technique, only the RINm5F cell line contained functional $GABA_A$ receptors (Hales et al., this meeting). We are currently investigating, using transfection and knockout strategies, the subunit requirements for functional channels in these cells.

In conclusion, these cell lines transcribe $GABA_A$ receptor subunits and therefore provide a tissue source for regulation and pharmacological studies. (Supported by MRC of Canada (RFT), NS22256 (AJT), NS28772 (RWO)).

469.2

RINm5F: A PANCREATIC CELL LINE WITH FUNCTIONAL $GABA_A$ RECEPTORS.

T.G. Hales*, R.W. Olsen, A.J. Tobin and R.F. Tyndale. Depts Anesthesiology, Pharmacology and Biology, UCLA, LA, CA 90024.

We have used the patch-clamp technique to characterize $GABA_A$ receptors expressed by an insulin-secreting cell line (RINm5F). γ-Aminobutyric acid type A ($GABA_A$) receptors are expressed throughout the mammalian nervous system. The receptor is also found in peripheral tissues e.g. the adrenal medulla and the islets of Langerhans. Despite the abundance of $GABA_A$ receptors *in vivo* and in primary cultured cells there has only been one report of a cell line expressing functional $GABA_A$ receptors (Hales et al., Mol. Pharm. 42: 197, 1992). The occurrence of 13 $GABA_A$ receptor subunits has been investigated by RT-PCR in this cell line and 12 others (Tyndale et al., this meeting).

Whole-cell GABA-evoked currents were recorded using the patch-clamp technique. RINm5F cells were voltage-clamped at −60 mV with CsCl and NaCl based internal and external solutions. GABA (100 μM) was applied transiently by pressure ejection (1.4×10^5 Pa for 0.01-1 s).

GABA activated inward currents in all cells tested (n=40). With a Cl^- reversal potential of 0 mV, GABA-evoked currents reversed at 1.3 ± 4.9 mV (± sem, n=4). GABA-activated currents were abolished (n=4) by bicuculline methiodide (10 μM) and potentiated by pentobarbital (100 μM) to 317 ± 89% of control amplitude (n=6). GABA-evoked currents were unaffected (n=4) by diazepam (10 μM) and were inhibited by 55 ± 10% (n=4) by Zn^{2+} (10 μM), in accordance with the RINm5F cell $GABA_A$ receptors lacking γ subunits.

RINm5F cells are the second cell line demonstrated to have functional $GABA_A$ receptors. We are now investigating the subunits required for functional receptors in cell lines using antisense and transfection paradigms.

469.3

CHLORIDE CHANNELS ACTIVATED BY GABA IN HIPPOCAMPAL SLICES AND IN SF9 CELLS INFECTED WITH RECOMBINANT VIRUSES.

B. Birnir, A. Everitt, A.C. Field*, M.L. Tierney, S.M. Howitt, G.B. Cox and P.W. Gage. John Curtin School of Medical Research, Australian National University, Canberra ACT 2601, Australia.

We have recorded single channel chloride currents activated by GABA in the dentate gyrus of rat hippocampal brain slices for comparision with events in cells expressing known subunits of the $GABA_A$ receptor. The brain was immersed in artificial cerebrospinal fluid and 400 μm thick slices, cut with a standard vibrating microslicer, were incubated for an hour at 35 °C. The slices were then kept at room temperature for the remainder of the day and patched using the "blind" patch clamp method. No enzymes were applied. The single channel current-voltage relation showed outward rectification in cell-attached and inside-out patches. Subconductance states were present and were more numerous at depolarized than at hyperpolarized potentials. Conductance states up to 65 pS were observed at 80 mV. We have also expressed the α_1,β_1 and γ_2S subunits of the $GABA_A$ receptor in the Sf9-baculovirus expression system. The formation of functional channels was found to be dependent on subunit composition. Properties of reconstituted receptors are being compared with native $GABA_A$ receptor.

469.4

PROTEIN KINASE C INCREASES RECOMBINANT α1β1γ2L $GABA_A$ RECEPTOR CURRENTS EXPRESSED IN L929 CELLS.

Y.-F. Lin[1*], M.D. Browning[3] and R.L. Macdonald[1,2]. Depts. of Physiology[1] and Neurology[2], Univ. of Michigan, Ann Arbor, MI 48109, and Dept. of Pharmacology[3], Univ. of Colorado, Denver, CO 80262.

During whole-cell recording from mouse fibroblast L929 cells which were transiently transfected with recombinant α1β1γ2L $GABA_A$ receptor subunit cDNAs, intracellular dialysis with intrapipette solutions containing catalytically active protein kinase C (c-PKC, 1-2 μM) enhanced the $GABA_A$ receptor currents and reduced the rate of current run-down during repetitive GABA (10 μM) application. Addition of PKC-inhibitory peptide (PKC-I, 4 μM) to the c-PKC resulted in GABA responses which were similar to those of control cells. Peak and normalized peak GABA-evoked currents ran-down faster over 12 minutes in both control cells (1124 ± 231 to 532 ± 135 pA, 100% to 58 ± 7%, mean ± SEM, n = 14) and c-PKC- plus PKC-I-treated cells (1075 ± 282 to 395 ± 120 pA, 100% to 57 ± 16%, n = 6), than those of c-PKC-treated cells (1287 ± 240 to 1012 ± 187 pA, 100% to 86 ± 7%, n = 20). Consecutive recordings from individual cells with a pipette containing control internal solution alone followed by another pipette containing c-PKC in addition to the control internal solution further confirmed that PKC increased GABA-evoked whole-cell currents. These data suggest that the function of $GABA_A$ receptors is enhanced by PKC, most likely by direct PKC phosphorylation of the $GABA_A$ receptors, or indirectly through phosphorylation of other intracellular components. Data from single-channel recordings are under analysis.

Supported by a Markey Foundation grant to R.L.M.

469.5

5-HT_3 RECEPTOR ANTAGONISTS AND $GABA_A$ RECEPTORS: A FUNCTIONAL STUDY.

R.L. Klein, E. Sanna, S.J. McQuilkin, J.M. Sikela*, P.J. Whiting and R.A. Harris. Dept. of Pharmacol., Univ. of Colorado & Denver VAMC, Denver, CO 80262, and Merk, Sharp & Dohme Res. Lab., Essex, U.K.

5-HT_3 receptor antagonists and drugs acting on $GABA_A$ receptors share the property to modulate anxiety and to alter some behavioral effects of ethanol. Based on these similarities, we tested the ability of different 5-HT_3 receptor antagonists to affect GABA-gated Cl^- currents recorded in *Xenopus* oocytes injected with human $GABA_A$ receptor subunit cDNAs. In oocytes expressing α1β1γ2S receptors MDL 72222 and LY 278584 inhibited GABA-gated currents, while zacopride was ineffective. ICS 205-930 induced a biphasic effect. At lower concentrations (0.1-5 μM), it potentiated, in a dose-dependent manner, GABA responses (44%, at 2.5 μM), whereas higher concentrations (50-100 μM) produced about 50% inibition. Ro15-1788 (1 μM) antagonized the ICS 205-930-induced increase of GABA-gated currents. Similarly, when oocytes were injected with α1β1 (but not the γ2S) subunit cDNAs, the stimulatory effect of ICS 205-930 could no longer be observed, suggesting that ICS 205-930 might interact with the benzodiazepine site. Consistent with these results is the finding that ICS 205-930 displaced ^{3}H-flunitrazepam binding in mouse cortical membranes and HEK 293 cells transfected with α1β1γ2S subunit cDNAs, with Ki values of about 1 μM. MDL 72222 and LY 278584 displaced this binding with Ki values around 20 μM. On the contrary, the inhibitory action on GABA-gated currents induced by higher concentrations (25-100 μM) of ICS 205-930 was not prevented by Ro15-1788. These results indicate that ICS 205-930 and other 5-HT_3 receptor antagonists modify GABAergic function, an effect that may be important for some of the actions of these drugs.

469.6

INTERACTIONS BETWEEN Zn AND THE GABAA RECEPTOR/IONOPHORE COMPLEX EXPRESSED IN XENOPUS OOCYTES.

D. A. Gurley*, V. Stürling, C. Hartnett, P.C. Ross, G. White. Neurogen Corp., Branford, CT 06405.

Benzodiazepine insensitive αβ subunit combinations are affected in a non-competitive manner by Zn (Draughn et al. Neuron 5:1990). αβγ combinations are insensitive to Zn and are sensitive to benzodiazepines (Ibid). However, in benzodiazepine sensitive cultured neurons Zn produces a non-competitive block of GABA current. This block is not altered by benzodiazepines (Celentano et al. Mol. Pharm. 40:1991), suggesting that Zn can act independently of the γ subunit. In addition, Zn reduces synaptic responses in cultured neurons (Mayer and Vyklicky, J. Physiol. 415:1989), but not in brain slices (Xie and Smart, Nature 349:1991). The lack of effect in brain slices could result from high GABA concentrations at the synapse overcoming the block by Zn in a competitive manner.

Xenopus oocytes were injected with poly-A mRNA coding for different GABAA subunits or with poly-A selected cortical message with and without anti-sense to γ1, γ2, and γ3 mRNA. Zn decreased current amplitude evoked by 10 μM GABA for cortical mRNA (IC50=9±1 μM, n=5), cortical+anti-sense mRNA (IC50=7±1 μM, n=4), and α1β1γ2 mRNA (IC50=1±1 μM, n=5). This interaction appeared competitive because the maximum amplitude of GABA current and the Hill coefficients were not altered (p>0.2). The shift in EC50 (log μM GABA) was larger in cortical and cortical+anti-sense cells than in α1β1γ2 cells (0.17±.02 (n=6) and 0.22±.03 (n=5) vs 0.09±.03 (n=5), respectively, p=.01). For α1β2δ subunits, interactions were non-competitive. 40 μM Zn inhibited saturated GABA (1mM) responses by 50±5% (n=4). Anti-sense+ cortical mRNA and α1β2δ injected cells were insensitive to benzodiazepines. Other cells were sensitive.

In summary, reduction of GABA current by Zn can appear competitive. Blocking γ expression in the presence of cortical mRNA does not alter sensitivity to Zn. δ in combination with α1β2 subunits does not mimic the γ2 subunit in terms of Zn pharmacology. We conclude that Zn is not a reliable probe for identifying the γ subunit. More than one factor contributes to inhibition of the GABAA complex by Zn and the to nature of the inhibition.

469.7

PROPOFOL MODULATES $GABA_A$ RECEPTORS WITH AND WITHOUT γ2 SUBUNITS. E.P. Greenblatt, A.R. Brooks-Kayal*, D.B. Pritchett, N.L. Harrison, M.V. Jones, T.G. Hales. Depts Anesth, Pediat, Pharmacol & Neurol Univ Penn, PA 19104, Dept Anesth & Crit Care & Dept Pharmacol & Physic Sci, Univ Chicago, Il 60637 & Dept Anesthesiol, UCLA, CA 90024.

The anesthetic propofol potentiates GABA responses and directly activates $GABA_A$ receptors. Some $GABA_A$ receptor properties are subunit specific; we have compared GABA and propofol modulation of α2β1 and α2β1γ2 combinations.

The calcium phosphate method was used to transfect 293 cells with pCIS2 plasmids containing α2, β1 and γ2 $GABA_A$ receptor subunit cDNAs. Patch-clamp recordings at -60 mV, with CsCl and NaCl based internal and external solutions, were made 48-74 hr after transfection. GABA and propofol (PROP) were applied by pressure (28 kPa for 0.1-80 s) from low (~5 MΩ) resistance pipettes.

PROP (100 μM) activated currents in cells with α2, β1 (n=12) and α2, β1, γ2 (n=13) cDNAs. Zn^{2+} (5 μM) inhibited PROP-activated currents in cells transfected with α2, β1 cDNAs by 74% (n=2). 50 μM and 100 μM Zn^{2+} reduced PROP-evoked currents in cells with α2, β1, γ2 cDNAs by 18 ± 7% (± sem, n=4) and 66% (n=2). To abolish a contribution of α2β1 receptors in cells with α2, β1, γ2 cDNAs, we included Zn^{2+} (100 μM) in the external solution. Single-cell dose-response curves of current activation by GABA and PROP gave EC_{50} values of 9.4 and 8.3 μM for cells with α2, β1 cDNAs and 16.6 and 17.9 μM for cells with α2, β1, γ2 cDNAs. In these cells peak current amplitudes evoked by PROP were 42% (α2β1) and 200% (α2β1γ2) of those evoked by GABA, suggesting that the efficacy of PROP relative to GABA may be influenced by the γ2 subunit. Dose-response curves of potentiation by PROP of GABA (3 μM)-evoked currents had EC_{50} values of 2.5 μM (α2β1) and 4.8 μM (α2β1γ2). Propofol enhances GABA responses and activates $GABA_A$ receptors in cells with or without the γ2 subunit.

469.8

GABA ρ 1 RECEPTOR: MUTAGENESIS OF BASIC EXTRACELLULAR RESIDUES. *T. Kusama, C.E. Spivak*, J.B. Wang, and G. Uhl.* Mol. Neurobiol. Br.,ARC/NIDA & Depts. Neurol. & Nsci., JHUSM, Box 5180, Baltimore, MD 21224.

The GABA ρ 1 subunit, enriched in retina, can function as a homooligomeric GABA-gated chloride channel. ρ 1 may recognize $GABA_A$ agonists in conformations different from those in which they are recognized by other $GABA_A$ receptors. We sought sites corresponding to these pharmacological differences between the ρ 1 and α_1 subunits by constructing 18 mutations in basic, putative extracellular ρ 1 residues and testing expression in voltage-clamped *Xenopus* oocytes.

Mutations in residue 141H diminished the affinity of GABA or abolished GABA responses. Four mutations increased affinity by up to 3-fold. Mutants displayed Hill coefficients as low as 1.24 and as high as 3.9 (wildtype ρ 1 n_H = 2.26). No mutant enhanced the characteristically low ρ 1 responses to THIP; each mutant receptor retained ρ 1-like agonist selectivity. These results support significant roles for basic, extracellular residues of this GABA receptor in agonist recognition and in the subunit-subunit interactions thought to mediate cooperativity.

467.9

CLONING AND TISSUE SPECIFIC FUNCTIONAL CHARACTERIZATION OF THE RAT DBI PROMOTER. M. Kolmer[1], L. Pani[2], P. Longone[2], E. Costa[2] and H. Alho[1]*. [1]Dept. of Biomed. Sci., University of Tampere, 33101 Tampere, Finland, [2]Fidia Georgetown Institute for Neurosciences, University of Georgetown, Washington DC 20007, USA.

Diazepam binding inhibitor (DBI) is a 10 kDa polypeptide with diverse biological actions. This protein regulates mitochondrial steroidogenesis, glucose-induced insulin secretion, metabolism of acyl-CoA esters and GABA action on $GABA_A$ receptors. Three positive clones were isolated from a rat genomic library. One of the clones contained DBI genomic DNA fragment, encompassing 4 kb of the 5' untranslated region, the first two exons and part of the second intron of the DBI gene. Two other overlapping clones contained one of the DBI processed pseudogene. Several transcription initiation sites were detected using RNase protection and primer extension assays. Different tissues exhibited clear differences in the efficiencies of transcription site usage. Transient expression experiments, with DNA fragments of different length from the 5' untranslated region of the DBI gene showed that basal promoter activity requires -146 bp of the proximal DBI sequence, while full activation is achieved with 423 bp of the 5' untranslated region. DNaseI footprint experiments with liver nuclear proteins demonstrated three protected region between nucleotides -387 to -333; -295 to -271 and -139 to -176 from the ATG. The most proximal region (-139 to -176) in gel shift assays binds several general transcription factors (Sp1 and Ap1) as well nuclear restricted proteins which may be related to specific regulatory ptterns in different tissues. The DBI gene possesses some features of a housekeeping gene but also includes a regulation depending on the function that it subserves in different cell types.

467.10

ALCOHOL MODULATION OF GABA-INDUCED CHLORIDE CURRENTS IN TRANSFECTED HUMAN KIDNEY CELLS. W. Marszalec[1], Y. Kurata[1], B.J. Hamilton[2], D.B. Carter[2] and T. Narahashi*[1]. [1]Dept of Pharmacol., Northwestern Univ. Med. Sch., Chicago, IL 60611 and [2]CNS Diseases Research, The Upjohn Company, Kalamazoo, MI 49001.

The modulation of GABA-induced Cl^- currents by ethanol and octanol was studied in A293 cells expressing either the $\alpha_1\beta_2\gamma_{2S}$ or $\alpha_6\beta_2\gamma_{2S}$ $GABA_A$ receptor subunit combination. GABA evoked currents in $\alpha_1\beta_2\gamma_{2S}$ and $\alpha_6\beta_2\gamma_{2S}$ cells with ED_{50}s of 9.1 and 1.7 μM, respectively. Co-applications of 100 μM octanol increased the current amplitude in both combinations, shifting the dose-response curve to the left. Although ethanol (3 to 100 mM) did not increase the currents of either subunit combination, it significantly decreased the current desensitization time constant in $\alpha_6\beta_2\gamma_{2S}$, but not $\alpha_1\beta_2\gamma_{2S}$. Ethanol perfusion (100 mM, 1-10 min) decreased the average desensitization time constant from 7.9 to 4.3 sec in $\alpha_6\beta_2\gamma_{2S}$. Octanol perfusion, however, decreased the desensitization rates of both receptor combinations. Thus, ethanol has a differential effect on the desensitization rates of $\alpha_6\beta_2\gamma_{2S}$ and $\alpha_1\beta_2\gamma_{2S}$ $GABA_A$ without increasing the current amplitude in either combination. Supported by NIH grant AA07836.

GABA RECEPTORS: FUNCTION—BENZODIAZEPINES

470.1

A COMPARISON OF $GABA_A$ RECEPTOR FUNCTION AND SUBUNIT DISTRIBUTION IN THE CEREBRAL CORTEX AND HYPOTHALAMUS. Inglefield, J.R.[1]*, Sieghart, W.[3], and Kellogg, C.K.[2] Depts. of Neurobiology and Anatomy[1] and Psychology[2], Univ. of Rochester, New York, 14642 and [3]Biochem. Psychiatry, University Clinic for Psychiatry, Vienna, Austria.

Allosteric interactions between binding sites on the $GABA_A$ receptor complex are thought to implicate functional changes in the complex. Of current interest is the determination of subunit compositions that mediate specific receptor functions. We compared subunit distribution, by immunoreactivity to specific antipeptides, and function, measuring chloride (Cl^-)- facilitation of clonazepam-displaceable [^{3}H]-flunitrazepam (Flu; 1 nM) binding, between the cerebral cortex and hypothalamus (HYPO) in the rat. The α-1 subunit was of the highest density in both regions and prevalent throughout both regions. However, the α-2 and B2/B3 subunits, while of intermediate density in cortex, were of low density (or absent) in the HYPO. This difference in subunit distribution between regions corresponded to the functional reactivity of the $GABA_A$ receptor. Flu binding in the cortex was facilitated by increasing concentrations (12.5-500 mM) of Cl^-, and this facilitation was potentiated following 15 min. of restraint. In the HYPO, Flu binding was not responsive to increasing [Cl^-] in either the basal or restraint-stress conditions. As a result, there were no stressor-induced changes in Flu binding. Analysis of other subunits and functional indices of the complex are underway. The data indicate that the $GABA_A$ receptor differs in composition between the two regions, thereby yielding receptors with different functional properties. Supported by Grant No. DA 07080.

470.2

REGIONAL EXPRESSION OF $GABA_A$ SUBUNIT mRNA MEASURED *IN SITU* AFTER CHRONIC FLURAZEPAM (FZP) TREATMENT. E.I. Tietz*, X. Weng, H.C. Rosenberg and T.H. Chiu, Department of Pharmacology, Medical College of Ohio, Toledo, OH 43699

Chronic flurazepam (FZP) treatment results in BZ tolerance, $GABA_A$ agonist subsensitivity and a reduction in $GABA_A$-mediated IPSP amplitude in CA1 region of *in vitro* hippocampus. Regulation of $GABA_A$ receptor subunits may be associated with reduced GABA-mediated inhibition in hippocampus of FZP-treated rats. Expression of α_1 and α_5 subunit mRNAs were measured *in situ* in brain sections (10 μM) from 1 week FZP-treated (100 mg/kg X 3 days; 150 mg/kg X 4 days, p.o. in .02% saccharin) and control rats (6 rats/group) 2 days after the end of treatment. Post-fixed, dehydrated and defatted sections, handled in parallel, were hybridized (42°C, 4XSSC, 50% formamide, 100 mM DTT) with 45-base α_1 (342-356) or α_5 (355-369) oligoprobes, 3'-tailed with [^{35}S]dATP. Sections were washed (2XSSC @ 22°C to 0.5XSSC @ 55°C), dehydrated and dipped in NTB2 emulsion. Grains/1000 μM^2 were counted over cresyl violet-stained neurons in hippocampal regions CA1, CA3, CA4 and dentate gyrus, from digitized, microscopic (500x) images. For α_1, grains were also measured in frontoparietal cortex and dorsolateral and ventromedial substantia nigra pars reticulata. Expression of α_1 was significantly reduced 30% (CON:50.2±3.5; FZP:34.3±3.9) only in CA1 region of hippocampus ($p < .01$). Expression of α_5, which is primarily localized to hippocampus, was not changed. The discretely localized decrease in α_1 mRNA expression in CA1 region may explain the inability to detect changes in hippocampus previously and may contribute to GABA inhibitory dysfunction in CA1 region. The lack of change in α_1 expression in some areas, i.e. cortex, may reflect limited sampling. Additional subunits and brain regions are under study. Supported by NIDA grant RO1-DA04075 and RSDA K02-DA00180 to EIT.

470.3

TRANSCRIPTIONAL REGULATION OF $GABA_A$ RECEPTOR ALPHA-1 SUBUNIT GENE EXPRESSION. I. Kang*, D. Linquist, T.B. Kinane, L. Ercolani and L.G. Miller. Dept. of Pharmacology and Experimental Therapeutics, Tufts University, Sch. of Med., Boston, MA 02111, Renal Unit, Massachussettes General Hospital, Boston, MA 02114.

We have previously demonstrated that murine $GABA_A$ receptor alpha-1 and gamma-2 subunit mRNA levels are regulated by chronic administration of benzodiazepine in vivo (Kang & Miller, Br. J. Pharmacol. 1990, 103, p1285). To determine the molecular basis for this regulation, we screened a human genomic library to obtain DNA segments encoding the alpha-1 subunit gene. These DNA segments were then fused to a firefly luciferase reporter gene and transfected into primary cultures of chick cortical neurons to determine if they contained the 5' regulatory region of this gene. A strong orientation specific firefly luciferase activity was detected in these segments indicating they contained the gene promoter. Successive deletions indicated the minimal promoter to be encoded by a 50 nucleotide segment that lacked a TATAA sequence. Following chronic benzodiazepine treatment of chick neurons, the transcriptional activity of the gene was repressed in a dose dependent manner. We are currently determining the protein factors that act in "trans" which are required for the coordinated regulation of this gene.

470.4

SUBTYPE SPECIFIC CHANGES IN BENZODIAZEPINE NUMBER FOLLOWING SUBACUTE EXPOSURE TO ALPRAZOLAM. R.J.Primus, J. Yu, M. Lee and D.W. Gallager*. Neurogen Corp., 35 N.E. Industrial Rd., Branford, CT 06405.

We have previously reported significant decreases in $\alpha 1$ and $\gamma 2$ subunit mRNA levels of the GABA/BZD receptor complex in cortical membranes from rats continuously exposed to the BZD agonist diazepam (DZ). While these changes accompany behavioral signs of tolerance and physical dependence, surprisingly no change in ^{3}H-Flunitrazepam (Flu) or ^{3}H-RO151788 binding site density or affinity have been observed. Such discrepancies may suggest that the decrease in mRNA levels reflect receptor subtype-specific effects or that mRNA levels may not accurately reflect receptor protein. In the present study, we have examined the effects of alprazolam (ALP) using a short exposure course (3 days) of high p.o. doses (100 mg/kg, b.i.d.) on ^{3}H-Zolpidem binding. This treatment schedule was sufficient to produce both tolerance to the bicuculline seizure threshold elevating effects of ALP and physical dependence as demonstrated by precipitated withdrawal after BZD antagonist administration in ALP-treated rats. Zolpidem has previously been shown to be selecitive for the Type I BZD receptor, binding to $\alpha 1$-containing constructs expressed in mammalian cells. A significant decrease in B_{max}, but not K_D, for ^{3}H-Zolpidem binding was measured in cortical membranes from rats treated chronically with ALP as compared to membranes from vehicle-treated rats. While a decrease in GABA enhancement of ^{3}H-Flu binding has previously been shown in cortical membranes from chronic DZ-treated rats, no change in GABA enhancement of ^{3}H-Zolpidem binding was observed in cortical membranes from chronic ALP-treated rats. Results will be presented to demonstrate whether differences in chronic ALP and chronic DZ treatments reflect agonist- or receptor subtype-specific changes in the GABA/BZD receptor complex.

470.5

CHRONIC ALPRAZOLAM ADMINISTRATION DECREASES GABAA RECEPTOR ALPHA1 mRNA.
G.A. Prithcard,*B. Laurijssens and L.G. Miller.
Deparment of Pharmacology and Experimental Therapeutics, Tufts University School of Medicine, Boston, MA 02111.

Chronic benzodiazepine administration is known to be associated with the functional downregulation of the GABAA/Benzodizepine receptor complex. The receptor downregulation is responsible for the tolerance and dependence associated with chronic benzodiazepine administration. Previously we have demonstrated in an mouse model of chronic benzodiazepine administration that receptor downregulation, as indicated by behavioral and biochemical, data occurs between days four and seven. However, Northern analysis indicate that the time period for mRNA downregulation is not observed until day 14 of benzodiazepine administration. The present study utilized chornic administration of alprazolam (2 mg/kg) or vehicle for 1,7 ,14 and 28 days. In Situ hybridization histochemistry was utilized to quantitate mRNA levels with the cerebral cortex (layers II-IV, V and VI) and within the hippocampus (CA1 and CA3) and dentate gyrus. No effect of alprazolam was observed at days 1 and 7 of alprazolam administration, but at days 14 Alpha1 mRNA levels were significantly reduced in all levels of the cerebral cortex and within CA1 and the dentate gyrus but not CA3. Decreased levels of mRNA were also observed at day 28 of Alprazolam administration but to a lesser degree compared to day 14. These data indicate that chronic benzopiazepam administration significantly attenuates mRNA levels within various regions of the CNS and show regional and temporal specificity .

470.6

CHARACTERIZATION OF A NOVEL BENZODIAZEPINE RECEPTOR IN THE RAT SPINAL CORD USING [^{3}H]Ro15-1788 AND [^{3}H]Ro15-4513. P.A. Maguire*, M.F. Davies and G.H. Loew. Molecular Research Institute, Palo Alto, CA 94304.

We have recently detected a relatively low affinity binding site for [^{3}H]Ro15-1788 in the rat spinal cord that was insensitive to the imidazopyridine, alpidem (65 μM). We have begun to characterize this site by competitive binding of BDZR and nonBDZR ligands using both [^{3}H]Ro15-1788 and [^{3}H]Ro15-4513 at 0°C in the presence of 65 μM alpidem. Nonspecific binding was determined in the presence of 100 μM Ro15-1788 or Ro15-4513, respectively. The BDZR ligands Ro15-1788, Ro15-4513, diazepam, clonazepam, chlordiazepoxide, triazolam, alprazolam and brotizolam were used in competitive binding assays against [^{3}H]Ro15-4513. The rank order of affinity of these ligands was: Ro15-4513 (IC_{50} = 4 nM; nH = 1.1) > brotizolam (IC_{50} = 14 nM; nH = 0.8) > Ro15-1788 (IC_{50} = 20 nM; nH = 1.0) > triazolam (IC_{50} = 26 nM; nH = 0.5) > alprazolam (IC_{50} = 88 nM; nH = 0.8) > clonazepam (IC_{50} = 141 nM; nH = 0.7) > diazepam (IC_{50} = 208 nM; nH = 0.6) > chlordiazepoxide (IC_{50} > 10,000 nM). This rank order confirmed that found in preliminary studies using [^{3}H]Ro15-1788. Several additional ligands (zolpidem, prazepam, Ro5-4864 and PK11195) were unable to displace specific [^{3}H]Ro15-1788 binding from the alpidem-insensitive site at concentrations of up to 10 μM. It is clear that this BDZ binding site is not the peripheral BDZ receptor that is present in the spinal cord. However, it is equally clear from the receptor binding characteristics of BDZR ligands that this site is unlike any cloned receptor so far reported.
Supported by NIDA Grant DA 06304.

470.7

LABELLING OF DIAZEPAM-INSENSITIVE BENZODIAZEPINE RECEPTORS WITH A NOVEL SELECTIVE RADIOLIGAND. G. Wong[1], Z.-Q. Gu[1,2], B. de Costa[2], D. Matecka[2], L. Possom[1*], K. Rice[2], P. Skolnick[1]. Laboratories of [1]Neuroscience and [2]Medicinal Chemistry, NIDDK, NIH, Bethesda, MD 20892.

A diazepam-insensitive (DI) benzodiazepine receptor (BzR) subtype has been described in cerebellar tissues from a range of species including humans, rats, mice, cows, and pigeons. This subtype is distinguished by high affinity binding of the putative ethanol antagonist Ro15-4513, and some other imidazobenzodiazepines, pyrazoloquinolines, and β-carbolines, but not diazepam, flunitrazepam, or other 1,4-benzodiazepines. Molecular biology and expression studies have determined that the DI binding site can be reconstituted with $\alpha 6$ or $\alpha 4$, $\beta 2$, and $\gamma 2$ subunits. Novel 8-substituted imidazo[1,5-a][1,4]benzodiazepine esters were recently synthesized and evaluated for their affinities at DI. Among these compounds, tert-butyl 8-chloro-5,6-dihydro-5-methyl-6-oxo-4H-imidazo[1,5-a][1,4]benzodiazepine 3-carboxylate(ZG-63) was tritiated, and radioligand binding studies were performed in adult rat cerebellar and cortical tissues. Saturation analyses of [^{3}H]ZG-63 binding to cerebellar and cortical DI receptors revealed K_d values of 2.6 ± 0.2 nM and 8.5 ± 4.3 nM respectively, and B_{max} values of 609 ± 66 and 224 ± 93 fmol/mg protein, respectively. The high affinity and selectivity of this ligand for DI suggests this compound will be useful for elucidating the function(s) of this benzodiazepine receptor subtype.

470.8

FAST MONOSYNAPTIC INHIBITION IS REDUCED IN CA1 REGION OF HIPPOCAMPUS AFTER CHRONIC FLURAZEPAM TREATMENT. Xu Zeng*, X-H. Xie and E.I. Tietz, Department of Pharmacology, Medical College of Ohio, Toledo, OH 43699

Chronic flurazepam (FZP) treatment reduces GABA-mediated feedforward and recurrent inhibition in CA1 region of hippocampus and decreases $GABA_A$-mediated IPSP amplitude. The role of inhibitory interneurons in mediating reduced GABA inhibition in FZP-treated rats was assessed indirectly by activation of monosynaptic inhibition in *in vitro* hippocampal slices from rats made tolerant to FZP (100 mg/kg X 3 days; 150 mg/kg X 4 days in 0.02% saccharin). Slices (400 μM) were cut from rats sacrificed 2 days after treatment, when residual BZs are not detected in hippocampus. CA1 pyramidal cells were recorded intracellularly with glass microelectrodes (3M KAc, 60-80 MΩ, 33°C). Resting membrane potential was not different between groups and was adjusted to -60 mV for all cells during subsequent PSP measurements. As reported previously for cells recorded at 22°C, fast IPSPs elicited with just-subthreshold Schaffer collateral stimulation were significantly reduced (CON: 6.8±0.6; FZP: 2.2±0.3, $p < .01$). Pure monosynaptic IPSPs were activated in stratum radiatum <0.5 mm from the recording electrode in the presence of the excitatory amino acid receptor antagonists APV (50 μM) and DNQX (10 μM). Slow monosynaptic IPSPs were selectively depressed by addition of the $GABA_B$ receptor antagonist CGP (25 μM). The fIPSP was decreased during superfusion with APV and DNQX (CON: 6.7±0.9 mV; FZP: 3.7±0.7 mV $p < .01$) and with CGP (CON: 6.4±1.2; FZP: 3.4±0.8 mV $p < .05$). Reduction of interneuron firing rate may not be related to decreased GABA-mediated inhibition in hippocampus after chronic BZ treatment. Supported by NIDA grant RO1-DA04075 and RSDA K02-DA00180 to E.I.T.

470.9

[^{3}H]ZOLPIDEM BINDING TO DIAZEPAM AND MIDAZOLAM TOLERANT RAT BRAIN BENZODIAZEPINE RECEPTORS. Y. Wu, T.H. Chiu and H.C. Rosenberg*. Dept of Pharmacology, Medical College of Ohio, Toledo, Ohio 43699.

Treatment of rats with flurazepam causes tolerance to several benzodiazepines (BZs) and can decrease the Bmax of [^{3}H]flunitrazepam (FNP) binding in cerebral cortex (CT) and hippocampus (HP), but not in cerebellum (CB). Binding assays using [^{3}H]zolpidem (Zol), a selective agonist for BZ_1 receptors, revealed an even greater down-regulation in all areas, including in CB. Since treatment with diazepam (DZP) or midazolam (MDZ) also produced tolerance, it was hypothesized that down-regulation of BZ_1 receptors might also be produced during chronic treatment with these BZs. [^{3}H]Zol binding was studied in DZP and MDZ treated rat brain. Male, Sprague-Dawley rats were treated 3 wk with DZP released from s.c. silastic reservoirs, or MDZ (40 mg/kg/day) in drinking water, and then sacrificed immediately after stopping treatment. Results of [^{3}H]Zol binding to homogenates from CT, CB and HP were analyzed by Scatchard analysis. In DZP tolerant rats, no change in Kd or Bmax of [^{3}H]Zol binding was found in any of these three regions. No change in [^{3}H]Zol binding was found in CB of MDZ tolerant rat brain. These results suggest that tolerance in DZP-treated rats is not mediated by down-regulation of BZ_1 receptors, and that tolerance in MDZ-treated rats is not mediated by down-regulation of BZ_1 receptors in CB. Studies of [^{3}H]Zol binding to CT and HP of MDZ tolerant rat brain are under way. Supported by RO1-DA02194

470.10

COMPARISON OF THE ANTICONVULSANT ACTION OF THE BENZODIAZEPINES NNC 13-8119 AND DIAZEPAM : TOLERANCE AND CROSS-TOLERANCE STUDIES IN MICE. H. C. Jackson*. (SPON: Brain Research Association). Novo Nordisk A/S, Måløv, DK-2760, Denmark.

NNC 13-8119 (3-(5-cyclopropyl-1,2,4-oxadiazol-3-yl)-5,6-dihydro-5-methyl-6-oxo-4H-imidazo(1,5-a)(1,4) benzodiazepine) is a more potent anticonvulsant than diazepam in mice (ED50 values against clonic seizures induced by pentylenetetrazol (PTZ, 160 mg/kg s.c.) are 0.2 and 1.3 mg/kg i.p., respectively). It also produces less motor impairment than diazepam (ED50 rotarod >10 c.f. 1.3 mg/kg i.p.). This study investigates whether tolerance develops to the anticonvulsant action of NNC 13-8119 and also whether cross-tolerance occurs between NNC 13-8119 and diazepam. Male NMRI mice (20g; n=10) were treated twice a day for 3 days with NNC 13-8119 (1 or 3 mg/kg i.p.) or diazepam (10 mg/kg i.p.). On day 4 animals were given the same drug treatment (or the other benzodiazepine) and 30 min later clonic seizure thresholds were determined following i.v. infusion of PTZ (15 mg/ml; 12 ml/h). Control groups were given vehicle for 3 days and drug on day 4 or vehicle throughout. Clonic seizure thresholds of mice treated acutely with the benzodiazepine agonists were over 3 times higher than those of the vehicle-treated controls. Tolerance did not occur to the anticonvulsant effects of either dose of NNC 13-8119 in this model. However, the increase in seizure threshold of animals given chronic diazepam was reduced by 63% c.f. the acute group. The effects of NNC 13-8119 were significantly reduced (70%, 1 mg/kg; 59%, 3 mg/kg) in mice given chronic diazepam. In contrast the anticonvulsant effects of diazepam (10 mg/kg) were not altered by chronic NNC 13-8119 (1 mg/kg) and only slightly reduced (25%) by chronic NNC 13-8119 (3 mg/kg). These results suggest interesting differences between the effects of NNC 13-8119 and diazepam that warrant further investigation.

470.11

MODULATION OF MUSCIMOL STIMULATED ^{36}CHLORIDE INFLUX INTO BRAIN MEMBRANE VESICLES BY ANTICONVULSANT AND ANTISPASTIC TRIAZOLES. J.A. Miller*, D.L. Braun and P.A. Chmielewski, Marion Merrell Dow Research Institute, Cincinnati, OH, 45215.

Two classes of novel triazoles have previously been identified as either potent broad spectrum anticonvulsants or selective in protecting against strychnine seizures and hyperreflexia, a model of spasticity. Neither class of compound displaces the binding of [^{3}H]muscimol, [^{3}H]flunitrazepam or [^{35}S]TBPS to the $GABA_A$ receptor. We examined the effects of the anticonvulsant triazoles, MDL 27,266 and MDL 27,192 and the antispastic triazole MDL 27,531 on muscimol stimulated $^{36}Cl^-$ influx into brain membrane vesicles. Vesicles were prepared from rat brain by the method of Harris and Allan (Science 228: 1108, 1985).

MDL 27,531 (100 nM) enhanced muscimol stimulated $^{36}Cl^-$ influx into cerebellar but not cortical membranes. This effect was attenuated by the benzodiazepine antagonist flumazenil (10 μM). MDL 27,266 had no effect on either cortical or cerebellar muscimol stimulated $^{36}Cl^-$ influx but inhibited influx in the presence of flumazenil (10 μM) in cerebellar membranes. MDL 27,192 (10 - 10000 nM) had no effect on $^{36}Cl^-$ influx in cortical and cerebellar tissue. These data (and see Ogden and Rogers, Soc. Neurosci. Abstr., 1993) suggest that MDL 27,531 and MDL 27,266 selectively interact with some $GABA_A$ receptor subtypes in a novel manner.

470.12

GABA-ACTIVATED CHLORIDE CURRENTS FROM CULTURED RAT NEURONS ARE SELECTIVELY MODULATED BY ANTICONVULSANT AND ANTISPASTIC TRIAZOLE COMPOUNDS. C.J. ROGERS* AND A.M. OGDEN, Marion Merrell Dow Research Institute, Cincinnati, OH 45215.

Two classes of triazole-derived compounds have been identified previously, one a broad spectrum anticonvulsant, the other an antispastic selective for protection against strychnine-induced seizures and hyperreflexia. These compounds did not displace binding of [^{3}H]muscimol, [^{3}H]flunitrazepam or [^{35}S]TBPS to the $GABA_A$ receptor. We sought to determine whether the anticonvulsants MDL 27266 and MDL 27192 as well as the antispastic selective compound MDL 27531 modulate GABA-activated chloride currents. GABA-activated chloride currents are modulated via allosteric receptor interactions.

Single channel recordings were obtained from cultured rat hippocampal- or spinal cord motor-neurons in response to the pressure application of the neurotransmitter gamma aminobutyric acid (GABA 2uM) alone or GABA(2 uM) plus either MDL 27531(100nM), 27266(100nM) or 27192(100nm).

Each of these compounds increased total pooled GABA-activated chloride current. MDL 27531, the antispastic compound increased GABA-activated chloride current in spinal cord motorneurons but did not increase chloride current in hippocampal pyramidal neurons suggesting a $GABA_A$ subtype selective effect. MDL 27266 produced a small enhancement of total chloride current in hippocampal pyramidal neurons while MDL 27192 greatly enhanced total chloride current. These data indicate (and see Miller et.al., Soc. Neurosci. Abstr., 1993) that MDL 27531, 27266 and 27192 modulate $GABA_A$ receptor chloride currents in a receptor subtype specific manner.

470.13

THE EFFICACY OF IMIDAZENIL ON ISONIAZID-INDUCED DOWNREGULATION OF GABAERGIC TRANSMISSION. M. Serra*, C.A. Ghiani, C. Motzo, P. Giusti and G. Biggio. Depts. Experimental Biology and Pharmacology, Universities of Cagliari and Padua, Italy.

In rats and mice, the effect of the imidazobenzodiazepine imidazenil (IMZ), a new ligand for benzodiazepine (BZ) receptor possessing anxiolytic and anticonvulsant properties, was evaluated on the function of central γ-aminobutyric acid $(GABA)_A$ receptor complex, both "in vitro" and "in vivo". IMZ displaced [^{3}H]Flumazenil binding with a IC_{50} of 0.9 nM showing that this compound binds with high affinity to BZ receptors. Added "in vitro" to mouse cortical membrane preparation, IMZ like the antagonist flumazenil, failed to modify [^{3}H]Muscimol binding, muscimol-stimulated $^{36}Cl^-$ uptake and [^{35}S]butylbicyclophosphorothionate ([^{35}S]TBPS) binding. After i.p. injection to mice IMZ failed to change [^{35}S]TBPS binding measured "ex vivo" in cortical unwashed membrane preparations. On the contrary, the i.p. administration of lorazepam (LZ) and abecarnil (AB) induced in 30 min a marked reduction of [^{35}S]TBPS binding in the same tissue preparation. In contrast to the effect in control animals, IMZ reduced in a dose-dependent manner the increase of [^{35}S]TBPS binding elicited by isoniazid (200 mg/kg s.c.), an effect mimicked by LZ and AB while bretazenil (BTZ) failed to do it. Moreover, IMZ at very low dose (0.05 mg/kg i.p.) delayed the onset of convulsions and death elicited by isoniazid and reduced significantly the number of mice presenting seizures. Thus, IMZ showed an efficacy much greater than BTZ and lower than LZ and AB. On the other hand, IMZ had a potency almost similar to AB. Accordingly, IMZ showed also a great potency in antagonizing the convulsion induced by pentylenetetrazole (PZ). In fact, at the dose of 0.8 mg/kg p.o. it antagonized the 50% of convulsions induced by 70 mg/kg (i.p.) of PZ while LZ and BTZ had the same effect at higher doses (3.8 and 2.4 mg/kg, respectively). Finally, IMZ antagonized completely the increase of [^{35}S]TBPS binding induced by both foot shock and exposure to carbon dioxide. The results demonstrate that the efficacy of IMZ on $GABA_A$ receptor function is greatly enhanced when the GABAergic transmission is reduced. This unique property of IMZ may give to this drug an efficacious anticonvulsant and anxiolytic action with a reduced incidence of unwanted side effects.

470.14

ZINC MODULATION OF $GABA_A$ RECEPTOR FUNCTION IN BRAIN MICROSACS. M. Li, T.H. Chiu* and H.C. Rosenberg. Dept. of Pharmacology, Med. College of Ohio, Toledo, OH 43699.

Previous studies suggested that zinc inhibited the effect of GABA at $GABA_A$ receptors. Most of the evidence was obtained from electrophysiological experiments in cultured cells. In the present study, we examined zinc modulation of $GABA_A$ receptor function using GABA-stimulated $^{36}Cl^-$ influx in rat brain microsacs. $ZnCl_2$ (1-100 μM) did not affect basal Cl^- influx, but inhibited the 30 μM GABA-stimulated Cl^- influx in a dose dependent fashion. Zinc (30 μM) inhibition could be partially, but not completely, surmounted by increasing the GABA concentration (e.g., 55.0% inhibition at 5 μM GABA, 17.6% inhibition at 500 μM GABA). Zinc produced differing degrees of inhibition in different brain regions. The order of sensitivity to inhibition of 30 μM GABA-stimulated Cl^- flux was hippocampus (48.3%) > cerebral cortex (34.9%) > cerebellum (18.8%). These regional differences may reflect the structural heterogeneity of $GABA_A$ receptors among brain areas. In the presence of zinc, the percent enhancement of GABA-stimulated Cl^- influx produced by diazepam was increased, showing that zinc did not interfere with the ability of benzodiazepine (BZ) to enhance GABA-stimulated Cl^- flux. This suggests that $GABA_A$ receptors possessing BZ sites may be less susceptible to zinc inhibition. Studies are in progress to determine if zinc modulation of GABA function may be a useful tool in studying BZ tolerance. (Supported grant RO1-DA02194)

470.15

Effects of propofol and midazolam on $GABA_A$ receptor-mediated responses in rat hippocampal neurons *in vitro* and pars reticulata neurons *in vivo*. **R.F. Cox*, D.G. Lang, and C.M. Wang. Burroughs Wellcome Co., Research Triangle Park, NC 27709.**

Propofol and midazolam are induction agents whose effects have been associated with activation of the $GABA_A$-chloride channel complex. We characterized the mechanisms of propofol and midazolam effects on this complex in cultured hippocampal neurons *in vitro* and on firing rates of substantia nigra pars reticulata (SNR) neurons *in vivo*.

Propofol potentiated GABA-induced chloride currents in a dose-dependent manner in hippocampal neurons with an EC_{50} of 41 μM. Propofol alone activated a current that showed ionic selectivity for chloride like the current activated by GABA. The potency of propofol was one-tenth that of GABA. The GABA-mimetic action of propofol was not affected by diazepam or flumazenil. Both propofol- and GABA-induced currents were inhibited by bicuculline and picrotoxin. Midazolam, inactive alone, potentiated GABA with an EC_{50} of 3.8 μM, an effect blocked by flumazenil and picrotoxin. Neither propofol or midazolam affected glycine-induced chloride currents.

Propofol (0.3-5 mg/kg, iv) inhibited SNR firing rates in a dose-dependent manner with an ID_{50} of 1 mg/kg. This action was partially blocked by bicuculline (5-10 mg/kg iv) and reversed by picrotoxin (4-8 mg/kg iv), but was unaffected by flumazenil (1 mg/kg iv). Low doses of propofol potentiated inhibitions of SNR firing rate induced by iontophoretically applied GABA. Midazolam alone inhibited SNR firing with an ID_{50} of 50 μg/kg, an effect which could be reversed by flumazenil. Like propofol, midazolam enhanced the activity of GABA applied by iontophoresis.

These results indicate that propofol has a direct GABA-mimetic effect and also potentiates GABA at the $GABA_A$-chloride channel. The activity of midazolam is dependent upon endogenous or applied GABA.

470.16

REGIONAL VARIATIONS OF Zn-SENSITIVITY OF $GABA_A$ RECEPTORS IN RAT BRAIN. **M. Gordey, I. Spigelman, T.DeLorey and R.W. Olsen*. Department of Pharmacology, School of Medicine, University of California, Los Angeles, CA 90024.**

Zn^{2+}, known to play an essential role in neuronal excitability, inhibits $GABA_A$ receptor function in rat brain invitro and the effect varies with brain region. Zn^{2+} is widespread throughout the brain, concentrated highly in hippocampal mossy fibers, cortex and cerebellum, but is absent in other nontelencepha-lic regions. Recombinant $GABA_A$ receptors lacking γ-subunits are susceptible to Zn^{2+} inhibition. We have found that $ZnCl_2$ (100 μM) decreases muscimol (50 μM)-stimulated $^{36}Cl^-$ efflux in slices of rat thalamus and hippocampus, with similar sensitivity in DG and CA1 regions. Inferior colliculus and cerebellum appear to be insensitive to Zn^{2+}. By contrast, in microsac assays, muscimol (10 μM)-stimulated $^{36}Cl^-$ flux was inhibited by Zn^{2+} in all four regions: hippocampus (72±4%), cerebellum (58±4%), thalamus (45±3%), inferior colliculus (54±7%). According to previous observations, Zn^{2+}-sensitive receptors are devoid of benzodiazepine stimulatory effects. Our experiments with simultaneous application of diazepam (10 μM) and $ZnCl_2$ (100 μM) during 5 sec incubation of μsacs with $^{36}Cl^-$ and muscimol (10 μM) appear to show the possible existense of a $GABA_A$ receptor subtype that is sensitive to both Zn^{2+} and benzodiazepines.

Supported by NS28772.

470.17

FUNCTIONAL AND PHARMACOLOGICAL PROPERTIES OF $GABA_A$ RECEPTORS IN ACUTELY DISSOCIATED RAT CEREBELLAR PURKINJE CELLS. P. Avenet*, V. Itier, P. Granger, B. Biton, B. Scatton, and H. Depoortere. Dept. of Biology, Synthélabo Recherche (L.E.R.S), 31 Ave. P.V-Couturier, 92220 Bagneux, France.

The distinct pharmacological properties (affinity and efficacy) of the omega (BZ) site agonists zolpidem, diazepam and zopiclone has been used to identify the subtype(s) of $GABA_A$ receptors present in native Purkinje cells. Cerebellar Purkinje cells were isolated from 7-11 day-old rat cerebella using a trypsin/mechanical dissociation protocol and GABA-induced currents studied using the whole cell configuration of the patch-clamp technique. All Purkinje cells responded to GABA and GABA-induced currents could be recorded for more than an hour without sign of run-down when the standard CsCl ATP containing pipette solution was used. With a -20 mV holding voltage, the current induced by 1 μM GABA averaged 107 ± 14 pA (n=29). Desensitization was slow (time constant > 60 sec) for GABA concentrations below 10 μM. The GABA concentration dependence reached saturation at 30 μM GABA with an EC_{50} value of 2.9 μM and a Hill coefficient of 1.8. Zolpidem, diazepam and zopiclone potentiated the GABA (1 μM)-induced current with EC_{50} values of 94, 50 and 22 nM and potentiation factors (at 1 μM) of 2.48, 1.91 and 2.16, respectively. These results are similar to those previously obtained with $\alpha_1\beta_2\gamma_2$ recombinant receptors transfected in 293 kidney cells (Avenet et al. 1992, Neurosci. abstr. 18:402) and taken together indicate the expression, in native Purkinje cells, of this $GABA_A$ receptor subtype, the exclusive presence of which was previously suggested by *in situ* hybridization experiments.

470.18

CHRONIC NEUROSTEROID TREATMENT PRODUCE UNCOUPLING OF THE GABA-BENZODIAZEPINE RECEPTOR COMPLEX IN MAMMALIAN CORTICAL NEURONS. Maharaj K. Ticku* and Rong Yu, University of texas Health Science Center, San Antonio, TX 78284-7764.

The effect of chronic 5α-pregnane-3α-ol-20-one (neurosteroid 5α3α) treatment was investigated on the binding of [^{3}H]flunitrazepam and its coupling to various sites associated with the $GABA_A$ receptors in cultured cortical neurons. Chronic treatment with neurosteroid 5α3α did not alter the basal [^{3}H]flunitrazepam binding to the intact neurons. However, 5α3α treatment produced a decrease in the E_{MAX} value of GABA enhancement (determined with 500 μM GABA) on [^{3}H]flunitrazepam binding from 118% (control cells) to 85% and 31% after 5α3α treatment (2 μM for 3 and 5 days, respectively); to 106%, 91%, 105%, 85% and 31% (5α3α 5 days with concentrations of 0.1, 0.2, 0.5, 1 and 2 μM). Studies of enhancement of [^{3}H]flunitrazepam binding by various modulators showed that the EC_{50} value of GABA, pentobarbital sodium and 5α3α were unaltered, however, their E_{MAX} value decreased. The E_{MAX} value of GABA decreased from120% to 27%; while the EC_{50} (12 μM v/s 9 μM) value was not altered after chronic treatment (5α3α; 2 μM for 5 days). The E_{MAX} value of pentobarbital sodium decreased from 155% to 40%; while the EC_{50} was not altered (210 μM v/s 160 μM). The E_{MAX} of 5α3α decreased from 60% to 0. The results indicate that chronic neurosteroid treatment produces a functional uncoupling of allosteric sites of GABA receptor complex in the mammalian cortical neurons. This uncoupling appears to be heterologous in nature. The molecular basis of the uncoupling phenomenon is under investigation. (Supported by NIH-NINDS grant # NS-15339)

GABA RECEPTORS: FUNCTION—$GABA_B$, $GABA_C$

471.1

The $GABA_B$ Receptor Agonist Baclofen and Antagonist CGP-35348 Modulate Synaptically-Evoked Responses in the Rat Dentate Gyrus, In-Vivo. F.H. Brucato[1], W.A. Wilson[1,3*], H.S.Swartzwelder[2,3] Duke Univ. Depts. of Medicine[1], Psychiatry[2], and VA Med Center[3] Durham NC 27705

There is now much evidence from hippocampal slices that $GABA_B$ receptors inhibit single and paired-pulse evoked responses in the hippocampus/dentate gyrus network. However, few in-vivo studies have demonstrated the role of $GABA_B$ receptors in modulating synaptic responses. We have previously shown that the $GABA_B$ receptor antagonist, CGP-35348, attenuates paired-pulse disinhibition in this network in-vivo, in the absence of any significant effect on baseline population spikes (PS) or pEPSPs.

We report here that the $GABA_B$ agonist baclofen significantly effects synaptically evoked responses in the dentate gyrus in-vivo. Extracellular recordings were made in the dentate gyrus of urethane anesthetized rats following stimulation of the angular bundle. At a dose of 10mg/kg, baclofen produced a significant enhancement of single pEPSP durations. In addition, baclofen attenuated paired pulse (150 ms) disinhibition of pEPSP durations. Paired pulse disinhibition of PS amplitude was also reversed by baclofen when paired pulses were presented at 150 ms interstimulus intervals. Baclofen also reversed recurrent inhibition at 25ms interstimulus intervals. CGP-35348 reduced this effect of baclofen on recurrent inhibition in addition to reversing baclofen's enhancement of a single pEPSP duration. We conclude that $GABA_B$ receptor activation produces mixed inhibitory and disinhibitory effects in the dentate gyrus in-vivo, and that these effects are prominent at interstimulus intervals which mimic endogenous theta activity.

Supported by VA Merit Review Grant to HSS.

471.2

BACLOFEN INHIBITS GLUTAMATE-MEDIATED EXCITATORY POSTSYNAPTIC CURRENTS IN RAT SUPRACHIASMATIC NEURONS IN VITRO Z.-G. Jiang* and R.A. North Vollum Institute, Oregon Health Sciences University, Portland, OR 97201.

Membrane currents were recorded with whole-cell patch clamp methods from cells in horizontal slices (500 μm) of brain with optic nerves attached. Single stimuli to the optic nerve evoked inward synaptic currents (EPSC, 5 - 120 pA at -60 mV) in almost all cells. Focal stimulation of the slice surface evoked a combined EPSC and outward synaptic current (IPSC). Spontaneous EPSCs and IPSCs persisted in tetrodotoxin; they were up to 60 pA and occurred at 0.2 to 60 Hz. Evoked and spontaneous EPSCs were blocked by CNQX (10 μM); evoked and spontaneous IPSCs were blocked by bicuculline (30 μM). Baclofen (1 μM) reduced the amplitude of the evoked EPSC by 20 - 60 % and reduced the frequency of spontaneous EPSCs by 15 - 55%; these effects were blocked by 2-hydroxysaclofen. Baclofen did not change the amplitude of spontaneous EPSCs, and did not affect evoked or spontaneous IPSCs. It is concluded that activation of $GABA_B$ receptors inhibits the release of excitatory amino acids at synapses within the suprachiasmatic nucleus.

471.3

ACTIVATION OF $GABA_B$ RECEPTORS INDUCES A TRANSLOCATION OF PROTEIN KINASE C IN NEONATAL RAT HIPPOCAMPAL SLICES. M.P.Roisin, E. Tremblay* and Y. Ben-Ari, U29, 123 Bd Port-Royal, 75014 Paris, France.

Protein phosphorylation constitutes an important mechanism in the tranduction of extracellular signals that modulate neuronal processes. GABA receptors which mediate most of the inhibitory drive in the adult CNS play an important role in the early postnatal life (Cherubini et al. TINS 14, 515-519, 1991). We now report that GABA acting on $GABA_B$ receptors induces in neonatal but not in adult hippocampus a translocation of protein kinase C (PKC).

PKC activity was determined in cytosolic and membrane fractions, using a PKC enzyme assay kit (Amersham). Application of GABA (300 μM) caused a rapid increase in PKC activity in the membrane fraction (by 30-50%) and a decrease in the cytosol (by 30-50%). These effects of GABA 1) were neither blocked by the $GABA_A$ antagonist bicuculline (10 μM) and not mimicked by the $GABA_A$ agonist isoguvacine (10-100 μM); 2) were reproduced with the $GABA_B$ agonist baclofen (30 μM), an effect prevented by the $GABA_B$ antagonist saclofen (200 μM) ; 3) were not observed with slices from adult rats, even with 3 mM GABA. Using antibodies of various PKC isoforms present in the neonatal hippocampus (α, β, ε, ζ), we found that PKCα, P KC β and to a less extend PKCε but not PKCζ, are increased in the membrane fraction after $GABA_B$ receptor stimulation.

These results suggest that GABA acting on G-protein-linked GABA receptors modulates the activation of some PKC isotypes in the developing neonatal rat hippocampus.

471.4

PAIRED-PULSE DEPRESSION OF IPSCs IN RAT HIPPOCAMPAL PYRAMIDAL CELLS. T.A. Pitler* & B.E. Alger, Dept. of Physiol., Univ. of MD Sch. Med., Baltimore, MD 21201.

Paired-pulse stimulation results in depression of the second evoked GABAergic IPSC in hippocampal neurons (PPD), an effect believed to involve inhibition of transmitter release by a presynaptic $GABA_B$ autoreceptor. Previous work, using pharmacological methods, has not determined whether or not pre- and post-synaptic $GABA_B$ mechanisms are the same, and indeed, in some cases, PPD may result from "synaptic fatigue," independent of $GABA_B$ receptors. Using whole-cell voltage clamp in thick, rat hippocampal slices, we studied PPD of evoked monosynaptic IPSCs. Low-intensity stimulation produced little PPD, while higher intensities increased PPD, in apparent agreement with the "fatigue" hypothesis. However, while alterations of extracellular [Ca^{2+}] and [Mg^{2+}] changed the extent of synaptic fatigue, they did not affect PPD. Moreover, the specific $GABA_B$ antagonist CGP 35348 completely blocked PPD; thus, in our hands, PPD is mediated by $GABA_B$ receptors.

To determine if the $GABA_B$ mechanism of PPD was the same as that responsible for the postsynaptic $GABA_B$ response, we tested Ba^{2+}, pertussis toxin and phorbol esters and confirmed previous reports that each of these virtually abolishes postsynaptic $GABA_B$ responses. In contrast, in the identical cells, neither Ba^{2+} nor phorbol esters had any effect on PPD, and pertussis toxin only partly reduced it. These data provide the first clear distinction between physiologically activated post- and pre-synaptic $GABA_B$ receptors.

471.5

PHARMACOLOGICALLY DISTINCT RELEASE-MODULATING $GABA_B$ AUTORECEPTORS IN RAT BRAIN CORTEX AND SPINAL CORD. G. Bonanno, G. Bozzo Costa, F. Donadini, A. Fassio and M. Raiteri*. Istituto di Farmacologia e Farmacognosia, Viale Cembrano 4 - 16148 Genova (Italy).

GABA receptors inhibiting [^{3}H]GABA release have been characterized pharmacologically using rat brain cortex and spinal cord synaptosomes depolarized in superfusion with 9 mM KCl. Exogenous GABA concentration-dependently inhibited the release of [^{3}H]GABA both in cortex and spinal cord (EC_{50}: 1.23 and 1.01 μM, respectively). The effect of GABA was not mimicked by muscimol nor it was antagonized by bicuculline and picrotoxin. Similarly to GABA behaved the $GABA_B$ receptor agonist 3-aminopropylphosphonous acid (EC_{50}: 0.095 μM, cortex, and 0.075 μM, spinal cord). (-)-baclofen was equipotent to GABA in the cortex (EC_{50}: 1.37 μM) whereas it was almost inactive in the spinal cord (EC_{50}: 425 μM). (+)-baclofen was extremely weak in both brain areas. Phaclofen antagonized the effect of GABA in the cortex (IC_{50}: 47.9 μM) but not in the spinal cord (IC_{50}: >1000 μM). Opposite results were obtained with another $GABA_B$ receptor antagonist, CGP 35348, which antagonized GABA in the spinal cord (IC_{50}: 1.07 μM) but was inactive in the cortex (IC_{50}: > 300 μM). It can be concluded that pharmacologically distinct GABA autoreceptors exist in the central nervous system of the same animal species. The present results, compared to those obtained previously (Bonanno and Raiteri, J. Pharmacol. Exp. Ther. 262, 114-118, 1992), suggest the existence of at least four pharmacological subtypes of the $GABA_B$ receptor.

Supported by Italian MURST and CNR.

471.6

$GABA_B$ RECEPTOR-MEDIATED REGULATION OF TUBEROINFUNDIBULAR DOPAMINERGIC NEURONS IN THE MALE RAT. E.J. Wagner*, J.L. Goudreau, K.E. Moore and K.J. Lookingland. Dept. of Pharm. & Tox., Michigan State Univ., E. Lansing, MI 48824.

Dopamine released from the terminals of tuberoinfundibular dopaminergic (TIDA) neurons in the median eminence inhibits the secretion of prolactin from the anterior pituitary. The purpose of the present study was to examine the effects of $GABA_B$ receptor activation and blockade on the activity of TIDA neurons in the male rat, and to correlate changes in the activity of these neurons with alterations in plasma levels of prolactin. The activity of TIDA neurons was estimated by measuring the concentration of the dopamine metabolite 3,4-dihydroxyphenylacetic acid (DOPAC) in the median eminence. Plasma concentrations of prolactin were determined by double antibody radioimmunoassay. Intraperitoneal administration of the $GABA_B$ receptor agonist baclofen decreased DOPAC concentrations in the median eminence, and increased plasma concentrations of prolactin, in a dose- and time-dependent manner. Intracerebroventricular administration of the $GABA_B$ receptor antagonist 2-hydroxysaclofen had no effect on TIDA neurons *per se*, but did block the baclofen-induced decrease in DOPAC concentrations in the median eminence. These results reveal a lack of $GABA_B$ receptor-mediated tonic inhibition of TIDA neurons, but activation of these receptors following the administration of the $GABA_B$ agonist baclofen inhibits these neurons and thereby increases the secretion of prolactin. (Supported by NIH Grant MH 42802.)

471.7

$GABA_B$ RECEPTOR-MEDIATED REGULATION OF PERIVENTRICULAR-HYPOPHYSIAL DOPAMINERGIC NEURONAL ACTIVITY AND THE SECRETION OF α-MELANOCYTE STIMULATING HORMONE. J.L. Goudreau*, E.J. Wagner, K.E. Moore, and K.J. Lookingland, Department of Pharmacology and Toxicology, Michigan State University, East Lansing, MI 48824

Periventricular-hypophysial dopaminergic (PHDA) neurons tonically inhibit the secretion of α-melanocyte stimulating hormone (αMSH) from the intermediate lobe (IL) of the pituitary. The purpose of the present study was to examine $GABA_B$ receptor-mediated regulation of PHDA neuronal activity and the secretion of αMSH in the male rat. To this end, the effects of the $GABA_B$ agonist baclofen, and antagonist 2-hydroxysaclofen on PHDA neuronal activity and αMSH secretion were characterized. PHDA neuronal activity was estimated by measuring the concentrations of 3,4-dihydroxyphenylacetic acid (DOPAC) in the terminals of these neurons in the IL of the pituitary. Intraperitoneal administration of baclofen produced a dose- and time-related decrease in IL DOPAC concentrations and a corresponding increase in plasma αMSH concentrations. Intracerebroventricular administration of the $GABA_B$ antagonist 2-hydroxysaclofen did not alter basal IL DOPAC concentrations or plasma αMSH concentrations *per se* indicating that GABA does not tonically inhibit PHDA neuronal activity or αMSH secretion via $GABA_B$ receptors. 2-Hydroxysaclofen, however, did block the baclofen-induced decrease in IL DOPAC concentrations and increase in plasma αMSH concentrations. These results indicate that $GABA_B$ receptor activation inhibits PHDA neuronal activity and thereby increases the secretion of αMSH. (Supported by NIH grant, NS 15911)

471.8

A NOVEL GABA-INDUCED CURRENT IN SUBSTANTIA GELATINOSA NEURONS. M. Yoshimura, Y. Yajiri, K. Morita* and S. Nishi, Dept. of Physiology, Kurume Univ. Sch. of Med., Kurume, 830 Japan.

Substantia gelatinosa (SG) neurons have been thought to play a critical role in modulation of nociceptive transmission in the spinal cord. Intracellular recordings revealed in a subset of SG neurons that the GABA induced-response is partially insensitive to bicuculline and phaclofen. To study the property of this bicuculline and phaclofen insensitive fraction, blind patch clamp recordings were made from SG neurons in the transverse spinal cord slice of the adult rat. Bath applied GABA (100-500 μM) produced an outward current consisted of an initial peak and a slowly decaying plateau. The peak current was depressed by bicuculline (10-20 μM), but the plateau current was hardly affected. $GABA_B$ receptor antagonist, phaclofen (500 μM) also had no obvious effect on the plateau current. Both the peak and plateau currents were reversed in polarity near the Cl^- equilibrium potential. It appears, therefore, that the plateau current is similar to the recently reported $GABA_C$ receptor-mediated current. The $GABA_C$ receptor analog, *trans*-4-aminocrotonic acid (CACA; 0.5-1 mM) was, in fact, found to produce in SG neurons a response similar to the GABA response. The CACA-induced current was, however, completely abolished by bicuculline (20 μM). These findings provide evidence that SG neurons may express a novel GABA receptor which is not likely to be identical to $GABA_C$ receptor found recently in a visual pathway and that the receptor might be involved in modulation of nociceptive transmission in the spinal dorsal horn.

471.9

CRUSTACEAN THORACIC NEURONS IN CULTURE SHOW GABA RESPONSES WITH $GABA_C$ PHARMACOLOGY. C. Jackel, W. D. Krenz and F. Nagy*. LNPC, Univ. Bordeaux I and CNRS, 33120 Arcachon, France.

Two main classes of γ-aminobutyric acid receptors ($GABA_A$ and $GABA_B$) have been described in central nervous system of vertebrates. In invertebrates, most GABA receptors are "non-A" and "non-B". Here, we describe in crustacean neurons a GABA response with characteristics similar to the novel $GABA_C$ pharmacology described in the vertebrate retina (Feigenspan et al., 1993, Nature *361*:159-161; Qian and Dowling, 1993, Nature *361*: 162-164).

Neurons were dissociated from thoracic ganglia of *Homarus gammarus*, and cultured for 3-4 days. GABA was applied by pressure ejection and whole-cell currents were recorded with patch pipettes. Under voltage-clamp conditions, GABA evoked inward current at potentials more negative than -49 mV, together with an increase in membrane conductance. Reversal potentials determined with various intracellular chloride concentrations corresponded nicely to the chloride equilibrium potential. 70% of the GABA current was blocked by picrotoxin (50 μM). These data indicate that the GABA response is mediated by Cl^--channels. The effects of GABA were mimicked by the $GABA_A$ agonists muscimol and isoguvacine with a potency gradient of muscimol> GABA>isoguvacine, but were totally insensitive to the $GABA_A$ antagonists bicuculline and SR95531. Diazepam (1 μM) and phenobarbital (100 μM) had no effect on the GABA response, which was also insensitive to $GABA_B$ receptor agonists (baclofen, 3-APA) and antagonists (phaclofen). Finally we tried *cis*-4-aminocrotonic acid (CACA), a folded analogue of GABA that is diagnostic for a novel $GABA_C$ receptor (Drew et al., 1984, Neurosci. Lett. *52* :317-321). As in the vertebrate retina, it evoked membrane currents mediated by Cl^--channels as exemplified by the reversal potential value and picrotoxin sensitivity. Furthermore it was insensitive to bicuculline and baclofen. In conclusion, GABA receptors of thoracic ganglia neurons in *Homarus* and $GABA_C$ receptors in vertebrates have similar pharmacological profiles.

471.10

Evidence of GABAergic Neurons and GABA Receptors in Limulus CNS. R.F. Newkirk,* M.A. Blackshear, C. Lawrence, and B. Washington. Department of Biological Sciences, Tennessee State University, Nashville, TN 37209-1561.

Recently, emphasis has been focused on GABA as an inhibitory transmitter in both vertebrates and invertebrates. Most of the invertebrate work has been concentrated on Crustaceans' peripheral motor neurons and large neurons of the abdominal muscle receptor organs of lobsters and crayfish. In Limulus, GABA has been shown to inhibit the rhythmic activity of the isolated heart and cardiac ganglion (Burgess and Kuffler, 1957; Van Burg & Corning, 1971) and to inhibit dorsal nerve activity and neurons in central ganglia (Walker & Robert, 1982; Washington et al, 1990). Also, Benson (1989) has identified a novel GABA receptor in Limulus heart. This study demonstrates the presence of GABA immunoreactive neurons in the post esophageal ganglia of Limulus. This distribution is consistent with axonal outputs in the 6th, 7th, and 8th dorsal nerves which give rise to nerves innervating the heart. Additionally, our results provide evidence of [^{3}H] GABA binding sites and endogenous GABA levels in these ganglia. These results support the contention that GABA serves as a neurotransmitter in Limulus and strongly suggests that the function is mediated through the 7th and 8th dorsal nerves. (NIH S06 GM08092 and 5G12 RR03033.)

GABA RECEPTORS: DEVELOPMENT

472.1

GABA RECEPTOR-COUPLED Cl^- CHANNELS EXHIBIT HETEROGENEOUS ELECTRICAL PROPERTIES IN EARLY EMBRYONIC NEUROEPITHELIAL CELLS. R. Serafini*, W.Ma, K. Tang, and J.L. Barker. Lab. Neurophysiology, NINDS, NIH, Bethesda MD 20892.

In situ hybridization histochemistry of embryonic (E) rat spinal cord probed for 13 $GABA_A$ receptor subunit mRNAs has shown that only $\alpha_4\beta_1\gamma_1$ subunit mRNAs can be detected in the ventricular zone (vz) over E13-17. We have isolated vz cells at E13 from the dorsal cervical spinal cord and studied the electrical properties of GABA receptor-coupled Cl^- channels by perforated-patch whole-cell recording. Dose-response curves showed that 1) 5-10 μm was the minimal effective dose, 2) 200 μm evoked the maximum effects, 3) $E.C._{.50}$ was 33 μm and 4) the Hill coefficient was 1.3. Fluctuation analysis of currents evoked by 10 μm GABA led to estimated preferred conductances of 7-8 pS(n=2), 12-15 pS (n=3), 18-19 pS (n=2) and 27-28 pS (n=2). Single channel level of activity directly recorded in whole cell yielded conductances of 23-25 pS (n=8) and 28 pS (n=1). Neither 100 μm bicuculline nor 100 μm picrotoxin, nor 100 μm zinc completely blocked these channels while 1 μm 5β3β pregnane-20-one was ineffective. Benzodiazepine drugs modulated GABA-activated channels in each cell tested. The results indicate that E cells in the vz of the rat spinal cord exhibit heterogeneous electrical properties, which may reflect co-expressions of $\alpha_4\beta_1\gamma_1$ subunit combinations.

472.2

ACCUMULATION OF SPLICE VARIANTS OF $GABA_A$ RECEPTOR ß2 AND ß4 SUBUNIT mRNAs DURING MATURATION OF CORTICAL NEURONS IN VITRO. B.J. Baumgartner and E.M. Barnes, Jr.* Dept. of Biochemistry, Baylor Col. of Med., Houston, TX 77030.

The isolation and sequencing of the chicken $GABA_A$ receptor ß2 and ß4 subunit cDNAs revealed that two transcripts are produced by alternate splicing of the primary transcripts (splice variants differing by 51 and 12 nucleotides, respectively, for ß2 and ß4) from each of two ß subunit genes (M. Darlison, personal commun., and Bateson et al., (1991) *J. Neurosci.* 56:1432, respectively). We have used quantitative RT-PCR to measure the relative levels of the ß2 and ß4 subunit transcripts in embryonic chick cortical neurons from days 2-8 in vitro. Previous experiments have shown that the level of $GABA_A$ ligand binding increases 4-fold during this period. The efficiency of amplification (R) was found to be equal for cDNAs of transcripts from the ß2 and ß4 genes (R = 0.81 ± 0.01). The extent of amplification was constant up to 27 cycles for 50 ng total RNA. Therefore, the amount of PCR product after 25 cycles was used to determine the relative levels of the ß subunit mRNAs. In neurons 2 days in vitro, PCR products from cDNAs of the smaller splice variants for the two ß subunit genes were more predominant (ß2L = 8.0 ± 2.7, ß2S = 63 ± 20; ß4L = 98 ± 20; ß4S = 122 ± 29; fmol PCR product; n = 4). By day 6, the amount of the products from the ß2L transcript increased 3.3 fold and the ß2S products increased 2.5 fold (to 27 ± 2.9 and 155 ± 13 fmol, respectively). In contrast, products of the transcripts of the ß4 subunit genes only increased 40 to 50% by 6 days (ß4L = 141 ± 13, ß4S = 179 ± 22). In general, the relative ratio of the large/small variants of ß4 is much greater than ß2 (ca., 0.8 for ß4L/ß4S and 0.15 for ß2L/ß2S). During the in vitro maturation period, the large/small ratio for the ß2 variants increased from 0.13 to 0.22, while the corresponding ratio for the ß4 variants remained essentially unchanged.

Supported by NIH grants MH47715, DK17436, and NS 11535.

472.3

NEURONS DERIVED FROM EMBRYONAL CARCINOMA (P19) CELLS EXPRESS MULTIPLE $GABA_A$ RECEPTOR SUBUNITS AND FULLY FUNCTIONAL $GABA_A$ RECEPTORS J. Reynolds*, A. Prasad and G. Paterno. Faculty of Medicine, Memorial University, St. John's, NF, Canada A1B 3V6.

Embryonal carcinoma (EC) cells have frequently been used as a model system to investigate events involved in embryonic development and cellular differentiation. The EC cell line P19 was induced to differentiate into neurons and glia by exposure to 0.5 μM all-trans retinoic acid for 5 days. At 7 days after retinoic acid treatment, neurons derived from P19 cells were examined for (i) GABA immunoreactivity, (ii) $GABA_A$ receptor-activated Cl^- currents (I_{GABA}), (iii) modulation of I_{GABA} by flurazepam, (iv) expression of $GABA_A$ receptor subunit mRNA, (v) expression of neurofilament mRNA. I_{GABA} and modulation of I_{GABA} by the hypnotic benzodiazepine flurazepam were determined using whole-cell voltage-clamp recording. Messenger RNA for $GABA_A$ receptor subunits and for neurofilament were detected after reverse transcriptase PCR amplification.

P19 EC cells were found to constitutively express some, but not all, $GABA_A$ receptor subunits. Seven days after retinoic acid treatment, all $GABA_A$ receptor subunits examined could be detected. Of particular interest, both γ2L and γ2S mRNA were expressed in differentiated P19 cells, but only γ2L mRNA was detected in undifferentiated cells. A large proportion of the neuronal cells also contained GABA immunoreactivity. I_{GABA} in differentiated P19 cells had an EC_{50} value of 12 μM, and a Hill slope of 1.3. I_{GABA} in differentiated P19 cells was strongly potentiated by flurazepam (1μM). Including cAMP (2 mM) in the recording pipette resulted in a slowly developing inhibition of I_{GABA}. Thus, P19 EC cells provide the advantages of a stable cell line which express fully functional $GABA_A$ receptors.

Supported by the Medical Research Council of Canada

472.4

ONTOGENY OF THE MODULATORY EFFECTS OF ANXIOLYTIC DRUGS ON TYPE I AND TYPE II $GABA_A$ RECEPTORS IN THE CEREBRAL CORTEX OF THE RAT.

M.G. Corda*, D. Lecca, R. Loi and O. Giorgi.
Department of Toxicology, University of Cagliari, Italy.

The ontogeny of $GABA_A$ receptors was studied by measuring ^{35}S-t-butylbicyclophosphorothionate (^{35}S-TBPS) and ^{3}H-Fluni-trazepam (^{3}H-FNT) binding in the cerebral cortex of the rat at intervals (1-90 days) after birth. The density of ^{35}S-TBPS binding sites increased approximately 4-fold from day 1 to day 15 after birth and progressively decreased thereafter (-30 % by postnatal day 45, when it returned to the adult value). The postnatal changes in the density of ^{3}H-FNT binding sites had a very similar time course. In addition, competition binding studies using ^{3}H-FNT and ligands with preferential affinity for type I sites (Zolpidem, 2-Oxoquazepam and Cl 218 872) showed a very low density of these sites at birth (< 10 %) followed by a rapid increase during the postnatal development (60 % of type I sites in adult rats). Although the allosteric modulation of ^{35}S-TBPS binding by GABAergic drugs was already detectable at birth, the IC_{50} for GABA was 3- fold larger in 1 day old rats as compared to adult counterparts. Moreover, a rightward shift was observed in the concentration-response curves for the inhibitory effects of type I selective ligands on ^{35}S-TBPS binding in newborn versus adult rats.

472.5

TRANSIENT EXPRESSION OF A NOVEL TYPE OF GABA RESPONSE IN HIPPOCAMPAL NEURONS DURING DEVELOPMENT. F.G.Strata and E.Cherubini*. Biophysic Lab., Sissa, via Beirut 2-4, 34013 Trieste, Italy.

Intracellular recordings were performed with 3M KCl filled microelectrodes on hippocampal CA3 neurons in slices obtained from neonatal (P0-P9) rats. As already reported (Cherubini et al., TINS, 515-519, 14, 1991) GABA (0.1-0.5 mM) elicited a dose-dependent depolarization from -65±5 mV, associated with a conductance increase. This effect was mimicked by the $GABA_A$ agonist isoguvacine (10μM) but not by the $GABA_B$ agonist baclofen (30μM), which at same potential induced a hyperpolarization. Bicuculline (50μM) partially reduced the effects of GABA but it abolished the response to isoguvacine, without affecting baclofen induced hyperpolarization. In the presence of bicuculline, the fractional conductance increase (R/R'-1) induced by GABA (300 and 500 μM) was respectively 1.16±0.29 and 1.76±0.34 (mean ± s.e.m., n=22). The bicuculline-insensitive GABA response was Cl^- dependent, as E_{GABA} changed at different $[Cl^-]_o$ concentrations (isethionate substitution). The bicuculline-insensitive GABA-response was blocked in an uncompetitive manner by picrotoxin (30-100μM) and was mimicked by the GABA analogue *cis*-4-aminocrotonic acid (CACA; 1mM). The same concentration of CACA had no effect on adult CA3 neurons. The GABA response was enhanced in the presence of a low Na^+ medium (choline substitution) or nipecotic acid (1mM). The bicuculline-insensitive GABA response seems to be mediated by activation of a novel type of GABA receptor, termed as $GABA_C$, and recently described in retinal horizontal and bipolar cells.

472.6

$GABA_A$ RECEPTOR-MEDIATED, Cl^--DEPENDENT POTENTIAL CHANGES AND Ca_i^{2+} RESPONSES IN CHICK EMBRYONIC TELENCEPHALIC CELLS CHANGE WITH TIME IN CULTURE. K. Torimitsu*[1], A. Kawana[1] and J.L. Barker[2]. [1]NTT Basic Research Labs., Musashino-shi, Tokyo 186, Japan., [2]Lab. of Neurophysiology, NINDS, NIH, Bethesda, MD 20892 , USA.

GABA and the $GABA_A$ agonist muscimol induce depolarizing responses of acutely dissociated embryonic chick telencephalic cells studied using voltage-sensitive dyes and flow cytometry. However, the ionic mechanism of the depolarization is not known. Here we report transient intracellular calcium (Ca_i^{2+}) and chloride ion (Cl_i^-) changes caused by GABA in cultured chick embryonic telencephalic cells, which were studied using the fluorescent Cl^- indicator MQAE and the fluorescent Ca^{2+} indicator Fura 2. Cells were dissociated from embryonic day 6 and grown in an MEM-based, serum-containing medium, then stained with MQAE and Fura 2. Transient Cl_i^- and Ca_i^{2+} responses were recorded after adding 10μM GABA or muscimol, which depended on the number of days in culture. At early times in culture (<7 days), there were transient increases in Ca_i^{2+} and decreases in Cl_i^-. Thus, net Cl^- efflux occured at a time when neurons could be depolarized by muscimol, suggesting that depolarizing responses could be due to Cl^- efflux. The Ca_i^{2+} increase was only observed at a time when neurons exhibited depolarizing $GABA_A$-type responses to muscimol. Since the Ca_i^{2+} responses were inhibited by T-type Ca^{2+} channel blockers (ethosuximide, Ni, amiloride), the transient Ca^{2+} increase induced by muscimol could be explained by Ca^{2+} influx through voltage-gated Ca^{2+} channels, which would be activated by depolarization due to Cl^- efflux. Some cells expressed Ca_i^{2+} signals in response to muscimol that were insensitive to these Ca^{2+} channel blockers. After 7 days in culture, muscimol decreased Ca_i^{2+} and increased Cl_i^-, indicating that $GABA_A$ receptor functions had changed in vitro as they do in vivo and suggesting that changes in Cl_i^- and Ca_i^{2+} are interdependent.

472.7

EXPRESSION OF $GABA_A$ RECEPTOR mRNAs IN CULTURED CEREBELLAR GRANULE NEURONS IS DEPENDENT ON DEVELOPMENTAL SIGNALS. K. Behringer and R.E. Siegel*. Dept. of Pharmacology, Case Western Reserve University, Cleveland, OH 44106.

$GABA_A$ receptor subunit mRNA expression in the CNS is developmentally regulated in a region-specific manner. We demonstrated previously that the mRNAs encoding α1, β2, and γ2 subunits in the rat cerebellum rise several fold during the second postnatal week. A similar developmental pattern for the β2 and γ2 subunit mRNAs is found in cultured cerebellar granule neurons prepared at P10. In contrast, the mRNAs remain constant in cultures prepared at E19, a relatively immature stage of cerebellar development. These studies suggest that subunit mRNA expression is not intrinsic to the neurons but may be regulated by cues received during cerebellar maturation. To determine the developmental stage at which subunit mRNA expression is initiated, cultures of rat cerebellar granule neurons were prepared at 2-day intervals from P2 through P8, and the relative levels of the α1, β2, and γ2 subunit mRNAs were assessed over time in culture by PCR. In cultures prepared at P2, P4, and P6, the subunit mRNAs were present at constant levels throughout the 14-day culture period. In contrast, in cultures prepared at P8, the β2 and γ2 subunit mRNAs rose approximately 7-fold between 2 and 7 days in culture in a pattern similar to that observed in P10 cultures. These findings suggest that the program of $GABA_A$ receptor subunit mRNA expression has been initiated by P8, a time at which extensive cell migration from the external germinal layer to the internal granule cell layer has begun. The exact point along the differentiation pathway at which receptor gene expression is triggered and the nature of the regulatory cues remain unknown.

472.8

CHRONIC EXPOSURE OF CHICK EMBRYOS TO $GABA_A$ AGONISTS IN OVO DOWN-REGULATES CEREBELLAR $GABA_A$ RECEPTORS. P.A. Calkin* and E.M. Barnes, Jr. Dept. of Biochemistry, Baylor Col. of Med., Houston, TX 77030.

Chick embryos with an undeveloped blood brain barrier were used to examine the down regulation of $GABA_A$ receptors in vivo. Eggs containing 8-day embryos were windowed and the $GABA_A$ agonist isoguvacine (5 mmol) was applied to the vascularized chorioallantoic membrane. The isoguvacine treatment was repeated on days 11, 14 and 17, and the embryos were sacrificed on day 18. Washed cerebellar membranes from isoguvacine-treated 18-day (stage 42) embryos showed a reduction of 30.2 ± 6.4 % in clonazepam-displacable binding of [^{3}H]flunitrazepam (5 nM final concentration) compared to vehicle-treated or unwindowed controls. Reductions of lower magnitude were observed in the level of [^{3}H]flunitrazepam binding to membranes from the cerebral cortex and optic lobes. The reduction in cerebellar membranes was dose-dependent, allowing a value for the half-maximum reduction in [^{3}H]flunitrazepam binding to be estimated at 8 μM isoguvacine. Binding of the $GABA_A$ channel ligand [^{35}S]-t-butylbicyclophosphorothionate (TBPS, 10 nM) was measured in the presence of R5135 (1 μM) to eliminate interference by agonist carry-over. The standard isoguvacine treatment produced a 43.6 ± 7.6 % reduction in the specific binding of [^{35}S]TBPS to cerebellar membranes compared to controls. However, this treatment produced no change in total embryonic, whole brain, or cerebellar wet weights, or in the specific binding of [^{3}H]-N-methylscopolamine to muscarinic acetylcholine receptors on cerebellar membranes. Saturation binding studies with [^{3}H]flunitrazepam revealed that isoguvacine exposure reduced the B_{max} value by 27.5 ± 7.3 % or 31.7 ± 6.9% compared to vehicle-treated or unwindowed embryos, respectively. The results indicate that chronic treatment in ovo with a $GABA_A$ agonist produces a down-regulation of $GABA_A$/benzodiazepine receptors in the cerebellum of chick embryos.

Supported by NIH grants MH47715, DK17436, and NS11535 .

472.9

PEPTIDES FROM RED ALGAE AND CYANOBACTERIA THAT INDUCE METAMORPHOSIS OF MARINE INVERTEBRATE LARVAE ARE LIGANDS FOR MURINE GABA RECEPTORS. **H.G. Trapido-Rosenthal*[1] and A.N.C Morse[2]. [1]Bermuda Biological Station for Research, St. George's GE01, Bermuda, and [2]Marine Biotechnology Center and Marine Science Institute, University of California, Santa Barbara, California 93106.**

The effectiveness of a new class of small peptides as novel ligands for pharmacologically distinct GABA receptors from both invertebrate and vertebrate organisms has been investigated. Larvae of the gastropod mollusc *Haliotis rufescens* are induced to settle from the plankton and begin metamorphosis specifically in response to this class of compound, initially identified and biochemically characterized from the crustose corraline red alga, *Lithothamnium californicum* (Corallinaceae). Although only peptides from members of this algal family are ecologically significant in the recruitment process, metamorphosis can be mimicked experimentally by GABA and a number of GABA analogs, as well as by peptides of the same class isolated from foliose red algae (Rhodophyta), and cyanobacteria (Cyanophyta). The peptide isolated from *Lithothamnium* competes for the binding of the radiolabeled GABA analog baclofen at the metamorphosis-mediating chemoreceptor of abalone larvae. In addition, this peptide competes for specific binding of radio-labeled muscimol at $GABA_A$ receptors from murine brain, as do peptides of the same class isolated from other algal and bacterial sources. These latter neural binding sites have a higher affinity for the algal and bacterial peptide ligands than they do for GABA itself. These peptides thus appear to comprise an interesting class of GABA-mimetic compounds that may be of pharmacological significance.

473.1

IMMUNOCYTOCHEMICAL LOCALIZATION OF CORTICOTROPIN-RELEASING FACTOR IN THE HUMAN BRAINSTEM. *V. Arango*, P.M. Rice, R.W. Smith, M.C. Austin and J.J. Mann.* Laboratories of Neuropharmacology, University of Pittsburgh, Pittsburgh, PA 15213.

Corticotropin-releasing factor (CRF) plays an important role in the stress response. We showed by immunocytochemistry and *in situ* hybridization (Austin *et al.*, 1992) that the locus coeruleus (LC) of the human receives a dense CRF innervation, suggesting a CRF modulatory role on the LC. In this study we mapped the location of CRF-immunoreactive (CRF-IR) cells and fibers in the human midbrain and pons, relative to tyrosine hydroxylase (TH-IR) and serotonergic (PH8) immunoreactivity.

Human brainstems (n=3) were blocked to include the midbrain and upper pons, sectioned (50 µm) coronally and processed for immunocytochemistry (every 500 µm) using CRF antisera, preadsorbed with melanin. We plotted the outline of each section and the location of CRF-IR cells and fibers.

A dense population of CRF-IR cells appears anterior to the pedunculopontine tegmental nucleus (PPTg) in a continuous column that first corresponds to the PPTg and extends medially towards the dorsolateral medial longitudinal fasciculus. CRF-IR neurons are also seen ventromedial to, and within the LC, on neurons devoid of TH-IR. The CRF-IR column of cells remains in that position throughout the caudal extent of the pontine tegmentum. CRF-IR neurons correspond to distinct cell groups as seen with Nissl. The serotonergic nuclei (dorsal raphe, caudal linear and median raphe) receive a strong CRF innervation. The paramedian raphe, which contains PH8-IR fibers, also has dense CRF fibers. The dorsal tegmental n, which is devoid of PH8-IR, exhibits strong CRF-IR throughout its rostrocaudal extent, mostly in fibers. The strong CRF innervation of serotonergic nuclei provides evidence for a neuromodulatory role of this peptide on the serotonergic system. Similarly, this CRF system may represent the basis for neuromodulation by CRF of noradrenergic neurons in the LC. (Supported by MH40210, MH46745 and AA09004)

473.2

PREPROTACHYKININ B [NEUROKININ B (NKB)] IMMUNOREACTIVE (IR) NEURONAL PROCESSES IN HUMAN BRAIN. D. Mileusnic,[1]* D.J. Magnuson,[2] S.A. Lorens[1] and J.M. Lee.[1,2] Departments of Pharmacology[1] and Pathology,[2] Loyola University Chicago Medical Center (Bldg. 135), Maywood IL 60153.

NKB is a mammalian tachykinin which is predominantly localized in the CNS. The distribution of NKB-like IR and NKB mRNA positive perikarya and fibers in the rat CNS have been described using immunohistochemical and *in situ* hybridization techniques (*J. Comp. Neurol.* 317:341, 1992). Ligand-binding studies [using (^{3}H) eledoisin; In: Henry, J.L. et al. *Substance P and Neurokinins*. Springer-Verlag:NY, 1987, pp. 363-365] and Northern blot analyses (*FEBS Lett.* 299:90, 1992) have documented the presence of NKB receptors (NK3) in both the rat and human brain. Although the functional role(s) of CNS NK3 receptor mediated events have yet to be firmly established, the available evidence suggests that NKB modulates midbrain raphe serotonergic neurons involved in the regulation of behavioral arousal (*J. Pharmacol. Exp. Ther.* 251:388, 1989; *Brain Res.* 517:11, 1990). In order to visualize NKB-like IR perikarya and fibers in the human brain, we employed rabbit polyclonal antibodies (AB) directed against NKB(1-10) and against amino acids 50-79 of preprotachykinin B [peptide 2 (P2); generously provided by Dr. J. Krause, Washington Univ., St. Louis] using the avidin-biotin (ABC; Vector) technique with nickel intensification. Control procedures included both the omission of the primary AB and the preincubation of the AB with NKB(1-10) or P2 (10 µM, 24 h at 4°C). We have observed P2 IR neurons and/or fibers in several human CNS regions (including the mesencephalic raphe, the granular layer of the cerebellum, the hippocampus, and the entorhinal, cingulate and frontal cortices). The NKB-like perikarya and fibers appear to have a distribution similar to that found in the rat brain. The human NKB neuronal processes also exhibit morphological properties similar to those discerned in the rat CNS: punctated or evenly stained perikarya and dendrites, as well as branched beaded axons. Pursuant to these observations, we are conducting a comparative neuroanatomical analysis of NKB systems in the mammalian CNS.

473.3

INTERLEUKIN-1ß-LIKE IMMUNOREACTIVITY IN CSF IN NORMAL, ALZHEIMER'S DISEASE AND OTHER NEUROLOGICAL DISORDERS: METHODOLOGICAL CONSIDERATIONS. S.N. Nair, S. Wahhab, D. Kapkov, J.C.Lehmann*, Hahnemann University School of Medicine, Philadelphia PA

The role of cytokines in neurodegenerative disorders of the central nervous sytem has become a topic of intense investigation. The interest in cytokines and CNS neurodegenerative disorders stems partly from analogies between Alzheimer's disease (AD) and the acute phase response. Therefore we set out to measure IL-1ß levels in CSF of AD patients, with a view to explaining disparate results obtained in various laboratories, using two commercially available EIAs, one commercially available RIA, and Western blotting with yet another antibody. CSF was used from 10 patients with Alzheimer's disease, 9 patients with acute relapsing multiple sclerosis in exacerbation, and 10 control subjects, obtained from the National Neurological Research Specimen Bank (Los Angeles, CA).

No EIA assay reliably detected IL-1ß-like immunoreactivity, and one commercially available IL-1ß antibody failed as well to detect IL-1ß-like immunoreactivity. The Advanced Magnetics (Boston MA) RIA successfully detected IL-1ß-like immunoreactivity, and the polyclonal antibody moreover successfully detected IL-1ß-like immunoreactivity at 14 kDa in Western blots. This study shows that detection of IL-1ß in CSF is probably disrupted by an endogenous substance, and that post-mortem CSF samples are not acceptable for valid IL-1ß determination.

473.4

LOCALIZATION OF ANGIOTENSINOGEN mRNA IN RAT BRAIN *IN SITU* HYBRIDIZATION. B. Kimura, L. Shen, V. Cook* and M.I. Phillips. Dept. of Physiology, University of Florida, Gainesville, FL 32610.

The renin-angiotensin system (RAS) in the brain is involved in cardiovascular control and fluid volume homeostasis. All components of the system have been identified in the brain, but angiotensinogen mRNA has been localized in glial cells and not in neurons. We have studied the distribution of angiotensinogen mRNA in the rat brain using in situ hybridization. The results show high concentration of mRNA in the paraventricular nucleus (PVN) which is mainly neuronal and in other areas involved in cardiovascular control such as nucleus tractus solitarius (NTS), organum vasculosum of the lamina terminalis (OVLT) and supraoptic nucleus (SON). There was also high amounts of angiotensinogen mRNA in areas with different functions, including the inferior olive (IO), the periventricular nucleus (PeVN) and the suprachiasmatic nucleus (SCN). The signal for angiotensinogen mRNA was localized to a few nuclei in the brainstem, however in the hypothalamic region the distribution was both in nuclei and diffusely spread in the preoptic area, the anterior hypothalamic and thalamic area and the amygdaloid nuclei. The distribution and density we found corresponds well with the distributon of angiotensinogen found with immunohistochemistry and indirect radioimmunoassay (Naruse et al, 1985). This study also corroborates and refines a previously published preliminary study by Younge et al 1991. Our results indicate that both neurons and glia contain angiotensinogen mRNA because there were neuronal nuclei with strong signals and more diffuse areas which contain glial cells. The results could also explain why the highest levels of angiotensin II in the brain are in the brainstem and hypothalamus since both contain considerable angiotensinogen mRNA compared to other regions.

473.5

DIFFERENTIAL DISTRIBUTION OF NEUROTENSIN-IMMUNOREACTIVE NEURONS IN THE FOREBRAIN OF SHR AND WKY RATS. R.S.Canbeyli, L. Skaredoff & B.G.Yongue*, Dept. of Developmental Psychobiology, New York State Psychiatric Institute, New York, NY 10032.

Neurotensin (NT) and Neuropeptide Y (NPY), implicated in cardiovascular and fluid/electrolyte regulation, were measured immunohistochemically in spontaneously hypertensive rats (SHR) and normotensive Wistar-Kyoto rats (WKY). To minimize variability, aged-matched SHR and WKY (♂, 5-10 mo) were perfused in pairs and alternate 50-micron sections were marked and processed together for immunohistochemical staining for NPY or NT.

Preliminary work indicates that there is a slightly increased density of NPY terminal staining in the hypothalamic paraventricular nucleus of SHRs compared to WKYs (9/11 pairs). No NPY-immunoreactive perikarya were observed subcortically in the two strains. There were significantly ($p<0.01$) more NT-immunoreactive neurons in the central nucleus of the amygdala (CeA) in SHR ($3.5 \pm .4$; mean $\pm$ sem, per CeA) than in the WKY ($1.2 \pm .3$) with no strain difference (8 pairs) in the density of NT immunostaining. There were very few immunoreactive cells in the rostral CeA; most were observed mid rostrocaudally. SHRs also had significantly ($p<0.01$) more NT-cells ($1.7 \pm .2$) than WKYs ($0.4 \pm .1$) in the rostral bed nucleus of the stria terminalis (BST). These results do not indicate general differential staining between strains, and may point to specific functional differences since SHRs and WKYs had similar numbers of NT-immunoreactive neurons in the medial amygdala and the intraamygdaloid division of BST, the only other structures in which clusters of immunoreactive perikarya were observed. A study is under way to determine distribution of NT messenger RNA expression in the two strains. (MH00803 & MH45951)

473.6

GALANIN IMMUNOREACTIVITY AND BINDING SITES IN THE NUCLEUS TRACTUS SOLITARII OF THE CAT. B.E. Maley*, C. Colson, J.P. McGillis and J.F. Hyde. Dept. of Anatomy & Neurobiology and Dept. of Microbiology and Immunology, Univ. of Kentucky Med. Ctr., Lexington, KY 40536

Galanin is a 29 amino acid polypeptide isolated from the intestine. It has a wide spread distribution throughout the central nervous system where it has a variety of functional roles. Galanin has been localized in the nucleus tractus solitarii (NTS), suggesting it plays a role in the regulation of autonomic function. It was the purpose of this study to investigate the distribution of galanin in the various subdivisions of the feline NTS.

Cats were perfused either with phosphate buffered 4% paraformaldehyde (immunocytochemistry) or buffered 0.1% paraformaldehyde (receptor binding). Sections for galanin immunocytochemistry were immunostained with the peroxidase, anti-peroxidase method, while galanin binding sites were labeled with [^{125}I]galanin.

The NTS had a dense plexus of galanin-like immunoreactive fibers throughout its rostral to caudal length. The majority of immunostaining was localized to the medial, dorsal, commissural and interstitial subdivisions. Lesser amounts were present in the parvicellular, ventrolateral and intermediate subdivisions. Galanin binding sites had a similar distribution throughout the various subdivisions of the nucleus.

Results of the present investigation provide a morphological basis for galanin's role in the regulation of autonomic functions, since it is localized in areas of the NTS involved in control of respiration and cardiovascular function. The presence of galanin binding sites indicates that it may be acting as a neuromodulator at synaptic junctions.

437.7

DISTRIBUTION AND mRNA EXPRESSION OF A NOVEL NEURON-SPECIFIC 14 kDa PROTEIN. S. Nakajo[*1], S. Shioda[2], Y. Nakai[2] and K. Nakaya[1]. Showa Univ., Schs. of Pharmaceut. Sci[1]. and Med[2]. Shinagawa-ku, Tokyo 142, Japan.

A neuron-specific protein with a molecular mass of 14 kDa, phosphoneuroprotein 14 (PNP 14), is phosphorylated by EGF receptor and $pp60^{v\text{-}src}$ on tyrosine residue(s) and by CaM-kinase II on a serine residue in vitro. Here we show the distribution and mRNA expression of PNP 14 in rat brain. PNP 14 immunoreactivity was observed in the nerve fibers and their terminals distributed in the brain including telencephalon, diencephalon, cerebellum, and cerebrum. In the cerebellum, PNP 14 immunoreactive fibers were numerously distributed in the molecular layer and in lesser amounts in the granular layer. In the cerebrum, the immunoreactivities were observed throughout the cerebral cortex and their terminals were found to be in contact with the pyramidal cells in the layer V. In situ hybridization revealed that PNP 14 mRNA was expressed particularly rich in granular layer of the cerebellum, in the hippocampus, and in the pyriform cortex of the cerebrum. These results suggest that PNP 14 might play an important role in the terminus of neuronal cells, and its function may be modulated by the protein phosphorylation reaction.

437.8

CALRETININ-CONTAINING PATHWAYS IN THE CENTRAL NERVOUS SYSTEM OF THE RAT. P. Krzywkowski, M.C. Senut, D.M. Jacobowitz and Y. Lamour*, INSERM U161, Paris, France, and Lab. Clinical Science, NIMH, NIH, Bethesda, USA.

The pattern of projection of central neurons containing the calcium-binding protein calretinin might provide clues as to the function of this protein. In order to identify the calretinin-containing pathways, immunohistochemical techniques using a well characterized polyclonal antibody were combined with the retrograde transport of a WGA-apoHRP-colloidal gold complex injected in various brain regions in the adult male rat. Double-labelled neurons were consistently found in the paraventricular nucleus of the thalamus, hilus of the dentate gyrus and lateral hypothalamus (medial septal injections), paraventricular nucleus of the thalamus, lateral hypothalamus (diagonal band injections), lateral mammillary nucleus (antero-dorsal nucleus of the thalamus injections), septo-fimbrial nucleus, nucleus triangularis, nucleus reticularis thalami, nucleus reuniens, anterior amygdaloid nucleus (habenula and medio-dorsal thalamic nucleus injections), substantia nigra, ventral tegmental area (caudate and globus palllidus injections), medial habenula (interpeduncular injections) zona incerta, ventral tegmental area (cortical injections). In contrast no double-labelling was found in other populations of calretinin-positive neurons. For instance no double labelling was observed in the cerebral cortex after thalamic, cortical or striatal injections. Our results demonstrate that subpopulations of calretinin-positive neurons in the central nervous system are projection neurons. Projections arising from the substantia nigra-ventral tegmental area complex, the lateral mammillary nucleus, caudal septum, medial habenula, zona incerta and several thalamic nuclei seem especially rich in calretinin.

437.9

NEUROACTIVE PEPTIDES EXIST IN THE MIDBRAIN DOPAMINERGIC NEURONS THAT CONTAIN CALBINDIN-D_{28K}. C.-L. Liang and D.C. German*. Dept. of Psychiatry, UT Southwestern Medical Center, Dallas, TX 75235-9070.

Calbindin-D_{28k} (CaBP) is a 28 kDa calcium-binding protein that is found within the cell bodies, dendrites and axons of specific nerve cells, and it binds micromolar concentrations of intracellular calcium. CaBP has been found within a subpopulation of midbrain dopaminergic (DA) neurons in several species, including man (e.g., Gerfen et al., 1985; German et al., 1992). The neuroactive peptides, cholecystokinin (CCK) and neurotensin (NT), have also been colocalized within a subpopulation of midbrain DA neurons (e.g., Hokfelt et al., 1980). The purpose of the present study was to determine whether CaBP is colocalized within the midbrain DA neurons that contain the peptides. Double and triple-labeling immunocytochemical staining techniques were used in the rat. Virtually all of the cells that contained CCK also contained CaBP, and most of the cells that contained NT also contained CaBP; such cells were found in the ventral tegmental area, substantia nigra pars compacta (dorsal region), and substantia nigra pars lateralis. CaBP may play a role in the co-release of neuroactive peptides, with dopamine, from the midbrain DA neurons.

437.10

LOCALIZATION OF ACIDIC FIBROBLAST GROWTH FACTOR mRNA IN THE MOUSE BRAIN. A.E. Kresse*, A.J. Bean, and T. Hökfelt. Department of Histology and Neurobiology, Karolinska Institute, Stockholm 104 01, Sweden.

The anatomical localization of acidic fibroblast growth factor (aFGF) mRNA in the adult male mouse brain was examined in situ histochemically using a radiolabelled 51-mer synthetic oligonucleotide probe. Consistent with recently published immunocytochemical data (Fallon et al., *Growth Factors* 6: 139-157, 1992), it could be shown that aFGF is expressed in several defined functional systems of the brain, e.g. the auditory (superior olive, cochlear, lateral lemniscal and trapezoid nuclei) and vestibular (vestibular nuclei) systems, somatosensory (spinal and mesencephalic trigeminal nuclei, cuneate nucleus) systems as well as autonomic-related structures (hypothalamus, laterodorsal tegmental nucleus, locus coeruleus, raphe nuclei, dorsal motor nucleus of the vagus). Unlike the reported distribution of aFGF peptide, the highest levels of aFGF mRNA were found in large somatomotor (nucleus of the oculomotor nerve and hypoglossal nucleus, ventral spinal cord) and visceromotor neurons (motor nucleus of the trigeminal nerve, facial and ambiguus nuclei). On the other hand, the reported immunocytochemical data as well as an in situ histochemical study concerning the aFGF mRNA expression in the young adult rat brain (Wilcox and Unnerstall, *Neuron* 6: 397-409, 1991) also revealed some other systems (pyramidal neurons of the neocortex, dentate granule cells of the hippocampus, external and internal granule cell layer and mitral cell layer of the olfactory bulb, striatum), where no aFGF mRNA was detectable in the present study in mouse. This might be due to methodical conditions (immunocytochemistry versus in situ hybridization), and species (rat versus mouse) and age (young adult versus adult) differences.

Though there is evidence for aFGF as a mitogen for a wide variety of cultured cells, its physiological significance is still unknown. However, its defined expression in several brain areas suggests that it may have an important neurotrophic or neuromodulatory function within these systems.

437.11

NITRIC OXIDE SYNTHASE IMMUNOREACTIVITY AND NADPH-DIAPHORASE LOCALIZATION IN MONKEY CEREBRAL CORTEX. T. Hashikawa*, M. G. Leggio, R. Hattori and Y. Yui. Lab. for Neural Systems, F.R.P., RIKEN, Wako 351-01, Japan, Exp. Neurology Lab., Catholic University, Rome, Italy, and Dept. of Internal Medicine, Kyoto University Hospital, Kyoto 606, Japan.

Neurons that contain the enzyme involved in NO synthesis, nitric oxide synthase (NOS), have proven readily identifiable in the central nervous system because of the apparent identity of NOS with nicotinamide adenine dinucleotide phosphate diaphorase (NADPH-d), an enzyme that can be revealed by a histochemical stain. However, there has been no systematic study of these neurons in the primate cerebral cortex. Moreover, although it seems likely that NADPH and NOS will invariably be colocalized in neurons, this has not been the subject of a detailed study. The present work was aimed at studying systematically the pattern of NOS immunoreactive and NADPH-d histochemical staining in the cerebral cortex of Japanese macaques. NADPH-d and NOS positive cells presented a similar uniform distribution in all cortical areas although significant differences were found in the pattern of distribution of fiber plexuses. Plexuses of fine beaded fibers were clearly identifiable with NADPH-d staining while only isolated fibers of similar type could be found in the NOS immunoreactive sections. In all areas there was a dense plexus in layer I composed of fibers mainly oriented parallel to the pial surface. Labeled fibers were also always present in layer VI and the underlying white matter. Other plexuses tended to vary from area to area. For instance, a relatively dense plexus of irregularly oriented fibers in layer II and the adjacent part of layer III was seen in many areas while a prominent layer IV plexus was evident almost exclusively in area 17. The present results indicate that NOS, as revealed immunohistochemically, and NADPH-d, as revealed histochemically, characterize the same population of cortical neurons in monkeys, and provide evidence of areal specificity in the distribution of NADPH-d containing fibers in the primate cortex.

474.1

A NOVEL METHOD FOR THE COLOCALIZATION OF mRNA AND PROTEIN IN MAMMALIAN BRAIN. N.B. Hastings, D.S. Albeck, and B.S. McEwen*. Laboratory of Neuroendocrinology, The Rockefeller University, New York, NY. 10021.

In situ hybridization histochemistry (ISHH) studies have demonstrated that corticosteroids can negatively regulate levels of arginine vasopressin (AVP) mRNA in the rat paraventricular nucleus (PVN). Double immunocytochemical (ICC) studies have indicated that type II glucocorticoid receptors (GR) are colocalized with AVP in the PVN. However, there is no technique readily used to simultaneously demonstrate colocalization and regulation. We have developed a method for the cellular colocalization of AVP mRNA with GR-ir using quantitative *in situ* hybridization histochemistry (ISHH) and immunocytochemistry (ICC). This methodology employs low salt ISHH washes that are stringent enough to destabilize non-specific RNA-DNA duplexes but wash temperatures low enough to spare antigenicity. Using this method, we have confirmed the presence of AVP mRNA in GR positive cells within the rat PVN. Control experiments indicate that labeling of mRNA and protein is specific and quantifiable. We believe that such a methodology, in conjunction with appropriate hormonal manipulations, may be readily used to identify sites of steroid hormone action and quantify changes in the mRNA of target genes.

474.2

ELECTRONMICROSCOPIC IMMUNOCYTOCHEMISTRY STUDY ON THE NEUROPEPTIDES IN THE HYPOTHALAMUS OF THE CAT

J.J. Kim*. Dept. of Anat., Chosun Univ., Kwang-joo, Korea 501-759.

This study was carried out to investigate the morphology and distribution the neuropeptide-Immunoreactivities in the hypothalamus of the cat.

The neuropeptide-immunoreactivities were identified in the hypothalamus immunocytochemically with antisera raised against somatostatin(SOM), corticotropin releasing factor(CRF), Neuropeptide-Y (NPY), Vasopressin (VP) and Oxytocin(OT).

Generally most SOM, CRF, NPY, VP and OT-neurons were found in the anterior periventricular, arcuate, paraventricular and supraoptic nuclei in the hypothalamus. Neuropeptides neurons were round, oval, spindle in shape, and the diameters of the were SOM, CRF and NPY neurons were 20-25 um, but VP and OT neurons were 30-40um in the cat, respectively.

Under the electronmicroscopic observations,the rough endoplasmic reticulum, mitochondria, Golgi apparatus and microtubules were all neuropeptide -immunoreactivity neurons. Also afferent axo-dendritic, axo-axonal and axo-somatic synapes were appeared symmetrically and asymmetrically.

474.3

COEXISTENCE OF CORTICOTROPIN-RELEASING HORMONE (CRH) AND GABA IMMUNOREACTIVITY IN LAMINA I OF THE FELINE CORTEX.

Y. Tizabi*, P.J. Gatti and V. J. Massari. Dept. Pharmacology, Coll. Med. Howard Univ. Washington, D.C. 20059.

It is well known that CRH is localized in the hypothalamus, where it serves to regulate the pituitary-adrenal axis. However, CRH immunoreactive cells are also distributed in many other areas of the brain. Previously (Neurosci.Abst.17:969,1991), we have reported the existence of CRH containing cells in lamina I of the feline cerebral cortex. Since this lamina also contains numerous GABAergic cells, we sought to determine whether CRH and GABA are colocalized in this lamina. Frozen 10-20u thick sections from colchicinized cats were processed by an indirect immunofluorescence method. Double labeled cells containing CRH and GABA, as well as single labeled cells containing either CRH or GABA, were detected in lamina I of the cortical areas examined. The number of single labeled GABA cells was much higher than the number of cells containing CRH. Double labeled CRH-GABA immunoreactive cells were seen more frequently than cells containing CRH alone. The physiological significance of these observations in the control of cortical function remain to be elucidated. Supported in part by grants from the American Heart Association / N.C.A.

474.4

LOSS OF CCK mRNA IN DORSOLATERAL THALAMUS AFTER CORTICAL LESIONS. M.K.Panni*, U.Capoor†, M.V. Sofroniew and M.A.N. Rattray†. Department of Anatomy, University of Cambridge, Cambridge CB2 3DY, U.K. and †Division of Biochemistry, UMDS, Guy's Hospital, London SE1 9RT, U.K.

Traumatic or stroke-like injuries of the cerebral cortex result in the retrograde degeneration of thalamic relay neurons that project to the damaged area. The basis for the extreme sensitivity of relay neurons to axotomy and the mechanisms involved in the degenerative process are not well understood. We have analysed the effects of cortical lesions on several messenger RNA's which are expressed at high levels in neurons of the thalamus and cerebral cortex, including a mRNA encoding a neuropeptide, cholecystokinin (CCK), and a growth associated protein, GAP-43. Under full anesthesia, male Wistar rats were given unilateral suction lesions of the fronto-parietal region of the cerebral cortex. After 24 hours, 2 weeks and 4 weeks survival, the animals were killed, their brains removed, rapidly frozen and sectioned. Antisense oligonucleotides were end-labelled with ^{35}S-dATP and used for *in situ* hybridisation. Film autoradiography revealed that after cortical lesions, CCK mRNA levels were markedly reduced in the dorsolateral thalamus at 2 and 4 weeks, but not at 24 hours. In contrast GAP-43 mRNA levels were relatively unchanged. Analysis of Nissl stained sections revealed that the decline in CCK mRNA was accompanied by the appearance of degenerating cell bodies. These results suggest that CCK mRNA levels are a sensitive marker for neurodegeneration and that GAP-43 is made by non-neuronal cells during retrograde degeneration. (Supported by MRC, UK).

474.5

CHOLECYSTOKININ (CCK) MESSENGER RNA (mRNA) IS EXPRESSED IN CORTICO-THALAMIC PROJECTION NEURONS: *IN SITU* HYBRIDIZATION COMBINED WITH RETROGRADE TRACING WITH RHODAMINE LATEX MICROSPHERES (RLM).

V.V. Senatorov*, V. Trudeau, B. Hu. *Loeb Research Institute, Ottawa Civic Hospital, Ottawa, Ontario, Canada, K1Y 4E9.*

Neocortical neurons reportedly contain a high density CCK mRNAs. Using combined retrograde tracing and *in situ* hybridization we now report that a large subpopulation of cortical CCK neurons send their axon projections to the thalamus.

Anaesthetized Long-Evans rats received a stereotaxic injection of RLM (0.03-0.20 μL) into the left ventrolateral (VL) thalamic nucleus and/or the medial geniculate body (MGB). After 7 days animals were anaesthetized and sacrificed by transcardial perfusion with 4% paraformaldehyde. Coronal sections (6-20 μm) were cut with cryostat and mounted on slides. The 24-base oligonucleotide probe complementary to the 3'coding region of the rat preprocholecystokinin mRNA was labelled with Fluorescein-dUTP or Digoxigenin-dUTP for direct or indirect (immuno-fluorohistochemistry) hybridization, respectively.

High levels of CCK transcripts were revealed in selected brain regions including the neocortex. CCK-hybridized perikarya were found in all cortical layers with moderate labelling in layers V-VI. Signals using control probe (oxytocin) were absent. A dense population of corticothalamic neurons was retrogradely labelled by RLM in the superficial/deep layer V and layer VI of the parietal-temporal cortices [Paxinos & Watson, 1986]. Co-localizations of CCK mRNAs and RLM were found in most corticothalamic neurons in the superficial layer V and in 65-80% of the cells located within deep layer V and layer VI. The present study suggests that CCK can be used as a neuromodulator in corticothalamic pathways. ***Supported by Parkinson Foundation of Canada, Ontario Mental Health Foundation and MRC of Canada.***

474.6

NEUROPEPTIDE EXPRESSION IN TARGET SPECIFIC NEURONS IN THE RAT SUPERIOR CERVICAL GANGLION. A.F. Elshaar* and L.L. Wright. Dept. Anatomy/Neurobiology, BUSM, Boston, MA 02118.

This study was to compare the pattern of neuropeptide Y-like immunoreactivity (NPY-li) and dynorphin A-like immunoreactivity (DYN-A) in adult and developing rat sympathetic superior cervical ganglion (SCG) neurons projecting to purely vascular targets, and those projecting to "mixed" vascular and glandular targets. Adult and postnatal day 5 (P5), P15, P25 male rats were used. The retrograde tracer Flurogold (FG) was injected into the temporalis muscle or frontal cortex to label SCG neurons projecting to a "purely vascular" target, and into the submandibular gland, to label neurons projecting to a "mixed" end organ. Immunofluorescence was utilized to compare the relative proportions of each population of SCG neurons that contained NPY-li or DYN-A-li.

Analysis of the results showed that nearly all (98.7%) of temporalis projecting neurons contain NPY, most (80.2%) of cerebral blood vessels projecting neurons contain NPY, and few (6.8%) of submandibular projecting neurons contain NPY. In contrast, few (7.8%) and (8.6%) of the temporalis and cerebral blood vessel projecting neurons contain DYN-A, respectively, and most (94%) of of submandibular projecting neurons contain DYN. The results were similar at P5, P15, and P25, in that most of the SCG neurons projecting to the cerebral blood vessels and temporalis muscle contained NPY-li but few contained DYN. In addition, significantly fewer of those projecting to the submandibular gland contained NPY-li but most contained DYN. From these data, we conclude that the population of neurons projecting to these target organs are chemically coded by their target tissues because they differ significantly in the percentage of neurons displaying NPY-li or DYN-li in adult. This pattern is present after the fifth postnatal day of development. This work has been supported by a B.U. GSRA to AFE.

474.7

SUBSTANCE P (SP) FIBERS OVERLAP CALBINDIN (Cb) CONTAINING NEURONS IN THE RAT SPINAL CORD.

G. Battaglia*, C. Colacitti, C. Lizier, A. Princivalle. Neurological Institute "C. Besta", Milano, Italy.

A selective anatomical relationship between substance P immunoreactive fibers and Calbindin immunoreactive perikarya was recently demonstrated in the rat thalamus (Battaglia et al., '92). The same methodological approach was employed in the present work to study the distribution of SP and Cb immunoreactivities (SP-ir and Cb-ir) in the rat spinal cord. The distribution of Cb-ir perikarya is similar at different spinal cord levels. Small Cb-ir neurons are densely concentrated in the superficial layers of the dorsal horn. A few large fusiform neurons are evident in lamina I, with proximal dendrites parallel oriented to the white matter border. Large multipolar Cb-ir neurons are evident in the lateral part of lamina V, and in the lateral spinal nucleus. In the ventral horn, large Cb-ir neurons are sparsely distributed in lamina VII and VIII. Small darkly stained Cb-ir neurons, with long proximal dendrites, are located around the groups of motorneurons in lamina IX. Pale stained neurons are present in the intermediolateral cell column (IML). The distribution of SP-ir fibers is very similar to that of Cb-ir perikarya along the extent of the cord, particularly in the superficial laminae of the dorsal horn, in lateral lamina V, and in lamina VIII. The soma and the proximal dendrites of lamina VIII neurons are frequently contacted by SP-ir puncta. SP-ir fibers are moreover concentrated over Cb-ir perikarya in the IML. The present data demonstrate that a selective anatomical relationship between SP-ir fibers and Cb-ir perikarya exists not only in the thalamus, but also in a subpopulation of spinal neurons.

474.8

EFFECTS OF INTRACEREBROVENTRICULAR ADMINISTRATION OF BOMBESIN ON LOCAL CEREBRAL GLUCOSE UTILIZATION IN THE RAT.

F. Wang, Z. Merali and P. Ramm*, Dept. of Biol. Sci., Brock Univ., St. Catharines, ON, Canada, L2S 3A1.

2-[^{14}C]deoxyglucose (2DG) was used to show regional cerebral functional activity (LCGU) in the rat brain after intracerebroventricular administration of bombesin (BN) (vehicle, 0.1μg, 0.5μg). At each dose condition, rats were run freely moving or restrained to determine whether alterations in cerebral function were the direct result of BN influences upon tissue, or were the result of BN-induced motor stereotypy (grooming). The anteroventral thalamic nucleus, ventrolateral part (AVVL), exhibited increased rates of metabolism ($p<0.01$) under both restraint conditions. Elsewhere in anterior thalamus, we observed increased metabolic rates in some regions in freely moving animals ($p<0.05$), but not in restrained animals. These effects were observed under both BN dose conditions. In sum, we observed a marked and highly localized alteration in cerebral metabolism within parts of the anterior thalamus. We failed to observe significant alterations in metabolism in any other of the 150 brain regions analyzed. These results suggest that BN exerts major effects on function in some components of anterior thalamus, and that in AVVL, at least, those effects do not reflect the emission of stereotyped motor behaviors. Rather, they reflect a direct influence of BN upon thalamic functioning.

474.9

PROJECTIONS OF HYPOTHALAMIC NEUROPEPTIDE FF-IR NEURONS TO LIMBIC AREAS IN THE RAT BRAIN.

A.A. Aarnisalo, L. Kivipelto* and P. Panula. Dept. Anatomy, University of Helsinki, 00170 Helsinki, Dept. Neurosurgery, Univ. Helsinki, and Dept. Biology, Åbo Akademi University, Biocity, 20520 Turku, Finland.

Neuropeptide FF (NPFF), a morphine-modulating neuropeptide, occurs in a prominent group of neurons between the dorsomedial and ventromedial hypothalamic nucleus. One projection of this cell group extends to the solitary tract nucleus, but other projections are unknown. Phaseolus vulgaris-leucoagglutinin (PHA-L) was injected iontophoretically between the dorsomedial and ventromedial nucleus of five rats. After 8-15 days, the rats were perfused with paraformaldehyde and the brains were processed for double immunofluorescence to visualize PHA-L and NPFF. Both markers were seen in the same fibers in the ventral and intermediate part of the lateral septal nucleus, in the medial amygdaloid nucleus and amygdalohippocampal area. No double-stained fibers were seen in the posterior pituitary. The results suggest that NPFF may function in the limbic system, where it is involved in connections between the limbic system and hypothalamus.

474.10

PEPTIDERGIC INNERVATION OF THE RAT CORNEA.

M.A. Jones, C.F. Marfurt AND S.F. Echtenkamp*. Northwest Center for Medical Education, Indiana University School of Medicine, Gary, IN 46408

The innervation patterns and densities of peptidergic nerve fiber populations within the rat cornea were determined by using an avidin-biotin immunohistochemical procedure and primary antisera directed against the neuropeptides calcitonin gene-related peptide (CGRP), substance P (SP), galanin (GAL), neuropeptide Y (NPY), met- enkephalin (ENK), vasoactive intestinal polypeptide (VIP), somatostatin (SOM), and cholecystokinin (CCK). Immunohistochemical processing was performed on 40μm thick sections cut tangential to the corneal surface. The distributions of peptidergic fiber populations within each corneal section were plotted onto line drawings using a camera lucida. CGRP-, SP-, and GAL-immunoreactive (-IR) nerve fiber populations were found in abundance within the corneoscleral limbus and cornea proper. NPY-, ENK-, and VIP-IR fibers were less numerous; however, moderate numbers of axons were found in the limbus and small numbers of fibers were consistently observed in the cornea proper. CCK- and SOM-IR corneal nerves were not seen in the present study. The origins of these peptidergic fiber populations were determined by selective ocular denervations. Transection of the ophthalmomaxillary nerve and/or extirpation of the superior cervical ganglion (SCG) revealed that all corneal CGRP- and SP-IR nerves, and most GAL-IR nerves, were sensory. Most VIP- and ENK-IR nerves, and occasional GAL-IR nerves survived the combined sensory and sympathetic denervations and were presumed to be parasympathetic. Following extirpation of the SCG, a small number of NPY-IR fibers were seen only in the limbus; however, after combined sensory and sympathetic denervations many NPY-IR fibers were observed in both the limbus and cornea proper, suggesting upregulation of NPY by parasympathetic nerves. WGA-HRP retrograde tracing experiments demonstrated that a few cells in the ciliary (but not the pterygopalatine) ganglion project to the cornea or corneoscleral limbus. In conclusion, these studies have demonstrated a complex peptidergic innervation of the rat cornea that includes a previously unrecognized parasympathetic contribution from the ciliary ganglion. (PHS EY05717 to C.F.M.)

474.11

DISTRIBUTION OF PROENKEPHALIN mRNA EXPRESSING CELLS IN THE AVIAN RETINA.

M. Molnar[1], G. Casini[2], N.C. Brecha[2] and P. Bagnoli[1]*. Dept; of Physiol. & Biochem., Pisa Univ., Italy 56127 and Dept. of Anat. & Cell Biol. Med., UCLA School of Medicine, Los Angeles, CA U.S.A.

Immunohistochemical studies provide evidences for the existence of enkephalin-like substances in the vertebrate retina. Using in situ hybridization technique. we examined the pattern of distribution of proenkephalin (PPE) mRNA containing cells in the retina of pigeons and chickens. Retinal sections, either perpendicular or parallel to the vitreal surface, were hybridized with a riboprobe synthetized from a 600 bp PPE cDNA. PPE mRNA was localized to numerous cells in both chicken and pigeon retinas. The hybridization signal was higher in the chicken although its pattern of distribution was comparable in the two species. A prominent population of PPE mRNA expressing cells was present in the amacrine region of the inner nuclear layer through the entire retina although its density was lower in the peripheral region. The above findings are in agreement with prior immunohistochemical studies and suggest a possible involvement of enkephalin peptides in the processing of the visual information.

474.12

IMMUNOHISTOCHEMICAL EVIDENCE FOR THE RELEASE OF GABA VIA HYDROLYSIS OF NEURONAL HOMOCARNOSINE (GABA-HIS).

M.C.Jackson, J.M.Hardman*, J.F.Lennev. Depts. of Pharmacology and Pathology, John A. Burns School of Medicine, University of Hawaii, Honolulu, HI 96816

Polyclonal antibodies to GABA-his and human serum carnosinase (HSC) were used to localize immunoreactivity in the cerebellum and other human brain regions. GABA-his was abundant in the cytoplasm of Purkinje cells, whereas HSC was localized in axons entering the folium in the medullary (M) layer, traversing the granule cell (GC) layer and terminating at the interface between the GC and M layers in the vicinity of the Purkinje cells. In other brain regions, HSC and GABA-his were also present in synaptically connected neuronal pathways or were co-localized in the same neuron. For example, HSC and GABA-his were both present in the retina, (rods and cones, ganglionic neurons) and the optic nerve.

Cerebellar and cortical homogenates contained a proteinase-sensitive, heat stable 1.25KD HSC inhibitor. Non-competitive inhibitory activity was abolished by pre-incubation of HSC with 0.8mM Cd^{2+}.

These results support the hypothesis that a glutamic acid decarboxylase-independent pathway exists for the release of GABA at synaptic terminals via the hydrolysis of GABA-his by HSC and that the release may be regulated by a peptide inhibitor.

475.1

PROBING THE OPIOID RECEPTOR COMPLEX WITH (+)-TRANS-SUPERFIT: RESOLUTION OF TWO δ_{CX} BINDING SITES. X.Y. Cha[1], H. Xu[1], K.C. Rice[2]*, C.-H. Kim[2], and R.B. Rothman[1]. [1]NIDA, ARC, NIH, Baltimore, MD 21224 and [2]LMC, NIDDK, NIH.

Previous studies support the existence of two δ receptor subtypes called δ_{CX} and δ_{NCX} for δ receptors which are and are not associated with the μ-δ opioid receptor complex.. The δ_{CX} site is optimally assayed with [^{3}H]DADL (10 mM TRIS-HCl, pH 7.4, 100 mM NaCl, 3 mM MnCl2, 2 μM GTP and 5 mM 2-mercaptoethanol) using membranes depleted of δ_{NCX} sites by pretreatment with the δ_{NCX}-selective acylating agent, (+)-trans-SUPERFIT. The present study examined the effect of a wide range of morphine (MOR) concentrations (0, 12.5, 25, 50,100,400, 800 nM) or DPDPE (0, 2, 20, 150, 1000 nM) on the Kd and Bmax of the δ_{CX} binding sites. The data resolved two binding sites: a δ_{CX1} site where MOR is a potent noncompetitive inhibitor, and a δ_{CX2} site where MOR was a weak competitive inhibitor. In contrast, DPDPE was a weak competitive inhibitor at the δ_{CX1} site , and a potent competitive inhibitor at the δ_{CX2} site. Ligand-selectivity analysis indicated that MOR and DAMGO were relatively selective for the δ_{CX1} site , and DPDPE,. DPLPE, DELT-II and [pCl]DPDPE had high affinity and selectivity for the δ_{CX2} site. Studies in progress will address the possibility that these the two δ_{CX} binding sites are two states of a single receptor and also explore the relationship between the δ_{CX2} site and the δ_{NCX} binding sites. Viewed collectively, these studies provide further evidence for heterogeneity of the opioid δ receptor.

475.2

DIFFERENTIAL BINDING OF OPIOID PEPTIDES AND OTHER DRUGS TO TWO SUBTYPES OF THE OPIOID δ_{NCX} BINDING SITE IN MOUSE BRAIN. H. Xu[1]*, J.S. Partilla[1], B.R. de Costa[2], S.N. Calderon[2], K.C. Rice[2] and R.B. Rothman[1]. [1]NIDA Addiction Research Center, PO Box 5180, Baltimore MD 21224. [2]LMC, NIDDK, NIH, Bethesda, MD 20892.

A variety of data support the hypothesis of a μ-δ opioid receptor complex. This model postulates two classes of δ binding sites: a δ binding site not associated with the opioid receptor complex, termed the δ_{NCX} site, and a δ site associated with the receptor complex, termed the δ_{CX} site. A major purpose of this study was to clarify the relationship between the δ_{NCX} binding sites and the δ_1 and δ_2 receptors. Mouse brain membranes were depleted of μ sites by pretreatment with the site directed acylating agent, BIT, and the δ_{NCX} binding sites were labeled with [^{3}H]DADL. Binding surface and ligand-selectivity analysis resolved two binding sites termed δ_{NCX-1} and δ_{NCX-2} which were not two states of a single receptor. Other studies suggested that neither δ_{NCX} binding site had the characteristics expected of the δ_2 receptor, and that the δ_{NCX-1} site, but not the δ_{NCX-2} site, was synonymous with the δ_1 receptor. The racemic non-peptide δ agonist, BW373U86, had high affinity and selectivity for the δ_{NCX-2} site, suggesting that this site may be a novel δ receptor which mediates some of the effects of BW373U86. Ligand-selectivity studies of resolved enantiomers of BW373U86 are now underway. These data provide additional evidence for heterogeneity of the opioid δ receptor.

475.3

ETONITAZENE PRODUCES POTENT, SUPRASPINALLY-MEDIATED ANTINOCICEPTION IN NORMAL AND IN OPIOID-μ_1 DEFICIENT (CXBK) MICE. Charlene D. Connelly, Rebecca P. Martinez, James J. Schupsky and Robert B. Raffa*. Drug Discovery, The R.W. Johnson Pharmaceutical Research Institute, Spring House, PA 19477-0776.

It has recently been reported (Moolten *et al.*, Life Sci./*Pharmacol. Letts.* 52, PL-199-203, 1993) that etonitazene displays selective affinity for opioid μ receptor types. Using specific radioligand binding protocols, these authors demonstrated K_i values (nM) for etonitazene of 0.00042 at μ_1 sites, 0.41 at μ_2 sites, 423.0 at δ sites, 900.0 at κ sites and > 1 μM at σ sites. These findings make etonitazene one of the most μ-selective compounds. Additionally, the approximately 10-fold greater potency of etonitazene than morphine at the μ_2 site suggests that it might be a valuable pharmacologic tool to investigate the possibility of μ_2-mediated antinociception. The purpose of the present study was twofold: first, to determine if high *in vitro* affinity would be translated into high *in vivo* antinociceptive potency and, second, to determine if etonitazene could produce antinociception through μ_2 receptors. Male, albino mice (Crl:CD-1®), 18-24 g, or CXBK mice were administered etonitazene by *icv* (intracerebroventricular) injection and then antinociception was assessed 15 min later using a standard tail-flick test or 55°C tail-immersion test. Antinociception was quantified by converting tail-withdrawal latency to a percent of the maximum possible effect (%MPE). In the albino mice, etonitazene produced dose-related antinociception in both the tail-flick and tail-immersion tests. Etonitazene-induced antinociception was blocked by naloxone (demonstrating an opioid mechanism) and by β-FNA (demonstrating involvement of μ receptors), but not by ICI-174,864 or naltrindole (demonstrating the lack of involvement of δ receptors). Identical results were obtained in the μ_1-deficient CXBK mice. These findings demonstrate that etonitazene produces potent, supraspinally-mediated antinociception, possibly through opioid μ_2 receptors.

475.4

MOLECULAR MODELING OF SIGMA RECEPTOR LIGANDS: DERIVATION OF A MODEL FOR THE BINDING SITE BASED ON CONFORMATIONAL CONSIDERATIONS. B. Cai*, R. B. Murphy and D. I. Schuster, Department of Chemistry and Center for Neural Science, New York University, Washington Square, New York, NY 10003.

The sigma receptor/binding site (SRBS) has attracted considerable interest due to its ability to bind with significant affinity to a variety of psychoactive compounds, including opiates of the benzomorphan type and compounds which show neuroleptic activity and little affinity for dopamine D2 receptor sites. The structural variety of potent SRBS ligands is very great, including haloperidol and other butyrophenones, disubstituted guanidines, phenylpiperidines, 1,2- diaminocyclo-hexanes, and a new class of tetracyclic ligands recently synthesized in our laboratory, the octahydronaphthoquinolizines. Earlier studies indicated a wide degree of tolerance by the SRBS for stereochemical and topographical demands of the ligands. The present study involved the search for low energy conformation of the major classes of sigma ligands using quenched molecular dynamics (MD) simulations. Structures were constructed using x-ray crystal coordinates or with the aid of the BUILDER module in INSIGHT II. MD simulations were performed using DISCOVER, version 2.9, running an a Silicon Graphics IRIS Crimson Elan workstation. Integrations used a leap-frog algorithm with a time step of 1.0 femtosecond at constant temperature. The calculations suggest there are two different pharmacophores for the SRBS, or alternatively two sites which may or may not be coupled allosterically. These will be described in detail. (Supported by NYU Research Challenge and Technology Transfer Funds)

475.5

SYNTHESIS AND PHARMACOLOGICAL CHARACTERIZATION OF SUBSTITUTED OCTAHYDRONAPHTHOQUINOLIZINES, A NEW CLASS OF LIGANDS FOR THE SIGMA RECEPTOR/BINDING SITE. D. I. Schuster, * B. Cai, M. Cornebise, G. Stoupakis and R. B. Murphy, Department of Chemistry and Center for Neural Science, New York University, Washington Square, New York, NY 10003.

We have recently described a method for synthesis of members of a new tetracyclic ring system, the octahydronaphthoquinolizines (OHNQs). (1) These compounds are analogs of 3-PPP and related tricyclic compounds which have high affinity for the sigma receptor /binding site (SRBS). The OHNQs are of particular interest in the elucidation of structure-activity correlations for the SRBS because they exist as eight stereoisomers, comprising four sets of enantiomers. We have now prepared additional members of this family with methoxy, hydroxy, fluoro, chloro and bromo substituents at three different positions on the aromatic ring. Using radioligand binding assays, we find that certain members of this group, particularly those with *trans,trans* ring fusions, have good affinity for the SRBS but are inactive toward dopamine D2 receptors. Some stereoisomers have no demonstrable activity at either of these receptor sites. Several of the sigma-active compounds have been additionally screened for activity at 40 other receptor sites under the NovaScreen program. Of these, activity was found only at the 5-HT2 receptor. Thus, the OHNQs appear to represent a new class of compounds with selective affinity for the SRBS. (Supported by NYU Research Challenge and Technology Transfer Funds)

(1) Cai, B., *et al. Tetrahedron Lett* **1993** *34*, 2067.

475.6

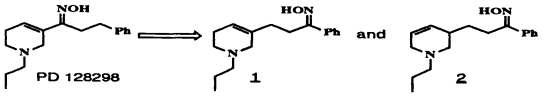
A STRUCTURE-AFFINITY STUDY OF SIGMA-1 RECEPTOR BINDING. S. Y. Ablordeppey†, J. B. Fischer¶,*K. J. Burke Howie¶, J. A. Dunn¶, R.A. Glennon†. †Medical College of VA/VCU, Richmond, VA 23298; ¶Cambridge NeuroScience, Cambridge, MA 02139.

We have previously characterized sigma subtype binding and demonstrated support for at least 2 sigma receptor binding sites (sigma-1 and sigma-2) in guinea pig brain membranes (*Soc. Neurosci. Abstr.* 1992, 18, 455). To understand the topographical requirements of sigma-1 binding sites, a structure-affinity study of sigma-1 ligand binding was undertaken. Based on these studies, we now report that binding of ligands at sigma-1 sites has considerable similarities to overall sigma binding. For example, increasing the alkyl spacer between the phenyl and N atom of phenylalkyl substituted phenethylamines from 3 to 5 increases binding affinity (Ki = 11 nM, 2.6 nM, and 0.17 nM, respectively), but affinity appears to drop off beyond a pentyl chain. Using 48 sigma-1 ligands (Ki = 0.2 to 4000 nM), a 3D QSAR study was performed using CoMFA. The binding model provides a very high correlation between observed and predicted affinities (r = 0.96, n=48, s = 0.31). To test the predictive validity, the model was used to predict the binding affinities of a newly synthesized series of 17 compounds with widely diverse structures. A plot of predicted versus observed affinities showed a high correlation (r = 0.76, n = 17; r = 0.95, n = 65, s = 0.40). The CoMFA steric contour map also suggests that sigma-1 sites may be quite tolerant of steric bulk.

475.7

IDENTIFICATION OF POTENT AND SELECTIVE SIGMA LIGANDS: DESIGN, SYNTHESIS AND STRUCTURE-ACTIVITY RELATIONSHIP OF PD 128298 REGIOISOMERS. H. Tecle, Y. Pei, J. C. Jaen*, W. H. Moos, L. D. Wise, Y. Shih, T. A. Pugsley, and T. G. Heffner. Parke-Davis Pharmaceutical Research Division of Warner-Lambert Company, 2800 Plymouth Road, Ann Arbor, Michigan 48105

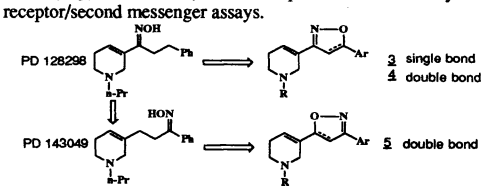
PD 128298 exhibits high affinity (IC_{50} = 1.55 nM) and selectivity for the sigma binding sites. It is at least 500 fold selective for sigma binding sites versus various other neurotransmitter receptors. Nevertheless, PD 128298 has appreciable affinity for muscarinic receptors. Consequently, biological results could not unequivocally be attributed to the interaction of PD 128298 with sigma receptors. Structural modification of PD 128298 led to the synthesis of sigma ligands 2 and 3, analogs of Pd 128298 devoid of affinity for muscarinic receptors. Ligands 2 and 3 are regioisomers of PD 128298. These sigma ligands are as potent and selective as PD 128298 in receptor binding assays and possesses negligible affinity for the muscarinic receptor.



475.8

IDENTIFICATION OF POTENT AND SELECTIVE SIGMA LIGANDS : DESIGN, SYNTHESIS AND STRUCTURE-ACTIVITY RELATIONSHIP OF TETRAHYDROPYRIDINYL-ISOXAZOLINES AND -ISOXAZOLES AS CYCLIC ANALOGS OF PD 128298 AND ITS REGIOISOMERS. Yazhong Pei, Haile Tecle, Walter H. Moos, Lawrence D. Wise, Hyacinth Akunne, Thomas A. Pugsley, David A. Downs* and Thomas G. Heffner. Parke-Davis Pharmaceutical Research Division of Warner-Lambert Company, 2800 Plymouth Road, Ann Arbor, Michigan 48105

Tetrahydropyridinylisoxazoline 3 and tetrahydropyridinylisoxazoles 4 and 5 are potent and selective sigma ligands. Many of these ligands exhibit subnanomolar affinity for sigma binding sites. Structure-activity relationship work culminated in the synthesis of PD 144418 (5, R=*n*-propyl and Ar=4-methylphenyl). PD 144418 is a selective sigma ligand. It is at least 1500 fold selective for sigma binding sites labeled with [^{3}H]-(+)-3PPP (IC_{50} = 0.067 nM) when compared with its affinity in other receptor/second messenger assays.



475.9

THE EFFECTS OF CHRONIC SPINAL ADMINISTRATION OF SELECTIVE OPIOID ANTAGONISTS. B. J. Keck*, J. L. Stafinsky and T. Crisp. Department of Pharmacology, N. E. Ohio Universities College Of Medicine, Rootstown, OH, 44272.

Male Sprague-Dawley rats were chronically treated with selective opioid receptor antagonists for 7 days to determine if supersensitivity developed (i.e., enhanced analgesia) following spinal opioid agonist administration. The tail-flick measure was used to determine sensitivity to thermal nociceptive stimuli. Each rat was implanted with an indwelling intrathecal (i.t.) cannula which was later connected to a subcutaneously implanted Alzet osmotic minipump (model 2001). The minipumps continuously delivered the mu antagonist CTOP or the delta antagonist naltrindole (NTI) for 7 days. Following a 24 hour washout period, rats were tested for supersensitivity after receiving an i.t. bolus dose (ED_{50}) of either DAMPGO or DPDPE (i.e. the mu agonist DAMPGO was administered to the chronically treated CTOP rats or the delta agonist DPDPE was administered to the chronically treated NTI rats). Findings revealed that chronic spinal treatment with selective opioid antagonists did not induce supersensitive responses to selective opioid agonists on the tail-flick measure of thermal analgesia.

475.10

NERVE GROWTH FACTOR RAPIDLY PROLONGS THE ACTION POTENTIAL OF MATURE SENSORY NEURONS IN CULTURE AND THIS EFFECT REQUIRES ACTIVATION OF EXCITATORY Gs-COUPLED KAPPA OPIOID RECEPTORS ON THESE CELLS. K.-F. Shen and S.M. Crain*, Dept. of Neuroscience, Albert Einstein Coll. Med., Bronx, N.Y. 10461

NGF (pM-nM) rapidly prolongs the Ca^{2+} component of the action potential duration (APD) of mouse dorsal-root ganglion (DRG) neurons in long-term organotypic culture. NGF-induced APD prolongation is blocked by pretreatment of the DRG neurons with: 1) monoclonal antibodies to rodent NGF receptors; 2) the opioid receptor antagonist, naloxone and the specific kappa opioid antagonist, nor-binaltorphimine (but not by specific mu and delta opioid antagonists); or 3) cholera toxin. The results suggest that NGF stimulates the release of opioids from DRG neurons and that prolongation occurs secondarily by activation of excitatory Gs-coupled kappa opioid receptors on these same or nearby cells. Extremely low (fM-nM) concentrations of exogenous opioid agonists can elicit similar excitatory effects on these neurons (S&C, Br. Res. '89,'90,'92). These studies suggest that excitatory opioid receptor functions may mediate some types of rapid NGF-induced physiologic effects, e.g. hyperalgesia. (Supported by DA-02031 to SMC.)

475.11

STRUCTURAL DETERMINANTS OF THE 5-HT AND NE UPTAKE INHIBITING ACTIVITY OF OPIOID AGONISTS AND ANTAGONISTS. E. E. Codd* and R. P. Shank, CNS Research, Drug Discovery Research, The R. W. Johnson Pharmaceutical Research Institute, Welsh and McKean Roads, Spring House, PA 19477-0776

Compounds that inhibit the neuronal uptake of norepinephrine and/or serotonin are analgesic in some preclinical pain models and are used clinically in the treatment of certain chronic pain conditions. These compounds also enhance opiate-induced analgesia in an additive or synergistic manner. The results of the present study show that some opioids, in addition to binding to opioid receptors, also inhibit the uptake of norepinephrine and/or serotonin. Structure-activity analysis revealed that phenanthrene opioids that have an oxygen bridge between C4 and C5, such as morphine and naloxone, did not block norepinephrine or serotonin uptake, whereas phenanthrene opioids without the oxygen bridge, such as levorphanol and levomethorphan, did inhibit uptake, as did non-phenanthrene opioids, such as d-propoxyphene and methadone. Thus a clear structure-activity relationship underlies the presence of this additional activity in opioidergic compounds. Since uptake inhibiting activity contributes to the analgesic effect of at least some opioids (such as tramadol), the demonstration of a structure-activity relationship underlying the presence or absence of the activity advances understanding of the analgesic activity of opioid drugs.

476.1

EFFECT OF CHRONIC I.C.V. INFUSION OF ANTI-DYNORPHIN IgGs ON OPIOID MU AND DELTA RECEPTORS IN RAT BRAIN: AN AUTORADIOGRAPHIC STUDY. C.B. Goodman[1], M.H. Baumann[1], I.S. Partilla[1], J.L. Cadet[1], K.F.A. Soliman[2*] and R.B. Rothman[1]. [1]CPS, NIDA, ARC, Baltimore, MD 21224, [2]Florida A&M University, College of Pharmacy and Pharmaceutical Sciences, Tallahassee, FL 32307.

Several endogenous peptides are known to exhibit anti-opioid activity, including dynorphin, which is an endogenous ligand for the kappa opioid receptor. Preliminary binding studies showed that chronic i.c.v. infusion of anti-Dynorphin A antiserum decreased the Bmax of mu opioid receptors to 68% of control, and that infusion of 1 μg/μl control rabbit IgG did not alter mu receptor binding. The purpose of this study was to determine the effect of chronic i.c.v. infusion of anti-dynorphin A IgG or anti-dynorphin(1-8)-IgG on mu and delta opioid binding sites using the technique of in vitro autoradiography. ALZET 2002 osmotic minipumps (0.5 μl/hr for 13 days) were filled with saline, anti-dynorphin A IgG (1 μg/3.75 μl 0.9% NaCl) or anti-dynorphin(1-8) IgG (1 μg/3.75 μl 0.9% NaCl). The solutions were infused via i.c.v. cannula placed in the left lateral ventricle and attached to the osmotic minipumps. The rats were sacrificed on day #13 of the infusion, and prepared for in vitro autoradiograpy. Regional brain sections (20 μm in diameter) at the level of N. Accumbens, Substantia nigra, Thalamus, Hippocampus, and Periaqueductal Gray will be used to assess treatment effects on mu receptors labeled by [125I]DAMGO and delta receptors labeled by [125I]Deltorphin-I. These data will provide pertinent information as to the possible modulatory role of endogenous dynorphin on mu and delta opioid receptors in rat brain.

476.2

BIOCHEMICAL CHARACTERIZATION OF κ_1 OPIATE BINDING SITES IN R1.1 THYMOMA CELLS

G.P. Brown*, K.M. Standifer, and G.W. Pasternak. The Cotzias Laboratory of Neuro-Oncology, Memorial Sloan-Kettering Cancer Center and Depts. of Neurology/Neuroscience and Pharmacology, Cornell University Medical College, NY NY 10021.

κ_1 opiate binding sites have been demonstrated in the mouse thymoma cell line R1.1 *(Bidlack et al., EJP 227:257, 1992)*. Binding studies have indicated a single site. The potency of U50,488H (K_i 0.45± 0.06 nM) and poor affinity for DAMGO (K_i > 700 nM) and DSLET (K_i > 4 μM) strongly imply a κ_1 classification. We have confirmed the presence of opioid binding sites in this cell line in standard binding assays. Like the prior studies, we find evidence suggesting a single site with a selectivity profile consistent with κ_1 receptors. These sites bind an ^{125}I-opioid affinity ligand synthesized in our laboratory with high affinity (K_D 1 nM). UV irradiation covalently attaches the ligand to the binding site. CHAPS solubilized, photoaffinity labelled R1.1 membranes run over an FPLC Mono-Q anion exchange column reveal a single peak of specific ^{125}I-affinity labelled receptor in the R1.1 cell line which corresponds to a κ_1 opioid receptor.

476.3

REGULATION OF OPIOID RECEPTOR EXPRESSION IN STRIATUM AND VENTRAL MESENCEPHALON DISSOCIATED CELL CULTURES. J.A.M. Smith, S.E. Loughlin*, and F.M. Leslie, Depts. of Pharmacology, Anatomy and Neurobiology, College of Medicine, University of California, Irvine, CA 92717.

Recently, we have demonstrated that *in vivo* striatal opioid receptor expression is under transsynaptic regulation by dopaminergic innervation from the substantia nigra (Smith et al., Neuroscience, in press). To further investigate the factors controlling opioid receptor expression, both individual and combined dissociated cell cuture models of striatum and ventral mesencephalon were established. Striata and ventral mesencephali were dissected from rat brains at embryonic days 17 and 15, respectively. Cells were enzymatically and mechanically dissociated and plated in serum-containing media at a concentration of 1.3 x 10^6 cells/ml. For coculture conditions, equal volumes of striatal and ventral mesencephalic cell suspensions were mixed such that the final plating concentration was 1.3 x 10^6 cells/ml. At 8 days in vitro (DIV), in whole cell preparations of striatal cultures, [^{3}H]diprenorphine bound to a single site with Kd = 0.35 nM. Pharmacological studies indicated that this site was naloxone-sensitive, but exhibited low or negligible affinity for kappa or delta agonists. Naloxone-sensitive [^{3}H]diprenorphine binding sites were detected also in ventral mesencephalic cultures. In cocultures of striatum and ventral mesencephalon there was an approximately 3 fold increase in the level of opioid binding sites, as compared to the sum of that in the two individual cultures. This potentiation was apparent at 4 DIV but was most dramatic at 8 and 12 DIV. The pharmacology of the additional sites and the cell type(s) upon which they are expressed have yet to be determined. [^{3}H] Dopamine uptake was not potentiated in the cocultures suggesting that dopaminergic neuronal number/function had not been modulated under these coculture conditions. The precise mechanisms underlying the increase in opioid binding site expression in striatum/ventral mesencephalon cocultures are currently under investigation.

Supported by NS19319 and NS2622671.

476.4

EFFECT OF ESTROGEN ON μ-OPIOID RECEPTOR G-PROTEIN COUPLING IN GUINEA-PIG MEDIOBASAL HYPOTHALAMUS (MBH). G. Zhang*, T.F. Murray[1] and M.J. Kelly. College of Pharmacy, Oregon State Univ., Corvallis[1] and Dep. of Physiology, Oregon Health Sci. Univ., Portland, OR 97201.

Previous findings have shown that estrogen decreases the potency of μ-agonist Tyr-D-Ala-Gly-MePhe-Gly-ol (DAMGO) mediated hyperpolarization of MBH neurons (Kelly et al., J. Neurosci., 12:2745,1992). The aim of the present study was to explore the effect of estrogen on μ-opioid receptor/G-protein coupling in MBH. MBH were obtained from ovariectomized females treated with oil (n=8) or 17β-estradiol benzoate (25 μg, s.c., n=8) 24 hr prior to sacrifice. The total membrane particular fraction was subjected to NaCl (120 mM) + GTP (100 μM) + EDTA pre-incubation conditions prior to use. μ-Opioid receptor competition binding was performed using [^{3}H]diprenorphine as an opioid antagonist radioligand and DAMGO as a competitor in a 50 mM Tris buffer containing 6 mM Mg^{++} either with or without Gpp(NH)p (50 μM). Since we found that μ, δ and κ subtypes all exist in MBH, selective agonists were included in assays to block δ and κ receptors. In control animals, most of the μ receptors were in the high affinity state with an IC_{50H} value of 12.0 nM (95%CI:8.8-16.5), and this population of receptors was completely shifted to an intermediate affinity state with an IC_{50I} of 56.7 nM (38.0-84.6) by Gpp(NH)p. Similar populations and Gpp(NH)p effects were observed in estrogen-treated animals with IC_{50H} and IC_{50I} values of 8.9 nM (7.0-11.2) and 40.2 nM (32.9-57.1). Therefore, it appears that under the assay conditions employed estrogen does not attenuate the regulatory action of guanine nucleotide on opioid μ binding (supported by DA05158).

476.5

THE EFFECT OF CHRONIC FOOD RESTRICTION ON MU OPIOID RECEPTORS IN THE RAT: A QUANTITATIVE AUTORADIOGRAPHIC STUDY. T.D. Wolinsky*, K. Carr, J.M. Hiller, and E.J. Simon Dept. of Psychiatry, NYU School of Medicine, New York, NY 10016

We have previously shown that chronic food restriction produces opioid facilitation of lateral hypothalamic self-stimulation (*Brain Res* 607: 141-148, 1993). Recently, this effect has been attributed, in part, to mu opioid activity (see Carr et al., this meeting). While binding to opioid receptors has been shown to be modified by food deprivation, the receptor type(s) and brain regions involved in this phenomenon have yet to be defined. We are therefore using quantitative receptor autoradiography to address this issue. Brains from rats that had been on either an *ad lib* or a restricted diet (10 grams per day for 14 days) were sectioned from the level of the prefrontal cortex to the posterior hypothalamus and processed using standard autoradiographic methods. [^{3}H]-DAGO (4 nM) was used to identify mu receptors. Areas analyzed included the cingulate cortex, caudate, nucleus accumbens, bed nucleus of the stria terminalis, ventral pallidum, various regions of the hypothalamus and the habenular complex. Most regions were examined at at least three rostrocaudal levels.

Of the areas sampled thus far, only the habenula appears to be affected by chronic food restriction - with a 17 - 25% decrease in mu opioid binding. The effect on mu binding in other brain regions, as well as on kappa opioid binding is currently being investigated. Supported by NIDA Grant DA-03956 (K.C) and NRSA DA-05441 (T.W.)

476.6

MU, KAPPA, AND DELTA OPIOID RECEPTOR DISTRIBUTION IN THE SONGBIRD HIPPOCAMPUS.

P. Deviche* and C.C. Gulledge. Inst. Arctic Biology, Univ. Alaska Fairbanks, Fairbanks, AK 99775.

Previous research has found that the chick and pigeon hippocampal complex (hippocampus: Hp; parahippocampus: AHP) contains regionally located opioid peptide-like immunoreactivity and receptors, but no information on this subject is available for songbirds. We used *in vitro* autoradiography to localize μ, κ and δ opioid receptors in the Hp complex of an adult male songbird (dark-eyed junco, *Junco hyemalis*). Tritiated DAMGO, EKC, and pCl-DPDPE were used to label μ, κ and δ receptors, respectively. Specific binding was measured in the AHP and in the dorsolateral (D), dorsomedial, and ventral Hp divisions. Data show that the AHP contains a high density of all three receptor types. Low κ and high μ receptor densities are found in all Hp regions. Finally, δ receptors are more abundant in the D than in other Hp regions. Thus, each receptor type has a unique neuroanatomical distribution, which differs from that seen in other species. These data suggest that the avian Hp complex is a site where opioids exert physiological, possibly species-specific effects. Supported by NSF Award BNS-9121258.

476.7

CHARACTERIZATION AND LOCALIZATION OF DELTA OPIOID BINDING SITES IN THE BOVINE PINEAL. P.S. Pazdalski, W.P. Battisi and V. J. Aloyo*. Dept. of Pharmacology and Anatomy and Neurobiology, Medical College of PA, Philadelphia, PA

Opioid binding sites in the crude membrane fraction of bovine pineal were characterized using the highly selective delta opioid agonist, ^{3}H-[D-Pen^2, pCl-Phe^4, D-Pen^5]enkephalin (DPDP(CL)E). Pineal membranes possess a single class of high affinity binding sites for this delta ligand (Kd = 260 pM). The specific opioid antagonist, naloxone, dose dependently inhibited ^{3}H-DPDP(CL)E binding confirming that this ligand is indeed binding to opioid receptors. The delta selective ligands deltorphin and [D-Pen^2, D-Pen^5]enkephalin(DPDPE) were much more potent than the mu selective compounds dermorphin and [D-Ala^2, MePhe^4, Gly^5-ol]enkephalin (DAMGO) in inhibiting ^{3}H-DPDP(CL)E binding. These results demonstrate that in bovine pineal membranes, DPDP(CL)E binds to delta opioid sites. Autoradiographic studies showed that ^{3}H-DPDP(CL)E binding is uniformly distributed over the bovine pineal. Thus, ^{3}H-DPDP(CL)E binding is a general characteristic of all pinealocytes. This wide spread distribution of delta opioid receptors supports the hypothesis that endogenous opioid peptides modulate pineal function. Supported by MH16841.

476.8

CHARACTERIZATION OF [3H]4-PHENYL-1-(4-PHENYLBUTYL)-PIPERIDINE BINDING IN RAT BRAIN. K. Hashimoto*†and E.D. London†§¶. †Addiction Res. Ctr., NIDA, NIH, Balto., MD 21224; §Dept. Radiology, The Johns Hopkins Med. Inst., Balto., MD 21205; ¶Dept. Pharmacol. & Exper. Ther., Univ. of Maryland Sch. Med., Balto., MD 21201.

4-Phenyl-1-(4-phenylbutyl)piperidine (4-PPBP) is a novel ligand for σ receptors, and has a higher affinity for binding sites labeled with tritated di-*o*-tolylguanidine (DTG) than haloperidol (Glennon et al., *J. Med. Chem.* 34: 3360-3365, 1991). We have reported that 4-PPBP is the most potent drug tested in competition assays with [3H]ifenprodil binding to a subtype of σ receptors (Hashimoto and London, *Eur. J. Pharmacol.* in press, 1993). Ifenprodil, a noncompetitive antagonist of NMDA receptors, has been shown to be cytoprotective in animal models of focal ischemia. Therefore, 4-PPBP also may be active in animal models of brain ischemia. The present study was undertaken to examine specific binding of [^{3}H]4-PPBP in rat brain membranes. Binding of [^{3}H]4-PPBP (49.8 Ci/mmol) was examined in 50 mM Tris-HCl buffer (pH 7.4) at 25°C. Nonspecific binding was estimated in the presence of 10 μM haloperidol. [^{3}H]4-PPBP bound with high affinity to two populations of sites (high affinity K_d = 79.5 pM, B_{max} = 999 fmol/mg protein; low affinity K_d = 5.98 nM, B_{max} = 1826 fmol/mg protein). The rank order of affinities from competition experiments was: 4-PPBP > haloperidol > ifenprodil > SL 82.0715 > amiodarone > flunaridine > (+)-pentazocine > BMY 14802 > (-)-butaclamol > (-)-pentazocine > DTG > (+)-3-PPP > (-)-3-PPP > rimcazole > (+)-SKF 10,047 > (+)-butaclamol > (-)-SKF 10,047. The regional distribution of [^{3}H]4-PPBP binding in brain was as follows: medulla oblongata > midbrain > hypothalamus > cortex > cerebellum > hippocampus > striatum. Thus, [^{3}H]4-PPBP labels haloperidol-sensitive, binding sites, with a regional distribution that is unlike that of σ receptors. As a number of σ receptor ligands interact with these sites, [^{3}H]4-PPBP may label a novel subtype of σ receptors in rat brain.

476.9

HIGH AFFINITY BINDING OF (+)[^{3}H]−3−(3−HYDROXYPHENYL)−*N*−(1−PROPYL)PIPERIDINE ((+)−3−PPP) TO DOPAMINE RECEPTORS IN RAT AND BOVINE STRIATA. AG Hohmann*, SL Patrick, J Park, and JM Walker. Schrier Research Laboratory, Department of Psychology, Brown University, Providence, RI 02912.

Studies of the anatomical and biochemical pharmacology of (+)[^{3}H]−3−(3−hydroxyphenyl)−*N*−(1−propyl)piperidine ([^{3}H](+)3−PPP) have led to the hypothesis that the effects of this ligand are mediated predominantly by binding to sigma receptors. Nonetheless, *in vivo* evidence suggests a role for (+)−3−PPP as a dopamine autoreceptor agonist, despite findings that it only weakly displaces several dopaminergic ligands from their receptors *in vitro*. The present study was undertaken to directly examine whether (+)[^{3}H]−3−PPP binds to dopamine receptors in membranes from striatum, a structure that exhibits a much greater density of dopamine compared to sigma receptors. Rat and bovine striata were rapidly dissected, homogenized with a polytron and centrifuged at 1000 X g for 10 min. Following centrifugation of the supernatant at 31,000 X g for 15 minutes, the pellet was resuspended in the 10 mM Tris HCl pH 7.4 for binding studies. All assays were performed under sigma receptor binding conditions in the presence of 10 μM 1,3−di−o−tolylguanidine (DTG) to block binding to sigma sites. Nonspecific binding was determined in the presence of 10 μM apomorphine. Scatchard analyses in rat striata revealed a K_d = 21.0 ± 6 and a B_{max} = 144.7 ± 20.7 for three assays performed in triplicate. Competition curves performed in both rat and bovine revealed a rank order of potency consistent with binding to dopamine D_3 receptors: quinpirole was more potent than apomorphine, which was more potent than SCH23390 in inhibiting (+)[^{3}H]−3−PPP binding. Although the selectivity of (+)−3−PPP for D_3 receptors remains to be determined, the high potency of quinpirole (IC_{50} = 5.2 nM in rat striatum) in inhibiting (+)− 3−PPP binding is consistent with actions of this sigma ligand at dopamine autoreceptors.
(Supported by PHS DA04988 and MH48869).

476.10

14β-(THIOGLYCOLAMIDO)-7,8-DIHYDRO-N(CYCLOPROPYLMETHYL)-NORMORPHINONE LABELS KAPPA OPIOID RECEPTORS IN THE MOUSE THYMOMA CELL LINE. Q. Jiang* and J. M. Bidlack. Dept. of Pharmacology, Univ. of Rochester, Rochester, NY 14642.

The mouse thymoma cell line R1.1 expresses a brain-type κ opioid receptor with a sulfhydryl group at or near the κ opioid binding site. The present study was directed at labeling the κ opioid receptor in R1.1 cell membranes using the sulfhydryl-containing affinity ligand, 14β-(thioglycolamido)-7,8-dihydro-N(cyclopropylmethyl)-normorphinone (N-CPM-TAMO). The affinity of N-CPM-TAMO for κ opioid receptors on R1.1 cell membranes was compared to guinea-pig brain membranes. Incubating R1.1 cell or guinea-pig brain membranes with N-CPM-TAMO, followed by extensive washing, produced a concentration-dependent inhibition of the binding of the κ-selective ligand [^{3}H]U69,593. The concentration of N-CPM-TAMO that resulted in a 50% inhibition of 1 nM [^{3}H]U69,593 binding was 104 ± 14 and 162 ± 4 nM in R1.1 cell and guinea-pig brain membranes, respectively. The κ opioid ligand, U50,488 but not μ or δ opioids protected the binding site from N-CPM-TAMO alkylation. Incubating membranes with 100 nM N-CPM-TAMO significantly decreased the B_{max} values from 62.2 ± 1.7 to 7.9 ± 3.7 in R1.1 cell and from 62. 2 ± 1.7 to 16.8 ± 2.7 fmol/mg of protein in guinea-pig brain membranes, without changing the K_d value for [^{3}H]U69,593. The wash-resistant inhibition of [^{3}H]U69,593 binding by N-CPM-TAMO was partially reversed by the addition of the disulfide-bond reducing reagent dithiothreitol. Thus, these results indicate that N-CPM-TAMO irreversibly bound to the κ opioid binding sites in R1.1 cell membranes, as well as in guinea-pig brain membranes, by forming a disulfide bond between the κ binding site and the affinity ligand. (Supported by grant DA04355)

476.11

POSITIVE SELECTION OF LEUKOCYTES EXPRESSING THE KAPPA OPIOID RECEPTOR. D.M.P. Lawrence* and J.M. Bidlack, Dept. of Pharmacology, University of Rochester Medical Center, Rochester, NY 14642.

The R1.1 mouse thymoma cell line expresses a high affinity κ opioid receptor that is negatively coupled to adenylyl cyclase through a pertussis toxin-sensitive G_i protein. To purify cells of the immune system that express opioid receptors, we have developed a method for magnetic separation using an affinity ligand for opioid receptors, 14β-(bromoacetamido)-7,8-dihydro-N(cyclopropylmethyl)-normorphinone (N-CPM-H_2BAMO). This compound bound with high affinity to the κ receptor on R1.1 cell membranes, as demonstrated by the IC_{50} value of 1.5 nM against the binding of 1 nM [^{3}H]U69,593. Under sterile conditions, N-CPM-H_2BAMO was covalently attached to iron oxide beads coated with sulfhydryl groups, forming a thioacetamido linkage. Using a bead to cell ratio of 5:1, the N-CPM-H_2BAMO-coupled beads were incubated with either R1.1 cells or a similar T-cell line that does not express opioid receptors. Optimal results were obtained with an incubation time of 30 min at 4°, using RPMI 1640 media with 15% bovine serum albumin to minimize nonspecific binding. After separation with a magnet, 70% of the R1.1 cells were associated with the magnetic beads, while less than 5% of cells from a T-cell line not expressing opioid binding sites bound to the beads. The beads were removed from the selected cells by incubation in media at 37° for 24 hr. We are currently attempting to positively select for cells of the mouse immune system which express the κ opioid receptor, in order to determine the phenotype of those cells as well as to identify the effector functions associated with leukocytic opioid receptors. (Supported by USPHS grants DA04355 and DA07232)

476.12

ALKYLATION OF KAPPA OPIOID RECEPTORS ON THE R1.1 THYMOMA CELL LINE BY β-CHLORNALTREXAMINE: EVIDENCE FOR SPARE RECEPTORS. D.B. Joseph* and J.M. Bidlack, Dept. of Pharmacology, Univ. of Rochester, Rochester, NY 14642.

The κ opioid receptor expressed by the murine thymoma cell line R1.1 is coupled through a G_i-protein to adenylyl cyclase. Evidence for downregulation of this receptor was obtained from binding studies, which showed that the B_{max} value for the κ-selective ligand [^{3}H]U69,593 was reduced in membranes from R1.1 cells cultured for 24 hr with the κ agonist U50,488. Since receptor downregulation is usually accompanied by receptor desensitization, studies were performed to determine if κ-opioid inhibition of forskolin-activated adenylyl cyclase activity was attenuated following chronic exposure of R1.1 cells to U50,488. R1.1 cells were exposed to 100 nM U50,488 for 24 hr, a treatment which produced a 50% reduction of the B_{max} value for [^{3}H]U69,593. Surprisingly, inhibition of adenylyl cyclase activity by U50,488 was unchanged in membranes from 24 hr-treated cells, compared to control membranes from 15 min-treated cells. To determine if spare receptors were involved in the preservation of the κ-agonist response following downregulation of κ receptors, membranes from 24 hr-treated R1.1 cells were exposed to 50 μM of the irreversible opioid antagonist β-chlornaltrexamine (β-CNA), followed by extensive washing. A complete loss of [^{3}H]U69,593 binding and of U50,488 inhibition of cAMP production was observed in β-CNA treated membranes, whereas treatment with the reversible antagonist naltrexone had minimal effect on these parameters. The results provide evidence for the presence of spare κ opioid receptors on the R1.1 cell line, which appear to maintain the functional response following receptor downregulation. (Supported by USPHS grants DA04355 and DA07232.)

476.13

A NEW ASSAY FOR MU$_1$ BINDING IN CALF THALAMIC MEMBRANES USING A NOVEL, HIGHLY SELECTIVE DELTA LIGAND. L.M. Visconti, K.M. Standifer, P. Schiller, G.W. Pasternak.* The Cotzias Laboratory of Neuro-Oncology, Memorial Sloan-Kettering Cancer Center, New York, NY 10021 and Lab. of Chemical Biology and Peptide Research, Institut de Recherches Cliniques de Montreal, Canada H2W 1R7

Previously, we reported a selective mu$_1$ binding assay. At low concentrations ^{3}H-DADL labels predominently mu$_1$ and delta receptors. The assay depends upon the selective blockade of the delta sites, leaving mu$_1$ binding. Bovine thalamus contains very low concentrations of delta binding sites, enabling DPDPE to lower delta binding to less than 10% of total binding without interfering appreciably with mu$_1$ binding. However, the assay is not valid in tissues with higher densities of delta receptors since it is not possible to adequately compete delta binding without also interfering with mu$_1$ binding due to the limited delta/mu$_1$ selectivity of DPDPE. TIPP[ψ] (H-Tyr-Tic-ψ-[CH$_2$NH]Phe-Phe-COOH) is a highly selective delta ligand. In standard binding assays, it competes delta binding with a K_i of approximately 0.2 nM while its K_i values against mu$_1$, mu$_2$, kappa$_1$ and kappa$_3$ sites are > 1 μM, leaving TIPP[ψ] with a delta/mu$_1$ selectivity of > 5,000, compared to only 25 with DPDPE. We have utilized this highly selective delta ligand to develop a mu$_1$ binding assay in bovine thalamus which is applicable to other tissues as well.

476.14

κ$_3$ OPIATE RECEPTORS ARE PRESENT IN SH-SY5Y NEUROBLASTOMA CELLS. Jie Cheng*, Kelly M. Standifer, J.L. Biedler, and Gavril W. Pasternak. Cotzias Lab of Neuro-Oncology and Laboratory of Cellular & Biochemical Genetics, Memorial Sloan-Kettering Cancer Center and Depts. of Neuroscience and Pharmacology, Cornell Univ. Medical College, New York, NY 10021.

The human neuroblastoma cell line, SH-SY5Y, has high levels of both μ and δ opioid receptors. However, the combined B_{max} values for μ and δ receptors constitute only about 60% of the B_{max} of the nonselective antagonist ^{3}H-diprenorphine. We now report that this remaining 40% of ^{3}H-diprenorphine binding corresponds to κ$_3$ receptors. The identity of the κ$_3$ sites was confirmed by competition studies. The poor affinity of DPDPE and U50,488H at the κ$_3$ site indicates that the binding was neither δ nor κ$_1$. The μ selective ligands DAMGO, morphine and fentanyl compete μ binding 4- to 10-fold more potently than κ$_3$ binding. In undifferentiated SH-SY5Y cell membranes, the ratio of μ δ κ$_3$ receptor subtypes is 3:3:4. Differentiation with retinoic acid for six days increases the binding of all classes. The levels of μ and δ binding doubled, while κ$_3$ binding increased 3-fold. All three receptor classes inhibit the accumulation of cAMP. Thus, SH-SY5Y cells contain a binding site with the binding specificity expected of κ$_3$ receptors. This site is upregulated to a larger degree than either μ or δ sites in the differentiated state, and is functionally active.

476.15

δ OPIOID RECEPTOR BINDING IN THREE HUMAN NEUROBLASTOMA CELL LINES. A.M. Babey*, J. Cheng, L. Visconti, K.M. Standifer, J.L. Biedler, and G.W. Pasternak. Cotzias Laboratory of Neuro-Oncology and Laboratory of Cellular & Biochemical Genetics, Memorial Sloan-Kettering Cancer Center and Depts. of Neuroscience and Pharmacology, Cornell University, New York, NY 10021

Advances in the characterization of opioid receptor subtypes have been markedly enhanced by the use of cultured cell lines. Recent progress in the application of molecular biology techniques to the study of opioid receptors has led to the cloning of the murine δ receptor. We would like to report that three human neuroblastoma cell lines, CHP-212, CHP-234 and SMS-KAN, express δ opioid receptor binding. Analysis of total opioid receptor binding, determined using the non-selective ligand ^{3}H-diprenorphine, revealed K_D values of 0.35 nM, 0.42 nM and 0.52 nM respectively and B_{max} values of 54 fmol/mg, 93 fmol/mg and 120 fmol/mg respectively, compared to δ binding in SH-SY5Y of 26.2 fmol/mg. The δ-selective ligand DPDPE competed well against ^{3}H-diprenorphine, with K_i values of 3.5 nM, 1.6 nM and 2 nM respectively. Attempts to identify μ, κ$_1$ and κ$_3$ binding using selective binding assays (^{3}H-DAMGO: μ; ^{3}H-U69,593: κ$_1$; ^{3}H-NalBzoH: κ$_3$) and competition studies against ^{3}H-diprenorphine failed to demonstrate the presence of μ, κ$_1$ or κ$_3$ receptor subtypes.

476.16

EXPRESSION OF κ$_3$ AND μ BINDING SITES IN *BUFO MARINUS* (GIANT TOAD) AND *CARASSIUS AURATUS* (GOLDFISH) BRAIN. A.I. Brooks, K.M. Standifer, G.R. Ciszewska*, J. Cheng, and G.W. Pasternak. The Cotzias Laboratory of Neuro-Oncology, Memorial Sloan-Kettering Cancer Center, New York, NY 10021.

Opiate receptor expression in phylogenetically different species has played an important role in the study of opioid receptor pharmacology. Previously it has been reported that *Bufo marinus* brains express a variety of opioid receptor subtypes. We now report that *Bufo marinus* and *Carassius auratus* brains express predominantly κ and some μ opioid binding. Total opioid binding measured with the non-selective ligand ^{3}H-diprenorphine reveals a B_{max} of 21.7 ± 1.37 fmol/mg protein and a K_D of 0.17 ± 0.03 nM in *Bufo marinus* and a B_{max} of 18.8 ± 0.41 fmol/mg protein and a K_D of 0.47 ± 0.18 nM in *Carassius auratus*. Binding of ^{3}H-DAMGO (μ) and ^{3}H-NalBzoH (κ$_3$) accounted for all of the binding observed with ^{3}H-diprenorphine. No specific binding of ^{3}H-U69,593 (κ$_1$), or ^{3}H-DPDPE (δ) was seen. In both species, ^{3}H-NalBzoH binding accounts for 90% of total binding with ^{3}H-DAMGO binding representing 10%. Competition studies confirm that ^{3}H-DAMGO is labelling a μ site. The selectivity of ^{3}H-NalBzoH is similar to previously reported κ$_3$ site. However, affinity labelled toad binding sites elute differently from a Mono-Q column than κ$_3$ sites in rat, mouse, or human tissue. While the κ binding in toad and goldfish appear to fall within a κ$_3$ classification, further studies are being conducted in order to more accurately define these sites.

OPIOIDS: ANATOMY AND PHYSIOLOGY II

477.1

OPIOID ACTIONS ON RAT CINGULATE CORTEX NEURONS E. Tanaka* and R. A. North. Vollum Institute, Oregon Health Sciences University, Portland, OR 07201.

Intracellular recordings were made from layer V pyramidal neurons of rat anterior cingulate cortex in vitro; biocytin labelling was used to identify cell types. [Met^5]enkephalin (ME; 30 nM - 30 μM) reversibly inhibited both fast EPSPs and bicuculline-sensitive IPSPs; half-maximal concentration was about 800 nM and 60 nM, respectively. These actions of ME were mimicked by DPDPE but not by DAMGO; they were blocked by naltrindole but not by CTOP. ME did not change the amplitudes of depolarizations evoked by glutamate or hyperpolarizations evoked by muscimol. Fifty percent of pyramidal cells were hyperpolarized by ME (through δ-receptors) and 70% hyperpolarized by ME (through μ-receptors). It is concluded that activation of δ opioid receptors hyperpolarizes some pyramidal neurons in the prefrontal cortex, and also inhibits presynaptically the release of excitatory amino acids and GABA at synapses onto pyramidal neurons. Activation of μ opioid receptors hyperpolarizes nonpyramidal neurons but does not have the presynaptic inhibitory effects.

477.2

MODIFICATION OF MEDIAL PREFRONTAL CORTICAL NEURONAL FIRING IN RESPONSE TO MORPHINE ADMINISTRATION J. L. Giacchino* and S. J. Henriksen. Dept. of Neuropharmacology, The Scripps Research Institute, La Jolla, CA 92037.

Studies of the medial prefrontal cortex (mPFC) and its connectivity to the nucleus accumbens suggest the importance of this area in reinforcement and drug-related behaviors in the rat. Compared to data on the effect of psychostimulants, less is understood regarding the mPFC contribution to the opiate reinforcement pathway. To address this issue, electrophysiological characterization of mPFC neurons was performed in twenty-five halothane-anesthetized rats. Morphine (2.5 mg/kg sq and naloxone (5.0 mg/kg sq) were administered after establishment of a 10 min stable baseline firing rate. Response to sq saline injection was also evaluated. In addition to evaluation of change in firing rate, the response to stimulation of the nucleus accumbens was investigated. Neurons encountered in the mPFC typically fired in bursts, with a baseline mean firing rate range of from 1 to 11 pulses/sec. Response to saline and/or drug was calculated as percent of mean baseline firing rate; data was collected over successive five minute epochs following injection. At 15 min post-morphine, there was a significant decrease in mPFC firing rate compared to that of the control period ($p<0.01$); the response was more marked at 20 min ($p<0.001$) with 14 of 24 rats having a significant decrease in firing rate at the end of this period. Naloxone appeared to reverse this change, at the end of 25 min, with 13 of 17 rats showing no significant difference in % mean firing rate from baseline. There was not a significant change in firing rate following saline injection. Only four mPFC neurons were found to be driven by stimulation of the nucleus accumbens, one of these cells was not spontaneously active. A relative inhibitory period (200-400 msec) followed stimulation of the nucleus accumbens; this inhibition was not altered by morphine. Preliminary data employing iontophoretic application of DAMGO suggest that mPFC firing rate is decreased by this selective μ agonist. (Supported by ARC grant #AA06420)

477.3

DOSE DEPENDENT EFFECTS OF SYSTEMIC MIRFENTANIL, SUFENTANIL, AND MORPHINE ON ANTINOCICEPTION AND CO2 RESPONSE FUNCTIN IN THE DOG. I Isackson, MS Wallace, TL Yaksh*. Dept. of Anesthesiology, Univ. of Calif. at San Diego, San Diego, CA 92093.

Several opioids were administered intravenously (IV) to compare analgesic effects with levels of depression of the CO2 response. Dogs were prepared with chronic tracheostomies and trained to stand in restraint. Analgesia was measured with skin twitch(SkT) (latency in seconds between placement of a 1cm^2 probe, heated to 60±0.5C, and SkT). CO2 response was measured with a rebreathing study (rebreathing a CO2 mixture and measuring end-tidal CO2 and minute ventilation). It was found that IV Morphine (M)(0.02-2.0 mg/kg), Sufentanil (S)(0.03-3.3 ug/kg),and Mirfentanil (Mi)(0.01-3.0 ug/kg) would induce dose dependent increases in SkT response latency and a reduction in the slope of the Ve/CO2 response curve. The relative potency for increasing SkT response latency was: S>Mi>M, while depression of respiration ordering was: S>M>Mi. Comparison of the slopes of the regression lines (plotting peak respiratory depression versus analgesia) for each agent revealed the ordering to be M>S>Mi, with Mi significantly different from M. Calculation of the ratio of the slopes of the dose response curves (Slope skin twitch/Slope CO2 response) revealed the ratios to be M 1.1±0.2, S 1.5±0.1, Mi 2.0±0.3. These data suggest that for a given degree of antinociception, there is a smaller decrement in respiratory function for Mi as compared to M and S. The analgesic and respiratory effects of all the drugs were antagonized by IV naloxone, excepting the respiratory effects of Mi. Sponsored by Anaquest and NIH GM454558 (MW).

477.4

THE ROLE OF μ, δ and κ OPIOID RECEPTORS IN VENTRAL PALLIDAL NEURONAL ACTIVITY. L Mitrovic* and T.C. Napier. Dept. of Pharmacology, Loyola University Chicago, Stritch School of Medicine, Maywood, Il 60153.

Enkephalin- and dynorphin-like immunoreactivity and μ, δ and κ opioid receptor subtypes are located in the ventral pallidal region of the basal forebrain (VP). Previous work in our laboratory demonstrated that (1) systemically administered, or (2) iontophoretically applied morphine alters firing rate of single neurons recorded in the VP, and (3) electrical stimulation of the nucleus accumbens, a source of VP enkephalinergic inputs, elicits responses that are modified by VP morphine and naloxone iontophoresis. Since these agents do not discriminate among opioid receptor subtypes, an evaluation of subtype-specific agonists was needed to provide a clearer profile of opioid physiology within the VP. Using chloral hydrate anesthetized rats, the present study characterized VP neuronal responses to microiontophoretic applications of DAMGO (μ; 10mM), DPDPE (δ; 10mM) and U50488H (κ; 200mM) to ascertain whether a differential sensitivity occurs. Approximately 57% of the 137 spontaneously firing neurons tested demonstrated a rate change to DAMGO, 38% of 105 were sensitive to U50488H, and 35% of 49 to DPDPE. Rate suppression was observed in 80-97% of the responding neurons. The extent of opioid-induced decreases was correlated with increases in the agonist ejection current, indicative of a "dose"-response relationship. Iontophoresis of CTOP, a μ antagonist (4mM), attenuated DAMGO-induced rate changes and nor-binaltorphimine, a κ antagonist (4mM), attenuated responses to U50488H. Intravenous injections of naloxone (0.1-1mg/kg) blocked DAMGO, but not U50488H. All three agonists were tested on 37 neurons and 70% of the opioid sensitive cells responded to one agonist only. These studies demonstrate, at the level of the single neuron, the functional consequences of activating specific opioid receptor subtypes, and suggest that many VP neurons may receive either dynorphin- or enkephalin-containing inputs, but not both. Work supported by DA05255 to TCN.

477.5

DIFFERENTIAL SUPPRESSION OF HIGH AFFINITY CHOLINE UPTAKE BETWEEN THE CORTEX AND AMYGDALA WITH μ, δ and κ OPIOID AGONIST INJECTIONS IN THE VENTRAL PALLIDUM. T.C. Napier*, F. Rehman and L.K.Gorman. Dept Pharmacol, Loyola Univ Chicago, Sch Med, Maywood, Il 60153; and Dept Psychol, Johns Hopkins Univ, Baltimore, MD 21218.

Cholinergic neurons that project to the frontal cortex (fCTX) and amygdala (AMG) are located in the ventral pallidal region of the basal forebrain (VP). Enkephalin- and dynorphin-like immunoreactivity and μ, δ and κ opioid receptor subtypes are located in the VP, and opioid terminals synapse onto VP cholinergic neurons. Microiontophoresis of DAMGO (μ), DPDPE (δ) and U50488H (κ) decrease VP neuronal firing, but many cells are sensitive to only one agonist (Mitrovic and Napier, this meeting). This suggests that VP neurons are differentially regulated by the various opioid receptors. Since VP cholinergic neurons projecting to the cortex are distinct from those projecting to the amygdala, the present study investigated whether these two systems exhibit dissimilar sensitivities to the opioid agonists. Changes in hemicholinium (HC-3) binding, a marker for high affinity choline uptake, was measured in fCTX, AMG, hippocampus and striatal tissue homogenates as an indicator for cholinergic neuronal function. Intra-VP microinjection of opioids were performed in rats previously implanted with guide cannulae, and the cholinergic terminal regions were dissected 30 min post treatment. DAMGO injected in doses known to induce locomotor behaviors (i.e., 33pmoles or 1.0nmole per VP) did not alter HC-3 binding; however, larger doses (33nmoles) decreased fCTX and AMG HC-3 binding by 33% and 39%, respectively. In contrast, DPDPE (10nmoles) and U50488H (33nmoles) decreased only AMG HC-3 binding (46% and 42%, respectively). None of the treatments affected hippocampal or striatal HC-3 binding. These results suggest that particular opioid receptor subtypes may be able to specifically regulate fCTX and AMG cholinergic projections. These data will be discussed in terms of behavioral and electrophysiological functions of VP opioids. Work supported by DA05255 to TCN.

477.6

DIFFERENTIAL NATURE OF DYNORPHIN ACTIONS ON CALCIUM- AND SODIUM-DEPENDENT ACTION POTENTIALS IN CA3 PYRAMIDAL NEURONS OF THE GUINEA PIG HIPPOCAMPUS. Z.J. Aguirre, N.A. Moy, M.R. Luna, M. Huerta* and M.F. Pacheco. Centro Universitario de Investigaciones Biomédicas, Universidad de Colima. Colima, Col. México.

The goal of the present study was to investigate the actions of dynorphin A 1-17 (DYN) on calcium- and sodium-dependent action potentials (APs) in hippocampal CA3 pyramidal cells. Transverse slices from hippocampus of adult guinea pigs were continuously perfused with artificial cerebrospinal fluid (ACF), and intracellular recordings with glass microelectrodes (30-18 MΩ, CsCl 2 M) were performed by means of a switched current-clamp amplifier. Bath-applied DYN (10-100 nM) gradually reduced the amplitude of sodium-dependent APs, elicited by depolarizing pulses (500 msec), and increased the stimulus strength necessary for initiation of the AP; attaining maximum effects 15 to 30 min after the beginning of the peptide application. These actions followed a concentration-response pattern; fully recovered after 30 to 80 min wash; were mimicked by dynorphin A 2-13; and were not prevented by naloxone 1 µM. Whereas the actions of DYN (10-100 nM) on calcium-dependent APs (recorded in ACF containing 1 µM TTX) affected the same parameters than for sodium-activated APs; such actions were naloxone-sensitive; and were not replicated by dynorphin A 2-13. These results are indicative that DYN exerts a potent modulation of the membrane excitability on CA3 pyramidal cells of the hippocampus, acting by mechanisms similar to the local anesthetic drugs on TTX-sensitive sodium-dependent APs, and inhibiting cadmium-sensitive calcium-dependent APs. Furthermore, DYN actions on calcium-dependent APs are mediated throughout opioid receptors; whereas the effects of this peptide on sodium-dependent APs are of nonopioid nature.
Supported by CONACYT DO780-M9201, México (M.F.P.)

477.7

DIRECT EXCITATORY OPIATE ACTION MEDIATED BY POSTSYNAPTIC DEPOLARIZATION ON RAT MEDIAL VESTIBULAR NEURONS. Y. Lin* and D.Q. Carpenter. Wadsworth Labs, NYS Dept. Health and School of Public Health, Albany, NY 12201.

Opiates increase firing of rat medial vestibular nucleus (MVN) neurons (Ann. NY Acad. Sci. 656: 668, 1992). We have attempted to determine the mechanism of these excitatory opiate actions by extracellular recording of neuronal activity with ionophoretic application of opiate agonists and bath application of antagonists. The spontaneous pacemaker activity of approximately 30% of MVN neurons, scattered throughout the nucleus, was increased by ionophoretic application of either morphine or enkephalin, implicating the presence of both mu and delta receptors. The excitatory responses to both were blocked by the opiate receptor antagonist naloxone. Most previous reports of direct opiate excitation have proven to be due to disinhibition. This is not the case here, since the excitatory opiate response was sustained when GABA receptors were blocked by bicuculline. Even in neurons whose spontaneous firing was almost totally depressed by adenosine, the opiate-induced depolarization was still evident. Perfusion of a 0.1 mM calcium/6.3 mM magnesium solution blocks synaptic transmission, but did not block the excitatory responses to both opiates. This result indicates that the excitation is neither due to disinhibition or a presynaptic opiate action. We conclude that medial vestibular neurons have postsynaptic opiate receptors which mediate a direct depolarization and excitation. Supported by the Aaron Diamond Foundation.

477.8

DYNORPHIN ACTIONS ON NMDA-ACTIVATED CURRENTS IN TRIGEMINAL NEURONS -- A SINGLE CHANNEL ANALYSIS. L. Chen[1]* and L.-Y.M. Huang[1,2] Marine Biomedical Institute[1] and Department of Physiology and Biophysics[2] The University of Texas Medical Branch, Galveston, Texas, 77555-0843.

Tissue injury or repetitive stimulation of small diameter primary afferent fibers triggers expansion of receptive fields and/or prolonged depolarization of nociceptive neurons in the spinal and medullary dorsal horns. This hypersensitivity of dorsal horn neurons requires the activation of N-methyl-D-aspartate (NMDA) receptors. The expression of dynorphin peptides increases many fold following peripheral injury. Application of dynorphin directly onto the surface of the spinal cord can cause enlargement of receptive fields. These observations have led to the suggestion that dynorphin plays a key role in neuronal plasticity in the nociceptive system. We have studied the effects of dynorphin on NMDA responses in trigeminal neurons (Neurosci. Abst. 18:1502, 1992). Unlike µ-opioid receptor agonist DAGO which causes a sustained increase in NMDA-activated currents (Neuron, 7:319-326, 1991), dynorphin was found to reduce NMDA responses. To further understand the mechanism of actions of dynorphin, we now examine the effect of dynorphin on single NMDA channels. The experiments were performed on trigeminal neurons acutely isolated from 9-15 day old rats. The single NMDA-receptor channel currents were measured from outside-out patches using the patch clamp recording technique. In the 14 patches we studied, dynorphin caused a 3-4 fold reduction in the frequency of the channel opening, but had no effect on single channel conductance, or the mean open time of the channel. A detailed kinetic analysis of the dynorphin blockade of NMDA-receptor channels will be presented. Supported by NIH grants NS30045, NS11255 and NS23061.

477.9

KAPPA1 OPIOID RECEPTORS ARE EXPRESSED ON PERFORANT PATH TERMINALS IN GUINEA PIG HIPPOCAMPUS. M.L. Simmons*, C.T. Drake, G.W. Terman[Y] and C. Chavkin. Depts. of Pharmacology and Anesthesiology[Y], Univ. of Wash., Seattle, WA 98195.

Kappa1 opioid receptors are thought to decrease granule cell excitation by decreasing glutamate release from perforant path terminals. Two additional points support the conclusion that kappa1 receptors are on the presynaptic glutamatergic terminals. First, paired pulse facilitation (PPF) of granule cell population spikes was examined, since manipulations that decrease glutamate release cause an increase in the PPF ratio. The stimulus intensity which produced a half maximal amplitude of the first spike (S1/2) was used, and paired pulses were delivered 20 msec apart. In the presence of 10 μM bicuculline, the kappa1 agonist U69593 (100 nM) caused a 52% decrease in the amplitude of the first spike. After readjustment of the stimulus intensity to the new S1/2, the PPF ratio was increased 27%, from 1.81 ± 0.11 to 2.30 ± 0.16 ($p<0.05$, n=7). The effect was reversible with washout of U69593 or addition of the kappa antagonist norbinaltorphimine (NorBNI, 100 nM). Second, electrolytic lesions of perforant path cell bodies in the entorhinal cortex resulted in a significant decrease in specific [^{3}H]U69593 binding in the ipsilateral hippocampus compared to the unlesioned side. These results suggest that kappa1 receptors are located on the perforant path terminals, and decrease excitatory transmission by presynaptic inhibition of glutamate release. Supported by NIH grant DA 04123 and a Merck Predoctoral Fellowship.

477.10

DYNORPHIN LOCALIZATION AND EFFECTS IN GUINEA PIG DENTATE GYRUS MOLECULAR LAYER CT Drake*[1], GW Terman[1,2], DD Kunkel[3], TA Milner[4], PA Schwartzkroin[3], and C Chavkin[1]. Depts of [1]Pharmacol., [2]Anesthesiol., [3]Neurosurg., Univ. of Washington, Seattle WA 98195 and [4]Dept of Neurol. & Neurosci., Cornell Univ. Medical College, New York, NY 10021.

In the guinea pig hippocampal slice preparation, endogenous dynorphin release stimulated by antidromic activation of dentate granule cells significantly depressed the extracellularly recorded population spike amplitude within 30 seconds of dynorphin release. These effects were completely blocked by 1 μM naloxone or 100 nM norbinaltorphimine, suggesting that the response was mediated by kappa1 opioid receptors. The rapid onset of effect indicated that dynorphin may have been released from a local source within the dentate molecular layer. Thus the distribution of dynorphin A(1-8) and dynorphin B(1-13) immunoreactivity (dyn-LI) in this region was examined with light and electron microscopy. In sections corresponding to those used for physiological studies, light microscopy revealed light dyn-LI distributed in a few scattered processes. Electron microscopic analysis revealed that dyn-LI was localized over dense-core vesicles in axons and spiny dendrites throughout the molecular layer. Significantly more molecular layer dyn-LI dense-core vesicles were observed in dendrites than in axons (74% vs 26% of 178). These results suggest that the granule cell dendrites may be a source of the endogenous dynorphin which acts to regulate excitatory perforant path inputs to the hippocampus, thus counteracting excitation-induced phenomena such as long-term potentiation. Supported by DA04123, GM07604, NS18895, DA08259.

477.11

ENDOGENOUS DYNORPHIN BLOCKS INDUCTION OF LTP IN THE GUINEA PIG HIPPOCAMPAL DENTATE GYRUS GW Terman*[1,2], ML Simmons[1], and C Chavkin[1]. Departments of Pharmacology[1] and Anesthesiology[2], University of Washington, Seattle, WA 98195.

We have recently reported that in the dentate gyrus both exogenous kappa opioids and endogenous dynorphin released by hilar electrical stimulation of granule cell axons can inhibit excitatory neurotransmission and long-term potentiation (LTP) at the perforant path/granule cell synapse. The present studies investigated whether the LTP inhibitory effects of dynorphin were primarily due to effects on LTP induction, maintenance or expression. Stimulating electrodes were placed in both the hilus and the perforant path of hippocampal slices and a recording electrode was placed in the granule cell layer. Long term potentiation was defined as the % increase from baseline population response seen 30 min after stimulation of the perforant path (PPHFS) with a 20 ms train of 100 HZ 300 μA 0.3 μs pulses given 3 times at 10 sec intervals. LTP seen in control slices (38%, n=6) could be blocked by 6, 1 sec 50 HZ trains of 300μA 0.3 μs pulses given at 10 sec intervals to the hilus immediately before PPHFS (0.2%, n=6). This inhibition was blocked by 100 nM of the kappa$_1$ opioid receptor antagonist, norbinaltorphimine (30%, n=6) and if the hilar stimulation was given immediately after, instead of immediately before, PPHFS (31%, n=6). Thus kappa opioids inhibit LTP primarily by interfering with mechanisms of induction - probably via presynaptic inhibition of glutamate release. DPLPE, a delta selective opiate agonist, also inhibited excitatory neurotransmission but failed to block LTP (n=6). Supported by DA04123 and the Foundation for Anesthesia Education and Research with a grant from Abbott Laboratories.

477.12

EXOGENOUSLY APPLIED AND ENDOGENOUSLY RELEASED OPIOIDS DECREASE SPONTANEOUS INHIBITORY CURRENTS IN GRANULE CELLS OF THE RAT DENTATE GYRUS. S.B. Bausch and C. Chavkin*. Dept. of Pharmacology, University of Washington, Seattle, WA. 98195.

Pharmacological studies have shown that exogenous application of opioids regulate excitability in the rat dentate gyrus. Moreover, Bramham, et.al. (1991) and Xie and Lewis (1991) have shown that endogenous opioids regulate LTP induction in this same region; however, the mechanism of the endogenous opioid effect is not known. To further define opioid actions on excitability, we investigated the effects of exogenous application of the μ and ∂ agonists DAMGO and DPDPE, as well as the effects of endogenously released opioids on spontaneous synaptic activity in dentate granule cells using the whole-cell voltage clamp technique. Bath application of DAMGO or DPDPE reduced bicuculline-sensitive sIPSCs, but did not change the input conductance of dentate granule cells. The reduction in sIPSCs could be reversed by the opioid antagonist naloxone. High frequency stimulation, using a paradigm previously shown to release endogenous opioids from the perforant path also decreased sIPSCs. The mechanism mediating the reduction in sIPSCs following high frequency stimulation is still under investigation. Thus, exogenously applied, and possibly endogenously released opioids acted to modulate the activity of GABAergic neurons as shown by a decrease in amplitude and frequency of sIPSCs. Supported by DA04123 and GM07750.

477.13

CHRONIC TREATMENT WITH MORPHINE IS ASSOCIATED WITH NON-SPECIFIC REDUCTIONS IN RESPONSIVENESS OF NUCLEUS TRACTUS SOLITARIUS NEURONS (nTS) IN VITRO. C.J. Malanga, W.W. Fleming, C.R. Craig* and D.A. Taylor. Dept. Pharmacol. and Toxicol., WVU Hlth. Sci. Ctr., P.O. Box 9223, Morgantown, WV 26506-9223.

Chronic treatment with morphine leads to the development of nonspecific subsensitivity in guinea-pig myenteric S neurons. To determine whether a similar nonspecific reduction in responsiveness occurs in a guinea-pig CNS preparation, the effect of chronic treatment with morphine on the sensitivity of nTS neurons to pharmacologically unrelated inhibitory agonists was studied using extracellular recording techniques to record spontaneous neuronal activity in an in vitro brainstem slice preparation. Muscimol (MUS), morphine (MOR) and 2-chloroadenosine (CAD) were applied by superfusion and changes in frequency of firing compared using interspike interval histogram analysis. Concentration-response curves were generated and EC20 or EC50 values determined by interpolation. MOR and CAD produced both excitation and inhibition of nTS neurons; furthermore, neither MOR nor CAD achieved greater than 60% inhibition of neuronal activity. MUS consistently produced inhibitions with near maximal (100%) responses. In preparations obtained from animals chronically treated with morphine, the responsiveness of nTS neurons to MOR was reduced approximately 2.5 fold at the level of the EC20 compared to naive controls. Similarly, the responsiveness to CAD was also reduced by 5.2 fold at the EC20. Neither of these reductions were statistically significant. However, the geometric mean EC50 for MUS was significantly reduced 5.2 fold in neurons from animals chronically treated with morphine. These data suggest that 1) the in vitro guinea-pig nTS preparation can serve as a model CNS system for the study of opioid tolerance, and 2) that the development of opioid tolerance in the nTS is nonspecific among pharmacologically dissimilar agonists and, therefore, appears similar to tolerance in the myenteric plexus. Supported by PHS grant DA 03773.

477.14

CHOLECYSTOKININ (CCK) INHIBITS MORPHINE-INDUCED EXCITATION OF PYRAMIDAL NEURONS IN THE CA1 REGION OF THE RAT HIPPOCAMPAL SLICE. Karen K. Miller* and Carl R. Lupica Dept. of Pharmacology, Univ. Colorado Health Sciences Center, Denver, Colorado 80262

Opioids have an excitatory effect on pyramidal neurons in the hippocampus, due to the inhibition of GABA release from interneurons. This opioid-induced excitation of CA1 pyramidal cells is manifest using extracellular recording as an increase in population spike amplitudes. Opioid effects in other areas of the CNS, such as the spinal cord, have been shown to be inhibited by the neuropeptide cholecystokinin (CCK) (Faris et. al., Science, 219:310-312, 1983). CCK is also found in high levels in the hippocampus, and several studies have reported conflicting effects of CCK in this brain region (MacVicar et. al., Brain Research, 406:130-135, 1987; Jaffe et. al., Brain Research, 415:197-203, 1987), but none have examined the interaction between CCK and opioids. In the present study, we tested the hypothesis that, as in the spinal cord, CCK could antagonize the actions of morphine in the CA1 region of the hippocampus. Dose dependent increases in population spike amplitudes were observed during bath application of morphine (1-500μM). The sulfated form of the CCK octapeptide (CCK-8s) (1μM) significantly blocked the population spike increases seen at all concentrations of morphine, through a non-competitive interaction. The antagonism of the morphine-induced excitation could be reversed upon washout of CCK-8s, or by co-administration of CCK-8s and the CCK_B receptor antagonist PD-135,158 (1.5μM). Neither PD-135,158 nor CCK-8s significantly affected baseline population spikes, in contrast to previous reports that CCK-8s directly affects extracellular hippocampal responses. The results of this study demonstrate that CCK-8s antagonizes the effects of morphine in the hippocampus through actions at a CCK_B receptor and suggest that CCK may function as an endogenous opiate antagonist in the brain as well as in the spinal cord. Supported by NIDA grant DA 07725 (C.R.L.).

477.15

MU AND DELTA OPIOID MODULATION OF EXCITATION AND MINIATURE GABA-MEDIATED IPSCs IN THE CA1 REGION OF THE RAT HIPPOCAMPUS.
Carl R. Lupica*, and Karen K. Miller, Dept. Pharmacology, Univ. Colorado Sch. Med. , Denver, CO, 80262.

Opioid peptides indirectly increase the excitability of hippocampal CA1 pyramidal cells through the inhibition of GABA release from interneurons. However, examination of μ or δ receptor selective enkephalin actions has revealed that while both receptors increased pyramidal neuron excitability (i.e. population spikes and EPSPs), GABA-mediated IPSPs evoked by moderate intensity stimulation of stratum radiatum were decreased only by μ agonists. In addition, both μ and δ opioid receptor activation significantly reduced spontaneous, action potential-dependent IPSPs **(Lupica and Dunwiddie, Synapse, 8:237, 1991; Lupica et al., Brain Research, 593:226, 1992)**. One hypothesis to account for these differences is that μ agonists modulate interneuron GABA release directly by a nerve terminal action, while δ agonists inhibit GABA release indirectly *via* hyperpolarization of the interneuron soma (by activating a K^+ channel). This hypothesis was tested by examining the effects of the enkephalin agonists DAGO (μ) and DPDPE (δ) on population spikes during application of the K^+ channel blocker BaCl, and by examining the actions of these peptides on miniature, TTX-insensitive spontaneous inhibitory postsynaptic currents (mIPSCs), recorded from pyramidal neurons. The dose-dependent increase of population spikes caused by DPDPE (EC_{50} = 6nM) was blocked by pretreatment with BaCl (500μM), while the effect of DAGO (EC_{50} = 43nM) was unaltered. In contrast to the differential effect of BaCl on μ- and δ-mediated population spike increases, both DAGO and DPDPE reduced the mIPSC frequency (55% and 57% of control, respectively) without affecting mIPSC amplitude distributions. These results suggest that both μ and δ agonists can act directly at interneuron nerve terminals to decrease GABA release, and that δ, but not μ, receptors utilize a BaCl-sensitive mechanism to increase excitability in CA1. Supported by NIDA grant DA 07725 (C.R.L.).

477.16

QX-314 BLOCKS THE POTASSIUM COMPONENT OF THE OPIOID-INDUCED OUTWARD CURRENT AND SPARES THE CATIONIC COMPONENT IN LOCUS COERULEUS (LC) NEURONS
M. Alreja* and G.K. Aghajanian. Depts. of Psychiatry and Pharmacology, Yale University School of Medicine, New Haven, CT 06508.

We recently reported that opiates hyperpolarize LC neurons by simultaneously opening K^+ channels and closing a resting Na^+-dependent cationic conductance, resulting in a reversal potential more negative than E_K. The K^+ component is partially blocked by external Ba^{2+}, and the cationic component is abolished by Na^+ substitution in the perfusate. We have now tested the effect of QX-314, which blocks G-protein-gated K^+ channels (Andrade, 1991), on the opiate response in LC neurons.

The effect of bath-applied met-enkephalin (200 μM) was studied in rat LC neurons under voltage clamp using intracellular electrodes filled with 2 M KCl alone or with 50 mM QX-314. In cells impaled with QX-314 electrodes, the enkephalin-induced outward current was reduced and parallel or divergent i-v curves were obtained in the -60 to -120 mV range, suggesting lack of a significant K^+ component. Furthermore, the QX-314-resistant current was virtually abolished following 80% substitution of Na^+ in the perfusate with TRIS, suggesting that the opiate current in QX-314 loaded cells is dependent almost entirely on external Na^+. Consistent with the presence of a cationic component, we have also found that the opiate current in LC neurons reverses not only in the hyperpolarizing but also in the depolarizing direction (~-20 mV).

We conclude that QX-314 blocks K^+ channels but spares the cationic component of the opiate current in LC neurons.

477.17

SPINAL ANTINOCICEPTION INDUCED BY THE SELECTIVE μ-OPIOID AGONIST TYR-D-ARG^2-PHE-SAR (TAPS)
J. H. Millison, S. Vonhof* and A-L. Sirén. Dept. of Neurology, Uniformed Services University of the Health Sciences, Bethesda, MD 20814.

Our previous studies have suggested that Tyr-D-Arg^2-Phe-Sar (TAPS), is a full μ_1-opioid agonist whereas it may act as a partial μ_2-opioid agonist. Intracerebroventricular (ICV) administration of TAPS at picomole doses induced respiratory stimulation, analgesia and catalepsy which were blocked by the μ_1-antagonist, naloxonazine (NAZ) (Paakkari P., et al., J. Pharm. Exp. Ther, in press). In the present study the antinociceptive and cataleptic properties of TAPS were examined after intrathecal (I.T.) administration in male Sprague-Dawley rats (275g-350g, n=31). An I.T. catheter (PE-10) was inserted into the lumbar subarachnoid space and a catheter was implanted into the right jugular vein 48 h prior to the experiment. Antinociception was measured by the radiant heat tail-flick latency method. TAPS (10, 20, 30 and 100 pmol/10 μl, I.T.) produced dose-dependent antinociception. The maximum antinociception after each dose was reached within 30 min. After the 100 pmol dose, the antinociceptive effect was observed for 3 h after administration. The calculated ED_{50} for the I.T. antinociception was 20 pmol (95% confidence limits of: 16-25 pmol). TAPS, I.T. did not produce catalepsy. The antinociception induced by TAPS (30 pmol, I.T.) was inhibited by naloxone (5 mg/kg, i.v.) whereas NAZ (10 mg/kg, i.v., 24 h before TAPS) did not block this effect. The data suggest that, in contrast to its supraspinal (ICV) effects, the antinociceptive effect of TAPS in the spinal cord does not involve activation of μ_1-opioid receptors.

477.18

HIGH-DOSE NARCOTICS CAUSE SEIZURES AND LIMBIC BRAIN DAMAGE IN VENTILATED RATS.
WA Kofke*, RH Garman, R Garman M Rose. Depts of Anes/CCM & Path, Univ. of Pitt, Pittsburgh, PA

High dose alfentanil (A), in rats causes hyper-metabolism seizure, and limbic system (LS) damage. We determined if A, sufentanil (S), and fentanyl (F), are neurotoxic. **Methods**: After animal Use Committee approval fed male SD rats, 300-450 gm, were anesthetized with halothane (halo) in O_2/N_2O 30/70, intubated and ventilated. After arterial/venous cannulation halo was stopped with O_2/N_2O 30/70 continued. Pancuronium was given. Temp was 38°C. At 60 m of no halo, opioid (with O_2/N_2 30/70) infusion was started. There were 2 opioid dosage groups (Hi, Lo) and 4 opioid drug groups (control (C), A, S, F). Half of the hi and all of the lo rats received hexamethonium (hex) iv 20 mg/kg then 40-120 mg/kg/hr. Opioid was infused for 2 h followed by extubation & then overnight in a cage. Each rat 18 h later underwent in vivo cerebral perfusion fixation followed by examination for pathology injury and grading from 0 (normal) to 4. **Results**: MAP was lower in hex-treated rats (145±3 vs 107±2 $p < .0001$). No C's sustained LS damage. LS lesions occurred in 4 A, 5 F, and 6 S treated rats. Hi dose opioid was associated with LS damage ($p = .009$). **Discussion**: All 3 clinically used fentanyl congeners cause LS damage in rats.

OPIOIDS: ANATOMY AND PHYSIOLOGY III

478.1

PREJUNCTIONAL INHIBITION OF VAGAL HEART RATE RESPONSE BY MET-ENKEPHALIN-ARG-PHE (MEAP).
B.A. Barron*, Z. Mateo, J.F. Gaugl, J.L. Caffrey. Dept of Physiology, Texas College of Osteopathic Medicine, Ft Worth, TX 76107.

We have identified proenkephalin-immunoreactive fragments in canine myocardium with an antibody directed against the C-terminal MEAP of proenkephalin. Ventricular MEAP-activity (104 fmol/mg protein) is 30 X greater than met-enkephalin (ME, 3.8 fmol/mg protein) and is 5 X higher than in atria (17.6 fmol/mg protein). Histochemical analysis of the heart with this MEAP antibody reveals immunoreactivity concentrated around the intercalated discs. Dogs were pretreated with atenolol (β1 adrenergic blocker) and the right vagus was stimulated (0.5-4 Hz) producing a 10-50 bpm decrease in heart rate. Infusion of MEAP (30-3000 pmol/kg/min) reduced the vagal response by 60%. The ED_{50} for MEAP (300 pmol/kg/min) corresponds to an EC_{50} of approximately 3 nM. The ED_{50} for ME was 3 X higher (900 pmol/min/kg). Both enkephalin effects were prevented by the opioid antagonist diprenorphine (100 μg/kg). The experiments were repeated with the infusion of methacholine (muscarinic agonist) to examine the possibility that MEAP exerted its effect postjunctionally at the SA node. MEAP did not inhibit the decrease in heart rate caused by methacholine infusion. These data suggest that local enkephalins inhibit vagal stimulation at either cardiac parasympathetic ganglia sites or presynaptically in the SA node by inhibiting ACh release.

478.2

MICROINJECTION OF NANOGRAM AMOUNTS OF MORPHINE INTO THE CAUDAL SUBRETROFACIAL AREA (CSRFA) OF THE VENTROLATERAL MEDULLA CAUSES APNEA.
S.M. Magee, R.A. Gillis and A.M. Taveira-DaSilva*. Departments of Medicine and Pharmacology, Georgetown University, Washington, DC., 20007.

Opioids produce respiratory depression because of an interaction with receptors located in the region of the ventrolateral medulla (J. Clin. Invest. 72:1209, 1983). However, the precise location of this site(s) has not been determined. A novel potential site of action for the respiratory depressant effects of opioids is a recently described area in the cat's medulla oblongata where bilateral microinjection of a non-specific universal excitatory amino acid antagonist causes apnea (J. Pharmacol. Exp. Ther. 259:1388, 1991). Amino acid transmission at this site, named caudal subretrofacial area (CSRFA), is required for breathing to occur. The purpose of this research was to determine whether bilateral microinjection of nanogram amounts of morphine into the CSRFA caused respiratory depression. Morphine (4,50 and 100 ng/40 nl) microinjected bilaterally into the CSRFA (7.6 mm caudal to foramen cecum; 4 mm lateral to midline; 1.5 mm below surface) of 17 chloralose-anesthetized cats while monitoring minute ventilation, tidal volume (Vt), respiratory rate (f), end-tidal CO_2 ($FECO_2$), heart rate and blood pressure, produced apnea and a disordered breathing pattern in 16 animals. Apnea was observed 20 $\pm$ 6 minutes after administration of the 100 ng dose and this effect was preceded by a significant decrease in Vt (-13 $\pm$ 1 ml $P<0.05$) and an increase in $FECO_2$ (+1.3 $\pm$ 0.2%, $P<0.05$) and f (+ 16 $\pm$ 3 breaths/min, $P<0.05$). A significant decrease in Vt was also observed after the 4 ng (-15 $\pm$ ml, $P<0.05$) and the 50 ng (-9 $\pm$ 2 ml, $P<0.095$) doses of morphine. We conclude that the CSRFA is an important site of action for the respiratory depressant effects of opioids.

478.3

INHIBITION OF ß-ENDORPHIN-1-31-INDUCED HYPOTENSION BY GLYCYL-L-GLUTAMINE. C.B. Unal, M.D. Owen-Kumer and W.R. Millington*. Division of Molecular Biology & Biochemistry, and Department of Anesthesiology, University of Missouri-Kansas City, MO 64108.

ß-endorphin-1-31 is post-translationally processed to ß-endorphin-1-27 and glycyl-L-glutamine (Gly-L-Gln; ß-endorphin-30-31) in the pituitary and certain brain regions, including brainstem nuclei involved in cardiovascular homeostasis. Like ß-endorphin-1-31, ß-endorphin-1-27 lowers blood pressure and heart rate when centrally injected, but the cardiovascular effects of Gly-L-Gln have not been investigated. The present study was undertaken to determine whether Gly-L-Gln modulates ß-endorphin-1-31 induced hypotension and bradycardia.

ß-endorphin-1-31 was administered intracerebroventricularly to pentobarbital anesthetized rats followed 15 min thereafter by either saline or Gly-L-Gln; mean arterial pressure (MAP) and heart rate were measured through a carotid artery catheter. ß-endorphin-1-31 (0.5 nmol/10 µl) lowered MAP (42.3 ± 6.2 mm Hg) and heart rate (154.0 ± 13.7 beats/min) within 60 min. Gly-L-Gln (0.3, 0.6, 1.0, and 10.0 nmol) inhibited ß-endorphin-1-31-induced hypotension, but not bradycardia, in a dose dependent manner; 1.0 and 10.0 nmol completely blocked the response. Co-injection of equimolar amounts of L-glycine and L-glutamine produced no effect indicating that Gly-L-Gln's inhibitory activity was not due to hydrolysis. α-N-acetyl-Gly-L-Gln also inhibited ß-endorphin-1-31-induced hypotension but Gly-D-Gln was ineffective, suggesting that the response was stereospecific. Gly-L-Gln injection alone at doses as high as 100 nmol produced no hemodynamic variation from baseline. Receptor binding studies demonstrated that Gly-L-Gln did not displace [^{3}H]naloxone binding to rat brain homogenates at concentrations of up to 10 mM. These data indicate that the effects of Gly-L-Gln are not mediated by either glycine or opioid receptors but suggest that Gly-L-Gln acts through a stereospecific binding site to inhibit the central hypotensive effects of ß-endorphin-1-31. Supported by USAMRDC DAMD17-90-Z-0022

478.4

HYPOTHERMIC EFFECT OF HIGH-DOSE CENTRALLY ADMINISTERED µ-OPIOID AGONISTS: SENSITIVITY TO NALOXONE. D. Mondgock, J.U. Adams*, E.B. Geller and M.W. Adler, Department of Pharmacology, Temple University School of Medicine, Philadelphia, PA 19140.

Centrally administered µ-opioid agonists, at analgesic doses, induce dose-related increases in core body (rectal) temperature (Tb) in rats. However, with 10-fold greater doses of the highly selective µ agonists, [D-Ala^2,NMe-Phe^4,Gly^5-ol]enkephalin (DAMGO) and [NMePhe^3,D-Pro^4]morphiceptin (PL017), hypothermia is the primary result. For example, with 6.25 µg icv DAMGO, Tb decreased by 1.19 (±0.58) °C, 45 min post-injection and returned to baseline within 2 hr. The same dose of PL017 induced a 0.56 (±0.25) °C decrease in Tb at 30 min, then a 0.44 (±0.38) °C increase above baseline Tb at 90 min. Is the hypothermic effect of these high doses mediated by opioid receptors, or are nonspecific or compensatory mechanisms involved? To test for opioid specificity, the competitive opioid antagonist naloxone (NLX; 10 mg/kg, sc) was injected at various intervals after agonist injection. At t=+15 min, NLX antagonized the hypothermia induced by 6.25 µg icv DAMGO. At t= +30 or +45 min, NLX failed to reverse the decrease in Tb. These data suggest that the effects of DAMGO were initially mediated by opioid receptors, but then a post-receptor cascade of events occurred which was not sensitive to NLX. Unlike the effects of DAMGO, at t=+15 or +30 min, NLX increased the magnitude and duration of the hypothermia induced by 6.25 µg icv PL017. This might suggest that NLX was selectively blocking the hyperthermic component of the change in Tb, resulting in a hypothermia which was not opioid-receptor mediated. It is not clear why NLX appeared to interact differently with the doses of DAMGO and PL017 tested. It is possible that these were not equi-effective doses and the interaction with NLX is a dose-dependent effect. Alternatively, the two agonists may differ in terms of 1) time course of opioid receptor occupation, or 2) nonspecific (non-opioid) effects. *NIDA Grants T32 DA07327, DA00376.*

478.5

NEUROTENSIN (NT) RELEASE FROM THE PERIAQUEDUCTAL GRAY (PAG) AND PREOPTIC ANTERIOR HYPOTHALAMUS (POAH) OF THE RAT: EFFECTS OF THE SELECTIVE µ and κ OPIOID RECEPTOR AGONISTS. L. Xin*, E. B. Geller and M. W. Adler, Dept. of Pharmacology, Temple University School of Medicine, Philadelphia, PA 19140

It has been presumed that there is a functional interaction between opioids and some neuropeptides in their effects on body temperature (Tb) or antinociception. We have reported that opioid receptor agonists decreased substance P release from the PAG caused by noxious cold. In the present study, we investigated the effects of selective opioid agonists on the release of neurotensin (NT) from different brain regions. Artificial cerebrospinal fluid was microdialyzed through a probe located in the PAG or POAH of S-D rats for 3 hours. Samples were collected every 20 min. NT-like immunoreactivity in the samples was measured by RIA. After a 60-min baseline collection period, the selective µ opioid receptor agonist PL017 (1.2 µg), κ receptor agonist dynorphin (Dyn, 5 µg), KCl (150 mM) or vehicle was microinjected into the regions where microdialysis probes were located. In the PAG, NT release was increased by 120% and 150% over baseline after PL017 and Dyn injection, respectively. In the POAH, NT release was increased by 210% and 120% above baseline after PL017 and Dyn injection, respectively. KCl produced a NT increase of 260% and 406% in the POAH and PAG, respectively. Microdialysis of NT (3 µg/µl) into the PAG for 1 hour produced 65% maximum possible analgesia in the cold water tail-flick test, which was not antagonized by the pretreatment of naloxone. There was no significant change in Tb. However, NT microdialyzed into POAH induced hypothermia (ΔTb: -1.44 ± 0.12 °C). These data indicate that the activation of opioid receptors within the POAH or PAG can increase NT release in these areas and that NT is involved in the effects of opioids on analgesia and Tb regulation. (Supported by NIDA grant DA 00376)

478.6

ENDOCRINE RESPONSE TO MU AND DELTA SPECIFIC OPIOID AGONISTS IN NEONATAL RATS. C. Kuhn*, P. Little, F. Porreca and R. Francis. Dep't of Pharmacology, Duke Medical Center, Durham, NC 27710.

Opiates are reported to stimulate prolactin (PRL) and corticosterone (CS) secretion in rats through actions on mu opiate receptors, while stimulation of GH is reported to reflect actions on both mu and delta receptors. However, previous studies have employed mu and delta agonists with poor selectivity for mu and delta receptors and no ability to differentiate between the newly described delta receptor subtypes. The purpose of the present study was to test the hypothesis that both mu and delta receptors contribute to control of anterior pituitary hormone secretion. Neonatal (day 10) rats were injected icv with saline, or various doses of the mu-selective agonist DAMGO, the delta-1 selective agonist DPDPE or the delta-2 selective agonist deltorphin, killed 20 minutes later, and serum hormone levels determined by RIA. As expected, DAMGO stimulated secretion of GH, PRL and CS. DPDPE stimulated only CS, while deltorphin significantly increased CS and PRL. Neither delta agonist increased GH. Similar results with GH were obtained in 20-day old pups. These results suggest that the delta receptor subtypes have a significant and specific role in endocrine regulation, although not in control of GH secretion. These findings further suggest that the delta-2 receptor may be the predominant delta receptor involved in endocrine regulation. Supported by DA02739.

478.7

ESTROGEN'S RAPID MODULATION OF μ-OPIOID POTENCY IN GUINEA PIG HYPOTHALAMIC ARCUATE NEURONS. A.H. Lagrange, O.K. Rönnekleiv, J.A. Resko* and M.J. Kelly. Department of Physiology, Oregon Health Sci. Univ., Portland, OR 97201-3098.

As a regulator of the hypothalamic-pituitary-gonadal axis, β-endorphin neurons are a site of 17β-estradiol (E_2) feedback action. We used intracellular electrophysiologic recording in *in vitro* hypothalamic slices taken from ovariectomized guinea pigs to show the direct and unusually rapid actions of E_2 to alter the response to μ-opioids. Cumulative dose-response curves (D/R) were made by measuring the hyperpolarization in response to increasing concentrations of the μ-opioid selective agonist, DAMGO. After washout of DAMGO and superfusion with 100nM E_2 for 20 minutes, a second DAMGO D/R was done. The EC_{50} shifted from 64 ±4nM (n=30) before E_2 to 239 ±9nM after E_2 (n=13,$p<0.001$). E_2 had no effect on the maximal hyperpolarization, RMP, τ, or the resting voltage/current relationships of these cells. There was also no change in the size of the DAMGO-induced conductance increase or the reversal potential of the current (-92mV). This E_2 effect is not homologous desensitization to DAMGO, as it is seen following E_2 in cells that have not been exposed to DAMGO previously. 17α-estradiol did not alter DAMGO potency (n=3), including a cell that subsequently responded to 17β-E_2. In contrast, 10 μM forskolin did decrease the potency of DAMGO, with an EC_{50} of 138nM ±13 (n=3). A subset of the E_2-responsive cells were histochemically double-labelled as β-endorphin neurons (n=3). Estrogen's effects on the potency of β-endorphin autoreceptors could have profound consequences for the opioidergic modulation of forebrain function as well as the reproductive axis. (Supported by PHS grants DA05158 to MJK and MH10327 to AHL)

478.8

CELLULAR MECHANISMS OF OPIATE TOLERANCE IN HYPOTHALAMIC ARCUATE (ARC) NEURONS. M.J. Kelly*, A.H Lagrange, G. Zhang, S.A. MacMillan and O.K. Ronnekleiv. Dept. Physiology, Oregon Health Sciences U., Portland, OR 97201.

The effects of the μ-opioid agonist Tyr-D-Ala-Gly-MePhe-Gly-ol (DAMGO) on ARC neurons was compared in hypothalamic slices prepared from ovariectomized guinea pigs (GPs) implanted with placebo or morphine (morph) pellets (10 x 75 mg) for one week. DAMGO caused a decrease in R_{in} and an outward current which reversed at $\approx E_{K+}$ and exhibited inward rectification ($I_{K+,ir}$). Membrane hyperpolarization in response to increasing concentrations of DAMGO was determined. Chronic morphine caused a rightward shift in the DAMGO dose-response curve: EC_{50} = 185.9 ± 21.9 nM (N=9) in morph GPs versus EC_{50} = 64.0 ± 4.4 nM (N=30) in controls. The maximum hyperpolarization (V_{max}) induced by DAMGO in morph GPs was 72% of the V_{max} of controls (9.2 ± 1.5 mV vs. 12.7 ± 0.9 mV). Since μ-opioid and $GABA_B$ receptors are coupled to $I_{K+,ir}$ in ARC neurons, the response to the $GABA_B$ agonist baclofen was also studied. Baclofen was occluded in ARC neurons from control GPs (V_{max} = 12.6 ±1.3 mV). However, the V_{max} for DAMGO was 50% of the V_{max} for baclofen in cells (N=4) from morph GPs. Therefore, chronic morphine decreases the number of functional μ-opioid receptors in ARC neurons possibly through a down regulation of μ-opioid receptors. (supported by DA05158)

478.9

KAPPA AGONIST BRL 52656 INHIBITS OSMOTICALLY-STIMULATED VASOPRESSIN SECRETION PREFERENTIALLY AT HYPOTHALAMIC SITES. NF Rossi* and DP Brooks, Wayne State U. Detroit, MI 48201 and SmithKline Beecham, King of Prussia, PA 19406.

Kappa opioid agonists provoke water diuresis. Besides inhibition of vasopressin (AVP) renal antidiuretic activity, kappa agonists that cross the blood brain barrier antagonize AVP secretion *in vivo*. We hypothesize the BRL 52656 [S(-)-2-(2-(1-pyrrolidinylmethyl)-1-((4-trifluoromethyl)phenylacetyl) piperidine hydrochloride] inhibits osmotically-induced AVP secretion by acting on hypothalamic (HT) opiate receptors. Explants of the rat hypothalamo-neurohypophysial system (HNS) were placed into compartmentalized static cultures with Ham F12 nutrient mixture, such that the only communication between the HT and the neurohypophysis (NH) was the infundibular stalk. A rise in HT medium osmolality of 15 mosmol/kg water with NaCl increased AVP release by 507 $\pm$ 139 %/HNS/h (n=6, $p < 0.02$). Increasing NH osmolality effected no change in AVP secretion. Osmotically-induced AVP secretion was significantly inhibited by 0.1 μM BRL 52656 at the HT site 87 $\pm$ 30 %/HNS/h (n=7, $p < 0.01$) and was suppressed by the agonist at NH sites 183 $\pm$ 49 %/HNS/h (n=4 $p = 0.10$). However, 0.01 μM BRL 52656 antagonized hormone release only at HT sites: HT 199 $\pm$ 36 %/HNS/h (n=4, $p < 0.05$); NH 488 $\pm$ 49 %/HNS/h (n=4, $p = 0.93$). The average rise in osmolality was 314 $\pm$ 1 to 329 $\pm$ 1 mosmol/kg water. Leaking between compartments was less than 1 %. These data are consistent with BRL 52656 inhibiting osmotically stimulated AVP secretion at both HT and NH opiate receptor sites, but that the HT mechanism is more sensitive to the kappa agonist by at least one order of magnitude.

478.10

SIGMA RECEPTOR ROLE IN MALE FERTILITY. X.-Z. Wu*, J.F. Siegel, M. Rich, B. Mellinger and L. Kushner, Department of Urology, Long Island Jewish Medical Center, New Hyde Park, NY 11041.

The sigma-1 (σ_1) receptor is highly dense in all male reproductive organs. Scatchard analysis of [^{3}H](+)-N-allylnormetazocine (NAMN) binding to membranes revealed a single class of binding sites in adult rat epididymis (K_d=66 nM, B_{max}=930 fmol/mg protein) at a density 3-fold that of rat brain. The rank order of potency of of drugs at this site was haloperidol>1,3-di(2-tolyl)guanidine(DTG)>(+)-NAMN>(+)-3-(3-hydroxyphenyl)-N-(1-propyl)piperidine>progesterone>>(-)-NAMN, with no significant binding of phencyclidine, opioid, adrenergic, or cholinergic receptor ligands to this site. Thus, this site is pharmacologically similar to the neuronal σ_1 receptor. Sigma receptors were present in the prostate (K_d=42nM, B_{max}=560 fmol/mg protein), seminal vesicle (K_d=41nM, B_{max}=510 fmol/mg) and testis (K_d=47nM, B_{max}=640 fmol/mg).

DTG (8 mg/kg/d), haloperidol (2.5 mg/kg/d), (+)- or (-)-NANM(10 mg/kg/d), or vehicle was injected IP to male Sprague-Dawley rats (450 g) for 8 d, after which caudal epididymal sperm were analyzed by computer-assisted semen analysis. DTG or haloperidol treatment caused a 50% ($p<0.001$) decrease in sperm concentration. A 10% decrease in motility with haloperidol and a 28% ($p<0.01$) decrease in motility with DTG treatment occurred. Neither (+)- nor (-)-NANM resulted in differences from vehicle in either fertility parameter.

Epididymides from these rats were analyzed for specific binding of [^{3}H](+)-NANM. Scatchard analysis revealed that DTG and haloperidol treatment resulted in 90-100% downregulation of σ receptors. No change in σ receptor density or affinity was detected in the epididymides from (+)- or (-)-NANM-treated animals. A correlation (r=0.93) between receptor downregulation and reduction of sperm count was demonstrated. This suggests that the σ receptor has a role in male fertility. (Supported by the Weingrow Family Foundation.)

478.11

OPIOIDS ENHANCE AND INHIBIT STIMULATED cAMP FORMATION. **L. Wang and A.R. Gintzler*. Dept. of Biochemistry, SUNY Health Science Center at Brooklyn, N.Y. 11203** **Electrically stimulated release of methionine-enkephalin from the myenteric plexus is regulated by a bimodal, opiate receptor-coupled mechanism. Low concentrations of opioid enhance release whereas higher concentrations inhibit the magnitude of evoked release. Indirect, pharmacological analysis suggested, but did not unequivocally establish, the involvement of the adenylyl cyclase/cAMP second messenger system in this bimodal regulatory mechanism. Accordingly, experiments were undertaken to directly ascertain the effect of opioids on stimulated cAMP accumulation in the myenteric plexus. The results demonstrate that a mu opiate receptor agonist, sufentanil, can regulate, in a bimodal fashion, the activity of adenylyl cyclase. The direction of the opioid modulation of this enzyme depends on the concentration employed. Low doses of opioid (10^{-10} M) enhance whereas higher concentrations (10^{-6} M) inhibit the magnitude of cAMP that is formed in response to electrical stimulation. Thus, opioid agonists can regulate, in a bimodal fashion, the accumulation of cAMP in the myenteric plexus. The parallel between these observations and those obtained for the opioid regulation of stimulated enkephalin release strongly suggest a causal relationship between opioid regulated cAMP levels and opioid regulated release of enkephalin. Thus, adenylyl cyclase is one biochemical substrate for the bimodal opiate receptor-coupled regulatory mechanism governing the stimulated release of enkephalin.**

478.12

ENHANCED PRODYNORPHIN PROCESSING DURING GESTATION. **V.M. Medina*, M.E. Dawson-Basoa and A.R. Gintzler. Dept. of Biochemistry, SUNY Health Science Center at Brooklyn, N.Y. 11203**

Behavioral and pharmacological experiments have demonstrated that during late gestation and parturition there is a significant activation of a maternal spinal cord dynorphin/κ opioid analgesic system. Consistent with these results, spinal levels of dynorphin (1-17 and 1-8) are elevated during the same periods of the gestational process, an effect restricted to the lumbar region of the spinal cord. In the present study, these observations of dynorphin modulation during pregnancy have been extended to include analyses of the spinal cord content of prodynorphin. The tryptic generation of leucine-enkephalin-arg was used as an indicator of dynorphin precursor content. The latter was quantified by RIA using an antibody specific for this hexapeptide. It is now demonstrated that late pregnancy is associated with a significant modulation of dynorphin precursor content; a marked decrease (50%) in the spinal cord content of prodynorphin occurs across all spinal regions. The amount of dynorphin represented by the decline in precursor content is about 3x greater in the lumbar cord than in cervical or thoracic areas. The decline in precursor content in the lumbar cord is about 10x greater than is the combined increase in content of dynorphin (1-17 and 1-8). The difference between the decrease in precursor content and the increase in dynorphin peptides suggests markedly enhanced release of this opioid peptide(s) in the spinal cord during the gestational period.

478.13

CONCOMITANT ACTIVATION OF MULTIPLE SPINAL ANALGESIC MECHANISMS DURING GESTATION. **M.E. Dawson-Basoa* and A.R. Gintzler. Dept. of Biochemistry. SUNY Health Science Center at Brooklyn, NY, USA 11203.**

Pain thresholds increase during pregnancy and parturition in both laboratory animals and humans. Previous studies in this laboratory have demonstrated that this analgesia is mediated, at least in part, by a spinal dynorphin/kappa opioid system. Intrathecal (i.t.) application of the kappa-selective antagonist, nor-binaltorphimine, significantly reduces nociceptive thresholds on gestational day 20, as does the i.t. administration of antibodies specific for dynorphin (1-17). In the current study, the first evidence is provided that multiple spinal analgesic mechanisms are responsible for the opioid-mediated analgesia of pregnancy. The involvement of additional types of opiate receptor in the analgesia of pregnancy was investigated using the delta-specific antagonist naltrindole (NTI). I.t. administration of NTI significantly reduces the threshold for responsiveness to aversive stimuli on day 20 of pregnancy. Maximum reduction in pain thresholds is observed twelve minutes after injection. In non-pregnant, ovariectomized rats i.t. NTI has no effect on analgesic thresholds. The activation of a spinal delta analgesic receptor during gestation is consistent with previous reports from this lab of a modest, but significant increase in spinal cord content of both met-enkephalin, and its precursor during this period.

479.1

DUAL MECHANISMS FOR THE RELEASE OF 3H-NE BY CLENBUTEROL. K. D. Murugaiah, M. R. Glover, J. D. Steketee*, and J. M. O'Donnell. Dept. of Pharmacology, Louisiana State University Medical School, Shreveport, LA 71130-3932.

The effect of clenbuterol (CLEN), a beta-2 adrenergic receptor agonist on 3H-NE release from brain slices was investigated. Tissue slices were incubated with 3H-NE for 30 min and 3H overflow determined during electrical stimulation (3 Hz, 20 mA, 2 ms, 2 min) at 20 (S1) and 65 (S2) min. Superfusion of slices from the cortex (CTX), hypothalamus (HYPO), or hippocampus (HPC) with CLEN (10 – 100 μM) for 15 min increased both basal and electrically induced overflow of 3H-NE in a concentration-dependent manner. 3H-NE efflux was increased to 185% (CTX), 186% (HYPO) and 155% (HPC) of control by 30 μM CLEN. CLEN-induced increases in 3H efflux were still evident in calcium-free medium. By contrast, electrically stimulated release of 3H-NE was not observed in the absence of calcium. Repeated administration of CLEN (1 mg/kg for 10 or 21 days or 10 mg/kg for 14 days) did not alter the effect of CLEN on basal release of 3H-NE despite a marked decrease in the density of beta-2 adrenergic receptors. However, the beta adrenergic receptor antagonists propranolol (0.5 μM) and ICI 118,551 (0.1 – 10 μM) partially antagonized CLEN-induced 3H-NE release. These findings suggest that CLEN may increase 3H-NE release via dual mechanisms: a) an amphetamine-like effect independent of synaptic transmission; and b) a beta adrenergic receptor-mediated facilitatory effect on stimulated release similar to that reported previously for isoproterenol. (Supported by USPHS Grant MH40694.)

479.2

INHIBITORY EFFECTS OF ETHAVERINE ON CATECHOLAMINE SECRETION AND $[Ca^{2+}]_i$ RISE IN DEPOLARIZED PC12 CELLS. Kyong-Tai Kim* and Byung-Chang Suh. Dept. of Life Science, Pohang Institute of Science and Technology, Pohang, Kyong Buk, 790-600 Korea.

Ethaverine, a derivative of papaverine, is used as a vasodilator and antispasmodic drug because of its inhibitory effects in arterial and cardiac muscle membrane Ca^{2+} channels. We have investigated the effects of ethaverine on neuronal cell line-PC12 cells depolarized by high K^+ treatment. Therapeutic concentration(1 μM) of ethaverine treatment with 70 mM K^+ decreases catecholamine secretion to 40%. Inhibitory mode of ethaverine showes taht its inhibition of secretion is dose-dependent and EC_{50} is about 2 μM. To study its inhibitory action mechanism on secretion, we examined intracellular Ca^{2+} level. Ethaverine pretreatment decreased the depolarized long-lasting phase with the concentration-dependent mode but still induced an initial phase of Ca^{2+} profile. General L-type Ca^{2+} channel blockers nifedipine, a dihydropyridine, and verapamil, a phenylalkylamine also inhibited high K^+-induced persistant phase of $[Ca^{2+}]_i$ rise. In contrast ω-conotoxin, N-type voltage-gated Ca^{2+} channel antagonist, pretreatment inhibited the initial phase but had no effects on the persistent phase. Ethaverine treatment at persistant phase decreased to the basal Ca^{2+} level like the action of L-type Ca^{2+} channel blockers, nifedipine and verapamil, and did not affect the initial phase of internal Ca^{2+} transient. We have concluded that treatment of ethaverine to PC12 cells resulted in reduction of catecholamine secretion through the blocking of L-type voltage-sensitive Ca^{2+} channel.

479.3

CYTOCHROME P-450 MEDIATED CATECHOLAMINE RELEASE FROM ADRENAL CHROMAFFIN CELLS T.L.Thompson* and R.L.Moss. Dept. of Physiology, Univ. of Texas Southwestern Med. Ctr., Dallas, TX 75235

Hormones which stimulate catecholamine (CA) release from chromaffin cells liberate arachidonic acid (AA) from the plasma membrane. A number of studies have shown that inhibition of AA liberation prevents stimulated CA release while direct application of AA can stimulate CA release from these cells. However, inhibition of AA metabolism attenuates secretagogue-stimulated CA release from chromaffin cells suggesting that a metabolite of arachidonic acid and not AA itself is responsible for the observed response. The metabolism of AA proceeds by cyclooxygenase and non-cyclooxygenase (lipoxygenase and cyto. P-450 monooxygenase) mediated pathways.

We have examined the role of cyto. P-450 mediated AA metabolism on stimulated CA release from resuspended cultured adrenal chromaffin cells using an in vitro voltammetric technique. CA release was measured in response to 2mM acetylcholine. Chromaffin cells were treated with cyto. P-450 inhibitors, piperonyl butoxide (PB) or YYB (a structural analogue of AA) for at least 15 min prior to stimulation. PB pretreatment produced a dose dependent decrease in stimulated CA release. 1.5μM PB was sufficient to cause a 94% inhibition of ACH stimulated release. Preliminary data suggest that YYB is also a potent inhibitor of release; 1.15μM YYB resulted in a 63% reduction in CA release. The ability of 5,6-epoxyeicostrienoic acid (5,6-EET), a synthetic product of AA metabolism formed by the activity of cyto. P-450, to overcome PB induced inhibition of CA release was examined. 5,6-EEt (.40-1.0μM) triggered a rapid, transient and massive release of a CA-like substance from the chromaffin cells in the presence or absence of PB. These data suggest that cyto. P-450 mediated oxidation of AA may play a vital role in secretagogue stimulated CA release from chromaffin cells. Supported by NIH grant MH47418.

479.4

INTRACEREBRAL MICRODIALYSIS IN THE HUMAN CEREBRAL CORTEX: ON-LINE MEASUREMENT OF NORADRENALINE DURING ANESTHESIA AND WAKEFULNESS.

Z.L. Rossetti*, G. Nurchi*, M. Coraddu*, and G. L. Gessa. Dept. of Neuroscience, Univ. of Cagliari and Neurosurgery Unit, Ospedale "Brotzu", Cagliari, Italy.

The changes in the extracellular concentrations of noradrenaline in the human cerebral cortex were investigated using in vivo microdialysis. Patients undergoing neurosurgery for tumor asportation, subarachnoid hemorrhage or severe head injuries, underwent implantation of a concentric dialysis probe into the parieto-occipital cortex. The probe was perfused for periods ranging from 6 to 24 h with sterile artificial CSF at a flow rate of 2 μl/min. Dialysates were analyzed for noradrenaline (NA) by HPLC-EC in 15 or 20 min samples. Neurochemical measurements were matched in time with the level of anesthesia and with systolic blood pressure. Following probe implantation, a relatively rapid decay in extracelluar NA levels was observed. Perfusate NA concentrations progressively decreased and reached stable levels (0.6 ± 0.12 pg/sample, mean ± S.E.M.) within 2 h following the implantion of the probe. Omission of Ca^{++} from the perfusion medium reduced NA output to undetectable levels, indicating the neuronal origin of the measured endogenous amine. During surgery, the changes in extracellular NA concentrations were temporally correlated with the levels of anesthesia. In the awakening phase, NA perfusate levels raised by about six-fold. These results provide the first direct biochemical evidence *in vivo* that in humans the waking state is associated with activation of the noradrenergic system. Thus microdialysis in neurosurgical patients could be valuable in detecting regional changes in brain activity and may open new fields of clinical research.

479.5

RELEASE OF HIPPOCAMPAL NOREPINEPHRINE IN VITRO FOLLOWING ISOLATION-REARING IN THE RAT

A.J. Fulford and C.A. Marsden (SPON: Brain Research Association) Department of Physiology and Pharmacology, Medical School, Queen's Medical Centre, Nottingham, NG7 2UH, U.K.

The changes in dopaminergic and serotonergic function following isolation from weaning are well documented. We have recently shown that α_2 adrenoceptor function is also affected (Fulford *et al* (1993) Br. J. Pharmacol., 103: 281P. Isolation at weaning induced an enhancement in presynaptic α_2 function and an elevation in hippocampal α_2 receptor number. In the present study we have examined the effect of isolation from weaning on the release of norepinephrine (NE) in hippocampal slices in vitro. At four weeks postweaning differentially reared male, Lister hooded rats were killed and the hippocampi removed and halved to provide paired control and test tissues. Tissue slices were prepared and incubated in Krebs at 37°C in a shaking water bath for 5 min prior to administration of either elevated K+ solution (30mM), idazoxan (10μM) or clonidine (10μM). After a further 20 min incubation, samples were centrifuged and the supernatants retained for the measurement of NE content by HPLC-ED. In addition the Ca^{2+} dependency of the release was investigated. The basal release of hippocampal NE tended to be lower in isolated compared to group-reared rats, although this did not reach significance. In both groups the release was Ca^{2+} dependent and potentiated by elevated K+. Idazoxan significantly increased NE release in isolated but not group-reared rats. Similarly, the reduction in release produced by clonidine was only significant in tissue from the isolation-reared animals. Preincubation of idazoxan blocked the reduction in release of NE induced by clonidine confirming α_2 receptor involvement. These results provide neurochemical evidence of a change in presynaptic α_2 adrenoceptor function following isolation-rearing in the rat. The significance of these changes remains to be determined.

479.6

HVA AND MHPG: CORRELATIONS BETWEEN SALIVARY AND PLASMA LEVELS. Ren-Kui Yang, Rachel Yehuda*Donna D.Holland, Robert A. Levengood and Peter J. Knott. Department of Psychiatry, Mount Sinai School of Medicine, NY 10029 and Department of Veterans Affairs, Bronx, NY 10468.

Plasma levels of 3-methoxy-4-hydroxyphenylglycol (MHPG) and homovanillic acid (HVA) are frequently measured as indicators of brain noradrenergic and dopaminergic activity, respectively. We now find that salivary levels of MHPG and HVA correlate significantly with plasma levels of these catecholamine metabolites.

Method: Salivary samples were obtained from volunteer subjects between 08:00 and 16:00 hours using Salivette (Sarstedt) collection tubes. Blood was collected concurrently into heparinized vacutainers by venipuncture. Saliva and plasma samples were stored at -20°C until assayed for MHPG and HVA by HPLC with electrochemical detection.

Results: Salivary HVA concentration correlated significantly with plasma HVA concentration (0.4231, n=25, P<0.0351). The saliva:plasma ratio for HVA was always less than 1 (0.53± 0.14). Similarly, salivary MHPG concentration correlated significatly with plasma MHPG concentration (r=0.7154, n=23, P<0.0001). However, the saliva:plasma ratio for MHPG was always greater than 1 (1.52± 0.23). Enzyme hydrolysis revealed that MHPG in plasma is both free (about 30%) and also conjugated with sulfate and glucuronide in roughly equal proportions but that it exists entirely free in saliva. Saliva did not hydrolyse glucuronide or sulfate conjugates and furthermore did not inhibit the actions of glucuronidase or sulfatase enzymes.

Conclusions: Significant correlations of salivary HVA and MHPG with plasma concentrations of these catecholamine metabolites suggests that salivary measurement may be of utility in situations where blood samples cannot be readiliy obtained.

479.7

DETERMINATION OF NOMIFENSINE AND DOPAMINE IN DIALYSATE USING GC/MS. T.L.Townsend and W.H.Church*. Dept. of Chemistry, East Carolina Univ., Greenville, NC 27858.

A GC/MS method is presented which allows for the simultaneous determination of striatal levels of dopamine (DA) and nomifensine (NOMI) in rats. Dialysate samples were collected every 30 minutes before and after i.p. injection of NOMI (10 mg/kg) using a microdialysis probe and then derivitized using TFE and TFAA. Samples were then injected onto a capillary GC column and individual components detected by quadrapole GC in SIM mode. DA increased in the first sample following drug injection. NOMI was detected in this sample also. Extracellular DA levels were elevated for the next 2 hours and then slowly decreased towards pre-drug levels. NOMI was detected in the dialysate for up to 4 hours following injection. Optimization studies of derivitive stability, signal-to-noise enhancement, and dialysate collection methods are presented.

479.8

A SIMPLE AND FLEXIBLE VERTICAL PROBE FOR BRAIN MICRODIALYSIS. E. Carboni.* Dept. Of Toxicology, University of Cagliari 09126 Cagliari Italy.

The construction, the implantation and the properties of a new vertical probe for microdialysis are described. A Hospal dialysis fiber closed at one end and covered with epoxy glue over its entire surface, except for 2.5 mm at its end was implanted in the dorsal caudate without any rigid connection with the skull in order to reduce brain damage. The fiber was perfused through a silica fused capillary tubing with Ringer. The dialysate was analysed for dopamine with an HPLC coupled with an electrochemical detector. A fiber as described above, implanted in the dorso-lateral caudate recovered 113 ± 12 fmoles (n=8) of dopamine in 20 ul of perfusate (1 ul/min). Basal dopamine output 24 h after the implant was calcium dependent and stimulated by the dopamine receptor blocker haloperidol. Histological analysis of a frontal section of caudate slice implanted with a probe, revealed minor damage along the fiber track. The technique described has several advantages, including simplicity, high recovery, and favorable histology.

479.9

GRAPHITE FIBER ELECTROCHEMICAL PROBE CONSTRUCTED WITH FUSED SILICA TUBING[1]. C. Ksir* and G.A. Gerhardt[2], Department of Psychology and Neuroscience Program, University of Wyoming, Laramie, WY 82071.

Graphite fiber working electrodes are commonly used for in-vivo electrochemical recording of dopamine and other neurottransmitters and/or metabolites. A fabrication technique is described that employs no electrode puller or other specialized equipment.

A 30 µm graphite fiber is passed through a 7 mm length of fused silica tubing (40 µm i.d., 150 µm o.d.). which is then inserted into a 1 cm length of fused silica tubing (200 µm i.d., 300 µm o.d.). 3 mm of the smaller tubing is left exposed. Epoxy is used to seal the two connections.

The open end of the larger tubing is packed with graphepoxy. A 36-ga copper wire is inserted into the graphepoxy to make a connection to the graphite fiber. The tip of the exposed graphite fiber is cut back so that approximately 100 µm is exposed as the working surface.

After applying 6 coats of Nafion, these electrodes were calibrated for dopamine recording. The electrodes showed selectivities of 1500 or more for dopamine relative to ascorbate, with oxidation/reduction current ratios of 0.40-0.50. Signal-to-noise during calibration indicates sensitivities of approximately 0.02 µM. Fused-silica electrodes have been used to record the clearance from rat striatum of dopamine microinjected from a pipette positioned 300 µm from the recording electrode tip.

[1]Supported by NSF Grant BNS 9110308 [2]Department of Psychiatry, University of Colorado Health Sciences Center, Denver. CO.

SEROTONIN RECEPTORS: MOLECULAR BIOLOGY II

480.1

MOLECULAR CLONING AND CHARACTERIZATION OF A RAT BRAIN cDNA ENCODING A 5-HT5B RECEPTOR. M.M. Voigt[1], E. Parker*[2], C. Mahle[2], D. Grisel[2], H. Nowak[2], F. Yocca[2], P. Seeburg[3] and W. Wisden[3]. [1]Dept. Pharmacol., Univ. Coll., Dublin, Ireland. [2]Bristol-Myers Squibb Co., Wallingford, CT 06492. [3]Center for Mol. Biol., Univ. Heidelberg, Heidelberg, FRG.

A gene encoding a novel serotonin (5-HT) receptor, termed 5-HT5B, was cloned from a rat forebrain cDNA library. The 5-HT5B receptor is moderately homologous to other cloned 5-HT receptors. The radiolabelled agonist [3H]5-carboxamidotryptamine (5-CT) bound to two affinity states of the 5-HT5B receptor in COS1 cell membranes (K_{DH}=0.8 nM, K_{DL}=31 nM). The proportion of receptors in the high agffinity state was reduced by the inclusion of guanine nucleotides in the binding assay, suggesting that the receptor couples to a G-protein in COS1 cell membranes. However, this receptor does not appear to alter either cAMP accumulation or phosphoinositide turnover in a variety of fibroblast cell lines. Several serotonergic ligands displayed affinity for the 5-HT5B receptor, with the following rank order potency: 5-CT > LSD > 5-HT > methysergide=methiothepin=8-OH-DPAT > cyproheptadine > sumatriptan. Ketanserin, pindolol, yohimbine, spiperone, mesulergine and zacopride were inactive. In the rat brain, 5-HT5B gene expression occurs predominately in the medial habenulae and hippocampal CA1 cells, with considerably lower levels detected in the entorhinal cortex and dorsal raphe. The ontogenic profile of this gene shows that low to undetectable levels are expressed prior to birth, with post-natal expression peaking in the adult. Based on its primary structure, pharmacological profile and signal transduction mechanism, the 5-HT5B receptor appears to be a member of a new family of G-protein coupled serotonin receptors.

480.2

A NOVEL ADENYLATE CYCLASE-ACTIVATING SEROTONIN RECEPTOR IMPLICATED IN THE REGULATION OF MAMMALIAN CIRCADIAN RHYTHMS

Timothy W. Lovenberg*[1], Bruce M. Baron[2], Luis de Lecea[1], Joseph D. Miller[3], Rebecca A. Prosser[3], Michael A. Rea[4], Pamela E. Foye[1], Margaret Racke[2], Amy L. Slone[2], Barry W. Siegel[2], Patria E. Danielson[1], J. Gregor Sutcliffe[1] and Mark G. Erlander[1]

[1]Dept. Molecular Biology, The Scripps Research Institute, La Jolla, CA 92037. [2]Marion Merrell Dow Res. Inst., Cincinnati, OH 45215. [3]Center for Sleep and Circadian Neurobiology, Stanford Univ. Sch. of Medicine, Stanford, CA 94305. [4]Circadian Neurobiology Group, Armstrong Laboratory, Brooks AFB, Texas 78235.

A polymerase chain reaction (PCR)-based strategy was used to identify a gene (REC20) encoding a novel G-protein-coupled serotonin receptor. REC20, encoding a putative 435 amino acid protein, exhibits greatest identity within the transmembrane domains to the 5-HT-dro1 receptor (54%). Transient expression of REC20 in CosM6 cells results in high affinity [^{125}I]-LSD binding which is antagonized by a variety of serotonin ligands including the 5-HT_{1A} agonist 8-OH-DPAT and the 5-$HT_{1C/2}$ antagonist ritanserin. In addition, these cells accumulate cAMP in response to 5-HT agonists with the same rank order of potency as their binding affinity vs. [^{125}I]-LSD. Northern blot hybridization detects two mRNA transcripts (3 and 4.2 kb) predominantly in the hypothalamus and thalamus. *In situ* hybridization studies indicate specific expression in CA3 of the hippocampus, several cortical layers, as well as thalamic and hypothalamic nuclei. We have also shown that the circadian phase of spontaneous neuronal activity in the rat suprachiasmatic nucleus advances in response to serotonin ligands with a pharmacological profile consistent with that of REC20. The pharmacology and tissue distribution of REC20 do not correlate with the known properties of the putative 5-HT_4 receptor, therefore we have provisionally identified it as the 5-HT_7 receptor.

480.3

CLONING OF A NOVEL HUMAN SEROTONIN RECEPTOR, 5-HT_7, FUNCTIONALLY COUPLED TO ADENYLATE CYCLASE ACTIVATION. J.A. Bard, J.M. Zgombick, N. Adham, P.J.J. Vaysse, T.A. Branchek*, and R. L. Weinshank. Synaptic Pharmaceutical Corp., Paramus, NJ 07652.

Primary amino acid sequence and signal transduction data are now published for 5-HT_1-like receptors, 5-HT_2 receptors and 5-HT_3 receptors, which couple to inhibition of adenylate cyclase, phosphoinositide hydrolysis and ligand-gated ion channel/Ca^{++} mobility, respectively. We report here the cloning of a human 5-HT receptor, distinct from 5-HT_4 (and from the recently cloned 5-HT_5 receptor), that couples to the stimulation of adenylate cyclase activity, which we propose to name 5-HT_7. This receptor was identified by low homology screening with transmembrane probes from the Drosophila serotonin receptor gene, 5-HT_{dro1}. This intron-containing gene exhibits greatest homology in the transmembrane regions with 5-HT_{dro1} (57%) and 39-53% with other serotonin receptor subfamilies. The pharmacological binding profile obtained with membranes derived from transiently transfected Cos-7 cells showed high affinity, saturable [^{3}H] 5-HT binding (Kd=8.5±0.8 nM; B_{max}=6.6±0.8 pmol/mg protein) with the rank order of binding potency: 5-CT > methiothepin > metergoline > 5-HT > 8-OH DPAT > sumatriptan > ketanserin > zacopride. Functional responses of 5-HT_7 indicated coupling to stimulation of adenylate cyclase: both 5-HT and 5-CT elicited a 20-fold increase in cAMP release over basal levels. The mRNA encoding the 5-HT_7 receptor, analyzed by reverse transcriptase-polymerase chain amplification, is expressed in human brain and a variety of human peripheral tissues. The divergent amino acid sequences of the 5-HT_6 and 5-HT_7 receptors, combined with their pharmacologic divergence from the 5-HT_4 receptor, indicates an unusual complexity in the structures of adenylate cyclase stimulatory 5-HT receptors.

480.4

PHARMACOLOGICAL PROPERTIES AND LOCALIZATION OF A NOVEL CLONED RAT SEROTONIN RECEPTOR (5HT_7) M. Ruat, E. Traiffort, J.M. Arrang,R. Leurs, G. Diaz, A. Enjalbert*,J. Tardivel-Lacombe and J.C. Schwartz. U.109 de l' INSERM, Centre Paul Broca, 75014 PARIS.

We have isolated a cDNA encoding a novel serotonin (5-HT) receptor, termed 5HT_7. This 448 amino acid protein displayed limited homology with previous cloned 5-HT receptors belonging to the superfamily of G-protein coupled receptors except (38%) with a drosophila Dro1 5-HT receptor (Witz et al., 1990). The 5HT_7 receptor is characterized by the presence of an unusual hydrophobic domain located at its long N-terminal end, and by at least two introns in its coding sequence, located at the end of the third hydrophobic domain and in its C-terminal end. Stable expression of the 5HT_7 receptor led to the appeareance of high affinity [^{3}H]5-HT binding sites (Kd=1nM) on membranes of transfected CHO cells. The pharmacological profile of the 5HT_7 receptor was clearly distinct from that of any other serotoninergic receptor as indicated by Ki's of a series of serotoninergic agents. 5-HT (EC_{50}=1nM), lysergic acid diethylamide (LSD,EC_{50}=10nM) and several serotoninergic agonists potently stimulated cAMP accumulation in transfected CHO cells. Northern blot analysis of a variety of rat tissues revealed two mRNAs of 3.9 and 3.1kb. These transcripts were highly expressed in hypothalamus, brainstem and hippocampus whereas the signal was the lowest in stomach and ileum. Detailed *in situ* hybridization with a cRNA probe revealed a highly heterogeneous distribution of transcripts in rat brain, where the highest levels were found in the retrosplenial cortex, hippocampus, tenia tecta, posterior hypothalamus, amygdaloid complex, thalamus, and pontine nuclei. The 5HT_7 receptor seems to be mainly associated with limbic structures, suggesting its potential role in control of functions such as learning, mood or neuroendocrine regulations.

480.5

CHARACTERIZATION AND LOCALIZATION OF mRNA FOR A NOVEL SEROTONIN (5-HT) RECEPTOR POSITIVELY COUPLED TO ADENYLYL CYCLASE IN GUINEA PIG BRAIN L.B. Jakeman*, D.W. Bonhaus, I.S.Ramsey, E.H.F. Wong, H.Chan, C. Bach, R.M. Eglen, and A.P.Tsou Inst. Pharmacology and Bio-Organic Chemistry, Syntex Discovery Research, Palo Alto, CA 94304.

Using homology cloning with a human 5-HT_{1A} receptor probe, a novel cDNA was isolated from a guinea pig hippocampal library. The sequence contains an open reading frame of 1338 base pairs encoding a 466 amino acid protein with 7 hydrophobic sequences representing putative transmembrane domains. Homology mapping to known receptors showed closest resemblance to 5-HT_{DRO1}(46%). Transient expression in COS-7 cells revealed specific, high affinity binding to [^{3}H] 5-HT. The binding was displaced by 5-CT>5-HT>methiothepin>8-OH-DPAT>spiperone >ketanserin. Functional expression in CHO-K1 cells showed positive coupling to an adenylyl cyclase, with a rank order of potency for agonists of 5-CT>5-HT=5-MeOT>DP-5-CT>8-OH-DPAT. Spiperone and methiothepin antagonized the response to 5-HT in a competitive manner (K_i = 56 nM and 36 nM), whereas pindolol was inactive. This pharmacological profile does not correspond to that of previously described 5-HT receptors including 5-HT_4, 5-HT_5 or 5-HT_6. In situ hybridization using a cRNA probe directed against the coding region of the gene revealed mRNA in guinea pig hippocampus (DG>CA3>CA2>>CA1), periventricular thalamus and superficial cortex (layers 2-3). These results demonstrate the isolation of a unique receptor with high affinity for 5-HT, which is found in cortical and limbic brain areas that receive serotonergic projections. Further studies are underway to determine the relationship between this receptor and other operationally defined 5-HT receptors.

480.6

ORGANIZATION OF THE MURINE 5-HT3a RECEPTOR GENE AND INVESTIGATION OF ITS SPLICE VARIANTS. P. Werner, Y. Humbert, F. Boess, J. Reid, K. Jones*, E. Kawashima. Glaxo Institute for Molecular Biology, Geneva, Switzerland

The 5-HT3 receptor has recently been cloned (Maricq et al., Science, 254: 432, 1991) and shown to be a member of the ligand-gated ion channel superfamily. We have isolated from an NG108-15 cDNA library a molecular variant (5-HT3aS) of the original cDNA (5-HT3aL), which is missing 18 base pairs. This variant lacks a casein kinase II phosphorylation site in the second cytoplasmic loop that is predicted for 5-HT3aL. Electrophysiological measurements in HEK-293 cells transfected with 5-HT3aS show a rapidly desensitizing response to 5-HT (EC_{50} = 1.8μM) that is reversibly blocked by ICS205-930 (IC_{50} = 0.3nM). These properties are similar to those previously observed for 5-HT3aL. To investigate further the physiological roles of the two variants we studied their relative abundance in a variety of tissues. In NG108-15 cells approximately 80% of the 5-HT3a mRNAs code for 5-HT3aS. We have also looked for and found 5-HT3aS mRNA in areas of the mouse nervous system that are known to have 5-HT3 receptors, such as cortex, hippocampus and superior cervical ganglion. We are currently quantifying the ratio of the two forms in these tissues, and preliminary results show that the predominant from is 5-HT3aS. The isolation of a mouse genomic λ phage clone, which spans the entire 5-HT3a receptor gene, allowed us to sequence the boundaries between coding exons and the respective introns. This analysis suggests that 5-HT3aS and 5-HT3aL mRNAs are generated by an alternative use of 3′ splice sites.

480.7

CLONING AND EXPRESSION OF THE OPOSSUM KIDNEY (OK) CELL LINE 5-HT_{1B} SEROTONERGIC RECEPTOR. D.R. Cerutis*, L.J. Iversen and D.B. Bylund. Dept. of Pharmacol., Univ. Neb. Coll. Med., Omaha, Nebraska 68198-6260.

Previous studies from our laboratory have demonstrated the presence of the 5-HT_{1b} receptor in the OK cell line, an established renal proximal tubule epithethial cell line. Using primers directed at the first and sixth transmembrane domains of the 5-HT_{1b} receptor for reverse-transcriptase PCR (RT-PCR) on OK cell cDNA, we obtained a 780 bp product having > 94% similarity with the corresponding region of the rat and mouse 5-HT_{1b} receptors. 5' and 3' RACE (Rapid Amplification of cDNA Ends) clones were obtained using internal sequence information from this 780 bp clone and adapter primers (BRL). (Cerutis *et al.*, FASEB J. 7(4)A694, 1993). The 5' and 3' RACE clones were sequenced and were shown to contain sequence from the 780 bp clone as well as approximately 250 bp of 5' noncoding region upstream from the start codon and the stop codon with 3' untranslated ~ 200 bp sequence. In order to acquire a full-length 5-HT_{1b} receptor cDNA clone, we performed PCR on OK cell cDNA using oligonucleotide primers for the receptor's N and C-terminus designed from the 5' and 3' RACE clones. The product obtained was cloned into pRc/CMV (Invitrogen) for sequencing and expression. The OK cell 5-HT_{1b} receptor is 80.1% identical and 12.8% similar to the mouse and human 5-HT_{1b} receptors at the amino acid level. Transient transfection of this cDNA (OKc1) into COS-1 cells gave high affinity binding of [^{3}H]5-HT and saturation studies using [^{125}I]iodocyanopindolol bound a single saturable site with a K_D of 0.20 nM. Inhibitions were performed with the following drugs: 5-HT, cyanopindolol, methiothepin, RU24969, sumatriptan, methylsergide, isoproterenol, and 8-OH-DPAT. These all yielded K_i values consistent with 5-HT_{1b} pharmacology. (Supported by NIMH grant MH47354).

480.8

TRANSCRIPTION OF A HUMAN SEROTONIN 5-$HT_{1D\alpha}$ PSEUDOGENE. S.P. Nawoschik, J.A. Bard, L.A. Borden*, T.A. Branchek and R.L. Weinshank. Synaptic Pharmaceutical Corp., Paramus, NJ 07652.

Molecular biological tools have permitted cloning and expression of a number of serotonergic receptor genes and demonstrated the presence of an array of genes within this G protein-coupled receptor gene family. Recently, two subtypes of 5-HT_{1D}, 5-$HT_{1D\alpha}$, and 5-$HT_{1D\beta}$, have been cloned and shown to display similar pharmacological profiles; the human 5-$HT_{1D\alpha}$ corresponds to gene RDC4 in the dog. To search for related serotonin clones, the canine RDC4 clone was used as a probe to screen a human genomic library. One genomic DNA clone exhibited greatest homology to the 5-$HT_{1D\alpha}$, exhibiting 76% identity (over 1088 nts.) to 5-$HT_{1D\alpha}$ and only 67% identity to 5-$HT_{1D\beta}$. Several structural features of this DNA sequence, including multiple in-frame termination codons and insertion of 45 bp contiguous to an *Alu* repetitive element in the third extracelluar loop (between transmembrane domains VI and VII), suggest that this gene is not translationally active and, is therefore, a pseudogene (ψ5-$HT_{1D\alpha}$). Confirmation of the existence of this pseudogene in the human genome was obtained by human genomic Southern blot analysis and genomic PCR cloning and sequencing. mRNA expression of both the 5-$HT_{1D\alpha}$ gene, as well as the pseudogene in human peripheral tissue, by reverse transcriptase-polymerase chain amplification (RT-PCR), demonstrated that the pseudogene is transcribed and has a similar tissue distribution as the 5-$HT_{1D\alpha}$ receptor. This serotonergic pseudogene most likely arose from a gene duplication or transposition event. Use of molecular tools may lead to the discovery of other G protein-coupled pseudogenes and may provide insight into the genetic variation and evolutionary diversity that has occurred at this multi-gene superfamily.

480.9

A SINGLE POINT MUTATION INCREASES THE AFFINITY OF SEROTONIN 5-$HT_{1D\alpha}$, 5-$HT_{1D\beta}$, 5-HT_{1E} AND 5-HT_{1F} RECEPTORS FOR β-ADRENERGIC ANTAGONISTS. N. Adham*, J.A. Tamm, J.A. Salon, R.L. Weinshank, and T.A. Branchek. Synaptic Pharmaceutical Corp., Paramus, NJ 07652.

The serotonin 5-HT_{1A} and 5-HT_{1B} receptors bind certain β-adrenergic antagonists, such as propranolol and pindolol, with high affinity. This pharmacological convergence between adrenergic and serotonin receptors can be attributed to conservation of a key asparagine (N) residue in the seventh transmembrane domain of receptors that bind β-antagonists with high affinity. Other 5-HT_1 receptors display very low affinity for these ligands and have either a threonine (T; 5-$HT_{1D\alpha}$, 5-$HT_{1D\beta}$ and 5-HT_{1E}) or an alanine (A; 5-HT_{1F}) residue in the homologous position. Replacement of this T in 5-$HT_{1D\beta}$ with N, increases its ability to bind β-adrenergic antagonists (Oksenberg et al.,1992). To evaluate the generality of these observations, we have used site-directed mutagenesis to replace the T or A in 5-$HT_{1D\alpha}$, 5-HT_{1E} and 5-HT_{1F} receptors with the corresponding N. Radioligand binding studies were performed by using [^{3}H]-5-HT and [^{125}I]-iodocyanopindolol on transient transfectants in COS 7. Using [^{3}H]-5-HT, we found that the affinities of all the mutant receptors for propranolol and pindolol, but not 5-HT, were significantly increased by 100-10,000 fold, the 5-$HT_{1D\alpha}$ receptor showing the highest and the 5-HT_{1E} receptor displaying the lowest affinity. All mutant receptors bound [^{125}I]-iodocyanopindolol with high affinity [K_d values in nM: 0.029, 5-$HT_{1D\alpha}$; 0.10, 5-$HT_{1D\beta}$; 0.52, 5-HT_{1E}; 0.42, 5-HT_{1F}]. Wild-type receptors failed to show any specific binding. Therefore, the presence of this key asparagine residue confers binding for β-antagonists even in serotonin receptors which differ substantially in molecular structure.

480.10

PROBING THE SIGNAL TRANSDUCTION DOMAINS OF SEROTONIN 5-HT2 RECEPTORS BY MUTAGENESIS. Y. Sheu*, D. Pritchett. Children's Seashore House; Depts. of Ped. and Pharm., University of Pennsylvania; Phila., PA 19104

Serotonin 5HT2 receptors are members of the G-protein coupled receptor family. Mutant or chimeric receptor studies with adrenergic and muscarinic receptors suggest that the third intracellular loop plays a role in signal transduction. To identify consensus sequences and pinpoint the specific amino acids necessary for signal transduction in 5HT2 receptors, we used polymerase chain reaction to create a restriction site cassette flanking the third intracellular loop, and replaced the third loop with oligonucleotides to create deletion mutants. These mutant receptors were expressed in human embryonic kidney 293 cells, and screened with ketanserin binding assay, phosphoinositol hydrolysis assay, and aequorin expression assay. Aequorin expression assay is a method to measure intracellular calcium increase, based on bioluminescent aequorin expressed in 293 cells. A mutant deleting 49 amino acids from the center of the loop and leaving 7 amino acids on the N-terminal and 9 amino acids on the C-terminal can be expressed and has significant ketanserin binding and intracellular calcium increase upon serotonin treatment. All of the remaining amino acids in the loop can be deleted or non-conservatively substituted without destroying coupling to phosphoinositol hydrolysis. Three amino acids in the C-terminal appear to be required for receptor assembly as assayed by binding of ketanserin. No consensus sequence or specific amino acids have been identified through these intensive deletion studies to be absolutely necessary for signal transduction of 5HT2 receptor. The lack of required amino acids in the third intracellular loop is consistent with the lack of a consensus amino acid sequence among other receptors coupled to phosphoinositol hydrolysis. Both of these observation call into questioning models which require protein-protein contact between the receptor and G-proteins in this area. Experiments demonstrating changes in coupling specificity due to changes in the third loop sequence are not inconsistent with models in which there are no direct contacts between G-proteins and the third intracellular loop of the receptor.

480.11

THE ALA/SER^{242} SPECIES VARIATION RESULTS IN SPECIES SELECTIVITY WITH CERTAIN SUBSTITUTED ERGOLINES AND TRYPTAMINES FOR 5-HT_2 RECEPTORS. M.P. Johnson[1]*, D.B. Wainscott, V.L. Lucaites, J.D. Kursar, L. Mercurio, R.J. Loncharich Jr., M. Baez and D.L. Nelson. Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, IN 46285; [1]Current address: Marion Merrell Dow Research Institute, CNS Research, Cincinnati, OH, 45215.

Previously it had been reported (Nelson et al., *J. Pharmacol. Exper. Ther.*, In press, Johnson et al., *Euro. J. Pharmacol.*, In press) that N(1) alkyl substitution alters the species selectivity of selected substituted ergolines and tryptamines at 5-HT_2 receptors. With the ser^{242} containing human 5-HT_2 receptor N(1) unsubstituted ergolines and tryptamines have a higher affinity, while with the ala^{242} containing rat 5-HT_2 receptor the N(1) alkyl substituted analogs have a higher affinity. Using mutagenesis of the rat 5-HT_2 receptor the importance of the ala/ser^{242} species variation was examined. All mutations were confirmed by sequencing, showed no decrease in affinity for [^{125}I]DOI or [^{3}H]ketanserin, and stimulated PI hydrolysis. The mutant A242S (ala^{242} mutated to ser) showed a increase in affinity for the N(1) unsubstituted analogs and a decrease in affinity for the N(1) alkyl analogs as compared to the wild type rat receptor clone. The affinities of the test compounds for the A242S mutant and the human cloned 5-HT_2 receptor were very similar. The mutant A242T (ala^{242} mutated to thr) resulted in a higher affinity for the N(1) unsubstituted ergolines, similar to what was seen with A242S, and a substantial decrease in affinity for the N(1) alkyl ergolines, significantly lower than seen with any other form of the 5-HT_2 receptor examined. The mutant A242V, resulted in no change in affinity for the N(1) isopropyl ergolines but significantly decreased the affinity of N(1) unsubstituted ergolines versus the wild type receptor. The results suggest that this position is in close contact with the some of 5-HT_2 ligands and that a H-bond occurs between the N(1) position on some indole containing ligands and the ser^{242} of the human 5-HT_2 receptor. Preliminary data with two other mutants of the rat 5-HT_2 receptor (S207A and S239A) will also be presented.

480.12

Site-Directed Mutagenesis of the 5-HT2 Receptor: Identification of Amino Acids Involved in Ligand Binding and Receptor Activation by Agonists and Antagonists. Cheng-Dian Wang and Jean C. Shih*. Dept. of Mol. Pharm. and Tox., Sch. of Pharmacy, Univ. of Southern California, 1985 Zonal Avenue, Los Angeles, CA 90033

The 5-HT2 receptor belongs to the G protein-coupled seven transmembrane domain receptor family. Stimulation of 5-HT2 receptor by serotonin or other agonists activates phosphoinositide(PI) formation. The amino acids involved in the ligand binding domain and PI coupling of 5-HT2 receptor were studied by site-directed mutagenesis. Mutants were assayed in stably transfected NIH3T3 cells by radioligand binding using [^{3}H]ketanserin as the 5-HT2 receptor specific ligand. Binding constants and the displacement profile of agonists and antagonists were compared to wild type receptor. The response to agonists stimulated PI turnover for each mutant and wild type 5-HT2 receptor were also determined. Our results indicated Asp120 is involved in G protein-coupling whereas Asp155 is involved in ligand binding for 5-HT2 receptor. Additional site-directed mutations were performed for aromatic amino acids(tryptophan, tyrosine and phenylalanine) and proline. The role of these residues in ligand binding and PI coupling to 5-HT2 receptor will be discussed.(Supported by Merit Award R01 MH37020, R37 MH39085 and Research Scientist Award K05 MH00796, from the National Institute of Mental Health and Welin Professorship).

480.13

Site-specific mutagenesis demonstrates that Arginine 195 in TMD 4 is a critical determinant for agonist affinities at the 5-HT_{1C} and 5-HT_2 receptors. S.Leonhardt*, K. Herrick-Davis, B.D. Wilcox, B.J.Hoffman@, and M. Teitler Dept. of Pharmacology (S.L.,K.H.D., M.T.) and Dept. of Medicine, Albany Medical College, Albany, NY 12208; @ Laboratory of Cell Biology, NIMH, Bethesda, MD 20892

5-HT_2 and 5-HT_{1C} receptors appear to be closely related, displaying a similar pharmacological profile, the same second messenger system (PI metabolism) and a high degree of sequence homology. However, there are striking differences in the interactions of serotonin with the 5-HT_{1C} and 5-HT_2 receptors. 5HT binds with a 22-fold higher affinity to radiolabeled 5-HT_{1C} receptors than to radiolabeled 5-HT_2 receptors; 5HT_{1C} receptors can be directly radiolabelled with ^{3}H-5HT while 5HT_2 receptors cannot. 5HT is 12-fold more potent in 5HT_{1C}-mediated stimulation of PI metabolism than in 5HT_2-mediated stimulation of PI metabolism. In order to explore the molecular basis for the higher affinity and potency of 5HT at the 5HT_{1C} receptor, we investigated the role of arginine 195 (at the postulated interface of trans-membrane domain IV and the second extracellular loop) in the interaction of 5HT with the 5HT_{1C} receptor. This residue is present in all mammalian 5HT receptor sub-types displaying high affinity for 5HT; in the 5HT_2 receptor a glutamine is substituted at this position. We report that mutating ARG 195 of the 5-HT_{1C} receptor to the corresponding residue of the 5-HT_2 receptor (GLN 195) decreases the affinity of 5HT for the mutated receptor by 8-fold, and for the agonist DOI by two-fold. Antagonist affinities were not affected by the mutation. This specific receptor domain has previously not been implicated in 5HT binding to the 5-HT_2 receptor family. The effect of this mutation on 5HT potency in second messenger studies is being investigated. (Supported by MH40716 (M.T.)

480.14

A RECOMBINANT HERPES SIMPLEX TYPE 1 VIRUS EXPRESSING ANTISENSE 5-HT2 RECEPTORS. William W.-G. Jia*[1], Leone Gatzke[1], Xiaoning Wu[1], Yihong Wang[2], Frank Tufaro[1] and Max Cynader[2]. [1]Department of Microbiology and Immunology, [2]Department of Ophthalmology, University of British Columbia, Vancouver, B.C. Canada

Herpes simplex type-1 virus (HSV-1) possesses a great potential as a vector to transfer and stably express genes in neurons as it can efficiently infect neurons and establish latency, in which the viral DNA persists without causing detectable cytopathetic effects in host cells. In the present study, a recombinant HSV-1 virus vTKSR2(-) was constructed by homologous recombination. The viral genome contains an antisense rat 5-HT2 receptor sequence and a poly A addition sites driven by the CMV major immediate early promoter. Since it was inserted in the coding region of the viral thymidine kinase gene, the virus is replication defective in postmitotic cells. The insertion was confirmed by restriction enzyme digestion, PCR and southern blots. The generation of antisense transcripts in infected Green monkey kidney cells by 5 to 20h PI was detected by RNase protection assay. The TKSR2(-) virus was also used to infect primarily cultured cerebral cortical cells at MOI=1 and the expression of 5-HT2 receptors in these cells was measured by binding of [3H]Ketanserin, a 5-HT1C/2 antagonist. Specific binding to the cells infected with vTKSR2(-) was reduced by 66% at 16 hours postinfection compared to the control cells infected with the parental virus. The results show that foreign gene(s) can be carried by a HSV-1 virus and can be efficiently expressed in the nervous system. The TKSR2-2 recombinant virus may be useful for functional studies of 5-HT2 receptors in development and plasticity of CNS by "knocking out" expression of the receptors in neurons in a relatively permanent manner.

481.1

PHARMACOLOGICAL CHARACTERIZATION OF A SEROTONIN RECEPTOR FROM MOLLUSCAN EMBRYOS. T.J. Diefenbach*, N.K. Koehncke, K.J. Christopher, C. Neumann and J.I. Goldberg. Dept. of Zoology, U. of Alberta, Edmonton, AB, Canada T6G 2E9.

Vertebrate serotonin (5-HT) receptors show broad diversity in tissue distribution, signal-transduction coupling, pharmacology and molecular structure. However, little is known about molluscan 5-HT receptors. *Helisoma trivolvis* embryos display a cilia-driven rotational behavior that is regulated by endogenous 5-HT. In this study, the receptors which mediate the cilio-excitatory action of 5-HT were pharmacologically characterized. Time-lapse video microscopy was used to quantify the effects of 5-HT agonists and antagonists using two assays: whole-embryo rotation rate and cilia beat frequency of isolated ciliated cells. In rotation experiments, 5-carboxamidotryptamine and methysergide had effective agonist activity in dose ranges similar to that of 5-HT (1-100 μM). In contrast, 8-hydroxy-DPAT displayed agonist activity of lower potency and effectiveness. Several compounds displayed antagonist activity in the 1-100 μM dose range, including mianserin, spiperone, ritanserin, 1-(1-naphthyl)piperazine and propranalol. α-methyl 5-HT had mixed agonist-antagonist activity. Metoclopromide and MDL-72222, antagonists of vertebrate 5-HT_3 receptors, displayed neither agonist nor antagonist activity. In experiments on isolated cells, the dose-dependency of the 5-HT stimulation of cilia beat frequency, and the effective antagonism by mianserin, were similar to those observed in the whole-embryo experiments. These results implicate the presence of a novel 5-HT receptor on ciliated cells of *Helisoma* embryos that share some pharmacological characteristics with the 5-HT_1 and 5-HT_2 vertebrate receptor families.

This research was supported by NSERC Canada.

481.2

CHARACTERIZATION OF THE INTERACTION OF (R) AND (S) RS-56812 AT 5-HT_3 RECEPTORS IN VITRO AND IN VIVO E.H.F. Wong*, D.W. Bonhaus, E. Leung, K.H. Lee and R.M. Eglen. Institute of Pharmacology, Syntex Discovery Research, Palo Alto, CA 94304, USA.

5-HT_3 receptor antagonists have been reported to have a multitude of pharmacological effects in behavioral models for emesis, anxiolysis, cognition and psychosis actions (Costall et al., 1991) . The availability of potent and selective antagonists for this receptor should help to establish mechanism(s) involved in conferring the above actions. The enantiomers of RS-56812 3-(1-azabicyclo[2.2.2]octan-3-yl) aminocarbonylcarbonyl-1-methylindole.hydrochloride) exhibit high affinities for the 5-HT_3 receptor (pKi vs [^{3}H]quipazine for (R) and (S) isomers were 9.3 ± 0.1 and 7.8 ± 0.1, respectively), and weak affinity at 30 other receptor binding assays (pKi < 6.0). In the guinea-pig ileum functional assay for 5-HT_3 receptors, (S) RS 56812 was inactive as an agonist up to 100 mM, while (R) RS-56812 acted as a partial agonist (pEC_{50}: 6.3; intrinsic activity: 0.45). Both the (R) and (S) isomers of RS 56812 induced the von Bezold-Jarisch reflex with EC_{50} of 1 and 100 mg/kg (i.v.), respectively. Evidence of partial agonism was indicated by a maximum response which was only 50 % of that observed with 2-methyl-5-HT. Indeed, the bradycardia elicited by 2-methyl-5-HT was inhibited by (R) and (S) RS-56812 (IC_{50} of 7.4 and 553 mg/kg, i.v., respectively). These in vitro and in vivo studies indicated that (R) and (S) RS 56812 were potent and selective 5-HT_3 receptor partial agonists. These compounds will facilitate investigation into the neurochemical basis of the behavioral effects of 5-HT_3 receptor antagonists.

481.3

DO NOVEL [^{3}H]5-CT BINDING SITES IN RAT HABENULA REPRESENT 5-HT_{5A} OR 5-HT_{5B} RECEPTORS?

L.G. Iben *, H.P.Nowak, C.D. Mahle, and F.D.Yocca , CNS Drug Discovery, Bristol-Myers Squibb Co., Wallingford, CT 06492.

Novel 5-HT_5 receptors have been cloned from mouse (5-HT_{5A} and $_{5B}$; Matthes et. al., *Mol. Pharmacol*, 1993) and rat (5-HT_{5B} ; Wisden et. al., submitted) brain. [^{3}H]5-carboxamidotryptamine ([^{3}H]5-CT) displays high affinity for these receptors. *In situ* hybridization discretely localizes detectable levels of message for these receptors in the medial and lateral habenula for both species. Due to this specific localization, we sought to determine if these receptors could be identified in native tissue. To this end, we used saturation analysis and competition studies, while selectively masking 5-HT $_{1A, 1B, 1C,}$ and $_{1D}$ binding sites, to identify the [^{3}H]5-CT sensitive binding sites present in these tissues. Saturation analysis of [^{3}H]5-CT in rat habenula indicates the presence of two binding sites, both of which appear to be Gpp(NHp) insensitive. Competition studies performed using [^{3}H]5-CT (1nM) reveal the following rank order potency: 5CT > 5-HT > dihydroergotamine > LSD > methiothepin > sumatriptan > 8-OH-DPAT > cyproheptadine. These data indicate the presence of two distinct [^{3}H]5-CT sensitive binding sites in rat habenula. While the high affinity site may represent a 5-HT_5 "like" receptor, the low affinity site may represent a potentially novel [^{3}H]5-CT sensitive receptor.

481.4

DETERMINATION OF THE 5-HYDROXYTRYPTAMINE (5HT) RECEPTOR SUBTYPES MEDIATING THE EFFECTS OF 5HT ON FOS-LIKE IMMUNOREACTIVITY IN THE PARAVENTRICULAR NUCLEUS OF THE HYPOTHALAMUS. S. Bovetto, C. Rouillard, J. Gervais, and D. Richard.* Department of Physiology, Faculty of Medicine, Laval University, Québec G!K-7P4, CANADA.

This study was carried out to determine the 5-hydroxytrytamine (5HT) receptor subtypes mediating the stimulating effects of 5HT on the expression of the primary response gene *c-fos* in the paraventricular nucleus (PVN) of the hypothalamus. In a first series of experiments PVN FOS-like immunoreactivity (FLI) was measured in *dl*-fenfluramine-treated rats that were concomitantly injected with either i) the 5-HT-1,2 receptor antagonist methysergide (5 mg/kg), ii) the 5HT-1B receptor S(-)-propranolol (3mg/kg), or iii) the 5HT-1C,2 receptor antagonist ritanserin (2 mg/kg). In a second series of animals, PVN FLI was determined in rats which were treated with i) the 5HT-1a receptor 8-OH-DPAT (2.5 mg/kg), ii) the 5HT-1b receptor CGS-12066B (10 mg/kg), iii) the 5HT-1C,2 receptor DOI (2.5 mg/kg), or iv) the 5HT-3 receptor 1-phenylbiguanide (5 mg/kg). Sixty minutes following the injections, rats were perfused via an intracardially inserted canulla with a 4% paraformaldehyde solution. FLI was measured in 40 mm-thick slices using the peroxidase-avidin:biotin complex method. Methysergide prevented the marked increase in PVN FLI induced by the serotonin indirect agonist *dl*-fenfluramine whereas both propranolol and ritanserin blunted the effect of *dl*-fenfluramine without completely preventing it. DOI and 8-OH-DPAT led to a noticeable increase in FLI in PVN whereas CGS-12066B and 1-phenylbiguanide had no effect. The present results provide evidence for the involvement of 5HT-1A and 1C,2 receptor in the the stimulating effects of 5HT in the expression of the early response gene *c-fos* in PVN.

481.5

THE SELECTIVE 5-HT_{1A} RECEPTOR AGONIST (R)-8-OH-DPAT EXERTS A PREFERENTIAL STIMULATORY ACTION ON THE MESOCORTICAL DOPAMINE (DA) SYSTEM L. Arborelius*, G.G. Nomikos, U. Hacksell and T.H. Svensson. Dept. of Pharmacology, Karolinska Institutet, Box 60400, S-10401 Stockholm, Sweden.

In the present study we have examined the effects of (R)-8-OH-DPAT on the neuronal activity of midbrain DA cells using extracellular single cell recording in anesthetized rats. (R)-8-OH-DPAT in low doses (2-32 μg/kg i.v.) caused an increase in the firing rate of DA neurons in the ventral tegmental area (VTA), an effect that was more pronounced in the parabrachial pigmentosus nucleus (PBP) than in the paranigral nucleus (PN), two major subdivisions of the VTA. In contrast, (R)-8-OH-DPAT in this dose range did not affect the firing rate of DA neurons in the substantia nigra (SN). We have also used in vivo microdialysis in freely moving animals to study the effects of (R)-8-OH-DPAT on extracellular concentrations of DA, DOPAC and HVA in three dopaminergic terminal areas; the prefrontal cortex (PFC), which is predominantly innervated by DA neurons in the PBP; the nucleus accumbens (NAC), which receives DA projections from both the PBP and PN; the dorsal striatum (STR), which is mainly innervated by DA neurons in the SN. (R)-8-OH-DPAT (25 μg/kg s.c.) significantly increased DA concentrations in the PFC to approximately 140% and DOPAC and HVA to 120% of basal values, but had only minor effect on DA, DOPAC and HVA concentrations in the NAC or in the STR. These data support a preferential, stimulatory action of low doses of (R)-8-OH-DPAT on the mesocortical DA system. Since 5-HT_{1A} receptor agonists may possess antidepressant activity and depression seems associated with dysfunction of the PFC, our finding that (R)-8-OH-DPAT preferentially increases DA release in the PFC is of considerable interest.

481.6

PHARMACOLOGICAL CHARACTERIZATION OF LY293284, A SELECTIVE AND POTENT 5-HT_{1A} AGONIST. MM Foreman*, RW Fuller, DL Nelson, K Rasmussen, DT Wong, DO Calligaro, CJ Paget, ME Flaugh, RN Booher, JE Barrett[1] and L Zhang[2]. Lilly Res Labs, Eli Lilly Corp Ctr, Indianapolis, IN, 46285; [1]CNS Res Dept, Lederle Labs, American Cyanamid Co, Pearl River, NY, 10965; [2]Dept of Psychiatry, Uniformed Serv Univ Hlth Sci, Bethesda, MD, 20814.

LY293284, (-)4R-6-acetyl-4-(di-n-propylamino)1,3,4,5 tetrahydrobenz[c,d]indole, is a rigid tryptamine derivative with an acetyl group serving as a protophilic substitution for the hydroxyl in serotonin. In ligand displacement assays, LY293284 had a Ki of 0.13 nM for the 5-HT_{1A} receptor but no significant affinity for other monoaminergic receptors. In rats, LY293284 reduced hypothalamic 5-HIAA levels at 3 μg/kg sc, increased serum corticosterone levels at 30 μg/kg sc, reduced body temperature at 3 μg/kg sc, induced lower lip retraction and flat posture at 10 μg/kg sc, reduced ejaculatory latency at 10 ng/kg sc and suppressed firing rate of raphe neurons with an ED_{50} of 8.5 ng/kg iv. LY293284 increased punished responding in pigeons at 3 μg/kg im and decreased immobility time in the rat forced swim test at 30 μg/kg sc, which are indices of potential anxiolytic and antidepressant activity, respectively. LY293284 is a novel selective 5-HT_{1A} receptor agonist that is 10-100 X more potent than 8-OH-DPAT in these tests.

481.7

[I-125]*trans*-8-OH-PIPAT: A NEW 5-HT1A RECEPTOR LIGAND. H.F. Kung*, M-P Kung and Z-P. Zhuang. Departments of Radiology and Pharmacology, University of Pennsylvania, Philadelphia, PA 19104

In order to prepare tracers with higher specific activity for *in vitro* and *in vivo* evaluation of 5-HT1A receptor subtype, we have prepared a new iodinated ligand, (R,S)*trans*-8-hydroxy-2-(N-n-propyl-N-3'-iodo-2'-propenyl)aminotetralin (*trans*-8-OH-PIPAT), to supplant the currently used tracer [H-3]8-OH-DPAT. Binding studies with rat hippocampal membrane preparations showed that the iodinated compound exhibited a Ki value of 0.92 nM against (R,S)[H-3]8-OH-DPAT. Radiolabeled racemic [I-125]*trans*-8-OH-PIPAT was prepared from the corresponding tri-n-butyltin precursor via an iododestannylation reaction with sodium [I-125]iodide and hydrogen peroxide as the oxidant (purity >98%, sp. act. 2,000 Ci/mmole). Saturation studies in the hippocampal homogenates revealed that [I-125]*trans*-8-OH-PIPAT bound to a single population of high affinity site with a K_d of 0.38 ±0.03nM. The B_{max} value (310±20 fmol/mg of protein) was comparable to those measured by (R,S)[H-3]8-OH-DPAT under the same assay conditions. Competition binding experiments clearly indicated that the ligand displayed the 5-HT1A receptor binding profile. The rank order of potency was: (R,S)*trans*-8-OH-PIPAT > (R,S)8-OH-DPAT> WB4101> 5-HT > (R,S)*trans*-7-OH-PIPAT > (R,S)7-OH-DPAT > (R,S)propranolol > spiperone >> ketanserin >> dopamine > atropine. Recently, the racemic radoiodinated ligand was successfully separated into two isomers by high pressure liqiud chromatography using a chiral column (retention time 19 and 24 min, respectively). The optically resolved ligand may prove to be more potent than the racemic mixture. This new iodinated ligand is an excellent probe for the investigation and characterization of 5-HT_{1A} receptors due to its high specific activity, high binding affinity and low nonspecific binding. Acknowledgement: Support from PHS (NS-24538 and MH-48125).

481.8

PHARMACOLOGICAL CHARACTERIZATION OF A NOVEL [^{3}H]DPAT BINDING SITE IN THE DORSAL RAPHE NUCLEUS. R.G. Johnson*, C.M. Severin and R. A. Rabin. Dept. of Pharmacology, SUNY BUFFALO, Buffalo, NY 14214.

This study was undertaken to characterize the properties of the [^{3}H]DPAT binding site in the dorsal raphe nucleus (DRN). In DRN membranes, [^{3}H]DPAT bound to a single homogeneous population of binding sites (Hill coef.~1) with a K_D of 16 nM. Unlike other 5HT receptor subtypes, the inclusion of magnesium decreased specific [^{3}H]DPAT binding, while GTP had no effect. Similar magnesium and GTP effects were obtained in the DRN with [^{3}H]5HT, indicating that these effects are not ligand specific. Of the various adrenergic, dopaminergic, and serotonergic ligands tested, only serotonin, spiperone, methiothepin, fluoxetine and citalopram showed appreciable affinity for the [^{3}H]DPAT binding site in the DRN (K_i values 100-500 nM). However, the pharmacological profile of this binding site does not correspond to a current serotonergic receptor subtype or to the serotonergic transporter. Thus the present study describes a high affinity [^{3}H]DPAT binding site in the DRN whose properties are not consistent with any currently recognized serotonin receptor subtype.

481.9

DISPLACEMENT OF 5-HYDROXYTRYPTAMINE BINDING IN THE SPINAL CORD BY SELECTIVE 5-HT RECEPTOR LIGANDS: AN AUTORADIOGRAPHIC ANALYSIS. L.M. Pubols* and S.D. Dawson R.S. Dow Neurological Sciences Institute, Portland, OR 97209.

Previous investigators have reported that high affinity (5-HT1) 5-HT binding to spinal cord is not fully displaceable by any combination of known specific 5-HT receptor ligands. This study was undertaken to re-examine this question using a 5-HT uptake blocker, as well as selective 5-HT1 agonists and antagonists. Quantitative receptor binding autoradiographic methods (Pubols et al., Neurosci. Lett., 142:111, 1992) were used to evaluate displacement of 2 nM [^{3}H]5-HT binding to individual rat spinal cord laminae. Blockers included 8-OH-DPAT, TFMPP, mesulergine(MES), zimelidine (ZIM), and unlabelled 5-HT. Unlabelled 5-HT displaced an average of 82% of total binding, while a combination of DPAT, TFMPP, and MES displaced an average of 70%. Displacement was not increased by the addition of ZIM (500 nM). A low concentration (100 nM) of ZIM alone failed to displace binding. Thus, the amount of unidentified 5-HT1 binding is less for these data than reported previously, but some binding is still not accounted for. Differences were observed in the laminar pattern of displacement caused by the individual ligands, suggesting that 5-HT1A and 1B sites predominate in the dorsal horn, and 1A and 1C sites in the ventral horn. (Support: NIH: NS19523)

481.10

IDENTIFICATION OF mRNA ENCODING 5-HT RECEPTORS IN THE ENTERIC NERVOUS SYSTEM (ENS) E. Fiorica-Howells and M.D. Gershon*, Dept. Anat. & Cell Biol, Columbia Univ. Coll., of P & S. New York, NY 10032

5-HT plays roles in the initiation of peristaltic reflexes and in neurotransmission in the ENS. Responses of the gut to 5-HT are difficult to analyze because subtypes of 5-HT receptor are present that have not yet been fully characterized. Pharmacological data suggest that the small intestine contains the 5-$HT_{1A, 1P, 2, 3, 4}$ and an additional receptor positively coupled to cAMP; all, except the 5-HT_3, are coupled to G proteins and thus are members of the 7-transmembrane domain receptor family. The current experiments were carried out to identify the 7-transmembrane domain 5-HT receptors expressed in the ENS of the guinea pig and rat small intestine. The polymerase chain reaction (PCR) was used. The PCR strategy takes advantage of the fact that highly conserved regions are present within the transmembrane domains of these receptors. Three different sets of degenerate primers were designed based on sequences found in the 3rd and 6th transmembrane domains. One set had maximum homology to the 5-$HT_{1A, 1B}$, and $_{1D}$ receptors, the second with the 5-$HT_{2, 2F}$ and $_{1C}$ receptors, and the third with the 5-HT_{1E} receptor. The PCR reaction was carried out with total RNA or with poly A+ RNA from mucosa-free rat small intestine or from longitudinal muscle + myenteric plexus dissected from guinea pig ileum. As a positive control, RNA from the brain of each species was also studied using the same oligonucleotides. Many products of the expected size (400-650 bp) were obtained. Electrophoretic patterns of the products were tissue- and species-specific. PCR products were subcloned (pT7Blue plasmid) and sequenced. Several 7-transmembrane domain receptors were identified. These include the 5-HT_{2F} receptor, which was found in the rat small intestine, a receptor in guinea pig ileum that is 90% homologous to the human 5-HT_2 receptor, and a receptor in guinea pig brain that is 85% homologous to the human 5-HT_{1C} receptor. Each of these was obtained with 5-HT_2 receptor-based primers. This is the first report of a small intestinal 5-HT_{2F} receptor and of a 5-HT_2-related receptor in the guinea pig ileum. Finally, a substance P receptor was detected in the guinea pig ileum, using 5-HT_{1A} -related primers. It is concluded that PCR will enable enteric 7-transmembrane 5-HT receptors to be identified and cloned. Supported by NIH grants NS07062, NS12969 and NS 22637.

481.11

IMMUNOLOCALIZATION OF 5-HT_{1A} RECEPTORS IN THE NERVOUS SYSTEM OF THE BOWEL AND PANCREAS. A.L. Kirchgessner*, M.-T. Liu, J.R. Raymond and M.D. Gershon, Dept. of Anat.& Cell Biol., Columbia University, New York, NY 10032.

5-HT_{1A} receptors have been demonstrated in the guinea pig bowel by electrophysiological techniques and specific binding of ^{3}H-5-HT_{1A} agonists has been observed, both in the enteric plexuses and in the ganglionated plexus of the rat pancreas. mRNA encoding the 5-HT_{1A} receptor has also been detected in enteric neurons by *in situ* hybridization. Immunocytochemistry was employed to determine which neurons of the gut and pancreas express 5-HT_{1A} receptors. Antibodies generated against a peptide found in the third intracellular loop of the human 5-HT_{1A} receptor were utilized. 5-HT_{1A} receptor immunoreactivity was found on the surfaces of a subset of neurons in both the submucosal and myenteric plexuses in all regions of the rat and guinea pig bowel and in the guinea pig gall bladder. Immunolabeling was blocked by the peptide used to generate the antibody, but not by a control peptide. 5-HT-immunoreactive (ir) axons were found in close proximity to sites of 5-HT_{1A} immunoreactivity. Calbindin (CBP)-ir neurons (Dogiel type II cells) of the guinea pig myenteric plexus expressed 5-HT_{1A} immunoreactivity. Dogiel type I myenteric neurons that did not contain CBP immunoreactivity were also 5-HT_{1A}-ir. 5-HT_{1A}-ir myenteric neurons became doubly labeled following injections of the retrograde tracer, FluoroGold into the submucosal plexus. These injections also caused 5-HT_{1A}-ir neurons in distant submucosal ganglia to become doubly labeled. In the pancreas, 5-HT_{1A} immunoreactivity was observed on nerves, islet cells and exocrine ducts. These observations suggest that 5-HT_{1A} receptors are present on Dogiel type II myenteric neurons, which are known to be innervated by serotonergic neurons, and on submucosal interneurons. Pancreatic 5-HT_{1A} receptors may be located on the targets of serotonergic enteropancreatic axons. The abundance of 5-HT_{1A} receptor immunoreactivity on nerves of the gut and pancreas suggests that drugs designed to interact with these receptors may have visceral actions. Supported by grants NS27645, NS01582, NS30927, and NS12969.

481.12

BIOCHEMICAL HETEROGENEITY OF [^{3}H]8-OH-DPAT BINDING TO CNS 5HT1A RECEPTORS: STUDIES USING THIOL REACTIVES.
E.K. Nénonéné*, F. Radja and T.A. Reader. *Centre de recherche en sciences neurologiques, Université de Montréal (Québec) CANADA.*

The heterogeneity of 5HT1A receptors was investigated biochemically in rat cerebral cortex (Ctx) and hippocampus (Hip), by using *L*-dithiothreitol (L-DTT) to reduce disulfide brigdes, or *N*-ethylmaleimide (NEM) to alkylate free sulfhydryls. L-DTT caused significant decreases of 2n*M* [^{3}H]8-OH-DPAT binding in a dose-dependant manner. The inhibition curves for L-DTT were monophasic, with half maximal effects (IC_{50}) at 0.89 ± 0.12 and 0.27 ± 0.08 m*M*, for Ctx and Hip, respectively. These changes were fully reversible after one wash and could be prevented by previous occupancy of receptor sites by the ligand. The effect of NEM was also a dose-dependant inhibition of [^{3}H]8-OH-DPAT binding but, contrary to L-DTT, the changes were irreversible and the curves biphasic. In Ctx the inhibition of binding by NEM showed a *high-affinity* IC_{50} of 18.8 ± 4.5 μ*M*, and a *low-affinity* IC_{50} of 8.7 ± 2.9 m*M*. For Hip the IC_{50} values were of 18.7 ± 1.95 μ*M* and 14.4 ± 3.4 m*M*, respectively. After a 200 μ*M* treatment with NEM, differences in *spared* [^{3}H]8-OH-DPAT binding sites could be documented by autoradiography for several regions; the decreases in binding were greater in the CA1 region (32%) than in the Dentate gyrus (18.5%) or in the septum or Raphe nuclei (15%). These results show the role of disulfide and sulfhydryl groups in the *primary ligand recognition site* in Ctx and Hip and provide further evidence on 5HT1A receptor heterogeneity. *[Supported by the Savoy Found., the FRSQ and the MRC].*

481.13

RECEPTOR RESERVE FOR 5-HT$_{1A}$ RECEPTOR-MEDIATED INCREASE IN SERUM CORTICOSTERONE (CORT) IN THE RAT.

K. Bohmaker* and E. Meller. Dept. of Psychiatry, NYU Medical Center, New York, NY 10016.

5-HT$_{1A}$ receptor stimulation elicits increases in plasma CORT, ACTH, prolactin and growth hormone. These effects appear to be mediated by postsynaptic receptors in the hypothalamus which display high sensitivity to 8-OH-DPAT, and desensitization produces a large rightward shift in the dose-response curve for elevation of serum CORT (Kelder & Ross, 1992), suggesting the presence of a large receptor reserve (RR). The extent of RR was directly determined by the method of partial irreversible receptor inactivation. In control rats, 8-OH-DPAT maximally elevated serum CORT about 10-fold (ED_{50}=65 μg/kg). After treatment with the receptor inactivating agent N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ) (6 mg/kg), the dose-response curve for 8-OH-DPAT was shifted 4.3-fold to the right (ED_{50}=282 μg/kg) and the maximal response was reduced (to 7.5-fold increase). Analysis of the data yielded a hyperbolic receptor occupancy vs. response curve; half-maximal response occurred at 5.5% receptor occupancy. A RR of 75-80% was apparent. Since serum CORT is also regulated by 5-HT$_2$ and D2 receptors, and these receptors are also inactivated by EEDQ, the specificity of 5-HT$_{1A}$ receptor mediation by 8-OH-DPAT was assessed. The attenuated response to 0.3 mg/kg 8-OH-DPAT in EEDQ-treated rats was almost completely prevented by pretreatment with the 5-HT$_{1A}$ receptor antagonist pindolol (30 mg/kg) but was not affected by pretreatment with a drug cocktail (drug, mg/kg) to prevent inactivation of D1/D2 (cis-flupenthixol, 2), α_1 (prazosin, 5), α_2 (idazoxan, 1.25) and 5-HT$_2$ receptors (ketanserin, 10). Studies assessing the 5-HT$_{1A}$ RR for other neuroendocrine hormones are in progress.

481.14

[3H]-SEROTONIN BINDING TO 5-HT1D RECEPTORS IN THE FERRET CORTEX. N.T. Brammer, C. Ennis and M.C.W. Minchin*.

Biomedical Research Dept. Wyeth Research (U.K.) Ltd. Taplow, Maidenhead, SL6 OPH England.

5-HT1D binding sites have been reported in a number of non-rodent species (Bruinvels et al, 1992). This study was performed in order to investigate if the ferret could be used as an alternative small laboratory animal to guinea-pig in which to study 5-HT1D receptors. Under conditions where the binding of [3H]-serotonin to 5-HT1A, 5-HT1C and 5-HT2 sites was blocked with 8-OH-DPAT (100 nM) and mesulergine (100 nM) a single binding site KD = 7.4±0.5 nM; Bmax = 138±5 fmoles/mg protein was observed in ferret cortical membranes.

In competition studies serotonin displaced [3H]-serotonin in a monophasic manner whereas biphasic curves were obtained with 5-CT, sumatriptan and CGS 12066B, all with a high affinity component in the nM range. Idazoxan, prazosin and R,S-zacopride did not compete with the radioligand for this binding site.

In order to discriminate between 5-HT1D and possible 5-HT1B binding sites competition curves were constructed using known 5-HT1B ligands and compared to the binding profile in species previously shown to possess 5-HT1D sites (pig, Beer et al, 1992) or 5-HT1B sites (rat, Hoyer et al, 1985). The compounds (-)propranolol, (±)pindolol and CP93,129 produced monophasic competition curves for the pig and ferret (IC50 values = 2318, 4355 and 6166 nM for the pig; 2197, 2344 and 4755 nM for the ferret). However, in the rat a biphasic curve was obtained for all three compounds with an IC50 for the high affinity site = 6.47, 6.87 and 12.87 nM respectively, for the three compounds examined.

481.15

DISTRIBUTION OF 5-HT$_{1B}$ AND 5-HT$_{1D}$ SEROTONIN RECEPTOR mRNA IN THE RAT BRAIN. J. F. Neumaier*, D. M. Dorsa, and M. W. Hamblin. GRECC, SVAMC, and the Department of Psychiatry and Behavioral Sciences, University of Washington, Seattle, WA 98108.

While the 5-HT$_{1B}$ and 5-HT$_{1D}$ (5-HT$_{1D\beta}$ and 5-HT$_{1D\alpha}$) serotonin receptors serve as presynaptic terminal autoreceptors for serotonin, postsynaptic functions of these receptors have also been suggested. To determine the location of cells expressing these genes in the rat brain, we have undertaken a detailed mapping of these two closely related receptors.

A "cocktail" of three oligonucleotides complimentary to different portions of either the 5-HT$_{1B}$ or 5-HT$_{1D}$ serotonin receptor mRNAs were end-labeled by the TDT reaction using ^{33}P-dATP. Standard *in situ* hybridization techniques were used on both coronal and sagittal rat brain sections. Hybridization of the probes to the tissue sections was detected using either film or emulsion dipped slides analyzed microscopically.

Hybridization signal for 5-HT$_{1B}$ receptor mRNA expression was detected in a number of brain regions, most notably the hippocampal CA1 field, the striatum, nucleus accumbens, ventral tegmental area, purkinje layer of the cerebellum, dorsal raphe, and central gray. There was less prominent hybridization in a number of other regions, especially in nuclei of the thalamus and hypothalamus.

Hybridization signal for 5-HT$_{1D}$ receptor mRNA expression was more scarce. It was prominent in the olfactory bulbs, major cell body layers of the hippocampal formation, dorsal raphe, central gray, and the granular layer of the cerebellum. It was also expressed in several other discrete brain regions at lower levels.

These overlapping, but distinct, distributions for the 5-HT$_{1B}$ and 5-HT$_{1D}$ receptors have physiological implications for understanding the roles of these receptors and the adaptation of the serotonergic system to pharmacologic challenges.

481.16

IN SITU HYBRIDIZATION EVIDENCE OF THE SYNTHESIS OF 5-HT$_{1B}$ RECEPTORS IN CENTRAL SEROTONINERGIC NEURONS. E. Doucet, M.B. Emerit, M. Pohl, S. ElMestikawy, J. Adrien, B. Berger*, M. Hamon. INSERM U288, CHU Pitié Salpétrière, 91 Bd de l'Hôpital, 75013 PARIS, FRANCE.

The regional distribution of the mRNA encoding the serotonin 5HT$_{1B}$ receptor was studied in the rat brain by in situ hybridization histochemistry and Northern blot analysis. A 200 bp probe, corresponding to a highly selective portion of the third intracellular loop of the rat 5HT$_{1B}$ receptor, was used. In most brain structures a single ~5kb message was found by Northern analyses. However, two additional bands (~2.5 and 4kb) were detected in the striatum. The rank order of 5HT$_{1B}$ mRNA abundance was as follows : striatum > septum = substantia nigra = colliculi = hypothalamus > hippocampus = cerebellum = brainstem > dorsal horn of the spinal cord > cerebral cortex > ventral horn of the spinal cord > olfactory tubercle. This general pattern of distribution was confirmed by in situ hybridization, but with a higher degree of resolution. In particular the 5HT$_{1B}$ mRNA was visualized in the layer IV of cerebral cortex, the Purkinje and molecular cell layers of the cerebellum, the pyramidal neurons in the CA1 area of the hippocampus and the dorsal raphe nucleus, (DRN). In situ hybridization was also performed in nomifensine (10mg/Kg i.p.) pretreated rats whose 5HT neurons were extensively and selectively lesioned by the microinjection of 5,7-dihydroxytryptamine (5,7-DHT, 8μg/1μl) directly in the antero-ventral vicinity of anterior raphe nuclei three weeks before sacrifice. In 5,7-DHT-lesioned rats, 5HT$_{1B}$ mRNA was present in the same areas as in control rats, exept in the DRN where it was no longer detected. These data provided the first demonstration of the synthesis of 5HT$_{1B}$ receptor within DRN 5HT neurons, as expected of their presynaptic autoreceptor function at the level of serotoninergic terminals.

481.17

ONTOGENETIC DEVELOPMENT OF 5-HT$_{1D}$ RECEPTORS IN HUMAN BRAIN: AN AUTORADIOGRAPHIC ANALYSIS. A. Pazos[1*], E. del Olmo[1], C. del Arco[1], A. Díaz[1], G. Mengod[2], J. Pascual[1] and J.M. Palacios[3]. [1]Dept. Physiol. Pharmacol., Univ. Cantabria, Santander; [2]Dept. Neurochem., Cent. Invest. Des., CSIC, Barcelona; [3]Res. Inst. Almirall Lab., Barcelona, Spain.

The pattern of ontogenetic development of brain 5-HT$_{1D}$ receptors in the human brain was studied by autoradiography in tissue from 11 fetal and newborn cases (gestational ages between 25 and 40 weeks) and 4 infants. ^{125}I-GTI was used as a ligand. At gestational ages under the 32nd week, the density of these receptors was low in general terms though the substantia nigra and the basal ganglia already exhibited relevant densities of specific binding. In newborns, 5-HT$_{1D}$ receptors were present at high or very high densities in the globus pallidus (lateral and medial parts), substantia nigra and visual cortex. Lower but still important levels of ^{125}I-GTI specific binding were on the caudate and putamen and, at a lesser degree, over the frontal cortex. This distribution is in good correlation with that previously reported in the adult. A progressive increase in the amount of these receptors was observed during the postnatal period, reaching adult levels at the first decade of life. These results demonstrate the existence of a clear delay in the ontogenetic appearance of 5-HT$_{1D}$ receptors in the human, when compared to that found for 5-HT$_{1A}$ receptors

(Supported by DGICYT, Ministry of Education, PM88-0170)

481.18

MOLECULAR AND PHARMACOLOGICAL CHARACTERIZATION OF HUMAN 5-HT RECEPTOR SUBTYPES. N. Stam*, P. Vanderheyden, Th. de Boer, C. Van Alebeek, J. Klomp and W. Olijve. Scientific Development Group, Organon Int. B.V., P.O. Box 20, 5340 BH Oss, The Netherlands.

Pharmacological and molecular studies have revealed the existence of multiple receptor subtypes for the neurotransmitter serotonin (5-HT) both in the central and peripheral nervous system. These subtypes are divided into four major pharmacological classes (5-HT1-like to 5-HT4-like, based on their pharmacolocical and functional characteristics. Molecular cloning and expression of the distinct receptor subtypes in Swiss 3T3 cells allows a detailed pharmacolgical and functional characterization of the individual receptor subtypes.

The coding sequences for the 5-HT$_{1A}$, the 5-HT$_{1D\beta}$, the 5-HT$_{1C}$, and the 5HT$_2$ receptor subtypes were obtained by screening genomic and cDNA libraries with probes corresponding to transmembrane regions, under low stringency conditions. Cells transfected with the 5-HT$_{1A}$ receptor displayed a single high affinity site for the agonists [^{3}H]-OH-DPAT and [^{3}H]-5-HT (Kd= 0.7 and 2 nM, respectively). Exposure of these cells to 5-HT resulted in a dose dependent inhibition of forskolin-stimulated cAMP levels. The 5-HT$_2$ transfectants expressed a single high affinity binding site for the antagonist [^{3}H]-ketanserin (Kd= 1.7 nM) and showed a pharmacological profile very similar to that of the rat 5-HT$_2$ receptor in frontal cortex. High affinity binding sites were found in cells expressing the human 5-HT$_{1C}$ receptor for [^{3}H]-mesulergine (Kd= 3 nM) and [^{3}H]-5-HT (Kd= 5 nM). Whereas the 5-HT$_{1A}$ and 5-HT$_{1D\beta}$ receptors were negatively coupled to adenylate cyclase, both the 5-HT$_2$ and 5-HT$_{1C}$ receptor subtypes were functionally linked with phospho-lipase C. These data, together with data on the genomic organization of these receptors indicate that the 5-HT$_{1C}$ receptor subtype belongs to the subclass of 5-HT$_2$-like receptors, rather than being a member of the 5-HT$_1$-like receptor subclass.

481.19

DIFFERENTIAL DOSE-DEPENDENT INACTIVATION OF BRAIN SEROTONIN RECOGNITION SITES BY EEDQ: LACK OF INACTIVATION OF THE 5-HT TRANSPORTER. W. Pinto, D. Guran, A.G. Karczmar* and G. Battaglia. Department of Pharmacology and Neuroscience Program, Loyola University of Chicago, Stritch School of Medicine, Maywood, IL 60153.

In vivo, EEDQ (N-ethoxycarbonyl-2-ethoxy-1,2,-dihydroquinoline) can inactivate several brain monoamine receptors by affecting the ligand recognition site. Following irreversible inactivation by EEDQ, receptor recovery can be monitored and the data used to determine receptor turnover parameters (i.e. receptor production and degradation rate constants). The present study investigates the feasibility of using EEDQ as a tool to inactivate various brain 5-HT recognition sites. Adult male Sprague-Dawley rats were given a single s.c. injection of vehicle (1:1 EtOH/H_2O) or EEDQ (1, 3, 6 and 10 mg/kg) and sacrificed 4 hours post-injection. Brain regions were dissected and frozen until assay. Saturation studies of 5-HT uptake sites (^{3}H-paroxetine), 5-HT_{1A} (^{3}H-DPAT), 5-HT_{1B} (^{125}I-CYP), 5-HT_2 (^{3}H-ketanserin) and 5-$HT_{2/1C}$ (^{125}I-DOI) receptors were carried out in cortical homogenates. EEDQ dose-dependently reduced the Bmax of each of the receptors (76-89%) but was without effect on the 5-HT transporter recognition site, even at the highest dose. The K_D of 5-HT_{1A} receptors was increased, but only at the higher doses of EEDQ. There were no significant effects of any dose of EEDQ on the affinity of the 5-HT_{1B}, 5-HT_2 or 5-$HT_{2/1C}$ receptors. The present data indicate a differential dose-dependent inactivation of 5-HT recognition sites by EEDQ with the following rank order of sensitivity: 5-HT_{1A} > 5-HT_2 ≈ 5-$HT_{2/1C}$ > 5-HT_{1B} >>> 5-HT uptake sites. These findings demonstrate: (1) EEDQ can differentially inactivate 5-HT receptor subtypes and therefore can be a useful tool to study 5-HT receptor turnover kinetics and (2) EEDQ does not inactivate the ligand recognition site of the 5-HT transporter. (Supported by Loyola University Potts Foundation)

481.20

RECEPTORIAL PROFILES OF ATYPICAL ANTIPSYCHOTICS AT CLONED, HUMAN DOPAMINE D_2 AND D_3 RECEPTORS AND AT RAT D_2 AND 5-$HT_{1C/2}$ RECEPTORS *IN VITRO/EX VIVO*. V. Audinot*, H. Canton, V. Jacques, L. Verrièle and M.J. Millan. I.D.R.S., 7 rue Ampère, 92800 Puteaux - France.

Whereas the typical neuroleptic, haloperidol, interacts primarily with dopamine D_2 receptors, the atypical antipsychotic, clozapine, also displays marked affinity for 5-$HT_{1C/2}$ receptors. As the novel antipsychotics, risperidone, sertindole, amperozide and ocaperidone possess an atypical profile in preclinical studies, we evaluated their actions at 5-$HT_{1C/2}$ and D_2 receptors in the rat *ex vivo*. The tracers were: D_2([^{3}H]-spiperone, striatum); 5-HT_{1C} ([^{3}H]-mesulergine, choroid plexus) and 5-HT_2 ([^{3}H]-ketanserin, cortex). In view of the putative relevance of D_3 receptors to schizophrenia, we also examined actions at cloned, human D_2 and D_3 receptors: ([^{3}H)-iodosulpiride, transfected CHO cells). Results are given as pKis (*in vitro*) and % displacement (*ex vivo*) c.f. vehicle (= 0%) at the dose specified in mg/kg, s.c.. Drugs were given 60 min pre-sacrifice.

DRUG	DOSE (*ex vivo*)	5-HT_{1C} pKi/%	5-HT_2 pKi/%	D_2 pKi/%	D_2(h) pKi	D_3(h) pKi
HALOPERIDOL	(2.5)	5.2/0	7.1/34	8.7/69	9.2	8.5
CLOZAPINE	(10.0)	8.1/62	7.6/78	6.6/18	7.1	6.6
RISPERIDONE	(2.5)	7.5/10	9.2/79	8.3/7	8.5	8.0
OCAPERIDONE	(0.63)	7.7/0	8.9/70	9.0/23	9.2	8.6
SERTINDOLE	(2.5)	9.0/85	8.8/81	8.3/14	8.6	8.1
AMPEROZIDE	(40.0)	5.7/0	7.1/49	6.1/14	6.2	6.2

As compared to their actions at D_2 receptors, all antipsychotics except haloperidol exerted pronounced actions at 5-HT_2 or 5-$HT_{1C/2}$ receptors. However, no ligand showed preferential activity at D_3 versus D_2 receptors. These data suggest that actions at 5-$HT_{1C/2}$ receptors may contribute to the distinctive profiles of atypical antipsychotics.

SEROTONIN: NEUROTOXINS, BEHAVIOR AND PHYSIOLOGY

482.1

EVIDENCE FOR SEROTONIN NEUROTOXICITY IN RECREATIONAL MDMA ("ECSTASY") USERS: A CONTROLLED STUDY. U.D. McCann,* A. Ridenour, Y. Shaham, and G.A. Ricaurte. Department of Neurology, Johns Hopkins Medical Institutions, Baltimore, Maryland 21224

3,4-Methylenedioxymethamphetamine (MDMA), an increasingly popular recreational drug, is known to damage brain serotonin neurons in animals, including nonhuman primates. Whether MDMA is neurotoxic in humans has not been established. Twenty-nine MDMA users and 24 controls were admitted to a controlled inpatient setting for measurement of biological and behavioral indexes of serotonin function. Outcome measures, obtained after at least two weeks of drug abstinence, included concentrations of monoamine metabolites in cerebrospinal fluid (CSF), prolactin responses to L-tryptophan, nociceptive responses to ischemic pain, and personality characteristics in which serotonin has been implicated (i.e., impulsivity and aggression). MDMA subjects had lower levels of 5-hydroxyindoleacetic acid (5-HIAA, the major metabolite of serotonin) in CSF than controls ($p=0.008$). Although not different from controls in their prolactin response to L-tryptophan, MDMA users had lower tolerance to ischemic pain ($p=0.05$) and lower scores on assaultiveness ($p=0.04$) and indirect hostility ($p=0.02$) personality scales. These results suggest that serotonin neurotoxicity is a potential complication of recreational MDMA use. Further studies of MDMA-exposed individuals could help elucidate the role of serotonin in normal brain function as well as in neuropsychiatric disease states. [Supported by DA05938 and MO1RR02719]

482.2

REORGANIZATION OF ASCENDING 5-HT PROJECTIONS AFTER MDMA INJURY. G.A. Ricaurte*, C.A. Fischer, G. Hadtzidimitriou, J. L. Katz. Department of Neurology, Johns Hopkins Medical Institutions, Baltimore, MD 21224.

3,4-Methylenedioxymethamphetamine (MDMA), a synthetic amphetamine analog with abuse potential, induces a chemical axotomy of central 5-HT neurons in experimental animals and, possibly, humans. Presently there is a lack of consensus regarding the fate of 5-HT neurons following MDMA injury. In rodents, there is evidence that 5-HT projections recover, but the extent and duration of the recovery remain uncertain; in nonhuman primates, the data indicate that some 5-HT projections recover (e.g., those to hypothalamus), while others do not (e.g., those to dorsal neocortex). To further study 5-HT axonal recovery after MDMA injury, we have examined ascending 5-HT projections in rats and squirrel monkeys treated with MDMA 1-2 years previously. Neurochemical and quantitative autoradiographic studies indicate that in most MDMA-treated rats, ascending 5-HT axons recover and reinnervate their targets in a pattern approaching that observed in controls. In some MDMA-treated rodents (approximately 25%), 5-HT axonal recovery does not take place, at least not in brain regions that are: a) initially very severely affected by MDMA, b) distant from the rostral raphe nuclei and/or c) far removed from a main 5-HT axon bundle (e.g., MFB). Interestingly, in these same animals, brain regions that originally sustain less severe MDMA injury, are closer to the rostral raphe nuclei and/or in proximity to the MFB recover substantially, often excessively. Such brain regions include the hypothalamus, amygdala, olfactory tubercle and septum. In the MDMA-treated primate, this pattern of persistent denervation in some brain regions with hyperinnervation of others is the rule rather than the exception. These results, which are reminiscent of previous findings with 5,7-DHT, indicate that in some rodents and in most nonhuman primates MDMA injury leads to a lasting reorganization of ascending 5-HT projections. [Supported by DA05707]

482.3

RELATION OF BRAIN LEVELS OF DEXFENFLURAMINE (DFEN) TO DEPLETION OF SEROTONERGIC (5HT) MARKERS AFTER CHRONIC TREATMENT IN GENETICALLY OBESE MICE. **N.E. Rowland***. Psychology, Univ. of Florida, Gainesville FL 32611-2065.

DFEN, and its metabolite dexnorfenfluramine (DNOR), produce long-lasting depletions of brain 5HT markers in animals. The relationship between brain concentrations of DFEN or DNOR and 5HT during chronic administration is unknown. Mice were used because, like humans, they metabolize DFEN to DNOR slowly. Obese (ob/ob) and lean mice were given a 2 wk regimen of DFEN (0,6 or 24 mg/kg/day via SC-impanted minipump) and were killed either on the last day of infusion, or 3 or 14 days thereafter. Hemibrain and plasma samples were assayed for DFEN and DNOR. The other hemibrain was assayed for [3H]-paroxetine binding, an index of 5HT uptake capacity. Mice treated with 24, but not 6 mg/kg/day had 40% depletions of paroxetine binding at all 3 survival times. Brain concentrations of DFEN and DNOR increased about 6-fold with the 4-fold increase in dose. Concentrations of less than about 10^{-5}M were not associated with loss of 5HT uptake sites. Dose-dependency of DFEN and DNOR action on 5HT cells will be discussed. [Supported by I.R.I.SERVIER. DFEN was assayed by D.B.Campbell (Servier UK)].

482.4

NMDA- AND AMPA- GLUTAMATE RECEPTORS AND 5-HT DEPLETION INDUCED BY REPEATED ADMINISTRATION OF HIGH DOSES OF DEXFENFLURAMINE IN RAT BRAIN. A.M. Gardier*, C. Rocher and C. Jacquot. Dept. Pharmacol. Fac. Pharmacie, Chatenay-Malabry 92290, France.

Dexfenfluramine (d-fen) is an indirect serotoninergic agonist which releases serotonin (5-HT) and blocks the neurotransmitter reuptake. The reason why repeated administration of high doses of d-fen reduces brain 5-HT is still unclear. We recently shown that autoreceptors located at nerve terminals are involved in this phenomenon (Gardier et al., *Brain Res.*, 588: 67-74, 1992) but heteroreceptors may also. Since a glutamate receptor-mediated control of brain 5-HT release has been described (Whitton et al., *J. Neurochem.*, 58: 1573-75, 1992), we tested the effects of two antagonists of either NMDA or AMPA type of excitatory amino acid receptors (dizocilpine=MK-801: 0.5, 1, 2.5 mg/kg i.p. or 2,3-dihydroxy-6-sulfamoyl-benzo(F)qinoxaline=NBQX: 3, 10, 30 mg/kg i.p., respectively) on d-fen-induced depletion of brain 5-HT. 5-HT and DA metabolisms were studied after treatment with d-fen (5 injections of 1.3, 2.5, 5 & 10 mg/kg i.p., at 2 h intervals) in conjunction with 2 injections of MK-801, or NBQX administered 15 min. before and 3 h after the first d-fen injection. 3 days post-treatment, d-fen dose-dependently reduced 5-HT levels (by between 40 and 70% depending on the region examined). 2.5 mg/kg MK-801 alone decreased striatal HVA (-36%) and hypothalamic DOPAC (-21%) levels, while 10 mg/kg NBQX alone did alter DA metabolism but increased hypothalamic 5-HT levels (+43%). MK-801+d-fen did not change the d-fen-induced 5-HT depletions in all brain areas studied. At the highest dose, NBQX enhanced hypothalamic depletion of 5-HT induced by 10 mg/kg d-fen. Thus, central effects of NBQX, a selective antagonist at AMPA receptors, need to be confirmed in view of recent data showing that probenecid, by inhibiting the transport of NBQX out of brain tissue, may prolong its central effects (Taylor and Vartanian, *Eur J.Pharmacol.*, 213:151-153, 1992).

482.5

SHORT TERM MDMA AND METHAMPHETAMINE ADMINISTRATION IN RATS FAILS TO REDUCE NEUROENDOCRINE RESPONSES TO A 5-HT RELEASER. G. Battaglia*, F. Tung, L.D. Van de Kar and D. Guran, Department of Pharmacology, Loyola University of Chicago, Maywood, IL 60153.

Short term administration of MDMA (3,4-methylenedioxymethamphetamine) or methamphetamine (METH) produces neurotoxic effects on brain serotonin (5-HT) axons and terminals. We hypothesized that functional changes in 5-HT mediated plasma hormone responses to a 5-HT releaser, such as p-chloroamphetamine (PCA), may provide a peripheral marker of central 5-HT neurotoxicity. Male Sprague-Dawley rats (175-200 g) were administered saline (1ml/kg), MDMA (20 mg/kg equiv. as free base) or METH (20 mg/kg) s.c. b.i.d for four days. Two weeks post-treatment, rats from each group were administered saline or PCA (8 mg/kg, i.p.) and sacrificed 1 hour later. MDMA markedly reduced 5-HT uptake sites in cortex (-61%), hippocampus (-52%), striatum (-56%), midbrain (-59%) & hypothalamus (-64%), confirming the loss of 5-HT axons and terminals. In contrast, METH significantly decreased 5-HT uptake sites in cortex, hippocampus and midbrain, but not in hypothalamus or striatum. Functional alterations in 5-HT systems were determined by changes in the stimulation of plasma hormones by PCA. Neither MDMA nor METH attenuated the PCA stimulation of plasma ACTH, renin or prolactin. The corticosterone response to PCA was increased (+42%) by METH. There were no apparent compensatory changes in 5-HT receptors, as cortical 5-HT_{1A}, 5-HT_{1B} or 5-HT_2 receptors were unchanged. These data indicate that plasma hormone responses to a 5-HT releaser may not provide an index for gross central neurotoxicity. The lack of reductions in all neuroendocrine responses following PCA suggests that (1) 5-HT projections to hypothalamic nuclei mediating these responses are unaffected or (2) that the remaining 5-HT terminals are sufficient to produce a maximal response. **(Supported by Loyola University Potts Foundation and MH 45812).**

482.6

REGIONAL CNS MONOAMINE METABOLISM AND SEROTONIN (5HT) IMMUNOREACTIVE (IR) NEURONS IN MALE F344 RATS EXPOSED TO REPEATED HIGH DOSES OF (+)FENFLURAMINE (d-FEN): EFFECTS OF PRETREATMENT WITH THE MONOAMINE OXIDASE INHIBITOR (MAOI) TRANYLCYPROMINE (TCP). M. George, J.M. Lee* and S.A. Lorens, Departments of Pharmacology and Pathology, Loyola University Chicago Medical Center (Bldg. 135), Maywood IL 60153.

Immunohistochemical (IHC) studies which have examined the effects of d-FEN, a 5HT releaser and reuptake inhibitor, on 5HT IR neuronal processes have employed a MAOI in order to increase the concentration of the antigen (5HT) to microscopically detectable levels, particularly in axons and terminals. Our objective was to document the effects of a MAOI on the regional CNS concentrations of 5HT, norepinephrine (NE) and dopamine (DA), and their metabolites, in vehicle (VEH) and d-FEN treated rats. We thus assayed by HPLC the concentrations of these monoamines and their metabolites 2 weeks after d-FEN (12 mg/kg, s.c., b.i.d. x 4 consecutive days) or VEH (1.0 ml/kg, s.c., b.i.d. x 4 days) treatment, and 2.0 h after TCP (10.0 mg/kg, i.p.) or saline (SAL, 1.0 ml/kg, i.p.) administration. Although d-FEN reduced regional CNS 5HT and 5HIAA concentrations, pretreatment of the d-FEN rats with TCP resulted in substantial elevations in 5HT content with concomitant decreases in 5HIAA levels. Similarly, the MAOI also increased the concentrations of both NE and DA while reducing the levels of their metabolites. These data indicate that rats treated with repeated high doses of d-FEN are quite capable of synthesizing 5HT. This view is supported by our 5HT IHC results which showed *regionally dinctinctive* enhanced 5HT IR in both the d-FEN and VEH rats. These findings may explain why other laboratories have reported aberrent and/or reduced 5-HT IR after d-FEN in certain brain areas. Our results clearly suggest that although daily high doses of d-FEN can produce significant reductions in CNS 5HT and 5HIAA concentrations, consistent with its mechanism of action, it does *not* produce neurotoxicity.

482.7

DIFFERENTIAL REGULATION OF 5-HT_{1A}-MEDIATED RESPONSES. P.A. Scott*, J. Chou, & A. Frazer, Dept. of Psychiat., Univ. of Pa. Sch. of Med. and Vet. Affairs Med. Ctr., Phila., PA 19104.

In the rat, 5-HT_{1A} receptor activation causes hypothermia and the 5-HT syndrome (flat body posture, forepaw treading, side-to-side body movement, and resting tremor). Of these latter symptoms, forepaw treading in rats pretreated with reserpine seems most directly linked to activation of 5-HT_{1A} receptors. We have previously reported the effects of three structurally dissimilar types of 5-HT_{1A} agonists on these two responses (Soc. Neurosci. Abstr., 18: 1383, 1992). The three agonists (the tetralin derivative, 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT); the substituted azapirone gepirone; and the methoxy-chroman derivative (+) S-20499) all induced comparable degrees of hypothermia, albeit with different potencies (8-OH-DPAT > gepirone > (+) S-20499). By contrast, only 8-OH-DPAT induced the 5-HT syndrome. We now report that (+) S-20499 is unable to either induce or antagonize the forepaw treading caused by either 8-OH-DPAT or 5-methoxy-N,N,-dimethyltryptamine (5-MeODMT) in reserpine pretreated rats (1.0 mg/kg s.c.; 18-24 hrs prior to experiment). Treatment with either 8-OH-DPAT (4.0 mg/kg) or 5-MeODMT (5.0 mg/kg) induced high levels of forepaw treading, whereas treatment with either gepirone (10 mg/kg) or (+) S-20499 (at doses up to 75 mg/kg) did not. These doses of gepirone and (+) S-20499 are about 5-10 fold higher than their respective EC_{50} doses in causing hypothermia. Pretreatment with gepirone (5-10 mg/kg) attenuated the forepaw treading induced by either 8-OH-DPAT or 5-MeODMT. By contrast, pretreatment with (+) S-20499 (75 mg/kg) did not attenuate the forepaw treading induced by these drugs. The inability of (+) S-20499 to either induce or antagonize forepaw treading is unlikely to be due to its inability to penetrate into brain (calculated partition coefficient: log p > 2). One possible explanation for the actions of (+) S-20499 is that there are subtypes of the 5-HT_{1A} receptor that differentially mediate hypothermia and the 5-HT syndrome and that (+) S-20499 is capable of discriminating between these putative subtypes. (Supported by Research Funds from the VA, USPHS Grant MH48125, and IRI Servier).

482.8

EFFECT OF 5-HT_{1A} AGONISTS ON THE LOCOMOTOR ACTIVITY OF GUINEA PIGS. J.L. Evenden*, Department of Behavioural Pharmacology, Astra Arcus AB, Södertälje, S-151 85, Sweden.

The prototypical 5-HT_{1A} agonist, 8-OH-DPAT, generally reduces the locomotor activity of naive rats and mice. However, species specific differences have been seen in the effects of this drugs on other aspects of behaviour e.g. the 5-HT syndrome (rats vs mice), or body temperature (rats vs guinea pigs). The present study was conducted to examine the effects of 8-OH-DPAT on locomotor activity (LA) in the guinea pig in an 80 by 80 cm open field, and to compare the effects of this drug with other 5-HT_{1A} agonists. In this species, doses of 1.0 to 10.0 mg/kg of the drug increased LA to a level of about 5 times that seen in control. Similar effects were seen with 5-MeODMT (1.0 - 10.0 mg/kg) and flesinoxan (3.0 and 10.0 mg/kg). Buspirone (10.0-100.0 mg/kg) also marginally increased LA, whereas ipsapirone (up to 30 mg/kg) had no significant effect. Dopamine D2 receptor blockade using the selective antagonist, raclopride (0.1 - 3.0 mg(kg) neither increased nor decreased LA. The increase in LA induced by 1.0 mg/kg 8-OH-DPAT was attenuated by ipsapirone (30 mg/kg) and raclopride (3.0 mg/kg) but was, if anything, enhanced by pretreatment with the beta-blocker and 5-HT_{1A} antagonist (-) alprenolol (15 mg/kg). However, the most notable blockade was obtained with the relatively selective 5-HT_{1A} antagonist (-)-UH-301, which reduced LA to control levels. These results suggest that hyperactivity is a major component of the 5-HT syndrome mediated by 5-HT_{1A} receptors in the guinea pig. This hyperactivity is only seen after administration of relatively full agonist, and is only weakly detectable after partial agonists, which may themselves antagonise the effects of 8-OH-DPAT. The relatively low levels of locomotor activity shown by guinea pigs in this test may underlie these effects of the drugs, since 8-OH-DPAT can increase activity in rats which have been habituated to the apparatus (Evenden and Ängeby-Möller, 1990)

Reference

Evenden J, Ängeby-Möller K, 1990, Psychopharmacology 102:485-491

482.9

PHARMACOLOGICAL DIFFERENCES BETWEEN 5-HT_{1A}-INDUCED DEFENSIVENESS AND INHIBITION OF VOMITING IN CATS. J.B. Lucot*, Dept. Pharmacol., Wright State Univ. Dayton, OH 45435.

5-HT_{1A} agonists exert a broad-spectrum antiemetic effect. Individual agonists differ in the doses that elicit defensiveness compared with those which suppress vomiting; e.g. buspirone elicits defensiveness at doses that suppress motion sickness while flesinoxan and DPAT elicit defensiveness at doses higher than those which suppress motion sickness. The defensive behavior elicited by flesinoxan and DPAT is blocked by NAN-190, whereas it was more effective at reversing the antiemetic effect of flesinoxan than of DPAT. (-)Propranolol was less effective at reducing defensive behavior. LY 285271, which is a selective, high affinity full agonist on standard measures of 5-HT_{1A} receptor function, was remarkably weak at suppressing motion sickness but elicited very strong defensive behavior. It also did not reverse the antiemetic effect of flesinoxan or DPAT suggesting that it bound weakly to the antiemetic site. The 5-HT_{1A} sites which elicit defensive behavior are pharmacologically different from those which are antiemetic.

482.10

QUIPAZINE-KETANSERIN DRUG DISCRIMINATION PROVIDES A BEHAVIORAL BASELINE SENSITIVE TO CHANGES IN SEROTONIN-2 RECEPTOR ACTIVITY. R.L. Smith*, E. Sanders-Bush and R.J. Barrett, Dept. of Pharmacology, Vanderbilt University and V.A. Medical Center, Nashville, TN 37232.

The present study was conducted to determine first, whether animals could be trained to discriminate a serotonin-2 ($5HT_2$) agonist from a $5HT_2$ antagonist, and second, the usefulness of this model for studying adaptive changes in the $5HT_2$ receptor system and for characterizing novel serotonin compounds. Rats were trained to discriminate quipazine (QUIP) (0.5 mg/kg) from ketanserin (KET) (1.0 mg/kg) on a VI-20 schedule of reinforcement. Training doses were adjusted so that when the animals were tested on saline 48 h after the last acquisition session, they made 42% of their responses on the QUIP lever. Following acquisition, a QUIP-KET dose-response function was found to be orderly and reproducible. Additional $5HT_2$ agonists (DOI and MK 212) and antagonists (pizotifen and cyproheptadine) were tested for generalization and found to substitute for QUIP and KET, respectively. A single large dose of QUIP (20 mg/kg) produced a rebound KET-like effect 20 h following administration; however, a single large dose of KET (10 mg/kg) did not produce a rebound QUIP-like effect. Additional novel serotonin compounds are presently being tested against this unique behavioral baseline. (Supported by USPHS Grant MH34007 and Fellowship DA05413)

482.11

ROLE OF SEROTONERGIC RECEPTORS IN SPINAL CONTROL OF MICTURITION IN CAT. M.J. Espey*, H.-J. Du, J.W. Downie. Departments of Pharmacology and Urology, Dalhousie Univ., Halifax, NS. B3H 4H7

Our previous study implicated spinal serotonin (5HT) as an inhibitory neurotransmitter in the spinal control of micturition (Espey et al. 1992 EJP 221:167). The present studies were performed to investigate the site and receptor subtypes involved in the spinal action of 5HT. In chloralose-anesthetized cats, an action on the afferent limb of the micturition reflex was studied by recording,with a tungsten microelectrode, ascending multiple or single unit activity in the thoracic spinal cord in response to pelvic nerve stimulation. In 3/7 animals intrathecal 5HT (0.3-6umol) diminished the total number of spikes elicited by pelvic nerve stimulation. In 4/7 animals 5HT was ineffective. Methysergide (60nmol), a 5HT1/2 receptor antagonist, increased ascending activity to 165% of control. 5HT3 antagonists MDL72222, ICS 205 930 and zatosetron increased pelvic nerve-evoked ascending activity. In addition, the 5HT3 antagonists decreased reflex discharge on the pudendal motor nerve in response to pelvic nerve stimulation. These data suggest that endogenous serotonin may suppress ascending signals from the bladder at the sacral spinal cord through 5HT3 and 5HT1/2 receptors. As well, serotonin may have an excitatory role, through 5HT3 receptors, in the pelvic-pudendal reflex pathway. (Supported by MRC)

482.12

CENTRAL 5-HT_{1A} RECEPTORS MODULATE THE BRADYCARDIA CAUSED BY THE "DIVING RESPONSE" IN ANAESTHETIZED RABBITS. A.G.Ramage*,M.P.Gilbey[1], J.G.P.Pires[2] & H.A.Futuro-Neto[2], Departments of Pharmacology & [1]Physiology, Royal Free Medical School, London, NW3, 2PF, U.K. & [2]Departmento de Ciencias Fisiologicas, Universidade do Espirito Santo, Vitoria, Brazil.

Stimulation of receptors in the nasal cavity with smoke excites cardiac vagal motoneurones (CVM's), raising BP and producing an apnoea. This apnoea then excites chemoreceptors causing a potentiation of this reflex excitation of CVM's. This combined effect is known as the "diving response". Drugs that act on 5-HT_{1A} receptors may modify this bradycardia as this receptor type affects the excitability of CVM's and modulates central respiratory drive.

In urethane anaesthetized, spontaneous breathing and atenolol (1mg/kg) pretreated rabbits recordings were made of BP, HR, renal nerve activity (RNA) and the duration of the apnoea. Cigarette smoke (10-30ml) was administered through the trachea to the upper airways. Test drugs (n=5-6) were given i.c. 8-OH-DPAT (50μg/kg) decreased while buspirone (200 μg/kg) potentiated the smoke induced bradycardia by -57 ± 12 and $+37 \pm 12$ bpm ($P<0.05$), respectively. 8-OH-DPAT also decreased the pressor effect by -15 ± 2 mmHg while buspirone potentiated the apnoea by 4 ± 2 s. 8-OH-DPAT tended to decrease the apnoea but this was not significant (ANOVA;least significant difference). Saline i.c. and i.v. 8-OH-DPAT had little effect on the changes caused by smoke. The increase in RNA was also unaffected by these drugs.

These data show that 5-HT_{1A} receptors play an important role in the central pathways involved in the "diving response".

482.13

SEROTONIN AND HPA AXIS REGULATION IN CONGENITAL LEARNED HELPLESSNESS. E. Edwards *and S.Y. Nguyen. Dept. Pharmacology & Toxicology, University of Maryland at Baltimore, MD 21201

Congenital learned helpless rats (cLH) and non learned-helpless rats (cNLH) can be differentiated by a dysfunctional HPA axis and an abnormal hippocampal serotonin (5-HT) system. These experiments examine whether 5-HT has a regulatory role in the stress activation of the HPA axis by comparing the plasma levels of adrenocorticotropin hormone (ACTH) and corticosterone (CORT) following a 30 min, 0.8mA footshock exposure in intact cLH and cNLH rats as well as in rats from either strains subjected to 5,7 dihydroxytryptamine (5,7-DHT) lesions of the fornix-fimbria region. The degeneration of hippocampal serotonergic neurons one week after the injection of 5,7-DHT (5 µg in 400 nl of 0.025% ascorbic saline; 15° angle from the vertical line, coordinates in mm: P 6.7, L 1.0, V 4.5 to bregma) was associated with a significant decrease in ^{3}H-serotonin uptake (- 70%) in cLH and cNLH rats. Footshock exposure resulted in significant increases in ACTH levels in intact, 5,7-DHT and sham-lesioned cLH rats (6-23 fold (+1). Similarly, ACTH levels were significantly elevated in cNLH rats under the same conditions (4-17 fold (+1). Since both cLH and cNLH sham-lesioned rats exhibited ACTH increases comparable to that of 5,7-DHT treated rats, the ACTH rise may be due to an additive effect of surgery and shock exposure. In addition, CORT plasma levels measured in the same rats were similarly elevated in cLH and cNLH rats under all three conditions. There was no differential effect in the stress response of either cLH or cNLH rats treated with 5,7-DHT.

These results do not support a regulatory role for 5-HT in stress activation of the HPA axis response of cLH rats.

482.14

COMPARISON OF 8-OH-DPAT AND CHLORDIAZEPOXIDE ON PUNISHED RESPONDING AND CONDITIONED EMOTIONAL RESPONSE. A.R. Allen* and I. Lucki. Departments of Pharmacology and Psychiatry, University of Pennsylvania, Philadelphia, PA 19104.

Antianxiety drugs with activity at 5-HT_{1A} receptors lack consistent effects on most animal models of anxiety, in particular punished responding. The conditioned emotional response (CER) may offer an alternative procedure for evaluating the effects of 5-HT_{1A} ligands on models of anxiety. In the present experiments, effects of the 5-HT_{1A} agonist 8-OH-DPAT and several 5-HT_{1A} partial agonists were compared to those of chlordiazepoxide (CDP) on the CER and punished operant responding in rats (N=8 per group). Both procedures included a suppressed and an unsuppressed schedule component. As expected, CDP increased previously suppressed responding in the punishment paradigm. On the CER, CDP increased the ratio of suppressed to unsuppressed responding (suppression ratio). 8-OH-DPAT did not share CDP's rate-increasing effect on punished responding, but did increase the suppression ratio. On the CER, 8-OH-DPAT also increased the suppression ratio. The effects of the 5-HT_{1A} partial agonists on suppression ratio varied across drugs. These results suggest that changes in the *relative* sensitivity to shock-induced suppression, rather than changes in response rate of individual components, may provide a more valid measure of anxiolytic activity of serotonergic drugs. Supported by USPHS grants MH 14654 and MH 36262.

482.15

SEROTONIN-DEGRADATIVE PATHWAYS IN THE TOAD BRAIN AND HUMAN PSYCHIATRIC DISORDERS. Naokuni Takeda*, Dept. of Biotechnology, COSMO Research Institute, Satte, Saimama, 340-01, Japan

Bufotenin (5-hydroxy-N,N-dimethyl tryptamine; BUTN)is a hallucinogen with psychotropic effects. High levels of BUTN and its precursor, N-methyl-5 -hydroxytryptamine(N-MET) are shown for the first time to occur and to accumulate in the brain during the degradation of serotoninin the central nervous system of the toad, Bufo bufo japonica. These compounds are concentrated in the hindbrain which includes the cerebellum and medulla oblongata. BUTN can also be detected in blood and urine specimens from the toad. In humans, autism is insome respect related to schizophrenia that appears to be a functional disease of the brain. BUTN can be detectged in urine specimens from infant autistic patients. Analysis by three-dimensional HPLC suggests that the presence and levels of BUTN may be important markers for the diagnosis of autism. Therefore, it appears that some aspects of the central nervous system of Bufo may provide useful pharmacological clues to the etiology of human psychiatric diseases, such as autism, that are known to be linked to the methylation of serotonin.

483.1

NITRIC OXIDE IN MOLLUSCS: LOCALIZATION AND PUTATIVE ROLE. K. Lukowiak*, L. Moroz, M. Gruhn[2], J. Jacklet[2], I. Roger, A.G.M. Bulloch and N.I. Syed Neuroscience and Respiratory Research Groups, University of Calgary, Alberta, Canada T2N 4N1 and [2]Department of Biological Sciences, SUNY, Albany, NY 12222.

NADPH-diaphorase (NADPH-d) histochemical staining is used to identify mammalian neurons containing nitric oxide synthase, which catalyzes the production of nitric oxide, a putative retrograde signalling molecule. To determine if nitric oxide might be used in neuronal signalling in molluscs, we performed NADPH-d histochemistry on central and peripheral neural tissues of *Aplysia californica*, *Bulla sp.*, *Phylaplysia sp.*, *Lymnaea stagnalis* and *Helisoma trivolvis*. In all of the species investigated, intense NADPH-d positive staining was observed in both peripheral and central structures. Staining was intense in some specific tracts, synaptic glomeruli (including the optic tracts lateral terminus), esophagus, gills, eyes and neuropil of cerebral, pedal and pleural ganglia of *Aplysia*. In addition, individual neuronal fibers, telodendria in the neuropil and individual cell somata located within the cerebral and other ganglia of *Aplysia* were also found to be NADPH-d positive. A similar pattern of staining was observed in *Phylaplysia*, whereas in *Bulla* only a few weakly stained cells were found in the central ganglia. In both *Lymnaea* and *Helisoma*, staining was predominantly confined to peripheral tissues such as salivary gland, esophagus, osphradium and pneumostome area. Furthermore, individual clusters of neurons that showed positive staining were also found in buccal, cerebral, pedal, pleural, parietal and visceral ganglia of these fresh water snails. The distribution of NADPH-d positive cells within the peripheral and central tissues suggests that nitric oxide (or a related substance) may be involved in neuronal signalling at molluscan synapses.

483.2

DISTRIBUTION OF PUTATIVE NITRIC OXIDE IN THE BRAIN OF CRAY FISH Cambarellus montezumae USING NADPH-DIAPHORASE HISTOCHEMISTRY. Martínez-Lorenzana G., Talavera E., Sánchez-Alvarez M., Sánchez-Islas E., Sitges M.* and Pellicer F. División de Neurociencias, Instituto Mexicano de Psiquiatría. Av. México-Xochimilco 101, CP 14370, México, D.F.

Recent studies have shown that nitric oxide (NO) functions as a neurotransmitter or neuromessenger in the central and peripheral nervous systems. The presence of NO in tissues of lower species is scarce. Thus, in this work the localization of NO producing cells in brain ganglia of the crayfish Cambarellus montezumae was determined. The ganglia of five freshwater crayfish were dissected and fixed for 2 h in 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.2, 4°C), placed in a sucrose solution (15%) for 24 h at 4°C. The brains were cut into 20 um thick sections and submitted to the NADPH-diaphorase reaction (Vincent and Kimura, Neurosci. 46:755, 1992). Positive fibers were localized in the olfactory and the accesory lobes, as well as, fibers along the deutocerebral commissure. A group of positive cells was mainly localized in the dorso-lateral region (Somata II). Its presence in the nervous system of this organism suggests an early evolutionary origin.

This work was partially supported by CONACyT grants MLO 1183-N9203 and FP N9111.

483.3

DISTRIBUTION OF PUTATIVE NITRIC OXIDE PRODUCING NEURONS IN PERIOESOPHAGEAL GANGLIA OF Helix aspersa. Sánchez-Alvarez M, León-Olea M, Talavera E, Pellicer F, Sánchez-Islas E, Alvarez-Leefmans FJ*, Martínez-Lorenzana G. División de Neurociencias, Instituto Mexicano de Psiquiatría, Av. México-Xochimilco 101, CP 14370, México, D.F.

Nitric oxide (NO) is a highly reactive molecule which participates in functions such as maintenance of blood pressure, muscle relaxation and neurotransmission. NO has been found in chicken, crab and toad tissues. Since Helix neurons are used as model systems in neurobiological research, we thought it interesting to look for the presence of NO. Perioesophageal ganglia of snails, Helix aspersa were used. After dissection, the ganglia (n=5) were fixed in 4% paraformaldehyde in 0.1 M sodium phosphate buffer (pH 7.4, 4°C), and cryoprotected in 15% sacarose during 3 h. Tissue sections (10 µm) were collected in cromoallumin gelatined slides. The reaction to detect the NADPH-diaphorase was as described by Vincent and Kimura (Neurosci. 46: 775, 1992). The results show a positive reaction in small cells (7.5 µm in diameter) from the lateral part of procerebrum. The mesocerebrum shows a few positive cells (between 27 µm and 50 µm major diameter). We also found positive fibers scattered in the neuropile of pro- and post-cerebra, and in the cerebral commissure, as well as in the neuropile of the suboesophageal ganglia.

The data suggest the presence of NO containing neurons in the nervous system of snails. Additional studies will be required to establish the role of this molecule.

483.4

NITRIC OXIDE SIGNALLING IN INVERTEBRATE NERVOUS SYSTEMS M.R. Elphick, I.C. Green+ and M. O'Shea*. Sussex Centre for Neuroscience and +Biochemistry Lab., Sch. of Biol. Sci., Sussex Univ., BN1 9QG, U.K.

Nitric oxide (NO) is a novel neuronal signalling molecule that exerts numerous physiological effects in mammals by causing an elevation of cyclic GMP in target cells. We have demonstrated that the nervous system of the locust *Schistocerca gregaria* contains the two key enzymes necessary for NO signalling: a NO synthase (NOS) and a NO-activated guanylyl cyclase. Since NO is synthesized in mammalian cells from L-arginine, and citrulline is a by-product, we assayed NOS activity in extracts of locust cerebral ganglia by monitoring conversion of [^{14}C]arginine to [^{14}C]citrulline. Locust neural NOS has similar properties to mammalian neural NOS; it is a NADPH requiring enzyme that generates citrulline from L-arginine upon activation by Ca^{2+}/calmodulin and it is inhibited by the N^{ω}-Nitro and N^{ω}-Methyl analogs of L-arginine. A guanylyl cyclase is the likely target of NO in the insect nervous system, as in mammals, since exposure of locust cerebral ganglia to the NO donors 3-morpholinosydnonimine (0.5mM), *S*-nitroso-*N*-acetylpenicillamine (0.5mM) and hydroxylamine (1mM) causes a four to eight fold elevation of cyclic GMP, but not cyclic AMP. We are using immunocytochemistry and NADPH diaphorase histochemistry to localize the sources and targets of NO in the nervous systems of *Schistocerca* and the snail *Lymnaea stagnalis*. Some of the neurons that appear to utilize the NO-cyclic GMP signalling pathway have been identified previously according to their transmitter content or physiological function. We are examining the role of NO in identified invertebrate neurons and exploring interactions between the NO-cyclic GMP pathway and other signalling systems. (Supported by S.E.R.C. grant GR/G52524 to M.O.)

483.5

ARE NITRIC OXIDE AND FREE RADICALS OF OXYGEN INTERCELLULAR MESSENGERS IN THE MOLLUSCAN CNS?
W.Winlow*, J.-H. Park and L.L.Moroz. Department of Physiology, University of Leeds, Leeds LS2 9NQ, UK.

Putative nitric oxide (NO)-containing neurones and NO synthase have been demonstrated in *Lymnaea stagnalis* and are predominantly localised in the buccal ganglia and pneumostome area, suggesting a regulatory role for NO in the feeding and respiratory programmes. On the other hand, NO and NO synthase itself are also involved in generation of the active radicals of oxygen. Thus, both NO and oxygen radicals could be intercellular messengers. To test this hypothesis we have investigated the effects of NO-generating substances and agents generating active forms of oxygen on the respiratory and feeding networks using semi-intact preparations consisting of the CNS, pneumostome and buccal mass. Up to four microelectrodes and 2 channels of optical recording of effector movements have been used simultaneously. We found that generation of NO, O_2^- and $\cdot OH$ both activated buccal and pneumostome movements and modulated the activity of neurones of the respiratory and feeding networks, including their central pattern generators. However, NO and oxygen radicals activated distinct, separate and specific motor patterns of both the pneumostome/mantle area and the buccal mass. We suggest that both NO and active radicals of oxygen may be involved as messengers in intercellular communications.

Supported by the Royal Society (London) and ESF (Strasbourg).

484.1

NEUROTENSIN-INDUCED FACILITATION OF 3H DOPAMINE RELEASE IN PRIMARY CULTURES OF MESENCEPHALIC CELLS: EFFECT OF CALCIUM CHANNEL BLOCKERS A. Brouard[1], D. Pelaprat[1], M. Vial[1], A-M. Lhiaubet[1], Y. Masuo*[2] and W. Rostene[1]. [1]U 339 INSERM, Hôpital Saint-Antoine, 75012 Paris, France.[2]Takeda Chemical Industries, Tsukuba-shi, Ibaraka-ken, Japan 300-42.

It was previously suggested that neurotensin (NT) increased dopamine (DA) release from mesencephalic DAergic neurons by opening voltage-sensitive Ca^{2+} channels. In the present work, this hypothesis was tested on primary cultures of rat mesencephalic cells. The potentiation by NT (10^{-7}M) of K^+-evoked ^3H DA release was abolished in the absence of extracellular Ca^{2+} and strongly diminished (-60%) by the non-selective Ca^{2+} channel blocker cadmium ($3x10^{-4}$M). However, NT effect was always observed in the presence of amiloride (10^{-4}M), omega-conotoxin (10^{-7}M) or nifedipine ($5x10^{-7}$M), respective blockers of T-, N- or L-type Ca^{2+} channels. Taken together, these results suggest that Ca^{2+} entry is indeed necessary for the NT-induced increase in ^3H DA release, but that the NT effect was not due to the opening of N-, L-or T-type Ca^{2+} channels by the peptide. Since NT was also previously shown to mobilize Ca^{2+} from intracellular pools, our results may further indicate that this process is unable to trigger DA release by itself and needs the simultaneous rise in intracellular Ca^{2+} through the Ca^{2+} entry induced by the potassium depolarization.

484.2

THERAPEUTIC LITHIUM LEVELS ALTER RAT BRAIN REGIONAL NEUROPEPTIDE CONCENTRATIONS. Garth Bissette*, Dan Griff, Michelle Melisko and Rita Clement. Department of Psychiatry, Duke University Medical Center, Durham, NC 27710.

We investigated whether a 3-week period of lithium (Li^{++}) treatment would alter concentrations of the neuropeptides corticotropin-releasing factor (CRF), somatotropin release inhibiting factor (SRIF, somatostatin), thyrotropin releasing hormone (TRH) and neurotensin (NT) in various brain regions of laboratory rats. These peptides are altered in diseases where Li_{++} is therapeutic. Groups of ten adult, male, Sprague-Dawley rats (225g) were given standard rat chow or a custom rat chow diet with Li^{++} (Teklad, Madison, WI). This treatment resulted in average Li^{++} levels of 0.8 mEq/liter/g protein in cerebellum samples of the Li^{++} treated rats and this compares favorably to the lower human therapeutic dose range. Brains were dissected into 16 discrete regions and acid extracted for neuropeptide quantification by radioimmunoassay. CRF, TRH and NT were elevated in the lithium treated group in all of the regions where significant alterations were noted with the exception of a TRH decrease in the amygdala. Conversely, SRIF was decreased in the Li^{++} group in all of the regions where significant changes were detected except for an increase in the hippocampus. TRH was significantly altered in the most brain regions (five), CRF and NT was significantly changed in 3 regions and somatostatin was significantly altered in 2 regions. CRF concentrations in the septal/diagonal band region was the most profoundly increased ($p<0.0009$, t-test) by Li^{++} treatment. An increase in peptide concentration can reflect increased release and synthesis or can be produced by decreased release relative to controls. These two completely different mechanisms cannot be discriminated without measurement of messenger RNA levels and/or receptor number and affinity. Supported by NIMH grant MH45975 to Garth Bissette.

484.3

EFFECTS OF DOPAMINE (DA) AGONISTS ON THE RELEASE OF NEUROPEPTIDE Y (NPY)-IMMUNOREACTIVITY FROM THE NUCLEUS ACCUMBENS AND STRIATUM OF RAT BRAIN. G.H. Cao and T.C. Westfall*. Dept. Pharmacol. Physiol. Sci., Saint Louis Univ. Sch. of Med., St. Louis, MO 63104

The caudate nucleus, putamen and nucleus accumbens contain a high density of NPY-immunoreactive cells. Moreover, DA axons are frequently found in close apposition to NPY neurons (Hendry, Biol. of NPY; Humana Press 65, 1993). Striatal injections of NPY has been shown to result in an increase in DA turnover (Beal et al., Neurosci Lett 71:118, 1986), while amphetamine administration has been observed to decrease NPY release (Tatsuoka et al., Brain Res 411:200, 1987). The purpose of the present study was to further investigate dopaminergic regulation of NPY release in the striatum and nucleus accumbens. Animals were sacrificed by decapitation and brains rapidly removed and placed (ventral side up) in a chilled rat brain matrix which allowed for reproducible sectioning of rat brains into 1 mm coronal slices. Dissects of the nucleus accumbens and striatum were removed from 1 mm brain slices and the release of NPY-immunoreactivity measured by radioimmunoassay (RIA). Basal release of NPY-IR could be measured in 10 min fractions and stimulation with a high K^+ buffer (40 mM) produced a 100-150% increase in NPY release. Apomorphine, a nonspecific dopamine agonist, produced a concentration dependent (10^{-6}-10^{-4}M) attenuation of K^+-evoked NPY release in both regions. In contrast neither the D_1 selective agonist SKF 38393 nor the D_2 selective agonist quinperole, when administered separately, altered the basal or K^+ stimulated NPY release when examined at concentrations up to 10^{-4}M. These results suggest that separate stimulation of either D_1 or D_2 dopamine receptors is not sufficient to alter NPY release from the striatum or nucleus accumbens. Stimulation of more than one receptor subtype may be necessary to influence NPY release in these brain regions. Supported by HL26319 and HL35202.

484.4

MODULATION OF DOPAMINE (DA) RELEASE BY THE CO-TRANSMITTER, NEUROPEPTIDE Y (NPY) IN PC-12 CELLS. X. Chen* and T.C. Westfall. Dept. Pharmacol. Physiol. Sci., Saint Louis Univ. Sch. of Med., St. Louis, MO 63104

We have previously shown that the NPY-Y_2 agonist, NPY13-36, inhibits the evoked release of dopamine (DA) in nerve growth factor (NGF) treated PC12 cells (Soc. Neurosci. Abs. 17:189, 1991). NPY13-36 also attenuated the K^+-evoked increase in intracellular calcium by decreasing the influx of extracellular calcium (Soc. Neurosci. Abs. 18:989, 1992). In the present study, we have further examined the concept that DA and NPY act as co-transmitters in PC12 cells and the mechanisms underlying the modulatory effect of NPY on DA release. PC12 cells were treated with NGF for 5-7 days and DA measured by HPLC-EC detection and NPY by RIA. Simultaneous basal release of both DA and NPY was observed with a significant increase in both following application of high K^+ or nicotine (NIC). NPY13-36 significantly attenuated both K^+ and NIC-evoked DA release. Nifedipine also attenuated the evoked release of DA in a concentration dependent manner. In the presence of maximum concentrations of nifedipine, NPY13-36 did not produce further inhibition of DA release while inhibitory effects of NPY13-36 and ω-conotoxin on DA release were additive. These results are consistent with NPY13-36 and nifedipine acting through a common pathway (inhibition of L-type Ca^{2+} channels). The inhibitory effect of NPY13-36 on the evoked release of DA was not altered by K^+-channel blockers (4-aminopyridine or TEA) suggesting that the effect was not through Ca^{2+}-activated K^+ channels. NPY13-36 induced inhibition of DA release was abolished by pertussis toxin pretreatment (PTX) suggesting the involvement of an inhibitory G-protein. Our results are consistent with a co-transmitter role for DA and NPY in NGF-treated PC-12 cells and that Y_2 receptor mediated inhibition of DA release acts by inhibition of L-type Ca^{2+} channels via a PTX-sensitive G protein. Supported by HL26319, HL35202 and NS07254.

484.5

EFFECT OF DOPAMINERGIC DRUGS ON THE RELEASE OF SOMATOSTATIN IN THE RAT STRIATUM.
J.M. Radke, C. Spyraki and K. Thermos*. Lab of Pharmacology, Dept. of Basic Sciences, Sch. of Medicine, Univ. of Crete, Heraklion, Crete, Greece

The neuropeptide somatostatin is found throughout the central nervous system where it is believed to act as a neurotransmitter. At present, its function in the brain and the mechanisms via which it interacts with other neurotransmitter systems remain unclear. In the rat striatum, somatostatin is contained within a specific population of interneurons. Pharmacological and behavioural studies have provided evidence suggesting a dopamine-somatostatin interaction. To provide a better understanding of how dopamine terminals in the striatum interact with the somatostatin containing interneurons, the effect of dopaminergic agonists and antagonists on the in vivo release of somatostatin was examined using intracerebral microdialysis in combination with a somatostatin radioimmunoassay. Using a trans-cerebral designed probe, basal levels of 10-15 femptomoles of somatostatin were measured per 20 µl sample (40 min). Previous studies in our laboratory have established that the levels of somatostatin, collected under these conditions, were increased by depolarization and found to be calcium dependent. Initial studies examined the effects of various doses of apomorphine (0.00; 0.05; 0.1; 0.5; 1.0 mg/kg s.c.) on somatostatin release in the unanaesthetized rat. Under these conditions, all doses of apomorphine were found to have no significant effect on the release of somatostatin in the rat striatum. To examine the effect of apomorphine more directly, we infused apomorphine directly into the striatum via the microdialysis probe (10^{-4}M; 10^{-5}M). As with the systemic administration, the infusion of apomorphine did not statistically alter the release of somatostatin. Furthermore, infusions of either the D1 dopamine antagonist SCH 23390 (10^{-4}M; 10^{-5}M) or the D2 antagonist sulpiride (10^{-4}M;10^{-5}M) failed to show changes in the release of somatostatin. The results from this in vivo study fail to support a direct interaction of the dopamine-somatostatin systems. We suggest that an intermediate neurotransmitter system may be responsible for mediating the dopamine-somatostatin interactions reported by other investigators. Studies are currently underway in our laboratory to ascertain this hypothesis.

484.6

CHOLINE ACETYLTRANSFERASE ACTIVITY AND MUSCARINIC RECEPTOR SUBTYPES IN ADULT SOMATOSTATIN TRANSGENIC MICE. R.Quirion*[1], I.Aubert[1], E.Moyse[2], S.Krantic[2], M.L.Oster-Granite[3] and S.L.Kinsman[4]. [1]Douglas Hospital Res. Ctr/McGill Univ. Dept. Psychiatry and Neurology & Neurosurgery, Montreal, Quebec, Canada; [2]Laboratoire de Physiologie Neurosensorielle, Lyon, France, [3]Dept. Anatomy, UC Riverside, CA and [4]Kennedy Krieger Inst. & Dept. Neurology, Johns Hopkins Medical Institute Baltimore, MD 21205.

In Alzheimer's disease, decrements of various cholinergic markers and somatostatin (SRIF)-immunoreactivity are among the most consistent neurochemical findings. However, it appears that SRIF is not colocalized with acetylcholine, at least in cortical and hippocampal areas. On the other hand, we have shown that SRIF can modulate the release of acetylcholine in the rat hippocampus (Araujo et al., *J.Neurochem.*, 55, 1546-1555, 1990). In order to investigate further the potential interaction between SRIF and cholinergic innervations, we studied one line of somatostatin transgenic mice [*Tgm Smst 13*] which carry extra copies of the mouse preprosomatostatin gene. Choline acetyltransferase (ChAT) activity, and quantitative *in vitro* receptor autoradiography of muscarinic M_1 ([^{3}H]pirenzepine, 15 nM) and M_2 ([^{3}H]AF-DX 384 2 nM) binding sites were determined. In transgenic (n=4) as compared to control (n=5) mice, no changes in cortical ChAT activity were detected. Similarly, the density of muscarinic M_1 binding sites was not altered in any brain regions of the transgenic animals. In contrast, a significant decrease in M_2 binding was quantified in the cerebellum (but not in the cortex or in the hippocampus) of the transgenic mice. This decrease in M_2 cerebellar sites may be inversely related to the marked increase in SRIF binding observed in this structure in transgenic animals (Moyse et al., this meeting). Supported by MH46529, K08 NS01455-01S1 and MRCC.

484.7

BRAIN SOMATOSTATIN RECEPTOR SUB-TYPES IN ADULT SOMATOSTATIN TRANSGENIC MICE. E.Moyse[*1], S.Krantic[2], R.Quirion[3], M.L.Oster-Granite[4], S.L.Kinsman[5]. [1]Labo. Physio. Neurosensorielle,Lyon,France; [2]Ecole Normale Sup., Lyon,France; [3]Douglas Hospital Res.Ctr./McGill Univ., Mtl,Qc, Canada; [4]Dpt.of Anatomy,UC Riverside,CA; [5]Kennedy Krieger Inst.&Dpt.Neurology,John Hopkins Univ.,Baltimore,MD.

Somatostatin (SRIF) is one of the most abundant neuropeptides of the brain. Its purported biological effects are numerous and include the modulation of the release of various neuroendocrine hormones, and the stimulation of learning behaviors. In order to investigate further the role of SRIF in the nervous system, transgenic mice carrying extra copies of the mouse preprosomatostatin gene were developed. Here we investigate one line of these transgenic mice (TginSmst13) for the quantitative radioautographic distribution of SRIF receptor sub-types. SRIF binding sites were labelled on frozen 20 µm-thick brain sections using 0.2 nM (^{125}I)Tyr^0-D-Trp^8-SRIF$_{14}$ in the absence (SRIF receptors$_{1,2,}$ and 3) or in the presence (R_1 and R_3) of 1 µM SMS-201995, and quantified by computerized film densitometry. In the control mice (n=5), the regional distribution of SRIF receptor sub-types differed from that seen in the rat and human brain by the absence of the R_2 sub-type in the cerebral cortex, and an overall lower density of labelling in the CA1 sub-field of the hippocampus. In the transgenic animal (n=5), specific (^{125}I)Tyr^0-D-Trp^8-SRIF$_{14}$ binding was significantly lower (17 to 53%) than in control animals in 22 areas including the anterior olfactory nucleus, the lateral septum, various diencephalic nuclei and the nucleus tractus solitarii. In contrast, specific SRIF binding was increased in the cerebellum (40 fold over control), frontal and cingulate cortices and in the claustrum. No significant alterations were detected in more than 60 other structures. These results demonstrate that SRIF receptors can be endogenously regulated by an over-expression of SRIF. Interestingly, the pattern of regulation is highly region-specific suggesting complex modulation.

This work was supported by MH46529 and K08 NS01455-0181 , and by CNRS.

484.8

ACTIVATION OF SEROTONIN$_3$ RECEPTORS ENHANCES THE ELECTRICALLY-EVOKED RELEASE OF [^{3}H]NOREPINEPHRINE IN RAT LIMBIC STRUCTURES. R. Mongeau*, C. de Montigny and P. Blier. Neurobiological Psychiatry Unit, McGill University, Montréal, Québec, Canada.

The ability of serotonin (5-HT) agonists to modulate the electrically-evoked (3 Hz, 20 mA, 2 min) release of [^{3}H]norepinephrine (NE) was tested on preloaded slices of the rat brain. The 5-HT$_3$ agonist 2-methyl-5-HT enhanced the electrically-evoked release of [^{3}H]NE in the hippocampus, hypothalamus and frontal cortex. The enhancing effect of 2-methyl-5-HT was blocked by the 5-HT$_3$ antagonist ondansetron. Elevated levels of endogenous 5-HT, achieved through selective reuptake blockade with paroxetine, as well as the addition of exogenous 5-HT in the medium, also enhanced [^{3}H]NE release. Furthermore, the effect of paroxetine was blocked by nanomolar concentrations of the 5-HT$_3$ antagonists ondansetron, ICS-205,930 and (S)-zacopride. The possibility that the enhancing effect of 2-methyl-5-HT could have been due to an antagonism of α_2-autoreceptors on NE terminals was ruled out by the unaltered effectiveness of the α_2-adrenergic agonist UK-14,304 to attenuate [^{3}H]NE release in the presence of 2-methyl-5-HT. Moreover, the activation of α_2-autoreceptors by endogenous NE reduced the effect of 5-HT$_3$ receptors activation. Indeed, there was an increase in the ability of 2-methyl-5-HT and of paroxetine to enhance the release of [^{3}H]NE in absence of α_2-autoreceptors activation, achieved in pseudo-one-pulse experiments (4 pulses, 100 Hz, 20 mA). The selective 5-HT$_{1A}$ and 5-HT$_{1B}$ agonists 8-OH-DPAT and CP-93,129, respectively, as well as the non-selective 5-HT$_1$ agonist 5-carboxyamidotryptamine, were devoid of effect on the release of [^{3}H]NE. Lesioning 5-HT fibers with the neurotoxin 5,7-dihydroxytryptamine did not alter the action of 2-methyl-5-HT on [^{3}H]NE release, indicating that this effect is not attributable to an action of this 5-HT$_3$ agonist on 5-HT terminals.

484.9

SEROTONIN-INDUCED HISTAMINE RELEASE FROM CORTICAL SYNAPTOSOMES MEASURED BY IN VITRO SUPERFUSION. L. Tuomisto*, H. Kosunen and K.S.M. Laitinen. Dept of Pharmacol & Toxicol, Univ of Kuopio, SF-70211 Kuopio, Finland.

Histamine (HA), serotonin (5-HT) and noradrenaline (NA) are all proposed to participate e.g. in the control of sleep-wakefulness, but their exact roles and interrelationships are far from clear. HA has been reported to inhibit 5-HT release from rat cortical slices through pre-synaptic H_3-heteroreceptors. On the other hand, there are facilitatory 5-HT receptors in the CNS, as evidenced by increased NA release from rabbit hippocampus. We studied whether 5-HT also influences histamine release. Rat cortical synaptosomes were purified using a Ficoll-gradient. Synaptosomes (4 mg of protein) were loaded in 300 µl chambers and superfused with artificial CSF (250 µl/min). After a 30 min stabilization period, 3 min samples of superfusate were collected. The synaptosomes were stimulated with 5-HT (1-300 µM) for 3 min. HA was analyzed by HPLC. The basal release of HA was 50-300 fmol/3min. 5-HT induced a dose related increase in HA release up to several pmol/3min. The release was Ca-dependent. The results suggest that there are facilitatory 5-HT receptors on cortical HA-ergic terminals. Together with other data this may imply reciprocal interactions between the HA- and 5-HT-ergic systems.

484.10

A METHOD FOR THE SIMULTANEOUS ASSAY OF NOREPINEPHRINE, DOPAMINE, SEROTONIN AND THEIR METABOLITES IN RAT HYPOTHALAMIC MICRODIALYSATES. Kenneth W. Perry and Ray W. Fuller*, Lilly Research Laboratories, Eli Lilly and Company, Lilly Corporate Center, Indianapolis, IN 46285.

Measuring both norepinephrine (NE) and serotonin (5HT) in microdialysates presents a challenging chromatography problem because in order to obtain adequate resolution of NE from the large void volume peak the retention time for 5HT is increased so much that the peak becomes very broad and difficult to quantitate. As a result of this problem, NE and 5HT values are rarely reported in the same experiment. We have devised one way to solve that problem and measure the metabolites as well. A ten-port HPLC valve with 5 and 20 µL sample loops was used to inject samples from the on-line dialysis system onto two separate HPLC columns. The columns were Spherisorb ODS2 3µ 2x150 mm, both housed in a column oven at 40°C. The 5 µL injection went onto the column with a mobile phase of 75 mM potassium acetate, 0.5 mM EDTA, pH 5.1, at a flow rate of 0.23 ml/min which was optimal for separation of the metabolites (DHPG, DOPAC, MHPG, 5HIAA and HVA). The 20 µL injection went onto the column with a mobile phase which resolved NE, DA and 5HT: 75 mM potassium acetate, 0.5 mM EDTA, 1.4 mM sodium octanesulfonic acid and 8% methanol, pH 4.9. The mobile phase for the amine column was delivered with a flow programmable pump at an initial flow rate of 0.2 ml/min increasing to 0.3 ml/min at 5 min then decreasing back to 0.2 ml/min at 26 min with a total run time of 30 min. Flow programming was used to elute the 5HT within a 25 min time period. The electrochemical detector (EG&G, Model 400) used for the metabolite column was set on a potential of 500 mV at a sensitivity of 5 nA/V, while a separate detector for the amine column was set at a potential of 400 mV and a sensitivity of 0.2 nA/V. This method has been useful in measuring the effects of uptake inhibitors on concentrations of NE, DA and 5HT, along with their metabolites, in dialysate samples from rat hypothalamus.

484.11

SEROTONIN-INDUCED HISTAMINE RELEASE IN THE RAT HYPOTHALAMUS MEASURED BY IN VIVO MICRODIALYSIS. K.S.M. Laitinen*, J.T. Laitinen and L. Tuomisto. Departments of Pharmacol & Toxicol and Physiology, Univ of Kuopio, 70211 Kuopio, Finland.

Histamine (HA) is a putative neurotransmitter in the mammalian brain and is assumed to modulate e.g. arousal and the sleep-wake cycle. In the rat hypothalamus, HA content is highest in the suprachiasmatic nucleus (SCN). One of the main inputs to the SCN is the serotonergic projection from the midbrain raphe cells. Hypothalamic serotonin (5-HT) is also thought to be involved in the regulation of biological rhythms. We used in vivo microdialysis to study the effects of 5-HT on hypothalamic HA release. Rats were anesthetized and a microdialysis probe was implanted into the anterior hypothalamus (aimed at the SCN). The probe was perfused with aCSF (2 µl/min) and samples were collected every 30 min. After 3-4 basal samples, 5-HT (10 or 100 µM) was delivered via the probe for 1 h. The dialysate HA concentration was analyzed by HPLC with fluorescence detection. Basal HA level was 104 ± 5 fmol/30 min. 5-HT infusion (10 and 100 µM) increased HA release up to 170 and 330 %, respectively, reaching maximal effect at 1 h. Methysergide (10 mg/kg i.p.), a nonselective 5-HT$_{1c/2}$ blocker, completely antagonized the response elicited with 10 µM 5-HT. Methysergide alone also decreased basal HA release by 20 %. These data suggest that 5-HT has a tonic stimulatory effect on the histaminergic terminals in the rat the hypothalamus.

484.12

THE EFFECTS OF CP-93129, A SELECTIVE 5-HT1B AGONIST, ON DOPAMINE RELEASE: MICRODIALYSIS STUDIES IN VIVO. Camille S. Suchowski, Michael J. Keegan and Matthew P. Galloway*. Cellular & Clinical Neurobiology, Wayne State Univ Sch Med, Detroit, MI 48207 USA

Using microdialysis, the facilitation of dopamine (DA) release was monitored in response to the co-perfusion of a selective 5-HT1B receptor agonist CP-93129 (3-(1,2,5,6-tetrahydropyrid-4-yl)pyrrolo[3,2-b]pyrid-5-one) into the anterior striatum of chloral hydrate anesthetized rats. Changes in extraneuronal DA levels were also assessed after systemic administration of either CP-93129 or m-CPP(1-(m-chlorophenyl)piperazine. Perfusion of CP-93129 facilitated DA release in a dose dependent manner. For example, local perfusion of either 0.4 nmol or 4.0 nmol CP-93129 increased extraneuronal levels of DA to 219±33% and 567±101% of control respectively (n=6). Systemic administration of 1mg/kg (i.v.) m-CPP caused a 7-fold increase in extraneuronal levels of DA whereas systemic application CP-93129 did not alter the extraneuronal levels of DA. Using striatal brain slices, in vitro, CP-93129 (1-30µM) did not increase extracellular DA levels, suggesting that CP-93129 is not an amphetamine-like substrate for the DA transporter. The results suggest that serotonergic innervation of the anterior striatum may exert a facilitatory influence on DA release. Further, the 5-HT1B selectivity of CP-93129 and its facilitatory influence on DA release suggest that 5-HT1B receptors may be involved in the control of DA release.

Supported by DA-04120 and Michigan Dept. of Mental Health.

484.13

MK-801-INDUCED STIMULATION OF STRIATAL DOPAMINE RELEASE: ANALYSIS OF POSSIBLE MECHANISMS. D.W. Miller* and E.D. Abercrombie. Center for Molecular and Behavioral Neuroscience, Rutgers University, Newark, NJ 07102.

A number of studies have shown that glutamate and dopamine (DA) interactions are important in regulating the behavioral outputs of the basal ganglia. We have observed that systemic administration of the non-competitive NMDA antagonist MK-801 significantly increases extracellular DA in striatum, measured with *in vivo* microdialysis in awake rats. At a dose of 0.2 mg/kg (n=7) a 53% increase in extracellular DA is produced by MK-801 and at 0.5 mg/kg (n=7) a 60% increase is observed. The present experiments are an attempt to analyze possible subtrates for this interaction. The increase in extracellular DA produced by systemic MK-801 was impulse-dependent, i.e., blocked by striatal infusion of TTX (10 μM or 1 μM). These data indicate that the substrate for MK-801-induced increases in striatal DA release is at least in part located within the striatum and that this interaction may be mediated indirectly via antagonism of NMDA receptors on striatal neurons that tonically inhibit striatal DA. Consistent with these suggestions, we have found, in preliminary experiments, that pulse infusions of NMDA (1 mM, 20 min; n=3) into striatum produce a 30% decrease in striatal DA levels. The ability for MK-801 to block this effect of NMDA is currently being tested. The typically reported NMDA-induced increases in striatal DA were only observed with prolonged infusion of 10 mM NMDA. The present data, as well as biochemical and electrophysiological data from other laboratories, suggest a working model to explain NMDA-mediated regulation of striatal DA release in which NMDA receptors activate the GABAergic striatonigral pathway which is, in turn, inhibitory to nigral DA afferents to striatum.

(Supported by USPHS Grant NS 19608)

484.14

CORTICO-STRIATAL REGULATION IN THE RHESUS MONKEY: *AN IN VIVO* NEUROCHEMICAL ASSESSMENT OF DOPAMINE AND GLUTAMATE INTERACTION. B.S. Kolachana*, R.C. Saunders, D.R. Weinberger. CBDB/NIMH Neuroscience Center at St. Elizabeths, Washington, DC 20032

Prefrontal and sub-cortical DA terminal field interdependence has been demonstrated in rodents (Louilot et al., Neuroscience 29:45-56, 1989). Previously we demonstrated this phenomenon in the rhesus monkey with dopamine (DA) agonists (amphetamine or cocaine) infused into the prefrontal cortex (Pfc) which resulted in reduced caudate DA levels as measured by *in vivo* microdialysis (Kolachana et al., Soc. Neurosci. Abst. 1992). The present study examines further the local Pfc neurochemical events involved in the regulation of the cortico-striatal pathway. Tetrodotoxin (TTX) was infused into the Pfc to block a broad spectrum of impulse dependent neuronal activity. Kynurenic acid (KYN), an antagonist of excitatory aminoacid receptor subtypes, was infused into Pfc to determine the effects of blocking local glutamatergic transmission on glutamate (GLU) levels in the caudate. During dialysis, rhesus monkeys were anesthetized using isofluorane gas and microdialysis probes positioned into the medial bank of sulcus principalis of Pfc and head of the caudate nucleus with the aid of previously implanted guide holders. Artificial cerebrospinal fluid, (pH 7.4) supplemented with ascorbate was continuously infused at 1ul/min flow rate. Dialysate (25ul) was collected every 25 mins and analyzed for DA and GLU using HPLC-ED. Samples were collected and baseline determined from the Pfc and caudate during the 3.5 hrs after probe implantation and before a 50 min infusion of TTX (10ng/ul) or KYN (100ng/ul) into the Pfc. In contrast to previously reported reductions in caudate DA levels seen after DA agonist or glutamate infusions into Pfc, TTX infusion greatly increased DA levels in the caudate (200-500%). KYN infusion, while reducing cortical GLU by 90%, increased caudate GLU levels by 300%. These data implicate complex interactions of excitatory and inhibitory events in Pfc, possibly involving local inhibitory circuit neurons in cortical regulation of striatal DA levels.

484.15

EFFECTS OF SIGMA LIGANDS ON THE EXTRACELLULAR CONCENTRATION OF DOPAMINE (DA) IN THE STRIATUM OF THE RAT. Gary A. Gudelsky*, Department of Psychiatry, Case Western Reserve University, Cleveland, OH 44106

Sigma ligands have been reported to increase the activity of striatal tyrosine hydroxylase in vitro and increase striatal DOPAC concentrations in post mortem tissue from rats. In the present study the effects of benzmorphan and non-benzmorphan type sigma ligands on the extracellular concentrations of DA in the striatum of rats were determined using in vivo microdialysis. Extracellular concentrations of DA in the striatum were increased approximately 40% for 120-150 min following the administration of (+)-N-allyl-normetazocine (NANM) (10 and 20 mg/kg, sc). This effect appeared to be stereoselective for NANM, since (-)-NANM (20 mg/kg, sc) did not significantly alter DA concentrations in dialysis samples of the striatum. (+)- Pentazocine (15 and 30 mg/kg, sc) also evoked a 40% increase in the extracellular concentration of DA. In contrast, (+)-3-hydroxyphenyl)-N-(1-propyl-piperidine) ((+)-3-PPP) (20 mg/kg, sc) reduced DA concentrations by 75%. Di-tolylguanidine (1 mg/kg, ip) did not alter striatal DA efflux. These results are consistent with the view that benzmorphan type sigma ligands can enhance the release of DA from terminals of nigrostriatal neurons.

SECOND MESSENGERS: NITRIC OXIDE AND CALCIUM

485.1

CEREBELLAR NEURON AND ASTROCYTE CULTURES EXPRESS BOTH THE CONSTITUTIVE AND THE INDUCIBLE NITRIC OXIDE SYNTHASES. D. Minc-Golomb* and J.P. Schwartz. Clinical Neuroscience Branch, NINDS, NIH, Bethesda, MD 20892

Nitric oxide (NO) has been identified as an important neuromodulator, as well as impicated in mediating neurotoxicity. NO is produced through the conversion of arginine to citrulline by two different forms of NO synthase (NOS): a constitutive form (cNOS) which generates NO tonically, and an inducible enzyme (iNOS), which mediates primarily toxic effects and requires gene transcription. The present study was designed to examine the expression of brain cNOS in neurons and astrocytes in culture. Since the granule cell layer in the cerebellum is the richest in cNOS in the brain, cultured cerebellar granule cells (CGC) were chosen as a model for this study. Furthermore, because of the potential role of iNOS in neurotoxicity, we wished to determine whether these neurons could be stimulated to produce iNOS by cytokines, which increase in the brain following various insults. Transcription of the genes encoding the two different forms of NOS was examined by RNA-specific reverse transcription followed by polymerase chain reaction (RS-PCR), and by fluorescent in situ hybridization. NOS enzymatic activity was assessed by the conversion of labeled arginine to citrulline. We show that CGC and type I astrocytes in culture transcribe the gene encoding brain cNOS, and in addition, can be stimulated by lipopolysaccharide (LPS) and interferon-γ (IFN-γ) to transcribe the gene encoding iNOS. The iNOS transcripts were not detected in untreated cultures. Both mRNAs are translated into active enzymes. Either LPS or IFN-γ alone increased the activity to a lesser extent than their combination. The demonstration that one type of neuron, the CGC, is capable of expressing two forms of NOS (cNOS and iNOS) raises the possibility that iNOS plays a role in neuronal damage following activation of brain microglia and production of cytokines such as interferon.

485.2

FURTHER CHARACTERIZATION OF NITRIC OXIDE SYNTHASE (NOS) ACTIVITY IN RAT FRONTAL CORTEX. S. Alagarsamy*, G. Lonart and K. M. Johnson. *The Department of Pharmacology and Toxicology, The University of Texas Medical Branch, Galveston, TX 77555-1031.*

We have previously shown that activation of classical N-methyl-D-aspartate (NMDA) receptors, 50mM K^+ and calcium ionophores can stimulate NOS activity in the frontal cortex. We now show that the K^+ response is blocked by 10μM N^{ω}-nitro-L-arginine, calcium free buffer, 100μM Cd^{2+}, 10mM Mg^{2+} and 10mM Co^{2+}. This suggests that the response to 50mM K^+ is due to calcium influx through voltage-dependent calcium channels. Pharmacological characterization of the specific calcium channel is inconclusive; however, 200μM Ni^{2+}, 10μM nifedipine or 100nM ω-conotoxin do not block the K^+ response. These data suggest that the voltage-dependent calcium channel involved in the K^+ response is not the T, L or N-type. The suspected channel is the newly described P-type channel.

Although NMDA can stimulate NOS activity there is no response observed with 300μM kainate or 30μM α-amino-3-hydroxy-5-methylisoxazole-4-propionate (AMPA). In addition, 300μM glutamate stimulates NOS and this response is blocked by 3μM phencyclidine and 1.2mM Mg^{2+}.

Further characterization of NOS activity has shown that the NMDA response can be blocked by 1mM carbamyl chloride and 30μM dopamine. The pharmacology of these interactions is yet unknown; however, future experiments will address these topics. Supported by DA-02073.

485.3

CHARACTERISATION OF RAT PINEAL NITRIC OXIDE SYNTHASE
N.C. Schaad*, D.C. Klein and P.E. Schulz Div. of Clinical Psychopharmacology, Dept of Psychiatry of the University of Geneva, 1225 Chêne-Bourg, Switzerland

The rat pineal gland respond to norepinephrine (NE) with a 100-fold increase in cGMP formation. Neither the function nor the molecular mechanism controlling its formation are fully understood. It is known however that the NO generator sodium nitroprussiate stimulates guanylate cyclase activity in pineal membrane and cytosolic preparations, and cGMP formation in intact pineal tissue. Accordingly, it appears that NO might be involved in the NE stimulation of pineal cGMP formation. To pursue this, we have determined if the pineal gland contains the enzyme which generates NO from arginine, NO synthase (NOS). A modification of a published method was used (PNAS, 86:9030-33). Pineal NOS activity was about 40 % of that in the cerebellum, 30 % higher than in the hippocampus and 80 % higher than in the cerebral cortex. Enzyme activity was cytosolic (95 % of total activity), Ca^{++}-dependent (EC_{50}=140 nM), and inhibited by trifluoperazine. These results indicate that a constitutive, Ca^{++}/calmodulin-dependent form of NOS is present in the rat pineal gland. Pineal $[Ca^{++}]_i$ is regulated via an α_1-adrenergic mechanism, which points to the probability that NE activation of the pineal gland elevates NOS activity trough elevation of $[Ca^{++}]_i$ and the resulting increase in $[NO]_i$ plays a role in the stimulation of cGMP formation.

Developmental studies indicate that NOS activity is present before the cGMP response to NE treatment is first detected and circadian studies indicate that it does not change significantly on a 24-hour basis in the adult.

485.4

EFFECTS OF PROTAMINE, HISTONE AND MYELIN BASIC PROTEIN ON NEURONAL NITRIC OXIDE SYNTHASE.
J. Hu*, J. Fridlund and E.E. El-Fakahany. Division of Neuroscience Research in Psychiatry, Uni. of Minnesota Medical School, Minneapolis, MN 55455.

Activation of neuronal nitric oxide synthase (NOS) converts L-arginine into L-citrulline and releases nitric oxide. To examine whether endogenous proteins whose amino acid sequences are rich in arginine affect the activity of NOS, we studied the effects of protamine, histone and myelin basic protein (MBP) on conversion of [^{3}H]L-arginine into [^{3}H]L-citrulline by NOS and on formation of cyclic GMP. In cytosolic preparations of rat cerebellum, protamine, histone and MBP all inhibited the formation of [^{3}H]L-citrulline in a concentration-dependent manner, with IC_{50} values of 12 μg/ml, 8 μM and 42 μg/ml, respectively. These proteins also stimulated the synthesis of cyclic GMP in neuroblastoma N1E-115 cells (EC_{50} values were 20 μg/ml, 7 μM and 396 μg/ml for protamine, histone and MBP, respectively). The stimulation of cyclic GMP formation by the basic proteins was blocked by N-nitro-L-arginine (but not by N-nitro-D-arginine) and hemoglobin, indicating an activation of the NOS/cyclic GMP pathway by these proteins. Moreover, equalizing extracellular and intracellular Ca^{2+} concentrations by EGTA abolished the effects of the proteins on cyclic GMP formation. Our results suggest that the endogenous basic proteins might be involved in the regulation of neuronal NOS activity.

485.5

METALLOPORPHYRINS INHIBIT NITRIC OXIDE-INDUCED ACTIVATION OF SOLUBLE GUANYLYL CYCLASE IN VIVO.
D. Luo and S.R. Vincent. Dept. of Psychiatry, The University of British Columbia, Vancouver, B.C. Canada

We previously showed using *in vivo* microdialysis, that activation of nitric oxide synthase results in a large increase in extracellular cGMP levels in the rat cerebellar cortex. We now use this technique to examine the direct activation of guanylyl cyclase by local application of sodium nitroprusside. Rats were implanted with a transverse microdialysis probe in the cerebellar cortex, and the probe was perfused two days later at 5 μl/min. Samples were collected every 20 min and assayed for cGMP by radioimmunoassay. Inclusion of sodium nitroprusside in the dialysate produced a dose-dependent increase in extracellular cGMP, with a 1600% increase at the ED_{50} of 25 μM. Perfusion with a calcium-free solution, or inclusion of N^G-nitro-L-arginine, both of which completely prevent the cGMP increases in response to activation of endogenous nitric oxide formation, had no effect on nitroprusside-induced increases in extracellular cGMP. Thus the nitric oxide-dependent increase in extracellular cGMP is calcium-independent.

It has been known for many years that certain metalloporphyrins which act as competitive substrates for heme in the heme oxygenase reaction, can also directly inhibit soluble guanylyl cyclase, and prevent its activation by nitric oxide. In this study, inclusion of Zn-protoporphyrin-IX (10 μM) in the perfusate attenuated the nitroprusside-induced increase in cGMP. Inclusion of Sn-protoporphyrin-IX also produced a dose-dependent inhibition of basal extracellular cGMP, and attenuation of the nitroprusside-induced increase in cGMP. These results indicate that metalloporphyrins can inhibit soluble guanylyl cyclase *in vivo*, and prevent the activation of this enzyme by nitric oxide. This agrees with *in vitro* results suggesting that metalloporphyrins with high affinity for soluble guanylyl cyclase can displace the heme, required for nitric oxide-induced enzyme activation.

485.6

PROTEIN TARGETS FOR ENDOGENOUS AND NITRIC OXIDE-DEPENDENT ADP-RIBOSYLATION IN NEURONAL TISSUE.
A. Surin* R. Raulli and J.T. Wroblewski. Fidia-Georgetown Institute for the Neurosciences, Georgetown University School of Medicine, Washington D.C. 20007.

In cerebellar granule neurons the activation of glutamate receptors leads to the activation of nitric oxide synthase and release of nitric oxide (NO). In addition to enhancing guanylate cyclase activity, NO may influence the ADP-ribosylation of intracellular proteins. In this study we compared the effects of NO on the endogenous ADP-ribosylation of proteins in homogenates obtained from adult rat brain and from primary cultures of cerebellar neurons and astrocytes. The incubation of rat brain homogenates with [^{32}P]NAD in ADP-ribosylating conditions caused the appearance of multiple radiolabelled proteins as visualized by autoradiograms of SDS-PAGE gels. In contrast, homogenates from cerebellar neurons exhibit a major radiolabelled band in the range of 36-39 kDa. In homogenates from cerebellar astrocytes we observed the appearance of three major bands with M_r of 37, 41 and 47 kDa. Incubations in the presence of sodium nitroprusside (SNP), a NO releasing agent, caused a strong increase in the ADP-ribosylation of the single protein band observed in neurons, but enhanced only ADP-ribosylation of the 37 kDa protein in cerebellar astrocytes. In view of the known ability of NO to enhance ADP-ribosylation of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) we investigated the effects of SNP on ADP-ribosylation of glutamate dehydrogenase (GDH) and alcohol dehydrogenase (ADH). Similarly to GAPDH, the ADP-ribosylation of ADH was enhanced by SNP, although to a lesser extent. The ADP-ribosylation of GDH enhanced by GTP and ADP, the allosteric regulators of this enzyme but, in contrast to GAPDH and ADH, SNP decreased these effects. The comparison of SDS-PAGE and isoelectrofocusing gels of neuronal homogenates and purified dehydrogenases indicates that the band of SNP-dependent ADP-ribosylated protein in neurons contains proteins corresponding to GAPDH, ADH, and an unidentified protein. Our results indicate that neuronal and glial cells contain different protein targets undergoing ADP-ribosylation. As shown in the case of ADH and GDH the ADP-ribosylation may be positively and negatively regulated by nitric oxide.

485.7

ACTIVATION OF STRIATAL NMDA RECEPTORS IN VIVO INCREASES HYDROXYL FREE RADICAL OUTPUT THROUGH A NITRIC OXIDE SYNTHASE-DEPENDENT MECHANISM.
Byron Hammer [1], Davis Parker [1,2] and James Bennett [1,3,4]* Departments of (1) Neurology, (2) Pediatrics, (3) Psychiatric Medicine and (4) Pharmacology. University of Virginia School of Medicine, Charlottesville, VA 22908.

NMDA receptor activation participates in excitotoxic neuronal death in acute paradigms (hypoxia-ischemia, hypoglycemia, status epilepticus) and may contribute to neuronal loss in chronic neurodegenerative diseases. NMDA receptor-mediated Ca^{+2} influx can activate nitric oxide synthase (NOS) through Ca^{+2}-calmodulin and protein kinase C, and resulting $NO^{\cdot}$ could be converted to destructive $OH^{\cdot}$ radical. We have used brain dialysis of dorsolateral striatum in awake rats to monitor $OH^{\cdot}$ production before and after NMDA receptor stimulation by perfusion with Mg^{+2}-free csf containing 5 mM salicylic acid (SA), an $OH^{\cdot}$ trap. Efflux of the SA derivatives (2,3- and 2,5- dihydroxybenzoic acid, DHB) was quantitated by HPLC-EC.

After 2-3 hours of stable baseline output of DHB's, the dialysis solution was changed to one containing 10 mM NMDA, yielding an average 4-fold increase in dialysate [DHB's]. The increased DHB output after NMDA infusion was reduced to baseline by 10 mM nitro-L-arginine, not altered by 10 mM L-arginine, and blocked by adding 100 uM MK801 before NMDA.

Our results demonstrate that local NMDA receptor activation in striatum increases production of destructive $OH^{\cdot}$ by a mechanism sensitive to nitro-L-arginine, suggesting that NOS activity is necessary for this process. (Supported by NIH NS30024).

485.8

NITRIC OXIDE TREATMENT OF TYPE I ADENYLYL CYCLASE CAUSES A REVERSIBLE LOSS OF CALCIUM/CALMODULIN SENSITIVITY VIA CYSTEINE OXIDATION.
R.J. Duhe, A. H. Dittman, E. C. Villacres*, E.J. Choi & D. R. Storm. Dept. Pharmacology, Univ. of Washington, Seattle, WA 98195

Type I adenylyl cyclase (ACI), overexpressed in Sf9 cell membranes via the baculoviral expression vector system (BEVS), was treated with reagents which selectively modify cysteine residues. N-ethylmaleimide treatment resulted in a rapid loss of all catalytic activity. Treatment with limiting amounts of o-iodosobenzoate (IBZ), which oxidizes vicinal sulfhydryls to disulfides, resulted in the complete loss of ACI stimulation by calcium/calmodulin (Ca^{++}/CaM), but not by forskolin. Ca^{++}/CaM stimulation was restored by treatment with dithiothreitol (DTT), which reduces disulfides to free thiols. Nitric oxide (NO) treatment resulted in a complete and DTT-reversible loss of calcium/calmodulin stimulation without significant impairment of either basal catalytic rates or forskolin stimulation of catalysis. NO also removed the calcium/calmodulin sensitivity of adenylyl cyclase activity in rat hippocampal membranes in a DTT-reversible process. These results implicate a novel role for NO in neuronal signal transduction.

485.9

NITRIC OXIDE GENERATORS CAN INDUCE BOTH SHORT- AND LONG-TERM CHANGES IN THE ACTIVITY OF RAT STRIATAL DOPAMINE TRANSPORTER. G. Lonart* and K.M. Johnson. *Dept. of Pharmacol. and Toxicol. Univ. of Texas Med. Branch, Galveston, TX 77555.*

We have recently observed that nitric oxide (NO) generator-induced dopamine (DA) release from striatal slices was reduced by nomifensine. Since this indicated a role for reverse DA transport, we tested the action of S-nitroso-L-cysteine (Cys-NO), a NO generator, on the DA transport by measuring the uptake of [^{3}H]DA into crude striatal synaptosomes. Five min treatment with Cys-NO (0.01-3 mM) concentration-dependently inhibited [^{3}H]DA uptake (complete inhibition at 3 mM, $IC_{50} \approx 500$ μM). Hemoglobin (300 μM) antagonized the Cys-NO effects. The major component (79%) of the effect of 3 mM Cys-NO was wash-resistant and long-lasting (> 20 min). The inhibition by 1 mM Cys-NO (78%) was completely reversible. Scatchard analysis of the [^{3}H]DA uptake revealed that Cys-NO reduced the capacity of the DA transporter. Cys-NO had no significant effect on [^{3}H]mazindol binding of the striatal membrane preparation. Collectively, these data suggest that NO regulates DA transport and that the site of action is not identical with the DA recognition site of the DA transporter. Supported by DA-02073.

485.10

MODULATION OF BASAL AND STIMULATED DOPAMINE RELEASE BY NITRIC OXIDE. P. Sun*, A. Kanthasamy, and G.E. Isom. Dept. of Pharmacol. & Toxicol., Sch. of Pharm. & Pharmacal Sci., Purdue Univ., West Lafayette, IN 47907

Previous investigations suggest the involvement of nitric oxide (NO) in the regulation of neurotransmitter release. This study characterized the effect of NO generating compounds on basal and potassium stimulated dopamine (DA) release in PC12 cells. The levels of DA released and DA remaining in cells were quantitated by HPLC-EC after 10 min of incubation. Although 5 mM isosorbide dinitrate (ISDN) increased DA release to 142.4% of basal levels, 1 mM and 5 mM ISDN attenuated KCl (56 mM) evoked release to 76.5% and 50.4%, respectively. In control studies, isosorbide, chemically similar to ISDN but lacking NO generating capacity, produced no effect on K^+ stimulated release. Also, s-nitrosol-acetyl-penicillamine, another NO donor, produced inhibition of K^+ evoked release. In additional studies, 5 mM ISDN potentiated DA secretion initiated by both the Ca ionophore A23187 and the Ca slow channel agonist Bay K 8644. 8-Br-cGMP, a phosphodiesterase resistant cGMP analogue, did not mimic ISDN on either K^+ or A23187 induced secretion. To study the mechanism underlying NO's action, cytosolic free calcium levels ($[Ca^{2+}]_i$) were measured; ISDN blocked the K^+ stimulated increase of $[Ca^{2+}]_i$ by 48.7%. These results suggest NO modulates DA release through a cGMP independent mechanism by affecting Ca mobilization during depolarization and/or possibly Ca^{2+} mediated exocytosis. (Supported by NIH Grant ESO4140)

485.11

SOMATOSTATIN INHIBITS TRANSMITTER RELEASE VIA NITRIC OXIDE AND cGMP-DEPENDENT KINASE IN CHOROID NERVE TERMINALS. D.B. Gray*, V. Scranton, and G. Pilar. Dept. of Physiology & Neurobiology, University of Connecticut, Storrs, CT 06269.

In hatchling chicks 50 nM somatostatin (SOM) inhibits 80 - 100% of Ca dependent, K-evoked ^{3}H-ACh release from ciliary ganglion nerve terminals in the excised choroid coat. This inhibition of transmitter release is thought to occur by modulation of N-type Ca channels (Gray et al., 1989, J. Neurosci., 9:1683). Incubation of choroid tissue with 10 uM 8-bromo-cGMP inhibits K-evoked ACh release by 83 ± 16%, mimicking the effect of SOM. Preincubation with 8-bromoguanosine 3',5'-monophosphorothioate, Rp isomer (BioLog), a specific inhibitor of cGMP-dependent kinase (PKG), has no significant effect on basal or control evoked ACh release. However, evoked release from choroids treated with the PKG inhibitor is no longer sensitive to SOM, suggesting that activation of PKG is involved in SOM induced inhibition of ACh release. Increased cGMP levels may be stimulated by production of nitric oxide (NO). Inhibitors of NO synthase such as nitroarginine or nitro-l-arginine mono-methylester (L-NMMA) reverse SOM-induced inhibition of ACh release while inactive enantiomers such as D-NMMA do not. Incubation of choroids with sodium nitroprusside, a NO producing compound, also inhibits ACh release. This data suggests that activation of the SOM receptor activates a series of intermediates including NO, cGMP, and PKG resulting in a decrease of both calcium influx into the nerve terminal and subsequent transmitter release. Supported by NSF #IBN-9213204 and NIH NS 10338.

485.12

A Modified, Sensitive Dual Label Assay for Nitric Oxide Synthase (NOS). Maria-Isabel Loza-Garcia[1], and Saul Maayani[2]*. Depts. of Pharmacology[1], University of Santiago, 15706 Santiago de Compostela, Spain (MILG) and Anesthesiology[2], Mount Sinai Medical Center, CUNY, NY, NY 10029.

Production of nitric oxide (NO) by constitutive or inducible cytosolic NOS has been described in a variety of tissues. NOS activity is assayed by measuring conversion of [^{3}H]-arginine (Arg) to [^{3}H]-citrulline (Cit) which is isolated with ion-exchange chromatography. Current protocols do not correct for recovery of [^{3}H]-Cit from the columns. In addition, the commercially available [^{3}H]-Arg has 2-5% of "[^{3}H]-Cit-like" material(s) and these contaminants increase up to 10% within two months. We have developed simple modifications which increase the sensitivity of the NOS assay. Stock [^{3}H]-Arg is purified at 4°C by applying 50 µCi of [^{3}H]-Arg to a 0.1 ml resin bed (AG-50X4, sodium form; Bio-Rad). The [^{3}H]-Cit-like materials are removed by washing the resin with 1500 µl H_2O and the purified [^{3}H]-Arg is eluted with 800 µl 0.01 N NaOH, into 28 µl of 1 M citric acid (final pH = 5-6; stability = 3 days). To protect the [^{3}H]-Arg during purification, all solutions contain 5% ethanol. The purification procedure reduces [^{3}H]-Cit-like materials present in stock solutions to 0.04-0.06% with a yield of 60-80%. To measure NOS activity, cytosolic fractions (40,000 x g x 10 min) from whole brain (rat or guinea pig) are prepared in 0.3 M sucrose, 20 mM HEPES and 1 mM DTT [pH=7.2). The final incubation mixture (500 µl, pH=7.2) contains 0.1 mM NADPH, 20 mM HEPES, 1 mM EDTA, 60 mM sucrose, 0.02 mM DTT, 0.2 mM $CaCl_2$ and 0.6 mg cytosol protein. The assay (10-30 min, 37°C) is initiated by the addition of 50-150,000 dpm of [^{3}H]-Arg and is terminated by addition of citric acid/EGTA to create pH=4.0. [^{14}C]-Cit (3000-5000 dpm in 10 mM Cit) is added (to monitor recovery) and each sample is transferred to a 1 ml bed of AG-50 x 4 (sodium form). The Cit is eluted with 2 ml of H_2O and counted in a scintillation counter (dual labeled dpm). (USPHS GM 34852; MH 48125).

485.13

PHARMACOLOGIC MODULATION OF REFILLING INTRACELLULAR CA^{2+} STORES IN NEURONAL CELLS. T. M. Lo* and S. A. Thayer Dept. of Pharmacology, Univ. of Minnesota, Minneapolis, MN 55455.

At least 3 intracellular stores of Ca^{2+} are present in NG108-15 cells. Caffeine (10 mM) and Bradykinin (BK, 30 nM) mobilize Ca^{2+} from the ryanodine- and 1,4,5-inositol trisphosphate (IP_3)-sensitive pools, respectively. These stores are distinct. The caffeine and BK releasable stores are subsets of a larger pool released by the ATPase inhibitors cyclopiazonic acid (CPA, 30 µM) and thapsigargin (TG, 10 nM). The effects of CPA on intracellular stores were reversible. In the absence of extracellular Ca^{2+}, depleting the stores with CPA prevented Ca^{2+} mobilization by BK or TG. Depletion of intracellular Ca^{2+} stores by CPA did not evoke Ba^{2+} or Ca^{2+} influx, indicating that capacitative Ca^{2+} entry does not occur in these cells. However, refilling the store did require extracellular Ca^{2+} and the Ca^{2+} influx required for refilling could be inhibited pharmacologically. Refilling of the BK-sensitive store was blocked completely by SKF96365 (20 µM) and econazole (10 µM). Multiple pharmacologic targets are present along the route by which Ca^{2+} re-enters the IP_3-sensitive store in neuronal cells.

485.14

2',3' DIDEOXYCYTIDINE ALTERS CALCIUM BUFFERING IN CULTURED RAT DORSAL ROOT GANGLION NEURONS. J.L. Werth* and S.A. Thayer. Department of Pharmacology, University of Minnesota Medical School, Minneapolis, MN 55455.

Depolarization of cultured rat DRG neurons with 50 mM K^+ elicits $[Ca^{2+}]_i$ transients with a prominent plateau phase that is due to mitochondrial Ca^{2+} sequestration. In cell culture 2',3' dideoxycytidine (ddC) increases lactic acid synthesis and decreases levels of mitochondrial DNA. We tested the possibility that mitochondrial Ca^{2+} buffering can be altered by chronic treatment with ddC. $[Ca^{2+}]_i$ transients were measured in single DRG neurons grown in primary culture with Indo-1 based dual emission microfluorimetry. Cultures were treated with 1µM ddC and challenged with 50 mM K^+ following 2, 4, 6, and 8 days of drug treatment. Following 4 days of ddC treatment $[Ca^{2+}]_i$ transients were shorter in duration (278 ± 59 s, mean ± SEM, N=12) compared to untreated cells (559 ± 101 s, N=7, $p < .05$). This trend was also apparent after 6 days of ddC treatment, $[Ca^{2+}]_i$ transients were shorter in treated cells (246 ± 47 s, N=10) compared to control cells (455 ± 88 s, N=10, $p < .05$). Changes in mitochondrial Ca^{2+} buffering may underlie the peripheral neuropathy produced by ddC. Furthermore, ddC promises to be a useful tool for studying the role of mitochondria in neuronal function.

485.15

INTRACELLULAR CALCIUM WAVES INDUCED IN ADULT MAMMALIAN NEURONS BY CAFFEINE. M.N. Rand*, S.G. Waxman, and J.D. Kocsis, Dept. Neurology, Yale School of Medicine, New Haven, CT 06510; and VAMC, West Haven, CT 06516.

Calcium participates in a variety of intracellular second-messenger pathways, including gene expression following injury. Caffeine is thought to release calcium from intracellular stores by increasing the sensitivity of the ryanodine receptor to cytosolic free calcium. Single waves of intracellular calcium were induced in dissociated adult rat DRG neurons and studied in real time with confocal microscopy and the calcium indicator dye, fluo-3. Brief application of caffeine (20 mM) to the edge of a neuron via micropipette resulted in the propagation of a calcium wave with an average velocity of 68 $\mu m\ s^{-1}$. Waves began ~ 400 ms after focal application of caffeine; during this latent period we frequently observed a reduction of the fluorescence signal within the area of caffeine stimulation. In some neurons, a slight decrease in calcium-induced fluorescence preceded the wave as it moved through the cell, suggesting a reduction in cytosolic free calcium at the leading edge of the wave. The magnitude of the calcium signal was relatively constant as it moved through the cytoplasm. Calcium signals moved through the nucleus concurrent with those in the cytoplasm; neurons were visualized with elevated calcium signals partially filling the nucleus and parallel with those in the cytoplasm. Calcium signals were high upon entering the nucleus (several-fold greater than cytoplasmic signals) and decreased their velocity to ~ 20 $\mu m\ s^{-1}$ within the nucleus. Nucleolar signals exceeded those of the nucleus. Calcium waves could be induced in neurons bathed in calcium-free solution, and could be induced in the same cell many times. These observations indicate that focal application of caffeine can trigger calcium waves generated by calcium-induced calcium release, and provide useful insights into mechanisms of calcium signal propagation in mammalian neurons.

Supported in part by the NIH and Department of Veteran's Affairs.

485.16

TRANSFECTED PARVALBUMIN ALTERS CALCIUM HOMEOSTASIS IN A CELL LINE. B.K. Mueller, P. Kabos, T. Neumann, B. Belhage, S.B. Kater* Anatomy and Neurobiology, Colorado State University, Ft. Collins, CO 80523.

Maintenance of calcium homeostasis is crucial for neurons. Indirect evidence supports a protective role of some EF-hand calcium-binding proteins against calcium induced neurotoxicity. Little is known about how these proteins influence cytosolic calcium levels. After cloning the parvalbumin gene into an expression vector, teratocarcinoma cells (PCC 7) were transfected. Transfected and untransfected cells were loaded with the calcium indicator Fura-2 and were exposed, in the same dish, to low concentrations of the calcium ionophore A23187. Calcium imaging technology and a computerized microscope stage enabled nearly simultaneous measurements of intracellular calcium. Before addition of A23187 both cell types showed no significant differences in rest calcium levels. However, 20 nM A23187 rapidly induced a very high increase of intracellular calcium in all the untransfected cells. In contrast, the majority of transfected cells showed only a modest, slow increase. A minority of transfected cells had similar calcium rises. One hour later these transfected cells had reduced their internal calcium levels much more efficiently than their untransfected neighbors. These results support the hypothesis that parvalbumin is able to contribute a powerful, stabilizing influence to calcium homeostasis.

485.17

REGULATION OF INTRACELLULAR CALCIUM IN CULTURED RAT DORSAL ROOT GANGLION NEURONS BY CALCIUM RELEASING AGENTS AND NEUROTRANSMITTERS. B.N.Christensen and M.D. Christensen*. Dept. Physiology & Biophysics and Dept. Int. Medicine, Univ. Tx. Med. Br., Galveston, TX 77555.

Dorsal root ganglion cells 1 - 4 days in culture were loaded with Fura-2am for measuring $[Ca^{2+}]_i$. The calcium releasing agent caffeine (10 mM) was pressure ejected onto individual cells and the change in fluorescence (340/380 nm) ratio measured. Caffeine produced a rapid increase in $[Ca^{2+}]_i$ that reached a peak within 2-3 sec. Ten second applications of caffeine produced a peak $[Ca^{2+}]_i$ response that decreased to a plateau and remained elevated for the duration of the application. In some cells, reduction of $[Na^+]_o$ (to 8 mM) markedly slowed the recovery from the caffeine-induced Ca^{+2} release suggesting that the Na^+/Ca^{2+} exchanger was important for clearing Ca^{2+} from the cytoplasm.

The glutamate metabotropic agonist (±)-1-aminocyclopentane-trans-1,3-dicarboxylic acid (ACPD) also was an effective releasing agent. Responses to ACPD were generally considerably longer e.g. a 10 sec application produced more than a 200 sec increase in $[Ca^{2+}]_i$. Supported by grant NS-29640.

485.18

CHANGES IN CALCIUM MOBILIZATION AND PHOSPHATIDYLINOSITOL TURNOVER BY AGENTS THAT ELEVATE CYCLIC AMP IN HUMAN NEUROBLASTOMA SK-N-SH CELLS. P.-S. Liu , H.-Y. Tu and L.-S. Kao*: Department of Microbiology, Soochow University; Institute of Biomedical Sciences, Academia Sinica, Taipei, Taiwan, R. O. C.

The effects of cyclic AMP-elevating agents on changes in cytosolic calcium concentration ($[Ca^{2+}]_i$) and phosphatidylinositol (PI) turnover induced by the activation of muscarinic receptors was studied in human neuroblastoma SK-N-SH cells. The carbachol-induced $[Ca^{2+}]_i$ rise was enhanced by forskolin or IBMX but was inhibited by caffeine if agents were added to the cells 30 sec before the addition of carbachol. The inhibitory effect of caffeine increased as caffeine concentration was increased. However, when the cells received longer pretreatment (30 min) with forskolin, IBMX, or caffeine, all three agents inhibited the carbachol-induced $[Ca^{2+}]_i$ transient rise and PI turnover. Caffeine caused the greater inhibition. It appears that forskolin and IBMX which elevate cytosolic cAMP concentration, exert a biphasic effect on $[Ca^{2+}]_i$ change. Carbachol-induced $[Ca^{2+}]_i$ change and PI turnover might be inhibited upstream from phospholipase C, while they be enhanced through a pathway downstream from IP_3 generation. Caffeine, in addition to its effect on cAMP, may inhibit the carbachol-induced $[Ca^{2+}]_i$ rise through the depletion of some internal Ca^{2+} pools. (Sponsored by National Science Council, NSC82-0211-B-031-003 and NSC82-0421-B-031-009-Z)

485.19

INTRACELLULAR AND INTERCELLULAR CALCIUM SIGNALING IN NEURONS AND GLIAL CELLS IN PRIMARY CULTURE A.C. Charles* Dept. of Neurology, UCLA School of Medicine, Los Angeles CA 90024 Neurons and glial cells in primary mouse cortical cultures showed distinct patterns of Ca^{2+} signaling as observed with fluorescence video imaging and fura-2. Neurons, identified with rhodamine- conjugated tetanus toxin fragment, showed spontaneous Ca^{2+} spikes with a periodicity of 0.5-10 sec. These Ca^{2+} spikes were most often asynchronous in individual neurons, although synchronous Ca^{2+} spikes in groups of 3-5 neurons were commonly observed. Ca^{2+} spikes in neurons had a rapid rise time (33 - 100 ms) and involved the entire cell simultaneously. Spontaneous neuronal Ca^{2+} spikes were abolished by 1 uM TTX. Glial cells also showed spontaneous Ca^{2+} spikes, but the periodicity was greater (5 - 60 sec) and the rise time was greater (0.5-3 sec) than those of neuronal Ca^{2+} spikes. TTX did not inhibit glial Ca^{2+} spikes. Glial Ca^{2+} spikes began in one part of the cell and propagated as a wave throughout the cell. In some cultures, spontaneous intercellular Ca^{2+} waves were observed in glial cells. These intercellular Ca^{2+} waves appeared to originate from sites of contact with neurons. Mechanical stimulation of a single neuron induced an increase in $[Ca^{2+}]_i$ in the stimulated neuron and some neighboring neurons, and frequently initiated an intercellular Ca^{2+} wave in surrounding glial cells. Mechanical stimulation of a single glial cell initiated an intercellular Ca^{2+} wave in neighboring glial cells, as well as a transient increase in $[Ca^{2+}]_i$ in some nearby neurons. These results suggest distinct mechanisms for intracellular and intercellular calcium signaling in neurons and glial cells, and provide evidence for direct communication between neurons and glia.

485.20

DYNAMIC REGULATION OF IP_3-DEPENDENT CALCIUM RELEASE BY EXTRAVESICULAR CALCIUM. E.A. Finch[1,3] and S. M. Goldin[2,3]. [1]Program in Neuroscience and [2]Biol. Chem. Dept., Harvard Med. Sch. Boston, MA; [3]Cambridge NeuroScience, Inc., Cambridge, MA.

We have used a rapid superfusion system to study the regulation and kinetics of IP_3-dependent ^{45}Ca release from brain microsomal vesicles. We previously reported that elevation of extravesicular [Ca] rapidly potentiates and more slowly inactivates IP_3-induced ^{45}Ca release (Finch et al., *Science* 252, 1991) and that ATP further potentiates this Ca release. This suggests that the IP_3 receptor may mediate IP_3-dependent Ca-induced Ca release (CICR), similar to CICR mediated by the ryandine receptor. Further investigation of the dynamic regulation of this Ca release supports this hypothesis.

The subsecond time resolution of our superfusion system and the ability to clamp extravesicular [Ca] with 5 mM EGTA or BAPTA permit pre-steady state measurements that result in temporal separation of the rapid activation and slow inactivation of IP_3-mediated ^{45}Ca release by Ca. Under these conditions, a biphasic [Ca] dependence with a maximum release rate at 3 - 10 μM Ca is observed. At steady state [Ca], release rate was also biphasically dependent on [Ca], with a maximum at 300 - 500 nM, reflecting the combined effects of both activation and inactivation by Ca.

The ability of the superfusion sytstem to effectively clamp the [Ca] during ^{45}Ca release prevented feedback regulation of the IP_3 receptor by released Ca. Under these conditions, exposure of the preparation to step changes in [Ca] in the continued presence of IP_3 rapidly modulated the rate of ^{45}Ca release, such that a rapid enhancement of the release rate was elicited by a step increase in [Ca] from 100 nM - 1 μM to 10 μM and an immediate decrease in release rate resulted from a step decrease in [Ca] from 10 μM to 100 nM - 1 μM. These data demonstrate that the rate of IP_3-mediated Ca release rapidly responds to changes in extravesicular [Ca]. Superfusion with buffers with decreased Ca buffering capacity (<1 mM EGTA or BAPTA), which resulted in changes in extravesicular [Ca] during ^{45}Ca release, directly illustrated that feedback regulation by released Ca altered the kinetics of ^{45}Ca mobilization by IP_3, further supporting the hypothesis that localized changes in cytosolic [Ca] in the vicinity of the receptor dynamically regulate IP_3-dependent Ca release.

486.1

DOPAMINERGIC PARTICIPATION IN TRIADIMEFON-INDUCED HYPERACTIVITY. R.C. MacPhail[1,3]*, J.D. Farmer[1], T.-H. Chu[2] and Q.D. Walker[4]. [1]Neurotoxicology Division, U.S. EPA, Research Triangle Park, NC 27711, [2]North Carolina School of Science and Mathematics, Durham, NC 27705, [3]Department of Psychology and Curriculum in Neurobiology and [4]Curriculum in Toxicology, The University of North Carolina, Chapel Hill, NC 27514.

Recent data indicate that triadimefon (a triazole fungicide) produces many behavioral effects, including hyperactivity, that resemble those produced by psychomotor stimulants. Since stimulant-induced hyperactivity is largely mediated by CNS dopamine receptors, this experiment determined the effect of dopamine-receptor blockade on triadimefon-induced hyperactivity. Thirty-six adult male Long-Evans rats were tested for four days during 30-min sessions in a photocell activity device. Before the fifth session, rats were divided into six groups (N=6/group) and received either: vehicle then vehicle or 100 mg/kg triadimefon, 15 µg/kg SCH23390 (a selective D_1 receptor blocker) then vehicle or 100 mg/kg triadimefon; or 0.1 mg/kg remoxipride (a selective D_2 receptor blocker) then vehicle or 100 mg/kg triadimefon. Treatments were given s.c. (1 ml/kg) 60 min and 30 min presession. Triadimefon increased motor activity, SCH23390 decreased activity and remoxipride had no effect on activity. Both blocking agents partially attenuated triadimefon-induced hyperactivity. A second experiment used a smaller dose of SCH23390 (5 µg/kg) and a larger dose of remoxipride (0.3 mg/kg). SCH23390 decreased activity, remoxipride slightly decreased activity and tradimefon increased activity. Compared to the first experiment, SCH23390 produced less attenuation, and remoxipride produced greater attenuation, of triadimefon-induced hyperactivity. In all instances, the attenuation of triadimefon-induced hyperactivity was greater than that predicted by effect additivity. These results indicate that triadimefon-induced hyperactivity requires dopamine-receptor activation.

486.2

CHARACTERIZATION OF THE STIMULUS PROPERTIES OF THE FULL D1 AGONIST SKF 82958. C.L. Zuch, S.C. Johnson, B. Weiss* and D.A. Cory-Slechta. Department of Environmental Medicine, University of Rochester School of Medicine and Dentistry, Rochester, NY 14642.

Previous studies of D1 receptor-mediated function have concluded that D1 receptors may play a less significant role in drug abuse than D2 receptors. These conclusions were based on studies using the partial D1 agonist SKF 38393. However, more recent studies indicate that unlike SKF 38393, the full D1 agonist SKF 82958 readily crosses the blood-brain barrier and is self-administered by rodents. Since the stimulus properties of drugs are important to their reinforcing properties, this study sought to determine whether SKF 82958 could serve as a discriminative stimulus. Rats were trained in a standard two-lever food-reinforced operant drug discrimination paradigm to discriminate 0.05 mg/kg SKF 82958 from saline with responding reinforced on an FR10 schedule. Acquisition of the discrimination was defined as 77% accuracy in 8 of 10 consecutive sessions. Generalization testing to other doses of SKF 82958, saline and the training dose followed acquisition. The average time taken to reach criterion was 44 sessions (s.e.m.=1.78). SKF 82958 engendered dose-dependent increases in drug lever responding, with an ED50 value of 0.041 mg/kg. Response rates in the presence of 0.025 mg/kg SKF 82958 were increased relative to saline response rates whereas at 0.031 mg/kg SKF 82958 and higher, response rates were decreased in comparison to saline. These data thus demonstrate that the selective D1 agonist SKF 82958 has discriminative stimulus properties. ES05017, ES05903.

486.3

AMPHETAMINE, COCAINE, AND DIZOCILPINE ENHANCE PERFORMANCE ON A LEVER-RELEASE, CONDITIONED AVOIDANCE RESPONSE TASK. I.M. White*, J.C. Christensen, K.M. Marley, G.S. Flory, and G.V. Rebec. Prog. Neural Science, Dept. Psychology, Indiana University, Bloomington, IN 47405.

A lever-release version of the conditioned avoidance response (CAR) task in rats is extremely sensitive to changes in forebrain dopamine transmission. In fact, CAR performance is impaired by relatively low doses of dopamine antagonists (White et al., Pharmacol. Biochem. Behav., 41:29-35, 1991), whereas apomorphine, a direct dopamine agonist, exerts the opposite effect (Wilcox and Spirduso, Psychopharmacology, 95:276-279, 1988). In the present study, we extended this line of research to amphetamine and cocaine, well-known psychomotor stimulants that facilitate dopamine transmission. We also assessed CAR performance in response to dizocilpine (MK-801), a behavioral stimulant that acts as a noncompetitive NMDA antagonist. Adult, male, Sprague-Dawley rats (n=32) were trained to make a lever-release response within 500 msec after the onset of an auditory cue (85 dB) in order to avoid shock (0.6 mA). All subjects, trained to avoid at least 70% of signalled shocks on two consecutive sessions, received 60 trials prior to drug injection followed by 60 post-drug trials. Amphetamine (0.1 and 0.25 mg/kg), cocaine (7.5 and 15.0 mg/kg), and dizocilpine (0.01 and 0.05 mg/kg) enhanced CAR performance relative to vehicle treatment. Thus, all doses both increased percent avoidance and decreased avoidance latency without altering the latency of the escape response. These results suggest that indirect dopamine agonists as well as NMDA antagonists facilitate CAR performance.

Supported by the National Institute on Drug Abuse, DA 02451.

486.4

APAMIN SELECTIVELY ATTENUATES THE PRESYNAPTIC BEHAVIORAL EFFECTS OF THE DOPAMINE D_2 AGONIST QUINPIROLE IN SQUIRREL MONKEYS. S. Rosenzweig-Lipson* and J.E. Barrett. CNS Department, Lederle Laboratories, American Cyanamid Company, Pearl River, NY 10965

The present study investigated the ability of apamin, a selective potassium channel blocker, to modify the pre- and postsynaptic behavioral effects of the dopamine D_2 receptor agonist quinpirole in squirrel monkeys. The duration of immobility and/or unusual postures indicative of catalepsy (presynaptic) or the duration of scratching (postsynaptic) were scored during 5 minute observation periods. Saline or incremental doses of quinpirole were administered 10 minutes prior to each observation period. Administration of saline did not increase the durations of catalepsy (2-16% of the observational period) or scratching (0-1% of the observational period) in individual monkeys. Low doses of quinpirole (0.003 - 0.03 mg/kg) dose-dependently increased the duration of catalepsy to approximately 18 - 84% of the observational period in individual monkeys. Higher doses of quinpirole (0.1 - 0.3 mg/kg) did not increase the duration of catalepsy, rather these doses increased the duration of scratching to approximately 30 - 80% of the observational period in individual monkeys. Pretreatment with apamin (0.003 - 0.03 mg/kg) 1 hr prior to the first observation period attenuated the effects of quinpirole (0.01 - 0.03 mg/kg) on catalepsy, with cataleptic postures maintained for 3 - 50% of the observational periods in individual monkeys. In contrast, pretreatment with apamin had little effect on the scratching produced by quinpirole (0.1 - 0.3 mg/kg) with scratching occurring for 60 - 80% of the observational periods in individual monkeys. The present results suggest that potassium channel blockade can differentially modify the pre- and postsynaptic behavioral effects of dopamine D_2 agonists in monkeys.

486.5

INTRANIGRAL INJECTIONS OF SCH 23390 INHIBIT AMPHETAMINE AND SKF82958-INDUCED ROTATIONAL BEHAVIOR. David M. Yurek* and Susan B. Hipkens Univ. of Kentucky College of Med., Div. of Neurosurgery and Dept. of Anatomy & Neurobiology, Lexington, Kentucky 40536.

Rats were given unilateral 6-hydroxydopamine lesions of the nigrostriatal pathway and permanent indwelling cannula were surgically implanted into the non-lesioned side of the brain; cannula were used for direct injections of dopamine antagonists into the pars reticulata region of the non-lesioned substantia nigra. The selective D1 receptor antagonist, SCH 23390, was injected intranigrally at various concentrations [3.0, 1.5, 1.0, 0.6, or 0.3 mM] just prior to an intraperitoneal injection of amphetamine. SCH 23390 dose-dependently inhibited amphetamine-induced rotational behavior with the highest doses completely blocking rotational behavior in some animals. An intranigral injection of the selective D2 receptor antagonist, (-) sulpiride [1.0 mM], did not produce a significant reduction in amphetamine-induced rotational behavior whereas an equivalent molar concentration of SCH 23390 [1.0 mM] produced a significant 62% reduction in amphetamine-induced rotational behavior. A concentration of SCH 23390 that produced a 50% reduction in rotational behavior when injected directly into the substantia nigra was unable to produce a significant reduction in rotational behavior when injected directly into the striatum. The effects of intranigral injections of SCH 23390 on apomorphine-induced rotational behavior were directly opposite to that observed for amphetamine-induced rotational behavior; contralateral rotational behavior increased relative to baseline measures.

SCH 23390 injected into the lesioned substantia nigra completely blocked rotational behavior induced with a subcutaneous injection of SKF 82958 [0.0075 mg/kg] whereas rotational behavior induced with quinpirole [0.2 mg/kg, s.c.] was not affected by even the highest doses of SCH 23390 pretreatment. These data support the hypothesis that dopamine release in the midbrain may act as a neuromodulator of motor behavior, and that D1 receptors play a functional role in this process. Supported by Univ. of Kentucky Med. Col. Research Fund and USPHS NS 29994.

486.6

INVOLVEMENT OF GLUTAMATE AND GABA IN TURNING BEHAVIOR CAUSED BY MICROINJECTIONS INTO THE DORSAL STRIATUM OF RATS. I.D. Smith*, K. Mitha and R.J. Beninger. Department of Psychology, Queen's University, Kingston, Canada.

Among the afferent systems projecting to the dorsal striatum are glutamatergic (GLU) inputs from the cortex, dopaminergic (DA) projections from the substantia nigra, and GABAergic input from local circuits within the basal ganglia. An imbalance in striatal DA transmission causes a directional bias in the rat. We have shown previously that activation of striatal NMDA or kainic acid (KA) receptors with sub-toxic agonist microinjections also causes rotation. Several experiments were carried out to further characterize the involvement of AMPA and $GABA_A$ receptors in circling behavior.

Male Wistar rats received 0.5µl drug injections into the dorsal striatum (A-0.3, L3.0, V4.5) and turning activity over 20 minutes was measured in an automated rotometer chamber. Injections of 200µM (21.3ng) KA induced significant contralateral turning and was blocked by co-injection of 7.9mM (20µg) of the DA antagonist *cis*-flupenthixol (*c*-flu). AMPA injections (50, 200, 400µM (4.7, 18.6, 37.2ng)) caused dose-dependent contralateral rotation and the effect of the highest dose was reversed by *c*-flu and by co-injection of 200µM (23.2ng) CNQX. The $GABA_A$ antagonist bicuculline (50, 200, 500µM (11.6, 46.2, 115.5ng)) also caused contralateral turning, whereas injections of the $GABA_A$ agonist muscimol (50, 200, 500µM (4.9, 19.5, 48.8ng)) had no significant effect.

These result show that blocking $GABA_A$ receptors and activating non-NMDA glutamate receptors causes contralateral rotation. The KA and AMPA effects seem to depend on intact DA neurotransmission, and may operate through increasing local DA levels. The bicuculline effect may involve a similar mechanism and may also increase striatal output unit discharge through direct postsynaptic disinhibition. (Supported by NSERC)

486.7

DEVELOPMENT AND REVERSAL OF SENSITIZATION TO AMPHETAMINE ANOREXIA: ROLE OF TEMPORAL, BEHAVIORAL AND PHARMACOLOGICAL VARIABLES. D.L. Wolgin*. Department of Psychology, Florida Atlantic University, Boca Raton, FL 33431.

Rats given 35 injections of either amphetamine (2.5 mg/kg; Group A) or saline (Group S) at 3-d intervals developed sensitization to the anorexic effect of the drug, as assessed by shifts to the left in dose-response (DR) functions conducted before and after the chronic treatment. Group A, but not Group S, also displayed sensitization of stereotypy. These results suggest that sensitization of anorexia developed as a result of the passage of time and/or the stress of repeated injections between the DR determinations. Subgroups from each group were then given daily injections of amphetamine (2 mg/kg) either before or after access to milk for 4 wk. Other subgroups were given injections of saline as a control. On a final DR determination, the control groups showed no further changes in milk intake. Groups given the drug after milk showed a loss of sensitization (DR 3 = DR 1), whereas groups given the drug before milk developed tolerance (DR 3 > DR 1). These results demonstrate that, unlike tolerance, the reversal of sensitization does not require access to milk while in the drugged state.

(Supported by NIDA grant DA 04592)

486.8

POSSIBLE ROLE OF A DA RECEPTOR RESERVE IN PREWEANLING RATS C.A. Bolanos and S.A. McDougall*. Dept. of Psychology, California State University, San Bernardino, CA 92407.

Evidence consistent with the presence of both a D1 and a D2 receptor reserve has been found using the preweanling rat, as 17-day-old rats treated with EEDQ (an irreversible DA antagonist) showed normal increases in behavior after challenge with the DA agonists NPA or quinpirole. To assess the receptor reserve idea, we treated 17-day-old pups with EEDQ and flupentixol (a reversible DA antagonist) and then challenged them with NPA. If a receptor reserve hypothesis is correct, pups receiving EEDQ should be more sensitive to the effects of a reversible antagonist than pups not receiving EEDQ. The 16-day-old pups were injected with EEDQ (7.5 mg/kg) after DA receptors were either unprotected or protected using sulpiride and SCH 23390. Flupentixol (0.1 or 0.2 mg/kg) was then administered to the pups 24 hrs after EEDQ. Locomotor activity and stereotyped sniffing were then measured during a 20 min testing session. NPA-induced sniffing was blocked in a dose-dependent fashion by flupentixol. Importantly, EEDQ did not differentially affect flupentixol's ability to block sniffing. Locomotor activity was also enhanced by NPA, but EEDQ and flupentixol did not combine to maximally antagonize this behavior. Therefore, these results suggest that the presence of a receptor reserve cannot explain EEDQ's inability to disrupt the agonist-induced responding of preweanling rat pups.

486.9

EVIDENCE OF ALTERED SENSITIVITY OF OPIATE RECEPTORS AFTER SEIZURES KINDLED BY PICROTOXIN. J.Thomas.* & W.L. Nores, Dept. of Psychology, Univ. of New Orleans, New Orleans, LA 70148.

The possibility that the development of increased sensitivity to picrotoxin-induced focal seizures as a result of previous seizure episodes was related to changes in the function of endogenous opiate mechanisms was investigated. Adult male rats were injected with saline or a convulsant dose of picrotoxin (3 mg/kg, SC). The latency to and number of focal seizure episodes was scored. This procedure was repeated twice at one week intervals for a total of three seizure induction days. Five months after the third seizure testing day, the saline and picrotoxin-treated rats were pretreated with morphine (.5 mg/kg, IP) and tailflick latencies were measured. The results showed that rats given the repeated picrotoxin treatment showed a greater incidence of focal seizures on Day 3 of testing relative to Day 1. Mean tailflick latencies after morphine were longer in the picrotoxin-treated rats than in the saline-treated controls. The results of the study suggest that alterations in the sensitivity of opiate mechanisms occur during the kindling of seizures with picrotoxin.

486.10

THE TEMPORAL ANALYSIS OF THE GENERALIZATION FUNCTION WITHIN THE CONDITIONED TASTE AVERSION BASELINE OF DRUG DISCRIMINATION LEARNING. A. L. Riley*, C. L. Wetherington, and B. X. Sobel. Psychopharmacology laboratory, The American University, Washington, DC 20016

Although the generalization gradient in drug discrimination learning is often described as being quantal in nature (Mathis and Emmett-Oglesby J. Neurosci. Methods 31:23-33, 1991), we have recently reported that within the taste aversion baseline of drug discrimination learning this function is more graded, i.e., individual subjects display intermediate drug control at intermediate doses of the training drug (Riley et al. Beh. Pharm. 2:323-334, 1992). In the present experiment, within-session licking patterns were observed for rats acquiring naloxone or pentobarbital discriminations within the taste aversion baseline. Although every individual subject displayed a graded dose-response function in subsequent assessments with varying doses of the training drug, i.e., consumption was intermediate at intermediate doses of the training drug, these intermediate levels of consumption actually reflected differences in onset and offset of drug action. Specifically, consumption was generally at control levels within the first five minutes of the 20-min session, followed by complete suppression and then rapid recovery to control levels near session termination. The averaging of these quantal effects (presumably reflecting drug onset and offset) appears to mediate the graded dose-response function with naloxone and pentobarbital within this baseline of drug discrimination learning.

486.11

NALORPHINE AS A STIMULUS IN DRUG DISCRIMINATION LEARNING. S.T. Smurthwaite* and A.L. Riley. Psychopharmacology Laboratory, The American University, Washington, D.C. 20016.

Utilizing the drug discrimination procedure, nalorphine has been reported to substitute for both mu and kappa agonists, as well as the mu antagonist naloxone. Given that the training drug has been reported to be an important factor in generalization patterns, in the present study animals were trained to discriminate nalorphine from distilled water. In subsequent tests for generalization, the mu agonist morphine substituted for the nalorphine stimulus in a dose-dependent manner, while the kappa agonist U50,488 and the mu antagonists naloxone and naltrexone failed to do so, suggesting that discriminative control was mediated by its agonist activity at the mu receptor. Although it remains unclear what specific mechanism underlies the different generalization patterns when different compounds are used as the training drug, it is clear that training drug is important in determining the generalization patterns with the opiate antagonists.

486.12

EVIDENCE FOR CROSS-TOLERANCE TO THE LOW EFFICACY AGONIST, NALBUPHINE, IN PIGEONS DISCRIMINATING AMONG TWO DOSES OF MORPHINE AND SALINE. S.A. Vanecek* and A.M. Young. Dept. of Psych., Wayne State University, Detroit, MI 48202.

Cumulative doses of nalbuphine (NB) were evaluated in pigeons trained to discriminate among 1.8 mg/kg (low dose) and 10 mg/kg (high dose) morphine (MS) and saline (SAL) under fixed-ratio 30 schedules for food reinforcement. At the lowest dose tested, 0.1 mg/kg, NB evoked SAL key responding. Higher doses (0.32-32 mg/kg) evoked low dose key responding, but did not engender high dose key responding prior to rate-suppressing doses. Suspending training and administering 56 mg/kg MS, b.i.d., a treatment that confers tolerance to MS itself, or 10 mg/kg MS, b.i.d., a treatment that does not confer tolerance to MS, produced cross-tolerance to the MS-like effects of NB and markedly increased the rate-decreasing potency of NB. In MS-treated birds, NB evoked only SAL key responding, and the doses required to suppress rates decreased 30- to 100-fold. An acute dose of 0.32 mg/kg NB during treatment with 10 mg/kg MS, b.i.d., or 1.0 mg/kg NB during treatment with 56 mg/kg MS, b.i.d., increased the doses of MS required to evoke either low dose or high dose key responding. In the absence of repeated treatment, these acute NB doses either evoked low dose key responding themselves or decreased the doses of MS required to evoke low dose key responding, and also increased the doses of MS required to evoke high dose key responding. These findings are consistent with the hypothesis that NB functions as a partial agonist at *mu* opioid receptors, and that it is less efficacious than MS. (Supported by DA03796 and K02 DA00132)

486.13

LONG-TERM EFFECTS OF d-FENFLURAMINE AND d-norFEN-FLURAMINE ON SEROTONIN AND 5-HIAA IN THE RAT HIPPOCAMPUS. M. Puig de Parada, M.A. Parada and B.G. Hoebel*, Dept. of Psychology, Princeton University, Princeton, NJ 08544 and School of Medicine, Universidad de Los Andes, Mérida, Venezuela.

This study compared chronic effects of a three week regimen of daily ip injections of saline, d-fenfluramine (d-FEN) (10mg/Kg/day) and d-norfenfluramine (d-norFEN) (5mg/Kg/day) on extracellular serotonin (5-HT) and (5-HIAA) in the rat hippocampus. Microdialysis was performed in the same awake animals during the first and last day of ip treatment, and two and four weeks after treatment ended. The first drug injection increased 5-HT (272% with d-FEN and 715% with d-norFEN). Only d-FEN decreased 5-HIAA. The last day of treatment basal 5-HT was normal. Basal 5-HIAA decreased to 41% of the first-day baseline with d-FEN and to 31% with d-nor-FEN. The last drug injection no longer released 5-HT. Two weeks after treatment basal 5-HT was still at or above the day-1 baseline. 5-HIAA was normal in the saline group and still depressed in the d-FEN group (57%) and specially the d-norFEN group (35%). A month after the treatment, 5-HT was still at the original baseline and 5-HIAA was low in all groups. High potassium (KCl, 65mM) infused at the end of the experiment released 5-HT in the control animals, but not in the d-FEN or d-norFEN groups. The results suggest that d-norFEN might account for the long term effects of d-FEN in rats.

Supported by Les Laboratoires Servier.

486.14

d-FENFLURAMINE REQUIRES CALCIUM TO INCREASE EXTRACELLULAR SEROTONIN IN VIVO. M.A. Parada*, M. Puig de Parada and B.G. Hoebel, Dept of Psychology, Princeton University, Princeton, NJ 08544 and School of Medicine, Universidad de Los Andes, Mérida, Venezuela.

It is known that d-Fenfluramine (d-FEN) releases 5-HT from an intravesicular pool while d-norfenfluramine (d-norFEN) releases mainly cytosolic 5-HT. d-FEN is a weak base and it could release intravesicular serotonin by a disruption of the pH gradient across the vesicle membrane (weak base mechanism) as ammonium chloride does, or it could promote an exocytotic mechanism by allowing calcium entry into the terminals. Reverse microdialysis was used to infuse drugs into the hippocampus of freely moving rats. Ammonium chloride (100mM) and d-FEN (1 mM) increased extracellular 5-HT in the same proportion (800%) when administered separately, and their effects were additive when administered together in the same infusion (2569%). Verapamil (1 mM) in calcium-free Ringer perfusate prior to the infusion of either 1 mM d-FEN, 1 mM d-norFEN or 100 mM ammonium chloride prevented the full increase in 5-HT by d-FEN (725% vs 2198%) but not ammonium chloride (525% vs 607%) or d-norFEN (1226% vs 1589%). EGTA with calcium-free Ringer also prevented the increase in 5-HT induced by d-FEN (635% vs 2929%). Tetrodotoxin (10μM) did not modify d-FEN release of 5-HT (1364% vs 1661%). These results suggest that d-FEN applied locally requires calcium to trigger the exocytotic process in 5-HT terminals in vivo.

Supported by Les Laboratoires Servier.

486.15

THE EFFECT OF ALPHA$_2$-ADRENOCEPTOR AGONISTS AND ANTAGONISTS ON THE STIMULUS PROPERTIES OF LSD. D. Marona-Lewicka*, R. Oberlender, D.E.Nichols Depts. of Medicinal Chemistry and Pharmacognosy, and Pharmacology and Toxicology School of Pharmacy and Pharmacal Sciences, Purdue University, West Lafayette,IN 47907

Substantial evidence suggests that the discriminative stimulus effect of LSD is mediated by actions at postsynaptic 5-HT receptors. However, in rats trained to discriminate LSD from saline complete generalization is reported with the alpha2-adrenoceptor antagonist, yohimbine, and partial substitution with the alpha2-agonist, clonidine. Rats were trained to discriminate LSD tatrate (0.08 mg/kg) from saline in a food-reinforced two lever procedure with FR=50. Partial generalization of the LSD stimulus was observed with clonidine (0.01-0.08 mg/kg) or yohimbine (1.0-3.0 mg/kg) 57% and 80%, respectively. Failure to achieve complete substitution of clonidine or yohimbine may be explained by the inability of subjects to complete the test session (high % of disruptions produced by moderate doses of clonidine and higher doses of yohimbine). Neither clonidine nor yohimbine were able to block the LSD cue. When clonidine (0.04mg/kg) or yohimbine (2 mg/kg) and various doses of LSD were given together, the LSD dose response curve was shifted significantly to the left. It is possible that serotonergic properties of yohimbine (e.g. nanomolar affinity at the 5-HT$_{1A}$ receptor), may be responsible for the potentiation of LSD. The role of alpha2 adrenoceptors in the stimulus effect of LSD was further examined using more specific alpha$_2$ antagonists, idazoxan and RS-26026-197.

486.16

DISCRIMINATION OF ELECTRICAL STIMULATION (ES) OF THE DORSAL RAPHE NUCLEUS (DRN), GENERALIZATION TO THE 5-HT$_2$ AGONIST DOI AND CORRELATION WITH CHANGES IN 5-HT LEVELS IN FOREBRAIN BY MICRODIALYSIS. **D.J. Mokler*, B. Whitten, A. Gully, S. Field and M. Dixon. Dept. Pharmacol., Univ. New England, Biddeford ME 04005.**

Sprague-Dawley rats were trained to discriminate ES of the DRN (200μA, 100μsec, 20Hz) by associating the ES with saccharin consumption made aversive by LiCl injection after the session. The discrimination was learned within 6 training sessions. Lowering the ES current to 50-150 μA resulted in non-stimulation levels of saccharin consumption. The 5-HT$_2$ agonist DOI (.5 -1 mg/kg) generalized to ES of the DRN; i.e., animals reduced saccharin consumption following DOI. In order to evaluate the neurochemical changes in 5-HT release following ES of the DRN, the dorsal hippocampus was sampled by microdialysis in awake animals. Absolute levels of 5-HT in dialysate were 23.8 ± 4.2 femtomoles/20μl (mean ±s.e.m.). ES (200μA) produced a decrease in 5-HT levels in the hippocampus to below levels of detectability (> 0.5 femtomoles/20μl), which was maximal 20-60 min after ES. DOI (1 mg/kg) also decreased 5-HT levels in the hippocampus to the same extent. This was maximal 60-120 min after ES. These studies suggest that the behavioral generalization between ES of the DRN and DOI is due to a decrease in 5-HT release in forebrain areas. This may be the result of inactivation of 5-HT neurons of the DRN caused by activation of somatic autoreceptors or through long-loop feedback mechanisms. (Supported by NIDA grant 07316.)

486.17

ANTIDEPRESSANT-LIKE EFFECTS OF CENTRALLY ADMINISTERED ISOPROTERENOL. J. M. O'Donnell*, S. Frith, and J. Wilkins, Dept. of Pharmacology, LSU Medical School, Shreveport, LA 71130.

Isoproterenol (1–30 μg, i.c.v.) reduced response rate and increased reinforcement rate of rats under a DRL 72-sec schedule. Propranolol (1 mg/kg, i.p.) antagonized this effect of isoproterenol. After propranolol administration, doses of isoproterenol up to 100 μg failed to affect DRL behavior. By contrast, following vehicle administration, doses of isoproterenol of 10 and 30 μg reduced response rate and increased reinforcement rate. The beta-1 selective antagonist betaxolol and the beta-2 selective antagonist ICI 118,551 antagonized, in a dose-dependent manner, the ability of 30 μg isoproterenol to alter DRL behavior. These antagonists exhibited similar potency suggesting that the effects of isoproterenol were mediated by both beta-1 and beta-2 adrenergic receptors. In order to further examine the role of the beta adrenergic receptor subtypes, the effects of i.c.v. administration of isoproterenol were determined in rats treated repeatedly with the centrally acting beta-2 adrenergic agonist clenbuterol in order to reduce the responsiveness of beta-2 adrenergic receptors. Following this treatment, isoproterenol still produced antidepressant-like effects on DRL behavior; there was, however, a tendency for slightly diminished responsiveness to isoproterenol in clenbuterol-treated rats compared to vehicle-treated rats. The present results indicate that stimulation of central beta adrenergic receptors by isoproterenol alters DRL behavior in a manner similar to that observed after administration of proven antidepressant drugs. This behavioral action of isoproterenol appears to be a consequence of stimulation of both beta-1 and beta-2 adrenergic receptors. (Supported by USPHS Grant MH40694.)

486.18

SEROTONIN UPTAKE INHIBITOR-INDUCED MOTOR BEHAVIORS-A POSSIBLE MODEL FOR SCREENING ANTIDEPRESSANTS? **C.L. Pinter, J.J. Rutter and S.B. Auerbach*. Nelson Biol. Lab., Rutgers Univ., Piscataway, NJ 08855.**

Several behaviors were consistently observed in rats shortly after the administration of 5-HT reuptake inhibitors. We hypothesized that these behaviors - flat body posture (FBP), hindlimb abduction/flexion (HA/F), and abdominal contraction (AC) - could be quantified and therefore serve as a screening test for other 5-HT uptake inhibitors. In the first set of experiments, duration of FBP, HA/F and AC was recorded for 30 minutes following peripheral administration of reuptake inhibitors selective for 5-HT (fluoxetine; FLU & sertraline; SER), NE (desipramine; DMI & maprotiline; MAP), DA (nomifensine; NOM), or the nonselective NE/5-HT uptake inhibitor (imipramine; IMI). Additional observations were made on rats receiving an MAOI (pargyline; PAR) or the 5-HT releaser fenfluramine (FEN). While all the compounds but NOM produced significant FBP, only the selective 5-HT and NE inhibitors produced significant HA/F and SC. In a second group of rats, pretreatment with the 5-HT synthesis inhibitor PCPA (100 mg/kg/day x 3 days) was used to test the hypothesis that decreased whole brain levels of 5-HT should attenuate expression of behaviors in response to FLU but not MAP. PCPA pretreatment decreased brain 5-HT levels by 90%. Compared to non-PCPA treated rats, those receiving FLU following PCPA exhibited significantly less FBP and more HA/F, while those rats receiving MAP showed no change in behavior. Future investigations will examine the effects of MAP and FLU on extracellular NE and 5-HT via microdialysis. Supported by a grant from NSF.

486.19

3β5α PREGNANOLONE IS NOT AN INACTIVE ANESTHETIC STEROID. C.Bukusoglu and N.R. Krieger.* Department of Anesthesia, Brigham and Women's Hospital, Boston, MA 02115.

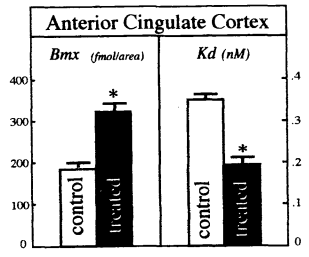
3β5α-pregnanolone (3β) has been studied for more than 50 years and is widely supposed to be an inactive anesthetic steroid. Here we re-evaluate the activity of this steroid. Following i.v. injection of 3β (12 mg/kg, cremophore/ saline) the times to LRR (tLRR) were 9, 6 and 2 min with 0.0, 0.5 and 1.1 g/kg ethanol respectively. Ethanol alone demonstrated less than 10 % LRR. Thus 3β could be supposed to have intrinsic anesthetic activity, or to be a precursor for an active metabolite or both. To demonstrate whether or not 3α has intrinsic activity, we co-injected 3β with subthreshold doses of 3α5α pregnanolone (3α). 3β (24 mg/kg) was co-injected with 3α (0.75 mg/kg) in the presence of ethanol (1.1 g/kg). tLRR values were reduced to 12 ± 2 sec (mean ± SEM, n=3). This time is too short to permit metabolite formation. Therefore, 3β itself must have intrinsic anesthetic potency. 3β was estimated to have 1/40th the potency of 3α.

RECEPTOR MODULATION, UP- AND DOWN-REGULATION III

487.1

EFFECT OF SERTRALINE TREATMENT ON BENZODIAZEPINE RECEPTORS IN THE RAT BRAIN. L. Giardino, M. Zanni, A. Velardo, L. Calzà*, Center of Pathophysiol. of the Nervous System, Hesperia Hospital, 41100 Modena, Italy.

The antidepressant drug sertraline also exhibits anxiolitic properties. In this study we investigated the effect of 8, 15 and 30 days sertraline treatment *(10 mg/kg day, ip)* on ^{3}H-flunitrazepam (^{3}H-FLU) binding sites in the rat brain by means of quantitative receptor autoradiography. After 8 days an increase of ^{3}H-FLU receptor density is found in the olfactory tubercle. This effect is reversed at 15 and 30 days. At 15 days, an increase is observed in the anterior cingulate cortex. This increase is still present after 30 days. At the same time, we also found an increase of ^{3}H-FLU in the frontoparietal motor cortex and in the septal nuclei. The Scatchard plots obtained from the saturation experiments indicate that this increase of the receptor density is due to an increase of both the receptor number and affinity *(see figure)*. All the other investigated areas are unaffected by the sertraline treatment. The benzodiazepine receptor modification described after the chronic block of 5-HT reuptake could be involved in the anxiolitic effects observed during sertraline treatment.



487.2

PARADOXICAL UP-REGULATION OF MUSCARINIC RECEPTORS BY AGONISTS IN NCB-20 CELLS. S. Gucker* and D.-M. Chuang, Biological Psychiatry Branch, National Institute of Mental Health, Bethesda, MD 20892.

Carbachol treatment (1mM, 24 h) of NCB-20 cells induces down-regulation (to 58 ± 2% of control level) of muscarinic receptors coupled to adenylate cyclase inhibition and stimulation of phosphoinositide turnover. 1 mM Oxotremorine-M and 0.1 mM McN-A-343, M_1-type agonists, induce down-regulation similar to carbachol (56 ± 11% and 71 ± 2%, respectively, of control, after 24 h). However, this classical agonist-induced down-regulation is not shared by all agonists. 1 mM Bethanechol treatment induces no down-regulation (92 ± 1%) after 24 h. Surprisingly, pilocarpine and oxotremorine induce up-regulation of [^{3}H]QNB binding to cell membrane homogenates from chronically treated NCB-20 cells. 1 mM pilocarpine or 1 mM oxotremorine induce 164 ± 14% or 157 ± 13%, respectively, of control binding after 24 h. Scatchard analysis of [^{3}H]QNB binding reveals K_d values of 48 ± 7, 63 ± 7, and 42 ± 10 pM for control, carbachol, and pilocarpine treated cell membranes, respectively, but B_{max} values of 76 ± 4, 40 ± 18, and 119 ± 16 fmol/mg protein. The effect of pilocarpine is blocked by atropine, indicating that the paradoxical up-regulation is not due to any antagonistic or partial agonist property of pilocarpine. Current studies seek to determine the specific receptor and mRNA subtypes involved in this phenomenon.

487.3

PARASYMPATHETIC DENERVATION SUPERSENSITIVITY TO MUSCARINIC AND PURINERGIC AGONISTS IN SINGLE PAROTID ACINAR CELLS. L. Tenneti* and B.R. Talamo, Neuroscience Labs, Tufts Med. Sch., Boston, MA.

Parotid acinar cells are innervated by both sympathetic and parasympathetic nerves and thus serve as a useful model to study neuronal modulation of target cell sensitivity and synaptic impulse trafficking. Although selective modulation of secretory response has been described in denervated animals, the mechanism of supersensitivity at the cellular level is largely unknown. Previously we observed that parotid cell responses to the muscarinic agonist carbachol and to extracellular ATP (P_{2Z} receptors) are sensitized after parasympathetic denervation but that the muscarinic receptor number was not altered. Fluorescence ratio imaging was used to examine cytosolic $Ca^{2+}(Ca_i)$ responses in single acinar cells prepared from control and parasympathetically denervated glands. In control preparations concentration response curves for carbachol and ATP showed that the threshold for activation varied in individual cells, indicating the presence of subsets of acinar cells with different sensitivities. Acinar cells prepared from denervated glands were sensitized to both agonists: the proportion of cells responding at low concentrations of agonists was 2-3 fold higher compared to control cells. A range of cell sizes was observed in both control and denervated cells. Following denervation there were more small cells and these cells tended to be more sensitive to agonist stimulation. Thus the lower cell volume in denervated cells which retain their full complement of receptors might in part explain the increased Ca_i response at very low concentrations of agonists. Supported by NS28556.

487.4

EFFECT OF LATE INSULIN TREATMENT INITIATED AFTER THE ESTABLISHMENT OF THE DIABETIC STATE ON RAT BLADDER DOME MUSCARINIC RECEPTORS. J. Latifpour*, Y. Fukumoto, M. Yoshida and R. M. Weiss, Yale Univ. Sch. of Med., New Haven, CT 06510.

To determine whether diabetes- and diuresis-induced alterations in muscarinic receptors in rat bladder dome are reversible, we administered insulin (started 8 weeks after the onset of diabetes) or removed sucrose from drinking water (begun 8 weeks after the onset of diuresis). Five groups of rats were maintained for 16 weeks: 1) STZ-induced diabetic (65 mg/kg i.v); 2) insulin-treated diabetic, 5-8 U/day s.c. ; 3) sucrose-fed, 5 % sucrose in drinking water throughout 16 weeks; 4) sucrose-removed, sucrose withdrawn from drinking water after 8 weeks; 5) age-matched controls. Saturation binding experiments with [^{3}H] quinuclidinyl benzilate showed an increase in the density of muscarinic receptors in bladder dome of diabetic and sucrose-fed rats compared with age-match controls. Removing the 5% sucrose from the drinking water of diuretic rats reversed the increased water intake and urine output and the upregulation of the muscarinic receptors. Late insulin treatment partially corrected the bladder enlargement, polydipsia, polyuria and the muscarinic receptor upregulation in the diabetic rat. Pharmacological evaluation indicated that the induction of diabetes did not affect muscarinic subtype specificity but did result in an alteration in muscarinic receptor-G protein coupling in rat bladder dome (*Supported in part by NIH grants DK 38311 and DK 42530*).

487.5

GABA$_A$ RECEPTOR MODULATION BY PROTEIN KINASE-A (PKA) AND β-SUBUNIT EXPRESSION IN CEREBELLAR PURKINJE CELLS. I.E. Cheun, E.V. Grigorenko and H.H. Yeh*, Dept. Physiology & Pharmacology, Bowman Gray School of Medicine, Wake Forest University, Winston-Salem, NC 27157.

Norepinephrine (NE) potentiates GABA-evoked current (I_{GABA}) in Purkinje cells acutely dissociated from the rat cerebellum. We have previously communicated preliminary data suggesting that this effect could be mimicked by components of the adenylate cyclase system, including PKA, and have postulated that this system participates in transducing the cross-talk between the β-adrenergic and the GABA$_A$ receptors. Here, we present new evidence that PKA is an intermediary in this transduction cascade. In addition, we used reverse transcription and polymerase chain reaction to profile in single Purkinje cells messages for the GABA$_A$ receptor β-subunits, one of which (β3) is a likely target for phosphorylation by PKA.

Catalytic subunit of PKA, introduced into acutely-dissociated Purkinje cells during whole-cell recording, potentiated I_{GABA} in a time-dependent manner. This effect was blocked by PKA inhibitor. Similarly, Rp-cAMP and the regulatory subunit of PKA blocked the NE-induced potentiation of I_{GABA}. In inside-out patches, PKA plus ATP increased GABA-evoked single channel activity by increasing the probability of channel opening. Under conditions that favor phosphorylation, closed time distribution shifted towards shorter durations, leaving unitary conductance level and open time distribution unaltered. Profiling GABA$_A$ receptor β-subunit mRNAs in individual Purkinje cells from which patch clamp data had been obtained revealed consistently messages for the β1 and β3 subunits but that for the β2 subunit was variable. In contrast, granule cells appear to express the β2 message exclusively.

Overall, electrophysiology combined with profiling of gene expression in single neurons is a promising approach for revealing the structural/functional bases underlying the modulation of the native GABA$_A$ receptor.

Supported by PHS NS24830, NS01340 and the Markey Pilot Project Fund.

487.6

DEPOLARIZATION UPREGULATES GABA$_A$ RECEPTOR NUMBER IN LIVING HIPPOCAMPAL SLICES. H. Tabuteau, N. de Lanerolle*, and M. Brines, Neurosurgery and Neuroendocrinology, Yale Medical School, New Haven, CT 06510

GABA is a predominant inhibitory neurotransmitter of the hippocampus. Regulation of the GABA$_A$ receptor in living preparations was studied to determine how depolarization affects the GABA$_A$ receptor and to delineate the ion dependency of this regulation. GABA$_A$ receptors were labeled using the antagonist [^{3}H]SR95531. Slices depolarized with K^+, veratridine, veratridine/glutamate (vera/glut) and ouabain produced average increases in binding of 72%, 113%, 127%, and 215% respectively. The approximate doubling of receptor number demonstrates a large reserve of GABA$_A$ receptors in the hippocampus. The response to vera/glut was accentuated in Ca^{++}-free medium (a tripling of binding), suggesting that Ca^{++} plays an inhibitory role. Depolarization with Na^+-free, 140mM K^+ medium resulted in an approximate doubling of receptor number. Thus upregulation is not Na^+-dependent. Octreotide, a stable somatostatin analog, and 6-cyclohexyl adenosine (CHA), a selective adenosine A1 agonist, blunted the vera/glut response (25% and 30% decrease in upregulation respectively). Octreotide and CHA also blunted the upregulatory response to 140mM extracellular K^+. Thus, octreotide does not decrease upregulation by hyperpolarizing the cell. Furthermore, this suggests that both octreotide and CHA inhibit an increase in GABA$_A$ receptor number by decreasing intracellular cAMP levels. Disruption of the cell membrane through freezing and thawing resulted in substantial radioligand binding but a lack of upregulation.

These data suggest that membrane potential determines GABA$_A$ receptor number. Although depolarization appears necessary for an increase in receptor number, octreotide and CHA do not inhibit up-regulation by changing membrane potential. No major extracellular ion is essential for upregulation. However, calcium, which has been implicated in excitotoxicity, inhibits upregulation.

Supported by NS27081 and NS30619.

487.7

THE PHOSPHOLIPASE A$_2$ INHIBITOR BROMOPHENACYL BROMIDE PREVENTS THE DEPOLARIZATION-INDUCED INCREASE IN ^{3}H-AMPA BINDING IN RAT SYNAPTONEUROSOMES. J. Bernard*, A. Lahsaïni, M. Baudry and G. Massicotte. Département de Chimie-Biologie, Université du Québec à Trois-Rivières, C.P.500, Québec, Canada, G9A 5H7.

The expression of long-term potentiation (LTP) in area CA$_1$ of hippocampus has been proposed to result from an increased sensitivity of the AMPA receptors. We previously demonstrated that potassium (KCl)-induced depolarization of synaptoneurosomes prepared from rat telencephalon increased ^{3}H-Amino-3-Hydroxy-5-Methylisoxazole-4-Propionate (^{3}H-AMPA) binding to the AMPA receptor. In the present study, we established optimal conditions to produce depolarization-induced increase in ^{3}H-AMPA binding in rat synaptoneurosomes and evaluated the effects of various inhibitors of calcium dependent enzymes on the regulation of AMPA receptors. The effect of depolarization on ^{3}H-AMPA binding was sensitive to temperature, as the increase in binding was present at both room (25°C) and physiological (37°C) temperatures but absent at 0°C. In addition, the effect was time- and calcium-dependent, with a maximal increase after 10-15 min in a physiological medium containing 0.5 mM calcium chloride. Treatment of intact synaptoneurosomes with the phospholipase A$_2$ (PLA$_2$) inhibitor, bromophenacyl bromide (BPB), produced a marked and dose-dependent reduction in KCl-induced enhancement in ^{3}H-AMPA binding. BPB had no significant effect on the ^{3}H-TPP accumulation in intact synaptoneurosomes, an index of membrane depolarization. In contrast, inhibitors of calcium-dependent kinases and proteases hade no significant effect on the KCl-induced increase in ^{3}H-AMPA binding. Since activation of phospholipases has been reported to play a critical role in the formation of LTP the results strengthen the hypothesis that phospholipases-induced modification of AMPA receptor properties is an important component of synaptic plasticity. This work was supported by NSERC Canada.

487.8

SELECTIVE ALTERATIONS IN [125I]IGF I AND [125I]IGF II SITES IN THE RAT HIPPOCAMPAL FORMATION FOLLOWING SYSTEMIC INJECTION OF KAINIC ACID. L. Srivastava*, S. Kar, D. Seto and R. Quirion. Douglas Hosp. Res. Ctr, Depts of Pharmacol. & Ther. & Psychiatry, McGill Univ., Montreal, CANADA H4H 1R3.

The insulin-like growth factors I and II (IGF I and IGF II) are similar in their structure with overlapping biological functions. These peptides are localized in distinct brain regions and their functions are mediated by IGF I and IGF II receptors. Both receptors are discretely distributed in various brain areas including the hippocampal formation. Functionally, IGFs are considered to play an important role in normal functions of the brain and also in response to pharmacological and surgical lesions. Following systemic injection of kainic acid (KA) to the adult rat (12 mg/kg; i.p.), the possible alterations in [125I]IGF I and [125I]IGF II binding sites, together with changes in neuronal and glial populations, were evaluated in the hippocampal formation at different time-periods (12h, 1, 2, 4 and 12 days). In the normal hippocampus, [125I]IGF I sites are concentrated primarily in the dentate gyrus (DG) and in the CA2-CA3 sub-fields whereas [125I]IGF II binding sites are discretely localized in the pyramidal layer and in the granular layer of the DG. Administration of KA, as indexed by GFAP immunostaining, induced proliferation of reactive astrocytes in all sub-fields of the hippocampal formation during 1-12 days periods. Severe neuronal damage followed by loss of neurones were evident particularly in the CA1-CA2 sub-fields during 4-12 days after injection. [125I]IGF I binding sites at early stages (12h- 2day) following KA injection were found to be decreased in CA1-CA3 sub-fields whereas in later periods (4-12 days) a marked increase was noted particulaly in CA1-CA2 sub-fields. As for [125I]IGF II binding sites, a marked depletion was noted in the pyramidal layer of CA1-CA3 sub-fields at all time periods studied. Granular layer of the DG however, did not exhibit any alteration in the density of IGF II binding sites. Thus, it appears that IGFs I and II receptors in the hippocampal formation are differentially affected following peripheral KA injection and the observed responses which coincide temporally with glial proliferation and/or neuronal degeneration possibly reflect the involvement of IGFs in kainic acid induced plasticity in this model. (Supported by MRCC).

487.9

EFFECT OF 15-HYDROPEROXYEICOSATETRAENOIC ACID (15-HPETE) ON PHOSPHOINOSITIDE (PID) TURNOVER IN SYNAPTONEUROSOMES (SN). M. M. Zaleska* and D. F. Wilson, Dept. of Biochem. and Biophys., Univ. of Pennsylvania School of Medicine, Philadelphia, PA 19104.

We have studied the interaction of lipoxygenasese metabolite of arachidonic acid (AA) with agonist-evoked PID turnover in rat brain SN. When SN are prelabeled for 60 min with [^{3}H]myo-inositol and incubation is continued in its presence, inositol phosphates (IP) accumulate linearly for up to 90 min with 1 mM glutamate (Glu), 100 uM quisqualate (Q) or 100 uM norepinephrine (NE). Accumulation of IP evoked by Glu and Q but not NE was significantly inhibited by 25 uM 15-HPETE. Concomitantly, the incorporation of [^{3}H]myo-inositol into PID was inhibited by 69% by 15-HPETE. Glu alone significantly inhibited the labeling of PID, while simultaneous presence of Glu and 15-HPETE resulted in 80% inhibition. Separation of individual lipids by HPTLC revealed that synthesis of [^{3}H] phosphatidylinositol (PI) and [^{3}H]PI monophosphate (PIP) was decreased by 60% and 50% by 25 uM 15-HPETE. Concurrent presence of Glu resulted in 77% and 50% inhibition of labeling of PI and PIP pools, respectively. The synthesis of [^{3} H]inositol lipids was not significanly affected by 100 uM NE. In order to separate the effects of 15-HPETE on PID hydrolysis from those on inositol lipid resynthesis, prelabeled SN were washed extensively before their exposure to agents. In such SN 25 uM 15-HPETE alone stimulated IP formation and did not inhibit agonist-evoked IP accumulation. Carbamylcholine-induced IP generation was not affected by 15-HPETE. It is suggested that AA oxygenation products may modulate the functional expression of excitatory amino acid metabotropic receptors by selectively reducing the availability of their lipid substrates. Supported by NS 10939.

487.10

AGING DECREASES THE DENSITY AND MODIFIES THE RESPONSIVENESS OF NICOTINE RECEPTORS IN RAT STRIATUM. Z. J. Yu, Y. N. Hsu and L. Wecker*. Department of Pharmacology, University of South Florida College of Medicine, Tampa, Florida 33612.

The effects of aging on the density and function of nicotine binding sites were studied in striata from male F-344 x BN rats 4 months, 14 months and 24 months of age. Rats received injections (s.c.) of saline or nicotine bitartrate (1.76 mg/kg) twice daily for 10 days. The affinity (Kd) and density (Bmax) of nicotine receptors were determined by measures of [^{3}H]cytisine binding to membranes and Rosenthal-Scatchard analyses; function was ascertained by measures of the ability of 10 µM nicotine and 25 mM KCl to release [^{3}H]DA from superfused striatal synaptosomes. Nicotine binding site density decreased by 20% and 30% in striata from rats aged 14 months and 24 months, respectively, as compared to tissue from 4 month-old rats; affinity was unaltered. Despite this decreased receptor density, the ability of 10 µM nicotine to release [^{3}H]DA from striata from rats aged 14 and 24 months increased by 100% relative to the response obtained in tissue from 4 month-old rats; release in response to 25 mM KCl was unaltered. The chronic administration of nicotine increased the density of nicotine binding sites by 30% in all age groups. In striata from 4 month old rats, this increased receptor density was accompanied by a 100% increased nicotine-induced release of [^{3}H]DA. In contrast, nicotine-induced [^{3}H]DA release from striata from 14- and 24-month old rats did not exhibit an enhanced response. Results indicate that normal aging is associated with a decreased density of nicotine binding sites in striatum, but an increased ability of nicotine to release [^{3}H]DA. In addition, although the up regulation of binding site density is not compromised in aged rats, there is a decrease in the ability of nicotine to release [^{3}H]DA. (These studies were supported by STRC, Inc. grant #0411.)

487.11

DIFFERENTIAL CHANGES IN D-2, D-3 AND D-4 DOPAMINE RECEPTOR mRNA LEVELS DURING AGING. A. Valerio, M. Belloni, C. Tinti, M. Memo, P.F. Spano*. Institute of Pharmacology, Department of Biomedical Sciences and Biotecnologies, University of Brescia, 25124 Brescia, Italy

One of the basic feature of aging brain is the decline of the postsynaptic decoding of dopamine signal. Particularly, several authors have reported age-related reductions of dopamine D-2 receptor number and function in various areas of rodent and primate brain. The reduction in dopamine D-2 receptors is associated with a fall by 30-40% in the dopamine content during aging. This is an intriguing finding since various experimental models have demonstrated that decreases in presynaptic input are followed by the induction of supersensitive receptors. Interpretation of these results is also hampered by the new knowledge in the dopamine receptor field. To date, dopamine receptors can be classified into two main families (D-1 and D-2), each of them including several variants. The D-2 like family comprises D-2 short, D-2 long, D-3 and D-4 dopamine receptor.

Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) technique was used to determine the steady-state levels of the mRNA encoding the different dopamine receptors in various brain areas of 6, 12, 18 and 24 month rats.

We found a progressive, age-dependent reduction in the mRNA levels of D-2 short and D-2 long dopamine receptor isoforms in corpus striatum, substantia nigra and frontal cortex. On the contrary, D-2 short and D-2 long receptor mRNA levels were unchanged in hippocampus while they increased in pituitary. D-3 receptor mRNA levels were reduced in olfactory tubercle and unchanged in striatum. D-4 receptor mRNA levels were unmodified in pituitary gland.

These data suggest that the mechanisms responsable for regulating the pattern of expression of the various dopamine receptor isoforms is differentially affected by aging.

487.12

DEVELOPMENTAL EXPRESSION OF THE A_1 ADENOSINE RECEPTOR AND G_i PROTEINS IN RAT CEREBRAL CORTEX. M. Wilson, T.W. Gettys, C.J. Moody*, V. Ramkumar. Dept. of Pharmacology, SIU School of Medicine, Springfield, IL 62794

Functions mediated via the A_1 adenosine receptor (A_1AR) are inhibited by caffeine, a methylxanthine and the active ingredient in common beverages such as coffee and cola. Methylxanthines can regulate the expression of both the A_1AR and its associated G_i proteins transducing protein, and can therefore alter their expression in the fetus and newborn during pregnancy and early postnatal development. Understanding how the A_1AR and G_i proteins are regulated during normal postnatal development is essential before the effects of methylxanthines could be explored. In this study, the developmental expression of the A_1AR and the G_i protein (α and ß subunits) in rat cerebral cortical plasma membranes were characterized using an A_1AR antagonist radioligand ([^{3}H]DPCPX). A_1AR increased from 76 ± 15 fmol/mg protein in 7 d old rats to 210 ± 48, 339 ± 42 and 348 ± 42 fmol/mg protein 17 d, 3 months and 19 months postnatally without changes in receptor affinity (K_d values ranging from 0.48 to 0.51 nM). No change in the A_1AR/G_i protein interaction was observed during development, as determined from computer modeling of R-PIA (agonist) competition curves. Western blotting for the $G_{\alpha i}$ and ß subunits indicate significant increases in the expression of these proteins during development. Levels of α_{i1}, α_{i2}, α_{i3} and ß subunits increased 5.2, 3.2 7.6 and 7.3 fold, respectively, from postnatal day 7 to 3 months. Interestingly, high levels of transcripts encoding these G_α subunits could be detected on postnatal day 7. These data suggest that the A_1AR and its associated G proteins are coordinately regulated during development in order to maintain efficient functional coupling during postnatal development.

487.13

DIFFERENTIATION OF NG-108 NEUROBLASTOMA CELLS VIA SERUM STARVATION OR DIMETHYL SULFOXIDE (DMSO) TREATMENT YIELDS MARKED DIFFERENCES IN ANGIOTENSIN II RECEPTOR EXPRESSION. K.J.N. Seidman*, J.H. Barsuk, R.F. Johnson, and J.A. Weyhenmeyer. Neuroscience Program, University of Illinois, Urbana, IL 61820.

Neuroblastoma cells can be differentiated by various methods, including serum starvation and dimethyl sulfoxide (DMSO) treatment. Differentiation of NG-108 cells under conditions of serum starvation results in extensive neurite extension that is not seen following DMSO treatment. Recently, angiotensin (ANG) receptor expression in NG-108 cells has been reported to be dramatically upregulated following differentiation with 1.5% DMSO and low serum (0.5%) (Bryson, S.E., *et al.*. Eur. J. Pharm. 225:119). In the present study we investigated whether serum starvation or DMSO/low serum, which give rise to very different culture morphologies, differentially influenced ANG receptor expression in NG-108 cells.

K_D values for all treatment conditions were not significantly different (0.64 ± 0.12 nM). Prior to treatment, the Bmax for undifferentiated cells was 26.8 ± 9.29 fmoles/mg. Treatment for 3 days with DMSO/low serum or serum starvation yielded Bmax values of 212 fmoles/mg and 24.1 ± 8.9 fmoles/mg, respectively ($p<0.005$), while treatment for 4 days yielded Bmax values of 607 ± 139 fmoles/mg and 50.33 ± 4.91 fmoles/mg, respectively ($p< 0.001$). Serum starved cells never showed the dramatic increase in ANG receptor expression (after serum starvation for 7 and 14 days Bmax values were 98.67 ± 13.5 fmoles/mg and 108 fmoles/mg, respectively). Previous studies indicate that most of the ANG receptor upregulation observed in DMSO treated NG-108 cells is accounted for by AT_2 receptor upregulation (Bryson, *et al.*), and we are currently investigating individual receptor subtype expression in DMSO treated and serum starved cells.

Clearly, the differentiation paradigm significantly influences ANG receptor expression. Whether these agents alter the synthesis of ANG receptors at the transcriptional, translational, or post-translational levels is under investigation.

K.J.N. Seidman is supported by NSF Fellowship GER-9253864.

487.14

EFFECT OF THE NEUROTENSIN RECEPTOR ANTAGONIST SR48692 ON THE EXPRESSION OF NEUROTENSIN AND NEUROTENSIN RECEPTOR mRNA IN RAT BRAIN.

C. Bolden-Watson*, M. Watson and E. Richelson, Dept. of Psychiatry and Psychology, and Pharmacology, Mayo Clinic Jacksonville, Jacksonville, FL 32224.

We examined the effect of chronic (two weeks) treatment with the selective, nonpeptide neurotensin receptor antagonist, SR48692 (Gully et al., PNAS, 90:65, 1993), on neurotensin (NT) and neurotensin receptor mRNA expression by *in situ* hybridization histochemistry. Quantitative optical density analysis showed SR48692 increased NTR mRNA levels in the substantia nigra/ventral tegmental area over that seen in control tissue. In contrast, the same treatment did not affect the expression of NT mRNA in rat brain. These results are similar to the effects observed in our previous study with haloperidol in rats. Two week treatment with haloperidol resulted in an increase in the expression of NTR mRNA but not NT mRNA. Others have shown acute treatment with haloperidol dramatically increases the expression of NT mRNA in rat brain. We are currently investigating the acute effects of SR48692 on NT and NTR mRNA expression. [Support: Mayo Foundation and U.S.P.H.S. grants MH10104 (C.B.) and MH27692 (E.R.); SR48692, batch no. 9200VI, from Sanofi Recherche].

487.15

EFFECT OF OROSENSORY DEPRIVATION ON NEUROPEPTIDE Y RECEPTOR POPULATION. M. C. Lee*, J. Grant, C. Clark, P. Mannon, T. N. Pappas. Departments of Surgery and Medicine, Duke Univ. Sch. of Med., Durham, NC 27710.

Centrally administered neuropeptide Y (NPY) is a known potent orexigenic, which produces a robust feeding behavior in satiated rats. Studies have demonstrated that NPY peptide levels in the paraventricular nucleus (PVN) fluctuate with different oral diets (eg. food-deprivation, hyperphagia). The purpose of our study was to examine whether orosensory deprivation alters NPY receptor levels in the rat brain. Fisher 344 rats were randomized to receive total parenteral nutrition (TPN) or saline as control. Half of the saline-infused rats were allowed to feed on standard rat chow to maintain their weights (sal-fed), and the other half was food-deprived (sal-dep). The third group of rats was orosensory-deprived, but they were given calories intravenously (tpn-dep). After 72 hours of continuous infusion, the rats were sacrificed and their brains were removed for NPY receptor autoradiographic studies. The NPY receptor population was measured by densitometry in four brain regions and is expressed in nCi/mg (*p<0.05; **p<0.005).

	paraventricular nucleus	thalamus	dentate gyrus	olfactory cortex
SAL-DEP	1.51±0.15	1.55±0.10	0.25±0.10	1.91±0.25
SAL-FED	1.40±0.20	1.60±0.13	0.41±0.13	2.15±0.33
TPN-DEP	2.29±0.19**	2.06±0.13*	1.01±0.13**	3.07±0.29*

NPY receptor density was significantly increased in TPN-infused rats compared to saline controls in the brain regions examined. We conclude 1) NPY receptor population is increased in these particular brain areas after 72 hours of orosensory deprivation accompanied by intravenous calorie infusion, and 2) orosensory deprivation without intravenous calories does not produce a significant change in NPY receptor number, perhaps due to diminished protein availability from increased catabolism. This data suggests that the absence of an oral diet with intravenous feeding results in an increase in NPY receptor number in the rat brain. This is consistent with the hypothesis that orosensory deprivation without concomitant weight loss may lead to decreased NPY peptide levels, resulting in an initial compensatory upregulation of NPY receptors in the rat brain.

487.16

HYPOTHALAMIC ESTROGEN RECEPTOR mRNA IN NORMAL AND NEONATALLY ANDROGENIZED FEMALE AND CASTRATED MALE RATS. L. Givon*, D.L. Hurley and A.A. Gerall. Depts. of Psychology and Cell and Molecular Biology, Tulane University, New Orleans, LA 70118.

Perinatal androgen exposure renders the female rat anovulatory and non-receptive, while early castration enables the male rat to manifest female sexual behaviors. These reproductive processes are mediated by the estrogen receptor (ER). Basal levels of ER mRNA in selected brain sites were higher in female than male rats and decreased by exogenous estrogen (Lauber et al., 1991; Simerly & Young, 1991).

The present study investigated the effects of adult estrogen on ER mRNA in female rats injected sc on day 4 with either 1.25 mg testosterone propionate (TP), 50 µg TP or sesame oil, and male rats castrated at 6 hr, day 5 or day 34. The females were ovariectomized postpuberally. When 5 mo. old, half of the rats were injected with 9.9 µg estradiol benzoate (EB) and the remainder with sesame oil, and 18 hr later all were anesthetized and their brains frozen at -100°C. *In situ* hybridization (Simerly & Young, 1991) was performed on 25 µm thick coronal brain sections using ^{35}S-labeled 850 nt antisense probe (provided by R. Simerly, ORPRC) to detect ER mRNA. Relative hybridization intensities were quantified from x-ray films in the anteroventral periventricular nucleus (AVPv), medial preoptic area (MPOA), arcuate nucleus and ventromedial nucleus (VMN).

Lordosis quotients for the 1.25 mg TP, 50 µg TP and control females were 0%, 85% and 100%, respectively; and for the 34 day, 5 day and 6 hr castrated males were 0%, 37% and 80%, respectively. In females, neonatal androgen decreased basal levels of ER mRNA in the AVPv and VMN. Adult EB injection decreased ER mRNA in the AVPv and MPOA. Analysis of data from males is in progress. Supported by grants from the Flowerree Fund to L.G. and a Faculty Development Grant to DLH.

487.17

REGULATIONOF RETINALPRESYNAPTIC EP_3-RECEPTORS BY ENDOGENOUS PROSTAGLANDINS. Al-Zadjali,K. and Ohia, S.E.*: *Dept. of Pharmaceutical Sciences, Creighton University, Omaha, Nebraska*

We have previously shown that exogenous prostaglandins (PG) inhibit dopamine (DA) release from the rabbit retina via an effect presynaptic EP_3 receptors. (Al-zadjali et al. Invest. Ophthal. Vis. Sci. 34:1047, 1993). Since there is evidence that endogenously generated PGs may regulate neurotransmitter release, the present study investigated the effects of intramurally produced PGs on the responsiveness of presynaptic EP_3-receptors to exogenous agonists. Neural retinas from albino rabbits were incubated in oxygenated Krebs solution containing 0.15 μM [^{3}H]DA at 37°C for 20 mins and then pre-pared for superfusion studies. Release of [^{3}H]DA was elicited by electrical field stimulation applied at 55 (S_1) and 91 (S_2) mins. after onset of superfusion. Application of the cyclo-oxygenase inhibitor, flurbiprofen (FBF, 3μM) (24 mins. before S_2), had no effect on basal or evoked [^{3}H]DA overflow. Inhibition caused by PGE_2 (3 nM) was increased from 31.9 ± 6.0% (n = 6) to 36.4 ± 3.2% (n = 4) in the presence of FBF (3 μM) (P = 0.6) We conclude that endogenously produced PGs are not involved in basal or evoked [^{3}H]DA release in the retina nor do they alter the responsiveness of presynaptic EP_3-receptors to exogenous agonists. (Supported by PMA Foundation)

487.18

INTRAOCULAR TETRODOTOXIN (TTX) REDUCES 2-[^{125}I]-IODOMELATONIN BINDING IN SELECTED VISUAL AREAS OF THE CHICK BRAIN.
M.L. Dubocovich[1]*, S. Iacob[1], F.S.-P. Yao[1], D.N. Krause[2], Depts. Pharmacol., Med. Sch., [1]Northwestern Univ., Chicago, IL 60611 and [2]Univ. of Calif., Irvine, 92717.

Unilateral transection of the chick optic nerve (ON) significantly decreases specific 2-[^{125}I]-iodomelatonin binding to ML-1 melatonin receptor sites in many, but not all, of the target brain retinorecipient areas and throughout the tectofugal visual pathway, i.e., optic tectum (OT), thalamic nucleus rotundus (ROT), and telencephalic ectostriatum (E) (Krause, Siuciak, Dubocovich, 1993). To determine whether these changes were the result of blockade of electrical activity in the ON, chicks (3 weeks old) were injected every 3 days with 6 ug TTX in one eye and saline/vehicle in the other eye. Chicks were sacrificed after a period of 7 days, during which the pupillary reflex remained suppressed in the experimental eye. Brain sections were processed for quantitative receptor autoradiography using 90 pM 2-[^{125}I]-iodomelatonin. Specific binding was defined using 1uM melatonin. Because the majority of the ON fibers cross in the chiasm, the areas ipsilateral to the TTX-injected eye served as a control. Inhibition of retinal electrical activity by TTX for 7 days significantly reduced specific 2-[^{125}I]-iodomelatonin binding in the contralateral OT (61 ± 3% of ipsilateral OT), the ROT (40 ± 1%), the isthmi nuclei (67 ± 2%) which also receive tectal projections, and the E (67 ± 5%). In contrast, TTX treatment had no effect on binding in the ON or primary retinorecipient areas, i.e., the ventrolateral geniculate nucleus and tectal gray, where ON transection dramatically reduced binding. The latter findings may reflect nerve degeneration resulting in loss of axonal presynaptic melatonin receptors, while the results with TTX suggest that expression of melatonin receptors in the tectofugal pathway and other tectal projections is influenced by the visual input to these pathways. [Supported by MH 42922 to MLD]

487.19

INDUCTION OF OLFACTORY RECEPTOR SENSITIVITY IN MICE.
H.-W. Wang, C. J. Wysocki and G. H. Gold*. Monell Chemical Senses Center, 3500 Market Street, Philadelphia, PA 19104.

Exposure to androstenone can induce sensitivity to that odorant in humans with specific anosmia to androstenone (Wysocki et al., 1989). Recordings of the olfactory receptor potential (EOG) were used to determine if induction can occur at the receptor cell level in mice. Androstenone-insensitive (NZB/B1NJ) and -sensitive (CBA/J) strains were chosen as animal models for androstenone-insensitive and -sensitive individuals, respectively, based on their behavior in conditioned aversion assays. In NZB/B1NJ mice, 2-4 weeks of 16 hr daily exposure to androstenone caused up to a 3.6-fold increase in the EOG response amplitude to androstenone, but did not significantly affect responses to isoamyl acetate. However, in CBA/J mice androstenone exposure had no effect on response amplitudes to either odorant. Similar induction of iso-valeric acid sensitivity was demonstrated in an iso-valeric acid-insensitive strain (C57BL/6J). Thus, induction of olfactory sensitivity in mice: 1) occurs at least in part at the receptor cell level, 2) occurs only in strains that initially have low sensitivity to the exposure odorant, and 3) increases sensitivity specifically for that odorant. In view of the odorant specificity of induction, it may reflect increased expression of olfactory receptor proteins with a high affinity for the exposure odorant. If so, olfactory induction may constitute a novel form of stimulus-regulated gene expression.

HYPOTHALAMIC-PITUITARY-ADRENAL AXIS REGULATION: POMC AND STEROID RECEPTOR STUDIES

488.1

THE CELLULAR REGULATION OF POMC: THE ROLE OF *c-fos* AND *c-jun*. **B.B. Ruzicka* and H. Akil, Mntl Hlth Res Inst, University of Michigan, Ann Arbor, Michigan, 48109-0720.**

In this study, a murine anterior pituitary cell line (AtT20) was used as the model system to examine the role of the immediate-early genes (IEGs), *c-fos* and *c-jun*, in the cellular regulation of pro-opiomelanocortin (POMC). AtT20 cells were plated in DMEM+10% FCS and grown until ~60% confluent. Subsequently, the medium was replaced with DMEM+2.5% FCS and the cells were incubated for a further overnight period. The cells were then exposed to corticotropin releasing factor (CRF, 10nM) or interleukin-1ß (IL1ß, 0.5nM) for various times (5min-48h) and analyzed for levels of ß-lipotropin (ßLPH) and ß-endorphin (ßE) secretion and content and POMC, *c-fos* and *c-jun* mRNA. Both CRH and IL1ß modulated ßLPH and ßE secretion and content in a time-dependent manner. CRH only weakly stimulated ßLPH secretion, but enhanced ßE release in a biphasic fashion: peak responses, reflecting 4- and 2-fold increases in ßE release occurred at 30min and 8h of CRH exposure, respectively. Generally, the CRH-evoked changes in peptide secretion were inversely related to the CRH-elicited changes in peptide content. In contrast, IL1ß preferentially stimulated ßLPH, rather than ßE secretion: the peak response, reflecting a 2-fold increase in ßLPH release, occurred after 24h of IL1ß exposure. IL1ß had little effect on peptide content. CRH and IL1ß produced no apparent change in POMC mRNA, but, at 1h, maximally elevated POMC heteronuclear RNA by 20% and 30%, respectively. Following a 1h exposure, CRH and IL1ß also maximally increased *c-fos* mRNA by 6- and 3-fold, respectively. Additionally, a 1h exposure to IL1ß, but not CRH, maximally elevated *c-jun* mRNA by 70% . These data show that CRH and IL1ß differentially modulate POMC peptide secretion, content and IEG expression in AtT20 cells. Experiments conducted with antisense *c-fos* and *c-jun* DNA should provide more conclusive data regarding the role that these IEGs play in the cellular regulation of POMC. Supported by NIMH grant PO1MH42251. B.B.R. is the recipient of a Medical Research Council of Canada post-doctoral fellowship.

488.2

REGULATION OF PITUITARY GLAND PRO-OPIOMELANOCORTIN IN HYPERTENSIVE AND HYPERACTIVE RATS. K. M. Braas*, E. D. Hendley and V. May. Depts. of Anatomy & Neurobiology and Molecular Physiology & Biophysics, Univ. of Vermont College of Medicine, Burlington, VT 05405.

Pro-opiomelanocortin (POMC) levels are changed in pituitary glands from spontaneously hypertensive rats (SHR) compared to normotensive Wistar Kyoto (WKY) animals. Previously, to determine whether altered POMC expression was associated with the hypertensive or hyperactive trait of the SHR, we examined peptide levels in two genetically related strains: WKHT is only hypertensive while WKHA is only hyperactive. Decreased anterior pituitary gland POMC peptide content was associated with the hypertensive trait, while increased intermediate pituitary gland levels of POMC peptides were associated with the hyperactive trait. We have further examined the cellular basis for these tissue-specific changes in POMC expression. Anterior pituitary corticotrope POMC mRNA levels, determined by Northern blot analysis, were decreased 55% in SHR and WKHT rats compared to WKY rats. Immunocytochemically stained corticotrope cell numbers were decreased 40 to 45% in SHR and WKHT tissues. Using *in situ* hybridization, the number of cells expressing POMC mRNA was decreased approximately 40% in the hypertensive SHR and WKHT rat strains, suggesting that the decreased POMC expression results primarily from decreased corticotrope cell number. Intermediate pituitary melanotrope POMC mRNA levels were increased approximately 300% only in the hyperactive SHR and WKHA strains. Morphological studies indicate that the increased intermediate pituitary POMC expression in the hyperactive rats may be the result of increased melanotrope number. Additional studies are required to determine whether there are changes in corticotrope or melanotrope responsiveness to secretagogues in the hypertensive and hyperactive rat strains. (PHS F07RR05429, NS26390, and NSF9010044)

488.3

REGULATION OF CYTOSOLIC CALCIUM IN FURA-2-LOADED CULTURES OF ANTERIOR PITUITARY CORTICOTROPE CELLS (AtT-20/D16v). J. F. Fiekers* Dept. Anatomy & Neurobiology., Univ. Vermont College of Medicine, Burlington, VT 05405.

Agonist-induced changes in $\{Ca^{2+}\}_i$ in neuroendocrine cells depend on either extracellular Ca^{2+} entry or release of Ca^{2+} from internal stores (Fiekers, et. al., Soc. Neurosci. Abstr., 1992). The present experiments examined the mechanisms of $\{Ca^{2+}\}_i$ regulation in single cells from cultures of anterior pituitary (AtT-20/D16v) cells. $\{Ca^{2+}\}_i$ was monitored in individual corticotropes by dual excitation microspectrofluorometry using fura-2. Elevated $\{K\}_o$ produced an increase in $\{Ca^{2+}\}_i$ which rapidly reached a peak and declined to resting levels following exposure. The response was abolished by either extracellular Co^{2+} (2mM) or antagonists of L-type voltage-gated Ca^{2+} channels but was unaffected by TTX. The amplitude, but not the duration, was significantly increased with elevated $\{Ca^{2+}\}_o$. Exposure of the cells to potassium cyanide produced a slow elevation of $\{Ca^{2+}\}_i$ which was independent of $\{Ca^{2+}\}_o$. 40% of the cells responded with an increase in $\{Ca^{2+}\}_i$ in response to 10 mM caffeine. Caffeine responses decreased with subsequent exposure even when primed by a previous K-depolarization. These results suggest that Ca^{2+} entry through voltage-gated Ca^{2+} channels is an important pathway for regulating $\{Ca^{2+}\}_i$, and that cyanide- and caffeine- sensitive intracellular stores are potential sites for Ca^{2+} release and sequestration in these corticotropes.

488.4

EFFECTS OF HEAVY METAL DIVALENT CATIONs ON TRANSCRIPTION OF PROOPIOMELANOCORTIN IN AtT-20 CELLS. R. A. Ashton, N. Levin* and J. L. Roberts. Dr. Arthur M. Fishberg Research Center for Neurobiology, Mount Sinai Medical Center, New York, NY 10029.

Corticotropin releasing hormone (CRH) induces a rise in the intracellular concentrations of cAMP and Ca^{++}, followed by an increased rate of proopiomelanocortin (POMC) transcription. In preliminary studies, this stimulation was abolished by lowering the intracellular concentration of Ca^{++}. Further lowering of intracellular Ca^{++} led to a decrease in the basal transcription rate of POMC, suggesting that the two Ca^{++}-dependent processes may be differentially regulated (Lorang and Roberts, submitted). Additional studies indicated that POMC transcription was also inhibited by Cd^{++} and Co^{++} through a mechanism that does not involve blockade of Ca^{++} channels. The purpose of the current work is to characterize the mechanism by which Ca^{++} ion modulates the basal level of transcription of the POMC gene in pituitary, using the AtT-20/D16-16 mouse corticotroph cell line as a model system. We have used RNase protection assays of POMC primary transcript as an indirect measure of the effects of divalent cations on POMC transcription rates, utilizing a probe that spans exon 2 of the mouse POMC gene, extending into introns A and B. Cells were incubated for one hour in serum-free media prior to addition of varying concentrations of ions (chloride salts). Incubation with Zn^{++} or Cd^{++} caused a dose-dependent decrease in the level of primary transcript (IC_{50} = 60 and 50 µM, respectively), with a greater than 90% decrease at 500 µM. Co^{++} was less potent, leading to a 25% reduction in primary transcript levels at 500 µM, the highest concentration tested. The reduction of POMC primary transcript levels following incubation with selected divalent cations suggests that the transcriptional complex has been inhibited.

488.5

NUCLEAR VS. CYTOPLASMIC GLUCOCORTICOID RECEPTOR IMMUNOREACTIVITY IN HIPPOCAMPUS OF ADRENALECTOMIZED AND INTACT RATS: DIFFERENT PROFILES WITH TWO DIFFERENT ANTIBODIES. R.L. Spencer*, A.H. Miller and B.S. McEwen. Lab. of Neuroendo., Rockefeller Univ., and Dept. Psychiatry, Mt. Sinai Sch. of Med., New York, NY.

A widely accepted, although not fully supported, model of glucocorticoid receptor (GR) function asserts that the unoccupied form of the receptor resides in the cytoplasm, whereas the hormone activated form of the receptor translocates to the nucleus. It may be possible to use the relative pattern of nuclear vs. cytoplasmic GR immunostaining present during different hormone conditions to assess the differential occupation/activation of GR by glucocorticoids. We have examined the patterns of hippocampal GR immunostaining (Vector Labs ABC kit) present in adrenal-intact and 3 d ADX male Sprague-Dawley rats. Two GR reactive antibodies were compared (GR#57 and BUGR2, Affinity Bioreagents). Interestingly, the two antibodies gave a different profile of immunostaining, with GR#57 exhibiting mostly nuclear immunostaining in the adrenal-intact rat and mostly cytoplasmic staining in the ADX rat. On the other hand, with this duration of ADX, BUGR2 exhibited predominantly nuclear immunostaining in both cases, although the immunostaining was stronger in the intact rats. This suggests that antibodies recognizing different epitopes of GR may differentially bind to the receptor depending, not only on the activation state of the receptor, but also on the nuclear vs. cytoplasmic location of the receptor. This prospect is being further explored with western blots and immunocytochemistry of cells treated with different hormone conditions in vitro. (Supported by MH47674 and the McArthur Foundation)

488.6

NGFI-A AND GLUCOCORTICOID RECEPTOR mRNA EXPRESSION IS INDUCED IN THE HIPPOCAMPUS AFTER ENVIRONMENTAL ENRICHMENT IN ADULT RATS. T. Olsson, A.K. Mohammed[1], K.L. French, and J.R. Seckl* Dept Med, West Gen Hosp, Edinburgh Univ and [1]Dept Ger, Huddinge Hosp, Stockholm.

Neonatal manipulations that permanently increase hippocampal glucocorticoid receptor (GR) expression prevent age-related cognitive deficits. In adult rats we recently found that chronic environmental enrichment increases hippocampal nerve growth factor (NGF) concentrations and also improves cognitive performance. We have now evaluated hippocampal GR and mineralocorticoid receptor (MR) gene expression in response to environmental manipulation. We also studied a possible mediator of changes in GR expression, the NGF-induced immediate early gene-transcription factor NGFI-A (zif268, krox24). Adult male Sprague-Dawley rats were housed in enriched or isolated environments for 30 days. Expression of mRNAs encoding GR, MR and NGFI-A was determined by *in situ* hybridization. The enriched environment was associated with significantly greater GR mRNA expression in pyramidal neurons of CA1 (28% rise), CA2 (76%) and CA4 (44%) but GR gene expression was not altered in other hippocampal subfields, including the dentate gyrus, or in parietal cortex neurons. MR mRNA expression was unchanged. There was a 55% increase in NGFI-A gene expression in the CA2 region ($p<0.01$) with non-significant increases in CA1 and CA4 regions. Thus, environmental enrichment increases GR gene expression in specific hippocampal subfields in adult rats. These effects may be mediated through NGF induction of transcription factors, including NGFI-A.

488.7

LOCALIZATION OF THE MINERALOCORTICOID RECEPTOR mRNA 5' SPLICE VARIANTS IN THE DEVELOPING HIPPOCAMPUS. D.M. Vázquez*, S.P. Kwak, J.F. López, S.J. Watson and H. Akil. Mental Health Research Institute and Department of Pediatrics, University of Michigan, Ann Arbor, MI 48109-0720.

Our laboratory has previously reported an MR cDNA clone from rat hippocampus (HC) which shares a high degree of homology with the kidney MR rat clone throughout the coding and 3' untranslated region (Patel et. al. 1992). However, these two clones differ significantly at the 5' untranslated region (5' UT). These clones have been named alpha 5' UT (kidney cDNA clone) and beta 5' UT (HC cDNA clone). Subsequent studies have shown that these 5' UT regions are located on separate exons of a single gene and give rise to two mRNAs that encode the same protein. Both alpha and beta MR mRNA forms are found in the adult HC and have a distinct regional distribution (Kwak et. al. 1993). We were interested in investigating the presence of these distinct MR mRNA forms in the developing rat HC. In situ hybridization with specific alpha and beta cRNA probes was performed in the HC of post natal day (PND) 10, 18, 28, 35 and 60 (adult) animals. We found that alpha is highly expressed in the CA2 subfield compared to other regions. This high expression is more evident in the PND 10 animal. The beta form is evenly expressed over all pyramidal cell subfields. A higher level of beta is evident over the granular cells of the dentate gyrus in all ages, except in PND 10. Our results, therefore, show that the alpha and beta 5' UT forms achive an adult pattern of distribution in the HC after PND 10. This coincides with the emergence of the developing animal from the Stress Hypo-Responsive Period (SHRP). Supported by NIMH MH09720 and MH422251.

488.8

ONTOGENY OF FORSKOLIN-STIMULATED CYCLIC AMP (cAMP)-DEPENDENT PROTEIN KINASE, AND OF DEXAMETHASONE SUPPRESSION OF FORSKOLIN-STIMULATED cAMP PRODUCTION IN RAT ANTERIOR HYPOTHALAMUS. S.-J. Yi and T.Z. Baram. Division of Neurology, Childrens Hospital Los Angeles and University of Southern California, Los Angeles, CA 90027.

An major regulatory mechanisms of corticotropin releasing hormone (CRH) synthesis in the adult hypothalamic paraventricular nucleus (PVN) is a negative feedback via local glucocorticoid (GC) receptors. The cAMP-cAMP-dependent protein kinase (cA-PK) second messenger cascade has been shown to participate in signal transduction of GC modulation of CRH gene expression. This GC-induced pathway, leading to suppression of the CRH gene promoter may not be operative in the neonatal rat, since we have previously shown that CRH gene expression was not regulated by GC before the 9th postnatal day, despite the presence of GC receptors in the PVN. This study was performed to examine whether GC altered cAMP accumulation and cA-PK activity in the anterior hypothalamus of neonatal and adult rats.

We found that forskolin (10 uM) increased cAMP production 10-fold in the adult rat and 3 to 6-fold in 3 to 13 day-old neonatal rats. Dexamethasone inhibited forskolin-stimulated cAMP accumulation in the anterior hypothalamus of adult rats. The suppression of forskolin-induced cAMP production by dexamethasone was considerably reduced in the anterior hypothalamus of 9 - 13 day-old rats, and was negligible in 3 - 4 day-old rats. A stimulatory effect of forskolin (10 uM) on cA-PK activity was seen in adult and 12 - 18 day-old rats, but not 4 -11 day-old neonatal rats. These results indicate that "uncoupling" of GC receptors with cAMP-cA-PK second messenger cascade, a crucial element of the signal transduction pathway between GC and the CRH promoter, may underlie the lack of down regulation of CRH synthesis by GC in the neonatal rat PVN.

488.9

GLUCOCORTICOID RECEPTOR mRNA ONTOGENY IN THE FETAL AND POSTNATAL RAT BRAIN. T.Z. Baram*, S.-J. Yi and J.N. Masters. Div. Neurology, Childrens Hospital Los Angeles; Dept. Neurology University of Southern California, Los Angeles, CA 900027 and Biotechnology Center, Ohio State University, Columbus OH 43210.

Glucocorticoid receptor (GR) localization has been well studied in postnatal rat brain. However, the ontogeny of GR distribution in fetal brain has not been reported. This study focuses on the distribution of GR-mRNA in the fetal and postnatal rat, with emphasis on hypothalamic and limbic structures. Time pregnant rats (day 0 defined by sperm-positive smear), were decapitated at 8:30-9:30 am on fetal days 14 (F14), F16, F17, F18, F19 and postnatal days 1,5,7 and 12. Fetuses were decapitated and heads quick-frozen. For postnatal rats, brains were removed from skulls, then frozen on dry-ice. Cryostat sections (20u) were subjected to in situ hybridization (ISH), using a cRNA probe directed to the GR-mRNA. GR-mRNA was detectible in the fornices as early as F14. By F16, GR gene expression was evident in the hypothalamic paraventricular nucleus (PVN). During late gestation (F17-F19), GR-mRNA was detectible also in thalamus, ventromedial hypothalamus amygdala and discrete cortical regions. Subsequent to birth, GR-mRNA was clearly localized to the CA1 hippocampal field, piriform cortex and dorsal endopiriform nucleus, and specific amygdaloid nuclei; the suprachiasmatic hypothalamic nucleus contained GR-mRNA as well. Semiquantitative analysis revealed much higher GR-mRNA abundance in the piriform cortex and PVN than in most other structures. In PVN, GR-mRNA is detectible prior to the onset of CRH gene expression (F17), which may suggest a role for GR in neuronal differentiation. [Supported by NIH NS 01307, 28912 (TZB) and AG 09425 (JNM)].

488.10

RAPID GLUCOCORTICOID EFFECTS ON CALPAINS IN RABBIT HIPPOCAMPUS. K. Ostwald* and J-O. Karlsson. Institute of Neurobiology, Medicinaregatan 5, S-413 90 Göteborg, Sweden.

Ten pairs of adult New Zealand White rabbits were given a single, intramuscular injection of betamethasone (0.3 mg/kg) or saline, and were sacrificed at various intervals between 20 min. and 86 h. after the injection. The soluble and particulate fractions from the hippocampi were assayed for calpain activity. The soluble fraction was also assayed for calpastatin activity, and subjected to hydrophobic interaction chromatography. All fractions were assayed for calpain activity and immunoreactivity (ELISA), and the total amount eluted was compared with the control animal. Major peaks were further subjected to anion exchange HPLC, revealing no changes in chromatographic behavior after glucocorticoid treatment. Just 20 min. after the injection, calpain activity in the soluble fraction was reduced to 80 % of control, but increased to 126 % in the particulate fraction, gradually returning to control levels after 4 h. The level of μ-calpain immunoreactivity decreased to 46 % of control after 20 min., and returned to control level after 80 min. The level of m-calpain immunoreactivity decreased at first to 80 % of control, increased to 150 % after 80 min., and returned to control level after 4 h. These results indicate that glucocorticoids have a rapid effect on the calcium-activated proteases in the hippocampus.

488.11

REGULATORY CHANGES IN CORTICOSTEROID RECEPTOR AND ACTH SECRETAGOGUE mRNA EXPRESSION WITH CHRONIC STRESS. C.M. Meyers*, D.H. Adams and J.P. Herman. Dept. of Anatomy and Neurobiology, University of Kentucky Medical Center, Lexington, KY 40536-0084.

Chronic stress affects stress-hormone secretion by the hypothalamic-pituitary-adrenal (HPA) axis in a long term fashion. Stress may exert these HPA actions via modulation of brain corticosteroid receptors. The present studies were designed to assess the effects of chronic stress on GR and MR mRNA in discrete hippocampal subfields and ACTH secretagogue biosynthesis in stress-integrative neurons of the paraventricular nucleus (PVN). Groups of rats were chronically stressed using variable stressor paradigms (bi-daily exposure to cold, warm water swim, cold water swim, crowding, vibration, isolation). Control rats were handled bidaily. Adrenal weight and basal corticosterone secretion were significantly increased in the chronically stressed animals , verifying the efficacy of the stress procedure. Semi-quantitative *in situ* hybridization histochemistry was used to assess regulatory changes in PVN and hippocampus. In response to chronic stress, GR mRNA was significantly down regulated in the dentate gyrus (DG) and the frontoparietal cortex. No change was seen in CA3 or ventral subiculum (VSub). MR mRNA was significantly down regulated in CA1, DG, and VSub. There was a marginal decrease in CA3, but no significant change was noticed in CA2 or frontoparietal cortex. CRH mRNA was upregulated in the PVN in chronically stressed animals (50% increase). Thus, chronic stress exerts an increase in ACTH secretagogue biosynthesis and a subfield-specific down-regulation of hippocampal corticosteroid receptor expression. The relationship between these stress-induced changes in corticosteroid receptor mRNA and HPA function remains to be determined. (Supported by MH 49698)

488.12

RAPID DOWN-REGULATION OF MINERALOCORTICOID RECEPTOR HETERONUCLEAR (hn) RNA BY ACUTE STRESS. J.P. Herman *, P.D. Patel and S.J. Watson. Dept. of Anatomy and Neurobiology, Univ. of Kentucky, Lexington, KY 40536-0084, and Mental Health Research Institute, Univ. of Michigan, Ann Arbor, MI 48109-0720.

The hippocampal mineralocorticoid receptor (MR) serves to interpret circulating levels of glucocorticoids into appropriate neuronal responses. In recent years, this receptor has been implicated in tonic regulation of hypothalamo-pituitary-adrenocortical stress (HPA) axis and in regulation of neuroendocrine stress responses. The present studies utilized an intronic *in situ* hybridization paradigm to assess regulation of the MR gene by stressful stimuli. *In situ* hybridization experiments were performed using cRNA probes complementary to a coding-region intron of the rat MR gene, or to the 3' coding region-untranslated domain of rat MR mRNA. In normal rats, the distribution of MR hnRNA overlapped that of MR mRNA completely. MR hnRNA was confined to the cell nucleus, and was easily detectable, suggesting either a high ongoing MR transcription rate or a stable MR splicing intermediate in the nucleus. For stress experiments, rats were placed in restraint cages and sacrificed 30 m, 1 h or 2 h later, with unstressed rats serving as controls. MR hnRNA was significantly decreased by some 50% in all hippocampal subfields following 1 h or 2 h of stress; in contrast, no changes in MR mRNA were observed during this period, suggesting a lag between transcription and changes in the cytoplasmic message pool. These results suggest a rapid decrease in MR gene transcription following stress; whether this decrease is associated with changes in circulating glucocorticoids is presently being determined. In all, the results indicate that stress can induce changes in corticosteroid receptor production and thereby, the manner in which hippocampal cells process steroid-related information. (Supported by MH49698 and MH42251).

488.13

EFFECTS OF AGING ON CORTICOSTEROID RECEPTOR, CRH AND AVP mRNA EXPRESSION IN HPA REGULATORY CIRCUITS. D.H. Adams, M.I. Morano, H. Akil, J.D. Porter* and J.P. Herman. Dept. of Anatomy and Neurobiology, Univ. of Kentucky, Lexington, KY 40536, and Mental Health Research Inst., Univ. of Michigan, Ann Arbor, MI 48109.

Aging has deleterious effects on regulation of the hypothalamo-pituitary-adrenocortical (HPA) stress response and on hippocampal corticosteroid receptor expression. The documented connection between the hippocampus and HPA regulation mandates careful correlation of age-related changes in hippocampal corticosteroid receptor regulation and expression of ACTH secretagogues. To approach this problem, the present studies used semi-quantitative *in situ* hybridization analysis to assess regulation of mRNAs encoding the mineralocorticoid receptor (MR), glucocorticoid receptor (GR), corticotropin releasing hormone (CRH) and arginine vasopressin (AVP) in HPA regulatory circuits of 26 mo. old (aged) and 9 mo. old (control) Fisher 344 rats. As previously documented, aged rats showed prolonged plasma corticosterone increases following acute restraint. CRH mRNA was significantly increased in the medial parvocellular paraventricular nucleus of aged rats (30%), whereas AVP mRNA was unchanged. In hippocampus, GR mRNA was significantly reduced only in the ventral subiculum, whereas MR mRNA was reduced in subfield CA3 and in the dentate gyrus. No other subfields were affected, nor was MR or GR mRNA in frontoparietal cortex. Results indicate an up-regulation of the HPA axis which is specific for the CRH component of this system, and down-regulation of corticosteroid receptor mRNA expression only in discrete hippocampal regions. The relationship between changes in corticosteroid receptor mRNA changes and age-related hippocampal/hypothalamic cell death is under determination. (Supported by MH49698 and MH42251).

488.14

INFLUENCE OF DOPAMINE ON BRAIN CORTICOSTEROID RECEPTORS AND CORTICOSTERONE SECRETION. **P. Casolini[1], M. Kabbaj[2], P.V. Piazza[2], F. Rouge-Pont[2], L. Angelucci[1], H. Simon[2], M. LeMoal[*2] and S. Maccari[2]. [1]Institute of Pharmacology II, University of Rome "La Sapienza" P.le A. Moro 5, 00185 Rome, Italy [2]INSERM U259 Rue Camille St Saëns 33077 Bordeaux, France.**

Central type I and type II corticosteroid receptors play a major role on corticosterone secretion. There is also evidence for a direct neural control of these receptors. We have previously shown that both noradrenaline and serotonin regulate brain corticosteroid receptors. However, the role of dopamine (DA) on these receptors is as yet unknown. In order to investigate the influence of DA we tested: i) the effects of 6-OHDA lesion of the ventral tegmental area on corticosteroid receptors in the dorsal striatum, ventral striatum and hippocampus, and on basal and restraint stress-induced corticosterone secretion; ii) the effect of intraperitoneal injection of D_1 (SKF38393) and D_2 (LY171555) DA agonists on corticosteroid receptors in the hippocampus and ventral striatum. Three weeks after the DA lesion was observed: i) an increase in type II receptor affinity in the ventral striatum but no effects in the dorsal striatum or hippocampus; ii) a reduction in basal and stress-induced corticosterone secretion after DA lesion; iii) a decrease in type II receptor affinity in the hippocampus after D1 agonist treatment but no differences in binding in the ventral striatum after injection of DA agonists. These studies suggest that i) DA is involved in the regulation of brain corticosteroid receptors and that it affects receptor affinity rather than number; ii) D1 receptor could be responsible for DA regulation; iii) DA may have a stimulatory influence on the HPA axis. In conclusion, these results shed light on mechanisms linking the DA and the HPA axis. (Supported by INSERM).

488.15

50% CORTICOSTERONE PELLET TREATMENT DOES NOT AFFECT GLUCOCORTICOID FAST FEEDBACK. E.A. Young*, K.Fried and E. Ho. Mental Health Research Institute, University of Michigan, Ann Arbor, MI 48109.

Our previous studies have demonstated that implantation of 50% corticosterone pellets raised the morning plasma levels of corticosterone to 4-5μg/dl and resulted in inhibition of evening corticosterone secretion. Implantation of these pellets was associated with a decrease in thymus weight, adrenal atrophy and a decrease in anterior pituitary proopiomelanocortin mRNA (ACTH pro-hormone). However, anterior pituitary ACTH content was normal. No changes were found in brain glucocorticoid receptors. To test whether this small increase in baseline corticosterone secretion would affect in vivo feedback, animals were subjected to 30 minutes of restraint stress and blood was sampled by tail nicks for measurement of plasma ACTH and corticosterone. Each rat was tested on 4 occassions, twice at baseline before corticosterone treatment and twice following implantation of corticosterone pellets. In each test session half the rats received saline and half the rats received a cortisol injection before the onset of the restraint stress. There was no difference in the plasma ACTH response at 5 or 30 minutes before and after the implantation of corticosterone pellets. Injection with cortisol significantly decreased the plasma ACTH response to restraint stress both before and after corticosterone pellet treatment. These data suggest that 50% corticosterone pellet treatment is able to inhibit circadian induced ACTH secretion but not stress-induced ACTH secretion. In addition, they suggest that glucocorticoid fast feedback is not affected by this low dose corticosterone treatment.

488.16

GLUCOCORTICOID RECEPTOR ACTIVATION DOES NOT ALTER SYNAPTIC POTENTIALS IN AREA CA1 OF RAT HIPPOCAMPAL SLICES. S. Birnstiel*, T. List and S.G. Beck. Dept. Pharmacol., Loyola Univ. Med. Ctr., Maywood, IL 60153.

Stressful stimuli increase corticosterone (CT) production by the hypothalamic-pituitary-adrenal (HPA) axis. One site of the feedback regulation of the HPA axis is the hippocampal CA1 subfield, which contains high densities of both mineralcorticoid (MR) and glucocorticoid (GR) receptors. Basal plasma CT levels occupy MR receptors; high concentrations of CT also activate the GR, which is implicated in the feedback control of the HPA. To investigate if the feedback control by CT is due to an action on fast amino-acid mediated synaptic transmission in area CA1, intracellular recordings of excitatory and inhibitory synaptic potentials were obtained from the CA1 pyramidal cell layer of transversal hippocampal slices. Male Sprague-Dawley rats (75-190 g at the beginning of treatment) were adrenalectomized (ADX) 14 days prior to recording. A second ADX group was chronically treated with a corticosterone pellet (12.5 mg) to mimic basal CT plasma levels. Perfusion with high concentrations of CT (100 nM for 30 min) did not change synaptic potentials in any group up to 60 min later, nor did it alter passive or active cell properties. These results argue against the hypothesis that HPA feedback control is mediated by an effect on amino acid transmission via GR receptors in area CA1 of the hippocampus. However, chronic treatment with CT seemed to prevent rundown of synaptic potentials over a 90 min recording period. A possible mediation of feedback control by other neurotransmitter systems is under current investigation. Supported by USPHS NS28512 and MH 00880.

488.17

SPLANCHNICECTOMY ALTERS DIURNAL SENSITIVITY TO ACTH AND ABOLISHES DIURNAL RHYTHMICITY IN CORTICOSTERONE SECRETION IN AWAKE RATS. M.S. Jasper* and W.C. Engeland, Dept. of Surgery/Sect. of Neurobiology, RI Hospital/Brown Univ., Providence, RI, U.S. 02903

An ultradian rhythm in adrenal secretion of corticosterone (CORT) has been described in awake rats using intra-adrenal microdialysis. To determine the role of splanchnic neural activity in diurnal changes in the CORT rhythm, CORT secretion was studied in intact (CTRL) and splanchnicectomized (SPLNX) rats during the inactive (AM) and active phases (PM) of the rat's circadian cycle. Experiments conducted between 1000 h and 0110 h, 5 days after surgery, consisted of continuous collection of dialysate at 10 min intervals. Discrete pulse detection revealed that the inter-pulse interval (IPI) was greater in the AM than in the PM in CTRL animals, but not in SPLNX animals. Power spectral analysis results supported this finding. CORT pulse amplitude was greater during the PM in both CTRL and SPLNX animals. Total integrated secretion was diurnally modulated in CTRL animals, whereas SPLNX animals secreted equal amounts of CORT during the AM and PM. To test whether neural modulation of pulsatile secretion during the AM was due to a direct effect of the splanchnic nerve on adrenal sensitivity to ACTH, rats treated with dexamethasone to suppress endogenous secretion of ACTH were repeatedly administered constant pulses of ACTH. Both CTRL and SPLNX rats were tested at 2 or 5 days after surgery. The results showed that adrenal sensitivity to ACTH was markedly reduced in CTRL animals compared to SPLNX animals at 5 days, but not at 2 days after surgery. This finding is consistent with the reduction in pulsatile secretion in CTRL animals during the AM, 5 days after surgery, which was abolished by SPLNX, suggesting that splanchnic neural activity provides inhibitory input to the adrenal cortex during the AM. The increased AM IPI observed in the CTRL group may result in part from a relatively insensitive adrenal cortex that responds to fewer of the endogenous ACTH pulses presented to it. The absence of AM neural inhibition of sensitivity to ACTH, 2 days after surgery, suggests that surgical stress obscures this splanchnic neural effect. Supported in part by NIH grant DK38951.

488.18

STRESS-INDUCED INCREASES IN ADRENAL SENSITIVITY TO ACTH ARE MEDIATED IN PART BY SPLANCHNIC NEURAL ACTIVITY. W.C. Engeland* and J.T. Walworth. Dept. of Surg., Univ. of Minn., Mpls, MN 55455.

Sympathetic neural input to the adrenal gland has been proposed as an extra-ACTH mechanism for the control of adrenal corticosteroid secretion during stress. To determine if splanchnic neural activity affects stress-induced adrenal responses by changing adrenal sensitivity to ACTH, adrenal sensitivity was tested in vitro after thoracic splanchnic nerve section (SPLNX). Rats (n=6/group) underwent unilateral SPLNX and at 6 days were killed in the AM by decapitation (non-stress) or after 5 min of continuous restraint (stress). Additional groups received dexamethasone (DEX) 2 h prior to stress to assess the role of ACTH in adrenal responses. Trunk blood was collected and plasma was assayed for ACTH and corticosterone by RIA; SPLNX and intact (CTRL) adrenals were removed, decapsulated and cortical cells from single adrenals were isolated with collagenase prior to in vitro testing with ACTH. Restraint stress resulted in increases in plasma ACTH and corticosterone, which were blocked by DEX treatment. Dose-dependent increases in corticosterone release were observed for all adrenals after in vitro exposure to ACTH. Stress increased in vitro adrenal sensitivity (CTRL: stress vs non-stress; $p<0.05$) and responses to low doses of ACTH were reduced by SPLNX (CTRL-stress vs SPLNX-stress; $p<0.05$). Following blockade of ACTH secretion by DEX, both stress-induced increases in adrenal sensitivity (CTRL/DEX: stress vs non-stress; $p<0.05$), and reduced responses observed after SPLNX (CTRL/DEX-stress vs SPLNX/DEX-stress; $p<0.05$) persisted. These data show that restraint stress induces a rapid increase in adrenal sensitivity to ACTH and that SPLNX reduces the response in the presence and absence of endogenous ACTH. These findings suggest that splanchnic neural activity augments adrenal sensitivity to ACTH and represents an extra-ACTH mechanism for augmenting stress-induced increases in adrenocortical secretion.

488.19

THE PREOPTIC AREA AS A SITE OF STEROIDAL REGULATION OF STRESS-RELATED HYPOTHALAMIC-PITUITARY-ADRENAL ACTIVITY. V. Viau* & M.J. Meaney. Developmental Neuroendocrinology Laboratory, Douglas Hospital Research Centr., Depts. of Psychiatry and Neurology/Neurosurgery, McGill Univ., Montreal H4H 1R3, Canada

Previous studies in our laboratory have indicated that male rats secreting high plasma testosterone (T) levels show reduced ACTH responses to stress. Furthermore, gonadectomized males replaced with elevated T levels show an increase in glucocorticoid receptor density only in the preoptic area (POA). These studies suggests a possible role for the POA in mediating glucocorticoid feedback inhibition of the HPA axis, which is perhaps regulated by T. This is consistent with previous electrophysiological studies implicating a role for the POA in regulating the HPA axis (Saphier and Feldman, 1986). To further examine the role of the POA on HPA activity, we examined the ACTH response to restraint in animals receiving either B (corticosterone) or T implants in the POA for 1 week prior to testing.

Animals with B or T implants in the POA showed significantly ($P< 0.05$) lower peak ACTH responses to 10 min of restraint compared to animals receiving cholesterol (CHOL) implants (539 ± 82, 656 ± 75, and 896 ± 89 pg/ml, respectively). B and T implanted animals also showed significantly ($P < 0.01$) lower ACTH levels up to 30 min following stress as indicated by area under curve analysis (211 ± 19, 263 ± 17, and 373 ± 28 pg/ml/min, respectively). No differences were found in pre-stress basal levels of ACTH. POA steroid implants did not affect median eminence CRH content, however, AVP content was significantly ($P < 0.01$) reduced in animals receiving B compared to T and CHOL implants (CRH= 14 ± 3, 16 ± 1, and 18 ± 2 pg/μg, respectively; AVP = 47 ± 5, 62 ±7, and 80 ± 6 pg/μg, respectively). These studies suggest that B and T inhibition of the HPA response to stress occurs at the level of POA, effects which may be related to the regulation of AVP synthesis. Thus, the POA appears to represent an important site within the brain mediating B and T regulation of the HPA axis.

488.20

HIGH-FAT FEEDING IMPAIRS BOTH BASAL AND STRESS-INDUCED HYPOTHALAMIC-PITUITARY-ADRENAL (HPA) RESPONSIVENESS. B.M. Tannenbaum*, D. Francis, G.S. Tannenbaum, D.N. Brindley, M.F. Dallman and M.J. Meaney. Dept. Neurol/Neurosurg.,McGill Univ., Montreal, Canada, H3A 2B4; Dept. Biochem., Univ. of Alberta, Edmonton, Canada, T6G 2C2; Dept. Physiol., UCSF, San Francisco, CA,94143.

Rats fed a high fat diet develop insulin resistance which could lead to elevated levels of circulating corticosterone (B). The present study was undertaken to determine whether dietary fat-induced increases in B, in turn, cause further alterations in the regulatory components of the HPA axis. Adult, male rats were maintained on a high fat (23%) or control (3%) diet for 1-3 weeks. Six hour plasma B profiles obtained from undisturbed, free moving rats revealed marked elevations in a.m. basal B levels as early as 7 days after fat diet onset compared to controls. In a second study, plasma B and ACTH levels were examined before, during and following a 20 min. restraint stress after 1, 9 or 12 weeks exposure to either the high fat or control diet. At 20, 60 and 120 minutes post-stress, all groups of rats fed the high fat diet had significantly elevated B and ACTH levels and showed a significant resistance to insulin compared to normal fed controls. To assess the mechanism, we examined glucocorticoid negative feedback in high fat and control animals. The inhibiton of basal ACTH levels by B was comparable in the two groups, providing little evidence for changes in negative feedback sensitivity in the high fat fed animals. Moreover, no differences in resting-state median eminence content of CRH or AVP were found in response to high fat feeding. Finally, animals maintained on the high fat diet for 3 weeks and exposed to chronic, intermittent cold stress during this period showed evidence of an enhanced HPA response to a novel stressor (restraint) compared to normal fed controls. Taken together, these results suggest that high-fat intake acts as a background form of chronic stress, elevating am basal B levels and enhancing HPA responses to stress. This latter effect may be associated with an augmented dietary fat-induced increased sensitivity to the neural signals associated with stress. (Supported by the MacArthur Foundation)

489.1

CORTICOTROPIN RELEASING FACTOR mRNA EXPRESSION IN THE FETAL SHEEP BRAIN: REGIONAL CHANGES AFTER MATERNAL RU486 TREATMENT. CJ Keiger*[1], JP Figueroa[1,2], G Brewer[3], MS Thomas[4] and JC Rose[1,2]. Departments of Phys/Pharm[1], Ob/Gyn[2], Micro/Immuno[3], and Comparative Med[4]. Bowman Gray School of Medicine of WFU, Winston-Salem, NC 27157.

Mifepristone (RU486), an antiprogestogen and antiglucocorticoid, is used to induce labor in early and late pregnancy. The fetal effects of maternal administration of RU486 are presently unknown. The aim of this study was to determine the effect of maternal RU486 on CRF mRNA expression in regions of the fetal brain associated with neuroendocrine and stress responses. Eight pregnant sheep (127-137 days gestation, term ~ 145 days) received either 100 mg RU486 or vehicle (i.p.) on two consecutive days. Fetal brain tissue was collected on day 3. CRF mRNA was quantified in the fetal hypothalamus (HYPO), hippocampus and amygdala (HA), frontal cortex (FC), and brainstem (BS) using a RNase protection assay with a sheep antisense probe. CRF mRNA is expressed as fg per μg total RNA (Table, mean ± SEM). Data were analyzed by ANOVA and two-sample tests.

GROUP	HYPO	HA	FC	BS
Vehicle	8.0 ± 1.7	3.2 ± 1.1	27.6 ± 4.8	8.9 ± 1.4
RU486	12.6 ± 6.2	18.6 ± 3.8	28.1 ± 11.3	24.0 ± 6.2

Maternal RU486 significantly increased fetal CRF mRNA expression in the HA and BS ($P<0.01$). In the vehicle group, CRF mRNA levels were greater in the FC than in the other regions ($p<0.001$). Combined neural and endocrine responses resulting from fetal hypoxia and somatosensory stimulation caused by RU486 induced uterine activity may be responsible for the observed changes in CRF mRNA. These differences in fetal CRF gene expression in response to maternal RU486 may reflect different interactions of intracellular messengers and transcription factors in various brain regions. Although unlikely, we cannot rule out direct fetal effects of RU486. Supported by grants HD 11210 and HD 25175.

489.2

CORTICOSTERONE EFFECTS ON CRH mRNA IN PARVOCELLULAR PVN AND THE CENTRAL NUCLEUS OF THE AMYGDALA S. Makino, P.W. Gold, and J. Schulkin* Clinical Neuroendocrinology Branch, National Institute of Mental Health, NIH, Bethesda, MD 20892.

The central administration of corticotropin releasing hormone (CRH) to the rat facilitates pathways subserving arousal and cautious avoidance and inhibits pathways subserving vegetative functions such as feeding, growth, and reproduction. It has been hypothesized that the principal role of glucocorticoid secretion during stress is to counter-regulate the major effectors of the stress response, including CRH. This has been previously shown for paraventricular nucleus (PVN) CRH, which is likely to mediate the inhibition of vegetative functions, but not for extrahyopthalamic CRH, which is thought to be more influential in promoting arousal. We report here a study of effects of low (1 mg/kg/day) and high doses (5mg/rat/day) of corticosterone (CORT) on CRH mRNA levels in the PVN and central nucleus (medial-caudal region) of the amygdala (CEA). We found that low and high doses of CORT given for 2, 4, 8 and 14 days significantly diminshed CRH mRNA levels in PVN at all time points; however, high dose CORT increased CRH mRNA expression in CEA at 4 and 8 days. These data suggest that glucocorticoid secretion during stress may restrain the impact of CRH on inhibiting vegetative functions, while enhancing the arousal producing effects of extrahypothalamic CRH.

489.3

CHRONIC DEXAMETHASONE TREATMENT ALTERS ARACHIDONIC ACID-STIMULATED PRODUCTION OF *EX VIVO* PROSTAGLANDIN E_2 PRODUCTION IN SELECTED BRAIN REGIONS. **T.M. Rampy*, F.A Fitzpatrick and G.M. Rose. Dept. of Pharmacology and Neuroscience Training Program, UCHSC, and Medical Research, VAMC, Denver, CO 80220**

Prostaglandins (PG) are an important class of neuromodulators. In many peripheral tissues, corticosteroids suppress the production of PGs. However, little is known about the effects of steroids upon PGs in the CNS. Thus, we examined the effect of chronic dexamethasone phosphate (DEX) treatment on prostaglandin E_2 (PGE_2) synthesis in several brain regions. Male Sprague Dawley rats received daily injections of DEX (30 mg/kg, i.p.) for two weeks. After sacrifice, levels of basal and arachidonic acid-stimulated *ex vivo* PGE_2 production were determined using quantitative enzyme immunoassays. In work to this point, we have found that chronic DEX pretreatment did not alter basal levels of PGE_2 in any brain region examined. By contrast, arachidonic acid-stimulated PGE_2 synthesis was reduced by DEX in hippocampus and prefrontal cortex, but not cerebellum. These results indicate that corticosteroids can modulate CNS prostaglandin production. The pattern of results suggest that this is accomplished through inhibition of cyclooxygenase (COX) enzymes, perhaps the recently described, steroid-suppressible, COX-2. Our observations may provide an explanation for the behavioral abnormalities sometimes observed with chronic steroid treatment (i.e. the clinical Steroid Psychosis syndrome).

489.4

GLUCOCORTICOIDS ACT VIA TYPE II RECEPTORS TO REGULATE VASOPRESSIN mRNA IN THE PVN. D. Albeck*, N. Hastings and B. McEwen. Lab. of Neuroendo., Rockefeller University, NY NY 10021.

Adrenal steroids mediate neuropeptide synthesis by acting via intracellular receptors. Two types of glucocorticoid receptors are expressed in the paraventricular nucleus (PVN): type I (MR) and type II (GR). GR receptors predominate in the PVN, and in the PVN of adrenalectomized (ADX) rats the GR agonist dexamethasone is more potent than MR agonists in restoring vasopressin immuno-reactivity to baseline level (J. NSci., 7: 1093-1106, 1987). Vasopressin mRNA also increases after ADX and dexamethasone reverses this increase (PNAS, USA, 83: 1145-1149, 1986). Using *in situ* hybridization we have examined the effect of several adrenal steroid agonists in preventing the ADX induced increase in vasopressin mRNA within the parvocellular PVN. ADX rats showed an increase in vasopressin mRNA ($p<.002$, n=4). Physiological (n=4) and supra physiological (n=4) doses of corticosterone prevented the ADX induced increase in vasopressin mRNA, as did the selective GR agonist RU28362 (n=4). The selective MR agonist aldosterone (n=5) did not prevent the increase. These results indicate that adrenal steroids regulate vasopressin mRNA in the PVN through GR receptors. Currently we are examining steroid mediated regulation of vasopressin synthesis in extra hypothalamic nuclei. [Grant support: 1 F32 MH10232-01A1 (D.A.) and MH 41256 (B.Mc.)]

489.5

EFFECTS OF ADRENAL STEROIDS AND REPEATED STRESS ON DYNORPHIN MRNA EXPRESSION AND KAINATE RECEPTOR BINDING IN THE HIPPOCAMPUS. Y. Watanabe*, N.G.Weiland and B.S. McEwen. Lab. of Neuroendocrinology, The Rockefeller Univ. New York, NY 10021.

Adrenal steroids and repeated stress cause atrophy of apical dendrites of CA3 neurons, and they also act in dentate gyrus neurons to regulate long-term potentiation. In the former actions, excitatory amino acid transmission via mossy fiber may have an important role. At mossy fiber terminals, dynorphin(DYN) and glutamate autoregulate their own release. In this study, we investigated the effects of adrenal steroids and repeated stress on DYN mRNA expression in dentate gyrus and kainate receptors in the hippocampus, by in situ hybridization and receptor binding autoradiography, respectively. ADX decreased DYN mRNA levels(25.8%) and corticosterone(CORT) replacement restored it, in agreement with other reports. In addition, CORT treatment of intact rats(40mg/kg, 21d) elevated DYN mRNA levels(31.7%). Kainate receptors in stratum radiatum, which may be associated with mossy fiber terminals, are decreased after ADX(18.6%) and restored by CORT replacement. However, CORT treatment of intact rats failed to elevate kainate receptor binding above control levels. Neither 3h/d nor 6h/d restraint stress for 21days affected DYN mRNA levels, and 3h/day restraint stress for 14d did not alter kainate receptor binding in the hippocampus.Since hippocampal pyramidal neurons and dentate gyrus granule neurons contain both type I and type II adrenal steroid receptors, the role of both receptor types in DYN mRNA expression and kainate receptor binding are being explored.(Supported by MH41256)

489.6

CHROMAFFIN AND ADRENOCORTICAL CELL INTERACTIONS in vivo and in vitro. M.A. Holzwarth*. Department of Physiology and Neuroscience Program, University of Illinois, Urbana-Champaign IL 61801.

Recent studies show that adrenocortical cell secretion is modulated by adrenal medullary cells and hence, indirectly by splanchnic nerve activity. We have shown that a network of VIP-containing nerve fibers in the glomerulosa of the rat adrenal originates from a subpopulation of medullary cells and that adrenocortical cells are responsive to VIP (Ann NY Acad Sci 512:449). We are currently using the frog adrenal as a model to study the chromaffin cell modulation of adrenocortical secretion because chromaffin and adrenocortical cells are interspersed in the frog. We show that like in the mammal, frog chromaffin cells express catecholamines, VIP, leu-enkephalin and substance P and networks of catecholaminergic and substance P containing nerve fibers are distributed throughout the adrenal. Chromaffin cells extend processes which appear to contact both cortical and other chromaffin cells. Steroidogenesis is stimulated by VIP, carbachol, norepinephrine and is comparable to ACTH, AII and forskolin as measured in vitro. To facilitate the investigation of chromaffin-cortical cell interaction, we have established primary cell cultures of frog adrenal cells. Growth and long term survival in our cultures are optimal using Pronectin-F plus laminin as a substrate and 55% L-15 medium supplemented with insulin, transferrin, ascorbate and α-tocoferol. NGF (50 ng/ml) and bFGF (10 ng/ml) enhance the neurite extension by chromaffin cells; these fine processes project to cortical and other chromaffin cells. The cultured chromaffin cells retain their expression of catecholamines and neuropeptides for several weeks. The cortical cells continue to proliferate and secrete both aldosterone and corticosterone. These co-cultures provide a useful model to study chromaffin-cortical cell interactions.

489.7

THE EFFECTS OF CORTICOSTERONE ON BRAIN AMINE OVERFLOW IN THE AWAKE AND FREELY MOVING RAT. D.N. Thomas*, R.M. Post and A.Pert. Biological Psychiatry Branch, NIMH, Blg. 10, Rm 3N212, Bethesda, MD 20892

There is evidence to suggest important modulatory control of brain amine function by corticosteroids. The perikaryal regions of all three major brain amine systems, for example, contain surprisingly high concentrations of glucocorticoid receptors. Furthermore, corticosterone and dexamethasone have both been found to alter brain amine metabolism and turnover. The purpose of these studies was to evaluate the effects of corticosterone on brain amine function in the awake freely moving rat using in vivo microdialysis. Male Sprague Dawley rats were implanted with guide cannulae into the frontal cortex (Fcx), striatum (Str) and the nucleus accumbens (Nac). Following recovery from surgery a dialysis probe was introduced into one of the structures on the day of the experiment. After stabilization of the baseline, corticosterone was administered (5mg/kg, s.c) and the extracellular levels of the monoamine was monitored for a further 2hrs. Basal extracellular DA in the Str, Nac and Fcx were 11.6 ± 0.3, 2.2 ± 0.2 and 1.1 ± 0.04 pg/sample (n=3-5) respectively. Following corticosterone, extracellular DA in the Fcx and the Nac decreased by 45% and 38% respectively, whilst there was no change in the Str DA. Basal extracellular 5-HT in the Fcx, Nac and Str were 6.4 ± 0.7, 0.77 ± 0.05 and 0.5 ± 0.06 pg/sample (n=3-5) respectively. Upon administration of corticosterone, 5-HT decreased in a manner similar to DA in the Fcx with a 68% decrease relative to the baseline, whilst in the Nac and Str no effects were observed. Preliminary studies suggest that corticosterone applied focally to the ventral tegmental area produces effects on Nac DA consistent with those seen following systemic injections. Studies are underway to more clearly define the effects of corticosterone on the noradrenergic and serotonergic systems.

489.8

BEHAVIORAL CHARACTERIZATION OF A TRANSGENIC MOUSE MODEL OF IMPAIRED TYPE II GLUCOCORTICOID RECEPTOR FUNCTION. S. Beaulieu*, I. Rousse, A. Gratton, N. Barden[1] and J. Rochford. Department of Psychiatry, McGill University, Montreal, and Department of Physiology[1], Laval University, Quebec.

A transgenic mouse model of impaired type II glucocorticoid receptor function has been developed recently (Pepin et al., Nature, 355, 1992; 725-728). The locomotor activity of transgenic mice (1st generation: C57BXC3H = B6C3F1, subsequent generations: B6C3F1XB6C3F1) and control non-transgenic mice (B6C3F1) were compared. Locomotor activity was recorded in chambers equipped with photoelectric cells. The activity was recorded in both dark and light phases of the circadian cycle in different groups of transgenic and control mice. Results indicate that the transgenic animals are more active than the controls in the first 30 min of testing during the light phase. However, no difference could be observed between the groups during the dark phase of the circadian cycle. These results parallel endocrine data indicating that during the dark phase period, the corticosterone and ACTH plasma levels of control mice reach similar high values to those observed in transgenic animals. However, when injected i.p. with d-amphetamine (1 mg/kg) during the dark phase period, the transgenic animals demonstrated a greater level of locomotor activity than the controls. In parallel, the transgenic mice demonstrate a decreased immobility time in the Porsolt swim test compared to controls. These results may provide some insight into the behavioral sequelae resulting from alterations in glucocorticoid receptor function. (Supported by the FRSQ and the MRC)

489.9

TYPE II GLUCOCORTICOID RECEPTOR MEDIATED REGULATION OF HYPOTHALAMIC NPY EXPRESSION. P. Ponsalle* and J.D. White. Dept. Neurobiology and Behavior, SUNY Stony Brook, Stony Brook, NY 11794

Neuropeptide Y (NPY) is the most potent orexigenic agent known and hypothalamic NPY synthesis and secretion is modulated by peripheral metabolic status. Food deprivation (FD) increases hypothalamic NPY mRNA content 2.5 fold over fed controls, and glucocorticoids are required for this response. The present studies sought to determine if the effect of glucocorticoids on hypothalamic NPY gene expression is mediated via the type I and/or type II glucocorticoid receptor. Animals underwent adrenalectomy (Adx), or not. All Adx animals received the type II agonist RU28362 (25μg/kg/d) via Alzet minipumps, implanted at the time of surgery. 24 hr later, food was removed from half of the AdxRU and Con groups; 72 hr later all animals were sacrificed. Nuclease protection analyses revealed that hypothalamic NPY mRNA content in the AdxRU-FD group was increased 3.5 fold over fed controls. Preliminary studies with aldosterone replacement suggest that type I receptor stimulation only partially restores increased hypothalamic NPY gene expression observed in intact FD animals. These findings suggest that increased hypothalamic NPY gene expression with FD occurs primarily via the type II receptor, but that there may be more than one pathway through which glucocorticoids modulate NPY expression.

489.10

GLUCOCORTICOIDS INHIBIT D_1 BUT NOT D_2 RECEPTOR MEDIATED ANF PRODUCTION IN HYPOTHALAMIC NEURONS A.T.Lim, D.Lee, W.Huang, Z.Yang, D.Copolov and J.Boublik*. Cell Biology Unit, Mental Health Res. Inst., Melbourne, 3052 and *Baker Medical Res. Inst., Melbourne, 3004, AUSTRALIA.

Recent evidence suggests that dopamine (DA) modulates the function of hypothalamic atrial natriuretic factor (ANF) neurons in a bimodal manner acting directly through D_2 and D_1 receptors which have very different affinity for DA (Lee, D., et al. submitted). Employing well characterised radioimmunoassay and colorimetric Northern blot analysis with synthetic oligonucleotide probes complementary to pro-ANF mRNA (Huang W *et al.* 1992 *Endocrinology* **131**, 1562), we have examined the effect of glucocorticoids on DA-stimulated ANF neurons in long term primary cultures of neonatal rat hypothalamic cells. Although dexamethasone (DM) alone did not affect basal secretion of immunoreactive (ir)ANF, it reduced D_1 agonist, (SKF38393)-induced irANF secretion to half (P<0.001). This effect of DM was both time-dependent and dose-related with an EC_{50} of 0.1 nM; it was blocked by 100 nM of RU38486 (P<0.05), a glucocorticoid receptor antagonist, but not by 100nM RU28318, a mineralocorticoid receptor antagonist. In addition, the effect of DM was mimicked by corticosterone (EC_{50} 10nM), but not by progesterone. The increased expression of pro-ANF mRNA signal induced by D_1 agonists in culture was also suppressed by DM in a similar manner. In contrast to the D_1 receptor effect, DM failed to modulate ANF production and secretion induced by D_2 agonist, quinpirole. Consistent with this finding, whereas DM abolished the D_1 receptor mediated stimulation of irANF release induced by high doses (10^{-6} and 10^{-5}M) of DA, it had no detectable effect on modulating D2 receptor mediated changes produced by low concentrations of DA (10^{-9} and 10^{-8}M). We thus conclude that in monolayer cultures of rat hypothalamic neurons, glucocorticoids selectively abolish D_1 but not D_2 receptor mediated stimulation of irANF secretion and proANF mRNA expression.

489.11

CYCLIC-AMP-DEPENDENT STEROIDOGENESIS OCCURS IN RAT BRAIN VIA ACTIVATION OF THE MITOCHONDRIAL DBI RECEPTOR (MDR). M.L.Barbaccia*, G.Roscetti, M.Trabucchi, C.Ambrosio§ and M. Massotti§. Dept of Exp.Medicine,Univ.of Roma "Tor Vergata" and § Lab. of Pharmacology, Istituto Superiore di Sanita',Roma-Italy.

In brain, glial cells are endowed with the enzymatic machinery that synthesizes steroids from cholesterol (P.N.A.S. 84,8215,1987). MDR, so-called for its ability to bind the endogenous peptide DBI together with other selective "peripheral benzodiazepine" ligands, appears to mediate the cyclic-AMP-dependent and the ACTH-induced steroidogenesis in adrenocortical and Leydig cells (J.B.C.266(6),3682,1991).We have observed that minces (300x300 um) prepared from adult rat brain cortex are a suitable model to study the process of brain steroidogenesis. A 30 min exposure of brain cortical minces to forskolin (6-50uM) or cyclic-AMP (0.2-1.0 mM) elicited a significant increase in pregnenolone(PRE) production (+50% and +30%,respectively).The forskolin-stimulated PRE synthesis was abolished by coincubating the tissue with the isoquinoline-carboxamide Pk 11195, a selective antagonist at the MDR. Moreover in minces of intact or adrenalectomized/orchiectomized rats pretreated with the protein synthesis inhibitor cycloheximide the levels of PRE and some of its metabolites were significantly decreased. These results show that, also in brain, steroidogenesis is a protein synthesis-dependent process which can be rapidly activated, in a cyclic-AMP and MDR-dependent manner, and suggest that some PRE derivatives produced in brain may participate in the physiological modulation of fast synaptic transmission.

489.12

PROGESTERONE RECEPTOR MEDIATED EFFECTS OF 5ß-REDUCED STEROIDS. T. Trapp, R. Rupprecht, K. Damm, J.M.H.M. Reul, and F. Holsboer*. Max-Planck-Institute of Psychiatry, Clinical Institute, Department of Neuroendocrinology, Munich.

5ß-metabolites of progesterone modulate neuronal excitability by modifying membrane permeability. However, genomic effects of these steroids have not yet been demonstrated. We characterized the molecular effects of 5ß-pregnane-3,20-dione (5ß-DHP) mediated by the chicken (cPR_B) and human (hPR_B) progesterone receptor (PR). In transient transfection studies employing the human neuroblastoma cell line SK-N-MC and the mouse mammary tumor virus promoter fused to the luciferase gene as a reporter system, 5ß-DHP pontently induced gene expression via both cPR_B and hPR_B. The cPR_B responded to 5ß-DHP in the low nanomolar range, whereas concentrations in the upper nanomolar range were required to activate the hPR_B. As shown by gel shift analysis, DNA-binding of the cPR_B was induced by 5ß-DHP in a progestin-like fashion. 5ß-DHP bound to the cPR_B reconstituted in COS-1 cells with a k_i value of 1.1 nM and to the hPR_B with a k_i value of 35 nM. Thus, 5ß-reduced metabolites of progesterone may modulate neuronal function via both membrane and genomic mechanisms.

489.13

ESTROGEN MODULATION OF mRNA FOR TWO FORMS OF GLUTAMIC ACID DECARBOXYLASE (GAD) IN RAT BRAIN.

M.M. McCarthy*, L.C. Kaufman, P.J. Brooks, D.W. Pfaff and S. Schwartz-Giblin. Rockefeller University, New York., NY 10021

Two genes coding for separate forms of GAD (GAD65 and GAD67) have been identified (*Neuron* 7; 1991). Numerous studies indicate that mRNA levels for GAD67 are readily modifiable whereas GAD65 mRNA levels remain stable. We investigated the hormonal modulation of the two forms of GAD. Brains were harvested from ovx rats treated with estradiol benzoate (EB; 10μg/day; 2 days prior) or vehicle. Specific DNA probes for GAD65 or GAD67 were ^{32}P radiolabeled using amplified primer extension. Results of *in situ* hybridization were analyzed for magnocellular preoptic area (McPOA), zona incerta (ZI), dorsal medial hypothalamus (DMH), midbrain central gray (MCG) and several regions of the hippocampus. In most of these brain areas, Kolmogorov-Smirnov analysis indicated GAD65 mRNA levels were less than GAD67, regardless of endocrine condition. Levels of GAD65 were reduced by EB-treatment vs veh in granule cell layer ($p<.001$) and hilus ($p<.01$) of dentate gyrus and pyramidal cell layers of CA1 and CA2 ($p<.05$) in hippocampus. EB also reduced GAD65 in ZI and DMH ($p<.01$). An exception was McPOA where GAD65 increased and GAD67 decreased with EB-treatment. In MCG, K-S analysis showed no differences in GAD mRNA levels/cell, but the proportion of cells expressing detectable levels of GAD65 or GAD67 was greater in EB-treated rats (X^2;$p<.001$). The DMH was notable in that GAD65 was greater than GAD67 in veh-treated rats but EB treatment decreased GAD65 while simultaneously increasing GAD67 (ANOVA). In summary, EB acts to reduce GAD67 in the forebrain but increases it in the hypothalamus and midbrain, which is consistent with previous observations of regional effects of GABA neurotransmission on EB-dependent lordosis.

489.14

EFFECTS OF ESTRADIOL (E_2) AND PROGESTERONE (P_4) ON EXPRESSION OF mRNAS ENCODING $GABA_A$ RECEPTOR SUBUNITS.

S.L.Petersen*[1], A.Reeves[1], M.Keller[1], E.Gardner[1], L.C.Mahan[2] and S.McCrone[1]. Dept. of Anatomy and Neurobiology, Univ. Missouri, Columbia, MO 65212[1]; Lab of Neurochemistry, NINDS, Bethesda 20892[2].

E_2 and P_4 alter $GABA_A$ receptor binding in the CNS and GABA receptors play an important role in the control of sexual behavior and gonadotropin release. In the present study we examined the effect of E_2 and P_4 on levels of mRNAs encoding several $GABA_A$ receptor subunits in regions involved in the control of these functions and in the hippocampus. We implanted 16 one-week ovariectomized (OVX) rats with E_2 or oil capsules. On Day 2, we injected 8 E_2-treated rats with P_4 and 4 h later, collected and froze their brains. We hybridized cryosections from regions containing the organum vasculosum of the lamina terminalis (OVLT) and rostral preoptic area (rPOA), hippocampus, and ventral medial nucleus (VMN) to ^{35}S-tailed 48-base oligodeoxynucleotide probes specific for β_2-, β_3- or γ_2-subunit mRNAs. We found that in the region of the OVLT/rPOA neither E_2 nor P_4 altered levels of β_3-subunit and, although E_2 had no effect on levels of β_2-subunit mRNA, combined E_2 and P_4 decreased these levels. E_2 increased levels of the γ_2-subunit mRNA and P_4 reversed this effect in the OVLT/rPOA. We found no hormone-induced changes in levels of β_3-subunit mRNA in the dentate gyrus or CA1 region of the hippocampus, but in the VMN, E_2 increased these levels and P_4 had no effect. Our findings show that E_2 and P_4 have differential, neuroanatomically-specific effects on the levels of mRNAs encoding β_2-, β_3- and γ_2-subunits of $GABA_A$ receptors.

489.15

A SEX DIFFERENCE IN THE EFFECT OF 5α–DIHYDROTESTOSTERONE (DHT) ON OCCUPIED ESTROGEN RECEPTOR BINDING IN SPECIFIC RAT BRAIN NUCLEI.

T.J. Brown*, G. Adler, and N.J. MacLusky. Div. of Reprod. Sci., Department of OB/GYN, The Toronto Hospital, Toronto, ON, Canada

Androgens are known to oppose the actions of estrogen on a number of neuroendocrine functions in the rat. Although earlier attempts to relate these antagonistic actions of androgens with a possible down-regulation of neural estrogen receptors have been unsuccessful, we have used an *in vitro* autoradiographic assay combined with an ^{125}I-labeled estrogen receptor ligand to compare the effect of DHT on occupied estrogen receptor binding in the male and female rat brain. Gonadectomized/ adrenalectomized rats were divided into 2 groups per sex. All animals received daily estradiol benzoate injections (EB:40 μg/kg BW) for 4 days. Animals in the treatment group recieved DHT (2.5 mg/250 g BW) every 12 hr for 4 days while animals in the control group received vehicle injections. Animals were killed 4 hr after the final EB/DHT injection and their brains were frozen on dry ice. Frozen sections (20 μm) from the preoptic - hypothalamic areas were incubated at 37 C for 2 hr with 2.5 nM [^{125}I]MIE_2. Sections were washed at 4 C to remove unbound radioligand, dried, and placed against Amersham Hyperfilm for 24 hours. Resulting images were analyzed by computer-assisted densitometry. High levels of estrogen binding were observed in the periventricular and medial preoptic area (PVP and mPO), bed nucleus of the stria terminalis, arcuate nucleus, ventromedial nucleus (VMN), and cortical and medial amygdala. As previously reported, higher levels of estrogen binding were present in the PVP, mPO, and VMN of the female than of the male. In the female, DHT treatment reduced estrogen binding in the PVP and VMN by 30 and 38% respectively, whereas in the male no significant regional effects were observed. These results demonstrate that androgens can inhibit estrogen binding in regions of the female brain implicated in the control of gonadotropin secretion and sexual behavior. Supported by the MRC Canada.

489.16

HORMONAL INFLUENCE ON THE DEVELOPMENT OF GENDER-SPECIFIC SOCIAL INTERACTION IN FEMALE RATS.

B.A. Etzel* and C.K. Kellogg. Dept. of Psychology, Univ. of Rochester, Rochester, NY 14627.

Social interaction (SI) behavior is both age and gender specific. Adult male rats typically spend less time interacting with a stranger of the same sex in a neutral environment that is unfamiliar to both rats than they do in an environment that is familiar to both rats. On the other hand, adult females and prepubertal males (28 days old) make no such distinction between the two types of environment. Both pubertal males and females (35 days old), however, display environment-specific SI. We have also shown that males require testosterone (T) during puberty, but not during adulthood, for the emergence of adult-typical SI behavior.

The purpose of this study was to determine why females lose environment-related SI over adolescence. Three questions were addressed: (1) do adult females require ovarian hormones for maintenance of female-typical SI, (2) does SI in females change over adolescence due to increasing levels of ovarian hormones or because of the lack of T, and (3) will neonatal exposure to T masculinize adult female SI? Results indicate (1) ovariectomy at 60 days did not lead to environment-specific SI in females tested 4 weeks later, although it did increase SI overall, (2) exposure of intact females to testosterone proprionate (TP) via implanted silastic capsules over days 28-60 also increased overall SI without inducing environment-specific SI at 60 days, and (3) neonatal exposure to TP by injection within 5 hours of birth and again 24 hours later did not alter SI in gonadally intact 60-day-old females in any way. Thus, the hormonal milieu during puberty and adulthood, but not at birth, may be critical for the emergence of gender-typical SI. Supported by grant no. DA07080.

489.17

ALLOPREGNANOLONE MIMICS THE ACTION OF PROGESTERONE ON GLUTAMATE DECARBOXYLASE GENE EXPRESSION IN THE HIPPOCAMPUS.

N.G. Weiland*, M. Orchinik, P.J. Brooks, B.S.McEwen. Rockefeller Univ., New York, NY 10021.

Progesterone (P) inhibits the activity of glutamate decarboxylase (GAD), the rate limiting enzyme for the synthesis of GABA (Endocrinology 131:2697, 1992). P may act at the classical intracellular receptor (PR) or through one of its metabolites such as allopregnanolone (A; 3α-hydroxy-5α-pregnan-20-one) which interacts with the $GABA_A$ receptor rather than the PR. Because many of the effects of estrogen on the hippocampus are confined to the CA1 and estrogen induces PR in this region, we hypothesized that P is acting at the PR. However, if A treatment mimics P's action, then the P-induced suppression of GAD mRNA may be indirect and mediated by a metabolite-induced activation of the $GABA_A$ receptor. Adult female rats were ovariectomized for 1 wk, then adrenalectomized, and 2 days later injected with 3 mg/kg P, A, or oil (O) and killed 5 hr later. The brains were processed for *in situ* hybridization using specific DNA probes for GAD65 labeled with ^{32}P using amplified primer extension. Treatment with either progesterone or allopregnanolone decreased the levels of mRNA for GAD65 compared to vehicle-treated controls (P, 29.2 ± 1.4; A, 30.7 ± 1.3; O, 36.0 ± 2.0 specific gray levels). The steroid-induced suppression of GAD65 was limited to the CA1 region of the hippocampus. Interestingly, the action of A paralleled the action of P indicating that the actions of these steroids may not be exclusively through the PR.

490.1

CENTRAL PATTERN GENERATION IN *IN VITRO* BRAINSTEM-SPINAL CORD PREPARATIONS OF NEONATAL RATS.

A. Tarasiuk, Z. Siddiqi and A.L. Sica*. Albert Einstein Coll. Med., Long Island Jewish Med. Ctr., Departments of Medicine (Pulmonary Div.) and Pediatrics, New Hyde Park, NY 11042.

Synchronization of inspiratory (I) discharges has been attributed to modulating inputs from a central pattern generator (CPG) and is signalled by the presence of correlated frequencies in coherence spectra. Although I activities have been recorded from different motoneuron pools in *in vitro* preparations, there is no information regarding the presence of CPG modulation. Thus, in 0-3 d old pups (n=14), extracellular recordings (bandpass 1 Hz to 10 kHz) of spontaneous I activity were obtained from different pairs of nerve roots in normal Ringer's solution (27°C) and in an acidified (pH 6.8-7.0) solution which is known to augment respiratory activity. Power spectral analyses were carried out on low pass filtered (400 Hz) signals sampled at 1024 Hz; auto-power spectra (APS) were constructed from I and from expiratory (E) activities. I-triggered APS of cranial nerve roots (n11, n12) and spinal ventral roots (C1, C4, C5, T2) had prominent peaks in a low frequency band (2-6 Hz) during control conditions; such peaks were not found in E-triggered APS. The 2-6 Hz peaks were highly correlated as indicated by values of magnitude ranging from 0.28-0.98 in coherence spectra. Acidic stimulation produced increases in peak amplitudes, but no inceases of coherence or shifts beyond the 2-6 Hz band. In summary, we have found evidence of CPG modulation originating in the brain stem and distributed to a number of I motoneuron pools. Furthermore, the finding of correlated frequencies in a relatively low frequency band may be important for identifying neurons involved in the generation of central I activity.

490.2

EFFECTS ON BREATHING OF EXCITATORY AMINO ACID (EAA) ANTAGONISTS IN DEVELOPING OPOSSUMS.

J.P. Farber* and A.M. Hutson. Dept. of Physiol., Univ. Okla. HSC, Okla. City, OK, USA.

Medullary respiratory neurons in spontaneously breathing thiobarbiturate-anesthetized immature opossums (Didelphis virginiana) can be excited by kainic acid (KA) or n-methyl-d-aspartate (NMDA), but do EAAs play a role in setting breathing pattern during early development? To test this possibility, the above preparation was studied from 3-14 postnatal weeks of age. The ventral medulla was exposed from just ahead of C1 to the trapezoid body. Dura was removed typically from midline to 2/3 the distance to the left lateral margin of the medulla. Drugs were administered by droplet on the ventral surface of the medulla because of close proximity to regions presumably affecting cardiopulmonary functions. Raw diaphragm EMG and the moving average of the EMG (IEMG) were recorded; these values were used to obtain peak IEMG, Ti, and Ttot. Effects developed over 5-7 min. Except for the oldest animals, the NMDA antagonist, MK801 (1 or 5 mg/ml) significantly reduced respiratory output (peak IEMG/Ttot), most typically with an increase in Ttot. Ti was not consistently affected. The oldest animals showed a fall in peak IEMG, typically coupled to decreased Ttot. Responses to the KA antagonist, CNQX (0.1 or 0.5 mg/ml), were less consistent, although peak IEMG/Ttot was significantly reduced on average. Apnea rarely occurred with either drug. Since the opossum requires about 8-9 weeks to achieve the gross maturation of a newborn cat or rabbit,it appears that endogenous excitatory amino acid systems, accessible from the ventral medullary surface, participate in the expression of breathing pattern at relatively early stages of development. (Supported by HL37318 from NIH).

490.3

INCREASED AUGMENTING AND LATE INSPIRATORY MEDULLARY NEURONAL ACTIVITY DURING REM SLEEP IN THE ADULT CAT.

J. Orem* and R.H. Trotter. Physiology Department, Texas Tech Univ. Hlth. Sci. Cntr. Sch. of Med., Lubbock, TX 79430.

Breathing is variable; obstructive apneas may be prolonged; and central apneas can occur in REM sleep. The cause of these respiratory effects, whether due to changes in respiratory drive or nonrespiratory mechanisms, is unknown. One study suggests that central respiratory drive might be decreased in REM sleep (*Kubin et al., Brain Res., 592:91, 1992*), but another study gives mixed results (*J. Appl. Physiol. 48:54, 1980*). Augmenting inspiratory (I-aug), and possibly late inspiratory (I-late) medullary cells are predominantly premotor cells whose activities may be representative of central respiratory drive. We studied 15 I-aug and 12 I-late neurons during normal wakefulness (W), NREM and REM sleep in adult cats. All I-aug cells had high η^2-valued activity (>0.8) (*J. Neurophysiol. 50:1098, 1983*) and were more active in REM sleep than in W and, with one exception, than in NREM sleep. REM rates averaged 174% of W rates and 165% of NREM rates. For I-late cells (η^2 = 0.6-0.9), REM rates averged 216% of W rates and 423% of NREM rates, confirming an earlier study (*Brain Res. 458: 224., 1988*). These results suggest that central respiratory drive increases in REM sleep. *Supported by grant HL21257.*

490.4

SUPERIOR LARYNGEAL NERVE AFFERENT INPUTS TO THE DORSAL RESPIRATORY GROUP INVOLVE NON-NMDA RECEPTORS.

D.F. Speck* and D.R. Karius. Dept. of Physiology, Univ. of KY, Lexington KY 40536

Single shock stimulation of the superior laryngeal nerve (SLN) in cats elicits a transient excitation of the contralateral phrenic nerve activity which does not alter inspiratory timing. This excitation is believed to be the result of a monosynaptic activation of dorsal respiratory group (DRG) neurons (*Brain Res. 135:231-254, 1977*). In a previous study, it was noted that injection of excitatory amino acid (EAA) antagonists near the DRG consistently led to a slow decrease in the amplitude of the short-latency excitation (*J.A.P. 1993. In Press*). This study tested the hypothesis that the short-latency excitation produced by SLN stimulation in the cat is mediated by neurotransmission at non-NMDA EAA receptors within the DRG. Adult cats were anesthetized (sodium thiopental, 50 mg/kg i.p.), decerebrated, paralyzed, artificially ventilated and vagotomized. Inspiratory motor output was recorded from the phrenic nerve and one or both SLN were cut and placed on bipolar stimulating electrodes. After recording the control responses to SLN stimulation, injection of the non-NMDA antagonist NBQX (the kind gift of Novo-Nordisk; 1 mM; 100 - 460 nl) into the ipsilateral DRG led to a significant ($F(2,8) = 98.5$) decrease in the amplitude (measured from peri-stimulus-triggered histograms, 40 - 50 cycles) of the short-latency excitation of the phrenic nerve discharge 10 and 30 minutes after antagonism. Injection of the NMDA receptor antagonist AP5 into the DRG (n = 1) or injection of NBQX outside the DRG (n = 1) had no effect on the amplitude of the excitation. We conclude that non-NMDA receptors within the DRG are involved in the production of the short-latency excitation elicited by SLN stimulation in cats. (Supported by NIH PO1 40369)

490.5

INSPIRATORY SHORTENING ELICITED BY ARACHIDONIC ACID REQUIRES $GABA_A$-MEDIATED NEUROTRANSMISSION IN CATS.

D.R. Karius*, K.M. Krause and W.R. Revelette. Dept. of Physiology, Univ. of Kentucky, Lexington KY 40536

Delivery of arachidonic acid (AA) into the arterial circulation (in close proximity to the phrenic artery) elicits a shortening of inspiratory duration (T_I) in the cat. While it is believed that diaphragmatic afferents are responsible for this response, the neural pathways are unknown. This study tested the hypothesis that $GABA_A$-receptor mediated neurotransmission is required in the production of this response. Adult cats were anesthetized with pentothal (50 mg/kg, i.p. initially, supplemented i.v. as required). Inspiratory activities of the costal and crural diaphragm were recorded with EMG electrodes. In order to limit input from afferent fibers not arising from the diaphragm, the vagus nerves and brachial plexi were cut. The spinal cord was transected at C_7 and caudal segments destroyed. Arachidonic acid (6 - 10 mg in 100 mM $NaHCO_3$, total volume: 1 ml; n = 6) injected through a catheter positioned in the thoracic aorta shortened T_I by 21 ± 8.0% (mean ± S.D.). The $GABA_A$-receptor antagonist bicuculline (0.1 mg/kg) was given intravenously at least 25 minutes after the injection. Five minutes after bicuculline delivery, AA injected into the artery did not elicit a shortening of T_I in any animal (ΔT_I = +21 ± 21%). In most cases (4 of 6 experiments), the inspiratory duration was prolonged by stimulation with AA. Arachidonic acid injections were repeated at 30 - 40 minutes intervals thereafter. One to two hours after bicuculline delivery, AA injected into the artery again elicited some degree of inspiratory shortening in all animals tested (average ΔT_I -13 ± 3.3%; n = 5). These results indicate that the inspiratory shortening elicited by AA injected into the arterial circulation requires $GABA_A$-receptor mediated neurotransmission. (Supported by NIH PO1 40369)

490.6

RESPIRATORY NEURONS IN VENTROLATERAL MEDULLA OF NEONATAL RATS HAVE PACEMAKER-LIKE PROPERTIES IN LOW Ca^{++} SOLUTION *IN VITRO*.

S.M. Johnson*, J.C. Smith, & J.L. Feldman. Systems Neurobiology Laboratory, Dept. of Physiological Science, UCLA, Los Angeles, CA, 90024-1527

Respiratory neurons with voltage-dependent pacemaker-like properties are located in the pre-Bötzinger Complex (preBötC) of ventrolateral medulla (Smith *et al., Science* 254:726, '91). We tested whether preBötC neurons still exhibit oscillatory bursting following synaptic blockade in low Ca^{++} solution. Medullary slices (650 µm thick) from neonatal rats (0-4 days old) were prepared (*ibid.*). Control solution was (mM): 128 NaCl, 9-11 KCl, 1.5 $CaCl_2$, 1.0 $MgSO_4$, 21 $NaHCO_3$, 0.5 NaH_2PO_4, and 30 glucose (gassed with 95% O_2-5% CO_2 at 27°C). Low Ca^{++} solution was identical except for: 120 NaCl, 0.2 $CaCl_2$, 4.0 $MgCl_2$. Inspiratory rhythm was recorded with suction electrodes on the hypoglossal (nerve XII) roots.

In low Ca^{++} solution, we recorded extracellularly from 38 bursting cells [burst duration/frequency = 0.80±1.0 s / 0.38±0.27 Hz (mean ± SD)]. After switching to control solution, the respiratory rhythm was activated and these cells were identified as: I-cells (n=27, discharge coincident with nerve XII output), E-cells (2, discharge tonic except during nerve XII output), or tonic cells (9, discharge tonic). From a separate group of 11 I-cells, 7 E-cells, and 2 tonic cells that were initially identified in control solution, only 3 I-cells exhibited bursting in low Ca^{++} solution. We conclude that there are subpopulations of I-, E- and tonic cells that have intrinsic bursting properties under conditions of synaptic blockade. The relatively large fraction of I-cells with these properties supports the hypothesis that pacemaker properties may play a key role in respiratory rhythmogenesis. Supported by NIH Grants HL02204, HL40959 and HL08524.

490.7

ROLE OF EXCITATORY AMINO ACID (EAA) RECEPTORS IN RESPIRATORY RHYTHM GENERATION AND DRIVE TRANSMISSION *IN VITRO*. G.D. Funk*, J.C. Smith, & J.L. Feldman, Systems Neurobiology Laboratory, Department of Physiological Science, UCLA, Los Angeles, CA, 90024-1527.

Medullary slices from neonatal rat containing the pre-Bötzinger Complex (pre-BötC) generate inspiratory (I) oscillations in cranial nerves IX and XII (Smith *et al.*, *Science*, 254:726,'91). To determine the role of EAAs in rhythmogenesis and drive transmission, antagonists of NMDA (MK-801, 100 μM) and non-NMDA receptors (CNQX, 20 μM) were injected into the pre-BötC and rostral ventral respiratory group (rVRG). MK-801 had no effect. Injections of CNQX produced dose-dependent decreases in: i) I amplitude and frequency (*f*). Amplitude reductions were produced following injections in rVRG and pre-BötC. Perturbations in *f* only occurred following injections into a limited region of pre-BötC. We tested the effects of local application of CNQX, MK-801 and AP-4 on I-modulated synaptic inputs to XII motoneurons (XII MNs). CNQX (50 μM) reduced synaptic currents by >95%, MK-801 (1.0 mM) had little effect, and AP-4 (1.0 mM) produced a 25% reduction. Local application of NMDA and non-NMDA (Quis, AMPA, Kain) agonists to synaptically isolated XII MNs (1.0 μM TTX) induced large inward currents. We also examined the role EAA receptor desensitization in I drive transmission to XII MNs by blocking desensitization with locally applied cyclothiazide (50-250 μM). I currents increased 10-15%. Results indicate that: i) rhythm generation in the *in vitro* neonatal rat medullary slice is dependent on endogenously released EAAs acting at non-NMDA receptors within a specific region of the pre-BötC; ii) EAA transmission within rVRG is involved in transmission of I drive to motor or premotoneurons; iii) XII MNs possess NMDA and non-NMDA (AMPA and Kain) receptors, but an EAA-like substance acting primarily at non-NMDA receptors mediates transmission of I drive to XII MNs; iv) minimal desensitization of glutamate receptors occurs during I-mediated glutamate release at the XII MN. Supported by NIH Grants HL40959 & HL02204 and Parker B. Francis Foundation.

490.8

RETROTRAPEZOID NUCLEUS (RTN) GLUTAMATE INJECTIONS: LONG-TERM STIMULATION OF PHRENIC ACTIVITY. Eugene E. Nattie* and Aihua Li, Department of Physiology, Dartmouth Medical School, Lebanon, N.H., 03756-0001

In chloralose-urethane anesthetized, paralyzed, vagotomized, glomectomized, and servo-ventilated cats, we injected glutamate (10 nl; 10 mM, 100 mM, and 1M) unilaterally over 60 sec into the RTN. Seven 10 mM injections had no consistent effects. Ten 100 mM injections increased the integrated phrenic amplitude from a baseline average of 31 +/- 2 (SEM) % of maximum to a peak response of 50 +/- 3 % of maximum over a time period of 45.6 +/- 8.6 min. This response was significantly attenuated by prior injection of kynurenic acid. Seven 1 M injections decreased phrenic amplitude from 29 +/- 3 % to 20 +/- 5 % of maximum. Comparison of glutamate (10 nl; 100 mM) injected over 60 sec vs 30 msec showed that the prolonged increase in the integrated phrenic amplitude was present only with the more prolonged injection. On average the mean firing rate of 5 RTN single units increased significantly at 5 min following the 60 sec injection. One of these units had a prolonged increase in firing rate over 40 min and two others were increased for 10 and 15 min. Following the 30 msec injection, integrated phrenic amplitude and RTN single unit firing rate were increased for the first two breaths and at 5 min (less than after the more prolonged injection). With three 1 M injections the RTN unit activity was silenced. We conclude that glutamate injected into the RTN at a dose which avoids depolarisation block can produce a prolonged stimulation of phrenic activity associated with an increase in RTN single unit firing rate which is also prolonged in some cases. (Supported by HL 28066).

490.9

EFFECT OF ACETAZOLAMIDE ON CO_2 CHEMOSENSITIVITY OF CULTURED MEDULLARY NEURONS. A.A. Martin and J.A. Neubauer*. Department of Medicine, UMDNJ-Robert Wood Johnson Medical School, New Brunswick, NJ.

To determine whether inhibition of carbonic anhydrase alters the CO_2 chemosensitivity of dissociated medullary neurons, CO_2 sensitivity was characterized in neurons (4-6 days in vitro) before and during exposure to 10^{-5} M acetazolamide. Dissociated cell cultures were prepared from ventral medulla of neonatal rats (0-1 day old) and grown in defined medium. Membrane potential was monitored in spontaneously active cells using the nystatin perforated patch technique. pH was transiently decreased from 7.4 to 7.1 (τ on = 20 sec; τ off = 30 sec) by varying the PCO_2 of bicarbonate buffer solutions. In 12 cells mean membrane potential was -53.4 mV (SE 1.3) and mean firing frequency was 3.09 Hz (SE 0.38). Five cells decreased firing frequency and 5 did not change when exposed to high PCO_2. In 2 of 12 cells, acidosis produced a transient large depolarization, a partial repolarization and a smaller secondary depolarization and increase in firing frequency (+3.5 Hz) which decayed more slowly (τ = 70 s) than the initial transient. In the depressed cells acetazolamide delayed recovery of firing rate after acidosis. However, in the excited cells, acetazolamide was associated with a faster decay of the small sustained depolarization while the initial large transient was unaffected. We conclude that acetazolamide affects the CO_2 sensitivity of cells which respond to CO_2 and that in cells that are excited, carbonic anhydrase is important for a sustained membrane response to CO_2-induced acidosis. Support: HL07467, HL16022 and AHA-NJ.

490.10

EFFECTS OF ETHANOL ON HYPOGLOSSAL DISCHARGE IN THE NEONATAL RAT BRAINSTEM-SPINAL CORD PREPARATION. S. Iscoe*, E. DiPasquale, G. Hilaire, and R. Monteau, Biologie des Rhythmes et du Développement, URA CNRS 0205, Faculté des Sciences et Techniques St.-Jérôme, BP 332, 13397 Marseille cedex 13, France.

Hypoglossal and other cranial motoneurons are more susceptible than phrenic motoneurons to certain agents. We hypothesized that alcohol, previously shown to depress hypoglossal, but not phrenic, activity in adult anesthetized cats, would exert the same effect in the neonatal rat brainstem-spinal cord preparation. We added ethanol in concentrations between 1 and 12 mM (approximately 0.005 to 0.06 vol%) to the superfusate of these preparations from 1 to 3 day old rat pups. After 5 and 10 min, ethanol at all concentrations did not affect burst frequency or amplitude of cervical (phrenic) activity. In contrast, after 5 min, ethanol (6 mM, n=6; and 12 mM, n=4) significantly ($P < 0.05$) reduced peak integrated hypoglossal activity by 50 and 38%, respectively. After 10 min, all concentrations of ethanol reduced hypoglossal activity (-71% at 12 mM). Ethanol (6 and 12 mM) eventually abolished hypo-glossal activity which did not recover 30 min after removal of ethanol from the superfusate. If the ethanol-induced depression of hypoglossal motoneuron activity is characteristic of that of other cranial motoneurons, and higher structures, this preparation may be a useful model of Fetal Alcohol Syndrome. (Supported by CNRS and the Medical Research Council of Canada)

490.11

MEDULLARY RESPIRATORY NEURONS RESPONSE TO CEREBELLAR STIMULATION. Fadi Xu and Donald T. Frazier*, Dept. Physiology, University of Kentucky, Lexington, Ky. 40536.

Our previous reports suggest that the cerebellum modulates the ventilatory response to mechanical and chemical loading. Our hypothesis is that activation of cerebellar pathways can modulate both the pattern and timing of brainstem pre-motor inspiratory and expiratory neurons. Adult cats were anesthetized with pentothal, paralyzed and ventilated. Stereotaxic coordinates were used to position a bipolar stimulating electrode into the rostral fastigial nucleus (rFN) and recording tungsten micro-electrodes within the medullary respiratory groups (DRG,VRG,NA,NRA). Stimuli (150 μA) were applied between 5 and 200 Hz at different phases in the cycle. The results show that 1) <10Hz delivered during expiration did not alter expiratory neuronal activity, however, >20Hz led to termination of the expiratory burst and an earlier onset of the next inspiration (phrenic neurogram) which was enhanced in both duration and amplitude; 2) <10 Hz delivered at beginning of inspiration did not affect inspiratory neuronal behavior, but 20-100 Hz produced prolongation of inspiratory duration and enhancement of peak ∫PN; 3) reversing the function of stimulating and recording electrodes failed to demonstrate antidromic activation in the. rFN. We conclude that the cerebellum is capable of modifying both the pattern and timing of the respiratory output at the brainstem level utilizing polysynaptic pathways. (NIH PO1 40369).

490.12

REGULATION OF NEUROTRANSMITTER RELEASE BY GABA AND TRH WITHIN THE PHRENIC MOTOR NUCLEUS OF NEONATAL RATS. Y. Sun*, P.G. Wagner, and M.S. Dekin, Pulmonary Div., Dept. of Medicine, UMDNJ-Robert Wood Johnson Sch. of Medicine, New Brunswick, NJ 08903.

Presynaptic inhibition mediated by $GABA_B$ receptors is insensitive to blockade by Ba^{++} ions (Lambert *et al.*, 1991). We have described a Ba^{++} insensitive outward rectifying K^+ conductance (g_B) in bulbospinal respiratory neurons which is activated via $GABA_B$ receptors and inhibited by thyrotropin-releasing hormone (TRH; see Dekin *et al.*, this volume). The Ba^{++}-insensitivity and outward rectifying properties of g_B are consistent with a role in presynaptic inhibition. Because of this, we tested the hypothesis that GABA and TRH act in a reciprocal manner to regulate neurotransmitter release. All experiments employed spinal cord slices from neonatal rats and whole cell recordings from phrenic motoneurons. The Ringer solution contained 500 μM $BaCl_2$ to suppress Ba^{++}-sensitive conductances coupled to $GABA_B$ and TRH, high Ca^{++}-Mg^{++} to block polysynaptic pathways, and 10 μM bicuculline to inhibit Cl^- mediated IPSP's. Monosynaptic EPSP's were elicited by stimulation of bulbospinal axons in either the lateral or ventral funiculus and could be blocked by the excitatory amino acid antagonist CNQX (HBC complex; 200 μM). Bath application of the $GABA_B$ agonist, (±)baclofen (100 μM), caused a significant depression of the EPSP amplitude measured at -80 mV ($p<0.01$). Similar application of TRH (1 μM) resulted in a significant enhancement of the EPSP amplitude ($p<0.01$). Measurement of the V-I relationship using small current injections into the postsynaptic cell showed that neither drug treatment affected input resistance. We also observed that (±)baclofen and TRH could each antagonize the others action on EPSP amplitude in a reversible manner. These data provide direct evidence that GABA and TRH act antagonistically on a common presynaptic conductance(s) to regulate transmitter release from respiratory bulbospinal neurons.(Supported by NIH Grants HL40369 and HL02314)

490.13

THYROTROPIN-RELEASING HORMONE (TRH) INHIBITS OUTWARD RECTIFYING $GABA_B$ ACTIVATED K^+ CHANNELS VIA A PROTEIN KINASE C DEPENDENT PATHWAY. M.S. Dekin*, Y. Sun, and P.G. Wagner, Pulmonary Div., Dept. of Medicine, UMDNJ-Robert Wood Johnson School of Medicine, New Brunswick, New Jersey, 08903.

The $GABA_B$ receptor agonist, (±)baclofen, activates a Ba^{++}-insensitive outward rectifying K^+ conductance (g_B) in labeled respiratory bulbospinal neurons cultured from neonatal rats (Wagner and Dekin, *J. Neurophysiol.*, 69:286, 1993). It has also been demonstrated that g_B is inhibited by TRH (Dekin and Wagner, *Neurosci. Abstr.*, 18:488, 1992). In this study we examined the mechanism(s) by which TRH acted on g_B. All recordings employed the patch clamp technique in the cell-attached configuration. During bath application of 100 μM (±)baclofen alone, g_B exhibited bursts of repeated channel openings separated by short quiescent periods. Mean channel open time (τ_O) was described by a single time constant. Closed times for g_B exhibited two time constants; a fast component ($\tau_{C,F}$) for channel closings within a burst of activity and a slow component ($\tau_{C,S}$) which reflected the interburst interval. In the presence of TRH, the open time probability for g_B was decreased (>30% in control vs. <10% in TRH) and $\tau_{C,S}$ was lengthened. τ_O and $\tau_{C,F}$ were not altered. These effects of TRH on g_B were identical to those produced by 8-bromo-cAMP (see Wagner *et al.*, accompanying abstract). To determine if TRH increased cAMP levels we applied Rp-cAMP, a competitive inhibitor of cAMP-dependent protein kinases. In the presence of Rp-cAMP (1 μM in the pipette), inhibition of g_B by TRH was prevented. Exposure to the protein kinase C (PKC) inhibitor, H-7 (100 μM in the bath), had a similar protective effect. These data suggest that TRH stimulated PKC to increase cAMP levels which then caused phosphorylation of g_B channels via a cAMP-dependent protein kinase. (Supported by NIH Grants HL40369 and HL02314)

490.14

CYCLIC ADENOSINE MONOPHOSPHATE (cAMP) INHIBITS $GABA_B$ ACTIVATED OUTWARD RECTIFYING K^+ CHANNELS IN CULTURED BULBOSPINAL NEURONS OF THE NEONATAL RAT. P.G. Wagner*, Y. Sun, and M.S. Dekin, Pulmonary Div., Dept. of Medicine, UMDNJ-Robert Wood Johnson School of Medicine, New Brunswick, NJ 08903.

The $GABA_B$ receptor agonist (±)baclofen activates a Ba^{++}-insensitive outward rectifying K^+ conductance (g_B) in labeled bulbospinal respiratory neurons of neonatal rats (Wagner and Dekin, *J. Neurophysiol.*, 69:286, 1993). The biophysical properties of g_B are similar to those of the S-type K^+ conductance (g_S) found in invertebrates. In this report, we further characterized g_B by studying the effects of second messengers known to control g_S. Specifically, we tested the hypothesis that phosphorylation of g_B via a cAMP dependent protein kinase inhibits channel activity. All recordings were made using the patch clamp technique in the cell-attached configuration. (±)Baclofen (100 uM) and 8-Bromo-cAMP (1-5 mM) were applied via the bath. During exposure to (±)baclofen alone, g_B channels displayed bursts of activity with a high open time probability (>30%). Within a burst, the mean channel lifetime (τ_O) was described by a single time constant. Closed times for g_B were fit with both fast ($\tau_{C,F}$) and slow ($\tau_{C,S}$) time constants; $\tau_{C,F}$ corresponded to rapid closings within a burst while $\tau_{C,S}$ corresponded to silent periods between bursts of channel activity. During exposure to 8-bromo-cAMP the open time probability decreased (<10%) and $\tau_{C,S}$ was lengthened. Neither τ_O nor $\tau_{C,F}$ were affected. 1 μM Rp-cAMP, a competitive inhibitor of cAMP-dependent protein kinases, prevented inhibition of g_B. These data establish cAMP mediated phosphorylation of g_B (or an associated protein) as a regulator of this channel in vertebrates and indicates that such phosphorylation inhibits bursts of channel openings without altering the kinetics of channel activity within a burst. (Supported by NIH Grants HL40369 and HL02314)

490.15

INTERSPECIES AND AGE-RELATED MORPHOLOGIC DIFFERENCES OF VENTRAL MEDULLARY STRUCTURES INVOLVED IN CHEMOSENSITIVE CARDIORESPIRATORY CONTROL. C. Ovid Trouth*, Y. Pan, R. Douglas, L.M. Sexcius, R.M. Millis. Department of Physiology & Biophysics, Howard University, College of Medicine, Washington, D.C. 20059

Microscopic examination of the cat and rat caudal ventral medullary surface revealed age-related and interspecies differences. (1) Age-related differences: In both cats and rats, the marginal glia of newborns was smoother and synaptic vessicles more numerous at the ventral surface than in adults. (2) Interspecies similarities: Chemosensitive neurons were found to lie lateral to the medullary pyramids and ventral to the inferior olives in both adult rats and cats. Three types of chemosensitive neurons were identified. Type I in the marginal glia; Type II within the neuropil below the marginal glia; and Type III at the walls of blood vessels (neurovascular elements) traversing the chemosensitive zones from pia mater. The marginal glia was found to be thicker and a greater number of synapses were identified in the chemosensitive area than in other areas of the ventral medulla. (3) Interspecies Differences: Differences between adult cat and rat structures were found in localization of chemosensitive neurons relative to the hypoglossal nerve rootlets. The chemosensitive neurons in the rat were located lateral and in the cat medial to the exit of the hypoglossal rootlets from the brainstem. Three distinct zones having chemosensitive neurons were demarcated in the cat, while in the rat the chemosensitive neurons were found to lie in two areas. It is concluded that age-related interspecies and morphologic differences might be indicative of physiologic variations in cardiorespiratory control at the ventral medullary surface.
SUPPORT: NIH-NIGMS GRANT #SO6GMO8016

490.16

CONCURRENT CHANGES IN VENTRAL RESPIRATORY GROUP (VRG) NEURON DISCHARGE PATTERNS DURING FICTIVE COUGH. R. Shannon*, K.F. Morris and B.G. Lindsey, Physiol. & Biophy., Col. Med., Univ. South Fla., Tampa, FL 33612

VRG premotor inspiratory and expiratory neurons that provide descending drive during eupnea are also active during cough (Soc. Neurosci. Abst. 18:124, 1992). Experiments were conducted to test the hypothesis that medullary neurons implicated in generating and shaping the eupneic pattern of breathing also produce the cough motor pattern. Decerebrated, thoracotomized, paralyzed, phrenic-triggered ventilated cats (n=6) were used. Up to 15 neurons were recorded simultaneously in the rostral and caudal VRG with arrays of microelectrodes. Caudal VRG bulbospinal neurons were identified by antidromic stimulation. Single neuron activity and phrenic and lumbar efferent neurograms were monitored during fictive cough produced by mechanical stimulation of the intra-tracheobronchial tree. Virtually all "types" of previously identified respiratory- related discharge patterns were observed. Comparison of neurons with firing rate changes during eupnea and cough showed that: 1) there were concurrent alterations in patterns in both the rostral and caudal VRG, 2) some neurons with similar discharge patterns during eupnea, responded differently during cough, 3) most neuronal types responded as predicted by cough model network simulations. The results support the aforementioned hypothesis. (Supported by USF Research and Creative Scholarship Grant)

490.17

DORSOLATERAL PONS MODULATES RESPIRATORY RESPONSE TO UPPER AIRWAY STIMULATION. T.E. Dick* and J.S. Jodkowski. Dept. of Med., Case Western Reserve Univ., Cleveland, OH 44106.

We hypothesized that parabrachial nuclei modulate the reflex motor response to upper airway stimulation. In barbiturate anesthetized rats, electrical stimulation (.2-1 mA, 1ms, 1 pulse/breath) of the nasal mucosa induced a short-latency (7-8 ms) and transient (4-33 ms) inhibition in diaphragmatic EMG in spontaneously breathing rats (n=6) or in phrenic ENG in paralyzed, vagotomized, ventilated rats (n=6). Conditioning electrical stimuli (Pulse: 1ms, 2-100uA, 100Hz, train duration: 50 ms) were delivered to the parabrachial area with a tungsten unipolar electrode positioned stereotaxically. The averaged (n=50) motor responses to nasal stimulation alone were compared to those that were preceded (5-200ms) by conditioning stimuli to the pons. Stimuli were delivered in the same phase of inspiration. In all experiments, the evoked inhibition of motor activity diminished after conditioning stimuli. This decrease in the evoked response was time dependent but lasted 100 ms. Conditioning did not occur after lesioning the site. In three rats, the electrode was replaced with a glass micropipette and L-glutamate (70-200 nl, 10 mM) was injected in the same area. The nasal-evoked inhibition in phrenic ENG was diminished after these injections for a period up to 20 min. D-glutamate (200 nl, 10 mM) injections were ineffective (n=2). Analogous experiments with electrical stimulation in decerebrate cats (n=3) produced similar results. We interpret these data to indicate that neurons in the dorsolateral pons modulate the response of respiratory muscles to upper airway stimuli. (Supported by HL-42400.)

490.18

THE CHARACTERISTICS OF NEURONAL ACTIVITY THAT MEDIATED THE PONTILE OFF-SWITCHING MECHANISM. M.-L. Fung* and W. M. St. John. Department of Physiology, Dartmouth Medical School, Lebanon, NH 03756.

Pontile off-switching is an essential component of the neurogenesis of eupnea. We studied the topographical organization of the rostral pons and found that neural inspiratory duration increases dramatically after small chemical lesions were placed in an extreme rostrolateral region. We believed that neuronal activities in the region are involved in off-switching of ventilatory activity. The purpose of this study was to characterize these neuronal activities. We recorded the unit activity extracellularly in decerebrate, cerebellectomized, paralyzed and vagotomized cats. Phrenic nerve activity was taken as the index for defining the respiratory cycle. The activities of thirty-three neurons were characterized during hyperoxic normocapnia. All had activities spanning through the respiratory cycle. The level of respiratory modulation was quantified by the h^2 value (Orem and Dick, 1983). Units had h^2 values ranging from 0.67 to 0.02, and about one third (12/33) had h^2 values above 0.2. Most units had peak activities either during late inspiration (I) or early expiration (E). Progressive hypoxic was induced by inhalation of carbon monoxide (1%) during the recording of 15 neuronal activities, and the activities of all declined and ceased during hypoxia. Units with low h^2 value had peak activities temporally related to the I-E transition when the unit resumed activity during the recovery from hypoxia. The hypercapnic responses of eight units were evaluated by step increases of the inhalated carbon dioxide level. The h^2 values of all of the units increased and some of the units increased the discharge rate during hypercapnia. Injections of high magnesium solution (6.3 mM, 10 nl) decreased the unit activities. In contrast, glutamate (5 mM) increased the unit activities. We concluded that (1) the late I and post I activities in the rostrolateral region are involved in the pontile off-switching mechanism; (2) the neuronal activities in the rostral pons that are required for the termination of inspiration are depressed during hypoxia; (3) chemical transmission is important for the functional properties of the rostral pons. (Supported by HL 20574)

491.1

ALZ-50 IMMUNOSTAINS MAINLY PRIMARY SENSORY FIBERS IN THE RAT SPINAL CORD. P. Liberini*, P. Piccardo, W. Ma, A.C. Cuello and A. Ribeiro-da-Silva. Dept. of Pharmacology & Therapeutics, McGill University, Montréal, Québec, Canada H3G 1Y6.

The monoclonal antibody (mAb) Alz-50 has been proposed as a marker for cellular pathological changes in Alzheimer's disease. However, it has been reported that that this mAb also reacts with specific epitopes located in discrete hypothalamic regions in normal individuals. Furthermore, intense Alz-50 immunoreactivity (IR) has been recently described in the hypothalamus and spinal cord of rat and monkey. In the present study, we analyzed the distribution pattern of Alz-50-IR in the rat spinal cord. Male Wistar rats, 300-350 g in weight, were anesthetized with Equithesin and perfused through the ascending aorta with an aldehyde mixture. Cross-sections for all levels of the spinal cord were processed for light microscopic immunocytochemistry. The ultrastructural study was carried out at cervical levels only. Using light microscopy, immunostained fibers and varicosities were detected mainly in laminae I-II, although some immunostaining could be detected in deeper laminae. At the ultrastructural level, immunostained axonal varicosities could be detected in lamina I and outer 2/3 of lamina II. The varicosities appeared either scalloped or dome-shaped and contained numerous agranular synaptic vesicles and a few dense-core vesicles. Most varicosities were presynaptic to dendrites. A few immunostained dendrites were also observed, but glial cells were never immunostained. Some ultrathin sections were processed for postembedding immunogold detection of CGRP-IR and GABA-IR. Most of the varicosities which were immunoreactive for Alz-50 co-localized CGRP-IR. In contrast, GABA-IR was never co-localized with Alz-50-IR. These results indicate that, in the superficial dorsal horn, the epitope recognized by the Alz-50 antibody is located mainly, but not exclusively, in primary sensory fibers.

Supported by MRC (Canada) and the Canadian Network of Centres of Excellence for Neural Regeneration and Functional Recovery.

491.2

OPTICAL RECORDING OF DORSAL ROOT STIMULUS-INDUCED RESPONSES IN RAT SPINAL CORD SLICES STAINED WITH A VOLTAGE-SENSITIVE DYE. K. Murase*, H.Yamada, M.Tanifuji and P.D. Ryu. Dept. Information Science, Fukui University, 3-9-1 Bunkyo, Fukui 910, Japan.

A high-resolution optical recording of neuronal activities was performed in spinal cord slices, and mechanism of sensory signal transmission was investigated. Transverse and sagittal slices of the rat spinal cord with attached dorsal rootlets were stained with a voltage-sensitive dye NK-3630 (Nippon Kankoshikiso), and the light absorption was measured by an 128x128-pixcel image sensor (SD1001, Fuji Film Microdevices) at every 0.6msec. Single dorsal root stimuli evoked optical response consisted of at least two phases, fast and slow components: The fast excitation lasting for several milli seconds occurred predominantly at the laminae II-III. It was followed by the slower excitation lasting for up to .5sec occurring most intensely at the laminae I-II and less at deeper laminae. Both components are of neuronal origin since no optical response was recorded when TTX was added in the perfusate. The former component probably resulted from the afferent fiber activation and the latter from the following postsynaptic events, because a calcium-free perfusate eliminated only the latter. A bath application of CNQX (5-20μM), a selective antagonist of non-NMDA-type excitatory amino acid receptors, eliminated almost completely the presumable postsynaptic component. In contrast, APV (30-50μM), a selective antagonist of NMDA-type excitatory amino acid receptors, abolished only a part of the postsynaptic component, the long-lasting part, while the initial part within 10-30msec was unchanged. A substance P receptor antagonist, spantide (10-50μM), reduced the long-lasting part as well. Adding suramin or α,β-methylene ATP, the antagonist and agonist of ATP receptors, in the perfusate did not alter the spatio-temporal pattern of the response. Supported by grants from Jpn Min. Edu.

491.3

A COMPARISON OF CELL TYPES IN FETAL AND POSTNATAL SPINAL CORD PRIMARY CULTURES. L. Kehl*, G. Poliac and G. Wilcox. Program in Neuroscience and Dept. of Pharmacology, University of Minnesota, Minneapolis, Minnesota, U.S.A.

Studies underway in this laboratory have focused on the electrophysiological examination of a subset of postnatal spinal cord neurons. However, neurons in these cultures are not easily distinguishable on the basis of morphology or electrophysiology. Indeed, electrophysiological studies in other laboratories have attested to the "neuronal-like" excitability of certain glial cell subtypes in rat spinal cord. Therefore, the purpose of these studies was to characterize a cross-section of fetal and postnatal spinal cord cell types maintained in primary culture using electrophysiological, pharmacological and immunohistochemical techniques.

Primary cultures of spinal cord tissue were obtained from fetal or 14-15 day old Sprague Dawley rats in the following manner. Spinal cords were removed, treated with enzymes, mechanically dissociated, plated onto laminin-coated coverslips and co-cultured with cortical glial monolayers. These were incubated at 37°C (10% CO_2) in MEM with 10% fetal bovine serum. Patch clamp studies using the amphotericin B perforated patch technique were performed between 1 and 30 days after plating. Drugs were administered in close proximity to the cell being studied (100-150 μm) using a U-tube. Immunostaining was performed using antibodies to neuronal proteins (neuron-specific beta tubulin and MAP2) and glial proteins (GFAP and OX-1).

In the postnatal cultures, immunostaining with neuronal markers labeled predominantly bipolar cells. However, postnatal cells with varying morphologies demonstrated action potential-like responses when depolarized from rest (-48mV to -83mV). (Supported by NIH/K15-DE00225-04 to LJK and NIH/K02-DA-00145 and NIH/RO1-DA-04274 to GLW.)

491.4

Classification of Cellular Properties of Spinal Dorsal Horn Neurons. M.C. Jiang*, C.L. Cleland and G.F. Gebhart. Department of Pharmacology, University of Iowa, Iowa City, IA 52242

Cellular properties can generate endogenous neural activity and play a role in modifying synaptic input. In order to study the contributions of cellular properties to the processing of nociceptive and non-nociceptive synaptic input to spinal cord neurons, we have first identified and characterized the expression of cellular properties in spinal dorsal horn neurons.

Intracellular recordings were obtained from spinal dorsal horn neurons in the L6-S1 spinal segments of intact, pentobarbital anesthetized adult rats. To stabilize neural recordings, a platform was placed under the spinal cord and a pneumothorax was performed to reduce movements caused by arterial pulsation and respiration. These manipulations allowed us to maintain stable recordings for up to 2 hours. Natural stimulation was applied to identify the receptive fields of the neurons. Cellular properties were tested by intracellular current injection.

Dorsal horn neurons could be divided into two groups: one group (n=9) had predominantly single-component afterhyperpolarizations (AHPs) and the other group (n=34) had multiple-component AHPs. The duration and magnitude of AHPs varied between neurons. Neurons with single-component AHPs tended to have longer duration action potentials. Regarding spike frequency adaptation (SFA), nine neurons had fast SFA, five slow SFA, and eight no SFA at all. We also observed oscillations in five neurons. In one neuron, the existence and frequency of oscillation were voltage dependent, suggesting that the oscillations arose from endogenous mechanisms. In ten neurons, post inhibitory rebound was observed. Currently, we are investigating the relationship between these cellular properties and natural input.

491.5

PERIODIC AND APERIODIC ACTIVITY IN ISOLATED MAMMALIAN SPINAL CORD: POINCARÉ MAPS. A.J. Dekhuijzen* and J. Bagust. Dept. Physiology and Pharmacology, University of Southampton, Southampton SO9 3TU, England, U.K.

The description of neural activity by traces from the oscilloscope does not necessarily represent the long term behavior of the neurons. Methods using interspike interval (ISI) histograms were compared to an analysis with Poincaré maps. The Poincaré maps showed the time preceding a spike against the time following the spike. Controls and several experimental interventions, 4-aminopyridine (2 μM) and modulation of temperature were applied to study the neural output of dorsal roots in the isolated spinal cord of the rat and hamster. ISI histograms showed unimodal, bimodal and trimodal distributions, depending on the experimental intervention. Poincaré maps revealed interburst intervals, ISIs within bursts of activity, the extent of periodic or aperiodic activity obtained, and were useful to study the effect of using a specific drug. Using maps, continuous firing of action potentials (periodic activity), higher order periodic patterns and aperiodic behavior were shown in the results. Artificial signals revealed distinct patterns in the Poincaré maps. Thus, Poincaré maps disclosed more about the inherent properties of the neural activity in the recorded data, as compared to ISI histograms alone.

491.6

CLASSIFICATION OF THE RAT SPINAL CORD DORSAL HORN NEURONS BY CLUSTER ANALYSIS. J.W. Leem*, W.D. Willis and J.M. Chung. Marine Biomed. Inst., Depts. of Anat. & Neurosci. and of Physiol. & Biophys., Univ. Texas Med. Br., Galveston, TX 77555.

The aim of this study was to classify dorsal horn neurons in the rat spinal cord with the use of a previously defined natural stimulus set that activates differentially different kinds of modality-specific cutaneous afferents in the rat foot.

The mean discharge rates of 89 sampled units evoked by 6 different stimuli applied to the skin of the foot were measured in anesthetized rats. The data set was subjected to hierarchical cluster analysis.

Five clusters were defined by cluster analysis. Two clusters included units receiving inputs only from low-threshold mechanoreceptors (LTMs) and those only from mechanical nociceptors. Three clusters contained units receiving inputs from LTMs as well as from nociceptors, which could be categorized conventionally as a "wide dynamic range" (WDR) class: a) units receiving greater inputs from mechano-heat nociceptors than from LTMs; b) units having strong inputs from both mechanical nociceptors and LTMs; and c) units with weak inputs from mechanical nociceptors and LTMs.

The results suggest that WDR cells in the rat spinal cord dorsal horn can be divided further into several functional groups. (Supported by NIH grants NS21266, NS11255 and NS09743 and a grant from Bristol-Myers Squibb Co.)

491.7

MODULATION OF SENSORY INPUT IN SUPERFICIAL AND DEEP LAMINAE OF THE RAT DORSAL HORN. P. Bernardi, J.G. Valtschanoff, R.J. Weinberg and A. Rustioni.* Dept. Cell Biology & Anatomy, UNC, Chapel Hill, NC 27599.

Immunocytochemical investigation of excitatory amino acids in terminals of primary afferents in the spinal cord of rats suggests that glutamate may be released by all terminals in superficial (I and II) and deep (III and IV) laminae of the dorsal horn, whereas aspartate may be co-released by terminals in superficial laminae only.

In the present work we investigated whether modulation of sensory input to the dorsal horn may differ in superficial versus deep laminae. Anterograde tracer was injected into the sciatic nerve to identify primary afferent terminals (WGA-HRP to label superficial laminae, CTB-HRP to label deeper laminae). The tracers were revealed with TMB/tungstate histochemistry. Nitric oxide synthase (NOS) was visualized using preembedding immunocytochemistry. Thin sections were further stained using post-embedding immunocytochemistry for GABA or glycine.

In superficial laminae, about 15% of type I primary afferent terminals received synaptic contacts from GABA-positive terminals. A larger percentage of type II terminals (about 30%) received synaptic contacts from GABA-positive terminals. Terminals of type I but not of type II were presynaptic to vesicle-containing GABA-positive profiles, mostly identifiable as dendrites. In deep laminae, about 15% of primary afferent terminals received synaptic contacts from GABA-positive terminals. Primary afferent terminals in these laminae were not presynaptic to vesicle-containing profiles. Glycine-positive terminals (also enriched in GABA) contacted only type II primary afferent terminals in superficial laminae. Glycine-positive terminals in deep laminae did not establish synaptic contacts on primary afferent terminals. Dendrites contacted by primary afferents in superficial laminae could be NOS-positive, whereas those in deep laminae were often glycine-positive.

491.8

WIND UP MEDIATED BY L-TYPE CALCIUM CHANNELS IN TURTLE DORSAL HORN NEURONS.
R.E. Russo & J. Hounsgaard* Department of Medical Physiology, University of Copenhagen, DK-2100, Denmark.

Wind up of the response to repeated synaptic activation of dorsal horn neurons is an elementary type of plasticity in the spinal cord. We have found wind up in deep dorsal horn neurons in an in vitro preparation of the turtle spinal cord. The number of spikes elicited by ipsilateral dorsal root stimulation (0.5-1 Hz) increased over the first few stimuli, and a prolonged afterdischarge was observed when stimulation was terminated. A slow depolarization and an increase in background synaptic noise was evident when cells were hyperpolarized, but the duration of the response was longer at depolarized membrane potentials. In some cells it was also possible to elicit wind up with depolarizing current pulses. This phenomenon was due to the activation of a plateau potential that accumulated between pulses. The accumulation of the plateau potential decreased and eventually disappeared as the cell was hyperpolarized.

APV (100 uM) reduced the overall response to dorsal root stimulation but, it was still possible to elicit wind up of the response. The intracellularly-induced wind up was not affected by APV. Nifedipine (10-50 uM) reduced the plateau potential as well as both types of wind up. Therefore, we propose that a plateau potential mediated by the L-type Ca channel is involved in wind up generation.

Supported by grants from: EEC, Lundbeck Foundation and Danish Medical Research Council.

491.9

GABA IMMUNOREACTIVITY IS CO-LOCALIZED WITH ENKEPHALIN OR ChAT IMMUNOREACTIVITIES IN THE DORSAL HORN OF THE RAT SPINAL CORD. AN ULTRASTRUCTURAL DOUBLE-LABELING STUDY
A. Ribeiro-da-Silva*, M. Ballak and A. C. Cuello. Dept. of Pharmacology & Therapeutics, McGill University, Montréal, Québec, Canada H3G 1Y6.

In previous studies, we demonstrated the occurrence of choline acetyltransferase (ChAT) immunoreactive (IR) (J. Comp. Neurol. 295: 370-384, 1990) and enkephalin (ENK)-IR varicosities (J. Neurosci. 11: 1068-1080, 1991) which were presynaptic to primary sensory fibers in the superficial dorsal horn of the rat spinal cord. As GABA-IR profiles are also known to be presynaptic to primary sensory fibers in the spinal cord, we decided to investigate the possibility of a co-localization of the above neurochemicals. Adult male Wistar rats were anesthetized with Equithesin and fixed by vascular perfusion with an aldehyde mixture. Transverse sections of the cervical spinal cord were processed for either ENK or ChAT ultrastructural preembedding immunocytochemistry using monoclonal antibodies. GABA-IR sites were demonstrated using a postembedding immunogold protocol. For the simultaneous demonstration of ChAT- and ENK-IR sites, a combination of radioimmunocytochemistry and enzyme-based immunocytochemistry was used. Most of the ChAT-IR profiles were also GABA-IR. Some ENK-IR boutons co-localized GABA immunoreactivity. In synaptic glomeruli, GABA/ChAT co-localization was detected in boutons presynaptic to the central varicosities. Although less frequent, GABA/ENK co-localization was also found in some boutons which were presynaptic to the glomerular central varicosities. Interestingly, the radioautographic pre-embedding study did not reveal any ENK/ChAT co-localization. These results indicate that GABA participates in the presynaptic modulation of primary sensory information through at least two independent neuronal systems, one co-localizing ENK and the other co-localizing ChAT.

Research supported by Canadian Medical Research Council (MRC)

491.10

UNMASKING OF A NOVEL LONG-LASTING SLOW IPSP IN RAT SUBSTANTIA GELATINOSA NEURONS FOLLOWING BLOCKADE OF GLYCINE AND GABA RECEPTORS.
Y. Yajiri, H. Baba, M. Yoshimura* and S. Nishi, Dept. Physiol., Kurume Univ. Sch. Med., Kurume, 830 Japan.

Intracellular recordings were made from substantia gelatinosa (SG, lamina II) neurons in tissue slices of the adult rat spinal cord. A dorsal root was retained attached to the slice preparation. Stimulation of primary afferent Aδ fibers evoked in 40% of SG neurons glycinergic and/or GABAergic IPSPs with a duration less than 500 ms. After blocking these IPSPs by strychnine and bicuculline, an additional slow IPSP with an exceptionally long time course, lasting 30-120 s following a single stimulus, appeared. The new IPSP, hereafter, may be referred to as the long-lasting slow IPSP (l. s. IPSP). Stimulation of Aδ fibers alone could generate the l. s. IPSP of full size. The l. s. IPSP was associated with a decrease in input resistance and reversed in polarity near the potassium equilibrium potential. The l. s. IPSP was abolished by CNQX but not by catecholaminergic, serotonergic or cholinergic antagonists. Several peptides which are reported to be contained in spinal interneurons have been tested, but they showed no appreciable effects on SG neurons, except for somatostatin. Somatostatin produced in SG neurons a slow hyperpolarizing response that was electrophysiologically analogous to the l. s. IPSP. These observations suggest that glycinergic and GABAergic interneurons normally suppress a subset of interneurons or their terminals which liberate the transmitter responsible for production of the l. s. IPSP. The transmitter in question might possibly be somatostatin, but this remains to be investigated further.

491.11

NITRIC OXIDE SYNTHASE-CONTAINING NEURONS IN THE DORSAL ROOT GANGLION ARE SUSCEPTIBLE TO CAPSAICIN-INDUCED EXCITOTOXICITY.
K. Ren*, E.H. Franklin and M.A. Ruda, Neurobiology and Anesthesiology Branch, NIDR, NIH, Bethesda, MD 20892.

Reduced nicotinamide adenine dinucleotide phosphate-diaphorase (NADPH-d) activity has been localized to spinal sensory ganglia and may be used as a marker for nitric oxide synthase. To study the involvement of NADPH-d/nitric oxide synthase in functions of primary sensory neurons, NADPH-d activity in spinal lumbar dorsal root ganglia (DRG) was examined in neonatally capsaicin-treated (CAP), (50 mg/kg, s.c.) or vehicle-treated rats seven days after sciatic nerve cut using a standard enzyme histochemical technique. In vehicle-treated rats, 7.4% (167/2271) of L5 DRG neurons exhibited intense NADPH-d activity. Among the labeled neurons, 83.8% had cross-sectional areas less than 1000 μm^2 (small) and 16.2% were between 1000 and 2000 μm^2 (medium). In CAP-treated rats, the number of NADPH-d positive neurons, mainly small-sized, was significantly reduced to 2.2% (43/1935, χ^2, $p < 0.0001$). Following sciatic transection, NADPH-d positive neurons in ipsilateral L5 DRG were markedly increased to 14.1% (285/2026, χ^2, $p < 0.0001$). CAP-treated rats showed no significant induction of NADPH-d activity after peripheral axotomy. These results suggest that a large number of primary sensory neurons exhibiting NADPH-d activity are CAP-sensitive neurons. Nitric oxide synthase appears to be primarily involved in the functional activity of small-sized DRG neurons, many of which are nociceptive.

491.12

A BIOASSAY FOR NITRIC OXIDE RELEASE FROM SPINAL CORD IN RATS.
J.C. Eisenach,* P. Li, C. Tong. Dept. of Anesthesia, Wake Forest University Medical Center, Winston-Salem, NC 27157-1009.

The purpose of this study was to determine if NMDA caused nitric oxide release in spinal cord, as measured by a vascular bioassay system. Rat aortic rings were denuded of endothelium and mounted on transducers for tension recording with a polygraph. Perfusion of spinal cord with NMDA caused concentration-dependent relaxation of the detector aortic rings, while this agent applied to the aortic rings themselves had no effects. Preincubation of spinal cord with different NOS inhibitors and methylene blue attenuated NMDA's effects. Pretreatment with the specific NMDA receptor antagonist, MK-801 blocked NMDA induced aortic rings dilation. These results demonstrate the release of a vasodilating substance from spinal cord during incubation with NMDA acting on NMDA receptors. This substance is likely nitric oxide because (1) this dilation response could be blocked by NOS inhibitors and methylene blue; (2) prostaglandin production was blocked by indomethacin; (3) applying these agents alone had no effect on the aortic rings' tension. Further studies will determine more definitively whether this substance is nitric oxide and examine factors which increase or decrease nitric oxide release from the spinal cord.

Supported in part by grant GM35523 from the NIH.

491.13

BIPHASIC EFFECTS OF ENFLURANE ON DORSAL HORN NEURONS. T. Yanagidani, K. Ota, A. Hinds, S. Cannan, Y. Yamamori*, J.G. Collins. Dept. of Anesthesiology, Yale Univ. Sch. of Med., New Haven, CT 06510

The purpose of this study is to examine the effects of enflurane (E) on responses of spinal dorsal horn neurons to low intensity stimulation of their peripheral receptive fields (RFs).

This protocol was approved by the Yale Animal Care and Use Committee. Following isolation of single neurons in intact, awake, drug-free cats, RF area sensitive to light touch was mapped and the most sensitive area was stimulated by brushing, pinching and heating to define baseline responses. The short acting i.v. anesthetic propofol was then used to induce anesthesia, the trachea was intubated and lungs were ventilated with 2.1% E in 0_2. RF areas and responses to stimuli were again evaluated at 5, 30, 45 and 60 minutes after induction of anesthesia. Paired t test was used for analysis.

Ten low-threshold neurons and one wide dynamic range neuron have been studied to date. 60 minutes after induction, E significantly reduced the mean RF size to 76% of control. In spite of reducing RF sizes E significantly increased the mean neuronal response of those same neurons to RF brushing to 177% of control. The increased sensitivity to low-intensity stimulation in the remaining RF area was also evident in a reduction in von Frey thresholds.

As we have observed with other anesthetics (pentobarbital, halothane, nitrous oxide, propofol, dexmedetomidine) E reduces the low-threshold RF area of spinal dorsal horn neurons. Unlike those other drugs, however, it increases the response of the neurons to low-intensity stimulation in the remaining RF area. These results point to significant impact of anesthetics on low-threshold sensory processing in the spinal dorsal horn and may help to explain the cortical hyperexcitability seen during E anesthesia.

Supported in part by NIH GM 44954

491.14

HALOTHANE REDUCES SPINAL DORSAL HORN NEURONAL RESPONSE TO LOW-THRESHOLD RECEPTIVE FIELD STIMULATION K. Ota, T. Yanagidani, A. Hinds, S. Cannan, K. Kishikawa,* J.G. Collins. Dept. of Anesthesiology, Yale Univ. Sch. of Med., New Haven, CT 06510.

The purpose of this study is to evaluate the effects of halothane (H) on the response of spinal dorsal horn neurons to low-intensity receptive field (RF) stimulation.

This protocol was approved by the Yale Animal Care and Use Committee. Following isolation of single neurons in intact, awake, drug-free cats, RF areas sensitive to light touch were mapped and the most sensitive area was stimulated by brushing, pinching and heating to define baseline responses. The short acting i.v. anesthetic propofol was then used to induce anesthesia, the trachea was intubated and lungs were ventilated with 1.3% H in 0_2. RF areas and responses to stimuli were again evaluated at 5, 30, 45 and 60 minutes after induction of anesthesia. Paired t test was used for analysis.

To date 15 low-threshold (LT) neurons have been studied. 60 minutes after induction, a time when propofol effects are known to have dissipated, H significantly reduced the mean RF size to 59% of control. Neuronal responses to both static and dynamic low-threshold RF stimulation were also significantly reduced. Rapidly adapting LT neurons seemed to be less sensitive to the a reduction in RF size than slowly adapting neurons.

This study demonstrates the ability of H to reduce spinal dorsal horn neuron responses to low-intensity stimulation. As proposed by de Jong and Wagman,[1] these effects at the level of the spinal cord could contribute to anesthetic induced loss of sensation.

Supported in part by NIH GM48954

1. Exp Neurol 20:352-358, 1968

491.15

EFFECTS OF NITROUS OXIDE ON SPINAL DORSAL HORN NEURONAL RESPONSES TO LOW-INTENSITY RECEPTIVE FIELD STIMULATION IN CATS. J.G. Collins*, K. Ota, T. Yanagidani, A. Hinds, S. Cannan. Dept. of Anesthesiology, Yale Univ. Sch. of Med., New Haven, CT 06510.

Our laboratory is pursuing a better understanding of the effects of anesthetics on spinal sensory processing. Nitrous oxide (N_2O) is an important anesthetic agent but, because of low potency, is used in combination with other drugs. The purpose of this study is to examine the effects of N_2O on spinal dorsal horn neurons in the presence of halothane or enflurane.

This protocol was approved by the Yale Animal Care and Use Committee. The effects of halothane or enflurane on neuronal receptive field (RF) size and response to RF brushing, pinching and heating were first determined. We then added 70% nitrous oxide to the anesthetic mixture, allowed for a 15 minute period of equilibration, and re-evaluated neuronal responses. 15 neurons (4 enflurane, 2.1%, 11 halothane, 1.3%) have been studied to date.

In the presence of halothane anesthesia, 70% N_2O produced a 50% reduction in RF size from the baseline halothane level. Halothane had already reduced the mean RF area by 40% from the intact, awake, drug-free preparation. N_2O may produce less of a reduction in RF size in the presence of enflurane. None of the four RFs studied to date were reduced by greater than 20%.

The presence of nitrous oxide appears to contribute to the depressant effects of enflurane and halothane on spinal dorsal horn neuronal responses to low-threshold receptive field stimulation. It appears that most anesthetics depress such responses at the level of the spinal cord.

Supported by NIH GM 44954

491.16

SPINOTHALAMIC AND SPINOMESENCEPHALIC NEURONS WITH COLLATERALS TO THE MEDULLARY RETICULAR FORMATION IN THE SUPERFICIAL DORSAL HORN (SDH) OF THE RAT LUMBAR SPINAL CORD. T.N. Bice and J.A. Beal.* Department of Cellular Biology and Anatomy, Louisiana State University Medical Center, Shreveport, LA 71130.

Neurons in the SDH of the rat spinal cord have been shown to project to the medullary reticular formation and are thought to be involved in the relay of nociception to pain modulatory and autonomic centers, as well as to the cerebellum. It is not known, however, what percentage of these neurons project beyond the reticular formation to more rostral nociceptive relay centers. To make this determination, two fluorescent retrograde axonal tracers, Fluoro-Gold (FG) and True Blue (TB), were injected stereotaxically into specific CNS centers. TB was delivered into the right dorsal, lateral, and ventrolateral reticular nuclei, and FG was delivered into the dorsal thalamus (medial and lateral) and midbrain (cuneiform nucleus, parabrachial nucleus, and periaqueductal gray). After 14 days, animals were anesthetized and intracardially perfused with 4.0% paraformaldehyde. Spinal cord segment L1 was serially sectioned in the transverse plane and examined under the fluorescence microscope. Results showed that some SDH nerve cells were labelled with FG alone, but the majority were double labelled, i.e., labelled with both FG and TB. No SDH neurons were labelled with TB alone. Results indicate that 100% of the input to the medullary reticular formation from the SDH is comprised of collaterals of spinomesencephalic or spinothalamic neurons.

RETINA: INVERTEBRATE

492.1

NEURAL CODING EFFICACIES OF SPIKE GENERATING CELLS IN *LIMULUS* EYES: COMPARISON WITH PHOTORECEPTOR CELL RESULTS. Zixi Cheng and Gerald S. Wasserman.* Sensory Coding Laboratory, Department of Psychological Sciences, Purdue University, West Lafayette, IN 47907-1364

Biological signals found in nerve cells exhibit complex temporal characteristics and are very different in shape from the stimuli that evoke them. Two current neural coding theories address these phenomena: Multiple Meaning Theory holds that these patterns make statements about combinations of stimulus properties. More specifically, the Task Dependence Hypothesis suggests that different properties (i.e., neural codes) of identical neural responses might mediate different behavioral tasks. (Both theories reviewed in Wasserman, G. S., *Biological Signals*, 1992, **1**, 117-142.)

We have characterized neural responses intracellularly recorded from single spike generating cells in *Limulus* lateral eyes across a wide range of light adaptation states and stimulus intensities. Six candidate codes were measured and their efficacies were calculated using Signal Detection Theory (TSD) as an objective index. The results show that: A) Both adaptation state and intensity affect efficacy. B) The efficacies of these neural codes are significantly different and can be arranged in the order: Area ≥ Peak ≥ Mean ≥ Slope ≥ Duration-End = Duration-Drop. C) As light adaptation reduces detection sensitivity, it increases discrimination acuity as characterized by TSD.

Though these results are similar to our previously reported findings in *Limulus* photoreceptors (Cheng, Z. and Wasserman, G. S., *Soc. Neurosci. Abstr.*, 1991, **17**, 298), they are not identical. We found: A) All efficacies tend to reduce between neural levels. B) The reduction is code dependent; the Peak, in particular, is affected less than the Area. C) The code which transmits best between cells therefore appears to differ from the code which has the highest efficacy within a cell.

492.2

INCREASE IN THE LIGHT RESPONSE OF LATERAL EYE PHOTORECEPTORS IN *LIMULUS* PRODUCED BY TWO ENANTIOMERS OF OCTOPAMINE. George H. Renninger*, Biophysics Group, Physics Dept., Univ. of Guelph, Ontario N1G 2W1.

Octopamine (OCT) is synthesized in the eyes of the horseshoe crab, *Limulus polyphemus*. It is stored in the terminals of efferent nerve fibers originating in the central nervous system, and released when the fibers are depolarized. Activation of these fibers by a central circadian clock produces changes in the compound lateral eye, among which is an increased responsiveness of the photoreceptor cells to light. OCT applied to a preparation of the lateral eye *in vitro* partially mimics the effects of efferent activity on the light response (review: Battelle, 1991).

Commercially available OCT is a racemic mixture of the enantiomers D(-) OCT and L(+) OCT. Application of D(-) and L(+) OCT separately to the *in vitro* preparation reveals that the concentration of D(-) OCT producing a half maximal effect is about two orders of magnitude lower than that of L(+) OCT. Thus the effects of OCT on photoreceptor response show stereospecificity for the D(-) enantiomer of OCT, which is the naturally occurring form.

The steady-state light response generally begins to increase within 5 - 10 min after application of D(-) OCT, reaching a steady level within 0.5 - 0.8 h. The increase is difficult to reverse, requiring more than 1 hr when reversal occurs. These observations suggest that OCT produces relatively stable changes in photoreceptor response, which perhaps require other factors to reverse *in vivo*.

Battelle, B-A (1991) In *Prog. Retinal Res.* **10** : 333-355 (eds. N. Osborne, J. Chader)

Research supported in part by NSERC Canada.

492.3

NEURAL CODING IN THE *LIMULUS* VISUAL SYSTEM: COMPUTATIONAL AND ELECTROPHYSIOLOGICAL STUDIES

E.D. Herzog*, F. A. Dodge, and R.B. Barlow, Jr.
Institute for Sensory Research, Syracuse University, Syracuse, NY 13244

What is the neural code that underlies visual behavior? We address this question by recording from and modeling responses of the optic nerve of *Limulus*.

We record retinal responses to behaviorally significant scenes (Powers, *et al.*, 1991, *Vis. Neurosci.* **7:** 179-89) in order to determine the threshold and suprathreshold neural codes for objects of different contrast and size. We record from 1-3 optic fibers while the animal moves in the Atlantic Ocean and also in an "indoor ocean." Field experiments include parameters that are difficult to simulate in the lab. In both the lab and the field, modulation of the optic nerve activity is determined by 1) the animal's velocity relative to the object, 2) the object's size and contrast and 3) the properties of the retinal network.

We simulate modulation of the optic nerve activity by first computing how the optics of an ommatidium transform the visual scene and second how neural elements of the retina transform this information into a neural code. We have tested a variety of candidates for the neural code and find that several correlate well with the animals' behavior.

A highly simplified model of the retinal array of receptors suggests that the ensemble activity of only a few adjacent receptors may be sufficient to detect objects of interest to the animal.

Supported by NSF grant BNS 9012069 and NIH grant EY00667.

492.4

PROTEIN KINASE C ACTIVATES TRANSIENT MEMBRANE SHEDDING AND SCREENING PIGMENT MOVEMENT IN THE *LIMULUS* LATERAL EYE.

R.N. Jinks, J.N. Licameli, E.J. Wladis, and S.C. Chamberlain*, Bioengineering & Neuroscience & Inst. for Sensory Research, Syracuse University, Syracuse, NY 13244.

Phorbol ester activation of protein kinase C (PKC) has been shown to induce rhabdomeral disorganization and assembly in dark-adapted photoreceptors of the moth *Manduca sexta* (*ARVO Abstracts*, **33/4**: 239). Our detailed understanding of the nature of daily rhabdom shedding and its control in *Limulus* makes it an ideal system to examine possible roles of the 1,2 diacylglycerol/PKC second messenger cascade in mediating transient membrane shedding.

Animals maintained under natural lighting were placed in a light proof tank at sundown. After 12 h, one lateral eye of each animal was subretinally injected with phorbol ester (PE) or DL-erythro-dihydro-sphingosine, a semi-specific PKC inhibitor, while the other eye served as a control. Eyes treated with PE (and their controls) were fixed in the dark 30 min after injection. Sphingosine treated eyes (and their controls) were fixed after 30 min of post-injection light exposure.

Membrane whorls typical of daily shedding were present in abundance in the rhabdomeral arrays of the ommatidia of eyes treated with the PE, phorbol 12,13-diacetate (150 - 1500 μM), in *Limulus* Ringers. Untreated control eyes in these animals did not shed. Negative controls using the inactive ester, 4α-phorbol, did not shed. The PKC inhibitor, sphingosine (100μM in DMSO), appears to block light-driven shedding. Screening pigment granules, which have been shown to migrate rapidly into the rhabdomeral segment at light onset were found there in PE treated photoreceptors. These data suggest that phorbol esters mimic light onset by triggering transient membrane shedding and the movement of screening pigment granules through the activation of a diacylglycerol/PKC second messenger cascade.

Supported by NIH EY03446 and Syracuse University.

492.5

INOSITOL TRISPHOSPHATE RELEASE FROM THE MEMBRANE IN THE PHOTORESPONSE OF THE HERMISSENDA TYPE B PHOTORECEPTOR CELL.

M. Sakakibara 1, T. Kouchi 2, H. Inoue 2 and T. Yoshioka2.*
1 Department of Biological Science and Technology, School of High Technology for Human Welfare, Tokai University, Numazu, 410-03,
2 Department of Molecular Neurobiology, School of Human Sciences, Waseda University, Tokorozawa, 359, Japan

Despite a considerable number of studies, elucidation of the phototransduction mechanism in invertebrates remains out of reach. The major reason for this is the difficulty of identifying the second messengers involved. The present idea stems from two contradictory results; (i) phosphatidylinositol 4,5-bisphosphate (PIP2) specific phospholipase C (PLC) is necessary, (ii) but inositol trisphosphate (IP3), the hydrolyzed product of PIP2, is not necessary for the generation of photoreceptor potential. By introducing the idea that the invertebrate photoresponse may be triggered by perturbation of the membrane field change by the hydrolysis of PIP2, we are able to explain a variety of properties of the photoresponse. Although the PIP2 content is very low , the effect of IP3 release from the membrane on the membrane field around the Na+-selective channel underlying the photoresponse should be quite large, since IP3 has 5 negative charges in the physiological pH range. We hypothesized that the photoresponse is triggered by the conformational change of the Na+-selective channel induced by IP3 release. If this were the case, inhibition of PIP2 specific PLC by screening of the negative charges of PIP2 by positively charged compounds should suppress the photoresponse. In order to test this idea we injected substances with high positive charges into Hermissenda type B photoreceptor cell, and observed an effect on the photoresponse. Various kinds of negative charge chelator for PIP2 produced reversible block of the photoresponse, while injection of IP3, cAMP or cGMP had no significant effect.

492.6

THRESHOLD MEASUREMENTS IN A PHOTORECEPTOR.

E.P. Hornstein and T.E. Cohn*. Group in Vision Science, University of California, Berkeley, CA 94720.

The estimate of threshold in a neural system is a common measure of its performance. In psychophysics, threshold measurements are based on the ability of an observer to just detect the stimulus presented (via verbal communication or pressing a button). In neurophysiology, the quantification of threshold is not so clear; since there is spontaneous activity (noise) and variability of response associated with neurons. The purpose of this study is to explore a procedure whereby the effects of variability of response and randomness in the absence of a stimulus are explicitly accounted for in the efficient determination of threshold.

We have adapted a two alternative forced choice staircase procedure (Relkin & Pelli, J. Acoust. Soc. Am. **82**:1679, 1987) to measure threshold in the dark-adapted locust (Schistocerca americanus) photoreceptor. The stimulus used was a green current controlled LED. The time integral of the cell's intracellularly recorded membrane potential was computed, taking account of the latency and duration of response in each of the two intervals. The two integrals were compared and a decision was made by computer as to which interval the stimulus was presented in. Concurrently, a human decision based on the membrane potential was also made. In a given run, staircase rules were based on either computer or human decisions. There are instances in each type of staircase where computer decisions are correct and human decisions are incorrect, and vice versa. Thresholds using human decisions are more than 50% lower. Therefore, if likelihood ratios were to be adopted for both decision rules, an optimized joint decision rule could be produced. This should lead to an improved computer receiver for the photoreceptor response, and thus, to better threshold estimates. Supported by NIH EY07607.

492.7

THE EYE SPECIFIC GENE *inaD* ENCODES A NOVEL POLYPEPTIDE WHICH IS INVOLVED IN DEACTIVATION AND LIGHT ADAPTATION IN *DROSOPHILA* PHOTORECEPTORS.

Barbara Niemeyer[1], Bih-Hwa Shieh[2] and Charles Zuker[1]*. [1]Howard Hughes Medical Institute and Departments of Biology and Neuroscience, University of California at San Diego, La Jolla, CA 92093, [2]Department of Pharmacology, Vanderbilt University, TN.

Although the initial events leading to activation of the photo–transduction cascade are fairly well understood in the invertebrate photoreceptor, less is known about the mechanisms of deactivation and adaptation. The *inaD* gene was isolated by a subtractive hybridization scheme designed to identify genes preferentially expressed in the *Drosophila* eye. *inaD* flies show an abnormal electroretinogram phenotype, suggesting that *inaD* is involved in visual transduction. We further characterized the electrophysiology of *inaD* mutant photoreceptors using whole cell patch-clamp recordings, and found that the deactivation of the light response was dramatically slower in *inaD* flies when compared with wildtype.

The deactivation defect suggests that the *inaD* gene product is an important player in the regulation of the photoreceptor response. Indeed, when we generated transgenic flies that overexpress the wildtype copy of *inaD* under the control of a heatshock promotor in a wildtype background, the deactivation processes become faster.

Finally, we also constructed flies that express the mutant copy of *inaD* in a wildtype background and found that the deactivation kinetics were slower than wildtype, suggesting that *inaD* has an antimorphic effect.

492.8

THE trp MUTATION INHIBITS WHILE THE inaC MUTATION ENHANCES LIGHT-INDUCED Ca^{2+} INFLUX INTO DROSOPHILA PHOTORECEPTORS.

A. Peretz[1,2], C. Sandler[2], K. Kirschfeld[2], R.C. Hardie[3], E. Suss-Toby[1], S. Barash*[1] and B. Minke[1]. Physiology, Hebrew Univ.-Hadassah Med. Sch., Jerusalem 91010, Israel[1]; MPI für Biologische Kybernetik, D-7400, Tübingen, Germany[2]; Zoology, Cambridge Univ., Cambridge, UK[3].

The trp and eye-PKC (inaC) mutations in Drosophila both inhibit light adaptation and result in response inactivation. The trp gene encodes a light-activated Ca channel. We have shown using whole cell recordings that inaC has a functional light-activated trp channels. Light-induced Ca^{2+} influx can be monitored using extracellular Ca selective microelectrodes. Compared to wild type the Ca^{2+} influx is enhanced in inaC but largely blocked in trp or the double mutant trp;inaC. The large light-induced Ca^{2+} influx observed in inaC is associated with a Ca-dependent inactivation of the trp channels.

The fact that light adaptation is nearly absent in inaC, in spite of high intracellular Ca^{2+} level indicates that in the absence of protein kinase C (PKC) the well known coupling between increased cytosolic Ca^{2+} and light-adaptation is disrupted.

492.9

CIRCADIAN EFFERENT INPUT TO *LIMULUS* RETINA STIMULATES THE PHOSPHORYLATION OF A PROTEIN SIMILAR TO THE *NINA*C GENE PRODUCTS OF *DROSOPHILA*. W. C. Smith, B. Eschweiler, A.W. Andrews, R.M. Greenberg and B-A. Battelle*. Whitney Lab. and Dept. of Neuroscience, Univ. of Florida, St. Augustine, FL. 32086

Circadian efferent input to *Limulus* eyes increases retinal sensitivity and responsiveness to light at night and primes photoreceptors for photo-mechanical movements, including membrane shedding. Efferent input also stimulates the phosphorylation of a visual system-specific 122 kD protein (pp122) via a cAMP-dependent mechanism. Here we report that pp122 is similar to the *nina*C proteins of *Drosophila*.

Degenerate oligonucleotides, designed from peptide sequences of gel purified pp122, were used in PCR to obtain a product that was then used to screen a cDNA library from *Limulus* lateral eye. A full length cDNA sequence encoding pp122 was isolated. Its deduced amino acid sequence has 52% total similarity (38% identity) with the *nina*C proteins from *Drosophila*, and like the *nina*C gene products, pp122 has a kinase domain and a myosin heavy chain domain. Northern analysis and PCR studies suggest that there is one *nina*C-like gene product in *Limulus* - in *Drosophila* there are two - and the *Limulus* protein is smaller than the *Drosophila nina*C proteins. Numerous potential A-kinase phosphorylation sites are distributed throughout the length of pp122. Preliminary CNBr cleavage studies suggest the protein can be phosphorylated at multiple sites including more than one site in the myosin domain. Based on analogy with *Drosophila* we propose that *Limulus* pp122 is involved in the circadian changes in structure and function of the rhabdomere. [Supported by NSF(DIR8914602, BNS8909052, IBN9211327) NIH(EY06454) and the UF DNA Core Facility]

RETINA: CHOROID, PIGMENT EPITHELIUM, AND PHOTORECEPTORS

493.1

11-*CIS*- AND ALL-*TRANS*-RETINYL ESTER HYDROLASE ACTIVITIES IN THE RETINAL PIGMENT EPITHELIUM OF THE EYE. E.P. Rodriguez , A.T.C. Tsin*, N.L. Mata, S. Ziari, and J. Bustamante. Division of Life Sciences, The University of Texas at San Antonio, San Antonio, Texas 78249.

Preliminary investigation of 11-*cis*- and all-*trans*-retinyl ester hydrolase (REH) activities in bovine retinal pigment epithelium (RPE) microsomes suggests that the two hydrolytic activities may be distinct from each other (Mata et al., 1992, *J. Biol. Chem.* **267**: 9794). In order to further explore the possibility of distinct hydrolytic sites for the two retinyl ester isomers, we have conducted studies using inhibitors of serine and thiol hydrolases. Specifically, we have employed inhibitors which modify catalytic sites containing cysteine residues (*N*-ethylmaleimide, NEM and phenylmethylsulfonyl fluoride, PMSF), serine residues (PMSF), and histidine residues (*N*-tosyl-L-phenylalanine chloromethyl ketone, TPCK). Effects of these inhibitors on 11-*cis*- and all-*trans*-REH activities in RPE microsomes were determined by monitoring product formation with increasing protein concentrations in the absence and presence (0.25 mM and 0.50 mM) of inhibitor. Hydrolysis of all-*trans*-retinyl palmitate was significantly inhibited (60 - 70%) by NEM, PMSF, and TPCK while hydrolysis of 11-*cis*-retinyl palmitate was inhibited by PMSF (50%) and TPCK (40%) only. Inhibition of 11-*cis*-REH by NEM was less than 15% at the highest inhibitor concentration (p.50 mM). These observations suggest that 11-*cis*- and all-*trans*-REH do not utilize the same amino acid residues for catalytic activity. Thus, based upon differential inhibitions, our results support the hypothesis of distinct 11-*cis*- and all-*trans*-REH activities in bovine RPE microsomes. Supported by grants from the NIH and the San Antonio Area Foundation.

493.2

THE EFFECTS OF DIFFUSIBLE FACTORS FROM ADULT HUMAN RETINAL PIGMENT EPITHELIAL CELLS ON PHOTORECEPTORS AND MULLER CELLS IN VITRO. H.J. Sheedlo, A. Davis, C.D. Jaynes and J.E. Turner*. Department of Anatomy and Cell Biology and North Texas Eye Research Institute, Texas College of Osteopathic Medicine, Fort Worth, TX.

Rat RPE-cell diffusible factors promote photoreceptor survival and Muller cell proliferation (Sheedlo et al., 1992). This study was undertaken to determine if primary, passaged and immortalized adult human RPE secrete factors which cause a similar cell response. RPE cells were isolated from adult humans, cultured and passaged. Rat photoreceptors and Muller cells were separately isolated from retinas of day 2 rats and cultured in media conditioned by human RPE (CM). The number of opsin-stained photoreceptors and carbonic anhydrase-stained Muller cells were counted after 3 days in vitro. RPE-CM from a 32 year-old human promoted the survival of 10,780 ± 278 photoreceptors, which was equivalent to the maximal response in cultures in CM of neonatal rat RPE-CM (11,429 ± 3035). In contrast, 1,572 ± 161 cells were counted in 74 year-old human RPE-CM cultures, but CM of first- and second-passaged RPE of a 81 year-old human effected the survival of 11,131 ± 1829 and 15,823 ± 438 cells, respectively. CM of immortalized adult human RPE cells caused the survival of Muller cells, which died when grown in a defined medium. This study showed that factors of adult human RPE affected photoreceptor survival and Muller cell proliferation. Specifically, old RPE cells could be upregulated in culture to release such factors, which may mimick their glial cell activities in retinal pathologies such as proliferative vitreoretinopathy. Supported by NIH grant EY 04337.

493.3

PROTEASES ACTIVITY IN MICROVESSELS OF RETINA, CHOROID, CILIARY BODY AND IRIS OF RAT EYE. A. Mitro, L.E. De Bault* and R.E. Nordquist. University of Oklahoma Health Sciences Center and Dean McGee Eye Institute, Oklahoma City, OK 73104.

There is evidence which suggests that various types of brain microvessels have distinct compliments of membrane-bound proteases, which cleave peptide bonds and may have a major role in the activation, deactivation and/or modulation of blood-borne neurohormones. However, the precise functions of these proteases is not yet well understood. The present study was undertaken to determine the presence and localization of glutamyl aminopeptidase (EAP), microsomal alanyl aminopeptidase (mAAP), dipeptidyl peptidase IV (DPP-IV), and gamma-glutamyl transpeptidase (γ-GTP) in rat eye. Cryostat sections of the adult rat eye were stained histochemically for EAP, mAAP, DPP-IV and γ-GTP according to the methods of Lojda et al. (1979). Vessels of the retina were strongly positive for EAP and mAAP, but weak to negative for γ-GTP. Retinal vessels were negative for DPP-IV. In the case of choroid, ciliary body, and iris, positive reactions were observed for EAP, mAAP, and DPP-IV, while γ-GTP was negative. These results suggest that blood-borne peptide hormones or other substances may be affected by EAP, mAAP, and DPP-IV, but not by γ-GTP in choroid, ciliary body, and iris. The absence of DPP-IV in retinal vessels suggests a functional role of retinal endothelium that differs from the role of vascular endothelium of the above mentioned eye structures. Supported by NIH grant NS18775 and an unrestricted grant from Research to Prevent Blindness, Inc.

493.4

TOPOGRAPHICAL SEPARATION OF COLOUR SPECIFIC CONES IN THE RABBIT RETINA. B. Juliusson[1,2], A. Bergström[1], P. Röhlich[3], B. Ehinger[1*], T. van Veen[2], A. Szél[2,3]. [1]Dept of Ophthalmology, Univ. of Lund, Sweden; [2]Dept of Zoology, Univ. of Göteborg,; Sweden. [3]Lab. of Electron Microscopy, Semmelweis Univ. of Medicine, Budapest, Hungary.

Recently, a marked heterogeneity of the mouse retinal cone cell distribution has been reported (Szél et al., J. Comp. Neurol. **325**:327,1992). The presence of complementary cone fields in the mouse retina was considered as a unique feature of the mouse, since not even close relatives of this species (wood mouse, rat, gerbil, hamster) shows this special cone pattern. Mapping the distribution of the colour specific cones in the rabbit retina, we found a narrow crescent-shaped area with an unusually high number of shortwave-sensitive cones (S-cones) and what appeared to be a total lack of middlewave-sensitive cones (M-cones), thus resembling the S-field of the mouse retina. Although the extension of this peculiar cone field (tentatively called blue-streak) was considerably smaller than the S-field of the mouse retina, its position was similar to that, since it also occupied the lowermost part of the retina.

For the identification of the two different cone types, we used the monoclonal antibodies COS-1 and OS-2, specific for middlewave and shortwave sensitive visual pigments, respectively. Immunofluorescent staining was performed on whole mounts of adult rabbit retinas.

The majority of the retinal surface, including the visual streak, exhibited a dominance of M-cones over S-cones with a ratio of about 10:1. It was only the lower 5% of the total retinal area (blue-streak) that showed a complete lack of M-cones and a high frequency of S-cones.

The functional implications of this finding is as yet unknown, however, it can be assumed that the blue streak is designed for an enhanced perception of predators approaching from above.

Supported by grants from the Swedish Medical Research and the Swedish Natural Sciences Councils (projects #14X-2321 and #4642-130), the RP Foundation Fighting Blindness, the T and R Söderberg Foundation and the Riksbankens Jubileumsfond.

493.5

SPECTRAL SENSITIVITY OF RETINAL CONES IN THE GOLDFISH (*CARASSIUS AURATUS*): Adrian G. Palacios* & Timothy H. Goldsmith, Department of Biology, Yale University, New Haven, USA.

Spectral sensitivities of individual goldfish cones were investigated by drawing the outer segments into pipette electrodes and measuring the membrane photocurrents. The amplitude of the cone response as a function of intensity is represented better by a Michaelis-Menten than an exponential function. The maximal photocurrent observed in these experiments was 20 pA. We find four classes of cones with maximal sensitivity at 368 (near UV), 458, 535 and 616 nm. The spectral sensitivity functions are fit better by a porphyropsin than a rhodopsin template, but the long wavelength limbs of the sensitivity curves descend more gradually with wavelength than predicted by the function of Mooij and van den Berg (1983), an analytical treatment that attempts to describe sensitivity of porphyropsin-containing receptors at wavelengths where absorbance is too low to measure by microspectrophotometry.

Supported by NEI grant EY00222

493.6

COMPONENTS OF THE PHOTOTRANSDUCTION CASCADE IN CONES AND ROD-LIKE CELLS OF THE GROUND SQUIRREL RETINA. M. von Schantz, Á. Szél, T. van Veen and D.B. Farber*. Jules Stein Eye Institute, UCLA School of Medicine, Los Angeles, CA 90024, and Dept. of Zoology, University of Göteborg, S-413 90 Göteborg, Sweden.

We have studied the expression and distribution of phototransduction gene products in the cone-dominant retina of the ground squirrel *Spermophilus tridecemlineatus*. mRNA expression was studied with Northern blot hybridization, and the distribution of the gene products was investigated with immunocytochemistry.

Northern blot hybridization showed messages for the ß1- and ß3-subunits of transducin, and for arrestin, recoverin, and IRBP (inter-photoreceptor retinoid-binding protein), but was negative for opsin, α1-transducin, and the γ-subunit of cGMP phosphodiesterase. The rod-like cells were immunopositive for opsin and blue opsin. All photoreceptor elements were positive for the α- and ß3-subunits of transducin, the γ-subunit of rod phosphodiesterase, and IRBP, while no cells were labelled by antibodies against the rod α- and ß-subunits of phosphodiesterase or the rod cGMP-gated cation channel. Rod-like cells and blue cones were stained by antibodies against ß1-transducin, arrestin, and phosducin.

Our results indicate that blue cones and rods share a similar or identical phosphorylation-dependent signal quenching mechanism involving arrestin and phosducin. We also present evidence for new cone-like traits in the biochemical make-up of the rod-like cells, further emphasizing their deviation from the classical duplicity theory.

493.7

LOCALIZATION AND MODULATORY ACTIONS OF ZINC IN VERTEBRATE RETINA *S.M. Wu*, X. Qiao, X.L. Yang, and J.L. Noebels.* Cullen Eye Institute, Division of Neuroscience and Department of Neurology, Baylor College of Medicine, Houston, TX 77030.

Vertebrate photoreceptors are thought to use glutamate as their neurotransmitter and a number of glutamatergic neurons in the nervous system contain zinc (Zn^{+2}) in their presynaptic vesicles. We therefore studied the localization and the actions of Zn^{+2} in the larval tiger salamander retina. By using the Neo-Timm staining method which allows selective visualization of Zn^{+2} associated with synaptic vesicles, we observed heavy staining in the basal regions of cell bodies of both rods and cones. Little staining was observed elsewhere in the retina. At EM level, zinc-positive particles were located in synaptic vesicle-rich regions of the photoreceptor terminals. Application of 5-50 μM Zn^{+2} hyperpolarized the horizontal cells (HCs) and suppressed their light responses, an action similar to that exerted by application of Co^{+2}, which blocks presynaptic calcium channels. 5 μM Zn^{+2} partially, and 50 μM Zn^{+2} completely, blocked the depolarizing action of GABA, which was mediated by $GABA_A$ receptors. In contrast, application of Zn^{+2} (up to 100 μM) exerted no effect on the depolarizing action of glutamate. We have demonstrated that endogenous zinc is present in photoreceptors. The staining distribution pattern suggests that it is associated with synaptic vesicles. If Zn^{+2} is released from the photoreceptors, it may regulate the presynaptic release of glutamate, and may be involved in regulating the HC response kinetics by modulating $GABA_A$ receptors.

Supported by NIH EY04446, NIH NS11535, Retina Research Foundation (Houston), Research to Prevent Blindness, Human Frontier Science Program and Blue Bird Circle Foundation.

493.8

OPSIN PHYLOGENY AND EVOLUTION: A MODEL FOR BLUE SHIFTS IN WAVELENGTH REGULATION. B.S.W. Chang[1*], K.A. Crandall[2], J.P. Carulli,[1] and D.L. Hartl[1]. [1]Harvard University, Biological Labs, 16 Divinity Ave., Cambridge, MA 02138; [2]Washington University, Biology Dept., St. Louis, MO 63130.

Shifts in absorption maxima of opsins are thought to be a result of interactions between amino acid side chains extending into the opsin binding pocket, and the protonated Schiff base (SBH+) chromophore. We used a comparative approach to identify residues important in opsin wavelength regulation. Opsin sequences were obtained from the literature, and aligned according to current understanding of the structural and functional domains of the molecule. Several methods of phylogenetic analysis were used to construct an opsin tree. Amino acid substitutions were traced along the branches of the opsin tree, using evidence from structural and functional studies of opsins (and other receptors with seven helical transmembrane domains) to identify residues likely to reside within the chromophore-binding pocket. Functionally convergent, nonconservative amino acid substitutions in independently evolved opsins with similar shifts in spectral properties were especially of interest. The results of this comparative analysis are summarized in a model for blue shifts of opsin absorption spectra. This model may explain some of the diversity of short wavelength opsins apparent in both vertebrates and invertebrates.

493.9

PCR SEQUENCE OF LARVAL AND ADULT OPSIN PROTEINS IN WINTER FLOUNDER. B. I. Evans*, T. L. Kasten, M. Nadal-Vicens, and R. D. Fernald. Stanford University, Stanford CA 94305-2130.

At metamorphosis, the winter flounder retina undergoes an extensive structural transformation (Evans and Fernald, 1993) and the wavelength of peak absorbance also changes for the photoreceptors (Evans et al., 1993). Larval retinas have one photoreceptor type, a cone, arranged in a hexagonal array with its spectral absorbance peak at 519 nm ("green"). After metamophosis, the retina has rods, single and double cones. The three adult cone types are arranged in a square array differing from one another in peak spectral absorbance (single cone =457 nm; double cone members = 531 nm, 547 nm). All three cone absorbances are also unique from that of the premetamorphic cone. Using PCR, we have isolated and sequenced regions of the photoreceptor opsin genes presumed responsible for determining the wavelength of peak absorbance. The adult flounder retinal tissue yielded DNA sequences with high homology to blue and red absorbing cone opsins and to the rhodopsin found in adult rods. Using a similar approach, we obtained two putative cone opsin sequences for winter flounder larvae. The larval opsin partial sequences have high homology to the red and blue opsins found in the adult retina. Metamorphosis transforms existing cone photoreceptors from the single larval phenotype into three postmetamorphic phenotypes. Since the larval opsin sequences differ from those of the adult, such a process requires an exquisitely coordinated change in opsin gene expression across the retina. Supported by NIH EY F32 06325 to BIE & NIH EY 05051 and EY 08306 to RDF.

493.10

A QUANTITATIVE ACCOUNT OF THE ROLE OF Ca^{2+} IN LIGHT ADAPTATION OF ROD PHOTORECEPTORS. Y. Koutalos[1], K. Nakatani[2,*] and K.-W. Yau[1,3]. Dept. of Neuroscience, Johns Hopkins Univ. Sch. of Med.[1], Howard Hughes Med. Inst.[3], Baltimore, MD 21205, USA and Inst. of Biological Sciences, Univ. of Tsukuba[2], Tsukuba, Ibaraki 305, JAPAN.

cGMP, the intracellular messenger that mediates photo-transduction, is synthesized from GTP by a guanylate cyclase and is hydrolyzed to 5'-GMP by a light-sensitive phosphodiesterase. Both enzymes have been shown to be Ca^{2+}-sensitive, a property found to be important for the adaptation of photoreceptors to background light. We have used a truncated rod outer segment preparation from the tiger salamander to study these enzymes under conditions close to the physiological situation. The distal portion of a rod outer segment was drawn into a suction pipette to allow recording of membrane current, while its proximal portion was removed by truncation to allow internal dialysis. In this way the cGMP-gated channels of the outer segment membrane can be used to monitor the cGMP concentration. The truncated outer segment can then be used to study the *in situ* activities of the cGMP-metabolizing enzymes. Our preliminary results indicate, for the guanylate cyclase, an inhibition by Ca^{2+} with a $K_{1/2}$ of 100 nM and a Hill coefficient of 1.5; for the steady-state phosphodiesterase activity elicited by steps of light, the stimulation by Ca^{2+} shows a $K_{1/2}$ of 410 nM and a Hill coefficient of 1.0. On the basis of these measurements the steady-state step response-intensity relation of the intact rod can be predicted. The predicted relation is in close agreement with experimental data from intact cells.

493.11

RETINAL DEGENERATION IN THE MOTOR NEURON DISEASE MUTANT (*Mnd*) MOUSE. D.L. Price and C.A. Pardo*. The Johns Hopkins Sch. of Med., Balto., MD 21205

The *Mnd* mutant mouse was described originally as an animal model for motor neuron disease. Recently, we and others have demonstrated that *Mnd* mice show widespread neuronal and nonneuronal accumulation of lipofuscin-like material and retinal degeneration. The time course of degenerative changes in the retina in *Mnd* mice was studied by immunocytochemical and electron microscopic techniques. Retinal degeneration was detected as early as two months of age, with loss of the outer segment of photoreceptors and cytoplasmic accumulation of autofluorescent lipofuscin-like material in photoreceptor and ganglion cell bodies. By the age of four months, extensive degeneration of the outer and inner segments of photoreceptors, as well as reductions in the number of nuclei in the outer nuclear layer (ONL), was observed. At this age, a decrease in the ONL thickness and synapsis was demonstrated with antisynaptophysin and anti-MAP-2 antibodies. By six months, the photoreceptor, ONL, and outer plexiform layers had degenerated completely, with decreases in the thickness and cell density of inner nuclear, inner plexiform, and ganglion cell layers. Ultrastructurally, lipofuscin-like material was characterized by granular, multilamellar, fingerprint, and curvilinear profiles surrounded by a limiting membrane. Immunocytochemical and Western blot analyses demonstrated abnormal accumulation of subunit *c* of mitochondrial ATP synthase in *Mnd* as compared with wild-type mice. The neuropathological changes, accumulation of subunit *c*, and retinal degeneration suggest that the *Mnd* mouse is a form of neuronal ceroid lipofuscinosis (NCL) and demonstrated that, rather than a model for motor neuron disease, the *Mnd* mouse is a suitable model to study NCL or Batten's disease.

493.12

Basic FGF mRNA Expression during Retinal Development in Normal and *rd* C57BL/6J Mice H. Gao, S.F. Basinger* and J.G. Hollyfield, Cullen Eye Institute and Division of Neuroscience, Baylor College of Medicine, Houston TX 77030

We reported previously that bFGF immunoreactivity was localized to the outer retina and more bFGF was detected in *rd* than in the normal mouse retina at certain developmental stages. To examine bFGF gene expression and compare bFGF mRNA levels in normal and *rd* retinas, *in situ* hybridization and ribonuclease protection assay (RPA) were performed using radiolabeled riboprobes synthesized by *in vitro* transcription from a cDNA segment (480 bp) coding for the mouse bFGF open reading frame. Both methods demonstrated that bFGF mRNA expression could be detected in earlier stages and at higher levels in *rd* than in normal retinas. bFGF mRNA expression was not detected in normal retina by *in situ* hybridization until postnatal day 10 (P10), when the hybridization signal was first detectable in the photoreceptor inner segments (IS). In *rd* P10, however, strong signals were detected in the IS in the central retina. At P12, much higher level of bFGF mRNA expression could be detected in the IS in *rd* than in normal retina. At P15, the *rd* residual photoreceptors express high levels of bFGF mRNA, especially near the ora serrata area. In adult retinas, *rd* demonstrated much stronger mRNA signals in more INL cells than the normal retina. Some cells in the INL and GCL could also be labeled with the probe in the normal and *rd* retinas. Intense hybridization signals were detected in astrocytes localized in the optic disc area during development. When RPA was employed for quantitative studies, bFGF mRNA could be detected from P6 *rd* neural retina, but not from P6 normal retina. At P10, up to 5 fold more bFGF mRNA was present in *rd* as compared to normal retinas. Normal adult retina contains more bFGF mRNA than P10 retina using RPA. These results suggest that (1) bFGF mRNA expression is up-regulated during retinal development. (2) Photoreceptor degeneration in the *rd* retina is accompanied by elevated levels of bFGF mRNA expression in the photoreceptors. (3) Astrocytes in the optic disc area express high levels of bFGF mRNA during development. Supported by NEI Grant 02363, the Retina Research Foundation and RP Foundation.

493.13

ABNORMALITIES IN DIRUNAL RHYTHM OF DOPAMINE METABOLISM IN DYSTROPHIC RETINAS OF MUTANT *RDS* MICE. I. Nir*[1] and P.M. Iuvone[2] Dept. of Pathol., Univ. of Texas Health Sci. Ctr., San Antonio, TX[1]. and Dept. Pharmacol., Emory Univ. Sch. of Med., Atlanta, GA[2].

Retinitis pigmentosa (RP) is a neurodegenerative disease, manifested by progressive loss of photoreceptors and blindness. Recent molecular genetics analysis revealed a mutation in peripherin/*rds* gene in retinal degeneration slow (*rds*) mice and subsequently in humans with RP. In mice the mutation is noted by the absence of normal light sensitive outer segments. In prior studies of *rds* retinas we discovered loss of the diurnal rhythm in arrestin gene expression. This observation led us to study dopamine (DA), which was reported to affect diurnal rhythms in the retina. DA metabolism was studied in BALB/c and *rds* mice at various stages of retinal degeneration. Levels of catecholamines were determined by HPLC-EC. Steady state DA levels were essentially the same in light and dark adapted retinas of BALB/c and *rds* mice. Levels of DA metabolite, DOPAC, increased significantly in illuminated retinas of BALB/c but not *rds* mice, indicating limited release and degradation of DA in the mutant. DA synthesis, as estimated by accumulation of DOPA following inhibition of DOPA decarboxylase, was markedly higher in BALB/c than in *rds* retinas. Rate of DA turnover, as calculated in 1 month old mice treated with αMPT, was 8 fold lower in the *rds* retina in comparison to BALB/c. Thus, although DA synthesis is limited in the *rds* retina, relatively high steady state levels are maintained as a result of a concomitant low turnover rate. In conclusion, the relatively high level of DA which is maintained in the *rds* retina might not be available to exert its effect on retinal functions.

493.14

Is a Circadian Rhythm Expressed in the Structural Organization of the Outer Plexiform Layer in the Japanese Quail?

M.E. Kelly and R.B. Barlow, Jr.*
Institute for Sensory Research, Syracuse Univ., Syracuse, NY 13244

The Japanese quail retina exhibits a circadian rhythm in cone and pigment granule movement at the light microscopic level.[1] Cones contract and pigment disperses during the day while at night cones elongate and pigment recedes. Are circadian changes limited to the most distal, light sensitive cells of the retina? Can the clock modulate structural changes at the initial site of visual transmission - the photoreceptor-bipolar synapse?

In an attempt to answer this question, we discovered a two-tiered arrangement of photoreceptor terminals. Cone pedicles and rod spherules are found in both tiers. Cone pedicles contain traditional triad synapses which appear to be influenced by environmental lighting conditions. Specifically, the ribbon per pedicle ratio decreases with prolonged darkness (about 30 hrs).

Circadian changes in retinal structure are not unique in birds. Circadian retinomotor movements of cones and retinal pigment epithelium granules have been detected in fish as well as rhythms in ribbon and horizontal cell spinule formation.[2, 3]

1. Kelly, Buelow, and Barlow, (1993) *ARVO Abstr.* **34**:754.
2. Burnside, B and Nagle, B. (1983) *Prog.in Ret. Res.* **2**,67-109.
3. Douglas, R. and Wagner, H. (1983) *Cell Tiss. Res.* **229**:443-449.

493.15

CHARACTERIZATION OF L, M, AND S CONE PEDICLES IN PRIMATE FOVEA Pooneh Esfahani[1], Stan Schein[1]*, Karl Klug[1], Yoshihiko Tsukamoto[2], and Peter Sterling[3] Dept of Psychology, UCLA[1], Los Angeles, CA; Hyogo Coll Med[2], Hyogo, Japan; Dept of Neuroscience, Univ of Pennsylvania[3], Phila, PA

To determine characteristics of cone pedicles that might distinguish L, M and S types, we reconstructed 40 contiguous, complete pedicles in a small patch of foveal retina from electron micrographs of serial thin sections. We measured the volume of each pedicle and characterized its synaptic ribbons. Based on the pedicle's connections to bipolar cells (Klug et al.1992; Calkins et al. 1992), we identified 17 putative L cone pedicles, 6 putative M cone pedicles, and 4 S cone pedicles. The remaining 13 pedicles were non-S. The results were as follows:

i) Pedicle volumes increased from 480 to 640 microns eccentricity. S pedicles were among the smallest at any eccentricity, but five non-S pedicles were similarly small. The volumes of L and M pedicles were indistinguishable.

ii) All four S pedicles had 22 ribbons. The non-S pedicles had 16-21. L pedicles, with 16-20 ribbons and M pedicles, with 18-20 ribbons, were similar.

iii) Synaptic ribbons are flat rectangles characterized by a range of docking lengths (0.1 to 1.3 μm) and constant heights of approximately 0.3 μm.

iv) Total docking length of ribbon in each pedicle increased with eccentricity and ranged from 8 to 14 μm. L, M, and S pedicles did not differ in this regard.

v) The average ribbon lengths in S pedicles were among the smallest, but the average ribbon length in five other pedicles was similarly small.

Only the number of ribbons distinguished S from non-S pedicles, and that measure might fail with a larger sample. None of the measurements distinguish L from M cone pedicles. Connections with bipolar cells remain the only feature that distinguishes cone types. Supported by NIH grant EY06096

493.16

CORRELATED LIGHT AND ELECTRON MICROSCOPY OF OPSIN-IMMUNOREACTIVE NEURONS IN LIZARD BASAL BRAIN. M.S. Grace*, V. Alones, M. Menaker, R.G. Foster. Dept. of Biology, Univ. of Virginia, Charlottesville, VA 22903.

Photoreceptors mediating circadian responses to light are distinct from retinal rods and cones. In non-mammalian vertebrates, these cells are extra-ocular, since removal of eyes does not prevent light input to the circadian system. Opsin immunoreactivity (IR) and retinoid identification suggest that photoreceptors exist in the basal brain of iguanids (Foster et al., J. Comp. Physiol. A 172:33-45, 1993). We investigated the ultrastructure of these putative photoreceptors using combined light and electron microscopic (EM) immunocytochemistry. *Iguana iguana* and *Anolis carolinensis* were perfused, and their brains sectioned at 50μm. Free-floating sections were immunostained with anti-cone opsin CERN 874, and HRP/DAB. Sections were plastic embedded and viewed by light microscopy (LM). Immunostaining in brain was observed only among pinealocytes and CSF-contacting ependymal neurons of the basal portion of the lateral ventricles. Sections containing IR CSF-contacting neurons were excised, thin-sectioned, and viewed by transmission EM. Individual IR cells observed by LM were readily identified by EM. These cells are flask-shaped, with a single dendrite and an axon emanating from opposite sides of the soma. The dendritic process contains an axoneme, ends in a bulbous terminal protruding into the lateral ventricle, and receives synaptic input. Opsin-IR CSF-contacting neurons contain many 110-190nm diameter vesicles in the perinuclear region, dendrite, and bulbous terminal. These vesicles may be analogous to "lipochondria" or "photic vesicles" of extra-ocular photoreceptors in gastropod mollusks. Opsin IR may be concentrated within these vesicles, and is diffusely distributed throughout the bulbous terminal. Together, these results suggest that opsin-IR CSF-contacting neurons may be photoreceptors. Support: NIH 5R29MH49837 (RGF); NSF PCM8409010 (MM)

493.17

NADPH-diaphorase positive neurons and fibers in the ciliary ganglion and choroid of the pigeon. M.E.C. Fitzgerald* and A. Reiner. Dept. of Anatomy & Neurobiology, Univ. of TN, Memphis, TN.

Choroidal blood flow (CBF) in the avian eye is under vasodilatory cholinergic control by the ciliary ganglion (CG). Since nitric oxide has been implicated in many vascular beds as a vasodilator, we sought to determine if the neurons in the ciliary ganglion contained the necessary enzyme to produce nitric oxide by using NADPH-diaphorase histochemistry. Pigeons were transcardially perfused with a paraformaldehyde fixative, the eyes were removed with the ciliary ganglion intact, rinsed in buffer, cryoprotected in a sucrose-buffer solution, sectioned at 20µm in a cryostat and mounted on slides. The slide-mounted sections were stored in the freezer and later processed for diaphorase activity.

Within the ciliary ganglion, both choroidal and ciliary neurons stained positively for NADPH-diaphorase. The presynaptic endings on the choroidal neurons appeared negative for NADPH-diaphorase; however, some diaphorase positive structures appeared to be cap-like endings, which were presynaptic to weakly labelled ciliary neurons. Within the choroid numerous diaphorase positive nerve fibers were observed around blood vessels. Additional diaphorase positive cells were observed within the extracellular space of the choroid and within the walls of choroidal blood vessels. Bundles of diaphorase positive ciliary nerves were also observed within the choroid and in the ciliary body, where individual fibers were observed to extend into the ciliary processes. The existence of NADPH-diaphorase positive neurons within the ciliary ganglion helps clarify the dramatic choroidal vasodilation we have observed in pigeons with activation of input to the ciliary ganglion.

Supported by AG-10538 (MECF), EY-05298 (AR).

493.18

POLARIZATION SENSITIVITY IN SINGLE UNITS OF THE RAINBOW TROUT (*ONCORHYNCHUS MYKISS*) VISUAL SYSTEM. D. J. Coughlin and C. W. Hawryshyn* Dept. of Biol., Univ. of Victoria, Victoria, B.C. V8W 2Y2 Canada

Polarization sensitivity (PS) has previously been demonstrated in multi-unit responses from the optic nerve of rainbow trout (Parkyn and Hawryshyn, '93, J. Comp. Phys. A). Discrimination of the *e*-vector of a polarized light stimulus in rainbow trout requires the presence of the UV cone mechanism, and UV sensitivity in rainbow trout is found primarily in the torus semicircularis (TS), a sub-tectal midbrain region. This study investigated polarization sensitivity in single units from the trout TS, by isolating individual cone mechanisms with chromatic adaptation and by examining polarization opponency in the most common color-coded units of the trout TS. Under a UV cone mechanism isolating background, single units always gave an ON response and showed PS with a sensitivity peak for stimuli with vertical (0° and 180°) *e*-vector. Under middle (M) and/or long (L) isolating backgrounds, single units gave either ON responses or OFF responses. The ON response units generally showed no PS, while the OFF response units were polarization sensitive with a sensitivity peak for stimuli with a horizontal (90°) *e*-vector. Examination of PS in biphasic color-coded units, which have opponency between an ON response from the UV mechanism and OFF responses from both the M and L mechanisms, showed PS consistent with the responses of isolated cone mechanisms. Thus, biphasic units discriminate the *e*-vector of polarized light stimuli and permit polarized light vision by rainbow trout. Alternatively, other types of color-coded TS single units did not code polarized light information and were polarization insensitive. Supported by an NSF-NATO PDF to DJC and an NSERC operating grant to CWH.

493.19

CHARACTERIZATION OF POLARIZED-LIGHT SENSITIVITY FROM THE OPTIC NERVE OF THE RAINBOW TROUT (*ONCORHYNCHUS MYKISS*). D. C. Parkyn* and C. W. Hawryshyn. Dept. of Biol., Univ. of Victoria, Victoria, B.C. V8W 2Y2 Canada.

We investigated the optic-nerve ganglion responses underlying polarization sensitivity (P.S.) in rainbow trout, using a compound action potential recording technique. Chromatic adaptation allowed us to characterize two orthogonally-oriented classes of polarization sensitive cones and one polarization-insensitive cone type. Adapted Ultraviolet (UV)-sensitive cones were found to respond with peak sensitivity to vertically (0/180°) polarized light or e-vector, whereas isolated middle and long wavelength-sensitive cone mechanisms responded maximally to horizontal (90°) e-vector. Short wavelength-sensitive cones (as well as rods) were found to be insensitive to the plane of polarizartion. E-vector adaptation, a technique analogous to chromatic adaption can be used to reduce the relative sensitivity of one e-vector sensitive mechanism to its orthogonal mechanism. Differential sensitivity to various orientations of e-vector was greater following chromatic adaption than following e-vector adaptation. The presence of UV light is necessary for orientation of trout under a downwelling plane-polarized light field (Hawryshyn et al. '90, J. Comp. Physiol.) . We present evidence that P.S. is mediated by the comparison of the responses from the vertically- and horizontally-sensitive mechanisms in the UV region of the spectrum in a manner analogous to colour vision. Supported by a NSERC operating grant and Dept. of Fisheries and Oceans subvention grant to CWH as well as grants from the University of Victoria.

AUDITORY SYSTEM: CENTRAL ANATOMY—BRAINSTEM

494.1

TRANSNEURONAL BIOCYTIN DELINEATES SPECIES DIFFERENCES IN A BRAINSTEM VOCAL-ACOUSTIC CIRCUIT IN SOUND PRODUCING FISH. A. H. Bass*, M. A. Marchaterre and R. Baker. Neurobiology and Behavior, Cornell Univ., Ithaca, N.Y. 14853; Physiology and Biophysics, NYU Medical Center, New York, N.Y. 10016.

The closely-related toadfish and midshipman generate vocalizations with species-typical temporal and spectral properties by the simultaneous contraction of sonic swimbladder muscles. Intracellular staining and recording in both species show that each muscle is innervated ipsilaterally by motoneurons in a brainstem sonic motor nucleus (SMN). Individual pacemaker neurons (PMNs) lie lateral to sonic motoneurons, innervate them bilaterally, and establish their fundamental discharge frequency. Transneuronal biocytin transport has now identified species-typical pacemaker circuitries. In both species, labelling of the sonic nerve ipsilaterally resulted in biocytin-filled neurons bilaterally within and lateral to the SMN; the lateral neurons overlapped the location of previously-identified PMNs. In toadfish, the PMNs formed a dense network along the entire length of the SMN, while in midshipman they were clustered rostral to the SMN. PMN neurites branched extensively across the midline, either ventral (toadfish) or rostral (midshipman) to the SMN. In both species, PMN neurites joined a lateral fiber bundle containing axons that terminated in the statoacoustic area of the medulla, including the efferent neurons that innervate the sacculus, the largest division of the inner ear which is considered the major acoustic end organ. The lateral pathway is likely a part of the vocal circuitry that provides temporal information to acoustic afferent and efferent nuclei. Species diversity in pacemaker/command circuitry may underlie species-typical vocal signalling and acoustic recognition. Support from NSF and NIH.

494.2

CHAT-IMMUNOREACTIVE COCHLEAR EFFERENT NEURONS IN THE CHICK BRAINSTEM. R.A. Code* and C.E. Carr. Dept. of Zoology, Univ. of Maryland, College Park, MD 20742-4415.

We have studied cholinergic neurons in the chick auditory brainstem using an antiserum to choline acetyl transferase (ChAT), the biosynthetic enzyme for acetylcholine (Code and Carr, '93, ARO abstr. 16: 125). ChAT-immunoreactive (ChAT-I) neurons were found in a ventrolateral and a dorsomedial cell group. The ventrolateral group is a rostrocaudally directed column of cells which surround the superior olive (SO), are ventromedial to the ventral facial nucleus (VIIv), and are lateral to the nucleus pontis lateralis (PL) as far rostrally as the nucleus subceruleus ventralis. Cells in the dorsomedial group were found in the pontine reticular formation medial to the dorsal facial nucleus and lateral to the abducens nerve root.

Because cochlear efferents in the pigeon stain for acetylcholinesterase and efferent terminals in the chick cochlea are morphologically similar to those in the pigeon, we wished to determine which cholinergic neurons in the brainstem project to the chick cochlea. A double-labeling technique was used combining ChAT-I and the retrograde transport of biotinylated dextran amine (BDA) from the inner ear. About 61% of cochlear efferent neurons are located contralateral to the injected cochlea and 39% are ipsilateral. Double-labeled cells were found bilaterally in both the ventrolateral and dorsomedial cell groups, with the exception of large ChAT-I cells dorsal to the SO which do not appear to project to the cochlea. ChAT-I cells that project to the cochlea were classified into three morphological groups: multipolar, elongate and round-to-oval. Both the ventrolateral and dorsomedial cell groups appear to have a mixture of these different cell types. The average somal area of double-labeled cells was 246 mm^2. Only about 70% of the cochlear efferent neurons, however, are cholinergic.

The ChAT antiserum was kindly provided by Dr. Miles Epstein, University of Wisconsin, Madison. This work was supported by NIDCD grants R03 DC01867 to R.A.C. and DC00436 to C.E.C.

494.3

ENHANCED C-FOS GENE EXPRESSION IN THE COCHLEAR NUCLEUS BY SOUND STIMULATION IN CONJUNCTION WITH AN EPINEPHRINE ANALOG.

D.E. Hillman,*S. Chen, R. Winicki, R. Giacchi and J. Cousins
Dept. Otolaryngology and Physiol./Biophys. NYU Med. Ctr., New York, NY 10016

The proto-oncogene c-fos is rapidly and transiently expressed in response to increased neuronal activity following various types of experimental stimulations. In this study, c-fos was used as a marker for physiological activity and a test for drug responses. Dipivefrin, an epinephrine analog that crosses the blood-brain barrier, was injected intraperitoneally. The animals were exposed immediately to unfamiliar complex sound (UCS) for two hours at 40-90 dB. Five groups of rats (naive, dipivefrin, UCS, UCS+dipivefrin, and UCS+various dosage of 0.1% dipivefrin ranging from 0.25 mg/kg to 3 mg/kg) were analyzed for c-fos gene expression in the cochlear-nucleus complex. The results of quantitative analysis revealed that the number of c-fos neurons: 1) increased by 2-fold above basal levels as a result of dipivefrin, 2) increased by 5-fold following UCS as compared to the naive group, 3) was enhanced by dipivefrin to 35% above the UCS group, and 4) followed a linear response dependent on the dosage of dipivefrin. C-fos is believed to synthesize proteins that are involved with plasticity and learning. This drug enhances the activation of c-fos, and may facilitate learning. Supported by USPHS NS-13742 and AG-09480.

494.4

FOS IS INDUCED BY ACOUSTIC STIMULATION IN LIMITED CELL POPULATIONS.

J.C.Adams* Dept. of Otolaryngology, Mass. Eye & Ear Infirmary, Boston, MA 02114.

Unilateral tone bursts at moderate sound levels were used to induce the expression of Fos in auditory brainstem nuclei in urethane anesthetized cats. The numbers of labeled cells and the strength of their labeling was directly related to the stimulus intensity. Using an Oncogene Sciences antibody, bands of labeled cells were found in the ventral and dorsal cochlear nuclei, in the ipsilateral lateral superior olive, and in the contralateral inferior colliculus. The band of labeled cells in the colliculus continued across nuclear subdividsions from the central nucleus to the dorsal surface. Scattered positive cells were also present in periolivary regions. Counter-staining the immunostained sections for Nissl substance showed that in every region except the lateral nucleus of the trapezoid body, small cells were predominantly labeled. Preliminary results with double immunostaining for Fos and for nerve terminals indicate that in the ventral cochlear nucleus the predominant, if not the exclusive, non-granule cell class that expresses Fos is type 1 stellate cells. This finding indicates that Fos expression can be used as a powerful tool for mapping these cells' locations and for studying their physiology.

Another Fos antibody (UBI) showed similar results only in the cochlear nucleus. An antibody to the product of zif 268 showed similar results in the cochlear nucleus and superior olive. Both these antibodies showed high numbers of positive cells in the colliculus even without sound induction.

494.5

DIVERSE INPUTS TO MULTIPOLAR CELLS AND SMALL CELLS THAT RECEIVE MEDIAL OLIVOCOCHLEAR (MOC) SYNAPSES.

T.E. Benson*[1] and M.C. Brown[2,3,4]. [1]Dept. Biomedical Engineering, Boston University, Boston, MA 02215; Depts. [2]Cellular and Molecular Physiology and [3]Otology and Laryngology, Harvard Medical School, Boston, MA; [4]Eaton-Peabody Lab., Mass. Eye and Ear Infirmary, Boston, MA 02114.

Via branches to granule cell regions of the cochlear nucleus, MOC fibers synapse with large dendrites from a class of multipolar cells and with varicose dendrites from "small" cells (Benson and Brown 1990, J.Comp.Neurol. *295*: 52). What other inputs might these target dendrites receive? To begin answering this question MOC branches labeled by HRP injection in the spiral ganglion were studied by serial electron microscopy to identify labeled terminals, to reconstruct unlabeled target dendrites, and to identify other unlabeled terminals synapsing upon the targets.

The following terminal types were observed to form synapses in 3 mice (134 synapses/10 dendrites): labeled MOC (16%); unlabeled with small round vesicles (44%); unlabeled with large round vesicles (18%); unlabeled with pleomorphic vesicles (16%); unlabeled degenerating terminals (6%). Terminals with small round vesicles may represent additional unlabeled MOC input. Terminals with large round vesicles may be type I primary afferents. Terminals with pleomorphic vesicles suggest an inhibitory input. Judging by their similarity with HRP-labeled type II terminals and the known projection of type II axons into this region, degenerating terminals may be from type II primary afferents severed by the labeling pipette.

These data suggest that MOC targets in the cochlear nucleus may also be driven by auditory nerve input. Furthermore, functional linkage of MOC fibers and type II fibers may be multiple if they both share target dendrites in the cochlear nucleus and contact outer hair cells in the cochlea.

{Supported by: The Deafness Research Foundation and NIH DC 01089}

494.6

GFAP-POSITIVE PROCESSES ARE PARALLEL TO THE TONOTOPIC AXIS IN THE DORSAL COCHLEAR NUCLEUS OF THE PERINATAL HAMSTER.

G. Riggs* and L. Schweitzer Dept. of Anatomical Sciences and Neurobiology, Univ. of Louisville School of Medicine, Louisville, KY 40292.

The most salient feature of maturation of the dorsal cochlear nucleus (DCN) is the development of its laminar organization. The laminae are oriented parallel to the surface of the DCN and parallel to the underlying strial fibers. Cells sort into these laminae, axonal inputs distribute within the DCN obeying laminar borders, and developmental markers such as peanut agglutinin are organized with respect to the laminae. In contrast, the tonotopic axis of the nucleus is radial to the brainstem and is, therefore, orthogonal to the laminar axis. In the hamster, during the first postnatal week, cochlear fibers course under the nucleus and, beginning on PND 3, they begin to turn abruptly to grow into the nucleus. Their growth is oriented at right angles to the laminar axis. These radially-directed axons set up the tonotopic map of the nucleus.

With a polyclonal antibody to glial fibrillary acidic protein (GFAP), we have recently demonstrated that the organization of GFAP-positive processes is radial as well. The radial organization is especially visible perinatally, begins to disappear by about PND 4 and gives way to a more disorganized distribution of GFAP-positive processes, typical of the astrocytic label seen in much older animals. GFAP is the first marker we have studied that is distributed with respect to the tonotopic rather than the laminar organization in the nucleus. The radial pattern of GFAP-positive processes is observed at a time during which it could guide axonal ingrowth and early neuronal migration patterns.

Supported by NIH-NIDCD grant #DC00233.

494.7

GLYCINE-ERGIC NEURONS IN THE AUDITORY BRAINSTEM OF THE CHICKEN.

D.W.F. Schwarz*, I.E. Schwarz and B. Westerberg. Div. Otolaryng. & Dept. Physiol., U.B.C., Vancouver, B.C., Canada.

Retrograde transport of [^{3}H]-glycine was used in an attempt to identify glycine-ergic projection neurons in the auditory brainstem of the chicken. Glycine uptake was enhanced in the central nucleus of the inferior colliculus. Injections there led to retrogradely labeled cells on the ipsilateral side in the ventral nucleus of the lateral lemniscus, in the peripheral region of the oliva superior and in a newly identified nucleus of the trapezoid body. [^{3}H]-glycine also labeled the projection from the inferior colliculus to the thalamic nucleus ovoidalis on both sides as well as the commissural projection to the contralateral inferior colliculus. Injections of [^{3}H]- glycine into the oliva superior led to ipsilateral retrograde labeling in the nucleus of the trapezoid body, a minority of cells in the nucleus angularis, and, surprisingly, the nucleus laminaris. Anterograde label was found in n. magnocellularis ipsilaterally and the contralateral intermediate nucleus of the lateral lemniscus and inferior colliculus.

Supported by MRC, Canada and Lion's MD19 Fdn.

494.8

GABAergic AND GLYCINErgic PROJECTIONS OF THE AUDITORY MIDBRAIN.

K.A. Hutson, K.K. Glendenning, B.N. Baker and R.B. Masterton*. Dept. of Psychology, Florida State Univ., Tallahassee, FL 32306.

Glycine is the major inhibitory transmitter within the auditory hindbrain and, via ascending pathways to the IC, accounts for one source of inhibition at midbrain levels. However, our recent evidence has shown that the chief inhibitory transmitter in the ascending system changes abruptly at the DNLL and IC from glycine to GABA.

Antibodies directed against glycine label fibers and puncta but not cell bodies within the IC and DNLL, while antibodies against GABA label cells throughout the IC and DNLL as well as fibers and puncta. Further, injection of ^{3}H-glycine into the IC retrogradely labels many cells within the hindbrain but no labeled cells in the contra IC while its injection into MG does not retrogradely label cells in either the ipsi or contra IC. In sharp contrast, injection of ^{3}H-GABA into the IC retrogradely labels many cells in the contra IC and both DNLLs while its injection into MG vividly labels many cells in the ipsi and some cells in the contra IC.

Thus, the IC may be the last structure along the ascending auditory pathway where glycinergic inhibition directly participates in shaping neuronal responses. At the IC and above, it is Golgi Type I as well as Type II GABAergic neurons that assume the major inhibitory role.

Supported by NIH Grant DC00197.

494.9

EVIDENCE THAT CERTAIN COCHLEAR NUCLEUS EFFERENTS MAY USE AN EXCITATORY AMINO ACID TRANSMITTER. S.K. Suneja, C.G. Benson, J. Gross and S.J. Potashner* Department of Anatomy, University Connecticut Health Center, Farmington, CT, 06030.

This study attempts to determine if certain projections ascending from the guinea pig cochlear nucleus could be glutamatergic or aspartatergic. In anesthetized animals, one cochlear nucleus was ablated with multiple radio frequency lesions, using tungsten electrodes. Four days later, the brain stem was excised and cut transversely into 500 μm sections from which samples of the lateral (LSO) and medial superior olive (MSO), medial nucleus of the trapezoid body (MNTB), and ventral nucleus of the lateral lemniscus (VNLL) were punched or microdissected. Tissue samples were incubated with ^{3}H-D-aspartate before the electrically evoked release of radioactivity was assessed in a superfusion system (Suneja *et al.*, Neurosci. Abs. 18: 1036, 1992). Unilateral cochlear nucleus ablation depressed ^{3}H-D-aspartate release in the ipsilateral LSO and MSO as well as in the contralateral MSO, MNTB, and VNLL. A small deficit in release also appeared in the contralateral LSO. These findings suggest that the projections ascending from the cochlear nucleus to the LSO, MSO, MNTB and VNLL may use glutamate or aspartate as a transmitter. (Supported by DC00199 from NIH-NIDCD)

494.10

Three-Dimensional (3D) Analysis of the Medial Superior Olive (MSO). S. Schreiner, D. L. Oliver*, and T. Ju, Dept. of Anatomy, Univ. of Connecticut Health Center, Farmington, CT 06030-3405.

In the present experiment, we attempt to clarify the relationship between the anatomy of the MSO and its ability to code interaural time differences. We have constructed 3D images of the major nuclei within the superior olivary complex (SOC) and labeled axons which project from the cochlear nucleus to the MSO. Biotin-labelled dextran was injected into the anteroventral cochlear nucleus (CN) in two cats. After ten days, the brain was fixed in formaldehyde, frozen, and cut into either 60,100, or 150 μm-thick sections. The tissue sections underwent ABC histochemistry and were stained with neutral red. Drawings of the nuclei within the SOC and of the labelled axons projecting from the CN were made with a camera lucida at x25. Outlines of the SOC nuclei for each section were digitized and edited on an IBM-PC. Then, the data was transferred to a Silicon Graphics computer (310-4DGTX). Custom programs were used to connect the individual sections and display the model.

The reconstructions show the MSO to be a thin, oblong plane, running rostral-caudally, with slight lateral curvatures at both ends. The low-frequency, dorsal part of MSO is long, while the high-frequency, ventral MSO is short. Axons terminate in horizontal bands that may be related to the bandwidth of MSO cells. The symmetry of the projections and the fine structure of the axons as they terminate may reveal mechanisms related to coding for interaural time differences.

Sponsored by NIH grant DC00189.

494.11

TRANSPORT OF EXTRACELLULARLY INJECTED BIOCYTIN AND NEUROBIOTIN IN SUPERIOR OLIVARY TISSUE SLICES. N. Kuwabara* and J.M. Zook, *Dept. of Biol. Sci. and OUCOM, Ohio University, Athens, OH 45701.*

Previously, we have used *in vitro* intracellular labeling to show extensive interconnections between several major and periolivary cell groups within the mammalian superior olivary complex. Of particular interest were the extensive projections from the medial nucleus of the trapezoid body (MNTB) to the medial superior olive (MSO), as well as the projections from both the MNTB and the MSO to the superior paraolivary nucleus (SPN). In an attempt to establish the extent and distribution of these projections, we made extracellular, focal injections of Biocytin and/or Neurobiotin within the boundaries of individual nuclei of the superior olivary complex in an *in vitro* gerbil brainstem slice preparation.

Iontophoretic or pressure injections of Biocytin or Neurobiotin were made within the MNTB, the MSO, or both cell groups. At the injection site, cell bodies, dendrites and axons were stained in near-Golgi fashion. After MNTB injection, collateral axons and numerous terminal boutons were labeled within the lateral superior olive (LSO), the MSO and the SPN. Projections were more randomly distributed in the SPN than in the LSO. In the LSO, projections were organized in somewhat columnar fashion. After MSO injection, axon terminals were found in the SPN. Preliminary results indicate that the projection from the MNTB to the SPN is larger than the projection from the MSO to the SPN. We are combining local anterograde labeling with retrograde pre-labeling of cells to study relationships between labeled axons and identified post-synaptic cell populatioins. (Supported by DC01303, DC00038 and OUCOM)

494.12

GABA AND GLYCINE IMMUNOREACTIVITIES DEFINE PRINCIPAL SUBDIVISIONS OF THE CAT LATERAL LEMNISCAL NUCLEI. R.L. Saint Marie*, Neuroanatomy Department, House Ear Institute, Los Angeles, CA 90057.

The lateral lemniscal nuclei (NLL) are a major source of ascending projections to the auditory midbrain, second only to the cochlear nuclei in the size of their projections. Traditionally, the NLL have been divided into 3 subdivisions, dorsal (DNLL), intermediate (INLL), and ventral (VNLL), based on differences in cytoarchitecture, response type, and afferent and efferent connections. Although much has been reported about the transmitters of DNLL and VNLL, little is known about INLL. In the present study, immunoreactivities for the transmitters, GABA and glycine, are used to differentiate between the 3 subdivisions of the NLL. DNLL contains mostly large (≥28 μm, ave diam) and medium-sized (18-27 μm) neurons that are GABA-immunoreactive (GABA$^+$) and not glycine$^+$. Most of the principal neurons in VNLL are medium-sized and either glycine$^+$, GABA$^+$, or both. With few exceptions, however, principal neurons in INLL are medium-sized and neither glycine$^+$ nor GABA$^+$, but are completely encircled by glycine$^+$ boutons. The latter is consistent with reports that INLL receives up to 50% of its projections from VNLL and the medial nucleus of the trapezoid body, both of which contain mostly glycine$^+$ neurons. Glycine$^+$ perisomatic boutons are much less prevalent in DNLL and VNLL, as are GABA$^+$ perisomatic boutons in all 3 subdivisions. As defined, the boundaries between the subdivisions are clear and distinct. These observations suggest that neurons in INLL are strongly inhibited by contralaterally driven monaural inputs and, unlike neurons in DNLL and VNLL, probably do not provide inhibitory projections to the auditory midbrain. Supported by NIH grant DC00726.

494.13

NEURONS WITH INTRINSIC AXONAL TERMINATIONS IN THE GERBIL'S LATERAL LEMNISCUS. G. P. Tuck, J. M. Zook* and N. Kuwabara, *Dept. of Biological Sciences and OUCOM, Ohio University, Athens, OH 45701.*

The cell groups of the lateral lemniscus have often been viewed as simple relays of ascending auditory information from the cochlear nuclei and the superior olives to the inferior colliculi. We have begun to intracellularly label lemniscal neurons in a Gerbil brain slice preparation in order to reveal the detailed morphology and interconnections of lemniscal cells.

Our main finding is that all three lemniscal nuclei contain cells with intrinsic axonal terminations. In the dorsal, intermediate and ventral lemniscal nuclei, cells were found with single axons that branched immediately upon leaving the soma. One branch commonly could be traced out of the lateral lemniscus. The other axon branch terminated locally forming small *en passant* and terminal boutons. The results indicate that the nuclei of the lateral lemniscus can not be thought of as simple relays of ascending information. Rather, the local terminations indicate communication between various cells within the lemniscal nuclei and indicate a clear potential for modification or elaboration of information at this level.

(Supported by DC01303, DC00038 and OUCOM)

494.14

SUBCELLULAR LOCALIZATION OF THE Kv3.1 POTASSIUM CHANNEL PROTEIN IN THE RAT AUDITORY BRAINSTEM. T.M. Perney*[1], E. Meloni[2], P. Eager[2], L.K. Kaczmarek[1] and I. R. Schwartz[2], [1]Dept. of Pharmacology or [2]Dept. of Surgery/Otolaryngology and Section of Neurobiology, Yale School of Medicine, New Haven, CT 06510.

A polyclonal antibody specific for the *Shaw*-like Kv3.1α (Kv4) potassium channel was generated by immunizing rabbits with a synthetic peptide corresponding to the last 15 C-terminal amino acids. Using this antibody, we have demonstrated that the Kv3.1 channel protein is highly enriched in the nuclei of the auditory brain stem where it is found both outlining the cell somata and in the neuropil. We now describe the precise subcellular distribution of this K$^+$ channel in the rat auditory system using electron microscopic immunohistochemistry.

A nonhomogeneous distribution of HRP reaction product was observed. As expected, much of the label was seen associated with the cytoplasmic face of the neuronal surface membrane. However, significant patches of reaction product were seen associated with lamellae of ER and sometimes with cisterns of Golgi suggesting that there may be a high turnover rate for the Kv3.1α protein. Patches of reaction product were also observed in the cell nuclei of some neurons showing cell membrane staining, especially in bushy cells of the VCN. The significance of this finding is not known.

Reaction product associated with cell membranes was most often observed on cell somata, dendritic processes and spines. However, we also observed staining inside presynaptic terminals including the calyxes of the end bulbs of Held and sometimes of axonal fibers especially in the molecular layer of DCN. The cell surface labeling was usually found in patches on either side of both pre- and postsynaptic specializations. These results suggest that the Kv3.1α protein may be preferentially localized near synaptic sites of auditory neurons and may play a role in shaping the fast synaptic transmission seen in auditory pathways.

494.15

COMPARISON OF CALRETININ, PARVALBUMIN AND CALBINDIN STAINING IN AUDITORY NUCLEI OF THE MUSTACHED BAT. M.L. Zettel*, M. Gordon, and W.E. O'Neill. Dept. of Physiology and Prog. in Neuroscience, Univ. of Rochester Sch. of Medicine and Dentistry, Rochester, NY 14642.

There has been much interest in the role of calcium binding proteins as possible intraneuronal calcium buffers. In the auditory system of the echolocating mustached bat such a buffering system could be critical for the response to rapid temporal events. Cross-reactivity among various calcium binding proteins has been questioned. We have examined the staining of antibodies to calretinin (cr) and parvalbumin (pv) and compared it to previously reported calbindin D-28k (cabp) staining.

The cochlear nucleus and the inferior colliculus had similar staining patterns using all three antibodies. Cells of the medial nucleus of the trapezoid body were very darkly stained for cabp and pv, but only some cells were cr+. Calycean endings were seen with all antibodies. The cells of the lateral superior olive were cabp- and cr-, but were strongly pv+. The medial superior olive contained only a few cabp+ marginal cells, while cr stained all but the most central cells, and pv stained cells throughout. The ventral (columnar) division of the ventral nucleus of the lateral lemniscus (LL) had darkly stained pv+ and cabp+ cells with fine bouton endings. However, only some cells were cr+ and large calycean endings were prominent. Only cabp stained the granular cap in the intermediate nucleus of LL. The dorsal nucleus of LL was cabp-, partly cr+, and strongly pv+. The medial geniculate was strongly cabp+ and mildly cr+, whereas the suprageniculate was negative for both, but strongly pv+. The dorsal division of MGB was especially immunopositive for both cabp and cr, but was pv-. (Supported by NIDCD DC0267)

494.16

ACETYLCHOLINESTERASE STAINING IN THE AUDITORY BRAINSTEM OF A SYMMETRICALLY EARED OWL. J.A. Mazer* and M. Konishi, Division of Biology, Caltech, Pasadena, CA 91125

Electrophysiological studies of the symmetrically eared great horned owl, *Bubo virginianus*, have shown that cells in the external nucleus of the inferior colliculus (ICx) have auditory receptive fields restricted in azimuth but not in elevation. This is in contrast to the asymmetrically eared barn owl, *Tyto alba*, which uses ear asymmetry to convert elevation into interaural intensity differences. In the barn owl ICx cells have receptive fields restricted in both azimuth and elevation.

Acetylcholinesterase (AChE) histochemistry in the barn owl has been shown to distinguish two parallel pathways in the brainstem: nuclei that process intensity are AChE positive, while those processing time or azimuthal cues are AChE negative.

Despite the lack of asymmetry, AChE staining in the great horned owl is qualitatively similar to that seen in the barn owl. Both fibers and cell bodies in N. angularis, a cochlear nucleus (CN) primarily involved in encoding intensity information, stain strongly for AChE. In n. magnocellularis (NM), a monoaural CN in the time pathway, fibers show no staining and cell bodies stain only weakly. In n. laminaris, which receives bilateral input from NM, neither fibers nor cell bodies stain for AChE. At the level of the lemniscal nuclei, the posterior portion of n. ventralis lemnisci lateralis (VLVp), part of the intensity pathway, stains for AChE, while the anterior portion (VLVa), part of the time pathway, does not.

At the level of the inferior colliculus (IC), the medial and lateral shells of the central nucleus of IC (ICc) and ICx, those subdivisions which in the barn owl process intensity information, are AChE positive in the great horned owl, while the core region of ICc, which in the barn owl processes only temporal cues, is AChE negative.

Though it is not yet clear how intensity information is used by the great horned owl, it appears that there are two histochemically distinct auditory pathways in the brainstem which are anatomically similar to the two parallel pathways seen both physiologically and histochemically in the brainstem of the barn owl.

SENSORIMOTOR CORTEX: FUNCTIONAL STIMULATION, MODELS, AND BEHAVIOR

495.1

MOTOR CORTEX RESPONSIVENESS TO TRANSCRANIAL MAGNETIC STIMULATION FOLLOWING PERFORMANCE OF A FATIGUING MOTOR TASK

W.B. McKay*, S. Tuel, A.M. Sherwood, M.R. Dimitrijevic
Div. of Restorative Neurology and Human Neurobiology, Baylor College of Medicine, Houston, TX 77030

It is well established that volitional activation of a muscle lowers its threshold for elicitation of a motor evoked potential (MEP) by transcranial magnetic stimulation (TMS) of the motor cortex. However, the effects on MEPs recorded following the performance of a fatiguing exercise are not known. We recorded MEPs through surface EMG electrodes placed over both quadriceps, hamstring, anterior tibial and triceps surae muscles. MEPs were elicited by a 14 cm. diameter coil placed over the scalp vertex before and after the performance of maximal voluntary isometric contraction of one anterior tibial muscle until the force generated decreased to less than 50% of its initial value. To monitor neuromuscular excitability of the exercised anterior tibial muscle, we recorded supramaximal M-waves elicited by electrical peroneal nerve stimulation. In all 5 subjects, MEPs recorded from the exercised muscle dropped to well below pre-exercise control values while the M-wave remained unchanged or increased . MEPs from non-exercised muscles, particularly the contralateral quadriceps and hamstring, showed marked amplitude increases following the isometric dorsiflexion exercise period. These changes in the non-exercised muscle MEPs were short lasting (<1 min.) while the post-exercise depression of the MEP in the exercised anterior tibial muscle lasted from 3 minutes to more than 30 minutes. These results suggest that central and peripheral mechanisms used to generate movement (force) can be studied separately using TMS and peripheral nerve stimulation with appropriate motor task conditioning.

495.2

VARIABILITY OF SINGLE MOTOR UNIT RESPONSES TO TRANSCRANIAL MAGNETIC STIMULATION. J. Qiao and B. Brouwer* Department of Rehabilitation Therapy, Queen's University, Kingston, ON., Canada K7L 3N6.

The intrasubject and population variabilities of responses evoked in tibialis anterior (TA), medial gastrocnemius (MG) and soleus (SOL) motoneurons (MNs) by transcranial magnetic stimulation were examined in 33 normal subjects. A total of 3-7 low threshold motor units were identified and recorded from each subject from a given muscle. The magnitudes of the postsynaptic potentials (PSP) produced in spinal motoneurons were estimated from the changes in firing probability of voluntarily activated single motor units. Short latency facilitations were observed in 54/56 TA MNs, 23/65 MG MNs and 27/66 SOL MNs. The mean estimated PSP amplitude was approximately 3 times greater in TA MNs than that observed in MG or SOL MNs ($p < .001$). Short latency inhibitions were found in 1/56 TA MNs, 4/65 MG MNs, and 2/66 SOL MNs. The remaining motor units (a total of 76 from all muscles) showed no short latency responses, but 87% of these demonstrated longer latency effects. The variability in amplitudes of the short latency effects was high for a given species of MN (coefficients of variation, CV, ranging from 70% (TA) to 208% (MG)). Intrasubject amplitude variability reflected that of the population. TA MNs responded consistently within a given subject (mean CV = 46%) whereas variances were significantly higher for MG (mean CV= 166%; $p < .001$) and SOL (mean CV=172%; $p < .01$). Variability in the direction of the response (i.e. facilitation, inhibition or no response) contributed strongly to both intrasubject and population variances in response amplitude in MG and SOL MNs. These findings provide insight as to the probability of evoking a response of a given direction and relative amplitude in a specific MN pool. Similarly varied corticospinal effects are exhibited in other primates.

This project was funded by the Botterell Foundation of Queen's University.

495.3

LARYNGEAL MUSCLE RESPONSES TO TRANSCRANIAL MAGNETIC STIMULATION. C.L. Ludlow*, A. Pascual-Leone, C Cozens-Hoffman, T. Yamashita and M. Hallett. Voice and Speech Section, VSLLB, NIDCD and Human Motor Control Section, MNB, NINDS, NIH, Bethesda, MD 20892.

Our purpose was to map the right and left cranial regions producing laryngeal muscle responses to transcranial magnetic stimulation in normal right handed adults. A figure 8 coil was used to map all the scalp positions over each hemisphere from which contralateral and ipsilateral muscle responses were obtained in bipolar hooked wire recordings of the thyroarytenoid and cricothyroid laryngeal muscles. Surface recordings of the abductor pollicis brevis (APB) were also used to determine muscle responses. All responses were identified visually and the total area under the curve was computed after subtracting resting activity for the same duration. In contrast with APB for which only contralateral responses were obtained similarly in each hemisphere, both contralateral and ipsilateral laryngeal muscle responses were obtained in most subjects. The ipsilateral and contralateral responses had similar latencies, between 10 and 20 ms. Responses to peripheral stimulation on the neck had earlier latencies and were infrequent using this stimulator. In some subjects, the map of the laryngeal muscles in the left hemisphere extended beyond the margins of representation of the APB. The results suggest variability in the degree of bilateral representation of the laryngeal muscles in each hemisphere in humans, with some subjects having larger regions of representation in the left than in the right hemisphere.

495.4

THE EFFECT OF VOLUNTARY CONTRACTION ON CORTICO-CORTICAL INHIBITION IN THE CORTICAL MOTOR AREAS OF MAN. M. Ridding, J. Taylor and J.C. Rothwell*. MRC Human Movement and Balance Unit, Institute of Neurology, Queen Square, London WC1N 3BG, UK.

A single transcranial magnetic stimulus applied on the scalp over the motor area of cortex can evoke EMG responses in contralateral muscles of conscious subjects. If this stimulus is preceded by a smaller conditioning stimulus, the response can be dramatically suppressed if the interval between the stimuli (ISI) is 6ms or less. In an initial set of experiments on 4 subjects we found that the threshold for this effect was 28±3% (s.e.) less than the intensity needed to produce a response in active muscle. Since conditioning stimuli of this intensity produce no effects on either spinal H-reflexes or responses evoked during activity in the same muscle by low intensity anodal electric stimulation of the motor area, we presume that the effects on the test response occur through cortical mechanisms. In the main set of experiments on 6 subjects, the intensity of the conditioning stimulus was set at 5-10% below threshold for producing an EMG response in active muscle, and the time course of its effect on a subsequent test shock was examined either when the subjects were completely relaxed, or when they made a minimal tonic contraction of the first dorsal interosseous muscle. In order to produce responses of equal size in the two conditions, the intensity of the test shock was slightly larger when subjects were at rest than when they were active. When relaxed, test responses were suppressed by an average of 55±11% when the ISI was 2, 3 or 4ms. At an ISI of 10 and 15ms the responses were facilitated to 125±16% of their control value When subjects activated their muscle, the responses were inhibited by only 21±12% (paired t-test with relaxed condition, $P<0.01$) and there was no obvious excitatory phase at 10 and 15ms (78±5% ($P<0.005$)). These results suggest that cortical circuits which mediate inhibition at rest are suppressed during voluntary activity of the target muscle.

495.5

INHIBITION AND EXCITATION OF THENAR MUSCLES TO TRANSCRANIAL MAGNETIC STIMULATION DURING VOLUNTARY CONTRACTION IN CERVICAL SPINAL CORD INJURY PATIENTS. P.H.Ellaway*, N.J.Davey and D.W.Maskill. Dept. of Physiology, Charing Cross and Westminster Medical School, LONDON W6 8RF, UK

Electromyographic responses of thenar muscles to transcranial magnetic stimulation (TMS) of the motor cortex were recorded in patients with incomplete lesions of the spinal cord at the cervical level. During weak voluntary contraction we measured the threshold and latency for excitation, and for inhibition in the absence of preceding excitation, in response to TMS. As in normal man (Davey et al. 1992 J. Physiol. 446:447P) the inhibition had a lower threshold than excitation. On average, threshold (percentage maximum stimulator output) for inhibition in patients (44 ± 6, mean ± S.D., n=6) was greater than in control subjects (26 ± 3.5, n=6). Threshold for excitation was also greater in patients (58 ± 13.5, n=8) than in controls (33 ± 6, n=6). In all patients, the latency of inhibition was longer (40-66.5 ms, n=6) than in controls (28.5-34.5 ms, n=6). However, the latency for excitation in patients (22-45 ms, n=8) was greater than for controls (23-28 ms, n=6) in only 3 cases. Since the thenar muscles were active under voluntary drive it is likely that the differences seen in spinal injury reflect changes at the level of the motor cortex. We thank patients and staff at the National Spinal Injuries Centre, Stoke Mandeville, UK.

495.6

MOTOR EFFECTS OF FRONTAL AND CINGULATE STIMULATION IN HUMANS. F. Richer*, M. Robert, G. Bouvier, J.M. St-Hilaire. Dept. Neurologie, Hôpital Notre-Dame, Montreal, Que. Canada, H2L-4M1.

Motor phenomena were evoked by stimulation of frontal and cingulate regions in epileptic patients undergoing a presurgical investigation with chronic intracerebral electrodes. An incremental sequence of bipolar stimulation trains was delivered at each of 327 sites in 96 patients through horizontal multicontact electrodes while monitoring stimulus afterdischarge propagation at frontal and temporal sites. Stimulation of medial regions evoked movements more frequently than lateral stimulation. In the supplementary motor area, the phenomena evoked included contralateral head and/or eye deviations, and contralateral or bilateral arm movements. In the anterior cingulate, evoked phenomena included movements of the mouth, of the eyelids, and of the ipsilateral arm, whereas in the posterior cingulate, stimulation evoked bilateral movements of the legs or arms. In lateral premotor cortex, stimulation dorsal to the inferior frontal gyrus evoked movements of the contralateral hand or mouth. The results agree with evidence from non-human primates of multiple motor areas in frontal and cingulate cortex.

495.7

THE RELATIVE TIMING OF MOTOR EVOKED RESPONSES (MEPS) TO TRANSCRANIAL MAGNETIC STIMULATION (TMS) IN CONTRALATAERAL AND IPSILATERAL HUMAN MUSCLES. E. M. Wassermann*, A. Pascual-Leone and M. Hallett. Human Motor Control Section, National Institute of Neurological Disorders and Stroke, NIH, Bethesda, MD 20892

TMS can produce MEPs in voluntarily activated ipsilateral, as well as contralateral, muscles. It is not known how the ipsilateral MEPs are conducted or whether the same pathways serve proximal and distal ipsilateral muscles.

In 2 normal subjects, we stimulated the optimal scalp positions for producing MEPs in the ipsilateral and contralateral first dorsal interosseous (FDI) and deltoid muscles during activation of the ipsilateral muscles and made 10 trial averages of MEPs.

Responses in the contralateral muscles were always earlier. The difference in latency between the ipsilateral and contralateral MEPs in the FDI was 6.5 ms in subject 1, and 4.5 ms in subject 2. In the deltoid, the latency difference was 3.5ms in subject 1 and 0 ms in subject 2.

Contralateral MEPs in all muscles are thought to be conducted by fast axons in the corticospinal tract. Ipsilateral MEPs in distal and proximal muscles appear to be conducted by different pathways. The pathway for proximal muscles may be recruited faster by TMS or contain faster-conducting axons or fewer synapses.

495.8

CUTANEOUS AFFERENT CONDITIONING OF MEPS FOLLOWING TRANSCRANIAL MAGNETIC STIMULATION OF MOTOR CORTEX. D.L. Wolfe* and K.C. Hayes, Neuroscience Prog., U. of Western Ontario, London, Ont., Canada, N6A 5C2.

Conditioning of motor evoked potentials (MEPs) by a preceding afferent stimulation has previously been shown to be helpful in detection of corticospinal input compromised by spinal injury. In the present investigation we electrically stimulated the sural nerve (20 ms train - 500 Hz - 0.1 ms pulse width) of normal human subjects at 5 x sensory threshold and at varying time intervals prior to cortical stimulation. The purpose was to determine if stimulation of a purely cutaneous nerve in the lower limb resulted in a pattern of MEP modulation in the ipsilateral tibialis anterior similar to that seen with conditioning a nerve with both proprioceptive and cutaneous fibers. Condition-test (C-T) intervals from 0 to 150 ms were employed randomly and compared to control unconditioned MEPs. Two separate phases of MEP facilitation were evident at C-T:20-30 ms and C-T:60-140 ms. The time course of the later facilitation was similar to that we have previously reported following conditioning of the medial plantar nerve (combined cutaneous and proprioceptive). This result suggests that in the lower limb stimulation of cutaneous (and/or nociceptive) afferents per se results in MEP amplitude modulation. These observations contrast with earlier reports that cutaneous afferent stimulation does not facilitate MEPs evoked in the upper limb and may reflect a differential organization of afferent inputs between the upper and lower limbs in man.

495.9

MOTOR CONTROL AND GAIT PERFORMANCE IN CHRONIC INCOMPLETE SPINAL CORD INJURED PERSONS.
S.F.T. Tang, M.M. Dimitrijevic, S.M. Tuel, M.R. Dimitrijevic*. Div. of Restorative Neurology, Baylor College of Med., Houston, TX. 77030

Neurocontrol of movement following spinal cord injury (SCI) is often spared, but few studies have investigated the chronic incomplete SCI patient. Brain motor control assessment, a multi-channel surface electromyography (SEMG) recording that through a series of volitional and reflex events provides a profile that describes characteristics of neurocontrol. The relationship of these neurocontrol characteristics to clinical function is as yet incompletely described. This study retrospectively evaluated the relationship between neurocontrol patterns in the supine position and ambulation ability. The records of 15 neurologically healthy (9 male; 6 female) and 36 incomplete SCI persons (27 male; 9 female) (C2-T10) were used. SEMG was recorded from both quadriceps, hamstrings, anterior tibialis and triceps surae muscles and displayed on a stripchart for analysis. Patterns of activity recorded in the supine position during volitional, unilateral, multi-joint (hip and knee flexion and extension) movement attempts were characterized, divided into 7 groups, and compared to the subjects' ambulatory capabilities (independent, cane, crutches, walker or nonambulatory). The neurocontrol patterns recorded in the supine position correlated well with the SCI subjects ambulatory capabilities. Marked decreases in motor unit output and/or loss of motor organization were found in the nonambulatory group. Coactivation of proximal muscles, poor timing of muscle activity, and radiation of activity into contralateral muscles were also noted in subjects who required a walker or crutches. To a lesser degree, abnormal motor patterns were also noted in subjects who ambulated with a cane or independently.

495.10

UNCOUPLING OF SPATIAL CHARACTERISTICS IN CALLOSOTOMY PATIENTS DURING A BIMANUAL TASK. **J.C. Eliassen[1], E.A. Franz[2], R.B. Ivry[2], A.P. Shimamura*[2] and M.S. Gazzaniga[1]. [1]Center for Neuroscience, University of California, Davis, CA 95616 and [2]Department of Psychology, University of California, Berkeley, CA 94720.**

Studies of normal subjects have revealed spatial and temporal coupling between the limbs in bimanual tasks. The present study examined the possibility that spatial coupling (both movement amplitude and direction) results from communication between the two cortical hemispheres via the corpus callosum. Two split brain patients and two normals were tested on a bimanual drawing task. Subjects were seated in front of a video monitor and fixated a center point. Stimuli were presented 3 degrees or more from fixation, unilaterally or bilaterally, for 150 ms to assure lateralization in the callosotomy patients. The basic stimulus was a three segment figure equivalent to three sides of a rectangle opening up, down, right, or left. Subjects were instructed to draw the stimuli at a preferred pace without visual feedback. Subjects were videotaped (60 Hz)and data were recorded analog on two drawing tablets (78Hz). For bilateral presentation subjects were instructed to begin drawing with the two limbs simultaneously. Qualitative and quantitative analyses indicated that normal subjects exhibited greater accommodation of amplitude and direction between the two limbs than the patients. Displacement profiles of normals revealed larger temporal pauses between segments of the movements, which is taken as evidence of a strategy to overcome bimanual spatial coupling effects. One callosotomy patient exhibited no such pausing strategy while the other did on some trials. These results suggest that the spatial characteristics of amplitude and direction are coupled via the corpus callosum. Supported by NIH/NINDS P01 NS17778-11 and the McDonnell-Pew Foundation.

495.11

PREDICTING SYNAPTIC WEIGHT PATTERNS FROM SENSORIMOTOR COORDINATE TRANSFORMATIONS RELATED TO POPULATION CODING OF MOVEMENT DIRECTION IN MOTOR CORTEX. Gyöngyi Gaál* and John P. Donoghue, Dept of Neuroscience, Brown University, Providence, RI 02912

We compared four population coding algorithms to reconstruct stimuli in neural coordinate systems defined by preferred response vectors of neurons (such as receptive field profiles for sensory areas or preferred directions for motor areas, Gaál, Neural Networks, 1993a; J Theor Biol, 1993b). One such algorithm was designed by Georgopoulos et al. (1988, J Neurosci). Another was derived from the tensor network theory (Pellionisz & Llinas, 1979, Neurosci). A third was inspired by an iterative network using feedforward-feedback connections (Daugman 1988, IEEE Trans ASSP) for image compression and reconstruction. We tested also a fourth algorithm, a non-vectorial, non-linear mapping to estimate movement direction from responses of simulated neurons (Gaál 1993b). We show that each algorithm, although equally plausible in computer simulations, provide different predictions for the functional architecture of the coding network. In the temporal domain, the population coding algorithms are capable not only of stimulus reconstruction but also of short-term prediction. Georgopoulos et al. (1993, Science) reported experimental results that are consistent with our predictions regarding the neural implementation of their population code to estimate the direction of arm movement from responses of directionally tuned motor cortical neurons. They found that the angle between preferred directions of pairs of neurons and the synaptic strength of their interconnections are negatively correlated. In contrast, the synaptic weight patterns found by Fetz (1992, Brain Behav Sci) for neurons nearer to motor output might seem to be counterintuitive: neurons with similar response properties can also inhibit one another, while neurons with dissimilar responses can excite each other. We will show how such connection patterns might be interpreted in terms of sensorimotor transformations as the Jacobian matrix of transformations between different coordinate systems. We will also demonstrate that cells with nonlinear responses (e.g. complex cells in visual cortex) might calculate invariants that are independent of coordinate systems. Supported by Grant NS 25074

495.12

MODELING PERISPIKE FEATURES IN SPIKE TRIGGERED AVERAGES OF RECTIFIED EMG ACTIVITY. B. J. McKiernan* and P. D. Cheney. Dept. of Physiology and Smith Mental Retardation Research Center, Univ. of Kansas Med. Ctr., Kansas City, KS 66160.

Spike triggered averaging (STA) of rectified EMG activity has proven to be an effective means of identifying cortical and red nucleus neurons that are synaptically coupled to motoneurons (Cheney et al., *Prog. Brain Res.* 87: 213, 1991). Most STAs have one prominent peak corresponding to a transient period of postspike facilitation (PSF). In addition to the primary peak, STAs often exhibit additional secondary features that straddle the primary peak. One such feature is a trough, or period of low average EMG activity, that precedes and follows the primary PSF peak. The purpose of this study was to investigate the factors contributing to the production of troughs in STAs. A computer controlled premotor cell spike was used to evoke a discharge in one of many motor units contributing to the EMG record. The model enabled manipulation of several parameters including: 1) the probability that a premotor cell spike would evoke a discharge in a motoneuron, 2) the repetitive firing characteristics of the premotor cells, 3) the number of motor units in the EMG recording, 4) the fraction of recorded motor units coupled to the premotor cell, 5) the repetitive firing characteristics of the motor units, and 6) background noise levels. Repetitive firing characteristics of the premotor cells were quantified by computing autocorrelograms and interspike interval histograms. Results from the model show that the troughs straddling PSF peaks in spike triggered averages of EMG activity are linked to troughs in the autocorrelogram of the trigger cell which result from the cell's refractory period. In conclusion, a simple model is presented that exhibits many of the features evident in spike triggered averages of EMG activity. Supported by NIH grant NS25646.

495.13

A NETWORK MODEL OF DIRECTION TUNING AND MENTAL ROTATION. S J Lee* and D Zipser, Department of Cognitive Science, UC San Diego, La Jolla, CA 92093

Many neurons in the arm region of monkey primary motor cortex are tuned to the 3D direction of reaching. The tuning of these neurons is sensitive to the location of the starting point for a reaching movement, but the population vector calculated over all tuned neurons always indicates the direction of motion. Most interestingly, if the animal has been trained to reach in a direction rotated from that of a visual target, the direction of the population vector rapidly rotates from the target direction to the actual movement direction (Georgopoulos et al, *Science*, 243:234-236, 1989). To account for starting position dependent direction tuning, we assume that M1 receives ongoing afferent information about both current and target hand position, and continuously computes a transformation to the motion direction that will bring the hand to the target. To account for the observations about *mental* rotation we hypothesize that the motor cortex initially gets afferent location information from the visual target, and then gets afferent information about the location of the rotated target from some other brain area that has learned to associate visual with rotated targets. Under our assumptions, the observed rotation of the population vector then comes from the inherent dynamics of M1 provided with two successive target locations, one from vision and one internally generated from associative memory. To test the consistency of these assumptions, a computational model of M1 was generated by training a fully recurrent neural network to continuously track a moving target. Network inputs consisted of current and target hand positions, encoded as two sets of arm joint angles. Network output indicates the direction required to bring the hand to the target. Since the network was recurrent and the training task continuous, the model of M1 developed its own internal dynamics. When tested on simulations of physiological experiments, the hidden unit and population vector behavior matched the starting position dependent, 3D directional tuning of cortical neurons and their population vectors. In simulated mental rotation experiments in which the visual and rotated target positions were successively presented to the model, the population vector rotated with dynamics similar to those observed experimentally.

495.14

A LESION IN PRIMARY MOTOR CORTEX CAUSES WRIST MOVEMENTS TO BE MISDIRECTED. D.S. Hoffman* and P.L. Strick, Research Service, VAMC and Depts. of Neurosurgery and Physiology, SUNY-HSC, Syracuse, NY 13210.

We removed the contralateral arm area of primary motor cortex (M1) in a primate trained to make rapid wrist movements in 8-16 different directions. Prior to the lesion, the animal's movements were smooth and nearly straight in all directions. Five months after the lesion, the monkey's wrist movements remained markedly slowed, more curved and more variable than prelesion movements. Most remarkably, the monkey "tacked" to reach some targets; that is, movements to these targets were made using a zig-zag trajectory with 1-2 sharp changes in direction. Thus, in the absence of M1, it appears that some directions of wrist movement are inaccessible to the animal. EMG recordings using intramuscular electrodes showed that alternating agonist and antagonist bursts were not present in single forearm muscles after the lesion. The absence of these bursts is the likely cause of the decrease in movement velocity. The spatial patterning of agonist muscle activity also was markedly altered. Wrist muscles were more narrowly tuned after the lesion, and movements in each direction were performed by activating fewer muscles. We conclude that M1 contributes to the broad tuning of muscles and thereby provides fine control of movement direction by grading muscle synergies. Supported by VA Medical Research Service.

495.15

DIFFERENT REACHING BEHAVIOR IN RATS WITH MOTOR AND SOMATOSENSORY CORTEX LESIONS. M.Salinᵃ, T. Sitarova, M.R.Dimitrijevic Inst.Norm.Pathol.Physiol.,Bratislava, Slovakia, Restorative Neurology and Human Neurobiology, Baylor College of Medicine, Houston, TX.

Selective lesions of sensorimotor cortex in rats result in specific impairment of reaching task. The aim of presented work was to analyze the effect of discrete cortical lesions on reaching behavior in different reaching task. Reaching for food pellet into the tube and through the grid was studied in the rats with bilateral motor - MCL (AP 0.5- 3mm, L 1.5 - 3.5mm) and somatosensory - SCL (AP -1 - 1mm, L 3 - 4.5mm) cortex lesions over a prolonged recovery period. Continuous recording of movement based on magnetic induction was used to monitor reaching. The success of reaching was quantified. Reaching attempts (mainly into the tube) in rats with MCL were accompanied by "copying " movements of non-reaching forepaw. With recovery the " copying " movements were less expressed in reaching into the tube and disappeared in reaching through the grid. Permanent significant decrease of successfulness in rats with MCL was observed only in reaching into the tube. In rats with SCL the modulation of ongoing reaching execution by information about grasping pellet were lost. Loss of tactile information related to the grasping was substituted by "anticipatory reaching behavior ". The successfulness of reaching was persistently decreased in both reaching tasks. The results showed that the lesions resulted in different behavioral reactions.

495.16

ROLE OF THE VENTROLATERAL ORBITAL CORTEX IN SPATIAL LEARNING IN THE RAT. J.V. Corwin*, M. Fussinger, R.C. Meyer, V.R. King, and R.L. Reep. Depts. of Psychology, Northern Illinois University, DeKalb, IL 60115; and Univ. of Wisconsin, Madison, WI 53706; and Dept. of Physiol. Sciences, Univ. of Florida, Gainesville, FL 32610.

Our previous research has indicated that the posterior parietal (PPC), medial agranular (AGm), and ventrolateral orbital (VLO) cortices form part of an interconnected cortical network for spatial orientation. Unilateral destruction of each of these areas produces severe multimodal neglect. In learning tasks bilateral destruction of the PPC or AGm produces dissociable deficits for allocentric and egocentric spatial learning respectively. However, the role of the VLO in spatial learning has not been directly examined. Therefore, the present studies directly examined the effects of bilateral VLO destruction on performance in allocentric and egocentric spatial tasks.

The effects of bilateral VLO destruction were examined in two spatial contexts, a cheese board task (allocentric, n=23) in which a food reward is kept in a constant location relative to extramaze cues and in an adjacent-arm maze (egocentic, n=23) in which the location of a food reward varies depending on the subjects' location in space. Three surgical groups were examined in each task: a Sham group, a VLO group, and a group which experienced equivalent destruction of the laterally adjacent lateral orbital cortex (LO) which has a different pattern of connectivity than the VLO.

The results indicated that the VLO operates were significantly impaired in the acquisition of the cheese board (allocentric) task relative to both the Sham and LO operates. In the adjacent-arm (egocentric) maze the VLO group was not impaired relative to the Sham group, and was superior to the LO group. The LO and Sham groups did not differ in either task. The results suggest that the VLO is involved in allocentric spatial processing and support the contention that the PPC, AGm, and the VLO form part of the cortical circuitry for the processing of space in rodents.

496.1

• MAGNETIC SOURCE IMAGING FOR PRESURGICAL LOCALIZATION OF FUNCTIONAL CORTEX • C.C. Gallen*, B.J. Schwartz, H. Tung, B. Copeland. The Scripps Research Institute, La Jolla, CA 92037.

• Five patients with mass lesions near eloquent cortex were measured with Magnetic Source Imaging (MSI) preoperatively, and results were compared with intraoperative electorocorticography (ECoG). A Magnes™ 37-channel Biomagnetometer recorded evoked somatosensory fields, with functional localizations overlaid on MR slices. Localizations were depicted on the surface of a 3D reconstruction of the brain based on MR, complete with vascular landmarks derived from MR angiography. Accuracy of MSI was assessed against ECoG results: discrepancy was typically within several mm. MSI has potential utility in planning and facilitating intraoperative procedures, and may in some cases help in the selection of craniotomy and surgical approach.

496.2

HUMAN FRONTAL MOTOR CORTICAL AREAS RELATED TO MOTOR PERFORMANCE AND MENTAL IMAGERY USING MAGNETIC RESONANCE IMAGING. JN Sanes*, CE Stern, JR Baker, KK Kwong, JP Donoghue, BR Rosen Department of Neuroscience, Brown University, Providence, RI 02912 and MGH-NMR Center and Harvard Medical School, Charlestown, MA 02129.

Previous studies in humans indicated that imagery of sequential movements uniquely activated non-primary cortical motor fields. By contrast, studies in monkeys indicated that mental rotation before performance of simple movements activated neurons in primary motor cortex. We hypothesized that human non-primary and primary motor cortical fields are distinguishable during actual or imagined simple, repetitive movements by physiological activation patterns obtained by functional magnetic resonance imaging (fMRI).

Normal humans rested, performed index finger movements in a square-like form, or imagined performing the finger movements, each for 1 min and with the eyes closed. In other experiments, subjects fixated a spot and rested or performed or imagined performing index finger movements. High-speed, echo-planar fMRI techniques measured the paramagnetic state of dexoyhemoglobin every 3 sec during each behavioral condition across 14-20 brain slices (7 mm thick) roughly aligned with the plane of the central sulcus.

Several brain areas contralateral and ipsilateral to the moving finger showed enhanced activation during selective periods of the motor task. At the level of the central sulcus, regions contralateral to the moved finger exhibited enhanced activation during motor performance. These regions were consistent with the primary motor, premotor, and cingulate cortex. More rostral extensions of sites in the premotor and cingulate cortex active only during motor performance showed enhanced activation during both actual and imagined motor performance. As yet, no frontal sites have been identified that exhibit enhanced activation only during motor imagery.

These data suggest that premotor and cingulate motor areas have a greater role in higher order processing related to motor actions compared to the primary motor cortex. However, there may be segregation of function within non-primary motor areas. (Support: AG10634, McDonnell-Pew Foundation, MH50054, NS25074.)

496.3

ANALYSIS OF MOTOR AND PREMOTOR REGIONS IN HUMANS STUDIED ACROSS MULTIPLE PET EXPERIMENTS. H. Van Mier*, M. Corbetta, J.A. Fiez, F.M. Miezin, R.L. Buckner, M.E. Raichle and S.E. Petersen. Washington University, School of Medicine, Box 8111, St. Louis, MO 63110.

PET activation studies produce large amounts of information that might be beneficial beyond the original intentions of a single experiment. To examine the consistency of active regions across different conditions and subject groups, an analysis of activation foci was performed using eight groups of subjects (three keypress groups, and five verbal response groups) in 36 conditions (19 keypress and 17 verbal). Foci of activation along the central sulcus and in adjacent areas of frontal cortex were identified using a computer search algorithm and statistical screens. Extending from the medial surface to the operculum, several clusters were identified: 1) along the central sulcus that probably represent primary motor cortex (M1); 2) along the midline surface of the frontal cortex consistent with supplementary motor area (SMA); 3) anterior to M1 consistent with a localization to premotor regions; and 4) along the frontal operculum that is possibly in areas 44/45. In primary motor cortex, SMA, and premotor cortex, the localizations of the verbal and keypress activations were statistically distinguishable from each other consistent with somatotopic representation. While significant, the somatotopic organization was less straightforward in the opercular region, and appears to form more than one representation. These results show, that despite reports of significant intersubject variability in human cortical anatomy, central tendencies are strong enough to do analyses across subject groups and tasks. Such analyses are useful for guiding more targeted within-subjects experimental designs.

496.4

AN MRI STUDY OF THE SUPERIOR TEMPORAL SURFACE IN MONOZYGOTIC TWINS DISCORDANT FOR SCHIZOPHRENIA AND IN HEALTHY TWINS K.Vladar*, J.J. Kulynych, D. W. Jones, E. F. Torrey and D. R. Weinberger Clinical Brain Disorders Branch, IRP, NIMH, Neuroscience Center at St. Elizabeths, Washington, DC 20032

The superior surface of the temporal lobe consists of language related areas, including the transverse temporal gyrus of Heschl (HG) and the planum temporale (PT). The PT commonly exhibits asymmetry, the left side being larger than the right. Recent MRI studies of the PT reported the loss of this asymmetry in schizophrenia (Rossi et al, 1990). Moreover, Crow proposed that "schizophrenia is a disorder of the genetic mechanism to control the development of cerebral asymmetry"(Crow et al, 1989). We conducted an MRI study of the PT and HG of 8 pairs of right handed monozygotic (MZ) twins discordant for schizophrenia and of 10 healthy right handed MZ twin pairs. Using sagittal volume MRI (1.5 mm contiguous slices) we created 3-dimensional surface renderings of the supratemporal plane. The HG and PT area measurements were made on these renderings. 3-way ANOVA using diagnosis (healthy vs. unaffected vs. affected) and gender (male vs. female) as between group factors and side (right vs. left) as a repeated measure showed no significant main effects of the between factors and a significant diagnosis by gender by side interaction($p< 0.03$). Post hoc tests revealed no significant pairwise comparisons. In a separate analysis of the healthy twin pairs by 2-way ANOVA (gender as between factor and side as repeated measure) a significant gender by side interaction ($p <0.03$) was found. Post hoc comparison demonstrated a trend in the males ($p<0.07$) towards PT asymmetry, the left side being larger than the right, and no such tendency in the females ($p>0.98$). Similar ANOVAs indicated no significant effects of group or side and no interactions in any comparison of the HG areas. Our measurements of the supratemporal structures do not support theories linking schizophrenia to genetically determined asymmetries.

496.5

MAGNETIC FIELDS IN THE CEREBRAL CORTEX ASSOCIATED WITH VISUALLY INITIATED FINGER MOVEMENTS IN HUMAN SUBJECTS. A. Nambu[1]*, R. Matsuzaki[1], H. Gemba[2] and K. Sasaki[1]. [1]Dept. of Integrative Physiology, National Institute for Physiological Sciences, Okazaki 444, Japan and [2]Dept. of Integrative Brain Science, Fac. of Medicine, Kyoto Univ., Kyoto 606, Japan

Magnetic and electric fields were recorded simultaneously over the scalp by using a 37-channel first-order SQUID gradiometer system (BTi, CA) while subjects performed flexion of a unilateral index finger in reaction to visual cue stimuli. Two components of magnetic field change were observed over the contralateral scalp to the index finger. The first component which reversed its polarity between the medial and lateral parts of the scalp preceded the onset of movements. This component reached the peak amplitude of 100-200 fT immediately prior to EMG onset. The second component which reversed its polarity in the direction opposite to the first one followed the onset of movements. The estimated current dipole of the first component was directed anteriorly around the central sulcus. This implies the existence of intracellular current flow directed from the surface to the depth of the anterior bank of the central sulcus. This component appears to represent the activation of the hand area of the motor cortex through the cerebello-thalamo-cortical pathway as reported in monkeys (Sasaki, Gemba and Mizuno, Exp. Brain Res., 1982, 46: 29-36). The estimated current dipole of the second component was in the opposite direction and located slightly posterior and lateral to that of the first component, representing the activation of the somatosensory cortex. When the subjects performed unilateral toe movements in reaction to visual cue stimuli, current dipoles were estimated at the midline, which suggests the activation of the foot areas of the motor and somatosensory cortices respectively.

496.6

A SPATIAL FILTERING MODEL FOR COMPARISON OF THE FORWARD AND INVERSE PROBLEMS FOR THE ELECTRO-ENCEHPALOGRAM AND THE MAGNETOENCEPHALOGRAM. J. P. Wikswo, Jr.*, R. S. Wijesinghe and L. A. Bradshaw. Living State Physics Group, Vanderbilt University, Nashville, TN 37235.

Current sources in the brain produce magnetic and electric fields. Using a volume conductor model based upon a spatial filtering technique, we simulate measurement of the electroencephalogram (EEG) and the magnetoencephalogram (MEG) by calculating the electric and magnetic fields produced by current sources in the cortex. We examine the effects of skull and scalp geometry and conductivity on the EEG and the MEG. Spatial filters allow easy visualization of the effects of volume conduction on the signals. The inverse calculations then give an estimate of the original current source distribution from the EEG and the MEG, and we can consider the effects of noisy signals on current source estimation. By inward-continuing the fields, we can examine the effects of noise, source depth and spatial separation of measurements on imaging and localizing resolution.

We find that the inverse filter functions act as high-pass amplifiers and lead to instabilities at high spatial frequencies. Windowing the functions can reduce the instability, but may also reduce spatial resolution and signal-to-noise ratio. We can easily visualize the sensitivity of the spatial filters to errors in the choice of parameters by comparing them with actual spatial filters.

496.7

NARROW-BAND OSCILLATORY ACTIVITY IN THE HUMAN MOTOR SYSTEM. R. Kristeva, B. Feige, S. Makeig[1] and T. Elbert Inst. Exp. Audiology, Kardinal von Galen Ring 10, 4400-Münster, Germany; 1 - Naval Health Research Center, San Diego, CA 92186, USA; SPON: European Brain and Behaviour Society

Changes in spectral power associated with a motor task requiring a high level of sensorimotor integration (SMI) were investigated by analyzing spontaneous neuromagnetic activity during motor preparation (WAIT), SMI, and control conditions in four healthy, right-handed subjects. In the SMI condition, subjects attempted to rapidly identify four different plastic figures by touch. Periods in which subjects were waiting for instruction to begin SMI (WAIT) were compared with periods of task performance (SMI). As a cross-check, 'eyes closed' and 'eyes open' conditions with no manual task were also recorded.

Neuromagnetic fields were observed over the left sensorimotor cortex using a 37-channel first-order gradiometer. A total of 60 to 100 artefact-free periods (3-10 s lenght) per subject were analyzed in each condition.

Results showed a narrow-band rhythm at about 19 Hz which was prominent during the WAIT state in all four subjects, but was absent during SMI and no-task conditions. This rhythm thus appears to be a "motor preparation rhythm" of the brain.

During SMI only, high-frequency activity at 28-30 Hz occurred in one of the four subjects, suggesting that narrow-band gamma activity may accompany SMI in humans, but is not obligatory for coding of information in the human motor system.

496.8

ANALYSIS OF MOTOR FUNCTION BY MEG,fMRI AND TMS. J.D. Lewine*[1], J.A. Sanders[1], J.S. George[2], R.S. Astur[1] and W.W. Orrison[1]. [1]MSI Facility, VAMC, 2100 Ridgecrest Drive, SE, Albuquerque, NM 87108; [2]Mail Stop M715,Los Alamos National Laboratory, Los Alamos, NM 87545.

The ability to noninvasively map human motor cortex could be of particular utility in preoperative planning of neurosurgical interventions in patients with tumors or vascular malformations in the vicinity of the central sulcus. Several different biomagnetic techniques offer promise in this regard. These techniques include magnetoencephalography (MEG) which is capable of measuring the weak neuromagnetic signals generated by current flow within pyramidal cells of motor cortex; functional magnetic resonance imaging (fMRI) which is capable of measuring activity-induced changes in the hemodynamics and blood oxygenation of motor cortex; and transcranial magnetic stimulation (TMS) which is capable of activating motor cortex via the application of a strong magnetic pulse which generates eddy currents within the brain. Because each technique is based upon a different physiological mechanism, their combined use provides a richer picture of cortical organization than is attainable when each is used in isolation. The cortical substrates of hand movements were examined in one subject using each technique. As expected, dipole sources for the motor evoked magnetic field localized to the pre-central gyrus as identified by structural magnetic resonance imaging methods. Movement induced changes in fMRI hemodynamics which co-localized with these dipole sources to within 1 cm. Movement of the hand was evoked most effectively by placing the center of a butterfly TMS coil at a position where the line passing through the center of the head and the center of the coil passed within 1 cm of the regions identified by MEG and fMRI. The techniques appear to cross-validate each other with respect to localization abilities.

496.9

REGIONAL CEREBRAL BLOOD FLOW CHANGES DURING CONDITIONAL MOTOR LEARNING IN THE HUMAN MOTOR CORTEX. A.R. Mitz*, S.P. Wise and T.A. Zeffiro. Lab. Neurophysiol., NIMH, Poolesville, MD and Medical Neurology Branch, NINDS, NIH, Bethesda, MD.

Neurons in the dorsal premotor area (PMd) of monkeys significantly change their task-related activity during conditional motor learning (Mitz et al. J. Neurosci. 11:1855;1991). In this study, we measured regional cerebral blood flow (rCBF) with positron emission tomography while 8 normal human subjects learned a conditional motor task. We used bolus injections of H_2O^{15}, and each subject was scanned in 3 task conditions: (1) a movement control task, (2) a conditional motor task (CMT), and (3) a sensory control task. In task 1, the subjects moved a joystick in 1 of 4 directions as instructed by an arrow, which pointed in the correct movement direction. In task 2, the CMT, the subjects learned by trial and error which 1 of the same 4 movements was instructed by a colored geometric form (e.g., a red circle). Feedback was provided at the end of each trial: a green spot indicated correct performance. In task 3, the subjects were instructed simply to view the same stimuli. Scans were obtained every 10 min for a total of 40 min during the CMT. All subjects performed at >75% correct by the last CMT scan, but averaged only ~25% correct (chance level) during the first scan. After stereotaxic normalization, statistical parametric maps were generated by making planned comparisons of task and control conditions. Whereas movement-related rCBF increases (task 1 vs. 3 and task 2 vs. 3) were seen in the primary motor cortex (M1) and anterior cerebellum, the strongest learning-related increases (task 2 vs. 1) were observed in PMd and in one or more cingulate motor areas (CMA) ventral to the supplementary motor cortex. In both PMd and CMA, these learning-related rCBF increases were inversely correlated with task performance. These results further support PMd's role in the mediation of conditional motor behavior and suggest an important role for CMA, as well.

496.10

ACTIVATION OF CORTICAL MOTOR AREAS: AN INDIVIDUAL PET ANALYSIS. Uwe Knorr, Gottfried Schlaug, Harald Hefter*, Yanxiong Huang, Hans Herzog, Helmuth Steinmetz, Rüdiger J. Seitz. Dept. of Neurology, University of Düsseldorf and Institute of Medicine, Jülich, Germany.

The inter-individual variability of cerebral anatomy impairs the capability of identification and localization of regional cerebral blood flow (rCBF) changes in activation studies with positron emission tomography (PET). Additionally, the individual task performance will influence the rCBF changes also. To estimate those two aspects of anatomo-functional variability we studied simple and complex finger movements. In 12 blindfolded, right-handed subjects the rCBF was measured with [^{15}O-butanol] and the SCX PC4095/15WB camera during the execution of simple and complex finger, and right hand movements in comparison to a control condition. The movements were paced at low and high rates (between 0.8 and 3.0 Hz) internally, as well as externally. $PaCO_2$ corrected values of the rCBF were used. After spatial alignment and integrated display of PET and high resolution magnetic resonance (MR) images, task specific rCBF increases were identified using the individual response identification algorithm. Results were compared to inter-subject averaged images using pixel-by-pixel t-map analysis. At high movement rates task-specific rCBF increases were consistently observed in the contralateral primary sensorimotor cortex. Activations in premotor, supplementary motor areas and subcortical regions had an individual distribution reflecting the inter-individual anatomo-functional variability in task performance. The individual activation patterns are strong evidence for functional heterogeneity among different subjects.

496.11

EEG-BASED BRAIN-COMPUTER INTERFACE: ON-LINE METHODS FOR IMPROVING FREQUENCY RESOLUTION. D.J. McFarland*, L. McCane, T. Lefkowicz, and J.R. Wolpaw. Wadsworth Labs, NYSDOH/SUNY, Albany, NY 12201.

Individuals can learn to control the amplitude of 8-12 Hz mu rhythm activity in the EEG recorded over sensorimotor cortex and use it to move a cursor to a target on a video screen (Electroenceph clin Neurophysiol 78:252-259, 1991). Up to the present, mu rhythm amplitude has been assessed by Fourier analysis of successive time segments, which necessitates a trade-off between resolution in frequency and resolution in time.

In order to improve accuracy and rapidity of cursor control, we are exploring methods for improving frequency resolution while maintaining a close temporal relationship between EEG activity and cursor movement. Better frequency resolution is needed to distinguish between mu rhythm components, and good time resolution is needed to minimize delay between mu rhythm amplitude and cursor movement.

One method for improving frequency resolution without corresponding increase in delay is to apply Fourier analysis to overlapping, rather than successive, time segments. To date, we have implemented this method in 6 subjects. For example, analysis of half-second segments at 8 Hz provides 2 Hz frequency resolution and 8 cursor movements/sec. We are also implementing an autoregressive frequency analysis algorithm. In combination with greater topographic resolution, these methods should support improved two-dimensional cursor control.

(Supported by NIH HD30146.)

497.1

ANTEROGRADE TRANSNEURONAL SPREAD OF HERPES SIMPLEX VIRUS FROM THE MURINE TOOTH PULP TO TWO CORTICAL AREAS ASSOCIATED WITH JAW MOVEMENT. E. M. Barnett*[1], G. D. Evans[2], M. D. Cassell[1], and S. Perlman[1]. [1]Neuroscience Program, [2]College of Dentistry, University of Iowa College of Medicine, Iowa City, IA 52242.

Transneuronal spread of herpes simplex virus type 1 (HSV-1) into and throughout the brain was examined following inoculation into the murine tooth pulp. Openings were made into the pulps of left mandibular incisors of Balb/c mice. The pulp was partially removed, approximately $3x10^5$ PFU of HSV-1 strain H129 was placed into the opening using a micropipette, and the tooth was sealed. Animals were sacrificed 5-9 days post-inoculation (p.i.) and *in situ* hybridization for HSV-1 nucleic acid was performed using a [^{35}S]-labeled anti-sense RNA probe. The H129 strain of HSV-1 has previously been reported to spread anterogradely.

Within several days p.i. ipsilateral perioral, lingual, and eye lesions were noted, suggesting spread within the semilunar ganglion following infection of the inferior alveolar nerve. Virus first appeared at 4-5 days p.i. in the dorsomedial spinal nucleus of the trigeminal nerve, and the lateral nucleus of the solitary tract, consistent with entry via the mandibular division only. Over the next several days virus spread to infect the subcortical efferents of these two structures, such as the ventral posterior medial thalamus, lateral parabrachial nucleus, paraventricular hypothalamus, and central extended amygdala. As early as 6 days p.i., virus was detected in laminae II/III and V of ipsilateral agranular insular cortex and lamina IV of contralateral cortex corresponding to the primary jaw motor area. These two cortical areas have been shown to produce rhythmical jaw movements upon electrical stimulation. The pattern of spread was generally consistent with anterograde spread of virus. Thus, the pathways defined by viral spread are consistent with sensory input from the trigeminal mandibular division into motor areas controlling jaw movement.

497.2

ORIGIN, SOMATOTOPY AND DENSITY OF THE CORTICOSPINAL PROJECTIONS FROM THE MOTOR AREAS OF THE MESIAL WALL OF THE HEMISPHERE IN THE MACAQUE MONKEY.

M. Matelli , G. Luppino , R. Camarda , G.P. Basso and G. Rizzolatti *(SPON: European Brain and Behavior Society) Istituto di Fisiologia Umana Universita' di Parma Via Gramsci 14 I-43100 Parma Italy.

Recent microstimulation experiments showed that on the mesial cortical surface of the macaque monkey there are four nonprimary motor areas (Luppino et al. J. Comp. Neurol. 311:463-482,1991). Two of them occupy the mesial sector of area 6: F3 (SMA-proper) and F6 (pre-SMA). The other two areas are located in the dorsal part of cingulate area 24: area 24d and area 24c.

In the present experiments we matched the site of origin, the somatotopy and the density of the corticospinal (CS) projections from the mesial wall of the hemisphere with the location and the somatotopy of the four above mentioned motor areas. Neural tracers (HRP and DY) were injected at various levels in the spinal cord of two macaque monkeys and their retrograde transport plotted. The results showed that all the mesial motor areas have CS projections, although with marked differences in their somatotopy and density. The two caudal areas, F3 and 24d, have segregated projections to both cervical and lumbar levels. In F3 the density of CS neurons is slighly less than that of F1 (area 4) (243 vs. 273 cells/sq. mm) and higher than that of area 24d (223 cells/sq. mm). The two rostral areas, F6 and 24c, project mainly to the cervical level. The CS projections from F6 are scanty and limited to a small sector of it. In the agranular cingulate motor areas 24d and 24c the density of CS neurons is similar and markedly differs from that of the unexcitable granular area 23c (123 cells/sq. mm). These data are in good agreement with the parcellation of mesial agranular frontal and cingulate areas proposed in our previous electrophysiological studies.

(Supported by a Grant from Human Frontier Science Program)

497.3

SUPPLEMENTARY MOTOR AREA (SMA) INPUT TO THE CERVICAL CORD. R.P. Dum* and P.L. Strick. VA Research Service, VAMC and Depts. Neurosurgery & Physiology, SUNY Health Science Ctr., Syracuse, NY 13210.

In prior studies we demonstrated that a substantial number of corticospinal neurons originate in the SMA. In this experiment, we used anterograde transport of WGA-HRP in macaques to examine the pattern of terminations of efferents from the 'arm' area of the SMA within the cervical spinal cord. For comparison, we also examined the pattern of terminations from the primary motor cortex (M1). SMA efferents terminated densely in 4 regions of the gray matter of the cervical cord- a lateral portion of the intermediate zone (laminae V-VII), a medial portion of the intermediate zone (laminae VI-VII at the base of the dorsal columns), a ventromedial portion of the intermediate zone (laminae VII-VIII) and *a dorsolateral part of the motor nuclei (lamina IX).* Terminations in motor nuclei were primarily found in regions of lower cervical segments (C7-T1) known to contain motoneurons that innervate muscles of the fingers and wrist. M1 efferents terminated densely in the same regions of the cervical cord as efferents from the SMA. The terminations of M1 generally were more dense and more extensive than those from the SMA. M1 terminations extended further into the base of the dorsal horn (lamina V) and included a larger portion of lamina IX. In summary, terminations from the SMA and M1 are focused on largely the same regions of the cervical cord. Each cortical area has terminations that appear to directly innervate motoneurons, particularly those innervating hand muscles. These results further support the concept that corticospinal efferents from the SMA and M1 represent parallel channels for motor output. Contrary to some previous hypotheses, the SMA has the potential to directly control hand movements independent of M1. Support: VA Rehab. R&D and USPHS 24328 (PLS).

497.4

PREFRONTOPONTINE PROJECTIONS IN THE RHESUS MONKEY. Jeremy D. Schmahmann*, and Deepak N. Pandya Massachusetts General Hospital, Boston, MA, and E.N.R.M. Veterans Hospital, Bedford, MA, 01730.

The prefrontal connections to the basis pontis have been incompletely studied and a detailed understanding of their organization is not available. We injected tritiated amino acids into subregions of the lateral, medial, and ventral prefrontal cortices in 18 Rhesus monkeys and studied the projections to the pons using the technique of autoradiography. The dorsal lateral convexity and the medial prefrontal cortex provided the majority of the pontine efferents, whereas the ventral lateral convexity and the orbitofrontal cortex provided sparse, or no, pontine terminations. Projections from the lateral prefrontal convexity were derived from dorsal area 46, areas 8B, 9, and 10; and those from the medial prefrontal cortex originated from areas 9 and 32. A minor projection was noted from ventral area 46. Our studies to date have not revealed pontine projections from areas 11, 12, and 14 in the orbitofrontal region. The projections from areas 8B, dorsal area 46, and area 9 were distributed throughout the rostrocaudal extent of the pons. The projections from area 10, medial area 9, area 32, and ventral area 46 occupied the rostral one-third to one-half of the pons. The distribution of terminal label favored the medial, paramedian, and dorsomedial pontine nuclei, with some label present also in the nucleus reticularis tegmenti pontis, and medial aspects of the ventral and peduncular nuclei. More medial prefrontal cases resulted in label at the most medial pontine regions, whereas following lateral prefrontal injections the pontine terminations shifted away from the midline. The existence and organization of these differential prefrontopontine projections, including those from area 10 at the frontal pole, are consistent with the notion that the cerebellum has access to higher order information from the cerebral hemispheres (Schmahmann and Pandya, '87; Leiner et al., '88; Schmahmann, '91). *(Supported in part by NIH grant 16841 and the ENRM VA Hospital, Bedford, MA 01730).*

497.5

DIFFERENTIAL SPINAL PROJECTIONS OF SUBREGIONS WITHIN THE FORELIMB AREA OF CAT MOTOR CORTEX. J.H. Martin*, C.Ghez. Columbia Univ., Ctr for Neurobiology and Behavior, NY,NY 10032

Two subregions have been identified within the forelimb area of the cat motor cortex that are located rostral and caudal to the cruciate sulcus. Reversible inactivation of portions of the subregions in which the distal joints are represented produces different effects on performance of a prehension task (Exp Brain Res 1993). Within the rostral subregion, inactivation produces both deficits in the control of locally represented joints as well as more generalized deficits in aiming and adapting trajectories. Inactivation of the adjacent caudal subregion does not impair prehension *per se.* We now examine if these subregions have differential projections to the contralateral cervical spinal gray matter using anterograde transport of WGA-HRP.

Consistent with their common somatotopy, both subregions projected more densely to the cervical enlargement than to the upper cervical segments. However, there were striking differences in the dorsoventral pattern of projections of the two subregions to the spinal gray matter. Injections in the rostral subregion produced dense labeling of terminals in the intermediate zone. This was the case both at upper cervical segments, where the C3-C4 propriospinal neurons are located, and in the cervical enlargements, where other populations of premotor neurons are found. Injections in the caudal subregion preferentially labeled the deeper laminae of the dorsal horn, where mechanoreceptive afferent fiber terminals and ascending projection neurons are located.

The present findings suggest that the functions of the rostral subregion are expressed through projections to premotor neurons at both upper and lower cervical levels. The projection of the caudal subregion to portions of the cord that process somatic sensory inputs suggests that it may play a role in motor tasks in which such inputs are critical.

497.6

PALLIDOTHALAMOCORTICAL PATHWAY IN THE RAT: A LIGHT & ELECTRON MICROSCOPIC ANALYSIS. S.T. Sakai* & K. Bruce. Dept. of Anatomy, Michigan State University, East Lansing, MI 48824.

Pallidothalamic input to the supplementary motor area is thought to provide an important link in motor control pathways in primates. Our purpose was to determine if the medial agranular cortex (AGm) which is thought to contain the supplementary motor area in the rat also receives the pallidothalamocortical pathway. A double-labeling paradigm was employed whereby pressure injections of the retrograde tracer, Cholera toxin subunit B (CTB) were made into AGm combined with iontophoretic injections of the anterograde tracer, *Phaseolus vulgaris leucoagglutinin* (PHA-L) made into the entopeduncular nucleus (EP)(homologue of the primate globus pallidus). Vibratome sections were reacted using a sequential immunohistochemical protocol. The greatest coincidence of both CTB labeled cells and PHA-L labeled pallidothalamic fibers was observed in the anterolateral portion of the ventral anterolateral nucleus (VAL). Both labels were also observed in the ventral medial nucleus (VM). Electron microscopic analysis of serial sections of VAL revealed numerous PHA-L labeled terminals as well as CTB retrogradely labeled cell bodies and large dendrites. The PHA-L labeled boutons were generally large with numerous mitochondria and attached to the postsynaptic elements by synaptic junctions as well as several puncta adhaerentia. Synapses were observed onto CTB labeled somata as well as onto labeled and unlabeled dendrites. These results demonstrate for the first time the pallidothalamocortical pathway by way of VAL to the AGm in the rat. This pathway may provide an anatomical substrate for the motor control functions ascribed to the AGm in the rat. (Supported by the College of Human Medicine BRSG).

497.7

THALAMIC CONNECTIONS OF THE DORSAL AND VENTRAL PREMOTOR AREAS IN OWL MONKEYS. I. Stepniewska*, T.M. Preuss, and J.H. Kaas. Dept. of Psychology, Vanderbilt University, Nashville, TN 37240.

In owl monkeys, as in macaques, the lateral premotor cortex includes two separate dorsal (PMD) and ventral (PMV) areas (Stepniewska et al. 1993, *J. Comp. Neurol.*, 330:238-271). In this study, we investigated the thalamic projections to PMD and PMV, with injections of fluorescent dyes (Diamidino Yellow, Fluoro-Ruby, Fast Blue) and WGA-HRP. Microstimulation was used to localize injection sites in the forelimb representations of PMD and PMV in 6 adult owl monkeys (*Aotus trivirgatus*). Perfused brains were sectioned in the coronal plane, and alternate series of sections were stained for Nissl, acetylcholinesterase, and cytochrome oxidase to locate the borders of thalamic nuclei.

Both PMD and PMV are strongly interconnected with the nuclei of the ventrolateral complex and with the mediodorsal (MD) and intralaminar nuclei; the major connections of both areas were similar. Labeling in the ventrolateral complex was concentrated in the medial (VLx) and anterior (VLa) subnuclei, with only a few cells labeled in the dorsal (VLd) and posterior (VLp) subnuclei. Labeling in MD was concentrated in the most lateral and posterior parts of the nucleus. Within the VL complex and MD, labeling after PMV injections was concentrated ventrally, while PMD injections labeled more dorsal territories. Other connections of premotor cortex were with the ventral anterior (VA) and ventromedial (VM) nuclei. Our results suggest that PMD and PMV both receive inputs from the cerebellum (e.g., through VLx) and basal ganglia (e.g., through VLa). [Supported by NS 164445 and JSMF 90-35]

497.8

IPSILATERAL CORTICAL CONNECTIONS OF THE DORSAL AND VENTRAL PREMOTOR AREAS IN OWL MONKEYS. T.M. Preuss*, I. Stepniewska, and J.H. Kaas. Dept. of Psychology, Vanderbilt Univ., Nashville, TN 37240.

In owl monkeys (*Aotus trivirgatus*), we have identified separate dorsal and ventral premotor areas (PMD, PMV) based on responsiveness to microstimulation and architectonics (Stepniewska et al. 1993, *J. Comp. Neurol.*, 330:238-271). In the present study, we made tracer injections in the forelimb representations of PMD and PMV, identified using microstimulation. Injections were made in 6 animals (3 for PMD, 2 for PMV, and 1 with separate injections of both PMD and PMV), using Diamidino Yellow, Fast Blue, Fluoro-Ruby, and WGA-HRP. After sacrifice, the distribution of labeling (primarily retrograde) was examined in sections cut coronally or flattened and cut tangential to the cortical surface.

PMD and PMV receive projections from multiple somatic sensory and motor areas; some areas project predominantly either to PMD or PMV, and some project to both. PMD and PMV differ principally in their somatosensory connections, PMD receiving input mainly from the parietal convexity (presumptive area 5), whereas PMV receives projections from multiple areas along the frontoparietal operculum, including S2, the parietal ventral area (PV), and the "parietal rostral" area (PR). Areas which project to both PMD and PMV include the primary motor area (M1); dorsal and ventral segments of the supplementary motor area (SMAd, SMAv); cingulate cortex; a region of posterior parietal cortex that is probably homologous to macaque area 7b; and a region in the lateral sulcus caudal to S2. [Supported by NS 164445 and JSMF 90-35].

497.9

CORTICO-CORTICAL PROJECTIONS BETWEEN PRIMARY AND SECONDARY SOMATOSENSORY AREAS OF RATS: BACKWARD PROJECTIONS LABEL LAYER I MOST HEAVILY. Barbara Clancy and Larry J. Cauller*, GR41, Cognition and Neuroscience Program, University of Texas at Dallas, Richardson, TX 75083-0688.

Projections between somatosensory areas of rats were labeled by multiple injections of fluorescent retrograde (Fast Blue or Diamidino Yellow) and anterograde tracers (rhodamine-labeled Dextran 10kMW, lysine-fixable; Molecular Probes). Overlapping or contralateral injections of these retrograde AND anterograde tracers (R&A) were employed to demonstrate the reciprocity of these connections and to analyze the topographical relationships between these reciprocally interconnected areas. All tracers were injected by air pressure (General Valves). Small R&A injections (<0.5 mm diameter centered in layer IV) into the dorsal vibrissal somatosensory area (SI) retrogradely labeled cells in all layers at two sites in the lateral parietal area, including SII (Par II) and the lateral agranular insular cortex. In both of these retrogradely labeled areas, anterogradely labeled fibers from SI were found which terminated throughout all layers in register with the retrogradely labeled cells. Similar R&A injections into the lateral parietal area (SII) retrogradely labeled cells in layers III and V of SI. Anterogradely labeled "backward" fibers from SII traveled medially through layer VI to the site in SI where retrogradely labeled cells were found. These backward fibers terminated most heavily in layer I, less heavily in layer II, with thick terminal branches in layers III and V, and only thick fibers passing through layer IV. Many of the backward fibers that reached layer I extended horizontally within layer I for at least 1 mm. We conclude that reciprocal projections interconnect common neuronal populations and the backward limb extends horizontally to spread this process to adjacent populations in surrounding representations of SI.

497.10

GRAY MATTER TRAJECTORY OF CORTICOCORTICAL AXONS IN RATS: PATHWAYS FOR DIRECTED ATTENTION R.L. Reep*, I. L. Vandevelde and J.V. Corwin, Department of Physiological Sciences, University of Florida, Gainesville, FL 32610; and Dept. of Psychology, Northern Illinois University.

In rats, the medial agranular (AGm), ventrolateral orbital (VLO) and posterior parietal (PPC) cortical areas each play a role in directed attention and spatial orientation, and are highly interconnected. In anterograde tracing studies using Fluoro-Ruby, we have discovered that a large proportion of the axons linking these areas travel in the deep gray matter rather than in the underlying white matter. These axons are oriented rostrocaudally, and travel predominantly in layer VI, extending rostrally and caudally from the injection site to their cortical targets, where they ramify and terminate in more superficial layers. Axons directed subcortically are grouped as a compact bundle in the deep white matter which can be followed to terminal fields in the striatum, thalamus and brainstem.

In preliminary behavioral studies, we have found deficits in directed attention after knife cuts which interrupt these axons without causing appreciable cortical damage.

These findings suggest the hypothesis that directed attention behavior in rats may be mediated in large measure by corticocortical connections among AGm, VLO and PPC.

497.11

DIFFERENCES IN THE IPSILATERAL CORTICAL INPUTS TO SUPERIOR AND INFERIOR REGIONS OF THE POSTARCUATE PREMOTOR AREA: A QUANTITATIVE STUDY OF RETROGRADELY LABELED NEURONS IN MACACA FASCICULARIS. S. Ghosh*. Dept. of Physiology & Pharmacology, University of Queensland, St. Lucia, Qld 4072, Australia

In order to investigate cortical inputs to the Postarcuate Premotor Area (PMA) Wheatgerm Agglutinin conjugated with Horseradish Peroxidase (WGA-HRP) was injected into the forelimb region of PMA (identified by cortical stimulation) in 2 adult macaques: 0.3 μL of a 5% solution behind the superior limb of the arcuate sulcus (AS) in one animal (M2) and 0.3 μL of a 10% solution behind the inferior limb of the arcuate sulcus (AI) in the other animal (M3). Every fourth serial section (50 μm) was stained by Tetramethyl Benzidine and all labeled cells counted. Nearly 30,000 neurons were counted in the ipsilateral cortex of M2 and 75,000 neurons counted similarly in M3. The study reveals profuse connections within the posterior bank and posterior lip of AS or AI, but very few connections between AS and AI. Both areas are well connected with area 4 and areas 5 and 7. However, projections from SMA and SII are numerically greater to the inferior than to the superior part of PMA.

497.12

IDENTIFICATION OF A TRANSITIONAL (CINGULATE) FIELD IN THE CAUDAL PART OF THE HUMAN MEDIAL FRONTAL LOBE: A CYTOARCHITECTONIC STUDY. R. Morris*, T. Paus, M. Petrides and D.N. Pandya. Montreal Neurological Institute, McGill University, Montreal, Que, Canada and Edith Nourse Rogers Memorial Veterans Hospital, Bedford, MA, USA.

The architecture of the caudal portion of the medial surface of the frontal lobe was reexamined to establish whether there is a transitional field between the proisocortex of the cingulate gyrus and the dorsally located isocortical areas 4 and 6. Proceeding in a ventrodorsal direction, we observed gradual but stepwise changes in cellular organization characterized by an increase in overall cortical thickness and in the supragranular layers relative to the infragranular layers. A transitional field, sharing features of both the proisocortex below it and the isocortex above it was observed. Along the rostrocaudal axis, this field represents a caudal extension of the classically defined area 32. The cytoarchitecture of this field resemble the cortex found in the fundus of the monkey cingulate sulcus at corresponding rostrocaudal levels. Its position is consistent with the location of rCBF changes related to the control of manual responses obtained in our previous PET study (Paus et al. J.Neurophysiol., 70 (2), 1993).

497.13

A CYTOARCHITECTONIC INVESTIGATION OF THE HUMAN PREFRONTAL CORTEX. Chiavaras M, Petrides M, Pandya DN.* Edith Nourse Rogers Memorial Veterans Hospital, Bedford, MA, USA and Montreal Neurological Institute, McGill University, Montreal, Canada.

A cytoarchitectonic analysis of the human prefrontal cortex shows that its architectonic areas can be arranged into four major groups according to their laminar features. The **first** group consists of areas 24, 25, 32 and the caudal orbitofrontal region and is characterized by the absence of a granular layer IV, a cell sparse layer III, and prominent infragranular layers V and VI. The **second** group includes medial areas 9 and 8B and orbital areas 13, 11, and 14 (Brodmann's areas 11 and 12). These areas have an incipient layer IV, medium pyramidal cells in layer III, and decreased infragranular cell density. The **third** group is comprised of areas 10, 46, and 47/12 and is characterized by a better developed layer IV, increased size of layer III pyramidal cells, and equal cell density in both the infra- and supragranular layers. The **last** group consists of lateral areas 9, 9/46, 8A, and 45. These areas have a densely packed layer IV, large layer III pyramidal cells, and prominent supragranular layers.

According to progressive laminar differentiation, these prefrontal cortical groups can be arranged into two parallel trends. The *mediodorsal* trend begins in areas 24, 32, and 25, extends to medial areas 8B, 9, and dorsal area 10, passes to dorsolateral areas 10 and 46 and finally to dorsal areas 9, 9/46 and 8A. The *basoventral* trend extends from orbital proisocortex to areas 13, 14, and 11, progresses through areas 47/12, ventral areas 10 and 46 and finally reaches area 45 and ventral areas 9/46 and 8A.

The organization of cortical areas into two trends with sequential laminar differentiation may provide a framework for better understanding clinical disorders and frontal lobe functioning. *(Supported by NIH grant 16841)*

497.14

SOMATOSENSORY AND MOTOR REPRESENTATIONS IN THE GRAY SHORT-TAILED OPOSSUM (*Monodelphis domestica*) R.J. Nudo*, S.B. Frost, G.W. Milliken and R.B. Masterton. Department of Neurobiology and Anatomy, University of Texas Medical School, Houston, Texas, 77030 and Department of Psychology, Florida State University, Tallahassee, Florida 32304

Previous studies suggest that somatosensory and motor representations in neocortex of neurologically primitive mammals are completely overlapping in a sensorimotor amalgam (Lende, '63). To examine this question using contemporary techniques, we used multi-unit recording and intracortical microstimulation (ICMS) to examine sensory and motor representations in the gray short-tailed opossum. In ketamine-anesthetized animals, sensory receptive fields were derived by determining the skin surfaces over which cortical neurons were driven by cutaneous stimulation. Motor fields were derived using ICMS techniques to determine the movement(s) evoked by minimum current intensities ($\leq$ 100μA). Responsive sites typically evoked movement at <30μA.

The somatosensory map revealed a complete representation of the body surface, dominated by a large representation of the snout. In contrast, the motor representation was confined entirely to the face region, primarily the vibrissae, and overlapped the somatosensory face representation. Although extensively and repeatedly explored, movements could *NOT* be evoked in musculature caudal to the face.

These results confirm Lende's earlier reports of a sensorimotor amalgam in Marsupials. Further, if the motor cortex of *Monodelphis domestica* is representative of a primitive condition, these results suggest that motor cortex initially emerged to control muscles of the face. Supported by NIH NS27974 (RJN) and NS07726 (RBM).

497.15

CEREBELLAR AND PALLIDAL INPUTS, VIA THE THALAMUS, TO THE PRIMARY (M1) AND SUPPLEMENTARY (SMA) MOTOR CORTICAL AREAS: EVIDENCE FROM MULTIPLE NEUROANATOMICAL TRACING IN MONKEYS. E.M. Rouiller*, F. Liang, A. Babalian, V. Moret and M. Wiesendanger. Institute of Physiology, University of Fribourg, CH-1700 Fribourg, Switzerland.

In order to clarify whether or not the primary motor cortex (M1) and the supplementary motor cortex (SMA) are each addressed, via the motor thalamus, by cerebellar and basal ganglia outflows, the goal of the present study was to compare directly, in the same animal, the thalamic territories that: a) project to M1 and SMA, b) receive cerebellar-nuclear (CN) and pallidal (GP) afferents. The 4 territories were mapped in 3 monkeys by means of 2 retrograde tracers for M1 and SMA injections and of 2 anterograde tracers for CN and GP injections. All injections were conducted under electrophysiological control. Injections in M1 and SMA-proper were restricted to the hand/arm representation. The anterogradely and retrogradely labeled thalamic territories all formed a number of highly complex patches that were usually not confined to one cytoarchitectonically defined thalamic nucleus. The overlap of clusters of labeled terminals and perikarya was evaluated morphometrically (area measurements) at regular intervals along the anteroposterior extent of the motor thalamus. Confirming previous studies, the thalamic territories fed by CN and GP afferents rarely overlapped. The new finding is, however, that the territories projecting to M1 and to SMA included thalamic islands receiving CN- as well as GP-projections. These data support the previous conclusion (based on transsynaptic labeling) that SMA receives not only pallidal but also transthalamic CN inputs (Wiesendanger and Wiesendanger, 1985, Exp. Brain Res. 59, 105-117). Reciprocally, M1 receives not only cerebellar but also pallidal transthalamic inputs.

497.16

DISTRIBUTION OF THALAMIC CELLS PROJECTING TO THE PRIMARY MOTOR CORTEX AND THE SUPPLEMENTARY MOTOR AREA. K. Shindo, K. Shima and J. Tanji* Dept. of Physiology, Tohoku Univ. Sch. of Med., Sendai, 980, Japan

The location and spatial distribution of thalamic cells projecting to the distal (Th-DIST) and proximal (Th-PROX) forelimb areas of the primary motor cortex and the forelimb area of the supplementary motor area (Th-SMA) were investigated by a multiple retrograde labeling technique using five Japanese monkeys (*Macaca fuscata*). The distal (MI-DIST) and proximal (MI-PROX) forelimb areas of the primary motor cortex were identified by effects of intracortical microstimulation and somatosensory responses. Retrograde fluorescent tracers (diamidino yellow and fast blue) were injected into the centers of each area. Two weeks later, the forelimb area of the supplementary motor area (SMA) was identified physiologically. Horseradish peroxidase conjugated with wheat germ agglutinin was injected into the center of the SMA. Labeled thalamic cells distributed basically in a topographic manner: Th-SMA cells medially; Th-DIST cells centrally; and Th-PROX cells laterally. In nucleus ventralis anterior (VA) and the anterior part of nucleus ventralis lateralis pars oralis (VLo), only Th-SMA cells were found. Posteriorly, the distribution of Th-SMA cells overlapped with that of Th-DIST cells in the dorsal part of the VLo, while, in the ventral part, they were separated from each other. The degree of the overlap increased anteriorly and decreased posteriorly. Only a small percentage of cells projecting to both the SMA and MI-DIST (double labeled cells) was seen. The distribution of Th-PROX cells were separated from others. In nucleus ventralis lateralis pars caudalis (VLc), the overlap of each cell group was small. In nucleus ventralis posterior lateralis pars oralis (VPLo), both Th-DIST and Th-PROX cells, but no Th-SMA cells, were labeled. In nucleus centralis lateralis (CL), cells with three different labels were intermixed.

497.17

CYTO- AND MYELOARCHITECTURE AND TRANSMITTER RECEPTOR DISTRIBUTION IN HUMAN SENSORY-MOTOR CORTEX. K. Zilles*, S. Geyer, K. Amunts, H.-J. Bidmon and A. Schleicher. C. & O. Vogt-Brain Research Institute, H. Heine Univ., D-4000 Düsseldorf, FRG.

Varying numbers of areas and positions of the areal borders in the human motor and somatosensory cortex have been reported. Since MR and PET need precise information about the extent and interindividual variability of the motor and somatosensory areas, these aspects were studied with quantitative cyto- and myeloarchitectonic methods and with quantitative receptor autoradiography.

Nissl- and myelin-stained serial sections through the region of the central sulcus of human brains with no history of neurological diseases were used. M1, M2, $GABA_A$, $5\text{-}HT_1$, $5\text{-}HT_2$, α_1 and L-glutamate receptors were demonstrated at regional and laminar levels. Cyto- and myeloarchitectonic studies were performed in adjacent sections.

The primary motor cortex (Area 4 of Brodmann) can be subdivided into two areas on the basis of myeloarchitecture and receptor distribution. Area 4 can be reliably delineated from Area 6 (premotor cortex) on the basis of receptor autoradiography. The border between both areas is restricted to the precentral gyrus on the dorsolateral surface of the hemisphere. The somatosensory cortex shows a receptor pattern completely different from that of the motor cortex, and can be subdivided into different areas with architectonic and receptor techniques. The results were transferred to 3D-reconstructions of the human brain in order to demonstrate the interindividual variability of cortical areas in the region of the centra sulcus. Supported by a grant of the DFG (SFB 194/A6)

497.18

APICAL DENDRITES OF SAME PROJECTION CELL TYPES MAY ASSOCIATE TO FORM CLUSTERS. D. Lev, D. Czeiger, G. Benshalom* and E.L. White, Center for Brain Research, Faculty of Health Sci., Ben-Gurion Univ., Beer Sheva, ISRAEL.

Based on analyses of the 3-dimensional organization of the primary visual areas in the monkey (Peters and Sethares, 1991a and b), cat (Peters and Yilmaz, 1992) and rat (Peters and Kara, 1987), it has been proposed that neurons in these cortices are organized into modules that center on clusters of apical dendrites belonging to layer V pyramidal neurons. By examining MAP-2 reacted sections with the light microscope, we observed that mouse primary motor cortex (MsI) is also composed of pyramidal cell modules and that the organization of neurons in these modules is similar to that in visual cortex. Here we report on efforts to determine if dendritic clusters are composed of apical dendrites belonging to one or to several types of projection cell. Callosal neurons in MsI cortex were labeled by the retrograde transport of horseradish peroxidase that had been deposited as a 40 % aqueous solution onto callosal fibers in the contralateral hemisphere. Examination of tangential thin sections through layer IV of MsI cortex shows clusters of apical dendrites in which every dendrite is labeled with HRP. Co-existent with these clusters are clusters composed of unlabeled dendrites, suggesting that the apical dendrites of callosal neurons aggregate to form clusters that are composed exclusively of this type of projection cell. These findings suggest a hitherto unsuspected degree of specificity in the cellular composition of cortical modules, at least for MsI cortex. Supported by Binational Science Foundation 890005 and NIH 20149 to E.L.W.

498.1

D1 DOPAMINE ANTAGONIST SUPPRESSES NEURONAL ACTIVITY RELATED TO REACHING MOVEMENTS IN THE PREMOTOR CORTEX OF MONKEYS. T. Sawaguchi*, I. Yamane, and K. Kubota. Dept. of Behavioral and Brain Sciences, Primate Res. Inst., Kyoto Univ., Inuyama, Aichi 484, Japan.

To examine the roles of dopamine receptors in the premotor cortex, dopamine antagonists (SCH23390 for D1 receptors and sulpiride for D2 receptors) were applied iontophoretically to neurons of the premotor cortices (dorsal PM and ventral PM) of monkeys that performed a visual reaching task with a delay. The task was initiated when the monkeys pressed a central hold lever. This was followed by waiting (1 s), warning (a green lamp, 0.5 s), cue (a green lamp in either a left, upper, or right location, 0.5 s), delay (4 s), and go (<1.2 s) periods. During the go period, after the monkeys received a visual go signal (a red lamp), they released the lever, reached, and pressed a lever at the target location that had been indicated prior to the delay period. Iontophoretically-applied SCH23390 (usually applied with a current of 50nA) reduced the activity of most task-related neurons (72/108). By contrast, neither sulpiride nor an inactive analogue of SCH23390, SCH23388, had a significant effect (3/59 for sulpiride, 2/28 for SCH23388). Since it has been suggested that SCH23390 also binds 5HT-2 receptors, a selective 5HT-2 antagonist, ketanserin, was tested. This drug also had little effect on task-related activity (9/36). The effects of SCH23390 varied with the type of the task-related activity: i.e., while neurons which showed a peak activity during the go period were frequently suppressed by SCH23390 (64/79, 81%), those which showed a peak activity during the warning or cue periods were less frequently affected (5/22, 23%). Thus, the activation of D1 dopamine receptors appears to be critical for facilitating neuronal activity related to reaching movements in the premotor cortex.

498.2

CLOZAPINE INDUCES FOS EXPRESSION IN PYRAMIDAL CELLS AND INTERNEURONS IN THE PREFRONTAL CORTEX IN A REGIONALLY-SPECIFIC MANNER. D. S. Cameron*, R. S. Duman, and A. Y. Deutch. Departments of Psychiatry and Pharmacology, Yale University School of Medicine, New Haven, CT 06510.

Recent data suggest that all clinically effective antipsychotic drugs (APDs) increase expression of the immediate-early gene (IEG) c-*fos* in the shell compartment of the nucleus accumbens. However, the atypical APD clozapine is effective in the treatment of a group of patients that do not respond to pharmacotherapy with typical APDs, and clozapine ameliorates negative symptoms more effectively than do typical APDs such as haloperidol. It follows that there are CNS sites at which clozapine acts selectively. We therefore examined the effects of acute administration of clozapine (CLZ) or haloperidol (HPD) on Fos protein in the prefrontal cortex (PFC).

Clozapine selectively increased the number of neurons exhibiting Fos-like immunoreactivity in the medial prefrontal cortex (PFC); HPD had no effect. Western blot analyses suggested that the effect was mainly due to an increase in Fos (58 kD band), although certain FRAs were marginally increased. CLZ increased the number of Fos-ir neurons in the deep but not superficial PFC layers. The increase in Fos expression was observed in the infralimbic and prelimbic cortices, but not in the medial precentral (shoulder) cortex. Fos was expressed in both pyramidal cells and interneurons. Among the interneurons, Fos-ir was observed in a sub- set of calbindin but not parvalbumin neurons. Fos was not observed in NADPH diaphorase-positive neurons of the PFC or underlying white matter. These data indicate that clozapine selectively increases PFC Fos expression in pyramidal cells and a distinct subset of GABAergic interneurons. These data may explain the observation that patients who do not improve on clozapine exhibit frontal cortical atrophy.

498.3

FIELD POTENTIALS EVOKED IN HORIZONTAL PATHWAYS OF RAT MOTOR CORTEX CONTAIN AN APV-SENSITIVE COMPONENT K. M. Jacobs*, J. P. Donoghue and G. Hess Dept. of Neuroscience, Brown University, Providence, RI 02912.

The contribution of NMDA receptors to horizontal transmission within layers III and V (termed: III-III, V-V) as well as to oblique interactions across these layers (V-III, III-V) was investigated in slices of rat motor cortex. Electrical stimulation evoked short latency responses consisting of a prominent negativity (-0.4 to -2.0 mV) horizontally at a distance of 500 µm in both superficial and deep layers and in the oblique V-III pathway. The response in the III-V pathway was smaller (-0.1 to -0.6 mV) and of longer latency. Bath application of the NMDA receptor antagonist, 2-amino-5-phosphonovaleric acid (APV, 100 µM) consistently reduced all field potentials. Decreases were observed in all components of the waveform, however, the primary effect was on the late phase of the response. In the III-III, V-V and V-III pathways APV produced on average a 22% decrease in the area, 12% of initial slope and 11% of peak amplitude of responses. The III-V path showed a greater percentage APV-induced decrease. All but a small, early, and presumably nonsynaptic component of the response was blocked by combined application of APV (100 µM) and the non-NMDA glutamate receptor antagonist 6-cyano-7-nitro- or 6,7-dinitro-quinoxaline-2,3-dione (CNQX/DNQX, 10-20 µM). The relative contribution of the APV-sensitive component was unrelated to field potential magnitude in 22 out of 30 cases, while in the remainder this component was relatively smaller as the magnitude of response increased. It is concluded that glutamate is the major transmitter of local intracortical connections. Most glutamatergic transmission is relayed by non-NMDA receptors, however, NMDA receptor activation is a small but consistent part of ordinary transmission in these pathways. The results further suggest that a potential for NMDA receptor-mediated synaptic modification exists in intrinsic horizontal pathways of both superficial and deep layers of rat motor cortex.

Supported by NIH grant NS 22517.

498.4

CATECHOLAMINERGIC LESIONS IN MEDIAL PREFRONTAL CORTEX INCREASE RUNNING-WHEEL ACTIVITY IN THE RAT. S. Lotfipour, C.H. Woodworth* and R.G. Robinson. Dept. Psychiatry, Univ. of Iowa Coll. Med., Iowa City, IA 52252.

Frontal cortical dopamine (DA) systems may play an inhibitory role in motor behavior, possibly mediated through the nucleus accumbens (NAc). Bilateral lesions of DA terminals in medial prefrontal cortex (MPFC) and bilateral electrolytic lesions in NAc both induce hyperactivity in rats. However, *unilateral* NAc lesions elicit hyperactivity only from the right hemisphere. The purpose of the present experiment was to determine whether unilateral lesions of catecholamine (CA) terminals in MPFC elicit an asymmetrical increase in locomotor activity, and whether this effect is dependent on norepinephrine (NE) or DA terminals.

Male Sprague-Dawley rats were housed in running-wheel cages for 21 days before and 30 days after receiving intracortical injections of the CA neurotoxin 6-hydroxydopamine (6-OHDA), either with or without pre-treatment with desmethylimipramine (DMI), a noradrenergic uptake blocker (n = 7 and 5 pair, respectively). Rats were paired according to baseline activity, and alternate members of each pair lesioned in either the left or right MPFC.

Lesioned animals *not* pretreated with DMI were significantly more active than pretreated rats, indicating that NE terminals were relatively more important than DA terminals in determining the effect. A tendency for non-pretreated animals with right-sided lesions to show greater hyperactivity than those with left-sided lesions suggested that the effect was lateralized. The finding that DMI-pretreated rats were no more active following neurotoxin injections than vehicle injections supported the conclusion that hyperactivity following MPFC lesions was not primarily due to focal destruction of mesolimbic DA terminals.

498.5

Activation Of M_1 Muscarinic Receptors Excites GABAergic Interneurons In Piriform Cortex. RL Gellman*, Yale Univ. Dept. of Psychiatry, New Haven, CT 06508 and Harvard Univ., MGH Dept. of Psychiatry, Charlestown, MA 02129.

During a recent study demonstrating that 5-HT_2, alpha$_1$ adrenergic and DA receptor expressing GABAergic interneurons in piriform cortex synapse onto layer 2 pyramidal cells (Gellman RL & Aghajanian GK, 1993) I noted that many of these interneurons were also activated by the cholinergic agonist carbachol. Subpopulations of putative interneurons were identified using extracellular electrodes to record from *in vitro* slices of piriform cortex in the presence of low concentrations of different agonists. Of 34 interneurons identified when 5-HT (20 µM) was present in the bath, 94% were also excited by carbachol. Carbachol (10 µM) activated an even larger population than did 5-HT; only 60% of the carbachol- sensitive interneurons were also excited by 5-HT (n= 17). The carbachol-mediated excitation was concentration-dependent (1-100 µM). Muscarine (0.5-25 µM) was an even more potent agonist while (-)nicotine (up to 100 µM) had no effect. Similarly, atropine (1-2 µM) completely blocked the response (n= 4) while hexamethonium (10 µM) had no effect (n= 4). Dose response curves were constructed for more selective muscarinic receptor antagonists. Pirenzepine (0.01-1 µM) and 4-DAMP (1-100 nM) each inhibited the carbachol- mediated excitation with IC_{50}'s of ~80 nM and ~70 nM respectively (n= 4-5). Gallamine had little effect at concentrations up to 10 µM. The rank order of potency is consistent with mediation by the M_1 receptor subtype.

In approximately half (11/20) of intracellular recordings from layer 2 pyramidal cells, using KCl filled electrodes, carbachol induced postsynaptic potentials (PSPs). The frequency of PSPs was concentration dependent over the same dose range (1-100 µM) as excited the interneurons. It will be important to determine how activation of this local network contributes to the complex effects of ACh in the piriform cortex.

498.6

EFFECTS OF LESIONS OF THE ENTORHINAL CORTEX ON FOREBRAIN DOPAMINE SYSTEMS IN THE RAT. C. E. Lander* and A. Y. Deutch. Interdepartmental Program in Neuroscience and Departments of Psychiatry and Pharmacology, Yale University School of Medicine, New Haven, CT 06510.

The entorhinal cortex (ENT) projects to the medial prefrontal cortex (PFC), ventral striatum, and hippocampus, all regions in which changes in dopamine (DA) function have been suggested to occur in schizophrenia. Certain hypotheses of the pathogenesis of schizophrenia posit that changes in PFC DA function result in an increase in subcortical DA function. However, neuropathological and *in vivo* imaging studies suggest that there are changes in both temporal and prefrontal cortices in schizophrenia. We therefore examined the effects of ENT lesions on cortical and subcortical DA function.

6-hydroxydopamine (6-OHDA) lesions of the ENT resulted in a $\geq$ 80% decrease in DA concentrations of the ENT and a much smaller but significant loss of ENT norepinephrine levels. The lesions did not alter DA utilization, as reflected by DOPAC/DA or HVA/DA, in subcortical areas including the nucleus accumbens (NAS) core or shell and the medial or lateral caudatoputamen. 6-OHDA lesions of the ENT also did not change DA utilization in the PFC. These results are similar to those obtained after PFC 6-OHDA lesions, which do not alter basal DA utilization in subcortical regions (i.e., the NAS), but do augment DA utilization under challenge paradigms. NMDA lesions of the ENT significantly increased both DA and serotonin utilization in the PFC but did not alter dynamics of these transmitters in the NAS. These findings are similar to those observed after ibotenic acid lesions of the PFC, which do not alter subcortical DA function. However, the axon-sparing lesion of the ENT does appear to modify prefrontal cortical DA function, suggesting the presence of distributed cortical networks that may be involved in schizophrenia.

498.7

STRESS-INDUCED ALTERATIONS IN GAD_{67} AND GAD_{65} mRNAs IN THE PREFRONTAL CORTEX. A. C. Grobin*, A. J. Bourdelais, P. Z. Gallipoli, and A. Y. Deutch. Departments of Pharmacology and Psychiatry, Yale University School of Medicine, New Haven, CT 06510.

The benzodiazepine site on the $GABA_A$ receptor complex allosterically modulates GABA-induced Cl^- flux. Anxiolytic benzodiazepines (BZ) reverse both behavioral and biochemical indices of exposure to stress. Among the latter are the stress-induced activation of the prefrontal cortical dopamine (DA) innervation, which is blocked by pretreatment with BZ agonists, and mimicked by administration of BZ inverse agonists such as FG 7142. GABAergic neurons in the prefrontal cortex (PFC) have recently been shown to be regulated by DA, such that DA enhances GABA release from interneurons. We therefore examined the effects of chronic stress on glutamic acid decarboxylase (GAD) mRNAs in the PFC and striatum.

Adult male Sprague-Dawley rats were subjected to 30 min restraint stress for seven consecutive days. This stressor results in an acute increase in DA metabolism and response in the PFC but not striatum. At the conclusion of the stress period on day seven, animals were sacrificed and the PFC and striatum removed. Northern blot analyses using GAD_{65} and GAD_{67} cDNAs were performed. Stress resulted in a decrease in GAD_{67} (but not GAD_{65}) mRNA in the PFC; no change was observed in the striatum.

These findings suggest that stress may decrease GABA availability, and are therefore consistent with the fact that anxiolytic BZs, by enhancing the actions of GABA, augment the responsiveness of post-synaptic neurons to decreased GABA availability. Western blot analyses of GAD proteins are being performed. Since stress increases DA release, which in turn enhances GABA release from PFC interneurons, the present data may also suggest that DA regulation of GABA neurons is restricted to a distinct subset of interneurons.

498.8

HALOPERIDOL AND CLOZAPINE DIFFERENTIALLY REGULATE GABA TRANSPORTER mRNAs IN THE PREFRONTAL CORTEX OF THE RAT. P. Z. Gallipoli[1*], J. Clark[1], S. Amara[2], and A. Y. Deutch[1]. [1]Departments of Psychiatry and Pharmacology, Yale University School of Medicine, New Haven, CT 06510 and [2]Vollum Institute, Portland, OR.

Dopamine (DA)-containing afferents to the striatum synapse with and regulate the activity of GABAergic medium spiny neurons. Antipsychotic drugs that result in extrapyramidal side effects increase GAD_{67} expression in these striatal neurons. Recent data indicate that DA afferents to the prefrontal cortex (PFC) regulate the activity of pyramidal neurons both directly and indirectly, the latter mode occuring through facilitation of GABA release from interneurons. In addition, recent findings suggest a loss of a subset of frontal cortical GABA interneurons in schizophrenia. There have been no examinations of the effects of chronic DA receptor blockade on the expression of GABA transporters. We have therefore examined the effects of chronic administration of the typical antipsychotic drug (APD) haloperidol (HPD) and the atypical antipsychotic drug clozapine (CLZ) on two different striatal and cortical GABA transporter mRNAs.

Adult male Sprague-Dawley rats received daily injections of HPD (1.0 mg/kg, sc), CLZ (15 mg/kg), or pH-matched vehicle for three weeks. The prefrontal cortex and striatum were removed, RNA isolated, and GAT-A and GAT-B mRNA levels measured by Northern blot analyses. Both APDs significantly decreased GAT-B mRNA in the striatum; GAT-A mRNA levels were not altered. In the PFC, HPD but not CLZ significantly decreased GAT-A mRNA. There was a non-significant trend toward a decrease in GAT-B mRNA in the PFC after HPD treatment. These data suggest that APDs alter GABA uptake in a regionally specific manner, and also suggest that typical but not atypical APDs selectively regulate GAT mRNAs in the PFC.

498.9

MEDIODORSAL THALAMIC LESIONS DIFFERENTIALLY REGULATE DOPAMINE METABOLISM IN PREFRONTAL CORTICAL SUBFIELDS AND NUCLEUS ACCUMBENS COMPARTMENTS. D. Ongur* and A. Y. Deutch. Interdepartmental Neuroscience Program and Departments of Psychiatry and Pharmacology, Yale University School of Medicine, New Haven, CT 06510.

The prefrontal cortex (PFC) has been defined as the mediodorsal thalamic (MD) projection field. Dopaminergic projections onto the PFC overlap completely with the MD innervation, but also extend to include the infralimbic cortex (PFC_{il}). The PFC_{il} and the more dorsally situated prelimbic cortex (PFC_{pl}) project to the nucleus accumbens (NAS) shell and core, respectively. These accumbal compartments, in turn, have distinct projections to the medial and lateral ventral pallidum (VP). These VP sites project back to the ventral midbrain, where they appear to form relatively closed loops. However, these two parallel systems may interact via selective medial VP projections to the medial MD, which projects to the prelimbic but not infralimbic PFC, and thus provides an avenue of intercommunication between the two "loops".

We examined the effects of ibotenic acid lesions of the medial MD of the rat on regional PFC and NAS dopamine metabolism. MD lesions increased prelimbic but not infralimbic cortical DA utilization, i.e., altered DA function only in the projection target of the thalamic nucleus. In addition, the lesion increased DA utilization in the NAS_{core} but not NAS_{shell}, i.e., in the terminal field of the MD-recipient PFC_{pl}. Thus, lesions of the MD result in changes in DA function in the corticostriatopallidal loop that emanates from the MD, which in turn is selectively innervated by the parallel loop.

We have also observed that the atypical antipsychotic drug (APD) clozapine (but not typical APDs) increases expression of the immediate-early gene *c-fos* in the PFC and the MD. The effects of MD lesions on APD-induced changes in PFC and NAS expression of *c-fos* are under current investigation.

CEREBELLUM III

499.1

A QUANTITATIVE ANALYSIS OF SYNAPTIC INPUTS ON BASKET CELL SOMATA IN THE CAT'S CEREBELLUM. Georgia A. Bishop*, Yi Fei Chen, Richard W. Burry and James S. King. Dept. of Cell Biology, Neurobiology and Anatomy and Neuroscience Program, The Ohio State University, Columbus, Ohio 43210.

Although the efferent connections of basket cells in the mammalian cerebellum are well established, only a few studies have analyzed afferent inputs to these neurons. Physiologically, stimulation of the inferior cerebellar peduncle elicits a low amplitude EPSP followed by a powerful all-or-none IPSP, suggesting the presence of numerous inhibitory inputs on the basket cell body. In the present study, an antibody to glutamic acid decarboxylase (GAD) was used to distinguish excitatory from inhibitory inputs to the basket cell body. Three basket cells from the same animal were serially thin sectioned at 90-100 nm. Every section was collected and analyzed for the proportion of labeled versus unlabeled contacts on these neurons. A total of 179 profiles were in direct apposition to the somata. Of these, 31 were GAD immunolabeled and 148 were unlabeled. When measured at their greatest dimension, the GAD immunoreactive and unlabeled profiles had average sizes of 1.4 μm (range 0.4-2.8 μm) and 1.0 μm (range 0.4-2.4 μm), respectively. The majority of unlabeled profiles had cytological characteristics of parallel fiber terminals. The largest GAD positive terminals were likely derived from Purkinje cell collaterals and the smallest GAD terminals may be of stellate cell origin. 85 of the 179 profiles examined formed confirmed synaptic junctions with the basket cell body. Of these 85 junction, 13% (n=11) were GAD positive and 87% (n=74) were unlabeled. Although fewer in number, activation of the inhibitory terminals derived from a single Purkinje cell (O'Donoghue et al., 1989. J.Neurosci. 9:2141) perhaps superimposed on a tonic suppression mediated by stellate cells appears to have a more powerful influence on basket cells when compared to excitatory afferents derived from parallel fibers. (Supported by NSF grant BNS 8919796.)

499.2

THE PHYSIOLOGICAL EFFECTS OF SEROTONIN ON NEURONS IN THE CAT'S CEREBELLAR NUCLEI. P.H. Kitzman* and G.A. Bishop. Dept. of Cell Biology, Neurology and Anatomy, The Ohio State University, Columbus, OH 43210.

The indoleamine serotonin (5HT) is present in a dense beaded plexus within the cat's cerebellar cortex and deep nuclei (Kerr and Bishop, 1991, JCN 304:502). The intent of the present study is to determine the physiological effects of 5HT on neurons within the cat's cerebellar nuclei. Serotonin was iontophoretically applied to individual neurons within the interpositus nucleus and its effect on spontaneous firing rate was recorded. In addition, 5HT was applied in combination with quisqualate, aspartate, and GABA. The results showed that 5HT partially reduced the spontaneous firing rate of the cerebellar nuclear neurons in a dose dependent manner. Of the cells which were activated by either of the excitatory amino acids, 50% were completely suppressed by the application of 5HT, while 36% were only partially suppressed. The remaining 14% of the recorded neurons were not effected by 5HT. 57% of the neurons which demonstrated complete suppression of firing rate showed a delay of approximately 10 seconds before activity was modulated. 58% of the neurons demonstrated a partial recovery of firing activity during application of 5HT, while 14% of the neurons recovered to their control firing rate. 28% of the neurons demonstrated complete suppression during the entire time 5HT was administered. 5HT also was shown to potentiate the inhibitory effect of GABA on 50% of the spontaneously active cells and 62% of the neurons activated by the excitatory amino acids. The remaining neurons demonstrated an additive interaction between 5HT and GABA. These results indicate that 5HT may have a similar function in the cerebellar nuclei as was found in the cortex; even though the brainstem sources of 5HT afferents to the cortex and nuclei are different. Specifically, 5HT modulates the response of the deep nuclear neurons to excitatory and inhibitory neurotransmitters. (Supported by NSF grant 8919796).

499.3

THE DISTRIBUTION OF MONOAMINERGIC FIBERS AND CELL BODIES IN THE CEREBELLUM IS NOT UNIFORM BETWEEN SPECIES. T.E.Nelson*, G.A.Bishop and J.S.King. Neuroscience Program, The Ohio State University, Columbus, OH 43210.

The indirect antibody peroxidase-antiperoxidase technique was used to determine the distributions of serotoninergic and catecholaminergic afferents in the mouse, opossum, and cat cerebellum. Antibodies to either serotonin (5-HT) or tyrosine hydroxylase (TH) revealed a diffuse plexus of thin varicose fibers that exhibited a different density and distribution pattern for each species. The only species in which TH-positive Purkinje cells were found is the mouse. Their primary location is vermal lobules VI-X. In the mouse, 5-HT fibers were scattered sparsely within all lobules and laminae, as well as all cerebellar nuclei. TH fibers formed a dense plexus within all cerebellar lobules, laminae, and nuclei. In the opossum, scattered 5-HT fibers were present in the granule and Purkinje cell layers of all lobules; they were densest in vermal lobules VIII and IX, and sparse in lobules II-VII. By comparison, TH fibers were more uniformly and densely distributed in the same layers; they were more abundant in vermal lobules V-VI than in more anterior and posterior lobules, particularly I and X. Numerous 5-HT and TH fibers were found in all four cerebellar nuclei. In the cat, except for sparse labeling in lobule X, 5-HT fibers were uniformly distributed at a high density in the granule and Purkinje cell layers of all cerebellar lobules and were present in all four nuclei. In contrast, TH fibers were sparsely distributed to all lobules and nuclei except for an area of elevated density in ventral folia of lobules V and VI. The transmitter synthesized in the TH positive fibers is most likely norepinephrine. However, dopaminergic fibers and binding sites have been reported in the mouse and rat cerebellum (Panagopolous et al., 1991, Neurosci.Lett. 130:208). The present results show that although monoaminergic axons within the cerebellum have similar morphologies their relative densities within cortical layers, distribution patterns within lobules, and brainstem origins vary between species. (Supported by NS08798 & 18028.)

499.4

AUTORADIOGRAPHIC LOCALIZATION OF CORTICOTROPIN RELEASING FACTOR (CRF) BINDING SITES IN THE ADULT OPOSSUM CEREBELLUM. P.C. Madtes Jr.* and J.S. King. Neuroscience Program, The Ohio State University., Columbus, OH 43210

A previous study has shown that CRF immunoreactive climbing fibers, mossy fibers and beaded axons are present in all lobules of the opossum's cerebellar cortex (Cummings et al 1989; JCN 280:501). The intent of the present study is to localize CRF binding sites in the cerebellum. Serial frozen sections were cut in both sagittal and transverse planes, and analyzed using the protocol of DeSouza and Insel 1990 (CRF: Basic and Clin. Stud. Neuropeptide, CRC Press p.69). Specific binding was determined using [125]I-ovine labeled CRF by subtracting nonspecific binding (measured in the presence of unlabeled human/rat CRF) from total binding (measured in the absence of human/rat unlabeled CRF). Binding sites were localized by apposing Kodak X-OMAT AR film to slides for specific time periods. The films were processed and analyzed using a computer-based densitometry system. The K_D value (0.1nM) agrees with values reported for CRF receptors in other species. CRF binding sites are present in all lobules of the cerebellar cortex with the greatest density in lobules IX, X and the flocculus. Binding sites are present over the molecular, Purkinje cell and granular layers. Thus, the lobular and laminar distribution of CRF binding sites matches the CRF afferent fiber distribution. A previous report (Bishop and King 1992; Neuropeptides 22:167) based on the iontophoresis of CRF and extracellular recordings indicates that this peptide has a facilitatory effect on the glutamate- and aspartate-induced firing rate of Purkinje cells in the cerebellar cortex. The present data further support our hypothesis that CRF functions as a co-transmitter in two of the primary afferent systems (climbing fibers and populations of mossy fibers) to the cerebellar cortex. (Supported by NS 08798).

499.5

THE LOCALIZATION OF CORTICOTROPIN RELEASING FACTOR (CRF) BINDING SITES DURING PURKINJE CELL MIGRATION King, James S.* and Paul C. Madtes Jr. Neuroscience Program, The Ohio State University Columbus, Ohio 43210

The purpose of the present study is to determine the temporal and spatial expression of CRF binding sites during early stages of cerebellar development in the opossum. Serial frozen sections were analyzed at PD 3,4,6,8 and 10 using the protocol of DeSouza and Insel 1990 (CRF: Basic and Clin. Stud. Neuropeptide, CRC Press p.69). Specific binding sites were determined by using [125]I-labeled CRF and localized by apposing Kodak X-OMAT AR film to slides for specific time periods. The films were processed and analyzed using a computer-based densitometry system. An antibody to calbindin was employed at the same postnatal ages to identify Purkinje (PK) cells during early stages of cerebellar development. Cummings et al (1989, Neurosci. Abst. 15:405) reported the presence of CRF immunoreactive axons and their growth cones in the opossum cerebellum in areas through which PK cells are migrating between PD 4 and 14. They also reported the presence of CRF mRNA in the inferior olive on PD 2 suggesting the earliest arriving axons are olivary in origin. CRF binding sites could not be detected until PD 8 at which time they were located superficially in the cerebellar plate over the EGL and the nascent Purkinje cell layer. At this age Purkinje cells have begun, but not completed their migration from the ventricular layer. Thus, based on the distribution of silver grains at PD 8, CRF binding sites are located over Purkinje cells that have completed their migration. However, this is prior to the time that CRF afferents arborize and penetrate the nascent Purkinje cell layer. The temporal expression and location of the CRF binding sites suggest that they could function in the formation of the initial climbing fiber Purkinje cell synapses. (Supported by NS 08798.)

499.6

ULTRASTRUCTURAL STUDY OF CGRP IMMUNOREACTIVITY IN THE NEONATAL CEREBELLUM. S. Morara [1]*, J.J.L. van der Want [2], A. de Wolf [2], L. Provini [1,3] and A. Rosina [1], [1]Ist. Neuroscienze CNR, Milano, Italy, [2]Dept. Morph., Neth. Ophthalm. Res. Inst., Amsterdam, Holland, [3]Ist. Fisiol. Gen. Chim. Biol., Univ. Milano, Italy.

In the rat cerebellar cortex, that mostly develops postnatally, complex phenomena of synaptic rearrangement occur. Purkinje cells (PCs) are contacted at birth by perisomatic synapses of multiple olivary axons which undergo a process of retraction, to reach the adult one-to-one relationship with P cells at the end of the second postnatal week. During this period, CGRP immunoreactivity is transiently found in terminal-like boutons around PCs (Morara et al., Brain Res. 504,315, 1989).

An ultrastructural study was performed to get direct evidence for the presence of CGRP in the climbing fiber (CF) synapses on PCs. Vibratome sections from 0-15 day old rat cerebella were incubated in a polyclonal anti-CGRP antibody (Chemicon), visualized by means of DAB reaction followed by a gold-substituted silver peroxidase intensification (van den Pol and Gorcs, J. comp. Neurol. 252, 507, 1986). CGRP-IR terminals are found confined to PC layer. PC show an invaginated somatic outline with spine-like extrusions contacted by numerous terminals. Among them several CGRP-IR terminals are seen, forming asymmetric synapses on PC somata that show either a single membrane specialization or an interrupted synaptic complex.

We conclude that CGRP is present in CF synapses contacting PC during the period of transient perisomatic olivocerebellar synaptogenesis, suggesting that CGRP may play a role in the cerebellar maturation.

499.7

COMPARISON OF PURKINJE CELL ZEBRIN II ANTIGENIC BANDS AND THE CUNEOCEREBELLAR FIBER TERMINATION BANDS IN THE MOUSE. A. Akintunde* and L. M. Eisenman. Dept. of Anatomy , Thomas Jefferson Univ., Philadelphia, PA 19017

The parasagittal parcellation of the cerebellar cortex has been revealed using various techniques. Mossy fibers, apart from providing the largest afferent input to the cerebellum and originating from many sources in the neuraxis, have also been shown in some instances to terminate in longitudinal compartments. Several correlation studies have been carried out to determine whether a common organizational plan encompassing the various afferent, efferent and intrinsic maps may exist in the mammalian cerebellum. Unilateral injections of WGA-HRP tracer were made into the external cuneate nucleus (ECN) of adult mice. Adjacent frontal sections of the cerebellar cortex were processed either for Zebrin II antigenicity or WGA-HRP tracer anterogradely transported from the ECN injection site. Cuneocerebellar fibers in the mouse were found to project in a strictly ipsilateral fashion and in well-delineated parasagittal terminal distribution zones. This was particularly clear in the vermal regions of both anterior and posterior lobes. Comparison of the banding patterns in alternate sections revealed that the limits of the cuneocerebellar terminal fields in the granule cell layer obeyed the bounderies of some, but not all, of the Zebrin II antigenic bands in the molecular layer. NIH grant NS 16531 (LME) gratefully acknowledged.

499.8

COMPARISON OF ZEBRIN COMPARTMENTS AND VIBRISSAL RECEPTIVE FIELDS IN LOBULE IXa OF THE RAT CEREBELLUM

V. Chockkan and R. Hawkes* Dept. Anatomy and Neuroscience Research Group, U. Calgary, Calgary, Alberta T2N 4N1, Canada.

Zebrin II is a 35 kDa polypeptide antigen of the cerebellum that is confined to parasagittal bands of Purkinje cells. In lobule IXa of the rat vermis anti-zebrin II staining reveals 4 zebrin$^+$ bands on each side of the midline (P1$^+$ - P4$^+$), interposed by 3 zebrin$^-$ zones (P1$^-$ - P3$^-$). In previous studies, electrophysiological micromapping has revealed prominent vibrissal receptive fields in the dorsal surface of IXa and so zebrin II immunocytochemistry was combined with RF mapping to compare the molecular and functional maps. Nine adult rats were mapped electrophysiologically, then perfused, sectioned and immunostained by using *anti-zebrin II*. After 3-D reconstruction, the antigenic and functional maps were superimposed. There are 3 discrete vibrissal receptive fields each side of the midline. These align reliably with the zebrin compartmentation with one centered on each zebrin$^-$ band. However, the RFs consistently overlap the zebrin$^{+/-}$ borders at each side. A clear, reproducible relationship between the sensory and motor maps suggests that Purkinje cell compartmentation, which is present very early in cerebellar development, may serves as a substrate to organize cerebellar function into discrete modules.

Supported by grants from the MRC (Canada) and the AHFMR.

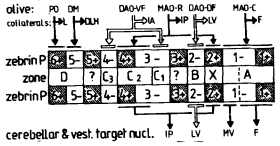
499.9

RELATION OF OLIVOCEREBELLAR PROJECTION ZONES TO ZEBRIN PATTERN IN RAT CEREBELLUM. J. Voogd* (1), L.M. Eisenman (2), T.J.H. Ruigrok (1) Depts. of Anatomy, Erasmus University Rotterdam, P.O. Box 1738, 3000 DR Rotterdam, The Netherlands (1) and Jefferson University Med. Coll. Philadelphia PA 19107 (2).

Projections were traced with Phasaeolus leucagglutinin (PHA-L). Cobalt-stabilized DAB-reaction product in PHA-L labeled fibers was combined with anti-zebrin I immunohistochemistry (Hawkes and Leclerc, J.Comp.Neurol. 256:29'86) on the same sections. DAO projects to B1,C1 and C3 zones in the lobules I-VI which correspond to zebrin-negative zones P2-, P3- and P4-respectively. Projections of MAO to the A, X and C2 zones include both zebrin-positive zones P1+, P2+ and P4+ and adjoining zebrin-negative regions. The C1/C2 border is located in the middle of P3-. Climbing fiber collaterals terminate in target nuclei of Purkinje cells they innervate. The same principles hold for the olivocerebellar projection to the posterior lobe.

Diagram of relation of zebrin pattern to olivocerebellar and corticonuclear (Voogd et al., Neurosci.Abstr.17:1573'91) projections in anterior lobe.

DAO-DF/VF: dorsal acc. olive, dorsal/ventral fold; DLH:' dorsolateral hump; DM: dorsomedial subnu.; F: fastigial nu.; IA: anterior interposed nu.; IP: posterior interposed nu.; L: lateral cereb.nu.; LV: lateral vest.nu.; MAO-C/R: medial acc.olive caudal/rostral; MV: medial vest.nu.; PO: principal olive.



499.10

FUNCTIONAL DIVISIONS OF CAT rMAO. C. M. Porter, P.L.E. Van Kan, K.M. Horn, J.R. Bloedel, and A.R. Gibson*. Barrow Neurological Institute, Phoenix, AZ, 85013.

Posterior interpositus (NIP) contains cells related to eye movements in its ventral, lateral and caudal region. Other areas of NIP contain cells related to limb movements. The rostral medial accessory olive (rMAO) has reciprocal connections with NIP. Do the connections of rMAO reflect the functional divisions of NIP?

We placed an injection (.008 ul) of 1% WGA-HRP into the rostral pole of rMAO. Placement was guided by recording responses to chin stimulation from overlying rDAO, since rostral rMAO cells did not respond to somatic stimulation. Retrograde transport revealed one brainstem input to rMAO, ventral-medial nucleus Darkschewitsch (ND). Cerebellar nuclear connections of rostral rMAO were confined to the ventral, lateral, and caudal regions of NIP.

In a second case, we injected the caudal half of rMAO where cells responsive to somatosensory stimulation were recorded. In ND only the dorsal-lateral region contained retrogradely labeled cells. Their locations had little overlap with that of the cells labeled by the first injection. Similarly, medial NIP was heavily labeled, but ventral, lateral and caudal NIP was devoid of label.

We conclude that rMAO contains functionally distinct regions, and we hypothesize that the rostral region is involved in the control of eye movements whereas the caudal region is involved in the control of limb movements. (Supported by NIH-NINDS PO1-NS30013-01A1)

499.11

NEURONAL SURVIVAL FOLLOWING 3-ACETYLPYRIDINE AND HARMALINE TREATMENT: SUPPORT FOR A COLUMNAR ORGANIZATION OF THE INFERIOR OLIVE. Helen H. Molinari*, Kathy A. Starr, and John A. Goncalves Jr. Dept. of Anatomy, Albany Medical College, Albany, N.Y. 12208

A host of recent studies suggest that the inferior olive is comprised of functional units that form rostro-caudally oriented columns. This study sought to determine whether columns characterize neurons in the inferior olive that survive treatment with the neurotoxin 3-acetylpyridine (3-AP). It also sought to determine whether columns respond differently to the tremorogenic drug harmaline, given after the 3-AP.

Male, Long Evans rats were given 3-AP at time 0, harmaline at 3 hrs, and Niacinamide at 4.5 hrs. Four days later the animals were terminated, the brains stained with cresyl violet, and the locations of surviving neurons plotted. Three-dimensional reconstructions revealed that the surviving neurons consistently formed rostro-caudally oriented columns. Furthermore, the columns paralleled those described by Apps (1990) for olivary efferents and by Bourrat and Sotelo (1991) for birthdates of olivary neurons. Some columns showed no consistent responses to harmaline; in others, with higher doses of harmaline, fewer neurons survived. These data support the notion that the inferior olive is organized in columns and suggest that neurons within a column share the same sensitivity to 3-AP and to harmaline.

Supported by NS29771.

499.12

REDUCED SENSITIVITY TO THE NEUROTOXIC EFFECTS OF 3-ACETYL-PYRIDINE IN THE INFERIOR OLIVE OF THE GENETICALLY DYSTONIC (dt) RAT. M.H. Thorstad and J.F. Lorden*. Dept. of Psychology, Univ. of Alabama at Birmingham, Birmingham, AL 35294.

The toxin 3-acetylpyridine (3AP) destroys the cells of the inferior olive (IO), although the caudal medial accessory olive (MAO) is generally found to be less sensitive than other regions. In dt rats, electrophysiological evidence suggests that there is an abnormality in the transmission of information between the caudal MAO and the cerebellar vermis. To further investigate this, the behavioral and neurotoxic effects of 3AP were examined in 15 dt and 17 normal rats given 75 mg/kg of 3AP followed 3 h later by 15 mg/kg of harmaline at 19 days of age. The twisting movements of the trunk and limbs typical of the dt rat persisted after 3AP; but, there was a small, significant decrease in overall activity. In normal rats, 3AP induced a characteristic motor syndrome.

Examination of the brains at either 72 h or 1 wk postlesion revealed near total destruction of the inferior olive in normal rats. The few healthy cells detected were found in subnucleus c of the caudal MAO. In all dt rats, olivary cells were numerous, but not uniformly distributed. A consistent pattern was observed with spared cells in subnuclei a and c of the caudal MAO, nucleus ß, and in the medial parts of the caudal dorsal accessory olive. Seven normal and 5 dt rats were given 65 mg/kg of 3AP without harmaline to increase the amount of sparing. This did not alter the pattern observed. The cells of the IO are organized into functional groups based on afferents and efferent projections. The observation that those parts of the IO projecting to the vermis are less sensitive to 3AP in the dt rat may indicate an intrinsic abnormality or may reflect a difference in afferent input. (Supported by a grant from the Dystonia Med. Res. Fdn.)

499.13

INFERIOR OLIVE SEROTONIN AND NOREPINEPHRINE LEVELS DURING DEVELOPMENT IN THE GENETICALLY DYSTONIC RAT. M.S. LeDoux, J.F. Lorden and J.M. Ervin. Dept. of Psychology, Univ. of Alabama at Birmingham, Birmingham, AL 35294

The dystonic(*dt*) rat is an autosomal recessive mutant with a motor syndrome that shares several features with idiopathic torsion dystonia in humans. In the *dt* rats, marked biochemical and physiological abnormalities have been localized to the olivo-cerebellar system. At the pharmacological level, the *dt* rats exhibit enhanced sensitivity to the behavioral effects of serotonergic(5HT) agonists, including quipazine, a drug that activates the neurons of the inferior olive(IO). High performance liquid chromatography with electrochemical detection was used to assay 5HT, 5-hydroxyindoleacetic acid(5HIAA), and norepinephrine(NE) in micropunches of the IO in normal and *dt* rats at 14, 18 and 22 days of age. Coronal sections(320 *um*) through the rostral frontal lobes were used as internal controls. Significant age-dependent effects were seen on 5HT and 5HIAA levels in the IO, but not the frontal cortex, in both groups. Although both groups reached similar 5HT levels by postnatal day 22, a significant interaction effect between age and phenotype indicated a difference in the pattern of development. Administration of quipazine(10 mg/kg, i.p.) to 18-day old normal and *dt* rats one hour prior to sacrifice caused significant reductions in NE, 5HIAA and the ratio of 5HIAA to 5HT in both groups. The findings do not suggest that the differential behavioral responses to 5HT agonists seen in normal and *dt* rats are the result of global abnormalities in 5HT systems. Nor do they suggest the presence of presynaptic defects in the IO. The age-dependent differences in 5HT levels in the IO may, however, indicate a maturational delay in the 5HT innervation of this structure in the *dt* rat. (Supported by BNS 90-10187, 1 K08 NS01593-01, Dystonia Medical Research Foundation and the American Association of Neurological Surgeons)

499.14

QUALITATIVE AND QUANTITATIVE ANALYSIS OF THE PC IN THE CEREBELLUM OF THE MEANDER TAIL MUTANT MOUSE. J.A. Napieralski* and L.M. Eisenman. Department of Anatomy/Developmental Biology, Thomas Jefferson University, Philadelphia, PA 19107.

The cerebellum of the meander tail mutant mouse (mea/mea) is characterized by an apparently normal cytoarchitecture posteriorly with an abrupt transition to an abnormal anterior region characterized by abnormal foliation, agranularity, Purkinje cell (PC) ectopia, and a paucity of Bergmann glial fibers. This study presents the results of a qualitative and quantitative analysis of the PC in the mea/mea cerebellum. Developmental and morphological analyses reveals that the PC in the anterior region do not form a monolayer during the first week of postnatal development and that the dendrites of these ectopic cells are atrophic and disoriented. Quantitative studies in adult animals reveal that the total number of these cells is normal, but the number of PC in the affected anterior region of the mea/mea cerebellum is greater than the number of PC in the anterior lobe of the normal animal. These data suggest that (1), the developmental morphology of the PC in the anterior region is abnormal, probably due to the lack of granule cells, while the total number of PC in the cerebellum is normal, and (2), the defect is not restricted to the anterior lobe but involves a portion of the posterior lobe. This latter possibility supports the notion that the mutant gene affects a unique developmental compartment in the cerebellum which does not coincide with the classic adult boundary, the primary fissure, between the anterior and posterior lobes. Supported by NIH grant NS22093.

499.15

SYNCHRONIZED OLIVARY OSCILLATIONS ARE TRIGGERED BY THE NEUROSTEROID PREGNENOLONE. Sheryl S. Smith*. Dept. of Anatomy, Inst. of Neurosci., Hahnemann Univ., Phila., PA 19102- 1192.

Elevations in circulating levels of sex steroids across the estrous cycle are associated with facilitation of rapidly alternating limb movements. One mechanism which may underlie this behavioral change is an increase in the sychronized oscillatory discharge of the rostral dorsal accessory olive (rDAO), a putative timing mechanism for rapid limb movements. Pregnenolone, a steroid elevated during estrous- enhanced performance state, was found in the present study to trigger this synchronized oscillatory discharge. This study was conducted in diestrous female rats chronically implanted with arrays of microwires (50 μ dia.) to record simultaneously from as many as 23 neurons within the rDAO, bilaterally, during locomotor paradigms or in the anesthetized state. During variable speed treadmill locomotion, pregnenolone (5 μgs, i.p.) increased the number of synchronized neurons by 150% and increased the amplitude of the 1-3 Hz oscillation by 60% compared to oil- injected controls. (n=10-23 neurons/rat recorded from 4 rats) This effect of the steroid was dose- dependent, with 100 ngs the minimal effective dose, and was not observed during non-movement. Under nembutal anesthesia (34 mg/kg, i.p.), pregnenolone triggered a fully synchronized oscillatory state from a previously non-synchronized population. This effect was specific for pregnenolone, as the two direct metabolites of this steroid, 17 α-OH-pregnenolone and progesterone, were not effective in this regard. Pregnenolone sulfate was slightly less effective than pregnenolone in triggering synchronization. The effect of pregnenolone may be due to its known actions at the level of the NMDA receptor (Wu et al, 1991) or voltage-sensitive calcium channel (ffrench-Mullen and Spence, 1991), as MK-801 (1 mg/kg, i.p.) and, to a lesser extent, octanol (0.2 mg/kg, i.p.), blocked the synchronizing action of pregnenolone. *(Supp. by USAFOSR GRANT# F49620-93-1-0136DEF)*

499.16

ANISOTROPIC AND HETEROGENEOUS DIFFUSION IN THE CEREBELLUM: IMPLICATIONS FOR VOLUME TRANSMISSION C. Nicholson*, Y. C. Okada and M. E. Rice. Dept. Physiology and Biophysics, New York University Medical Center, 550 First Avenue, New York, NY 10016.

Measurements of the extracellular diffusion parameters tortuosity (λ) and volume fraction (α) were made in three orthogonal axes of the molecular and granular layers of the isolated turtle cerebellum using ionophoresis of tetramethylammonium (TMA^+) combined with ion-selective microelectrodes.

Diffusion in the molecular layer was characterized by an anisotropic tortuosity with three distinct values. Along the x-axis (the parallel fibers) $\lambda_x = 1.44 \pm 0.01$ (mean $\pm$ S. E. M.), along the y-axis (perpendicular to parallel fibers and parallel to the pial surface) $\lambda_y = 1.95 \pm 0.02$, while $\lambda_z = 1.58 \pm 0.01$ (vertical axis). By contrast, the granular layer was isotropic with a single tortuosity value, $\lambda_{Gr} = 1.77 \pm 0.01$. There was a striking difference in α, for each layer. In the molecular layer $\alpha = 0.31 \pm 0.01$ while in the granular layer $\alpha = 0.22 \pm 0.01$.

One consequence of these results was demonstrated by measuring local changes in $[K^+]_o$ and $[Ca^{2+}]_o$ following micro-ionophoresis of the glutamate. The ratios of ion shifts in the x- and y-axes in the granular layer were 1.04 ± 0.08 for the rise in $[K^+]_o$ and 1.03 ± 0.17 for the decrease in $[Ca^{2+}]_o$. In contrast, ion shifts in the molecular layer had an x:y ratio of 1.44 ± 0.14 for the rise in $[K^+]_o$ and 2.10 ± 0.42 for the decrease in $[Ca^{2+}]_o$.

These data demonstrate that the structure of cellular aggregates can influence the migration of substances in the extracellular microenvironment and may play a role in volume transmission to extra-synaptic sites. This study was supported by NIH Grant NS 28642.

BRAIN METABOLISM AND BLOOD FLOW: MISCELLANEOUS

500.1

SPREADING DEPRESSION IS UNAFFECTED BY ANTI-MIGRAINE COMPOUNDS: A CEREBRAL BLOOD FLOW AND ELECTROPHYSIOLOGICAL STUDY Peter J. Goadsby*, Karen L. Hoskin and Holger Kaube Department of Neurology, The Prince Henry Hospital, Sydney 2036 AUSTRALIA

Leao's cortical spreading depression (SD) is often cited as the pathophysiological substrate for the neurological symptoms of migraine with aura. If this is the case it might be expected that drugs useful as anti-migraine agents, particularly those useful in prophylaxis, may alter or prevent SD. In this study we attempted to further investigate the effects of DHE and other anti-migraine drugs on SD by measuring cortical blood flow with laser Doppler flowmetry (CBF_{LDF}) and cortical single unit activity in the α-chloralose anaesthetised cat. The following substances were tested: DHE, acetylsalicylic acid, lignocaine, metoprolol, clonazepam and valproate. The NMDA-receptor blocker MK- 801 and halothane (1.5%) were used as reference substances that reliably block SD. The outcome measures were speed of propagation of the wave of SD across the cortex and the CBF_{LDF} increase during the hyperaemic phase of SD. Data were taken from three control episodes (60min apart) and after drug administration. The rate of propagation was significantly reduced from the first control period (3.0±0.3mm/min) to the subsequent 2 control observations (2.3±0.1mm/min) even without any drug treatment. Following the control observations the test drug was administered and a further SD elicited. This fourth SD was reliably blocked by MK-801 and halothane. None of other test drugs inhibited SD, reduced the rate of propagation or changed the amplitude of the CBF_{LDF} increase. It is therefore possible that anti-migraine drugs that are active in prophylaxis do not have their effect through a modulation of SD. Indeed if SD is the explanation for the aura phase of migraine it may be an epiphenomenon driven by some further central process rather than the causal initiator of the migraine attack.

500.2

CHRONIC HYPOXIA INCREASES CAPILLARY DENSITY IN MOUSE BRAIN

J. Boero,[1] A. Arregui,[1] J. Christensen[2]*, C. Rovainen[3] & T. Woolsey.[2] [1] Dept. of Neurology, Universidad Peruana "Cayetano Heredia", Lima, Perú, and Depts. of [2] Neurology and Neurological Surgery and of [3] Cell Biol. and Physiol., Washington University, St. Louis MO 63110

A mouse model was developed to explore cerebrovascular pathophysiology associated with life at high altitude. We report here substantial changes in the cerebral capillaries in this model. Weanling *Swiss Webster* mice were put in an intermittent hypobaric hypoxic chamber simulating an altitude of 4, 300 m. Littermate controls were at sea level. After 3 weeks, mice in both groups were anesthetized and perfused through the heart with photographic emulsion to fill brain capillaries (e.g., Wang et al, JCBF&M 12: 935 '92). Frozen 50 µm serial sections through the brain were cut in the coronal plane. Selected areas were measured through a microscope with a video camera and Macintosh Computer using Image (W. Rasband, NIH). Qualitatively, capillaries in all parts of the hypoxic brains appeared larger, and were more numerous and tortuous. Measurements of Lv (vascular length density) increased by 20+% in cortex, cerebellum and. brainstem and were significant in the striatum (e.g., $p \leq 0.01$). These substantial changes indicate a significant capacity of the maturing cerebral vascular system to compensate for decreased O_2 tensions to meet sustained metabolic demands of the brain.

Supported by Guggenheim Foundation Fellowship (AA) and NIH Grants HL 41075 and NS 28781, the McDonnell Center for Studies of Higher Brain Function and The Spastic Paralysis Rearch Foundation of the Illinois-Eastern Iowa District of Kiwanis International.

500.3

DISSIMILARITIES IN THE LINKAGE OF BLOOD FLOW AND GLUCOSE INFLUX TO GLUCOSE METABOLISM. J. Fenstermacher, L. Wei*, T. Otsuka, D. Bereczki, V. Acuff, K. Pettigrew and C. Patlak. Department of Neurological Surgery, SUNY at Stony Brook, NY 11794-8122

The coupling of local cerebral blood flow (LCBF) to local cerebral glucose utilization (LCGU) may vary fairly broadly among brain areas and between conditions, suggesting that the dependency of LCBF on LCGU differs considerably within the brain. To examine this suggestion LCBF, LCGU, and local 3-O-methylglucose (3MG) influx (3MGIn) were measured in control and hypercapnic (awake, breathing 8% CO2) rats. Hypercapnia sizably raised arterial CO2 and O2, significantly elevated LCBF (25-230%), significantly lowered LCGU, and did not change 3MGIn. In control rats, LCBF/LCGU and 3MGIn/LCGU ratios varied significantly (2-fold) among brain areas; these ratio also varied significantly (3-fold) among brain areas in hypercapnic rats. This suggests that the linkage of LCBF and of 3MGIn to LCGU differs among brain areas. The pattern of the differences in these ratios between control and hypercapnia groups was dissimilar, indicating that hypercapnia did not have a uniform effect on the coupling of LCBF and 3MGIn to LCGU. The sensitivity of parenchyma arteriole to blood gases and local metabolites or the ability of them to respond may vary among brain areas. The permeability-surface areas product of 3MG was identical in control and hypercapnic rats, which implies little or no capillary recruitment during hypercapnia and little regulation of 3MG influx.

500.4

MEASUREMENT OF BRAIN TEMPERATURE IN MULTIPLE SITES DURING COLD STRESS REVEALS DYNAMIC AND NONUNITARY CHANGES. **S.T. Ahlers*, C.L. Kish, and J.R. Thomas, Naval Medical Research Institute, Bethesda, MD 20889-5607.**

Exposure to environmental cold stress has been shown to impair working memory. Previous research has shown that the level of cooling in which the deficit is observed also produces a temperature decrease of approximately 1°C in the hippocampus (HIPP), suggesting that subtle changes in brain temperature may play a role in the impairment of cognitive function. In the present study we examined the effects of cold stress on brain and body temperature in order to determine how closely coupled temperature is in the brain as a whole and what relationship brain temperature has to "core" temperature as measured in the peritoneal cavity. Accordingly, rats were implanted with thermistors into the prefrontal cortex (PFC), the medial preoptic area of the hypothalamus (MPA), and the dorsal hippocampal formation (HIPP) of the rat. Probes were attached to a six channel receptacle affixed to the rats' skull. During measurement, a cable was connected to a swivel and attached to the receptacle to allow on line measurement of brain temperature in three discrete brain regions simultaneously. A radio-telemetry probe (Mini-Mitter, Sun River, OR) was implanted into the peritoneal cavity. One week after surgery the brain and body temperature were measured in rats during exposure to ambient temperatures of 24°C and 5°C. Analysis indicated nonuniform changes in the regions of the brain and in the body during both nonstressed (normothermic) and cold stressed conditions. These data demonstrate that brain temperature is not homogeneous and that temperature in various regions of the brain show unique response patterns. The extent that these nonuniform changes in brain temperature relate to specific cognitive deficits observed during exposure to cold stress remains to be determined.

500.5

IS THERE RECRUITMENT OF CAPILLARIES AND/OR GLUCOSE TRANSPORTERS IN RAT BRAIN DURING STRESS? J.-L. Chen, T. Otsuka, D. Bereczki, L. Wei, V. Acuff, C. Patlak, and J. Fenstermacher*. Div. Neurosurg., Tri-Service General Hospital, NDMC, Taipei, Taiwan, and Dept. Neurol. Surg., SUNY, Stony Brook, NY 11794-8122.

Local cerebral glucose utilization (LCGU) and blood flow (LCBF) are often changed during stress. In order to understand the mechanism of LCBF alteration and its linkage to LCGU during stress, LCBF and the permeability-surface area products (PS) of antipyrine (PS-AN) and of 3-O-methylglucose (PS-3MG) were measured in many areas of rat brain in four different conditions. With hypoxia and hypercapnia, LCBF and plasma glucose levels were markedly elevated, PS-AN was only slightly raised, and PS-3MG was slightly lowered (hypoxia) or unchanged (hypercapnia). With nicotine treatment, plasma glucose was normal, LCBF was greatly increased in some brain areas, and PS-AN and PS-3MG were virtually unchanged in these areas. In view of the effects of plasma glucose on PS-3MG, these data indicate little or no capillary recruitment when LCBF is increased with these three conditions. After 24 hr of dehydration, LCBF was slightly lowered, plasma glucose and PS-AN were unchanged, and PS-3MG was raised throughout the brain. Dehydration does not appear to alter the number of perfused capillaries but may raise glucose transport, possibly by recruiting glucose transporters in capillary endothelial cells.

500.6

RETINAL BLOOD FLOW FOLLOWING OCULAR ISCHEMIA IN CATS. S. Roth,* Z. Pietrzyk, Department of Anesthesia and Critical Care, University of Chicago, Chicago, IL 60637.

Retinal ischemia may occur after retinal vascular occlusion or may accompany systemic diseases such as diabetes mellitus. The mechanisms responsible for visual damage after retinal ischemia are not well understood. We hypothesized that retinal blood flow (RetBF) after ocular ischemia would be hyperemic, similar in time course and magnitude to that occurring after cerebral ischemia. Adult cats weighing 2.5-3.5 kg were anesthetized with chloralose 80 mg/kg and acepromazine 0.25 mg/kg IV. RetBF was measured using radioactively labeled microspheres injected into the left atrium with the reference blood sample method. Ocular ischemia was produced for 1 h by increasing intraocular pressure to 100 mmHg > systolic arterial blood pressure using a saline reservoir connected to an anterior chamber cannula. Blood flows were measured before and after ischemia in 6 cats. In 4 control animals, blood flows without ischemia were measured over the same time course. Results were analyzed using repeated measures ANOVA with $P < .05$ considered statistically significant. RetBF before ischemia was 17 ± 9 ml • g^{-1} • min^{-1} (mean ± SEM). Within 15 min of the return of normal IOP after ischemia, RetBF increased significantly to 52 ± 10 ml • g^{-1} • min^{-1} ($P < .0001$). By 1 h after ischemia, RetBF had returned to baseline. In control animals, RetBF did not change significantly over a comparable time course. We conclude that retinal ischemia is followed by hyperemia after reperfusion, but not by delayed hypoperfusion, as others have shown in the brain. Hyperemia after ischemia may contribute to blood-retinal barrier dysfunction and macular edema, and therefore we are currentlyinvestigating its mechanism.

500.7

THE ROLE OF CALCIUM-ACTIVATED PROTEOLYSIS IN VASOSPASM AFTER SUBARACHNOID HEMORRHAGE. P.L. Foley*, P. Vanderklish^, Y. Goto, G. Lynch^, N.F. Kassell, K.S. Lee. Dept. of Neurological Surgery, Univ. of Virginia, Charlottesville, VA 22908, ^CNLM, Univ. of California, Irvine, CA 92717.

A rise in cytosolic calcium levels in smooth muscle cells is believed to be a pivotal event in the development of chronic vasoconstriction after subarachnoid hemorrhage (SAH). Calcium-activated proteolysis via calpain may participate in vasospasm by damaging the function of key proteins regulating smooth muscle tone. The present studies investigated this issue by measuring levels of native calpain in the rabbit basilar artery following SAH. Two to four days following experimental SAH, basilar arteries were removed, thoroughly flushed with cold homogenization buffer to remove all blood, and placed into 100 µl of cold buffer. The tissue was subsequently homogenized using glass tissue grinders. Western blot analyses were performed using an antibody for calpain, and an antibody for spectrin. Spectrin is a cytoskeletal protein and a preferred substrate for calpain.

Vessels from normal (non-SAH) rabbits exhibited high levels of native calpain and native spectrin. Vessels removed 2 to 4 days after SAH showed significant reductions in the levels of native calpain. In addition, an elevation in spectrin breakdown products was seen. The molecular weights of the spectrin breakdown products were typical of those observed following calpain proteolysis.

The decline in native calpain levels after SAH suggests that autoproteolytic activation of this protease had occurred. This conclusion is supported by the finding that a preferred substrate of calpain, spectrin, is degraded at the same time. These findings indicate that calpain activation occurs following SAH, and that proteolysis could contribute to the pathophysiology of vasospasm.

500.8

INTERACTIONS BETWEEN MITOCHONDRIA AND CYTOSOLIC FREE CALCIUM IN DISSOCIATED RAT HIPPOCAMPAL NEURONS. A.V. Nowicky and M.R. Duchen*. Physiology Dept., Univ. College, London, U.K., WC1E 6BT.

Changes in mitochondrial function may play a central role in defining neuronal viability after metabolic disturbances such as hypoxia. We have examined the interrelationships between changes in mitochondrial energetics and $[Ca^{2+}]_i$ in central neurons. Cells were freshly dissociated from hippocampal slices of 14-22 day old rats. Fluorimetric measurements were made from cells loaded with the Ca^{2+}-sensitive dye Fura-2 and/or the lipophilic cation, rhodamine 123 (Rh 123), which selectively reports mitochondrial potential ($\Delta\psi_m$). Elevation of $[Ca^{2+}]_i$ by brief depolarization with 50mM K^+ depolarized $\Delta\psi_m$ in most cells. $[Ca^{2+}]_i$ rose on depolarization of $\Delta\psi_m$ with the uncoupler, FCCP. This response persisted in the absence of external Ca^{2+} suggesting that it reflects Ca_{2+} release from an internal store. This store could be loaded by K^+ induced depolarization, enhancing the response to FCCP. Dual loading allowed the direct examination of the temporal relationships between changes in $[Ca^{2+}]_i$ and of $\Delta\psi_m$. These data suggest that depolarization of the cell membrane is associated with mitochondrial Ca^{2+} uptake. (supported by Action Research)

500.9

DEVELOPMENT OF ACTIVITY STANDARDS FOR QUANTIFICATION OF CYTOCHROME OXIDASE HISTOCHEMISTRY D. Jones* and F. Gonzalez-Lima, Dept of Psych, Univ of Texas, Austin TX, 78712 USA

Taking advantage of quantitative image analysis systems we measured the enzyme activity in histochemically stained brain slices by calibrating the analysis system with internal standards of known amounts of cytochrome oxidase CO activity. Standards were prepared with homogenized brain or heart tissue. Different mixtures were obtained by adding to microwaved inactive paste increasing percent weight proportions of active paste. The mixtures were rehomogenized, placed in microcentrifuge tubes, frozen in isopentane, and sliced in a cryostat. Samples of each standard were prepared for enzyme assay according to Hess and Pope and the calculation of specific activity was performed spectrophotometrically based on Wharton and Tzagaloff. The staining procedure, a modification of Silverman and Tootell, involved cobalt preincubation, oxygenation and heating of reaction medium. Percent weight concentration was compared to spectrophotometrically assessed activity. The effects of length of incubation, section thickness, and activity on measured optical density (OD) were assessed. A regression curve for the known activities and the corresponding OD was matched to a regression curve for the section thickness and their corresponding OD to obtain predicted activity for the varying thicknesses of section. The effect of varying concentrations of heart or brain active tissue on measured activity and OD of histochemical staining were investigated. Validation of the procedure was assessed by testing the effect of varying each of the above parameters on measured activity and OD. The resulting quantitative CO method provides a superior approach for regional mapping studies of brain metabolic capacity. Examples of applications of this technique are presented using the auditory system of the gerbil. (supported by RO1 MH43353).

500.10

REGULATION OF NUCLEAR- AND MITOCHONDRIAL-ENCODED CYTOCHROME OXIDASE SUBUNITS AND ENZYME ACTIVITY BY NEURONAL ACTIVITY IN DORSAL LATERAL GENICULATE NUCLEUS OF ADULT MONKEYS. S. Liu* and M. Wong-Riley, Dept. Cellular Biology & Anatomy, Med. Coll. Wis., Milwaukee, WI 53226

Cytochrome oxidase (CO), a mitochondrial enzyme of oxidative energy metabolism, is composed of 13 subunits derived from both nuclear and mitochondrial genomes. The goal of this study was to determine whether the expression of these two genomes was regulated coordinately at the subunit protein level by functional activity in neurons. To clarify this question, we examined the level of CO activity histochemically, and protein levels of subunit II, II/III (mitochondrial-derived) and subunit IV (nuclear-encoded) immunohistochemically in dorsal lateral geniculate nuclei (LGN) of normal and monocular TTX-treated monkeys. In the normal LGN, a similar staining pattern was found for all markers examined. After 3 or 7 days of TTX treatment, levels of CO activity and subunit proteins reduced disproportionately in the deprived laminae of LGN. Changs in levels of CO activity and subunit IV was significantly greater than subunits II and II/III ($P<0.01$). We also found that the levels of CO activity and subunit proteins were up-regulated in cell bodies of the nondeprived laminae. These findinds demonstrate that at subunit protein levels, mitochondrial and nuclear genomes are somewhat disproportionately regulated by neuronal functional activity. Changes in subunit IV parallel most closely that of CO activity, suggesting that subunit IV may play an important role in governing the level of CO holoenzyme activity in response to changes in neuronal functional activity. (Supported by NIH grant NS 18122).

500.11

HEXOKINASE I mRNA IN THE CENTRAL NERVOUS SYSTEM OF THE RAT: AN *IN SITU* HYBRIDIZATION STUDY

G. Jacobsson[1], K. Uvnäs-Moberg[2] and B. Meister[1]*, [1]*Departments of Histology and Neurobiology, and* [2]*Pharmacology, Karolinska Institute, P.O. Box 60400, S-104 01 Stockholm, Sweden.*

After glucose entries the cell by the aid of a glucose transport protein, it is rapidly converted into glucose 6-phosphate with ATP serving as the phosphoryl donor. In mammals, the phosphorylation of glucose to glucose 6-phosphate is catalyzed by at least four different hexokinases (isoenzymes I-IV). Glucose 6-phosphate is a necessary substrate for several metabolic pathways, including glycolysis, glycogen synthesis and the hexose monophosphate pathway. Recently, the rat brain hexokinase I was cloned. In the present study we have synthesized two complementary 48-mer oligonucleotide probes to rat brain hexokinase I and studied the distribution of its mRNA in the rat central nervous system by *in situ* hybridization. Both probes showed a wide distribution of labelled neurons of the brain. Particulary strong expression was demonstrated in the cerebral cortex, in the CA1-3 regions and dentate gyrus of the hippocampus, in the amygdala, olfactory bulb, thalamus, cerebellum and several brainstem nuclei. Motoneurons of the spinal cord and dorsal root ganglion cells were strongly labelled. Within the hypothalamus, strong expression of hexokinase I mRNA was demonstrated in the supraoptic, paraventricular, arcuate and ventromedial nuclei. Ependymal cells of the choroid plexus expressed hexokinase I mRNA. Expression of hexokinase I mRNA in the central nervous system was also demonstrated in the E15 rat fetus, where dense labelling in addition was observed in the liver, intestine and heart. After adminstration of hyperosmotic stimluli (2% saline in the drinking water) an increase in hexokinase I mRNA could be observed in the hypothalamic supraoptic and paraventricular nuclei. Further studies may show regulation of hexokinase I mRNA in individual brain nuclei after changes in plasma glucose.

500.12

THE EFFECT OF AN ENDOTHELIN RECEPTOR ANTAGONIST ON ENDOTHELIN-1 - INDUCED CONSTRICTION IN CEREBRAL MICROVESSELS.

O. Sagher*, Y. Jin, Q-A. Thai, N.F. Kassell and K.S. Lee. Dept. of Neurosurgery. Univ. of Virginia, Charlottesville, VA 22908.

Endothelin-1 (ET-1) is a potent vasoconstrictor that has been implicated in the pathogenesis of cerebral vasospasm. ET-1 is thought to elicit constriction through activation of a membrane-bound receptor. Two receptor subtypes have been recognized, ET_A and ET_B. The effects of ET-1 and an endothelin receptor antagonist on rat cerebral microvessels were studied in submerged, *in vitro* brain slices using computerized videomicroscopy. Previous data have suggested that the effects of ET-1 on cerebral microcirculation are mediated by ET_A receptors. The effect of cyclo[D-Asp-L-Pro-D-Val-L-Leu-D-Trp] (ETant), an antagonist of ET_A receptors, on ET-1-induced vasoconstriction was examined. When vessels were pretreated with ETant (20nM), ET-1-induced vasoconstriction was effectively blocked. When ETant was administered following an established constriction with ET-1, it exerted a moderate vasodilatory response. These studies indicate that ETant is a potent competitive antagonist of ET-1 in cerebral microvessels, and that it may be able to reverse an established ET-1 induced constriction. Agents such as ETant may be useful in the treatment of ET-1-induced pathological vasoconstriction, such as cerebral vasospasm.

500.13

THE ROLE OF ENDOTHELIUM IN RAT PIAL ARTERIOLAR DILATORY RESPONSES *IN VIVO*

Q. Wang, D.A. Pelligrino*, H.M. Koenig. Dept. Of Anesthesiol., Univ. of Illinois at Chicago, IL 60616.

In fentanyl (25 $\mu g\ kg^{-1}\ hr^{-1}$)/70% N_2O/30% O_2 anesthetized, paralyzed (curare) and mechanically ventilated male Sprague-Dawley rats, we employed a light/dye (LD) *in vivo* endothelial injury model and studied pial arteriolar responses to adenosine 5'-diphosphate (ADP), S-nitroso-acetyl-penicillamine (SNAP), and CO_2. LD treatment consisted of intravenous injection of sodium fluorescein and the illumination (for a period of ~90 sec) of a discrete area of the arterioles with light from a mercury lamp. The diameter changes in 30-60 μm pial arterioles were examined using a closed cranial window system and intravital microscopy/videometry. Before LD treatment, suffusion of 5 x 10^{-6} M Ach, 10^{-4} M ADP, 10^{-5} M SNAP and inhalation of 10% CO_2 increased the pial arterioles by 27 $\pm$ 3 (n = 16), 30 $\pm$ 3 (n = 14), 33 $\pm$ 4 (n = 13) and 32 $\pm$ 3% (n = 16) (means $\pm$ SE). After LD injury, the response of pial arterioles to Ach was completely abolished and the ADP response was reduced to 18 $\pm$ 3% ($p<0.05$). In contrast, SNAP and CO_2 still produced diameter increases (26 $\pm$ 2 and 32 $\pm$ 3%, respectively) not different from control. It was previously reported that ADP induced vasodilation by a NO-dependent mechanism. The partial reduction in the response after endothelium damage suggests that there exists either a non-endothelial source of NO in ADP-induced dilatation or that a portion of the ADP response is NO-independent. The completely normal CO_2 response after endothelium damage suggests that the reported NO-dependence of hypercapnia-induced cerebral vasodilatation/hyperemia in rats cannot be attributed to an endothelial NO source. *Supported by the Juvenile Diabetes Foundation International.*

BRAIN METABOLISM AND BLOOD FLOW: BLOOD FLOW

501.1

METABOLIC CORRELATES OF INTRINSIC OPTICAL SIGNALS IN RODENT CORTEX.

S. Narayan*, A.I.Blood and A.W.Toga

Laboratory of Neuro Imaging, Dept of Neurology, UCLA, Los Angeles CA.

We have investigated the nature of optical intrinsic signals (activity-related optical reflectance changes) in rodent primary somatosensory cortex (S-I).

S-I was exposed in male Sprague-Dawley rats and illuminated at 550 nm, 610 nm and 850 nm. Images were obtained before, during and after contralateral vibrissal deflection or forelimb stimulation. After pixel-by-pixel division of images by controls, intrinsic signals were seen over the posteromedial barrel subfield and forelimb sensory cortex, and included a strong venous component. Signal characteristics differed between wavelength and paradigm. Electrophysiologic recordings were performed using bipolar SNEX electrodes (100 μm diameter), advanced stereotactically at sites inside and outside the zone of reflectance change. Evoked responses were maximal over cortex showing the greatest optical activity, and both measures diminished in tandem. We compared signal localization with the density of cytochrome oxidase staining in 50 μm sections from these subjects, and found that signals may be affected by the activity of this enzyme.

Intrinsic signals have been observed over electrophysiologically active cortex, but commence, peak and extinguish over seconds. In addition to other etiologies[1], our studies indicate that optical reflectance activity in rodent cortex is related to cytochrome oxidase density and microvascular changes.

[1]. Grinvald et al., 1986. Nature (324):36.

501.2

INCREASE IN BLOOD CELL FLUX RATE, VELOCITY AND DIAMETER OF CEREBRAL CAPILLARIES DURING HYPERCAPNIA.

U.Lindauer, A.Villringer*, U.Dirnagl. Dept.of Neurology, Univ.of Munich, 8000Munich 70,FRG

We studied blood cell flux rates, velocities and diameters of cerebral capillaries during normocapnia and changes of these parameters during hypercapnia. Male Wistar rats (n=6) were anesthetized with thiobutabarbiturate (100mg/kg), tracheotomized and artificially ventilated. The left femoral artery and vein were canulated. Systemic arterial blood pressure, endexspiratory pCO_2 and body temperature were monitored continuously and arterial blood gases were measured periodically. A closed cranial window (dura removed) was implanted over the parietal cerebral cortex. Na-fluorescein was injected intravenously (2mg/100g body weight as a bolus, following the same dosage per hour of continuous infusion). We used a confocal laser scanning microscope and measured cerebral capillaries up to 200μm beneath the brain surface. Changes in blood cell flux rates and velocities and capillary diameters were monitored during normocapnia and hypercapnia (5% CO_2 in inspired gas) as a global stimulation of cerebral perfusion. During normocapnia all physiological parameters were within normal ranges. For results during normo- and hypercapnia see table below. Earlier experiments during normocapnia showed, that all capillaries are plasma perfused all the time and only less than 5% are not perfused with blood cells intermittently. More than 50% of all capillaries show blood cell flux rates and velocities in the lower fourth part of the range. During hypercapnia this functional reserve capacity is used, that is the flux rate- and velocity-distribution curve is shifted to the right and capillary diameters are increased ($p<0.05$). Supported by the DFG and the Wilhelm Sander Stiftung.

	pCO_2 (mmHg)	flux-rate (n=36) (cells/s)	velocity (n=14) (μm/s)	diameter (n=38) (μm)
normocapnia	33.25	53.17 ± 48.46	990.29 ± 983.79	4.81 ± 1.10
hypercapnia	50.26	69.19 ± 49.50	1076.86 ± 888.30	5.28 ± 0.93

501.3

CHANGES IN BLOOD FLOW AND VASCULAR RESISTANCE OF CHOROID PLEXUSES OF POSTNATALLY DEVELOPING RATS. C.E. Johanson*, J. Szmydynger-Chodobska, and A. Chodobski. Program in Neurosurg., Dept. Clin. Neurosci., Brown University and R.I. Hospital, Providence, RI 02903.

Postnatal developmental changes in blood flow to choroid plexuses (CPs) of the lateral (LVCP) and fourth (4VCP) ventricles were studied in pentobarbital-anesthetized and artificially ventilated rats at the age of 2, 3, 5, and 7–8 wk. Blood flow was measured by the indicator fractionation method with ^{125}I-*N*-isopropyl-*p*-iodoamphetamine used as the marker. The period between the third and fifth week after birth was associated with a pronounced increase in choroidal blood flow. At the earliest stages of development analyzed in this investigation (between the second and third week postnatally), blood flow to both LVCP and 4VCP was low (ie, 2.5–2.8 ml g^{-1} min^{-1}) and did not change significantly. There was also no difference in blood flow values between the two CPs at 2 wk vs 3 wk. However, during this same period of development, the vascular resistance of both CPs increased by 62–65% (P<0.01), which was associated with concomitant elevation of arterial blood pressure (from 57±1 to 92±2 mmHg, P<0.01). From the age of 5 wk on, blood flow to 4VCP was higher than that to LVCP. These two CPs seemed to differ not only in regard to their absolute blood flow values, but also in regard to the rate of maturation of their vascular beds during ontogenesis. Thus, in 5-wk-old rats, blood flow to LVCP was similar to that of adult animals (3.29±0.09 vs 3.30±0.07 ml g^{-1} min^{-1}), whereas 4VCP at this postnatal age was still less perfused than fully matured tissue (3.74±0.11 vs 4.12±0.08 ml g^{-1} min^{-1}, P<0.05). The postnatal developmental changes in choroidal blood flow found by us correlate well with a progressive increase in choroidal ion transport and enzyme activity observed in rats after birth. These changes in choroidal blood flow represent a continuing adjustment of the choroidal vascular system to steadily increasing CSF secretory capabilities of maturing choroidal epithelium. The increase in choroidal vascular resistance observed between the second and third wk after birth may indicate the presence of the efficient blood flow autoregulatory mechanisms as the growth of choroidal vascular bed is probably slow during this period of development. Supported by NIH Grant NS 27601.

501.4

CORTICAL BLOOD FLOW (CBF) INCREASES ELICITED BY CONTINUOUS OR DISCONTINUOUS ELECTRICAL STIMULATION OF THE RAT SUBSTANTIA INNOMINATA (SI). F. Dauphin, P. Lacombe, E.T. MacKenzie* and J. Seylaz. CNRS ERS 19, Caen and CNRS URA 641, Faculté de Médecine Villemin, Paris, France.

Discrepancies on the inhibitory effect of muscarinic receptor blockade on CBF increases elicited by electrical stimulation of the SI (Dauphin et al., Brain Res 553, 75, 1991; Arneric, Soc Neurosci Abs 14:488.2, 1988) led us to postulate differences between the two stimulation procedures (continuous (CS): 100 Hz, 0.5 ms, 50 µA, 10 s; discontinuous (DS): 100 Hz, 0.5 ms, 1 s on/1 s off, 0-100 µA, 1-20 min) so far investigated. We thus compared the effects of the nitric oxide synthase (NOS) inhibitor L-NAME on the CBF increases, as measured by laser-Doppler flowmetry, induced by SI CS and DS in the anesthetized rat (chloralose-urethane). First, both intracerebral stimulation paradigms resulted in CBF increases, however with different magnitude (+312 % for CS and +137 % for DS). Second, consistently sustained vasodilatation was obtained in the DS protocol for durations up to 20 min; at the end of a stimulus, CBF rapidly decreased, reaching resting level within 20-30 s. Third, when applied repeatedly with interstimulus time < 15 min, a fatigue phenomenon to CS was observed with the CBF response (up to -80%) while this effect was much less intense (-10%) for DS. Crossed investigations revealed that DS reduced the effect of CS (up to -69%), while no changes could be evidenced for the reverse experiments. Fourth, L-NAME (10 mg/kg, i.v.) induced a major (~60%) and long lasting blockade (> 160-180 min) of both CBF increases. Such data demonstrate that although different cholinergic mechanisms seem to be involved in the two stimulation paradigms, blockade of NOS induces similar reductions of SI-elicited cortical vasodilatation.

501.5

CEREBRAL BLOOD FLOW REDUCTION AND TRANSIENT CBF INCREASES INDUCED BY GLUCOSE AND MANNITOL: A LASER-DOPPLER STUDY. Robert B. Duckrow.* Department of Neurology, The University of Connecticut School of Medicine, Farmington, CT 06030.

Cerebral blood flow (CBF) is reduced during acute hyperglycemia in awake restrained rats. Previous studies suggest that this reduction is not related to increased plasma osmolality or secondary to changes in hematocrit, viscosity, glucose utilization, or hyperinsulinemia. However, previous measurements of CBF were performed using iodoantipyrine, which provides a single measurement of flow integrated over a 30 s period. To remove this sampling restriction, CBF was measured during acute hyperglycemia in the anesthetized rat using continuous laser-Doppler flowmetry. After induction of pentobarbital anesthesia, the rat was fixed in a stereotactic frame and the parietal bone was thinned with a dental burr. Body temperature was maintained and arterial pressure, pH, gas tensions, hematocrit, and osmolality were monitored. A Vasamedic BPM^2 0.8 mm laser-Doppler probe was placed on the bone window away from dural vessels. Cortical CBF was measured relative to the zero flow reading obtained after death. Acute hyperglycemia was induced by intraperitoneal injection of 25% D-glucose, 4 gm/kg. An equal amount of mannitol was given to control rats. Maximum plasma osmolality was 349 mOsm. CBF decreased 19% 10 minutes after either glucose or mannitol injection. In addition, brief transient increases of CBF with peak magnitude 2 to 4 times baseline level occurred 25 s to 13 min following glucose or mannitol injection (mean latency: glucose, 6 min; mannitol, 8 min) These CBF transients occurred in 8 of 14 rats given glucose and in 9 of 10 rats given mannitol. They were not accompanied by EEG suppression, otherwise seen during spreading cortical depression induced by needle penetration of the cortex. They did not occur spontaneously and were not suppressed by pretreatment with indomethacin or Nω-nitro-L-arginine methyl ester. I conclude that reduced CBF measured during acute hyperglycemia in anesthetized rats may be directly related to increased plasma osmolarity. Also, transient CBF increases may confound discontinuous measures of CBF during acute hyperosmolar states. (Supported by PHS NS24109)

501.6

ESTIMATION OF RAT CEREBRAL BLOOD FLOW (CBF) DURING +G_z CENTRIFUGE EXPOSURES LEADING TO G-INDUCED LOSS OF CONSCIOUSNESS (G-LOC). P.M. Werchan[1], R.M. Echon[2], J.A. Barber[2], S. Galindo[2], A.R. Shahed[1].* [1]Armstrong Laboratory, Brooks AFB TX 78235-5104, [2]KRUG Life Sciences, San Antonio TX.

Research is currently in progress using a small animal centrifuge to identify causative factors leading to G-LOC (isoelectric EEG) during short duration high +G_z (head-to-tail inertial load) exposures. Previous measurements of the rat cerebral energy state at the point of G-LOC (10-15s) are consistent with static bench-top models of global cerebral ischemia (P.M.Werchan and A.R.Shahed. The Physiologist, 35(1); 1992.). However, it remains unclear if CBF completely ceases at the point of G-LOC. Four rats were chronically implanted with EEG electrodes and flowprobes on the common carotid artery (CA). Rats were given nine consecutive 30s exposures of 5 to 25 +G_z (increment of 2.5 +G_z). During all +G_z exposures, CA blood flow (CABF) was significantly reduced (45-100%) within the first 5 sec of onset and completely ceased by the end of the 20 to 25 +G_z exposures. G-LOC was observed only during the 22.5 and 25 +G_z exposures at 13.6 ± 3 sec. Hyperemia was observed within 5 sec after deceleration at all +G_z levels. The magnitude of hyperemia increased as the +G_z level increased up to 17.5 +G_z, but was significantly less at higher +G_z levels. It is concluded that a cessation of CABF of sufficient duration is required to elicit G-LOC in rats. Furthermore, the reduction in the magnitude of the hyperemic response at higher +G_z exposures indicates a compromised cardiovascular recovery.

501.7

QUANTITATIVE MATCHING AUTORADIOGRAPHIC IMAGES AND AVERAGING TECHNIQUES IN CEREBRAL BLOOD FLOW STUDIES W. Zhao*, K. Takagi, D.W. Smith and M.D. Ginsberg CVDRC (D4-5), Dept. of Neurology, Univ. of Miami, Sch. of Med., P.O. Box 016960 Miami, FL, 33101.

In the analysis of replicate autoradiographic studies of animals studied under controlled experimental conditions, the ability to average corresponding coronal levels from several animals would permit one to perform region-of-interest (ROI) analysis on the average image; this would increase the precision of analysis and diminish inter-pixel noise. We have employed a newly developed software package which provides efficient and accurate image-matching and alignment capability. Corresponding coronal sections from n animals are matched by a disparity analysis algorithm. Each section is linearly transformed using with bilinear interpolation to reduce quantitation errors. "Mean" and "standard deviation" images can then be easily obtained. We validated this approach in 3 control rats undergoing ^{14}C-iodoantipyrine cerebral blood flow studies. CBF values were compared from six regions read both in individual brains and on the computed mean and SD images (Table). This approach facilitates analysis while preserving accuracy and precision.

Region #	1	2	3	4	5	6
$\bar{X}_{1,2,3}$	1.37	1.23	1.48	1.29	1.51	1.54
$SD_{1,2,3}$	0.29	0.33	0.27	0.20	0.37	0.50
$X_{Avg.Img.}$	1.33	1.10	1.49	1.35	1.41	1.48
$SD_{Avg.Img.}$	0.25	0.27	0.17	0.12	0.57	0.65
% off $\bar{X}_{1,2,3}$ vs $X_{Avg.Img.}$	2.90	10.6	1.00	4.70	2.60	3.90

501.8

EFFECT OF ELECTRICAL STIMULATION OF THE DORSAL RAPHE NUCLEUS ON LOCAL CEREBRAL BLOOD FLOW IN RAT. *M.J. Bakalian*, M. D. Underwood, V. Arango, L. Peton, and J. J. Mann.* Laboratories of Neuropharmacology, University of Pittsburgh, Pittsburgh 15213.

Electrical stimulation of the dorsal raphe nucleus (DRN) elicits heterogeneous cortical blood flow responses as measured by laser-Doppler flowmetry; stimulation of rostral DRN decreases cortical blood flow and stimulation of caudal DRN increases cortical blood flow (*JCBFM.*, 1992;12:664-673). We sought to examine the distribution of local cerebral blood flow (LCBF) responses to electrical stimulation of the rostral DRN using quantitative autoradiographic methods.

Rats were anesthetized (α-chloralose), paralyzed and artificially ventilated. Arterial pressure (AP), heart rate and blood gases were continuously monitored and controlled. The rostral DRN was stimulated electrically (1s on/ 1s off, 200 Hz, 100 µA) and AP was maintained as needed by exsanguination. LCBF was measured in 32 regions autoradiographically using ^{14}C-iodoantipyrine (IAP) as tracer. Following infusion of IAP, the brain was quickly removed, frozen and sectioned (20µm). The stimulation sites were verified histologically from Nissl-stained sections.

In unstimulated controls (n=8), LCBF ranged from 139±11 ml/100g/min in the nucleus tractus solitarius to 49±5 ml/100g/min in corpus callosum. Following DRN stimulation (n=6), LCBF was significantly reduced in 17 of the 32 regions (p<0.05, grouped t) compared to controls and ranged from 61±7% of control in the presubiculum to 76±6% of control in corpus callosum. Greater LCBF changes were observed in forebrain (61-76% of control) than hindbrain (65-99% of control) regions. Increased LCBF was not observed in any region. Arterial pH, pCO_2, hematocrit and heart rate were not different between groups (p>0.05).

Electrical stimulation of the rostral DRN elicits widespread decreases in LCBF. DRN neurons, possibly serotonergic, may be involved in the neurogenic regulation of cerebral blood flow throughout brain. (Supported by NARSAD and MH46745.)

501.9

HUMAN RCBF CORRELATES OF EEG FREQUENCIES BEFORE AND AFTER IV PROCAINE. P. Parekh, J. Spencer, M.S. George, D.S. Gill, T. Ketter, and R.M. Post*. Biological Psychiatry Branch, NIMH, NIH, Bethesda, MD 20892.

Introduction: The regional metabolic and blood flow changes associated with EEG frequencies in man are poorly understood.

Method: In a single-blind fashion, we simultaneously sampled rCBF (using ^{15}O-water PET) and recorded quantitative EEG in 23 healthy controls during injection of saline (baseline) and intravenous procaine. Raters (JS, DSG, PP) then meticulously screened the EEG data, rejecting subjects with prominent muscle or movement artifact, and included for analysis only artifact-free epochs in 7 subjects. Quantitative spectral EEG data from leads O2 and T6 were then correlated with each subject's PET rCBF values on a pixel-by-pixel basis, both at baseline and after high dose procaine. PET scans had been normalized across subjects and non-linearly fitted to the Talaraich atlas.

Results: Injection of IV procaine increased omega activity and reduced alpha activity across all subjects, when compared with the baseline saline injection. At baseline, resting alpha and omega activity positively correlated with L frontal cortex rCBF. Following procaine, alpha activity failed to significantly correlate with rCBF, while omega activity positively correlated with increasing rCBF in the L caudate. The change in omega activity from baseline to high dose positively correlated with rCBF increases in the L mesial temporal cortex (amygdala). (All correlations are for both O2 and T6 and $p<0.001$).

Conclusion: Human EEG-rCBF correlational maps may aid in understanding the neurobiological basis of EEG at rest and after pharmacologic neuroactivation.

501.10

EVALUATION OF CEREBRAL BLOOD FLOW USING RADIOACTIVE Xe^{133} AFTER LONG TERM SURVIVAL OF ANIMALS EXPOSED TO PROFOUND HYPOTHERMIA AND COMPLETE BLOOD SUBSTITUTION. A.Elrifai, J.Bailes, S.Govindan, E.Teeple, M.Taylor, S.Shih, M.Leavitt, M.Adatepe and J.Maroon*. Allegheny-Singer Research Institute and Allegheny General Hospital, Pittsburgh, PA 15212.

rCBF was measured in dogs prior to and one week after exposure to a protocol of profound hypothermia $<10°C$ and complete blood substitution (Cryomedical Sciences, Inc.) to extend cardiac arrest time for >3hr. A group of animals ($n=6$) were observed for up to 80 weeks, 48 ± 20 (Mean$\pm$SD) & the rCBF was measured using a modified NOVO 10a Cerebrograph using two detectors on each side; the remaining detectors were shielded. Animals were anesthetized (Pentothal), endotrachealy intubated & connected to the cerebrograph. ECG, heart rate, respiration and tidal volume were monitored. The end tidal PCO_2 was kept at 35-40 mmHg. Radioactive Xe^{133} (Dupont Pharmaceuticals) was mixed with low dissolved oxygen saline (LDO). Following registration of background activity for 0.5 min, a bolus of Xe^{133} in saline, 15.5 ± 1.9 mCi, was injected in a cephalic vein. Xe^{133} clearance was recorded throughout 11 minutes and expired air was monitored to supply the air curve. CBF results were expressed in ml/100gm/min as the initial slope index between 30 and 90 sec, as CBF15 and as the fast compartment flow F1. Corrections for hemoglobin and CO_2 were not instituted. Hemispheric CBF was treated as the average value of the 2 detectors on each side & an average for each animal was calculated. ANOVA showed no difference between pre- vs early (≈1 week) post- or late (≈1 year) post- hypothermia CBF. $P>.05$ was considered not significant.

501.11

LOCAL CEREBRAL BLOOD FLOW IN RAT TRIGEMINAL (WHISKER) SYSTEM.
L. Wei,[1] J. Fenstermacher,[1] C. Rovainen,[2] T.Woolsey,[3] and R. Grubb[3]*
[1]Department of Neurological Surgery, SUNY at Stony Brook, NY 11794 and Departments of [2]Cell Biology and Physiology and of [3]Neurology and Neurological Surgery, Washington University School of Medicine, St. Louis Missouri 63110.

Changes in local cerebral blood flow(LCBF) with general physiological challenges are well known for different structures in the brain. However, relatively little attention has been paid to LCBF in or changes that occur in particular neural systems. We measured LCBF at rest, with hypoxia (10% O_2) and hypercarbia (8% CO_2) by ^{14}C iodoantipyrine autoradiography in the rat trigeminal (whisker) pathway (V). Structures measured include: V nerve and tract; V motor root and nucleus; V spinal nuclei and principal nucleus; V thalamus and cortex. Control values for white matter were $\approx$ 40 ml/100g/min; these increased 30-40% with hypoxia and from 2-3 fold with hypercarbia. Control values for LCBF in gray matter throughout the V system were $\approx$110-160 ml/100g/min which increased 60-120% with hypoxia and 3-4 fold with hypercarbia; flow changes were greatest for the cortex. Control values for cortex will be compared to LCBF measured from videomicroscopy and H_2 clearance. Their correspondence to changes in local metabolism, measured by glucose analogs, will also be considered. Such information is important for relating one-time measurements of LCBF throughout the brain to dynamic changes observed on the brain surface.

Supported by NIH Grants NS 26004, HL 41074 and NS 28781 and The McDonnell Center for Studies of Higher Brain Function.

501.12

LOCAL CEREBRAL BLOOD FLOW IN RAT SOMATOSENSORY CORTEX DURING WHISKER STIMULATION.
J. Dowling,[1] Y. Moskalenko,[2] D. Liu,[1] C. Rovainen[3]*, M.E. Spence[3], T. Woolsey [1]. Depts of [1] Neurology and Neurological Surgery and of [3] Cell Biol. and Physiol., Washington Univ. Sch. of Med., St. Louis, MO 63110 and [2] Sechenov Institute of Evolutionary Physiol. and Biochem., Acad. of Sci. of Russia, St. Petersburg, K-223, Russia.

H_2 clearance methods were used to demonstrate changes in local cerebral blood flow (LCBF) with CO_2 inhalation and whisker stimulation. An array of four small Pt electrodes were inserted into or around the rat barrel cortex. H_2 electrolytically generated at a constant level from one electrode was detected by the other three electrodes. Changes in LCBF produced inversely proportional changes in the level of detected H_2. These changes were calibrated against standard H_2 clearance curves to provide quantitative estimates of LCBF. CO_2 inhalation produced generalized LCBF increases, whereas contralateral whisker stimulation produced smaller increases more localized in the barrel cortex. Stimulation of one row of whiskers or a single whisker resulted in even smaller, but appropriately localized responses. Minimal latency in LCBF responsewas 0.75 s after stimulation (mean latency =2.70 s, SD=1.60, n=126). Thus, measuring H_2 clearance in the rat barrel cortex is a useful method for testing the coupling between blood flow and neural activity. In particular, it provides sensitive, continuous monitoring of focal CBF changes within brain parenchyma itself.

Supported by NIH Grants TW 00053, NS 17763, NS 28781, and HL 41074, the McDonnell Center for Studies of Higher Brain Function and an award from the Spastic Paralysis Foundation of the Illinois-Eastern Iowa District of the Kiwanis International.

501.13

CEREBRAL HEMODYNAMICS IN MOTOR AND SOMATOSENSORY STIMULATION MEASURED WITH TRANSCRANIAL DOPPLER SONOGRAPHY. R. J. Seitz*, M. Sitzer, R. Baumgartner. Department of Neurology, Heinrich-Heine-University, 4000 Düsseldorf 1, FRGermany.

We studied the time course of cerebral blood flow velocity (CBFV) in the middle cerebral artery (MCA) using transcranial doppler sonography (TCD) during physiological stimulation of the ipsilateral sensorimotor cortex. Twenty healthy male right-handed subjects (17 to 35 years) were examined during blind-folded performance of right hand somatosensory discrimination, finger sequences, and vibratory stimulation. After an initial five-mins episode of rest three stimulation sessions were alternated with rest of two mins each. Continuous TCD recordings were performed with a 2 MHz pulsed-wave range-gated Doppler device (TC 2000S, EME), the end-expiratory pCO_2 was measured on-line using a face mask and a DRÄGER CO_2-monitor. Somatosensory discrimination induced a significant increase of the mean CBFV (7.6 +/- 2.9 (SD) cm/sec, $p<0.05$) in the left MCA compared to rest (58.7 +/- 9.1 (SD) cm/sec). No significant changes of the mean CBFV occurred in finger sequences and vibratory stimulation. In all three activations, the CBFV raised initially with a maximum 3.3 +/- 1.6 (SD) seconds after stimulation onset. The first maximal CBFV increase (20.9 +/- 7.3 %) was significantly higher in the first stimulation session compared to the third ($p<0.02$), whereas the mean CBFV was not significantly lower in the third compared to the first stimulation task for each type of activation. In the two tasks involving finger movements the breathing and heart rates per minute accelerated significantly ($p<0.01$) but were unrelated to the mean CBFV. Our data agree with the time course of task-specific signal changes in deoxy-hemoglobin magnetic resonance imaging and substantiate measurements of regional cerebral blood flow with positron emission tomography.

502.1

BRAIN INCORPORATION OF ^{11}C-ARACHIDONATE IN NORMOCAPNIC AND HYPERCAPNIC MONKEYS: A PET STUDY. T. Arai, M.C.J. Chang, L.M. Freed, S. Wakabayashi, M.A. Channing, B.B. Dunn, M.G. Der, J.M. Bell, W.M. Williams*, P. Herscovitch, W.C. Eckelman and S.I. Rapoport. Lab of Neurosciences, NIA and PET Dept, CC; NIH, Bethesda, MD 20892.

Our lab has developed an *in vivo* method in rats to quantitatively study brain lipid metabolism following intravenous injection of radiolabeled long chain fatty acids (FAs). Arachidonic acid (AA) is selectively incorporated in the sn-2 position of phosphatidyl-inositol and -choline, and its metabolism and turnover in brain is correlated to functional activity. To extend the FA method to primates, we used ^{11}C-AA (33-72 mCi) with positron emission tomography (PET) to measure the regional rates of incorporation in isoflurane-anesthetized rhesus monkeys (n=6). For comparison, incorporation rate into brain, using ^{14}C-AA (170 μCi/kg), was determined biochemically in isoflurane-anesthetized rats (n=3). In paired studies (n=3), the incorporation rate constant (k) at normocapnia and hypercapnia (to increase cerebral blood flow, CBF) in monkey and rat was also determined. Brain radioactivity in monkey was constant after 5 min infusion of ^{11}C-AA (corrected for ^{11}C-CO2: 0.4 and 0.6x plasma concentration in normocapnia and hypercapnia, respectively). Plasma ^{11}C-CO2 was 6.5%$\pm$2.3 at 30 min. Mean$\pm$SEM k (10-30 min), for temporal-, occipital-, frontal-, parietal-cortex, white matter and whole brain was 1.2$\pm$0.1, 1.3$\pm$0.1, 1.2$\pm$0.1, 1.2$\pm$0.1, 0.9$\pm$0.1 and 1.2$\pm$0.1 x10^{-4} ml/sec.ml, respectively. No significant difference in k, for any regions, was seen between normocapnia and hypercapnia in the monkey. For the rat, whole brain k, 3.1$\pm$0.1 x10^{-4} ml/sec.g, was not significantly different from that determined with hypercapnia. These results indicate that the incorporation rate coefficients for AA in monkey and in rat brain are comparable and independent of CBF. The the FA method with PET has the potential to be used in humans.

502.2

EFFECT OF METHYL PALMOXIRATE (MEP) ON INCORPORATION OF PALMITATE INTO MONKEY BRAIN WITH PET. M.C.J. Chang, S. Wakabayashi, T. Arai, M.A. Channing, B.B. Dunn, M.G. Der, P. Baldwin, J.M. Bell, P. Herscovitch, W.C. Eckelman and S.I. Rapoport*. Lab of Neurosciences, NIA and PET Dept, CC; NIH, Bethesda, MD 20892.

Our lab has developed an *in vivo* method to quantify regional rates of incorporation into brain phospholipids (PLs) of intravenously injected radiolabeled long chain fatty acids (FAs). The rates of formation and turnover of brain PLs reflect functional activity and structural integrity of brain. Radiolabeled palmitic acid (^{11}C-PA), which is selectively incorporated into phosphatidylcholine in the sn-1 position, was used with positron emission tomography (PET) to measure regional rates of incorporation in the brain of isoflurane-anesthetized rhesus monkey (n=6). For PA, this method has limitations because the tracer is readily oxidized to produce unincorporated metabolites in brain (^{11}C-glutamine, ^{11}C-glutamate and ^{11}C-CO_2). In an attempt to increase the incorporation rate of ^{11}C-PA into brain lipids, an inhibitor of ß-oxidation (MEP, McNeil Pharm.), was administered (iv) 2 hr before infusion of the radiotracer. In rats, MEP has been shown to enter the brain and allow 85% of ^{14}C-PA to be incorporated into brain lipids. The incorporation rate constant (k) mean$\pm$SEM, calculated between 10-30 min, for temporal-, occipital-, frontal-, parietal-cortex, white matter and whole brain was 4.4$\pm$0.3, 3.6$\pm$0.3, 3.9$\pm$0.2, 4.2$\pm$0.2, 2.2$\pm$0.3 and 3.5$\pm$0.2 x10^{-5} ml/sec.ml, respectively. In a matched study, the percent plasma ^{11}C-CO_2 in vehicle- and MEP-treated monkeys reached 40.8%$\pm$3.3 and 19.1%$\pm$2.1, respectively, by 60 min. With the exception of white matter, k was significantly increased (35-40%) by MEP in all regions. These results demonstrate that regional incorporation rates of ^{11}C-PA in brain can be measured with PET, and that MEP may be useful with ^{11}C-PA for studying brain lipid metabolism *in vivo* in humans by PET.

502.3

REGIONAL BRAIN GLUCOSE METABOLISM IN HABITUATION TO REPEAT PET SCANS. H. Szechtman*, S. List, R. Kaplan, S. Franco, B. Szechtman, G. Firnau, C. Nahmias and E.S. Garnett. Depts of Biomedical Sciences, Psychiatry, and Nuclear Medicine, McMaster Univ, Hamilton, Ontario, CANADA, L8N 3Z5.

Positron Emission Tomography (PET) studies often involve repeated scans on the same individual to compare a test condition or intervention with a 'resting' or 'baseline' state (eg., Cleghorn *et al.*, Psychiatry Research: Neuroimaging, 40:135, 1991). The present study assesses what changes in relative regional brain glucose metabolism are attributable to experiencing successive scans *per se*. Cerebral accumulation of ^{18}F-fluorodeoxyglucose was measured by PET on two successive occasions 1 week apart in 8 healthy male volunteers lying quietly with eyes closed. Statistical analysis using the method of Friston *et al.* (JCBFM, 11:690, 1990) revealed that on the second scan, the left orbitofrontal cortex was significantly more active, and the hippocampus and hippocampal gyri (bilaterally) significantly less active, compared to activity on the first scan. No significant differences between two scans were observed when scan order was randomized. These findings suggest that a process of habituation is associated with repeat PET scans, possibly involving learning to suppress emotional-autonomic responses. (Supported by MRC and OMHF. HS is a Research Associate of the Ontario Mental Health Foundation).

502.4

DO WOMEN HAVE MORE ACTIVE BRAINS THAN MEN? A PET STUDY DURING COGNITIVE ACTIVATION. **G. Esposito*, D. R. Weinberger, J. D. Van Horn, J. L. Ostrem, K. F. Berman. Clinical Brain Disorders Branch, NIMH, Washington, D.C. 20032.**

A number of studies of sex related differences in cerebral blood flow or metabolism have suggested that women have higher values than men. However, not all studies agree. One explanation for these inconsistencies may be that cognitive conditions can influence the results. To examine this possibility we used the oxygen-15 water technique to study rCBF with positron emission tomography in fourteen normal volunteers, eight men and six women, during the performance of six tasks: three neuropsychological tasks linked to frontal cortex and three sensorimotor control tasks. The cognitive tasks were the Wisconsin Card Sorting Task (WCS), Delayed Alternation Task (DA) and Delayed Response Task(DR). A separate sensorimotor control task was designed for each frontal task. Data were collected on the Scanditronix 2048-15B scanner which has in-slice and axial resolution of 6-6.5 mm after reconstruction.

A main effect of gender on global CBF (ml/min/100 gm) was found (grand means across all conditions: women 60.9, men 53.2; ANOVA F = 9.35, p<.01). However, post hoc contrasts demonstrated that gender differences (women>men) significant at or above the p=.05 level were seen during the three neuropsychological tasks (WCS: 63.9$\pm$6 vs. 56.3$\pm$7; DA: 62.2$\pm$4 vs. 52.2$\pm$5; and DR: 62.2$\pm$6 vs. 51.5$\pm$6). Trends in the same direction were seen during two of the sensorimotor control tasks: WCS control (58.4$\pm$5 vs. 51.4$\pm$9; p=.10); and DA control (60.2$\pm$9 vs. 52.0$\pm$5; p=.06). No such trend was noted during the DR control (58.4$\pm$7 vs. 55.6$\pm$8; p=.52).

Data support the notion that higher rCBF levels in women compared to men can be demonstrated. However, these data also suggest that the cognitive state of the subject can affect the results.

502.5

A Linear Regression Approach to the Assessment of rCBF Change in Cognitive Paradigms John D. Van Horn* Alex Terrazas, Karen Faith Berman, Terry E. Goldberg, Daniel R. Weinberger, Neurosciences Center at St. Elizabeth's, 2700 Martin Luther King Jr. Ave. SE, Washington DC 20032

The search for optimal methods for the statistical comparison of positron emission tomographic (PET) data has generated much debate in the functional brain imaging literature. A particular problem has been that the large intra- and inter-individual differences in global blood flow or metabolism that are generally observed obscure more subtle regional changes. Therefore, data transformation is often needed in order to "correct" or "adjust" PET data for differences in global activity. While no "best" method has yet been clearly established, a number of techniques have been presented (e.g. ratio of CBF to the whole brain mean; ANCOVA; Scaled Profile; etc.). We present a statistical approach that uses linear regression to predict the relationship between rCBF and global blood-flow. The regression equations computed for subjects performing a control task may be used to predict rCBF during a cognitive task. Statistical differences in residual values between observed and expected rCBF are indicative of changes in rCBF due to task performance. The advantages of this method include its ability to compare several groups to a single predictor, its ability to contrast different tasks, and its suitability for examining within- and between- group rCBF data.

502.6

GLYCOLYSIS INDUCED DISCORDANCE BETWEEN DEOXYGLUCOSE-BASED AND GLUCOSE-BASED ESTIMATES OF LOCAL CEREBRAL METABOLIC RATE IN CORTICAL SPREADING DEPRESSION.

R.F. Ackermann* and J.L. Lear. Nuclear Medicine, UCLA School of Medicine, Los Angeles CA 90024; Nuclear Medicine, Health Sciences Center, Denver CO 08262.

We previously reported that radiolabeled glucose (GLC)-based metabolic rate estimates normally are similar to the corresponding radiolabeled deoxyglucose (DG)-based estimates, but are strikingly dissimilar in activated structures; DG-based labeling was markedly elevated, while GLC-based labeling was hardly elevated in the same structures. Here, we used the same dual-label autoradiographic method to study spreading depression induced with KCl applied unilaterally to the neocortex of rats. The resulting DG images revealed marked hypermetabolism in the KCl-treated cortex, accompanied by hypometabolism in the functionally deafferented ipsilateral striatum and thalamus. The corresponding GLC images also revealed hypometabolism in the same subcortical structures, but nevertheless failed to register the KCl-induced cortical hypermetabolism. Much literature suggests that the increased cortical DG labeling must be due to the accelerated Na/K ATPase activity induced by the high extracellular K ion levels, and also suggests that the ATP used by the Na/K pump is derived specifically from glycolysis. Thus, we and others have hypothesized that GLC fails to register this increased glycolytic flux due to "overproduction" of labeled pyruvate, with subsequent clearance of labeled lactate.

502.7

CMR_{glc} during cortical spreading depression: Assays with [6-^{14}C]glucose or [^{14}C]deoxyglucose. K. Adachi, N. Cruz, L. Sokoloff, and G. Dienel*, Lab. of Cerebral Metabolism, NIMH, Bethesda, MD 20892.

It is assumed that all metabolites of [6-^{14}C]glucose are trapped in the brain during the routine 5 min experimental period, and that [6-^{14}C]glucose is the preferred tracer to assay rates of glucose utilization ($lCMR_{glc}$) in brain because errors in the steady-state assumption are minimized (Hawkins et al., *Am. J. Physiol.* **248:** C170, 1985). To test these assumptions, rates of metabolism of ^{14}C-labeled glucose and deoxyglucose (DG) were compared in rats with unilateral spreading depression during a 5 min experimental period for both tracers. Spreading depression was induced by application of 5 M KCl to the intact dura at 15 min intervals. After the second application, [^{14}C]hexose was injected i.v., and samples of arterial blood were drawn at timed intervals for 5 min. Then the rats were anesthetized with thiopental (20 mg/kg), and their brains were frozen *in situ*. Portions of each hemisphere of each brain were dissected out in a cryostat at -25°C, weighed, extracted with ethanol, and assayed for their contents of labeled hexose and products by anion exchange HPLC. The remaining portions of each brain were cut into 20 μm sections, exposed to x-ray film, and their total ^{14}C contents assayed by quantitative autoradiography. Left-right differences in total ^{14}C contents of the cerebral cortex, whether measured by autoradiography or direct chemical measurement, were rather small when glucose was used as the tracer (10 ± 1%, n = 3) (mean ± SD). Increases in the ^{14}C contents of experimental hemisphere were, however, easily detected with DG (50 ± 13%, n = 4). When precursor and products were separated by HPLC and assayed, a small rise in the metabolism of [^{14}C]glucose was found in the experimental hemisphere (14 ± 16%, n = 6), but the increase was much smaller than that observed with DG (66 ± 46%, n = 5) (corrections were made for changes in the lumped constant). These results demonstrate that stimulation of $lCMR_{glc}$ is registered by DG within 5 min, and they suggest that labeled products of [6-^{14}C]glucose are lost from the brain.

502.8

A CORTICAL VOLUME PATTERN ANALYSIS OF "ON-OFF" HALOPERIDOL FDG PET DATA. E.A. Gastineau, C.A. Tamminga, H.H. Holcomb, B. Gordon*, H.L. Loats. Maryland Psychiatric Research Center, University of Maryland, Baltimore MD 21228, Loats Associates, Inc., Westminster, MD 21158

Traditional Region of Interest (ROI) sampling of functional images such as FDG-PET have been on acquired axial slices. Reconstructing these slices into a volume allows for dynamic interaction with the data to render slices through any plane and generate surfaces. Here, an MRI and 3 FDG-PET scans were acquired from 8 patients enrolled in an on-off-off haloperidol experiment. PET and MRI images were registered by overlaying the three orthogonal midplanes and adjusting the PET for X,Y,Z, translation and roll pitch and yawl, matching the functional image to the anatomy of the MRI. Lateral surfaces were created for both the MRI and PET data sets using methods previously described (Gastineau, Neurosci Abst. 18:726, 1992). Atlases were described for the surfaces and midbrain area using the anatomy of the reconstructed MRI and applied directly to the associated PET studies. Volume measurements from the atlases were correlated for "on" and "off" drug PET data. Spatial distribution of the rCMRglu values for a prescribed volume were compared across subjects for activity patterns. Registered "on" and "off" volumes were subtracted and the subsequent difference patterns analyzed. Anatomical sampling based on reconstructed volumetric MRI data provides precise location for functional image sampling and shows the variability of activity within a prescribed area. With the method described, volumetric as well as spatial relationships may be much more accurately reported.

502.9

THE RELIABILITY OF RELATIVE REGIONAL METABOLIC VALUES USING PET-FDG. D.R. Medoff, A. Summerfelt, H.H. Holcomb*, C.A. Tamminga. Maryland Psychiatric Research Center, University of Maryland, Baltimore MD 21228

One factor that makes the analysis of PET/FDG imaging data challenging is the influence of overall global metabolism. Global cerebral metabolism can be affected by a variety of factors both experimental and incidental. The appropriate statistical technique to control for this global factor depends on a number of factors, which may or may not always be discernible. In this study we have compared the reliability of four different methods of controlling the global factor. Global factors were controlled by: ratios of absolute regional level to whole brain grey, z-scores, residual scores, and difference scores. Test-retest reliabilities, interrater reliabilities and functional-anatomical sampling reliabilities were compared for fourteen regions of interest using the four different methods of transformations. Interclass Correlation Coefficients (ICC's) were compared across transformation method. For all regions, the ICC's for transformed scores were much lower than the ICC's for the absolute metabolic data. Although some regions had similar ICC's across transformation method, for certain regions (e.g. hippocampus, occipital cortex) the transformation method had a significant influence on the reliability. The reduced reliability of relative regional metabolic values and the influence of the type of transformation must be taken into consideration when attempting to interpret PET-FDG studies.

502.10

RESTING STATE BRAIN GLUCOSE METABOLIC CORRELATES OF SUSTAINED ATTENTION CAPACITY IN HEALTHY AGING. A. Berardi*, F. Gaillard, R. Parasuraman, P. Greenwood, C.L. Grady, J.V. Haxby. Lab. of Neurosciences, National Institute on Aging, NIH, Bethesda, M.D. 20892; Institute of Applied Psychology, Univ. of Lausanne, 1004 Lausanne-CH; and Cognitive Science Lab., Catholic Univ. of America, Washington, D.C. 20064.

Positron Emission Tomography (PET) brain activation studies of sustained attention in normal subjects using ^{15}O or ^{18}FDG have found consistent increases in regional cerebral blood flow (rCBF) or in regional cerebral metabolic rates for glucose (rCMRglc) in the right prefrontal cortex; rCMRglc decreases in the anterior cingulate region; and increases in rCBF or decreases in rCMRglc in the right superior parietal cortex. The objective of this study was to determine whether these regions are also related to sustained attention in subjects undergoing resting state PET. Forty-seven optimally health-screened subjects in 3 age groups (young, middle age, old) were studied (mean age ± SD: 51 ± 14.7; range = 22-75). Sustained attention was measured using a high-event rate visual digit-discrimination task lasting 7.2 min. (six 1.2 min. blocks) at six levels of stimulus degradation. A decrement score was computed for the highest level of degradation in which all groups showed significant decrements (blocks 5 and 6 minus blocks 1 and 2). Resting state rCMRglc in the homologous right and left superior parietal, prefrontal, anterior cingulate and sensorimotor (control) regions were measured at rest (eyes patched, ears plugged) using 18F-fluoro-deoxyglucose and the Scanditronix PC1024-7B PET scanner. Because there were no age-related changes in sustained attention and in rCMRglc, the relationship of sustained attention decrements to normalized rCMRglc rates (rCMRglc/mean neocortical gray matter CMRglc) was examined in all subjects combined using partial correlations factoring out age. Significant positive partial correlations were found between sustained attention decrements and rCMRglc rates in the right prefrontal ($r = .35$, $p < .05$) and right anterior cingulate regions ($r = .29$, $p < .05$), confirming previous studies. No significant correlations were found in the right superior parietal cortex ($p > .05$), which has been linked to the efficiency of disengagement of spatial attention, nor with the sensorimotor cortices ($p > .05$). Sustained attention is associated primarily with right hemisphere activity as no correlations were found with any left hemisphere region (all $p > .05$). Our results indicate that differences in sustained attention capacity in humans are associated with functional resting state brain activity levels of rCMRglc.

502.11

CEREBRAL METABOLISM IN ADULTS WITH GENERALIZED RESISTANCE TO THYROID HORMONE. **J.A. Matochik*, P. Hauser, A.J. Zametkin, M. Ernst, R.M. Cohen and B.D. Weintraub. NIMH, LCM, SCBI, Bldg. 10/4N317 and NIDDK, MCE, Bethesda, MD 20892.**

Generalized resistance to thyroid hormone (GRTH) is characterized by elevated T3 and T4 levels and inappropriately normal or elevated TSH levels and reduced responsiveness of pituitary and peripheral tissues to thyroid hormone action. GRTH has been linked to the human thyroid receptor-β gene on chromosome 3. Attention deficit and hyperactivity are the major behavioral symptoms of GRTH. To measure cerebral metabolism by positron emission tomography, 8 adults with GRTH (all off thyroid treatment) and 9 normal controls received 4-5 mCi of 18-fluorodeoxyglucose while performing an auditory continuous performance task. Whole brain and 60 regional measures of glucose metabolic rate were extracted. GRTH adults were greatly impaired in performance on the attention task as measured by correct identifications of the target tone. There were no differences in global metabolism. With normalized data, GRTH adults had significantly reduced metabolism in 5 regions, predominately in the orbitofrontal cortex. Metabolism was increased in 6 regions, primarily in frontal and rolandic regions. Performance on the attention task was highly correlated with glucose utilization in the left posterior frontal and right rolandic areas in GRTH adults. The increased metabolism in these regions may reflect neural inefficiency in performing the attention task.

502.12

PRELIMINARY RESULTS ON DEVELOPING A VOLTAGE SENSITIVE DYE FOR HUMAN BRAIN IMAGING WITH PET. R.M. Dasheiff and D.S. Sacks. Dept of Veterans Affairs, VAMC, University Dr., PGH, PA, 15240 and Univ. of Pittsburgh Epilepsy Center.

A significant advance in human brain imaging could be made if three dimensional computed tomography could be combined with EEG. Presently, topographic maps of human scalp EEG are limited by the number of electrodes, and the inability to record deep brain structures. Positron emission tomography (PET) produces functional maps of blood flow, metabolism and neuroreceptors/transmitters, but not electrical activity directly. We are exploring the utility of using voltage sensitive dyes as tracers for human PET, which would allow direct measurement of the brain's electrical activity throughout it's volume.

In vivo experiments in rats were conducted using our best voltage sensitive redistribution probes: diO-C(2)-5 and Rhodamine B. When mapping seizure pathways, the dye is injected via the carotid artery [Brain Res. 595:79-86,1992]. We measured the percentage of dye which entered the brain using our standard vehicle: 30% mannitol, 5% EtOH. We found that 17% of diO-C(2)-5 and 6% of Rhodamine B entered the brain. Using EtOH alone as the vehicle yielded 10% and 7%, respectively. Because intravenous routes would be better tolerated by patients, we determined the percentage of injected dye in the brain via that route. Using only EtOH as vehicle, we found .01% for both dyes. We are presently investigating other vehicles, dye kinetics, distribution to other organ systems, washout parameters, and radiolabeling sites, which will be presented.

502.13

GENDER DIFFERENCES IN PATTERNS OF HEMISPHERIC REGIONAL GLUCOSE METABOLISM (rCMRglc): A MULTIPLE REGRESSION/ DISCRIMINANT ANALYSIS OF POSITRON EMISSION TOMOGRAPHIC (PET) DATA. N.P. Azari*[1], K.D. Pettigrew[2], M.B. Schapiro[1], and B. Horwitz[1] [1]Lab of Neurosciences, NIA, [2]Division of Epidemiology/Applied and Services Research, NIMH; NIH, Bethesda, MD 20892

Gender differences in the pattern of hemispheric rCMRglc correlations were reported in a prior correlational analysis of resting (eyes/ears covered) rCMRglc PET data. To further explore the effect of gender on patterns of hemispheric rCMRglc interactions, we applied a multiple regression/discriminant analysis to resting rCMRglc PET data from 17 men and 17 women (age < 40 yr). Two separate discriminant functions distinguished men and women: the first reflected rCMRglc interdependencies between hemispheres (left posterior medial temporal, left prefrontal, right temporal, right parietal, right sensorimotor) and correctly classifed all women and 94% of the men; the second reflected rCMRglc interdependencies within the left hemisphere and correctly classified 82% of the women and 88% of the men. The results, which support and extend our prior correlational analysis, demonstrate gender differences in the pattern of hemispheric rCMRglc interdependencies.

502.14

GLUCOSE METABOLIC CORRELATES OF ATTENTIONAL PERFORMANCE IN ADULTS WITH A HISTORY OF INFANTILE AUTISM, SCHIZOPHRENICS, AND CONTROLS. B.V. Siegel, Jr.* and M.S. Buchsbaum. Dept. Psychiatry, Mt. Sinai School of Medicine, New York, NY.

Positron emission tomography (PET) was used to assess brain regions important in attentional deficits in autism and schizophrenia. 14 adults with a history of infantile autism, 25 schizophrenics, and 20 controls performed the degraded stimulus continuous performance test (CPT) during the 35 minute 18-fluoro-2-deoxyglucose uptake period preceding PET scan acquisition. CPT performance was impaired in schizophrenics compared to controls, but autistics did not differ from either group. In controls and schizophrenics, task performance correlated with medial frontal, medial temporal, and inferior temporal cortical metabolism, suggesting that activation of those regions is important in the normal performance of the task and that damage to those regions, which also showed low glucose metabolic rates (GMR) in schizophrenics, contributes to the attentional dysfunction in schizophrenia. In contrast, autistics showed negative correlations of medial frontal cortical GMR with attentional performance, suggesting that neuronal inefficiency in that region may contribute to poor performance. Positive correlations of performance with GMR in globus pallidus and midbrain suggest that damage to those regions contributes to impaired attention in autism.

502.15

BRAIN ACTIVITY DURING WORKING MEMORY ASSESSED WITH FUNCTIONAL MRI R.S.Sexton*[a], V.S.Mattay[b], F.A.Barrios[a,d], J.A.Frank[b], G.Sobering[c], C.T.W.Moonen[c], D.R.Weinberger[a]. CBDB, NIMH[a] Washington, DC. Laboratory of Diagnostic Radiology Research[b], In Vivo NMR Research Center[c], NIH, Bethesda, MD. Computational Physics, Inc[d]. Fairfax, VA.

It has been shown in prior blood flow studies with single photon emission and O-15 PET imaging techniques that activation of the dorsolateral prefrontal cortex (DLPFC) occurs while subjects perform the Wisconsin Card Sorting Test (WCST), a working memory cognitive task. We have explored whether similar regional activation can be seen using functional MR imaging, a rapidly evolving imaging modality with temporal and spatial resolution much superior to SPECT and PET.

5 subjects underwent Functional MR imaging on a 1.5 Tesla MR unit with a quadrature head coil during the WCST and a sensorimotor control task[BAR]. The functional images were obtained in an axial plane using a SPGR sequence and Blood "Oxygenation" Level Dependent technique without inflow suppression. Images were analyzed using a time-stimulus vs time-intensity cross-correlation method to detect the activated pixels. In all cases activated pixels were found with greater density and with greater frequency in the DLPFC during the WCST than during the BAR test. This is in agreement with previous findings obtained using radio nuclide imaging methods. The results suggest that assessment of the distribution of neural activity associated with higher order cognitive processing is possible with this technique.

BRAIN METABOLISM AND BLOOD FLOW: NITRIC OXIDE

503.1

COUPLING OF CEREBRAL BLOOD FLOW TO NEURONAL ACTIVATION: ROLE OF ADENOSINE AND NITRIC OXIDE K.Niwa, U.Lindauer, A.Villringer, U.Dirnagl*, Dept.of Neurology, University of Munich, 8000 Munich 70, F.R.G.

This study aims to evaluate the role of adenosine for coupling of neuronal activation and regional cerebral blood flow (rCBF) using a physiological somatosensory stimulation mode, and to investigate a possible correlation between the two putative mediators of coupling, adenosine and nitric oxide (NO). Somatosensory stimulation was carried out in α-chloralose anesthetized rats by stimulating the mystacial vibrissae for 60 seconds by manual deflection (2-3 s) while measuring the rCBF-response over the contralateral SI cortex with Laser-Doppler flowmetry. •Adenosine receptor blockade ($5x10^{-5}$ M theophylline in artificial cerebrospinal fluid, aCSF) or reduction of the extracellular adenosine concentration (adenosine deaminase, ADA, 1 U/ml in aCSF) reduced the rCBF response to whisker stimulation by approx. 40% (from 17.9 ± 3.0 % to 10.6 ± 2.7 % after theophylline, n=24; from 18.2 ± 0.7 % to 10.7 ± 1.9% after ADA, n=5) without affecting baseline CBF. •The reduction in the rCBF response to whisker stimulation afforded by theophylline was further reduced by coapplication of theophylline and the NOS blocker N$^{\omega}$-nitro-L-arginine (L-NA, 10^{-3} M in aCSF) (from 17.9 ± 3.0 % to 7.5 ± 1.3 %, n=24). •Blockade of NO synthase with L-NA (10^{-3} M in aCSF) reduced the reactivity of rCBF to adenosine (10^{-4} M in aCSF) by approx. 50 % (from 39.4 ± 10.4 % to 22.9 ± 10.5 %, n=7). This effect was stereospecific, since superfusion of D-NA (10^{-3}M in aCSF) did not affect the response to topical application of adenosine. We conclude that adenosine is involved in coupling of rCBF to physiological stimuli, and that in the cerebral circulation the vasodilator effects of adenosine are at least partially mediated by NO. Supported by the Deutsche Forschungsgemeinschaft.

503.2

NITRIC OXIDE SYNTHASE (NOS) BLOCKADE INDUCED CEREBRAL VASOMOTION: INFLUENCE OF HYPERCAPNIA AND EXTRACELLULAR ACIDOSIS U.Dirnagl, K.Niwa., U.Lindauer, G.-D.Borasio*, A.Villringer. Dept.of Neurology, University of Munich, 8000 Munich 70, F.R.G.

Blockade of the brain NOS blocks the rCBF response to CO_2 or H^+ and induces cyclic variations of regional cerebral blood flow (rCBF) with a frequency of 10/min and amplitudes up to 60 % of baseline. We investigated the influence of extracellular acidosis on NOS blockade induced vasomotion. Anesthetized (α-Chloralose) and ventilated Wistar rats were eqipped with a closed cranial window (dura removed) over the right parietal cortex. Vasomotion was induced by topical application of 1 mM N$^{\omega}$-nitro-L-arginine (L-NA, in artificial cerebrospinal fluid, aCSF) for 30 minutes. rCBF was measured continuously with Laser-Doppler flowmetry with two separate probes at cortical sites 1 mm apart (group II: one probe only). Rhythmic activity in the rCBF was analyzed for frequencies and amplitudes by Fast Fourier Transformation. In group I (n=5), CO_2 was added to the inspiratory gas, in group II (n=5) extracellular acidosis was produced by superfusion of acidic aCSF (pH 7.08 ± 0.06). Superfusion of L-NA reduced the rCBF to 86 ± 12 %. L-NA induced vasomotion had a mean frequency of 10.3 ± 1.1 % and a mean amplitude of 18.2 ± 4.3 %. Neither CO_2 nor acidosis affected baseline rCBF. However, the amplitude of vasomotion was significantly reduced by CO_2 (-72 ± 14 %) and by acidosis (-69 ± 17 %). Vasomotion frequency was unaffected. 76 % of the time studied vasomotion at different cortical sites showed phases of relative synchronicity, the rest of the time shifts between phases and frequencies occured. We conclude that CO_2/H^+ may also have NO independent actions on the cerebral vasculature, and that vasomotion is only partially synchronized on the cortical surface. Supported by the DFG .

503.3

IN VIVO SPIN TRAPPING STUDY OF NITRIC OXIDE FORMATION FOLLOWING GLOBAL BRAIN ISCHEMIA IN THE RAT. T.Tominaga†*, S. Sato†, T. Ohnishi and S. T. Ohnishi. Philadelphia Biomedical Research Institute, King of Prussia, PA19406, and †Division of Neurosurgery, Institute of Brain diseases, Tohoku Univ. Sch. of Med., Sendai, 980 JAPAN.

It has been suggested that Nitric Oxide (NO) is produced during brain ischemia. However, whether NO protects or injures the ischemic brain is a question that has raised controversy. Using electron paramagnetic resonance (EPR) with an in vivo spin trapping method, we attempted to detect NO in vivo during global brain ischemia and answer this question. Global brain ischemia in the rat was produced by the occlusion of the bilateral common carotid arteries and/or hemorrhagic hypotension. Diethyldithiocarbamate (DETC, 400mg/kg) and Fe-citrate (20mg $FeSO_4$ -100mg sodium citrate/kg) were used as spin-trapping reagents [1]. Under these ischemic conditions, NO signals were detected as NO-Fe-DETC spin adducts (triplet centered at g=2.039 with the hyperfine coupling constant a_N of 13 gauss). The size of NO signals increased as the extent of ischemia increased. During the early phase of ischemia, NO may play as an endothelium-derived relaxing factor to increase cerebral blood flow protecting the ischemic brain.

Reference: [1] Voevodskaya, N.V. and Vanin, A., Biochem. Biophys. Res. Commun., 186 (1992) 1423-1428.

(Supported by NIH Grant NS30186)

503.4

ROLE OF NITRIC OXIDE FOR CORTICAL BLOOD FLOW DURING SPREADING DEPRESSION. M.Fabricius, B.Pakkenberg* and M.Lauritzen. Dep. of Medical Physiology, Univ. of Copenhagen, DK-2200 Copenh., Denmark.

We examined the relation between nitric oxide (NO) synthesis and regional cerebral blood flow (rCBF) changes during spreading depression (SD) in the parietal cortex of halothane-anaesthetized rats. NMDA-receptor activation is essential for propagation of SD, which is associated with marked changes of rCBF. NMDA-receptor activation stimulates nitric oxide synthase (NOS), which in turn triggers vasodilation.

SD was elicited by needle stab in left frontal cortex and rCBF changes were measured by laser Doppler flowmetry. NOS was inhibited by i.v.(30 mg/kg) and/or topical (1 mM) application of N^G-nitro-L-arginine (NOLAG). L-arginine was given i.v. (50 mg bolus + 100 mg/kg/h) or topically (20 mM). TTX (20μM) was applied topically to test the role of perivascular nerves for the rCBF changes.

In untreated animals, the percentage rCBF changes accompanying SD was calculated at a) 1 min before SD: -13 ± 2%, b) SD (peak rCBF): +98 ± 7%, c) 1 min after SD: -23 ± 2%, d) 10 min after SD: -16 ± 3%, and e) 20 min after SD: -15 ± 3%. Pretreatment with i.v./topical NOLAG decreased a): -43 ± 7%* (*:p<0.05), and increased c): -2 ± 4%*, while b), d), and e) did not differ from controls. Effects of NOLAG were independent of application mode. Topical L-arginine did not affect rCBF changes during SD. I.v. L-arginine increased d): +16 ± 7%*, but caused no other changes. TTX increased c): +49 ± 12%* but caused no other changes.

The results show that NO modulates rCBF changes during SD, but NO synthesis cannot account for the massive flow rise during the NMDA-receptor activation.

503.5

NITRIC OXIDE AND ADENOSINE MEDIATE THE NMDA-INDUCED INCREASE IN HIPPOCAMPAL BLOOD FLOW. Michael G. Kaiser*, Matthew J. During. Dept. of Neurosurgery, Yale Univ. School of Medicine, New Haven, CT 06510.

With the increasing use of noninvasive functional brain imaging methods which measure local cerebral blood flow, determining those factors which control regional cerebral blood flow is an area of increasing interest. In the present study we investigated the role of nitric oxide(NO) and adenosine(ADO) in coupling neuronal activity to local blood flow. Rats underwent unilateral placement of paired guide cannulae into the CA1 region of the hippocampus, through which a microdialysis and laser doppler probe were inserted. Blood flow changes were recorded with the doppler, and microdialysis samples were analysed for citrulline, produced in a 1:1 molar ratio with NO, and ADO. The first group of animals (n=6) received increasing doses of NMDA, and responded with mean blood flow increases of 2.7%±2.9%, 6.9%±1.4%, 16.9%±6.6%, and 32.9%±4.0% at 50, 100, 200, and 500μM respectively. The microdialysis data from this group indicated significant increases in both citrulline and ADO during the stimulation periods. In a second group of animals (n=6) 1mM S-Nitroso-N-acetyl-penicillamine(SNAP), a direct NO donor, was infused through the microdialysis probe. The mean increase in blood flow produced was 36.6% ± 5.8%. A third group of animals (n=7) received nitro-arginine methyl ester(NAME), a competitive NO synthase inhibitor. 5mM NAME resulted in a mean decrease of 22.7% ±2.9% in basal flow, and attenuated the response of 200μM NMDA from 63.5%±17.8% to 24.8%±6.5%. The effects of 5mM NAME on basal flow were significantly reduced by infusing 100mM L-Arg, and 100mM L-Arg alone produced an mean increase of 13.3 % ± 2.9% in blood flow (n=6). Citrulline decreased during the infusion of NAME and the NMDA-induced response was antagonized by NAME. When the adenosine receptor antagonist 8-(p-Sulfonophenyl)theophylline was infused there were significant decreases in both basal blood flow as well as the response to 200μM NMDA. Preliminary results indicate that stimulation of the perforant pathway increases cerebral blood flow in CA1 hippocampus. Studies are presently underway to characterize the mediators of this response. Based on these results NO and ADO mediate in part the changes in regional cerebral blood flow associated with hippocampal excitatory neurotransmission.

503.6

NEUROVASCULAR INTERACTIONS OF NITRIC OXIDE SYNTHASE-POSITIVE NEURONS IN RAT HIPPOCAMPUS. F. Schottler, J. Collins, O. Sagher, D. Okonkwo, A. Martin, N.F. Kassell and K.S. Lee*. Department of Neurosurgery, University of Virginia, Charlottesville, VA 22908.

The anatomical relationship between nitric oxide synthase (NOS)- positive neurons and blood vessels was examined in the hippocampus of the rat. The distribution of NOS was evaluated using NADPH-diaphorase histochemistry. Many, but not all, NOS-positive neurons had processes located in close proximity with blood vessels. The somata and dendrites of neurons at times appeared to drape or engulf a vessel. With opportune section angles, neuronal processes were sometimes closely associated with vessels over several tens of micrometers. While NOS-positive processes were occasionally found near branching points of arterioles, no preferential relationship with arteriolar branching points was observed. Occasionally, axon-like processes originating in local NOS-positive neurons were also associated with vessels. In certain cases, axon-like processes generated varicosities around neighboring vessels.

The factors which couple local cerebral blood flow with changes in local neuronal activity remain a matter of considerable discussion. The present findings suggest the possibility of two distinct mechanisms of neurovascular signalling by local NOS-containing neurons: 1) direct vascular innervation by terminals generating nitric oxide, and 2) paracrine signalling from closely apposed somatic and dendritic neuronal elements.

503.7

REGIONAL CEREBRAL METABOLIC EFFECTS OF A1 AND A2 ADENOSINE RECEPTOR AGONISTS AND ANTAGONISTS. J.L. Daval* and A. Nehlig. INSERM U 272, 30 rue Lionnois, 54013 NANCY, FRANCE.

In order to study the specific metabolic responses mediated by A1 and A2 adenosine receptors in the brain, we investigated the effects of selective ligands for these receptors on local cerebral metabolic rates for glucose (LCMRglc) in rats, using chlorocyclopentyladenosine (CCPA, 0.01 mg/kg) and cyclopentyldipropylxanthine (DPCPX, 0.01 mg/kg) as A1 agonist and antagonist, and CGS 21680 (0.01 mg/kg) and dimethylpropargylxanthine (DMPX, 0.3 mg/kg) as A2 agonist and antagonist, respectively. These doses were chosen to selectively interact with each type of receptor without inducing any significant systemic or peripheral effects. The 2 agonists, CCPA and CGS 21680 induced decreases in LCMRglc. These were limited to globus pallidus and hypothalamus for CCPA whereas CGS 21680 affected LCMRglcs in 17 areas. The latter regions were mainly located in cerebral cortex, limbic and motor systems, and white matter. Conversely, the 2 antagonists had more variable effects, either decreasing or increasing LCMRglcs. With DPCPX, LCMRglcs were decreased in dentate gyrus and globus pallidus, and increased in 6 regions, i.e. thalamic, hypothalamic and cochlear nuclei. DMPX had more subtle effects, decreasing LCMRglc in globus pallidus and increasing it only in cochlear nucleus. In conclusion, although high-affinity A2 receptors are limited to striatum, accumbens and olfactory tubercles, metabolic effects of CGS 21680 are widespread. The reverse is true for CCPA, A1 receptors being distributed throughout the brain. The significance of metabolic increases or decreases induced by both antagonists remains to be elucidated.

503.8

THE ROLES OF ADENOSINE AND NITRIC OXIDE IN THE CEREBRAL BLOOD FLOW EFFECTS OF HYPOXIA AND HEMODILUTION. M.M. Todd*, B. Wu, D.S. Warner. Neuroanesthesia Research Lab, University of Iowa College of Medicine, Iowa City, IA 52242

Both adenosine and nitric oxide (NO) may be involved in the cerebral blood flow (CBF) changes produced by hypoxia. If the CBF increase which occurs during hemodilution is also related to arterial/tissue O_2 content or tension, these two compounds may also play a role. To examine this, male pentobarbital-anesthetized NZW rabbits were prepared for the measurement of CBF with radioactive microspheres. Arterial O_2 content (CaO_2) was then reduced in each animal to ≈6ml O_2/dL, either by reducing PaO_2 (to ≈24mmHg) or by hemodilution (hematocrit ≈14.5%). Baseline CBF was measured. Animals were then sequentially given 3, 10, and 30mg/kg of L-nitroarginine methylester (L-NAME) or 2, 10, and 20mg/kg of 8-phenyltheophylline (8-PT). CBF was measured ≈10min after each dose of drug. Baseline CBF was greater in hypoxic animals (124±37 ml/100gm/min, X±SD) than with hemodilution (73±16ml/100gm/min). These compared with normal CBF values (prior to hypoxia or hemodilution) of ≈38ml/100gm/min. The administration of both L-NAME and 8-PT resulted in dose related decreases in CBF in hypoxic animals, with a minimum CBF≈96±27ml/100gm/min with L-NAME and 91±30ml/100gm/min with 8-PT. By contrast, neither drug altered CBF in hemodiluted rabbits (minimum CBF's of 70±16 for L-NAME, 76±24 ml/100gm/min for PT). These results indicate that A) the CBF responses to an equal reduction in CaO_2 differ between hypoxia and hemodilution, B) both NO and adenosine play a role in the CBF response to hypoxia, and C) these two compounds play little or no role in the response to hemodilution, at least under the conditions studied here.

503.9

WITHDRAWN

LEARNING AND MEMORY: SYSTEMS AND FUNCTIONS IX

504.1

THE FUNCTIONAL ANATOMY OF AMYGDALA EFFERENT PATHWAYS
Larry Cahill * and James L. McGaugh, Ctr. Neurobio. Learning and Memory, Dept. Psychobio., UC, Irvine, CA 92717

It is well established that intra-amygdala (AC) injections of a variety of neurotransmitter agents can modulate memory processes. We have recently begun a series of experiments designed to map out the brain systems involved in the memory-altering effects of intra-AC injections. Our strategy is to combine intra-AC injections of specific neurotransmitter agents with either 2-deoxyglucose autoradiography or c-fos immunohistochemistry to identify brain structures affected by the injections. Male Sprague-Dawley rats receive unilateral intra-AC injections via implanted cannulae of a specific neurotransmitter agent, and control injections into the opposite AC. The agents used to date are N-Methyl-D-aspartic Acid, naloxone, and Beta-endorphin. After the injections, the brains are processed using standard techniques for either 2-DG autoradiography or c-fos immunohistochemistry. Sections from throughout the brains are taken for analysis. Assymetries in structure labelling between the brain sides were taken as evidence of activation resulting from the unilateral intra-AC injection of the active agent. The results show strong labelling assymetries in several brain structures for both 2DG and c-fos, although the affected areas are not identical for both techniques. Among the areas most clearly affected by the intra-AC injections are the piriform cortex, caudate nucleus, and dentate gyrus (seen with c-fos), dorsal thalamic nuclei (2DG) and the auditory system (colliculi, geniculate, cortex- 2DG). We expect that this approach will allow us to create a relatively complete map of the brain systems involved in memory modulation after intra-AC injections. [Supported by USPHS/NIMH MH 12526 (JLM), NIH/NIA T32AG00096-10 (LFC)].

504.2

EFFECTS OF POST-TRAINING D-AMPHETAMINE INJECTIONS INTO HIPPOCAMPUS, AMYGDALA, AND CAUDATE NUCLEUS ON RETENTION IN SPATIAL AND CUED WATER MAZE TASKS. M. G. Packard*, L. F. Cahill, & J. L. McGaugh. Center for Neurobio. of Learning & Memory and Dept. of Psychbio., U. of Calif., Irvine, CA 92717.

Extensive evidence suggests that different forms of memory involve different neural systems. In particular, the hippocampus and caudate nucleus appear to mediate and/or regulate different forms of memory. Other findings suggest a prominent modulatory role for the amygdala in memory storage processes. We examined the hypothesis that amygdala modulation of memory storage can be exerted independently of the "type" of memory required for task acquisition. Rats received an 8 trial (30 second ITI) training session on a single-platform spatial or cued water maze task. In the spatial task, a submerged escape platform was located in the same quadrant of the maze on all trials. In the cued task, a visible escape platform was located in a different quadrant of the maze on each trial. Following trial 8 in both tasks, the rats received a unilateral post-training intracerebral (hippocampus, amygdala, or caudate nucleus), injection of d-amphetamine (10 ug/0.5 ul) or saline. On a retention test session 24 hours later latency to mount the escape platform was used as a measure of memory. In the spatial task, the retention test escape latencies of animals receiving intra-hippocampal, but not intra-caudate injections of d-amphetamine were lower than those of saline injected controls. In the cued task, the retention test escape latencies of animals receiving intra-caudate, but not intra-hippocampal injections of d-amphetamine were lower than those of saline injected controls. Post-training intra-amygdala injections of d-amphetamine enhanced retention of both tasks. The double dissociation of memory modulating influences observed following hippocampal and caudate nucleus injections supports the hypothesis that these two structures are parts of systems mediating or regulating different forms of memory. In contrast, the finding that amygdala injections enhance memory in both tasks suggests that the modulatory role of this structure in memory can be exerted independently of the type of memory involved.

Supported by PHS grant 1 F32 NS08973-01 (MGP), and USPHS MH12526 from NIMH and NIDA (JLM).

504.3

EFFECTS OF NMDA LESIONS OF INSULAR CORTEX AND AMYGDALA COMPLEX ON THE RETENTION OF OVERTRAINED INHIBITORY AVOIDANCE TASK. I.B. Introini-Collison*, F. Bermudez-Rattoni, K. Coleman and J.L. McGaugh. Center for Neurobiol. of Learning & Memory and Dept. of Psychobio. U. of Calif., Irvine CA 92717 and Instituto de Fisiologia Celular, UNAM, Mexico, D.F. 04510.

Amygdaloid complex (AM) and insular cortex (IC) lesions produce severe impairment of the acquisition of aversively motivated tasks. The present experiment examined the effects of N-Methyl-D-Aspartate (NMDA) lesions on the retention of an overtrained step-through (two-compartment) inhibitory avoidance task. Male Sprague Dawley rats were trained in a multiple trial (10 trials) inhibitory avoidance task. After 7 days, animals were randomly divided into four groups, and were given NMDA injections (8.0 µg in 0.8 µl) in either the AM or IC, and two other groups received buffer injections in either the AM or IC as controls. Seven days after the injections the animals were tested in the same apparatus where they were trained. The results showed that the retention latencies for both the AM- and IC-NMDA lesioned groups were significantly lower than the buffer-injected groups. The number of crossings to the compartment where the footshock had been delivered and the time spent in that compartment were significantly increased as compared with the buffer controls. These results provide additional evidence that the AM and the IC are involved in long term retention of aversively motivated information.

Supported by PHS MH 12526, NIMH and NIDA (JLM) PHS training grant 2T32MH14599-16 (KC).

504.4

The Right and Left Amygdalae are Differentially Involved in the Expression of Memory for an Aversively Motivated Task K. Coleman* & J.L. McGaugh, Center for Neurobio. of Learning & Memory and Dept. of Psychobio. U. Of Calif., Irvine, CA 92717

Extensive evidence from studies of both humans and lower animals suggests that the two hemispheres contribute differentially to the evocation of emotional experience and emotional expression. That is, the brain mechanisms underlying negative emotion may be lateralized to the right hemisphere, and mechanisms underlying positive emotion may be lateralized to the left hemisphere. There is also evidence that emotionally influenced memory may be lateralized. That is, memory for affective aspects of an experience appear to be lateralized to the right hemisphere. Several lines of evidence implicate the amygdala in the integration and modulation of emotionally influenced memory. The purpose of this study was to investigate whether or not there is lateralization of amygdala involvement in the memory of an aversively motivated task. Male Sprague-Dawley rats (250-300 g) were implanted bilaterally with cannulae aimed at the amygdala. Nine days after surgery, rats were trained in a one-trial step-through inhibitory avoidance task with 1mA/1 sec footshock. After training, animals were returned to their home cages. 24 hours or 10 days later, animals received a pre-test microinjection of either buffer in both cannulae, 2% lidocaine in both cannulae, or buffer in one cannula and lidocaine in the other. Five minutes after the injections, retention was tested. The retention latencies of rats given lidocaine into the right amygdala and buffer into the left amygdala were significantly lower than rats in all other groups at both 24 h ($p < .01$) and at 10 d ($p < .05$). These results suggest that the right and left amygdalae may make differential contributions to the expression of memory, and that the contribution of the right amygdala may be more important to the expression of memory for an aversive task.

Supported by PHS training grant 2T32MH14599-16 (KC) & PHS MH12526 (NIMH and NIDA) (JLM)

504.5

VARIATIONS IN FOOTSHOCK INTENSITY AND THE RETENTION IMPAIRMENT PRODUCED BY POSTTRAINING AMYGDALA LESIONS. G.L. Quirarte*, M.B. Parent, & J.L. McGaugh. Center for the Neurobio. of Learning & Memory and Dept. of Psychobio., U. of Calif., Irvine, CA 92717-3800.

It is well established that retention of aversively-motivated learning is impaired by posttraining amygdala lesions. Previous findings from this laboratory indicate that the retention impairment is partially attenuated by extensive training prior to the induction of the lesions. The present experiment examined whether the degree of impairment is also affected by the footshock intensity used during training. Male Sprague Dawley rats were given one trial of inhibitory avoidance (IA) training using a footshock intensity of 0.45 mA, 0.75 mA, or 1.25 mA. One week later, large neurotoxic amygdala lesions were induced. Retention performance was assessed four days after surgery. The retention performance of amygdala-lesioned animals (AL) was significantly poorer than that of sham-lesioned animals (SL): AL-1.25 and AL-0.75 animals had significantly poorer retention performance than their respective SL controls. However, there was no difference in the retention performance of SL-0.45 and AL-0.45 animals. More importantly, variations in footshock intensity affected retention performance. The retention performance of SL-0.75 and SL-1.25 animals was significantly better than that of SL-0.45 animals. Likewise, the retention performance of AL-1.25 animals tended to be better than that of AL-0.45 animals. The retention performance of AL-0.75 animals was comparable to that of SL-0.45 animals; similarly, the retention performance of AL-1.25 animals was comparable to that of SL-0.45 and SL-0.75 animals. Thus, these findings indicating that the degree of impairment produced by amygdala lesions is influenced by the footshock intensity used prior to the induction of the lesion suggest that the amygdala is not a site essential for mediating the changes induced by IA training.

Supported by 1967 NSERC/Canada (to MBP), PHS MH12526 (NIMH & NIDA) (to JLM), & PADEP DMDE-9114 & MED-9210 (to GLQ, Dept. Fisio., UNAM).

504.6

TIME-DEPENDENT RETROGRADE IMPAIRMENT PRODUCED BY LIDOCAINE INJECTIONS INTO THE BASOLATERAL COMPLEX OF THE AMYGDALA. M.B. Parent* & J.L. McGaugh. Center for the Neurobio. of Learning & Memory and Dept. of Psychobio., U. of Calif., Irvine, CA 92717-3800.

Extensive evidence suggests that the basolateral complex (BL) of the amygdala is involved in the retention of aversively-motivated learning. Previously we reported that inhibitory avoidance retention performance is impaired by posttraining inactivation of the BL. The present experiment examined the temporal involvement of the BL in the consolidation of inhibitory avoidance learning. Male Sprague Dawley rats were implanted bilaterally with cannulae aimed at the BL (AP - 0.31; ML+- 0.51; DV - 0.5 cm). One week after recovery, the rats were trained in a one-trial inhibitory avoidance task (0.45 mA footshock; 1 sec) followed by infusions of lidocaine hydrochloride (Sigma; 0.01 mg; 40mg/ml; 0.25 µL/1 min) either immediately, 6 hr, or 24 hr after training. On a retention test 48 hr after training, the latency to enter the dark/shock compartment and the total time spent in the lighted/safe compartment were recorded for 600 sec and used as indices of retention. Immediate posttraining injections of lidocaine into the BL significantly impaired retention performance. This impairment was also observed when the injections were administered 6 hr, but not 24 hr, after training. These results support the findings of Bucherelli, Tassoni, and Bures (1992), who found that inactivation of the amygdala immediately following or 90 min following aversive training significantly impaired subsequent retention performance, whereas inactivation of the amygdala 24 hr after training had no effect. The results of the present experiment suggest that the retention impairment produced by inactivation of the amygdala may be mediated by the BL and that BL signals are critical for regulating the consolidation of inhibitory avoidance learning for at least 6 hr after training.

Supported by 1967 NSERC/Canada (to MBP) and USPHS grant MH12526 from NIMH & NIDA (to JLM).

504.7

A CONNECTIONIST MODEL OF THE THALAMO-AMYGDALA NETWORK MEDIATING CONDITIONED FEAR REACTIONS. J.L. Armony, D. Servan-Schreiber, J.D. Cohen, J.L. McClelland* & J.E. LeDoux. Center for Neural Science, New York Univ., NY, NY 10003.

Sensory information reaching the lateral nucleus of the amygdala (AL) is processed along two parallel and possibly competing pathways with different physiological and functional properties. We developed a connectionist model to study this circuit. In the model, auditory thalamic nuclei (MGm, MGv), auditory cortical areas and AL are represented as layers of units connected by feedforward parallel pathways of excitatory weights. Within a layer, units are organized into clusters with lateral inhibition. Information processing capacity in a layer is set by the number of units and cluster size. The network is initially trained on simulated tones of different frequencies using a Hebb-type learning rule. As a result of this training, MGm units develop broad receptive fields (RFs) whereas the RFs of MGv units become sharply tuned around their best frequency (BF), as observed in single-cell recordings. Conditioning is then simulated by pairing a fixed frequency CS (tone) with a UCS. Units receiving converging CS-UCS input show frequency-specific changes in their RFs: (1) units with a BF close to the CS "retune" their BF towards the CS frequency; (2) units with BFs distal to the CS do not modify their RF; (3) units with BFs in an intermediate frequency range develop a minor peak in their RF for the CS frequency and decrease their BF response; (4) plasticity of RFs is largest for units with broad tuning (MGm and AL). The model demonstrates that our assumptions about connectivity between and within layers, number of units in each layer, and a Hebb-type correlational learning provide a sufficient basis for the RF changes observed empirically during conditioning.

504.8

SENSORY-SPECIFIC CONDITIONED PLASTICITY IN LATERAL AMYGDALA NEURONS. F. Bordi * and J. E. LeDoux. Center for Neural Science, New York University, New York, NY 10003.

Projections from the acoustic thalamus to the lateral nucleus of the amgydala (AL) have been implicated in auditory fear conditioning, which involves the associative pairing of an auditory conditioned stimulus (CS) and a noxious somatosensory unconditioned stimulus (US). Since frequency receptive fields in the acoustic thalamus can shift towards the CS as a result of conditioning (Weinberger et al, 1990), we examined whether AL neurons, which also have frequency receptive fields (Bordi and LeDoux, 1992), exhibit similar changes.

Unit activity was recorded in AL from awake, restrained rats (n=2) or urethane anesthetized rats (n=11). White noise bursts were used to search for auditory responsive cells. Frequency tuning was evaluated by presentation of a series of tones (1-30 kHz, 50-100 msec, 2-3 Hz). Threshold and best frequency (BF) of the cell 30 dB above threshold were determined. During sensitization training, a block of 10-20 unpaired CS (30 dB above threshold, 2 sec) and US (3-4 mA, 10 msec tailshock) presentations was given. Conditioning consisted of 5 blocks of 4 trials involving CS-US pairing. Frequency receptive fields were reevaluated immediately after and 30-45 min after conditioning.

Cells in AL showed different degrees of changes as a consequence of learning. A group of cells (5 from anesthetized and 2 from awake animals) increased their response to the CS frequency, with 3 of these also exhibiting increases in a number of adjacent frequencies as well. The main changes in other cells (n=2) were specific decreases at the BF (n=2) or decreases at the CS (n=2) frequency. In general, conditioned increases in firing occured in the early latency responses (<25 ms) and increases were more common 30-45 min after conditioning than immediately afterwards. These changes in AL are similar to those reported for the thalamic neurons that project to AL.

Supported by MH38774 and MH46516 (JL) and NS9044 (FB).

504.9

LONG-TERM INCREASES IN AUDITORY-EVOKED RESPONSES ACCOMPANY TETANICALLY-INDUCED LTP IN THE THALAMO-AMYGDALA PATHWAY. M. Rogan*, F. Bordi, J.E. LeDoux, Center for Neural Science, New York University, NY, NY 10003

Projections from the medial geniculate body (MGB) to the lateral amygdala (AL) have been implicated in classical fear conditioning with an auditory CS. Previous studies demonstrated the induction of LTP in AL by stimulation of the MGB (Clugnet and LeDoux, 1991). The present study examined the effects of LTP induction in the thalamo-amygdala pathway on the processing of auditory stimuli as measured in the AL. Rats (n=4) were anesthetized with urethane and fixed in a stereotaxic frame equipped with a calibrated monaural sound source. Electrical stimulation was delivered through a concentric bipolar electrode placed in the MGB. A steel recording electrode was lowered into the AL until a position was reached where field potentials were recorded both in response to electrical stimulation in the MGB and a frequency modulated tone delivered to the contralateral ear. Baseline measures were taken of both the auditory-evoked and electrically-evoked responses prior to tetanic stimulation of the MGB (10 trains at 1Hz of 30 pulses at 300Hz; 3 repetitions, 5 min apart). After tetanization, evoked responses to the same auditory and electrical stimuli were measured every 15 minutes for an hour. Post-tetanus measures showed increases in both auditory-evoked and electrically-evoked field potentials. After 1 hour, auditory-evoked field potentials remained elevated 10-130% over baseline levels; electrically-evoked potentials remained elevated 13-202%. Across animals, increases in auditory evoked potentials were highly correlated with increases in electrically evoked potentials. These findings demonstrate a tetanically-induced change in the processing of natural auditory stimuli in a pathway known to be involved in classical fear conditioning with an auditory CS. Supported by MH46516 and MH38774.

504.10

DIFFERENTIAL CONTRIBUTIONS OF VENTRAL AND DORSAL AREAS OF MEDIAL PREFRONTAL CORTEX TO FEAR CONDITIONING. M.A. Morgan, E.E. Coons*, J.E. LeDoux, Center for Neural Science, New York University, NY, NY 10003

The medial prefrontal cortex (mPFC) includes cortical areas lying along the medial wall of the anterior frontal lobe, specifically the infralimbic (IL), prelimbic (PL), medial orbito-frontal (MO), and anterior cingulate (ACg) regions. Lesions of ventral portions of mPFC, particularly IL, PL, and MO, result in a greater resistance to extinction of conditioned fear but do not alter the amount of fear expressed on a given trial. We therefore examined the effects of lesions of areas which lie dorsally in mPFC and were, at most, minimally included in the previous lesions. Experimental rats received lesions of dorsal mPFC (n=8). Controls (n=8) received sham lesions or were unoperated. After 2 weeks, all rats were trained on a fear conditioning task that involved 2 pairings a day of a tone conditioned stimulus (CS: 10KHz, 80 dB) with a footshock unconditioned stimulus (US: 0.5mA, 0.5sec.) for 2 consecutive days, followed by 2 trials a day of the tone alone until extinction criterion was met. Dorsal mPFC lesioned rats exhibited more freezing than did controls and were in fact at ceiling on a far greater number of trials than controls. A second study was conducted employing the same experimental paradigm but using a weaker (0.3mA) footshock in an attempt to eliminate the ceiling effect. Dorsal mPFC lesioned rats (n=7) again exhibited more freezing than controls (n=10). Moreover, half of the lesioned rats were still at ceiling a much greater number of times than were controls. Thus, while ventral areas of mPFC appear to play a role in the regulation of fear extinction, the dorsal mPFC appears to be involved in the regulation of fear reactivity. Both of these functions may involve prefrontal projections to the amygdala. Supported by NSF9209646

504.11

NMDA RECEPTORS ARE INVOLVED IN SYNAPTIC TRANSMISSION IN THALAMO-AMGYDALA PATHWAYS. X.F. Li, R.G. Phillips, J.E. LeDoux,* Center for Neural Science, New York University, NY, NY.

Projections from the auditory thalamus to the amygdala have been implicated in fear conditioning. Previous studies suggest that the pathway, which exhibits LTP, utilizes an excitatory amino acid as a neurotransmitter. In the present study we examined whether synaptic transmission in this pathway is mediated by AMPA and/or NMDA excitatory amino acid receptors. Rats (n= 9) were anesthetized with urethane. Extracellular unit responses were recorded in the lateral nucleus of the amygdala (AL) in response to electrical stimulation (200-500 uA, 300-500 us, 0.5 Hz) of the acoustic thalamus. A total of 15 units were identified. The cells responded with latencies of 5-8 ms. Microiontophoresis of the NMDA antagonist, AP5 (50mM, pH 8.5), depressed the response in 12 units and had no effect on the remaining 3 cells. In contrast, the AMPA receptor antagonist, CNQX (1.95 mM, pH 5.0), depressed 4 cells, excited 6 cells, and had no effect on 5 cells. Three of the 4 cells depressed by CNQX were also affected by AP5. The excitation produced by AMPA antagonism may be due to the blockade of excitatory projections to inhibitory neurons and the consequent release from inhibition of cells postsynaptic to the inhibitory neurons. Thus, both NMDA and AMPA receptors appear to play a role in synaptic transmission in AL. The involvement of NMDA receptors rasises questions about the effects of NMDA receptor blockade in the amgydala on learning. Supported by MH38774 & MH46516.

504.12

INVOLVEMENT OF DIFFERENT SUBDIVISIONS OF THE MEDIAL GENICULATE NUCLEUS AND OF THE PERIRHINAL CORTEX IN FEAR POTENTIATED STARTLE TO ACOUSTIC AND VISUAL CONDITIONED STIMULI S. Campeau* and M. Davis, Dept. of Psychology and Psychiatry, Yale Univ. Sch. of Med., 34 Park St., New Haven, Ct 06508.

Prior studies in our laboratory have indicated that post-training lesions of the entire auditory thalamus specifically block fear-potentiated startle using an auditory conditioned stimulus (CS), and post-training lesions of the perirhinal cortex block fear-potentiated startle to both auditory and visual CSs. The goals of the present studies were: 1- to test the involvement of different auditory thalamic subnuclei in fear-potentiated startle and; 2- to test the effects of pre-training lesions or retraining in animals sustaining damage to different auditory thalamic subnuclei or the perirhinal cortex. Anatomically, the posterior auditory thalamic nuclei project directly to the perirhinal cortex, whereas the ventral and dorsal divisions of the medial geniculate nucleus project indirectly to the perirhinal cortex via an auditory cortical relay.

Rats received 10 noise-footshock pairings, mixed with 10 light-footshock pairings on each of 2 consecutive days. They were then matched into groups having equivalent levels of fear-potentiated startle to both auditory and visual CSs. Twenty-four hr later, rats were given neurotoxic NMDA lesions of either the ventral and dorsal divisions of the medial geniculate nucleus (n = 10), the posterior auditory thalamic nuclei (n = 7), the perirhinal cortex (n = 10), or sham lesions (n = 22). Ten days later, a startle test revealed that only lesions of the ventral and dorsal divisions of the medial geniculate nucleus, but not lesions of the posterior auditory thalamic nuclei, specifically blocked fear-potentiated startle to the auditory CS. Lesions of the perirhinal cortex completely blocked potentiated startle to the acoustic and visual CSs. Importantly, rats sustaining pre-training NMDA lesions of the perirhinal cortex (n = 10), or retraining the rats initially sustaining lesions of different subdivisions of the auditory thalamus, showed levels of fear-potentiated startle similar to those of sham-operated and unoperated rats to both auditory or visual CSs.

The post-training lesion results indicate that the ventral and dorsal divisions of the medial geniculate nucleus and the perirhinal cortex are relays along a pathway normally mediating the expression of auditory aversive memories. However, the pre-training and retraining results strongly suggest that subcortical pathways may be used to express aversive memories following injury to the cortical pathway, but does not appear to be the primary neural circuit that normally mediates aversive conditioning. These results thus indicate the importance of performing lesions both before and after training to determine the most likely neural circuits mediating fear conditioning.

504.13

MUSCIMOL INJECTED INTO THE BASOLATERAL AMYGDALA BLOCKS BOTH ACQUISITION AND PERFORMANCE OF CONDITIONAL FEAR

F.J. Helmstetter* & P.S. Bellgowan, Department of Psychology, University of Wisconsin, Milwaukee, WI 53201

Recent data indicate that the amygdala is critical for learning associations between an observation chamber and footshock as well as for the performance or expression of fear-related behavioral responses when the stimulus that has been paired with shock is presented after learning has occurred. Prior results from our lab (Physiol. Behav. 51:1271) showed minimal disruption of fear conditioning when the amygdala was temporarily inactivated with lidocaine prior to learning while the same treatment significantly blocked performance 24 h later. In the present study we injected muscimol (0.5μg, bilateral) into the basolateral amygdala of rats 1 hr prior to pairing an observation chamber with footshock (1.6mA x 3). This treatment resulted in a dramatic reduction in conditional freezing behavior 24 h later relative to saline controls. As with lidocaine, injections made prior to the test session also blocked performance. The different pattern of results obtained with muscimol vs lidocaine may relate to large differences in the duration of functional inactivation that would be expected given the present parameters. Although our preliminary findings also provide some evidence that muscimol's effects may be "state-dependent", the results are generally consistent with a role for the amygdala in both learning and performance of conditional fear.

504.14

INJECTIONS OF CALCITONIN GENE RELATED PEPTIDE IN THE AMYGDALA BLOCK FEAR CONDITIONING TO CONTEXTUAL BUT NOT DISCRETE STIMULI

L. H. Poore*, and F. J. Helmstetter, Department of Psychology, University of Wisconsin at Milwaukee, Milwaukee, WI 53201

Calcitonin gene-related peptide (CGRP) is localized in projections to the amygdala that may be important for fear conditioning. When injected intracerebroventricularly (ICV), CGRP produces freezing behavior and disrupts associative learning. CGRP was injected into the lateral nucleus of the amygdala in order to study its effects on fear conditioning using discrete and contextual conditional stimuli. Rats implanted with bilateral cannulae in the lateral amygdala were injected with either saline, 6.3nM or .63mM CGRP prior to fear conditioning using four white noise (72db,10 sec) shock (2mA, 1 sec) pairings. Twenty-four hours later, all animals were injected with saline and observed for freezing to the observation chamber in which training occurred. On the same day, rats were also observed in a novel chamber in which the white noise CS was presented. CGRP had no effect on freezing prior to foot shock and did not change the amount of freezing in the presence of the discrete auditory CS. However, freezing to contextual stimuli present at the time shock was delivered was dose dependently blocked by CGRP when applied to the lateral amygdala prior to training. These results indicate that the disruption of associative learning seen with ICV injections of CGRP may be mediated by receptors in the amygdala, while the fear evoking effect of CGRP injected ICV is not.

504.15

HYPOALGESIA ON THE FORMALIN TEST INDUCED BY NOISE STRESS IS ATTENUATED BY SYSTEMIC ADMINISTRATION OF NALTREXONE P.S. Bellgowan* & F.J. Helmstetter, Department of Psychology, University of Wisconsin at Milwaukee, Milwaukee, WI 53201.

Previous research has shown that a single presentation of moderately intense (75-95 dB) white noise (WN) is sufficient to induce opioid mediated hypoalgesia on the radiant heat tail flick test. The neural circuit responsible for noise stress hypoalgesia is also critical for the expression of hypoalgesia in response to an auditory stimulus that has previously been paired with footshock during Pavlovian conditioning. The presentation of such a conditional stimulus to a rat will result in an integrated pattern of defensive behaviors which includes freezing and hypoalgesia. The present study was conducted to further define behavioral responses to noise stress and to compare these responses with those observed during associative fear conditioning. Adult male rats received a 50 ul s.c. injection of 10% formalin solution in the right hind paw. Prior to being placed in a test chamber subjects were injected i.p. with either 3 mg/kg naltrexone or saline. Following four minutes of baseline behavioral observations subjects were exposed to a 60 second presentation of 0, 72, or 95 dB WN. Behavioral observations continued for five minutes following the onset of the noise. Presentation of WN induced a graded level of defensive freezing during noise presentation with the highest intensity of WN having the largest effect. Presentation of the 95 dB WN also induced a transient hypoalgesia that was attenuated by naltrexone. Non-stimulated controls showed no defensive behaviors throughout the test session. When returned to the test chamber 24 hrs later subjects showed no behavioral signs of Pavlovian fear conditioning to the context. This study, along with previous findings, suggests that noise stress hypoalgesia results from a state of sensitization or non-associative fear.

504.16

PRETRAINING INJECTIONS OF CORTICOTROPIN RELEASING FACTOR INTO THE VENTRAL PERIAQUEDUCTAL GRAY ENHANCE FREEZING TO AN AUDITORY CONDITIONAL AVERSIVE STIMULUS.

S. A. Tershner*, and F. J. Helmstetter, Department of Psychology, University of Wisconsin, Milwaukee, WI 53201.

The present study investigated the effect of corticotropin releasing factor (CRF) on fear conditioning using discrete and contextual conditional stimuli. Separate groups of rats received an infusion of CRF (.2ug) or isotonic saline into the ventral periaqueductal gray (vPAG). Twenty minutes after infusion, the rats were placed into observation chambers and their behavior was recorded for twenty minutes. This dose of CRF alone did not produce any significant behavioral changes during the observation period.

Later, all subjects were randomly reassigned to either the CRF (.2ug) or saline group. Twenty minutes after infusion, the rats were placed into the observation chambers where they received Pavlovian fear conditioning consisting of four presentations of white noise (74dB/10 sec) followed by footshock (1.6mA/ 1 sec).

On the next day, conditional fear responses to the observation chamber (context) and discrete auditory CS were measured. Rats were returned to the chamber in which conditioning was conducted and observed for twenty minutes. CRF given before training did not affect the amount of freezing behavior to this context. One hour later animals were given a single five minute presentation of the noise CS in a novel context. Animals given CRF prior to noise - shock pairings showed significantly more freezing to the CS. These results suggest that the vPAG may be an important site for the modulatory effects of CRF on fear conditioning and support the idea that different neural systems are involved in fear responses to discrete vs. contextual conditional stimuli.

504.17

INTEROCULAR TRANSFER IN THE SPLIT-BRAIN RAT. D.P. Crowne*, P. Forsyth, and J. Fitzgerald, Dept. of Psychology, Univ. of Waterloo, Waterloo, Ontario, CANADA N2L 3G1

Disruption of interocular transfer by division of the cerebral commissures was long ago established in cat and monkey with the evidence of unilateral engrams inaccessible to the isolated and untrained hemisphere. Interocular transfer was difficult to study in the rat because uniocular exposure of one hemisphere was impossible. The optic chiasm in the rat was inaccessible, and split-brain experiments were limited by transmission of visual information to both hemispheres. In this experiment, using a recent method to transect the chiasm, we investigated IOT in split-chiasm rats, comparing groups with corpus callosum section and callosum intact. 8 split-brain and 8 chiasm sectioned rats were monocularly trained on an orientation discrimination, trained on a reversal with the other eye, and retrained with the first eye on either the 2nd-eye S^+ (N = 4) or S^- (N = 4). ANOVA yielded a Groups X Tests interaction, $F(6,24) = 4.04$, $p = .006$). The critical comparisons are on retesting the original eye. We separately compared chiasm-section and split-brain groups given reversal or transfer tests. In rats with chiasm section alone, the 2nd-eye S^+ showed significant transfer to the first eye; the reversal group did not and differed significantly from the transfer group. In the split-brain groups, those given the 2nd eye S^+ showed a reversal effect with the original eye; the group retested with the original S^+ showed significant savings. We definitively establish interhemispheric communication via the callosum, unilateral engrams, and the disruption of transfer by callosal section.

504.18

VISUAL ACUITY IN PIGMENTED AND ALBINO RATS. Ian Steele Russell* & Susan E. Maier. Human Anatomy, Texas A&M University Health Science Center, College Station, TX, 77843-1114

Interocular transfer (IOT) of visual information following monocular training is found to occur readily in pigmented rats while equivocal results are derived for albino rats. The extent to which differences in visual acuity are responsible for this fact was investigated in this study. Visual acuity was examined for intensity and orientation differences in groups of albino and pigmented rats. All animals were trained in a two choice discrimination apparatus using shock motivation. The groups were trained to discriminate black versus white circles followed by vertical versus horizontal striations. All animals were then tested using either the constant stimuli method or the staircase method over a comprehensive range of stimuli to determine intensity and angular difference thresholds. The results showed that the psychophysical curves were identical for both albino and pigmented rats for intensity and orientation suggesting that the difference between these animals in IOT must be accounted for by some other mechanism.

504.19

SPATIO-TEMPORAL PATTERNS OF VISUAL, AUDITORY, AND SOMESTHETIC EEGs IN PERCEPTION BY TRAINED RABBITS W. J. Freeman*, J. M. Barrie. Dep't of Molecular & Cell Biology, University of California at Berkeley, CA 94720

Perceptual information has been found to exist in paleocortical EEGs in the form of spatial patterns of amplitude modulation of a broad-spectrum aperiodic wave form in the gamma range of the EEG (20 - 80 Hz, often referred to as "40 Hz"). These occur only in subjects that have been trained to discriminate and respond to selected stimuli. We tested the hypothesis that similar patterns would be found in the EEG of neocortex. Arrays of 64 electrodes (8x8 at 0.8 mm spacing) were surgically fixed onto the epidural surface of the visual, auditory or somesthetic cortex of 12 rabbits. After recovery, the subjects were trained to respond to simple CS (+ and - on interspersed trials) in a classical aversive paradigm. The 64 EEG traces were recorded in 6 sec trials, stored on disk, segmented, and decomposed by Fourier or PC analysis. Spatial pattern differences were expressed for 120 msec segments at 40 msec intervals as points in 64-space. Pattern differences were determined by a Euclidean distance in the SD metric and given a probability value. Differences between CS+ and CS- segments were found in narrow time windows near 40, 300, and 450 msec after stimulus onset. We concluded that the neural processes for perception are similar for sensory paleo- and neocortex. In all of these systems, perceptions are constructed by chaotic dynamics

Supported by the National Institute of Mental Health - MH06686.

LEARNING AND MEMORY: SYSTEMS AND FUNCTIONS X

505.1

DIFFERENTIAL EFFECTS OF INSULAR CORTEX AND DORSAL STRIATUM LESIONS IN A PLACE CONDITIONING TASK. A.L. Piña*, C.E. Ormsby, V. Ramírez-Amaya and F. Bermúdez-Rattoni. *Instituto de Fisiología Celular, UNAM, México, D.F. 04510.*

Insular Cortex (IC) and dorsal striatum (DS) lesions induce deficits in the acquisition of an Inhibitory Avoidance (IA) learning. This work examines the effects of IC and DS lesions on a positively motivated learning task.

Male Wistar rats were used. One half of animals were trained in the IA task and the other in a positively reinforced place conditioning (PPC). Subjets were divided in control (CON), IC and a DS lesioned groups. Electrolytic lesions were performed and all animals were trained in either of the two procedures, and tested 48 hrs later. All animals had restricted water access. Multitrial IA task was conducted conventionally. A modified PPC was developed for this work. The PPC was conducted in the same box, but 10 sec after the door was opened a drinking pipette was introduced in the lit compartment. The animal was allowed 3 sec of drinking, then the door was closed and the pipette retired. This procedure was repeated 12 times for each subject. The test session for both tasks consisted in the measurement of step through latency and time spent in the lit compartment during a 10 min period.

Both CON groups showed increased stay in the lit compartment in both tasks. In the IA task both IC and DS showed disruption of learning indicated by a short stay in this compartment. In the PPC task, however, the IC did not show any impairment at all, but the DS showed a markedly low stay. These results indicate that the IC is involved only in aversive motivated behaviors, whereas the DS is probably involved in both aversive and positive motivated conditioning. Supported by DGAPA IN204689 & CONACyT 0178

505.2

TASTE AVERSION LEARNING IN RATS LACKING GUSTATORY CORTEX USING MEASURES OF CONSUMPTION AND TASTE REACTIVITY. S. A. Bailey*, S. L. Rock, and S. W. Kiefer. Dept. of Psychology, Kansas State University, Manhattan, KS 66506.

The ability of GC rats to develop sucrose aversions after repeated sucrose-illness pairings was examined in the present study using concurrent measures of consumption and videotaped recordings of the rats' orofacial responses. Rats lacking GC (n=12) and control rats (n=14) were given three, 10 min trials with a sucrose solution followed by LiCl illness. Each training trial was videotaped and later scored for taste reactivity. GC rats showed significant deficits in acquiring avoidance of sucrose although consumption did decrease with repeated trials. However, after water consumption in the test chamber returned to normal levels, sucrose consumption (mean=8.0 $\pm$ 1.6) approached pretraining levels (mean=12.5 $\pm$ 1.1); at the end of training, aversive reactivity to sucrose was low for all GC rats (mean=1.3 $\pm$ 0.4). Although sucrose consumption by control rats (mean=12.7 $\pm$ 0.8) was initially high, they quickly learned to avoid consumption of sucrose in the test chamber and also showed high levels of aversive reactivity (last trial mean=13.5 $\pm$4.3). It appears GC rats show relatively nonspecific reductions in consumption when given illness experiences and that a hedonic shift to the illness-paired taste does not occur as it does in control rats.

505.3

THE EFFECT OF AMYGDALOID LESIONS ON THE ACQUISITION OF EEG AND HEART RATE CONDITIONED RESPONSES (CRs) IN THE RABBIT. B.S. Kapp*, P.J. Whalen, A.J. Silvestri, M.P. Jordan, and J.H. Fechter, Dept. Psychol., Univ. of Vermont, Burlington, VT 05405.

We have recently demonstrated that conditioned EEG desynchronization in the rabbit, as reflected in the inhibition of high voltage delta wave activity (1-4 Hz), is rapidly acquired via Pavlovian conditioning and that electrical stimulation of the amygdaloid central nucleus (ACe) produces inhibition of delta activity (Kapp et al. 1990; Whalen et al. 1991). The present study was designed to determine if damage to the region of the ACe, previously demonstrated to produce deficits in the acquisition of Pavlovian bradycardic CRs, would affect the acquisition of EEG CRs.

New Zealand rabbits received either bilateral ibotenic acid (0.2-.3ul; 1%) or vehicle injections aimed at the ACe and were administered either paired Pavlovian conditioning or pseudoconditioning trials using a tone as a conditioned stimulus and a brief electrical stimulus to the pinna as an unconditioned stimulus. A Fourier analysis of the cortical EEG demonstrated that animals receiving vehicle injections and paired conditioning trials demonstrated acquisition of EEG CRs at a rate similar to the acquisition of heart rate CRs. Animals with damage to the ACe and immediately adjacent regions of the dorsal amygdala and ventral globus pallidus/putamen demonstrated a significant attentuation in the magnitude of both EEG and heart rate CRs (p's < .05). The results are consistent with the notion that the ACe may contribute to the expression of CRs indicative of enhanced arousal in the rabbit (Kapp et al. 1991). Supported by NSF grant BNS 9010760.

505.4

COMPARISON OF LATERAL AND MEDIAL AMYGDALA LESION EFFECTS ON TWO-WAY ACTIVE AND PASSIVE AVOIDANCE. A. L. Beggs*, C. Burkhalter, and V. Melvin. Dept of Psychology, Univ. of Southwestern Louisiana, Lafayette, LA 70504.

Subjects in this experiment were 23 rats approximately one year of age weighing in excess of 550 g. The control group received sham surgery in which an electrode was lowered bilaterally proximal to the amygdala. The experimental groups received electrolytic lesions to either the lateral or medial amygdala. Subjects were placed in a two-way shuttle box and given adaptation trials consisting of a 10 s tone on two consecutive days. Intertrial interval crossings (ITCs) were recorded. This was followed by active avoidance training. This training was followed by 2 days of passive avoidance training. Analysis of the data indicated a significant group effect only during active avoidance training. Tukey's HSD comparison of the group means revealed that the medial lesioned group made longer latency responses than either the control or lateral lesioned group (ps<.01). This experiment indicates that the medial nuclei of the amygdala may play a more important role in acquisition of active avoidance responses.

505.5

DIFFERENTIAL *c-fos* EXPRESSION IN RAT DORSAL PERIAQUEDUCTAL GREY (PAG) AFTER 2-WAY AVOIDANCE. J.F. Brennan[1]*, C.A. COHEN[2] and L. KACZMAREK[3]. [1]Dept Psychology, Univ of Massachusetts/Boston, MA 02125, [2]Dept Psychology, Tufts University, Medford, MA 02155, [3]Dept Neurophysiology, Nencki Inst of Experimental Biology, Warsaw, Poland.

18 adult pigmented rats were trained for 100 daily trials in a 2-way shuttle box to avoid a footshock signalled by 5 s tones of 10 kHz, 62 dBC. 6 rats received a single session, were sacrificed and perfused 1 hr after the session. 2 other groups of 6 rats each were likewise treated after the 2nd or the 3rd training session. 6 additional rats served as controls and were handled briefly 1, 2 or 3 times, matching the respective avoidance training groups. Rats showed increasing proportion of avoidance responding with progressive training sessions. Preliminary data suggest that there is a significant increase in Fos immunopositive nuclei in the dorsal PAG after 2 sessions as compared to handled controls. No significant differences were found between the 3 avoidance training groups. The results support c-fos expression as an index of the emotional state underlying the transition from classically control escape responses to instrumental behavioral≡Xcontrol.

505.6

DIFFERENTIAL EXPRESSION OF *C-FOS*, *C-JUN* AND *JUN-B* PEPTIDES IN THE RAT BRAIN FOLLOWING EXPOSURE TO NOVELTY. H.Welzl[3]*, M.Papa[1] and A.G. Sadile[2]. [1]Ist. Anatomia Umana Normale; [2]Dip. Fisiologia Umana "F.Bottazzi", 2nd Univ. of Naples (SUN), Naples, Italy; [3]Inst. Behavioral Biology, ETH, Zürich, Switzerland.

The neural consequences of exposure to spatial novelty on expression of immediate early genes (IEG) were mapped in the rat brain by immunocytochemistry for *c-fos*, *c-jun and jun-B* peptides. Adult male Sprague-Dawley rats were tested for 10 min in a Làt-maze and sacrificed at different time intervals (0.5, 2, 6 or 24 hr) thereafter. Unexposed handled rats or rats exposed for three days to the maze were used as controls. The former showed a low and scattered basal positivity. In the *exposed* rats, extensive *c-fos* and *c-jun* positive cells were the granular and pyramidal neurons of hippocampus, and later neurons in all layers of somatosensory cortex and the granule cells of the cerebellar cortex. The positivity was stronger in rats exposed for the first time to the maze. The *fos* and *jun*-like immunoreactivity was *time-dependent*, since it was present between 2 and 6 h after the first exposure, and it was *NMDA-dependent*, being prevented by pretreatment with the competitive NMDA receptor antagonist CPP at a high dose (5mg/Kg). In contrast, *c-jun* and *jun-B* expressed in a non congruent manner, since *jun-B* was activated to a lower extent compared to *c-jun*. The results indicate that exposure to spatial novelty involves a differential expression of IEG in the hippocampus and in the cerebral and cerebellar cortex. Thus, IEG brain mapping reveals diffuse, distributed changes in IEG expression associated with a non-associative task. However, expression of some IEGs is not necessarily followed by gene expression that results from a complex orchestration of several transcriptional factors. **(Supported by CNR 92.01092.CT04, MURST 40% and NF 3100-9450 grants).**

505.7

LONG-TERM HABITUATION OF THE EMG STARTLE RESPONSE FOLLOWING LESIONS TO THE MESENCEPHALIC RETICULAR FORMATION IN RATS

W. P. Jordan*, C. J. Nelson, and T. B. Bodie, Psychology
St. Mary's College of Maryland, St. Mary's City, MD 20686

The reflex pathway of the acoustic startle response is a fast-latency system within the brainstem of the rat. Short-term habituation of startle occurs within the reflex pathway, but long-term habituation relies upon the inhibition of the response by neural mechanisms outside of the reflex circuit. Acoustic startle stimuli provoke a bimodal electromyographic (EMG) response in the spinotrapezius muscle in the rat. The early EMG component has a latency of about 8 ms from stimulus onset, while the later component occurs with a latency of about 18 ms.

During long-term habituation, the late EMG component shows a more robust decrease in amplitude than does the early component (Poore & Jordan, 1992). Lesions to the mesencephalic reticular formation (MRF) in rats attenuate long-term habituation of acoustic startle. If the decreasing amplitude of the late EMG component represents the effects of a long-term habituation mechanism exerting its influence on the startle circuit, then lesions to the MRF should attenuate habituation of the late EMG component of startle.

The present study tested this hypothesis in 13 sham-operated rats and 21 animals with bilateral lesions of the MRF. The early EMG component showed small but reliable response decrements in both groups of animals, but the late EMG component decreased across days only in control animals. This result is consistent with the hypothesis that long-term habituation of the acoustic startle response in the rat is expressed within the late, but not the early, EMG component of startle.

505.8

ASPIRATION LESIONS OF THE DORSAL AND POSTSUBICULUM ATTENUATE CLASSICALLY CONDITIONED BRADYCARDIA IN RABBITS. Mark Chachich*, Brian Maxwell & D.A. Powell, VA Medical Center, and University of SC, Columbia, SC.

Much evidence suggests the participation of the medial prefrontal cortex (mPFC) in classically conditioned bradycardia. The present study is part of a systematic exploration of the role that structures afferent to the mPFC might play in modulating mPFC neuronal activity responsible for conditioned bradycardia. A major mPFC input from the hippocampus, which is known to be involved in associative learning, originates in the dorsal and postsubiculum in rabbits. In a previous study we found that ibotenic acid lesions of these structures had no effect on classical heart rate (HR) conditioning, but in no animal was the subiculum completely destroyed. Thus, in the present experiment aspiration lesions were employed to completely destroy both the dorsal and postsubiculum. Animals with subicular lesions were compared with cortical and sham control animals on differential classical HR conditioning. Although the HR orienting reflex, which also consists of bradycardia, was unimpaired in animals with subicular lesions, the latter animals showed a severe attenuation of classically conditioned bradycardia, compared to both the cortical and sham lesion controls.

Supported by DVA Institutional Research Funds

505.9

LESIONS OF THE MEDIODORSAL NUCLEUS OF THE THALAMUS ATTENUATE CS-EVOKED NEURONAL ACTIVITY IN THE PREFRONTAL CORTEX, BUT HAVE NO EFFECT ON CONCOMITANTLY OCCURRING CONDITIONED BRADYCARDIA. Virginia Murphy*, Shirley Buchanan & D.A. Powell, VA Medical Center, and University of SC, Columbia, SC.

Much evidence suggests the participation of the medial prefrontal cortex (mPFC) in classically conditioned bradycardia. The present study is part of a systematic exploration of the role that structures afferent to the mPFC might play in modulating the mPFC neuronal activity responsible for conditioned bradycardia. Rabbits received ibotenic acid (IA) lesions of the mediodorsal nucleus of the thalamus, which is the thalamic projection nucleus to the mPFC. Chronic recording electrodes were also implanted in each animal for assessing multiple unit activity (MUA) in the mPFC. After recovery from surgery the animals were subjected to simple classical heart rate (HR) conditioning. CS-evoked increases in MUA recorded during conditioning were obtained in both the sham and lesioned animals, but they were significantly smaller in the lesion group. In spite of reduced mPFC MUA in the lesion group, the magnitude of the HR decelerative orienting reflex, as well as the decelerative classically conditioned HR response, was unimpaired compared to that of sham control animals.

Supported by DVA Institutional Research Funds

505.10

A CONDITIONING MODEL WHICH PROVIDES EVIDENCE FOR TWO MEMORIES, A CS AND A CS/US ASSOCIATION MEMORY. C. Rogers, V. Ghanta, S. Demissie, N. Hiramoto, and R. Hiramoto. Departments of Biology and Microbiology, University of Alabama at Birmingham, Birmingham, AL 35294.

Natural Killer (NK) cell activity can be conditioned with one trial learning with camphor odor as the conditioned stimulus (CS) and poly I:C as the unconditioned stimulus (US). Ablation of the main olfactory bulb (MOB) or peripheral anosmia produced by zinc sulfate irrigation of the nose, abrogates NK cell conditioning. The olfactory system must be intact in order for the CS odor signal to be perceived. We believe that the olfactory system not only recognizes and perceives the specific CS odor signal, but it also forms a memory for it. When the CS/US signals were overlapped, a conditioned enhancement of both NK cell activity and body temperature was observed. However, when the memory for the CS signal was established one day before administration of the US, a conditioned *enhancement* of NK cell activity was observed, whereas a conditioned *suppression* of core body temperature resulted.

Since the memory for the CS is established one day before exposure to the US for both NK and temperature conditioning, the CNS processes the US (IFN-β) differently for NK cell activity and temperature. This divergence in responses suggests differences in efferent mechanisms and memory circuits. The results suggest that the CS must have access to multiple response pathways and that the memory for the CS/US association is made centrally whereas the CS (camphor odor) memory must reside within the sensory circuits.

Supported by NIH CA 37570 and AG 10263.

505.11

FUNCTIONAL NETWORK INTERACTIONS AMONG LIMBIC CORTICES, BASAL FOREBRAIN AND CEREBELLUM DIFFERENTIATE A PAVLOVIAN CONDITIONED EXCITOR FROM AN INHIBITOR: FLUORODEOXY-GLUCOSE MAPPING AND COVARIANCE STRUCTURAL MODELING F. Gonzalez-Lima* and A.R. McIntosh[1], Dept of Psych, Univ of Texas, Austin TX, 78712 USA; [1] Lab of Neurosci, NIA, NIH, Bethesda, MD, 20892 USA

We examined how opposite learned behavioral responses to the same physical stimulus are represented by the pattern of interactions between neural networks. [^{14}C]2-fluoro-2-deoxyglucose autoradiography (FDG) was used to compare mean activity and interregional covariances resulting from presentation of a tone trained as either a Pavlovian conditioned excitor or inhibitor. Rats were trained with reinforced trials of the conditioned stimulus (A^+) intermixed with nonreinforced trials of a tone-light compound (AX^-). For Group CE, the tone was the excitor (A^+), while for Group CI the tone was an inhibitor (X^-). After conditioning, both groups were injected with FDG and presented with the same tone. Regional differences in FDG incorporation were noted in the sulcal frontal cortex (SFC), medial (MS/DB) and lateral septum (LS), retrosplenial cortex (RS), and deep cerebellar nuclei. Structural equation models identified two functional networks that differentiated the conditions. One involved basal forebrain regions (MS/DB, LS, and n. accumbens) and the other limbic thalamocortical structures (SFC, RS, perirhinal cortex and the anteroventral thalamic n.). Differences in basal forebrain interactions were mainly in sign and may relate to the opposite affective properties of the tone-CS for the two groups, while sign differences in the thalamocortical circuit may have reflected the associative effects. The functional influence from the basal forebrain to perirhinal cortex was greater in the Group CI model suggesting that one key site of convergence for the information about the associative and affective components of the tone-CS was the limbic cortical circuit through perirhinal cortex. (Supported by RO1 MH43353).

LEARNING AND MEMORY: SYSTEMS AND FUNCTIONS XI

506.1

PASSIVE AVOIDANCE LEARNING AND ANATOMICAL EFFECTS OF INTRA-VENTRICULAR IMMUNOTOXIN TO p75^{NGFr}. R.G. Wiley*, T.G. Berbos and D. Lappi. Lab of Experimental Neurology, DVAMC, Nashville, TN 37212-2637 and Whittier Institute, La Jolla, CA.

Cholinergic neurons of the basal forebrain (CBF) have been implicated in memory and attentional processes. However, all reported behavioral analyses of CBF lesions have been confounded by damage to non-cholinergic neurons. The immunotoxin, 192-saporin, selectively destroys CBF neurons that display the low affinity neurotrophin receptor, p75^{NGFr}. In the present study, we sought to determine the extent of forebrain cholinergic dennervation resulting from destruction of p75^{NGFr}-positive CBF neurons and the correlation between the anatomical findings and performance in a step-through passive avoidance task. 8 adult, male Sprague-Dawley rats received pressure microinjections of 2-3.4 μg of 192-saporin into the rostral left lateral ventricle. 8 rats received similar injections of 1.7-2.55 μg of the anti-Thy 1 immunotoxin, OX7-saporin, and 8 naive rats served as unoperated controls. One month later, animals were trained on a step-through passive avoidance task using single daily trials for one week. In this task, rats were placed into a brightly lit chamber with an opening into a dark chamber. After acclimation trials, rats received a foot shock every time they entered the dark chamber. Latencies to enter the dark chamber increased with successive trials. Naive controls and OX7-saporin treated rats showed significantly greater increases in latencies to enter the dark chamber than did the 192-saporin rats. After behavior testing, rats were sacrificed and brain sections processed for immunocytochemical demonstration of choline acetyltransferase (ChAT), p75^{NGFr} and acetylcholinesterase (AChE). There were no significant anatomical differences between OX7-saporin and control rats. All 192-saporin rats showed loss of CBF neurons with decreased AChE-positive fibers in the hippocampus and frontoparietal neocortex but not in the amygdala. Densitometric analysis revealed that cortical dennervation was more complete in 192-saporin rats that did not learn the avoidance task (N=4) compared to those that learned normally (N=4). The results support the conclusion that the CBF projection to the neocortex, but not the projections to hippocampus or amygdala, is critical for passive avoidance learning.

506.2

DOSE DEPENDENT EFFECTS OF IMMUNOTOXIC LESION OF THE RAT CHOLINERGIC BASAL FOREBRAIN ON LEARNING, ACTIVITY, AND CHOLINE ACETYLTRANSFERASE. J.J. Waite*, and L.J. Thal. Dept. of Neuroscience & Neurology, UCSD & VAMC, San Diego, CA 92161.

In the adult rat, NGF receptors are preferentially located on membranes of cholinergic neurons originating in the basal forebrain. The immunotoxin IgG192-saporin targets low affinity NGF receptors and destroys basal forebrain cholinergic neurons with a high degree of specificity. This toxin therefore produces a more specific cholinergic lesion than infusion of excitotoxins. IgG192-saporin was infused into the left lateral ventricle of rats in 4 doses: 4μg, 1.34μg, 0.34μg, or 0 μg in 10μl PBS. Beginning 9 weeks post-surgery, spatial memory acquisition in a water maze and open field activity tests were conducted. The activity of choline acetyltransferase (ChAT) was measured in homogenates of brain regions to assess lesion effects. Animals receiving 4μg of toxin were unable to learn the water maze task with either a hidden or visible platform. The other groups learned the task as well as controls (mean latencies of 20 trials, hidden platform, 4μg group: 89 sec; 1.34μg, 37 sec; 0.34μg, 34 sec; 0 μg: 32 sec). The 4 μg group exhibited hyperactivity in the open field test compared with all other groups ($p<.001$). Cortical ChAT activity was decreased in all groups infused with toxin in comparison with vehicle controls: 4μg: 18, 11, 15%; 1.34μg: 39, 21, 13%; 0.34μg: 84, 75, 48% of control activity remained in frontal, parietal, and occipital cortex, respectively.

506.3

PERFORMANCE IN A LATENT INHIBITION (LI) TASK BY RATS WITH QUISQUALIC ACID-INDUCED LESIONS OF THE NUCLEUS BASALIS MAGNOCELLULARIS (NBM). S.A. Welner*, A.P. Sen, Z.C. Koty and J. Rochford. Douglas Hospital Research Center, Department of Psychiatry, McGill University, Montreal, Quebec, Canada, H4H 1R3.

Rats with lesions of the NBM provide useful models with which to test the effects of treatments that can potentially ameliorate a lesion-induced behavioral deficit. As such, it is important to first characterize the deficits which ensue from such lesions. In the present experiments we assessed the effects of quisqualic acid NBM lesions in a LI paradigm. The LI phenomenon has been suggested to be an animal model of selective attention, insofar as it assesses the ability to ignore irrelevant stimuli.

Half of 38 male Sprague-Dawley rats were lesioned with 0.12M quisqualic acid bilaterally in the NBM; the remaining half were left unoperated. Choline acetyltransferase activity in the cortex was reduced 40% by these lesions, measured post-test. Following 7 days of recovery, animals were pretrained to bar press for saccharin solution reinforcement. Subsequently, rats in each condition were preexposed to either 40 (PE) or 0 (NPE) presentations of a tone conditioned stimulus. Over the next 2 days all animals received two tone-shock (0.6 mA, 0.5 sec) pairings, and the acquisition of conditioned suppression to the tone was measured. LI was demonstrated by the finding that non- lesioned PE animals took longer to acquire the conditioned suppression response than nonlesioned NPE animals. Both lesioned groups took longer to acquire the conditioned suppression response than did the nonlesioned groups, indicating that lesioning the NBM provoked a deficit in associative learning. More importantly, there were no significant differences in the rates at which the PE and NPE lesioned groups acquired the response. This latter result shows that lesioning the NBM with quisqualic acid abolishes the LI effect, suggesting that these lesions produce deficits in selective attention (Funded by MRCC and FRSQ).

506.4

PHAMACOLOGICAL EVIDENCE FOR LONG-TERM GRADUAL CHANGES IN THE INVOLVEMENT OF CHOLINERGIC MECHANISMS IN RETRIEVAL. S. Multon, J. Micheau* and R. Jaffard. Lab Neurosciences Comportementales et Cognitives URA CNRS 339 Av. des Facultés 33405 Talence cedex France.

Several studies have provided evidence for gradual and long-lasting changes in the neuronal substrates that subserve the retrieval of information. Among these studies, results from pharmacological investigations have suggested that synaptic efficiency of cholinergic neurones was altered as a function of time after learning and hence of the "age" of memory (Deutsch, 1971). The present study was aimed at determining the influence of individual differences in initial learning ability on subsequent sensitivity of short-term *vs* long-term retention performance to the antimuscarinic scopolamine. Mice were trained (one daily session for 12 days) in an appetitively reinforced conditional discrimination (if...then) task in a T-maze. Retention performance was assessed at either 1, 15 or 30 days after acquisition. For each training-to-test interval a different set of animals was tested under each of for drug treatments (0.1, 0.25 mg/kg scopolamine, 0.25 mg/kg methyl-scopolamine and saline,sc) administered 30 mn before each testing session. Following training, the animals were divided into 2 groups on the basis of their acquisition performance and refered as "good learners" and "poor learners". Results show that (i) under saline treatment, retention performance decreased as a function of time in good learners whereas it increased in slow learners (ii) whatever the retention interval, scopolamine (0.25 mg/kg) dramatically impaired retention performance in good learners whereas at 30 days post-training the treatment induced a slight improvement in poor learners (interaction group x scopolamine 0.25: $p < 0.001$). These results might be accounted for (i) by an imbalance between the use of a "cholinergic-dependent" (if...then) and a "cholinergic independent" (go-nogo) stategy at the time of initial acquisition; (ii) as time passes, there would be a general weakening of the bringing into play of cholinergic mechanisms at the time of retrieval; (iii) this would facilitate performance of poor learners by upsetting the imbalance to the "cholinergic-independent" go-nogo strategy that they would preferentially use, while disrupting performance of good-learners that are hypothesised to solve the task using the "cholinergic-dependent" alternative strategy.

506.5

SPATIAL WORKING MEMORY AT LONG RETENTION INTERVALS: DEPENDENCE ON MAINTAINED CHOLINERGIC ACTIVATION IN SEPTO-HIPPOCAMPAL OR NBM-CORTICAL PATHWAYS ? T.P.Durkin*. Lab. Neurosciences Comportementales et Cognitives, CNRS URA n° 339, Univ. Bordeaux I, Av. des Facultés, 33405 Talence, France.

Studies of dynamics of cholinergic activity in the septo-hippocampal (S-H) and NBM-cortical (N-C) pathways at various stages during repeated testing of mice with selective memory protocols (DURKIN and TOUMANE, Behav. Brain Res. 1992, 50: 43-52) showed that the durations of cholinergic activation in each pathway varied as a function of the type of memory tested and the level of task mastery. Since 1) the hippocampus may act as a temporary memory buffer and 2) working memory items are not thought to be consolidated, we postulated that the **duration of activation in the S-H pathway may govern the maintenance of the working memory trace over the retention interval.** C57 Bl/6 mice were extensively trained (1 trial/day, 25 days) on a DNMTS task to attain high levels of retention (>80% correct) using either 5min (Gp 1) or 60 min (Gp 2) retention intervals. At various times (30sec-70min) following initial acquisition cholinergic activity in the hippocampus and frontal cortex was quantified using high-affinity choline uptake. Whereas cholinergic activation was observed in both pathways at 30sec post-acquistion and throughout the 5min retention interval in Gp 1, the situation in Gp 2 is different, S-H activation being maintained only for 15 min, while N-C activation is maintained for the totality of the 1hr retention interval. The N-C pathway, in addition to its role in reference memory processes may, thus, also subsume a consolidation-like role in working memory situations requiring retention intervals of longer than 15 min. This "backup"function would thus liberate the the S-H complex from its active role in temporary trace maintenance in these cases.

506.6

MK-801 IMPAIRMENTS OF PATTERN TRACKING UNDER TRANSFER FROM HIGHLY STRUCTURED TO LESS STRUCTURED SERIAL PATTERNS IN RATS. J.D. Rowan*, P.S. Fowler, and S.B. Fountain. Department of Psychology, Kent State University, Kent, OH 44242.

The NMDA antagonist, MK-801, impairs some aspects of serial-pattern learning in rats. The effects of MK-801 exposure on serial-pattern learning in a transfer task were examined. Rats were trained to anticipate the correct sequence of leverpresses in an array of levers mounted on the walls of an octagonally-shaped operant chamber. The training pattern used in the first phase of the experiment was highly structured

123 234 345 456 567 678 781

where digits represent the positions of levers in the chamber, spaces indicate 3-s pauses, and other ITIs were 1 s. After rats learned the pattern, they were transferred to one of two new patterns that contained all elements of the first pattern and three additional elements. For the Perfect group, the added elements (812) were consistent with the structure of the training pattern. For the Violation group, the last of the added elements (818) violated the structure of the pattern learned in training. At transfer, rats were exposed to 0.0625 mg/kg MK-801 by i.p. injection 30 minutes prior to testing. Rats in the violation group that were injected with MK-801, unlike comparably exposed rats in the perfect group, demonstrated impaired performance throughout the entire pattern when compared to saline controls. These results support the view that MK-801 impairs the integration of new information that is not consistent with previously-learned pattern structure. However, MK-801 may have little effect on acquisition of responses consistent with previously-learned lower-order rules.
(Supported by NIMH MH48402.)

506.7

CHOLINERGIC INVOLVEMENT IN RETENTION OF SERIAL-PATTERN STRUCTURE IN RATS. M.O. Wollan* and S.B. Fountain. Department of Psychology, Kent State University, Kent, OH 44242.

Rats are sensitive to the rule-based formal structure of patterned sequences of responses (i.e., of "serial patterns"). For example, during acquisition rats make more errors at places where the rules change, namely, on the first elements of chunks, than on within-chunk elements. Atropine is a muscarinic cholinergic antagonist which impairs retention performance on a variety of cognitive tasks. We examined the effects of atropine on retention of serial patterns. Rats were trained in an octagonal operant chamber equipped with a lever on each wall. They learned to press the levers in a particular order (the serial pattern) for brain-stimulation reward (BSR) in a discrete-trial procedure with correction to a criterion of 10 percent or fewer errors on each trial. Two groups learned a pattern composed of eight 3-element chunks:

123 234 345 456 567 678 781 812

where the numbers represent positions of levers in the chamber, spaces indicate 3-s pauses (Phrased group) or 1-s pauses (Unphrased group), and other ITIs were 1 s. After criterion was reached, rats received i.p. saline injections alternating with 50, 25, and 100 mg/kg injections of either atropine sulfate or atropine methyl nitrate. Atropine sulfate caused significant impairments in retention performance at the first elements of chunks for both phrased and unphrased patterns. Atropine methyl nitrate caused no significant changes in performance. The results support the idea that intact central cholinergic systems are required for proper performance at places in sequences where pattern structure changes.
(Supported by NIMH MH48402.)

506.8

THE ROLE OF THE SEPTOHIPPOCAMPAL CHOLINERGIC PROJECTION IN CONTEXTUAL LEARNING AND SHORT-TERM MEMORY PROCESSES. G.M. McAlonan, G.R. Dawson, L.O. Wilkinson, L.S. Wilkinson, T.W. Robbins, B.J. Everitt. (SPON: European Neuroscience Association) Depts Anatomy and Expt Psychology, University of Cambridge, U.K. & Merck Sharp and Dohme, Neuroscience Research Centre, Harlow, Essex, U.K.

These experiments investigated the effects of lesions of the septohippocampal cholinergic projection on learning and memory. AMPA (0.015M) infused at two sites in the medial septum/diagonal band (MS/BD) markedly destroyed cholinergic neurons, while sparing completely the lateral septal nuclei. There was a concomitant 65% reduction of ChAT activity in the hippocampus, while acetylcholine release in the hippocampus measured by *in vivo* dialysis was negligible. These MS/vDB lesions resulted in a significant, delay-dependent deficit in a spatial, delayed non-matching to sample test that was ameliorated following systemic physostigmine treatment. Hippocampal dysfunction following cholinergic depletion was further investigated using the water maze and an aversive CS/contextual learning paradigm. Previous work has shown that lesions of the hippocampus itself disrupt both spatial navigation and contextual learning, while sparing conditioning to an aversive CS. However, AMPA lesions of the MS/vDB resulted in only a mild disruptive effect in the final stages of acquisition of the water maze, but significantly enhanced contextual conditioning at the expense of conditioning to a weak aversive CS.

Cholinergic deafferentation of the hippocampus may thus allow a competitive advantage of context-mediated responses over CS-mediated strategies. However, the conditional retrieval of information in a short-term spatial memory task is significantly impaired by such cholinergic loss.

506.9

ANTERIOR, BUT NOT POSTERIOR, CINGULATE CORTEX LESIONS FACILITATE ACQUISITION OF A CONDITIONAL VISUAL DISCRIMINATION TASK. T.J. Bussey*, J.L. Muir, B.J. Everitt+ and T.W. Robbins. Departments of Experimental Psychology and Anatomy+, University of Cambridge, Cambridge CB2 3EB, U.K.

It has been suggested that anterior and posterior cingulate cortices are involved in non-spatial and spatial forms of learning and memory. For example, it has been proposed that anterior cingulate activity predominates during the early stages of learning, while the posterior cingulate predominates in later stages.

The present study examined the effects of excitotoxic lesions of either anterior or posterior cingulate cortex on the acquisition and performance of a conditional visual discrimination task. Rats were trained in a Skinner box to respond by lever pressing to fast and slow flashing lights. The animals were required to learn a rule of the type 'if fast flash, go left; if slow flash, go right'.

Paradoxically, anterior cingulate lesions facilitated acquisition of the task relative to posterior cingulate lesioned animals or sham controls. Detailed analysis revealed that this facilitation occurred predominantly as a result of enhanced learning over trials in which the fast flashing stimulus was presented. The data will be discussed with respect to performance of these animals on the task following various manipulations of the basic paradigm, including interpolation of delays, variation of inter-trial intervals and modification of discriminanda.

506.10

DISSOCIABLE BEHAVIOURAL EFFECTS FOLLOWING EXCITOTOXIC LESIONS OF ROSTRAL AND CAUDAL ELEMENTS OF THE BASAL FOREBRAIN CHOLINERGIC SYSTEM. J.L. Muir*, B.J. Everitt+ and T.W. Robbins. Departments of Experimental Psychology and Anatomy+, University of Cambridge, Cambridge CB2 3EB, U.K.

The basal forebrain (BF) cholinergic system, comprising the nucleus basalis of Meynert (nbM), the medial septum and the diagonal band of Broca has been implicated in a variety of forms of learning and memory. The cortical targets of each of these components of the BF are dissociable and this anatomical dissociation may have important implications regarding the functions of these elements of the BF cholinergic system. The present study compared the effect of excitotoxic lesions of the nbM with those of the vertical limb of the diagonal band (VDB) on tasks assessing visual attentional function or conditional visual discrimination learning. The nbM lesions, which produced significant reductions in choline acetyltransferase (ChAT) activity in neocortex, had no effect on conditional learning, but profoundly affected attentional function. This impairment in task accuracy was reversed by physostigmine or nicotine, but not by the 5-HT_3 receptor antagonist, ondansetron. In contrast, lesions of the VDB, which cholinergically denervated the cingulate cortex, had no effect on attentional function but affected conditional visual discrimination.

Thus the consequences of lesions of BF cholinergic neurons depend initially on the functions subserved by the cortical area(s) to which they project. The VDB-cingulate cortex projections may subserve the learning and memory functions previously attributed to the nbM. The nbM-neocortical projection appears instead to be critical for visual attentional function.

506.11

IMPAIRMENT OF REACQUISITION AND PERFORMANCE OF DELAYED NONMATCHING-TO-SAMPLE FOLLOWING LESIONS TO THE MEDIAL SEPTUM AND DIAGONAL BAND IN RATS. T.J. Kornecook, T.E. Kippin, J.P.J. Pinel and E.R. Wood*. Dept. Psych., Univ. British Columbia, Vancouver, BC, Canada, V6T 1Z4.

Damage to the hippocampal complex and adjacent cortical tissue impairs object recognition-memory in rodents, monkeys, and humans. The neuronal cell bodies of the medial septal area (MSA) and vertical limb of the diagonal band of Broca (VDBB) are the primary source of cholinergic innervation of these medial temporal-lobe structures. We examined the effects of combined bilateral electrolytic lesions to the MSA and VDBB on object-recognition memory in rats using a nonrecurring-items delayed nonmatching-to-sample (DNMS) task that is similar to the task that is commonly used to study amnesia in monkeys.

Male Long-Evans food-deprived rats were trained to criterion on the DNMS task at a 4-sec delay, and then they were tested at retention delays of 4, 15, 30, 60, and 120 sec. Following surgery they were retrained and then tested again. Rats that received MSA-VDBB lesions (n=4) performed more poorly after surgery than did the sham-lesion controls (n=4). They acquired the task more slowly, and once they reached criterion, they performed more poorly at all of the delays. These results suggest that combined MSA-VDBB lesions produce retrograde amnesia for the reference memories involved in performing the DNMS task and anterograde amnesia for the retention of specific objects. However, it is also possible that either or both of these impairments reflect lesion-produced attentional deficits, motivational deficits, or changes in the strategy that was being used to solve the task. Lesions of the MSA and VDBB have previously been found to result in decreases in cholinergic activity at their target sites; such decreases at hippocampal and entorhinal target sites might underly the deficits observed in this experiment.

506.12

GENDER DIFFERENCES IN THE EFFECT OF NEONATAL BASAL FOREBRAIN LESIONS ON SPATIAL NAVIGATION. J.Berger-Sweeney, K. Meadows, J.Mills and C.F. Hohmann* Dept. of Biological Sciences Wellesley College, Wellesley, MA and The Kennedy Krieger Research Institute, Baltimore, MD.

We have previously shown that lesions of the basal forebrain [nBM] projections to cortex in neonatal mice result in persistent changes in cortical connectivity and cytoarchitecture. These persistent cortical abnormalities are correlated with dramatic behavioral deficits on a spatial navigation task (using the Morris water maze) in the adult mice. Since gender differences in performance have been observed in spatial navigation, we are interested in determining whether gender differences are present in this nBM lesion model.

A total of 22 BALB/cByJ mice were used in this experiment. We have analyzed data from 12 of these mice at this point. Mice received lesions to the nBM 12-24 hours after birth; litter mates served as controls. Spatial navigation testing began at 8 weeks of age and continued for 2 weeks. Following behavioral testing, mice were processed for Nissl and AChE histology to evaluate cortical morphology and lesion efficacy.

In the spatial navigation task, mean latencies to find the submerged platform decreased steadily during the first 3 days in the male controls to asymptotic performance by day 4. Female controls took longer to find the submerged platform on virtually all 9 days and their asymptotic performances were never as low as that of the control males. In the nBM neonatal lesion groups, both females and males performed worse (had longer latencies) than the control groups. In contrast to controls, the lesioned females performed better than the lesioned males on all 9 days of behavioral testing. Histological analysis confirmed lesion sites and cortical morphological abnormalities in the nBM groups. These data suggest that neonatal nBM lesions produce differential patterns of spatial learning in females and males. Cortically-projecting basal forebrain neurons could, therefore, play a critical role in the development of gender differences in spatial memory in adult mice.

506.13

BEHAVIORAL ALTERATIONS CAUSED BY NORADRENERGIC AND CHOLINERGIC DEPLETIONS IN RAT HIPPOCAMPUS AT NEONATE. H.-M. Hwang, G.-H. Yeh, S.-C. Weng, and E.H.Y. Lee*. Department of Anatomy, Chang Gung Medical College and Instit. Biomed. Sci., Academia Sinica, Taiwan, R.O.C.

Acetylcholine (ACh) and norepinephrine (NE) in the mammalian brain have been demonstrated to play a role in the hippocampal processing of sensory information. To elucidate their involvement, locomotor activity and spatial learning were measured in four groups of Long-Evans hooded rats: ACh-depleted, NE-depleted, ACh/NE-depleted, and control. ACh depletion was done by electrolytic lesions (DC current: 3 mA for 10 sec) of the medial septum 5-8 days after birth. NE was depleted by subcutaneous injection of DSP-4 (50 or 100 mg/Kg) at birth. Both depletions were confirmed at ages of 4-6 months. ACh/NE-depleted rats showed an increase in locomotor activity while ACh-depleted and NE-depleted rats did not show any activity change. In a series of spatial tasks with Morris's parameters, memory impairement was found only in rats depleted of both ACh and NE in the hippocampus. These results suggest that ACh and NE function integratively to modulate locomotion and spatial learning in rats.

506.14

EFFECTS OF SELECTIVE MODULATION OF SEPTAL NORADRENERGIC AFFERENTS ON ANXIETY AND SPATIAL WORKING MEMORY PARADIGMS IN MICE. M. Belotti, D. Galey*. Lab. de Neurosciences Comportementales et Cognitives. CNRS URA 339. Avenue des Facultés 33405 Talence Cedex. FRANCE.

Substantial data now provide evidence that the septal area is involved in the regulation of anxiety-related behaviors and memory processes. Using C57Bl/6 mice, we have attempted to elucidate the role of septal noradrenergic afferents in these two processes.

The anxiety level was evaluated by comparison between exploratory activities measured in an elevated plus-maze and in a four hole-board. The spatial working memory investigations, achieved in a radial-maze, were based on the acquisition of the delayed non-matching to place rule.

In these conditions, selective blockade of noradrenergic activation by bilateral intraseptal infusion of an α_1 antagonist , BE 2254 (500 ng/ 0,2 µl) did not induce changes in anxiety level. Similar lack of modification was observed on working memory performance evaluated in two different conditions. However, when the treatment was applied before the habituation session, when animals were initially exposed to the context, they were impaired in subsequent acquisition of the rule. An inverse effect was obtained by intraseptal infusion of idazoxan (0,5ng/0,2µl).

These data suggest that septal noradrenergic activation is essential at initial stages of learning, when animals process new environmental features, according to the Nadel and O'Keefe contextual cognitive map theory.This interpretation is presently under investigation.

506.15

NITRIC OXIDE IN BASAL FOREBRAIN NEURAL DEGENERATION. CA Harrington*, S Mobley and GL Wenk. ARL Div. of Neural Systems, Memory and Aging, University of Arizona, Tucson, AZ, 85724.

Degeneration of the basal forebrain (BF) cholinergic system may underlie the cognitive deficits seen in Alzheimer's disease. Animal models of this disease often employ Ibotenate (IBO) and Quisqualate (QUIS) to lesion the BF. While both neurotoxins destroy BF cells, only IBO impairs memory. One explanation for this differential effect on behavior may be that IBO destroys a neuronal population within the BF that QUIS does not. Recently it was shown that NADPH-diaphorase positive neurons in the BF are destroyed by QUIS, but not by IBO. NADPH-diaphorase is found in neurons that contain the nitric oxide (NO) generating enzyme, NO synthase. Therefore the memory deficit produced by IBO may be due to the differential sparing of NO generating neurons. Cell culture studies have shown that NMDA agonists, such as IBO, produce a NO-mediated neurotoxicity via the generation of high levels of cGMP. Therefore IBO, by generating NO, may be destroying a neuronal population that QUIS does not. We investigated whether excessive NO production is responsible for the memory impairment seen following IBO lesions. Rats were given injections of either IBO, IBO coinjected with the guanylyl cyclase inhibitor methylene blue, methylene blue alone, QUIS, QUIS coinjected with the NO generating compound nitroprusside, or nitroprusside alone. Injections of IBO, but not QUIS, elevated cGMP in the BF. A second group of rats with the same lesions were tested in a food motivated, continuous alternation task on a T-maze. Only the IBO, alone or coinjected with methylene blue, impaired performance on this task. Taken together these results suggest that the generation of NO by injection of IBO into the BF does not result in the loss of a neuronal population that is critical to working memory. Supported by NSF BNS 89-14941.

506.16

ALTERATION IN DENDRITIC MORPHOLOGY OF NEOCORTICAL NEURONS AFTER BASAL FOREBRAIN LESIONS IN ADULT RATS. C.L. Wellman* and D.R. Sengelaub. Program in Neural Science and Department of Psychology, Indiana University, Bloomington, IN 47405.

The nucleus basalis magnocellularis (NBM) is the major cholinergic projection to neocortex in the rat and appears to play a role in the modulation of cortical neuron activity. We have demonstrated that bilateral NBM lesions produce deficits in radial arm maze performance, reductions in laminar thickness, and decreases in average neuronal soma area in lamina II-III of frontoparietal cortex. In the present study, to test the hypothesis that decreased dendritic arborization could also contribute to reductions in laminar thickness, we have assessed dendritic morphology of neocortical neurons in adult rats with unilateral lesions of the NBM. Five rats received unilateral ibotenic acid lesions of either the left or right NBM, as well as sham lesions on the contralateral side. Three months after surgery, the rats were killed and their brains removed and stained using a Golgi-Cox procedure. Pyramidal neurons in lamina II-III of frontal cortex were drawn and morphology of basilar arbors quantified in three dimensions (Eutectic NTS), with the experimenter blind to condition. While average length of individual branches was unaffected by lesion, total dendritic length of neurons ipsilateral to the lesion was over 25% less than that of neurons from the sham-lesioned side ($p<.05$). This relative reduction in total dendritic length was due to significantly less branching on the lesioned side (over 20%; $p<.01$). Therefore, decreases in both neuronal soma size and dendritic branching contribute to reductions in thickness of lamina II-III after NBM lesion. Thus, the behavioral deficits seen after lesion of the NBM may be related to regressive changes in the morphology of lamina II-III neurons in frontoparietal cortex.

LEARNING AND MEMORY: PHARMACOLOGY—OTHER II

507.1

NEFIRACETAM (DM-9384): AN EFFECTIVE COGNITION-ENHANCING AGENT IN NM CLASSICAL CONDITIONING IN OLDER RABBITS. *D.S. Woodruff-Pak*, L.P. Conway, & Y.-T. Li.* Department of Psychology, Temple University, Philadelphia, PA 19122.

Previously, we reported that rabbits administered 10 mg/kg Nefiracetam (DM-9384) attained the highest conditioning rates that we had ever observed in older animals. Here, we report additional results with this nootropic compound. Nefiracetam was evaluated in 40 older rabbits in a 750 msec delay paradigm of classical conditioning of the nictitating membrane (NM)/eyeblink response. Rabbits (mean age = 30.3 months) were assigned in groups of 8 to one of five conditions: Paired tone conditioned stimulus (CS)-corneal airpuff unconditioned stimulus (US) presentations and 5 10, or 15 mg/kg Nefiracetam or sterile saline vehicle (Conditions 1-4, respectively); explicitly unpaired CS and US presentations and sterile saline vehicle (Condition 5). Rabbits in Conditions 1-4 received 15 daily sessions of 90 paired trials. In Condition 5 they received 15 daily sessions of 90 unpaired CS and US presentations. All dependent measures (percentage conditioned responses (CRs), trials to criterion, CR amplitude, response latency) indicated significantly better conditioning in Nefiracetam-treated rabbits in the paired CS-US condition. Whereas Nefiracetam did not elevate motor responding, it facilitated acquisition of NM/eyeblink classical conditioning in older rabbits. Eyeblink conditioning is performed poorly by older humans and is seriously impaired in Alzheimer's disease. These preclinical data in an animal model with clear parallels in humans suggest that Nefiracetam may prove effective as a cognition enhancer in clinical trials. *Supported by Daiichi Pharmaceutical Co., Ltd., Tokyo, Japan.*

507.2

EFFECTS OF T-588, A NOVEL COGNITIVE ENHANCER, ON VARIOUS AMNESIA MODELS IN RODENTS.
S.Ono*, T.Yamafuji, H.Chaki, M.Maekawa, K.Kitamura, K.Hirata, Y.Nakada, H.Hayakawa, T.Hiraiwa and H.Narita, Research Laboratory, Toyama Chemical Co. Ltd., Toyama 930, Japan.

We investigated the cognitive enhancing effect of T-588 ((-)-α-[[2-(Diethylamino) ethoxy] methyl] benzo [b] thiophen-5-methanol hydrochloride) on impairment of passive avoidance tasks induced by CO_2 and scopolamine in mice, on scopolamine-induced amnesia for the 8-arm radial maze task in rats and on acquisition of the active avoidance response of cerebral embolized rats in comparison with those of tacrine (THA). The impairment of passive avoidance tasks was more effectively ameliorated by T-588 (M.E.D.: 0.1-0.3 mg/kg, p.o.) than tacrine (M.E.D.: 1 mg/kg, p.o.). T-588 (3 and 10 mg/kg, p.o.) significantly improved the scopolamine-induced disruption of spatial cognition. Tacrine (3 mg/kg, p.o.) also improved. The cerebral embolization, produced by injecting 1000 microspheres into the internal carotid artery, caused marked impairment of the acquisition of the active avoidance response. In the embolized rats, significant decreases in ACh, DA, NA and 5-HT were observed in the left side of the brain. T-588 (3 and 10 mg/kg, p.o.) significantly improved the impairment of the acquisition of the active avoidance response. But, tacrine (0.3-3 mg/kg, p.o.) had no effect. After oral administration of T-588 (10 mg/kg) to rat, the serum and brain levels of T-588 at 30 min were 87 ng/ml and 1050 ng/g, respectively. The level in brain was 12 times higher than that in serum. These results suggest the usefulness of T-588 in clinical treatment of cerebrovascular disorders and Alzheimer's disease.

507.3

BEHAVIORAL PROFILE OF (R) and (S) RS-56812, NOVEL 5-HT_3 RECEPTOR ANTAGONISTS WITH COGNITION-ENHANCING PROPERTIES.
D. J. Fontana*, C. A. Henderson, S. Daniels, J. Nunes, E. H. F. Wong, R. L. Whiting, and R. M. Eglen. Institute of Pharmacology, Syntex Discovery Research, Palo Alto, CA 94304, U.S.A.

Using several tests of learning and memory, we studied the behavioral action of the (R) and (S) isomers of RS-56812 (1-azabicyclo[2.2.2.]octan-3-yl)aminocarbonyl-1-methylindole hydrochloride), potent 5-HT_3 receptor antagonists, in rodents. In a spatial memory task in the Morris Water Maze, both isomers (0.1-10 µg/kg, i.p.) partially reversed the atropine deficit in young adult rats. In addition, cognition-impaired aged rats treated with (S) RS-56812 (0.1-100 µg/kg, i.p.) performed better than impaired rats treated with vehicle. In behavioral tests of anxiety; such as the Conditioned Suppression of Drinking conflict test (CSD) in rats, neither isomer affected punished or unpunished drinking (3-1000 µg/kg, i.p.). In contrast, in the light:dark box, (S) RS-56812 reduced the behavioral suppression associated with aversion to the brightly illuminated side of the box (0.3 ng-30 mg/kg, i.p.). The (R) isomer did not affect activity in the light:dark box. (R) and (S) RS-56812 did not affect locomotor activity (0.01-1000 µg/kg, i.p.). Furthermore, they did not affect cocaine-induced or scopolamine-induced hyperactivity (0.1-1000 µg/kg, i.p.) and did not affect motor coordination in a roto-rod test (0.01 µg/kg-10 mg/kg, i.p.). In addition, neither isomer produced any changes in gross behavior in the Irwin Profile test (10 ng/kg-1 mg/kg, p.o.). Our results suggest that (R) and (S) RS-56812 are potent pro-cognitive agents which lack deleterious effects on the central nervous system. Thus, RS-56812 may be a valuable therapeutic agent for disorders associated with cognitive dysfunction (also see abstract by Terry et al., this meeting).

507.4

SPATIAL LEARNING PERFORMANCE IN CD1 MICE: EFFECTS OF CGP 36742. V. Libri, R. Carletti and N.G. Bowery*, Dept. Pharmacology, School of Pharmacy, 29/39 Brunswick Square, London WC1N 1AX.

CGP 36742 (3-aminopropyl-n-butyl phosphinic acid) is the first selective orally active $GABA_B$ antagonist which improves cognitive functions in experimental animals. Recent finding have shown that systemic injection of (-)baclofen decreases cGMP levels in several areas of the rat brain. This was antagonized by CGP 36742. In addition cGMP was increased by CGP 36742 and this was blocked by competitive inhibitors of nitric oxide synthase, suggesting a possible involvement of nitric oxide in $GABA_B$-mediated effects such as cognition. In the present study CGP 36742 was examined on the performance of CD1 mice in an 8-arm radial maze. Animals (8 per group, 25-30 g) were treated daily for 10 consecutive days with CGP 36742 (1, 10 or 100 mg/kg), (-)baclofen (2 or 4 mg/kg), scopolamine (1 mg/kg) or saline (10 ml/kg), given alone or in combination, and tested on the maze 15 min after the injection (i.p.). Differences between groups were determined by a one-way analysis of variance (ANOVA). In comparison with control animals injected with saline, CGP 36742 (10 and 100 mg/kg but not 1 mg/kg) significantly ($p<0.05$) enhanced the radial maze acquisition (occurrence of first error) whereas (-)baclofen (2 and 4 mg/kg) produced a significant impairment of performance. This depressant effect was completely reversed by pre-treatment (15 min before) with CGP 36742. On the contrary, CGP 36742 did not affect scopolamine-induced amnesia. In order to verify whether the amnesic action of (-)baclofen was related to possible muscle relaxation, animals treated with 2 and 4 mg/kg (-)baclofen were submitted to a rota-rod test (accelerating from 8 to 16 rpm over 360 s period) 15 and 30 min after injection. At these concentrations (-)baclofen failed to affect rota-rod performance. The results are consistent with the suggestion that $GABA_B$ receptor is involved in the control of learning and memory processes. Supported by Ciba Basel.

507.5

SCOPOLAMINE AS A PHARMACOLOGICAL TOOL TO STUDY MEMORY PROCESSES; APPLICATION OF A COGNITION ENHANCER.

T.Duka *, B. Redemann, T. Mager and B. Voet Department of CNS Human Pharmacology, Schering AG, Sellerstrasse 31, D-1000 Berlin 65, Germany

Given the current level of concern over cognitive dysfunction in old age and in certain pathological states, amnestic substances may be useful in characterising human models of cognitive disturbances or in allowing to draw paralles between neurotransmitter changes and changes in cognitive functions. We applied scopolamine as a pharmacological tool to investigate its effects on various stages of mental functioning. Tests used concern hierarchically arranged cognitive performance and included tests of vigilance, continuous attention, cognition and memory. In a double blind placebo controlled study 36 healthy volunteers were assigned to receive either scopolamine 0.5 mg or placebo subcutaneously. Shortly before and 60 min after scopolamine treatment the psychometric battery has been applied. Scopolamine impaired performance in tasks measuring vigilance and continuous attention; number of correct responses to a brief stimulus were decreased and the time required for a correct response was increased. Scopolamine on the other hand in a memory test in which immediate and delayed recall of a series of pictures was tested, impaired only the immediate recall. In a further study with the same design the effects of a possible cognition enhancer, ZK 93426 a ß-carboline benzodiazepine receptor antagonist, administered intravenously in the dose of 0.04 mg/kg, have been investigated in combination with scopolamine . ZK 93 426 has partially antagonised the effects of scopolamine on the attentional and vigilance tasks but not on the immediate recall in the memory test. It is assumed that this effect of scopolamine does not involve mechanisms wich can be influenced by a benzodiazepine receptor antagonist.

507.6

COMPARABLE MODULATION OF RECENTLY ACQUIRED MEMORIES BY GLUCOSE AND FRUCTOSE.

W.A.Rodriguez*, A.Horne, A.N.Mondragon, and D.Phelps. Dept. Behav. Sci., New Mexico Highlands Univ., Las Vegas NM 87110.

A passive avoidance to active avoidance negative transfer paradigm was used to investigate in rats the effects of glucose and fructose on recently acquired memories. Immediate post-passive avoidance conditioning injections of glucose, fructose, or saline were followed 24 h later by active avoidance conditioning. Equimolar 3.2 mg/kg SC doses of both glucose and fructose had no significant effect on acquisition of the reversal task, whereas 10, 32, 100, and 2000 mg/kg SC doses of both sugars significantly impaired subsequent performance and 320 mg/kg SC doses of both sugars significantly enhanced subsequent performance. The cubic trends for both dose-response functions were statistically significant and did not differ from each other. This is the first demonstration that glucose and fructose affect recently acquired memories in accord with similar cubic functions, and that both glucose and fructose can enhance and impair memory compared to saline treatment. The comparable cubic dose-response functions strongly suggest that the mechanisms of action for peripherally injected glucose and fructose are similar. In addition, because fructose does not readily pass the blood-brain barrier, our results suggest that these monosaccharides operate via a common peripheral pathway.

507.7

PARADOXICAL SLEEP PREDICTS MEMORY DEFICITS A YEAR LATER, WHICH ARE REVERSED BY GLUCOSE, IN RATS PRENATALLY EXPOSED TO ALCOHOL. W.S. Stone*, G. Arankowsky-Sandoval, H.J. Altman and P.E. Gold. Dept. Psychology, U. of Virginia, Charlottesville, VA 22903, and Inst. of Gerontology, Wayne State U., Detroit, MI 48202.

We previously demonstrated parallel deficits in paradoxical sleep and memory in young adult rats (6 months old). These results suggest the possibility that measures of paradoxical sleep could predict memory deficits later in life, and glucose, which we previously found attenuates memory deficits in old rodents and humans, could reduce the deficits.

To address this issue, sleep measures (3 hr records via cortical electrodes) were obtained from adult female rats (6 months old) either prenatally exposed to alcohol (their mothers received a liquid diet which included 35% ethanol-derived calories from gestation days 8-19; N=5), or prenatally exposed to an (pair-fed) isocaloric diet (N=6). One year later, rats from both groups were assessed for spontaneous alternation behavior in a Y-maze (8 min), 30 min after glucose (100 mg/kg) or saline administration (IP, using a counterbalanced, crossover design).

At 18 months of age, alcohol-exposed rats showed significantly lower alternation scores than did pair-fed controls. Glucose reversed this deficit. Paradoxical sleep levels at 6 mo. were significantly correlated with spontaneous alternation levels one year later in alcohol-exposed but not pair-fed rats. These findings provide evidence that sleep measures can predict cognitive deficits over large portions of the lifespan, and glucose can attenuate memory deficits in a variety of impaired populations. Supported by NSF BNS-9012239, ONR N0001489-J-1216, and NIA AG 07648. We gratefully acknowledge the Fetal Alcohol Res. Ctr. at Wayne State U. for providing the alcohol-exposed rats.

507.8

INHIBITORY AVOIDANCE DEFICITS RESULTING FROM INTRA-AMYGDALA PROPRANOLOL ARE NOT REVERSED BY GLUCOSE. R.C. Lennartz*, E.R. Mook, K.L. Hellems, and P.E. Gold. Department of Psychology, University of Virginia, Charlottesville, Virginia 22903.

Systemic glucose administration reverses neural and behavioral effects of drugs directed at several neurotransmitter systems. When injected directly into brain targets, intra-septal and intra-amygdala injections of glucose attenuate deficits for inhibitory avoidance learning produced by morphine injections into the same site. However, in contrast to results obtained with systemic injections, direct septal or amygdala injections of glucose do not appear to reverse the effects on learning and memory of other drugs such as scopolamine or AP5. This study determined whether intra-amygdala injections of glucose reverse inhibitory avoidance deficits produced by intra-amygdala injections of the β-adrenergic antagonist, propranolol. When administered 10 min before inhibitory avoidance training (2 ma, 1 s), bilateral intra-amygdala injections (0.5 μl) of propranolol (10, but not 1-5 μg) impaired retention on tests 24 hr later. The propranolol-induced deficit was not seen in rats with placements near but outside of the amygdala. Footshock (flinch, jump) thresholds were not significantly altered by propranolol. Glucose co-injected with propranolol did not attenuate the deficit at any dose tested (1.5, 3, 6 μg). Thus, in contrast to results obtained with intra-amygdala morphine, glucose does not attenuate inhibitory avoidance deficits produced by a noradrenergic antagonist. Such differences in interactions between glucose and neurotransmitter systems should help to define the actions by which glucose enhances memory. Supported by NIA (AG 07648), NIMH (5-T32-MH1811), and NSF (BNS-9012239).

507.9

THE EFFECT OF ETHYLESTRENOL ON ACQUISITION AND RETENTION. J.A. Varner, P.E. Yoder, and R.L. Isaacson*. Psychology Dept., Binghamton Univ., Binghamton, NY 13902.

Ethylestrenol, a synthetic steroid with testosterone and estrogen components, was administered to adult male Long-Evans rats to investigate its role in acquisition and retention. Animals were injected i.p. daily with either 100 µg of ethylestrenol dissolved in 10% ethanol further diluted with double-distilled water or with the vehicle alone (volume of 0.1 cc). Injections began one week prior to testing and continued throughout the study. A modified hole board task was used. The animals were required to find a food reward which was placed in a given hole. Acquisition of the task was measured as the time required to reach the food hole over each of five days, eight trials per day. Following a ten day rest period a one day, eight trials (only), retention test was given. Then, the rats were trained with a new food location hole for the two subsequent days. There were no differences between the groups in performance during either the original five day period or the final two day new hole period. During the retention test after the ten day interval indicated above, however, a significantly reduced time to reach the food reward was found for the ethylestrenol treated rats. This suggests that retention was enhanced by the administration of ethylestrenol.

507.10

NEUROSTEROIDS BLOCK ETHANOL-INDUCED AMNESIA. C.L. Melchior*, A. Glasky and R.F. Ritzmann. West Los Angles Veterans Administration Medical Center, Los Angeles, CA 90073

The neurosteroids (NS) dehydroepiandrosterone (DHEA), its sulfate (DHEAS), pregnenolone (PE), its sulfate (PS), and pregnanolone (PA) have been shown to improve memory in avoidance tasks and in the win-shift test of working memory. The win-shift test consists of placing a mouse in a T-maze with a reward in both goal boxes. The mouse is allowed to chose one of the goal boxes. On the next trial, if the mouse remembers which box it entered on the previous trial, it will enter the other box (correct). By increasing the delay between trials the length of time a mouse can remember can be determined. The longest delay at which C57BL/6 mice can remember is 120 seconds. At this delay, control mice went to the correct side 80% of the time. Ethanol (0.5 g/kg, IP), 10 minutes prior to testing, reduced the correct responses to 65%, without altering the time to leave the start box or the time to traverse the maze. When 50 µg/kg NS were given IP 20 minutes prior to ethanol, DHEA, DHEAS, PE, PS, and PA all blocked ethanol-induced amnesia. PA was found to block at doses from 15 mg/kg to 5 ng/kg; lower doses were devoid of activity. Doses of PA above 5 µg/kg also increased the latency to leave the start box and time to traverse the maze. Epipregnanolone (5ß-pregnan-3ß-ol-20-one), which has been shown to antagonize some biochemical effects of PA and was devoid of any effect on ethanol-induced amnesia at doses of 50 µg/kg to 1.0 mg/kg, when co-administered (50 µg/kg) with 5 µg/kg PA prevented PA from blocking the ethanol amnesia. However, it did not alter the effect of 50 µg/kg PE. Thus, the NS appear to act in more than one way to block ethanol-induced amnesia.

Supported by the Veterans Administration and NIAAA AA08709.

507.11

ESTROGEN/PROGESTERONE TREATMENT REVERSES THE EFFECT OF SCOPOLAMINE ON T-MAZE PERFORMANCE.

*A.J. Fader, D.J. Rawls, and G.P. Dohanich.**
Department of Psychology and Neuroscience Program, Tulane University, New Orleans, LA 70118.

Scopolamine, a muscarinic receptor blocker, inhibits behaviors associated with mating in female rats including the dorsoflexive posture lordosis. Recently, we reported that the inhibition of lordosis by scopolamine was reversed by repeated administration of high doses of estrogen in combination with progesterone. The purpose of the present experiments was to determine if hormone treatment might attenuate the effects of scopolamine on other behaviors. Ovariectomized rats were trained to complete a reinforced T-maze alternation task. Then, females were treated at 72, 48, and 24 hours before T-maze testing with estradiol benzoate (25 μg, i.m.) or oil vehicle and at 4 hours before testing with progesterone (500 μg, i.m.) or oil vehicle. Fifteen minutes following administration of scopolamine hydrobromide (0.2 or 0.4 mg/kg, i.p.), females were given the opportunity to alternate between the arms of the T-maze on 10 consecutive trials. In the absence of hormone treatment, females failed to alternate between the arms successfully, performing at only chance levels. Administration of estrogen in combination with progesterone prevented this effect of scopolamine on T-maze performance. Estrogen also attenuated the inhibitory effect of scopolamine when administered without progesterone, although its effectiveness was reduced. Similar results were obtained in two series of experiments. Gonadal hormones can prevent the impairment of T-maze performance induced by scopolamine. NSF-BNS9021447

507.12

NEUROSTEROIDS DECREASE HIPPOCAMPAL RECURRENT INHIBITION AND INDUCE UNIT SYNCHRONY TO THETA RHYTHM Young, W.G.*, Steffensen, S.C. and Henriksen, S.J., Scripps Research Institute, La Jolla, CA 92037

Several neurosteroids have been shown to have proconvulsant and memory enhancing properties and to be potent modulators of the GABA/benzodiazepine receptor-chloride ionophore complex. We evaluated the effects of the natural sulfate esters of the neurosteroids dehydroisoandrosterone (DHEAS) and pregnenolone (PREGS) as well as trilostane, an inhibitor of 3β-hydroxysteroid dehydrogenase, on EEG recordings, field responses and single-unit activity in the dentate gyrus and CA1 hippocampus of halothane-anesthetized rats. Microelectrophoretic application of DHEAS into CA1 had no effect on field EPSPs but increased population spikes (70%), whereas DHEAS increased dentate field EPSPs (50%) but population spikes were not significantly altered. Microelectrophoretic application of DHEAS abolished the early paired-pulse (PP) inhibition in both the dentate and CA1, whereas microelectrophoretic application of PREGS increased CA1 PP inhibition but had no effect on dentate PP responses. Intraperitoneal administration of DHEAS (2 mg/kg) markedly decreased dentate and CA1 PP inhibition similar to locally applied DHEAS. Application of the GABAb antagonists phaclofen or saclofen antagonized the effects of DHEAS on field potentials and PP responses in CA1, but not the dentate. Three days following intraperitoneal administration of trilostane (3-120 mg/kg), dentate PP inhibition was significantly reduced and CA1 PP inhibition was increased. In control animals under halothane anesthesia, the hippocampal EEG showed periodic episodes of theta rhythm. Neither DGCs, hilar cells, CA1 pyramidal cells nor alveus/oriens cells followed the theta rhythm with significant fidelity; however, all cell types synchronized to the theta rhythm following administration of trilostane Microelectrophoretic DHEAS markedly increased the firing rate of dentate granule cells (DGCs, 200%) and synchronized their firing to the theta EEG. These results demonstrate that the increased hippocampal excitability produced by the neurosteroid DHEAS is mediated by GABAb receptors and suggest that endogenously released neurosteroids may serve to entrain hippocampal neurons to the theta rhythm.

507.13

COMPARING THE EFFECTS OF COCAINE, LIDOCAINE AND COCAINE METHIODIDE ON ACQUISITION OF AN AUTOSHAPED LEVER-TOUCH RESPONSE. R.R. Rule*, P.H. Janak, and J.L. Martinez, Jr. Dept of Psychology, Univ. of California, Berkeley, CA 94720.

The effects of daily peripheral (i.p.) post trial injections of cocaine, lidocaine and cocaine methiodide were investigated. Male Sprague-Dawley rats received 10 daily pairings of a retractable lever (conditioned stimulus; CS) and food delivery (unconditioned stimulus; UCS). If the subject touched the lever, then the lever retracted and food delivery occurred immediately; otherwise the lever was retracted and food was delivered at the termination of the 10 second CS interval. Cocaine at a dose of 7.5 mg/kg, but not at a dose of 5.55 mg/kg, impaired acquisition of the lever-touch response, as compared to the saline-treated control group. Because the injections were given after each conditioning session, we suggest that cocaine affects the neural processes involved in consolidation of memory. Lidocaine administered at doses (5.97 and 4.42 mg/kg) which were equimolar to those of cocaine, did not affect lever-touch responding, as compared to the saline-treated control subjects. Because lidocaine has similar anesthetic properties to cocaine, we suggest that the impairment of acquisition observed in cocaine-treated rats is not due to it's anesthetic properties. Cocaine methiodide, a quaternary derivative of cocaine, which does not readily cross the blood-brain barrier does not effect acquisition when administered at a dose equimolar (9.82 mg/kg) to the effective dose of cocaine; however a higher dose (29.46 mg/kg) impairs acquisition. This may indicate that cocaine's effect on autoshaping are at least partially due to peripheral effects. (Supported by a Ford Predoctoral Fellowship and DA06192)

507.14

DAILY POSTSESSION COCAINE IMPAIRS DEVELOPMENT OF AN INSTRUMENTALLY-CONDITIONED LEVER-TOUCH RESPONSE IN RATS. P.H. Janak* and J.L. Martinez, Jr. Dept. of Psychology, University of California, Berkeley, CA 94720.

Previously, we found that postsession cocaine (5.55 mg/kg IP) impaired response development in an autoshaped lever-touch task that contains both classical and instrumental contingencies (Janak et al.,1992). Therefore, in separate studies, we examined the effects of daily postsession cocaine on the development of conditioned responding in tasks with either purely instrumental or purely classical contingencies. In each study male Sprague Dawley rats received 10 daily presentations of a retractable lever. In the instrumentally-conditioned task, if the subjects contacted the lever, then the lever immediately withdrew and food was delivered; however, if the subjects failed to contact the lever within 10-sec, the lever was retracted and no food was delivered. In contrast, in the classically-conditioned task, contact with the lever had no effect and food delivery always followed lever retraction. All subjects received a IP injection of cocaine or saline immediately after each session. Cocaine (5.55 mg/kg) impaired development of lever-touch responding under instrumental contingencies [$F(1,24)=4.64, p<.05$], but not under classical contingencies. These results suggest that cocaine-induced impairment of responding in the autoshaped task is due to disruption of the formation of the instrumentally-, rather than the classically-conditioned association. (Supported by DA06192.)

507.15

LEARNING DURING GENERAL ANESTHESIA: EVIDENCE FOR CLASSICAL CONDITIONING BUT NOT LATENT INHIBITION

R. Pang, H. Turndorf and D. Quartermain*.
Departments of Anesthesiology & Neurology, NYU Medical Center, 550 1st Ave. New York, N.Y.10016

Conditioned emotional response (CER) and latent inhibition (LI) were employed to test for the presence of memory formation during anesthesia in mice. In the CER, mice were given 3 CS-UCS presentations (ITI 5 min) while anesthetized with halothane. The CS was a 10 sec tone and the UCS 0.4mA shock delivered intramuscularly to the hind limbs via needle electrodes. Control groups received CS only, UCS only or CS followed by a 3 min delayed UCS. In the LI procedure, mice were exposed under halothane anesthesia to 3 sessions of 60 unreinforced tone presentations prior to being conditioned in the unanesthetized state. A control group was given 3 sessions of anesthesia without tone presentation. Strength of fear conditioning was assessed 24 hr later by measuring duration of suppression of drinking induced by the tone in a test session. Results showed that mice conditioned under anesthesia exhibited significantly more suppression than the 3 control groups. Mice preexposed to the tones during anesthesia showed no evidence of the weakened conditioning (LI) that was present in mice preexposed while awake. This suggests this form of learning cannot occur in unconscious subjects. The results were interpreted as evidence that implicit but not explicit memories can be formed under anesthesia.

507.16

CHRONIC ADMINISTRATION OF DOCOSAHEXAENOIC ACID REDUCES THE SPATIAL LEARNING DEFICIT FOLLOWING TRANSIENT FOREBRAIN ISCHEMIA IN RATS. M.Okada[1], Y. Ohgami[1], K.Yazawa[2], K.Mine*[3], K.Iwasaki[1], M.Fujiwara[1]
[1]Dept. Physiol.&Pharmacol., Fac. Pharmaceut. Sci., Fukuoka Univ., Fukuoka 814-01, [2] Sagami Chem. Res. Cent., Kanazawa 229, [3]Dept. Psychosom. Med., Fac. Med. Kyushu Univ., Fukuoka 812, Japan.

The present study is to determine whether oral treatment of purified docosahexaenoic acid (DHA) can reduce the spatial learning deficit following transient forebrain ischemia in rats.

Rats subjected to 10 minutes of transient forebrain ischemia by the method of four-vessel occlusion and then cerebral reperfusion were tested on a radial 8-arm maze task 24 hours after occlusion. DHA was administered singularly 1 hour before occlusion or chronically administered for 21 days before occlusion. The neuroprotective effect of chronic administration of 200 mg/kg of DHA was assessed by measurement of lactate dehydrogenase (LDH) release from the hippocampus slices exposed to the medium lacking oxygen for 60 minutes. Single treatment of DHA (1, 10, 100, 200 mg/kg) did not affect significantly any aspect of the spatial learning deficit following occlusion. On the other hand, chronic treatment of DHA (10, 100, 200 mg/kg) reduced significantly the spatial learning deficit following occlusion. The LDH release from the hippocampus slices induced by oxygen deprivation was almost completely blocked by chronic treatment of DHA.

From these results, the increase in the concentrations of DHA in the hippocampus may be occurred by chronic oral treatment of DHA and it might also play a role in the counteraction of spatial learning deficits induced by transient forebrain ischemia.

507.17

EFFECTS OF NEWLY DEVELOPED T-588 ON HIPPOCAMPUS OF THE RAT, *in vitro* AND *in vivo* STUDIES. T. Kimura , T. Ono, T. Kobayashi*, M. Fukuda and S. Ono . Department of physiology, Faculty of Medicine, Toyama Medical and Pharmaceutical University, Toyama 930-01, Japan. and Research Laboratory, Toyama Chemical Co. Ltd., Toyama 930, Japan.

The effect of newly developed T-588 ((-)- α -[[2-(Dietylamino) methyl] benzo [b] thiophen-5-methanol hydrochloride), which is reported to improve learning disorders and memory processes, on membrane potential, membrane resistance and current-voltage-relations of CA1 hippocampal pyramidal cells in rat brain tissue slices was studied by intracellular recording. T-588 had an ACh-like excitatory action on pyramidal cells, causing depolarization, increased firing, and increased membrane resistance. But this effect was not through cholinergic nor NMDA receptor activation, In another experiment, acquisition of spatial memory was evaluated in a hippocampal lesioned animal model of cerebral ischemia. Rats with selective neuronal death in the hippocampal CA1 subfield after 15 min transient forebrain ischemia were allowed to recover; and were then tested in a spatial navigation task. Postischemic rats tended to have a persistent spatial memory deficit with no motor behavior deficit compared to controls ($p<0.01$). This spatial memory deficit was effectively ameliorated by T-588 (3mg/kg, p.o.). The results suggest that T-588 has a direct excitatory effect on pyramidal cells in the hippocampal CA1 subfield, acting on a different site of action in a different way then cholinergic and NMDA agonists, and ameliorates memory deficit due to selective neuronal death.

507.18

PRENATAL ETHANOL EXPOSURE AND NONSPATIAL VERSUS SPATIAL LEARNING/MEMORY IN RATS. **C.K. Kim#, L.E. Kalynchuk, T.J. Kornecook, V.A. Redila, C.P. McIntyre, D.G. Mumby, J.P.J. Pinel and J. Weinberg*#. Departments of Anatomy# and Psychology, University of British Columbia, Vancouver, B.C., Canada.**

Fetal ethanol exposure (FEE) can cause cognitive deficits and behavioral problems in the offspring. This study examined the effects of FEE on learning/memory. The subjects were adult male offspring from prenatal ethanol exposed (36% EDC)(**E**), pair-fed (**PF**), and ad libitum-fed control (**C**) conditions.

We have previously reported that E rats do not show deficits on a nonrecurring-item delayed nonmatching-to-sample (DNMS) task at retention delays of 4 to 600s, or in relearning this task after a 10 week rest period. Furthermore, these same rats do not display increased distractibility or perseveration of response during the DNMS task. These data suggest that FEE does not affect nonspatial learning/memory. In the present experiment, these same rats were tested for spatial navigational learning/memory using the Morris water maze. The E rats displayed performance deficits on this task. This finding is consistent with other reports of spatial learning/memory deficits following FEE. Taken together, these data suggest that FEE may affect spatial but not nonspatial learning/memory in the same rats. (Supported by NIAAA to JW; NSERC and BCHRF to JPJP)

507.19

AUDITORY SIGNAL DETECTION IN RATS: EFFECTS OF DISTRACTORS, SIGNAL RATE, AND TOLUENE ON VIGILANCE. P.J. Bushnell*[1] and K.L. Kelly[2]. [1]Neurotoxicology Division, US EPA and [2]ManTech Environmental Technology, Inc., Research Triangle Park, NC 27711.

Vigilance involves maintaining attention to repeated stimuli over time; a vigilance decrement (reduced accuracy or speed over time) can be demonstrated under some testing conditions. Male Long-Evans rats performed a discrete-trial operant task in which an auditory signal (a 20-msec increase of 2, 4, or 6 dB(A) in the intensity of continuous 60-dB white noise) was presented on half of the trials. A food pellet was delivered if the rat pressed one of two retractable levers on a signal trial, and if it pressed the other lever on a blank (no signal) trial. Signal detection analysis showed that both sensitivity (sensitivity index, SI) and bias (response index, RI) increased with increasing signal intensity. Presentation of distractor events (intermittent decreases in the intensity of the background WN: -4 dB, 200-800 msec, 2 to 16 per trial) reduced SI at all intensities without affecting RI. Increasing the signal presentation rate from 4 to 13 signals/min reduced SI at 4 and 6 dB, increased RI at 2 dB, and decreased RI at 6 dB. No parametric manipulation induced a reliable vigilance decrement; however, inhalation of toluene during performance of the task did, by reducing SI and increasing response latencies across trial blocks. The toluene-induced vigilance decrement depended upon the concentration of inhaled toluene vapor but was independent of the duration of toluene exposure prior to testing. The toluene-induced reduction in SI was not exacerbated by high signal presentation rates, while its ability to slow responding was.

507.20

ANTAGONIST TIMING DETERMINES SPATIAL NAVIGATION PERFORMANCE FOLLOWING ENVIRONMENTAL NEUROEXCITOTOXIN EXPOSURE. B.F. PETRIE*(1), C. PINSKY(2), G. GLAVIN(2)
(1) Department of Psychology, Red Deer College, Red Deer, Alberta, Canada, T4N 5H5; (2) Department of Pharmacology, University of Manitoba, Winnipeg, Manitoba, Canada R3E 0W3.

The present study examines the effect of parenteral injection of the neuroexcitotoxin domoic acid (DOM), and the non-competitive NMDA antagonist dextromethorphan (DEX) on spatial navigation performance in mice. DOM impaired acquisition of the place task in the Morris water maze, while animals administered DEX subsequent to DOM performed at levels equal to those of saline controls. Animals administered DEX prior to DOM however, showed acquisition rates similar to those of DOM mice. The results indicate that the timing of antagonist administration following environmental neuroexcitotoxin ingestion appears to be a fruitful area of investigation in assessing functional and neuroanatomic deficits associated with neurodegenerative disorders, including senile dementia of the Alzheimer's type.

Thanks to A. Petrie for her skillful assistance in swimming the animals.

INGESTIVE BEHAVIORS IV

508.1

CALMODULIN ANTAGONISTS: EFFECTS ON ANTIESTROGEN BINDING IN BRAIN, FOOD INTAKE AND BODY WEIGHT. J.M. Gray* C. Monian, R. Seaman, J. Bogdany and T. Bialy. Program in Biopsychology, Vassar Coll., Poughkeepsie, NY 12601

Previous data from this lab demonstrated the presence of two distinct antiestrogen binding sites (AEBS) in brain and pituitary, as measured using in vitro binding techniques. In this study we explored the competion of several calmodulin antagonists for the AEBS, using [3H]tamoxifen as the ligand. The calmodulin antagonists all effectively competed with [3H]tamoxifen, with the order of efficacy being tamoxifen > fluphenazine > W-7> bepridil >> ACTH, $ACTH_{1-24}$.

In a second study we examined whether or not daily bepridil administration to ovariectomized rats affected food intake and body weight regulation in ways similar to previously studied drugs, estradiol benzoate, tamoxifen and fluphenazine. While daily administration for 2 weeks of 2 ug estradiol benzoate or 1 mg tamoxifen decreased both food intake and body weight gain, and daily administration of 0.05 mg fluphenazine decreased body weight gain but not food intake, daily administration of 1 mg bepridil had no effect on either food intake or body weight.

508.2

DIENCEPHALIC SITES MEDIATING PROLACTIN-INDUCED HYPERPHAGIA IN DOVES. R. M. Hnasko and J.D. Buntin*. Dept. of Biological Sciences, Univ. Wisconsin-Milwaukee, Milwaukee, WI 53201.

Microinjections of prolactin (PRL) into the ventromedial nucleus of the hypothalamus (VMN) or the preoptic area (POA) have been previously shown to increase food intake and body weight in ring doves. In order to provide a more complete map of PRL-sensitive brain sites mediating this response, we investigated the effects of delivering small volumes (10 nanoliters/injection) of PRL or saline vehicle to a variety of diencephalic sites in dove brain that had been previously demonstrated to contain high concentrations of PRL receptors. Food intake of cannulated male doves was monitored daily during a 6 day baseline period, an initial 4 day treatment period, a 6-12 day post-treatment recovery period, and a second 4 day treatment period. Approximately half of the birds received PRL first (50 ng/10nl twice-daily) and saline vehicle second (10 nl twice-daily), while remaining birds received these treatments in the reverse order. No significant changes in food intake were observed following PRL injections in the lateral POA, paraventricular nucleus of the hypothalamus (PVN), or the region between the tuberal hypothalamus (TU) and VMN. In contrast, injections of PRL into the VMN area, medial POA, or TU resulted in average daily food intake values that significantly exceeded those recorded during vehicle treatment. Nevertheless, the 58% increase in food intake observed following PRL injections into the VMN was significantly greater than the 26% increase recorded following medial POA injections and the 32% increase seen after TU injections. These findings suggest that the VMN may be a primary site of PRL action in promoting hyperphagia in this species, although PRL effects at other diencephalic loci, such as the medial POA and TU, may also contribute to the response. (Supported by grant MH 41447)

508.3

ESTRADIOL INTERACTS WITH GASTRIC OR POSTGASTRIC FOOD STIMULI TO INHIBIT FEEDING IN OVARIECTOMIZED RATS. N. Geary*, D. Trace and G.P. Smith. E. W. Bourne Behavioral Research Laboratory, New York Hospital-Cornell Medical Center, White Plains, NY 10605.

We tested estradiol benzoate's (EB) effects on sham feeding (SF). Ovariectomized rats with chronic gastric cannulas received 10 μg EB (n=6) or oil vehicle (n=6) sc on Tues and Wed mornings, a regimen that elicits behavioral estrus on Fri but does not produce the carry-over effects of continuous EB replacement (*Science 250*:691, 1990). Rats were adapted to daily real feeding (RF) of 0.8 M sucrose test meals after 21 hr food deprivation. Rats sham fed with open cannulas only twice, on two consecutive Fri. EB inhibited RF, but not SF. Inhibition of RF began within 6 min (see Table) and was maintained throughout the meal (for example; on a RF Thurs, wk 2, 45 min sucrose intake was 11.8 ± 1.6 ml in control rats *vs.* 8.0 ± 1.2 ml in EB-treated rats, t-test, $p < .05$).

Initial 6-min Sucrose Intake (M ± SEM)

	Control	EB
RF Thurs, wk 2	6.7 ± 0.8	5.0 ± 0.5*
SF Fri, wk 2	7.0 ± 0.8	8.0 ± 0.6
RF Fri, wk 3	8.0 ± 0.7	6.3 ± 0.4*+

*Different from control, t-test, $p < 0.05$.
+Different from SF, paired t-test, $p < 0.025$.

Because food does not accumulate in the stomach or enter the intestine in appreciable amounts during SF, these data indicate that EB must interact with gastric or postgastric food stimuli in order to inhibit intake of sucrose after 21 hr food deprivation. Further, food stimuli sufficient for this interaction occur within 6 min of meal onset.

Supported by NIH DK 32448, MH 15455 & MH00149

508.4

TCDD COMPLETELY REVERSES THE HYPERPHAGIA DUE TO VMH LESION. J. Tuomisto*, J.T. Tuomisto, M. Unkila and R. Pohjanvirta. Dept. of Toxicol., Nat'l Public Health Inst., POB 95, SF-70701 Kuopio, Finland.

Acute toxicity of TCDD (2,3,7,8-tetrachlorodibenzo-*p*-dioxin) seems to correlate with its ability to cause anorexia and a typical wasting syndrome. Han/Wistar (Kuopio) strain of rat (H/W) is remarkably resistant to TCDD, and survives a large dose, but seems to become hypersensitive to satiety signals and hyposensitive to hunger signals. In this study ventromedial hypothalamic nuclei (VMH) were lesioned electrolytically in eleven-week old H/W rats, and after a two-week recovery and weight gain period given a large dose (1000 µg/kg) of TCDD. The average starting weight was 194±8 g (mean±SD), feed intake after lesioning 23±5 g and after sham-lesioning 14±2 g, and weight gain 27±8% and 5±2%, respectively ($p<0.01$). After TCDD administration VMH lesioned overweight rats (N=6) stopped eating completely for at least 1 week. Three of the animals started to eat again, and their feed intake and body weight increased to the level of sham-operated TCDD-treated rats, but three succumbed after a total fast of about 3 weeks. The results suggest that TCDD regulates weight to a new level, and it is also able to completely reverse the effect of VMH lesion.

508.5

A MONOSODIUM GLUTAMATE (MSG)-INDUCED DELAY OF WEIGHT GAIN IN GENETICALLY OBESE MICE. F. A. Caputo*, G. L. Wolff and A. C. Scallet. FDA/National Center for Toxicological Research, Jefferson, AR 72079.

The yellow mouse is a model of obesity induced by the A^{vy} gene at the agouti locus on chromosome 2. Obesity also results from postnatal MSG administration. The present study was to determine if environmental factors such as MSG administration, imposed on a genetically susceptible model such as the yellow mouse, would result in an augmented form of obesity. Both yellow obese and black lean males (n's=8) were injected on postnatal days 1,3,5,7,9 with 2.0 mg/g body weight MSG sc. Control mice of both genotypes (n's=8) received an equal volume of saline. Mice were weaned on postnatal day 28 and given food and water ad libitum.

Paradoxically, MSG interacted with the yellow genotype to delay the rapid rate of weight gain characteristic of this model [$F(4,112)=4.4$, $p<0.05$]. By postnatal week 8, saline-treated yellows (S-Y) were 28% heavier than saline-treated blacks (S-B; means 32 vs 25g, $p<0.05$). However, prior MSG-treatment completely reversed this effect with MSG-treated yellows (M-Y) weighing less than S-Y (23 vs 32g, $p<0.05$). By week 24, the S-Y were up to 44% heavier than S-B (52 vs 36g, $p<0.05$), while M-Y compared to S-Y were still significantly lighter (49 vs 52g, $p<0.05$). Effects of MSG on body weight may depend on factors such as developmental stage and genetic context and warrants further consideration.

508.6

THE RELATIVE CONTRIBUTIONS OF BETA-1 AND BETA-2 ADRENORECEPTORS TO ISOPROTERENOL-INDUCED DRINKING C. Novak, M. Blumberg*, R. Thunhorst, A. Johnson, & R. Kirby. Dept. of Psychology, Univ. of Iowa, Iowa City, IA 52242.

The renin-angiotensin system has been shown to be a primary mediator of isoproterenol-induced drinking. As a mixed beta adrenoceptor agonist, isoproterenol can stimulate renin release via two independent mechanisms. First, isoproterenol acts directly on beta-1 adrenoceptors in the kidney to stimulate the release of renin. Second, isoproterenol decreases blood pressure by stimulating beta-2 adrenoceptors located in the skeletal muscle vasculature and the decrease in blood pressure stimulates the release of renin. Therefore the present study examined the relative contribution of beta-1 and beta-2 adrenoreceptor stimulation on drinking behavior and plasma renin activity induced by isoproterenol. Selective pharmacological antagonism of each adrenoreceptor type was achieved by administering atenelol (2.5 mg/kg), a beta-1 adrenoreceptor antagonist, or ICI 118.551 (1mg/kg), a beta-2 adrenoreceptor antagonist before treatment with isoproterenol (25µg/kg). Neither adrenoreceptor mechanism alone could account for all of the water intake due to isoproterenol treatment; plasma renin activity due to isoproterenol was similarly affected by selective beta-adrenergic antagonism. Cardiovascular recordings confirmed that the beta-adrenoreceptor antagonists were selective to their respective adrenoreceptors. The results suggest that both the direct stimulation of renin release and the indirect stimulation due to decreased blood pressure contribute to isoproterenol-induced drinking.

508.7

EVIDENCE THAT D_1 AND D_2 DOPAMINE RECEPTOR ACTIVATION ANTAGONIZES FEEDING ELICITED BY PERIFORNICAL HYPOTHALAMIC NEUROPEPTIDE Y. E.R. Gillard*, and B.G. Stanley. Depts. Neurosci. & Psych., Univ. Ca., Riverside, CA 92521, USA.

Recent evidence suggests that dopamine (DA) in the perifornical hypothalamus (PFH) may act to suppress neuropeptide Y-elicited eating. However, the identity of the DA receptor subtypes mediating this suppression is unknown. To begin to address this, the D_1 agonists SKF 38393 and SKF 82958 (0.01 to 100 nmol), and the D_2 agonists quinpirole (3 pmol to 300 nmol) and bromocriptine (3.3 to 248 nmol), or vehicle (0.3 μl) were injected prior to NPY (78 pmol/0.3 μl) into the PFH of adult male rats and subsequent food intake was measured. NPY alone stimulated feeding in all groups of subjects (5.0 g on the average, 1 hr postinjection) and each DA agonist reduced the response by at least 49% at the most effective dose. The D_1 agonists SKF 38393 (1.0 nmol) and SKF 82958 (0.1 nmol) reduced 1 hr intake by at least 73%; the D_2 agonist bromocriptine (33.0 nmol) reduced intake by at least 49%, and the D_2 agonist quinpirole (3 pmol) abolished peptide-elicited eating for up to 4 hrs. These findings may suggest that PFH DA antagonizes the eating-stimulatory effect of NPY via at least two distinct DA receptor subtypes.

508.8

α-METHYLNOREPINEPHRINE (α-MeNorEpi)--A POTENT ANORECTIC DRUG WITHOUT STIMULATORY ACTIVITY IN RATS. **S. Y. Yeh*, Lab. of Neurobiology, Addiction Research Center, NIDA/NIH, P. O. Box 5180, Baltimore, MD 21224.**

Reduction of body weight of rats treated with 3,4-methylenedioxamphetamine (MDA) and its metabolites, α-MeNorEpi, have been observed. The present study is to investigate the anorectic and stimulatory effects of dl-MeNorEpi and l-3,4-dihydroxynorephedrine (l-MeNorEpi) in free feeding rats. As compared with that of saline control rats, S.C. injection of dl-α-MeNorEpi at doses larger than 2.5 mg/kg significantly inhibited the food intake on days 1 to 3 from 3 to 24 hr intervals. Profound inhibition on food intake of rats was observed when the drug was given twice daily. Similar results were observed in rats treated with l-α-MeNorEpi. The ED_{50} (to reduce 50% of food intake) values of dl-α-MeNorEpi on inhibition of food intake at 1, 3, 14, and 24 intervals were estimated to be 0.76, 0.79, 1.96 and 2.6 mg/kg, respectively. The ED_{50} values of l-α-MeNorEpi on inhibition of food intake at 1, 3, 14, and 24 intervals were estimated to be 0.73, 0.95, 1.27 and 1.55 mg/kg, respectively. Body weight and locomotor activity, but not water intake, of rats treated with both dl-α-MeNorEpi and l-α-MeNorEpi, were decreased. It was concluded that both dl-α-MeNorEpi and l-α-MeNorEpi are potent anorectic drugs without stimulatory effects.

508.9

HYPOTHALAMIC 5-HT1A RECEPTOR BINDING IS INCREASED IN GENETICALLY OBESE AND DIABETIC RATS. J.A.Finkelstein* and M.Jhanwar-Uniyal. Northeastern Ohio Univ.Col.Med.,Rootstown OH 44272 and Rockefeller Univ.,New York NY 10021

Both genetically obese Zucker rats and streptozotocin-induced diabetic rats (STZ-D) have abnormalities in insulin levels and hypothalamic serotonergic (5-HT) activity which could play a role in their impaired feeding behavior and body weight regulation. Obese Zucker rats are characterized by hyperinsulinemia, whereas STZ-D rats are hypoinsulinemic. The 5-HT_{1A} receptor agonist, 8-OH-DPAT, induces feeding behavior and modulates circulating levels of insulin and glucose. Binding of [^{3}H] 8-OH-DPAT to 5-HT_{1A} receptors was measured in the medial (MH) and lateral hypothalamus (LH) of male and female, lean and obese Zucker rats, and of control and STZ-D Sprague-Dawley rats; the latter animals were sacrificed three weeks after IP injection of 60 mg/kg STZ. Non-specific binding was determined in the presence of 10 μM 5-HT, and the results expressed as fmoles/mg protein. Genetically obese rats of both sexes have higher binding in MH than lean rats (male: 84+9 vs. 38+4; female: 62+5 vs. 32+3). STZ-D rats have higher binding in MH than controls (48+5 vs. 22+3). There were no differences in LH for any of the groups. These results suggest that hyperphagia coinciding with abnormalities in peripheral insulin metabolism may operate via medial hypothalamic 5-HT_{1A} receptors.

508.10

EFFECT OF CHRONIC 8-OH-DPAT ON GENE EXPRESSION OF HYPOTHALAMIC NEUROPEPTIDES. A.H. Kahn, S. E. Bachus, R.C. Arora*, and M. Jhanwar-Uniyal, The Rockefeller Univ., NY 10021, NIH, Maryland and VA Hosp, Hines IL 60141.

Numerous studies have presented evidence for a strong relationship between the serotonergic (5-HT) and the hypothalamo-pituitary-adrenal (HPA) axis. This relationship has also been implicated in the regulation of insulin (INS) and glucose (GLU) levels. This study examines the levels of messenger RNA (mRNA) of corticotropin releasing factor (CRF), dynorphin (DYN) and galanin (GAL) in the paraventricular (PVN) and supraoptic (SON) hypothalamic nuclei, after the chronic administration of 5-HT1A receptor agonist, 8-OH-DPAT (60 μg/kg BW, IP for 10 days). Purina lab chow and water were available ad libitum to male Sprague-Dawley rats (325-375 g BW). Rats were sacrificed 24 h after the last injection. Levels of blood INS were assessed by RIA, and GLU levels were estimated by YSI glucose analyzer. *In situ* hybridization histochemistry was used to determine the area and density of mRNA for these neuropeptides in the PVN and SON. The results demonstrate that, as compared to the saline treatment, chronic 8-OH-DPAT-treatment caused: 1) an increase in the circulating levels of GLU (123%;$P<0.05$), and no change in INS levels; 2) a significant reduction in density for CRF mRNA in PVN and not in SON; 3) an increase in DYN mRNA area in both PVN and SON, but decreased density in PVN, resulting in no net change in label in PVN, but an increase in SON; and 4) a significant increase in area for GAL mRNA, in the PVN, but not in the SON. Thus, 8-OH-DPAT treatment influences the gene expression of certain neuropeptides in specific hypothalamic regions that regulate HPA and INS/GLU levels.

508.11

PERIPHERAL SEROTONIN (5-HT) REDUCES FEEDING IN RATS WITH AREA POSTREMA LESIONS. V. Adipudi* and K. J. Simansky. Department of Pharmacology, Medical College of Pennsylvania, Philadelphia, PA 19129 USA.

Peripherally administered serotonin (5-HT) reduces food intake in rats. Both 5-HT_1 and 5-HT_2 type receptors have been implicated in this serotonergic action but the neural substrates for 5-HT-induced anorexia remain to be defined. The area postrema (AP) is a circumventricular organ of the hindbrain that provides a potential target site for 5-HT in regulating food intake. This study assessed the effect of ablating the AP on the acute anorectic action produced by 5-HT (0, 1.6, 4.0 and 10.0 µmol/kg, i.p.) in 11 lesioned rats (APX) and 10 sham-operated controls (APC). The rats were maintained on standard pelleted chow and deprived at 09.30 h each day for testing 3 h later during a 30-min period with access only to a sweetened mash. Experiments were conducted with each rat receiving each dose of 5-HT in a randomized order beginning 6 wk postoperatively. Lesioned rats weighed less than controls at this time (APC, 472±9 g vs. APX, 343±26 g, $p<0.01$) but ate more than controls during the 30-min after injection with vehicle (0 dose)(APC, 4.8±0.4 g; APX, 11.0±1.2 g; $p<0.01$). 5-HT reduced intake in a dose-related fashion: Two-way ANOVA on the actual intakes revealed significant effects of Lesion ($p<0.01$) and Dose ($p<0.01$) but no interaction between these factors ($p>0.10$). Conversion of the data to percentages demonstrated that 5-HT produced similar anorectic actions in the two groups (APC, -32%, -45% and -69%, for the 3 doses, respectively, from baseline; APX, -27%, -46% and -52%). These data establish that peripherally administered 5-HT decreases food intake in rats with AP lesions. The apparent blunting of the effect at the highest dose suggests further studies testing whether AP damage might alter anorexia produced by more selective 5-HT_1 and 5-HT_2 analogs. Supported by NIMH Grant MH41987 (KJS).

508.12

DULOXETINE, A MIXED INHIBITOR OF SEROTONIN (5-HT) AND NOREPINEPHRINE (NE) UPTAKE, LOWERED MEAL-FEEDING AND BODY WEIGHT AND IMPROVED GLUCOSE TOLERANCE IN OBESE ZUCKER RATS. D.T.Wong* and W.N.Shaw. Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, IN 46285

Duloxetine, an antidepressant drug candidate, suppressed food intake in normal rats after 20 hr of fasting (1) and of nocturnal feeding, which was persistently reduced during chronic drug treatment (15 mg/kg i.p. daily up to 11 days) and resulted in lowering body weight (2). In obese Zucker rats, duloxetine and its racemate, during chronic administration (10 mg/kg i.p. daily), lowered meal-feeding and body weight. After 7 days of treatment, increase of oxygen consumption and decrease of epididymal adipose tissue mass became evident. Clearance of blood glucose after an oral glucose bolus was progressively improved between day 6 and 21 of duloxetine administration. An exogenous dose of insulin (0.25 unit/kg s.c.) produced changes in basal levels of blood glucose in rats chronically treated with duloxetine compared with the vehicle-treated group. There was no evidence of change in glucose intolerance in streptozotocin-induced diabetic obese Zucker rats after 7, 14 and 26 days of duloxetine administration. Thus, long-term treatment with duloxetine lowered food intake and body weight of obese Zucker rats, which show improved glucose tolerance possibly resulting from an increase of sensitivity to insulin. Moreover, besides the known involvement of central 5-HT and NE neurons in regulation of food intake, the present findings suggest their involvement in improving glucose utilization and insulin sensitivity in obese Zucker rats.
Ref.: 1) D.T.Wong, F.P.Bymaster, D.A.Mayle et al., Neuropsychopharmacology (1993) 8:23-33; 2) D.T.Wong, L.R.Reid, D.A. Mayle, Amer. Coll. Neuropsychopharmacol. Abst. (1992), p. 192.

508.13

SEROTONIN ATTENUATES FEEDING INDUCED BY INFUSING NEUROPEPTIDE Y INTO THE PERIFORNICAL HYPOTHALAMUS C.M. Brown and D.V. Coscina*. Sect. of Biopsychology, Clarke Inst. of Psychiatry, 250 College St., Toronto, Ontario, CANADA M5T 1R8.

Recent research has shown the perifornical hypothalamus (PFH) to be particularly sensitive to the feeding-stimulatory effects of locally infused neuropeptide Y (NPY). Conversely, earlier work suggested that PFH infusion of exogenous serotonin (5-HT) may suppress feeding, although some controversy exists about these findings. Furthermore, recent work shows that endogenous 5-HT is released in this site during meal-taking behaviors, making unclear the exact role 5-HT may play in PFH feeding controls. To determine what effects PFH 5-HT infusions might have on NPY-induced feeding, unilateral cannulae were implanted into freely-feeding adult male Sprague-Dawley rats. Using a within-subjects, repeated-measures design, NPY (0 or 235 pmol in 0.4 ul saline) was infused into the PFH, followed 15 min later by infusions of 5-HT (0, 6.3, 12.5, 25 or 50 nmol in 0.4 ul in saline). Food intake was measured 1 and 2 hrs after the second injection. NPY alone elicited strong, reliable ($p < .001$) feeding as expected. 5-HT attenuated this response, largely blocking it at the highest dose tested. These data confirm that infusing exogenous 5-HT into the PFH can suppress intake. Extending this observation to feeding stimulated by NPY implies that these two neuroactive agents may normally interact at this site to modulate ingestive behaviors. (Supported by NSERC of Canada)

508.14

DIFFERENTIAL EFFECTS OF THE 5-HT_{1A} AGONIST 8-OH-DPAT ON NOCTURNAL FEEDING IN THE RAT. P.J.Currie* and D.V. Coscina. Section of Biopsychology, Clarke Institute of Psychiatry, 250 College Street, Toronto, ON, M5T 1R8 CANADA.

Intra-raphe injection of 8-OH-DPAT preferentially stimulates somatodendritic 5-HT_{1A} receptors leading to a suppression of 5-HT cell firing, transmitter synthesis and terminal release. Since other evidence suggests that 8-OH-DPAT may also exert both excitatory and inhibitory effects on feeding, we examined the impact of this compound administered at various times throughout the nocturnal/active cycle. Rats with cannulae implanted in either the dorsal (n=9) or median (n=9) raphe were injected with 8-OH-DPAT (0.4, 0.8 and 1.6 nmol) or saline immediately following dark onset, or during the mid or late portions of the nocturnal cycle. Food intakes were measured 1.5 h postinjection. Analysis of baseline feeding across the nocturnal period indicated that rats exhibited an increase in spontaneous feeding at the onset of the dark period compared to a reduced intake found later in this cycle. Injection of 8-OH-DPAT potentiated feeding but only when injected in the mid or late-dark cycle when baseline feeding was low; whereas treatment during the early dark period actually suppressed feeding. These findings suggest that 8-OH-DPAT's effects on feeding may be dependent on the animal's motivational state and are consistent with recent reports of a biphasic action of this agent on ingestive behaviour. (*Supported by NSERC and NIN of Canada*).

508.15

ROLE OF CHOLINERGIC AND ADRENERGIC PATHWAYS OF THE MEDIAL SEPTAL AREA ON THE PRESSOR AND DIPSOGENIC RESPONSES INDUCED BY CENTRAL CHOLINERGIC ACTIVATION AND ANGIOTENSIN II IN RATS. S.P. Barbosa, W.A. Saad*, L.A.A. Camargo, A. Renzi, L.A. De Luca Jr., J.E.N. Silveira and J.V. Menani. Dept. of Physiology, School of Dentistry, UNESP, Araraquara, SP 14801-903, Brazil.

In the present study we investigated the effect of previous injection of atropine (cholinergic antagonist) or prazosin (alpha$_1$-adrenergic antagonist) into the medial septal area (MSA) on the pressor and dipsogenic responses induced by intracerebroventricular (ICV) injection of carbachol (CARB, cholinergic agonist) and angiotensin II (ANGII). Male rats with canullae in the lateral ventricle and MSA were used. Atropine (0.05 nmol to 5 nmol) reduced the pressor and dipsogenic responses induced by ICV carbachol (7 nmol), but not the responses induced by ANGII (25 ng). Prazosin (40 nmol) reduced only the dipsogenic response induced by ANGII. The results show the involvement of alpha$_1$-adrenergic pathways of the MSA in the dipsogenic response to ICV ANGII, while cholinergic pathways of the MSA mediate the pressor and dipsogenic responses to ICV cholinergic activation. Therefore, they suggest a dissociation of the pathways of the medial septal area involved with the responses induced by central cholinergic activation and ANGII in rats.

Research supported by CNPq and FAPESP.

508.16

PERIPHERAL ATROPINIZATION DOES NOT CHANGE MEAL SIZE CONTROLLED BY PREPYLORIC MECHANISMS. E.Rauhofer, G.P. Smith, J.Gibbs,* and D.Greenberg. E.W. Bourne Behavioral Research Laboratory, New York Hospital-Cornell Medical Center, White Plains, NY 10605.

When gastric emptying is prevented by a pyloric cuff, meal size (MS) is not different from when gastric emptying occurs. Thus prepyloric mechanisms are sufficient for the normal control of MS. To determine if peripheral cholinergic, muscarinic mechanisms are necessary for prepyloric control of MS, we compared MS in rats (n=7) administered atropine methyl nitrate (AMN, 3mg/kg, ip, at -30 min) with MS in rats (n=6) administered 0.15 M NaCl. There were 3 tests of 30-min access to 0.8M sucrose after 1 hour food deprivation. The pylorus was closed prior to all tests by inflating a pyloric cuff. AMN did not change meal size (Table).

TREATMENT	TESTS 1	2	3
SALINE	5.3±2.1	5.7±1.9	6.7±1.9
ATROPINE	6.1±2.5	6.7±2.7	4.9±2.9

Note: Data are mean ± SEM 30-min intakes (ml).

These results suggest that peripheral cholinergic, muscarinic mechanisms are not necessary for the prepyloric control of meal size.

Supported by MH15455 and MH00149.

508.17

ACETYLCHOLINE IS INCREASED IN THE NUCLEUS ACCUMBENS AND PREFRONTAL CORTEX DURING OPERANT RESPONDING FOR FOOD. G.P. Mark*, P.V. Rada & B.G. Hoebel. Dept of Psychology, Princeton University, Princeton, NJ 08544

We previously reported that free feeding was accompanied by an increase in ACh in the nucleus accumbens (NAC)[1]. In the present experiments, microdialysis was used to determine the impact of operant responding on ACh release in the NAC and prefrontal cortex (PFC). Microdialysis probes (2 mm tip for NAC, 3 mm for PFC) were perfused with a neostigmine Ringer (0.3 µM) and ACh was measured in 10 min intervals before, during and after responding for food (fixed ratio 1: FR1) in mildly food-deprived rats. ACh was increased in both sites (48% in NAC, 73% in PFC; $p<.05$) during 30 min bar pressing for food pellets. In rats trained to bar press on an FR25 schedule, the ACh response in the NAC was dampened (35% increase) whereas PFC ACh was increased to 90% ($p<.05$). Response extinction was accompanied by a transient 24% increase in extracellular ACh in the NAC. In contrast, cortical ACh increased by 127% during extinction. These data suggest that accumbens ACh may be released as a concommitant of reinforcement whereas ACh release in the prefrontal cortex may be more closely related to motor activity.

Supported by USPHS grant NS 30697

[1] Mark, G.P. et al., *J. Neurochem.* **58**, 2269-2274 (1992)

508.18

THE LATERAL HYPOTHALAMUS: A PRIMARY LOCUS FOR EXCITATORY AMINO ACID-ELICITED EATING. B.G. Stanley*, H.W. Donias, V.L. Willett III, L.H. Ha, L.C. Spears, Depts. Neurosci. & Psych., Univ. California, Riverside, CA 92521.

We have recently demonstrated that an intense transient eating response is elicited in rats by lateral hypothalamic (LH) injection of glutamate or the excitatory amino acid (EAA) agonists kainic acid (KA), D,L-α-amino-3-hydroxy-5-methyl-isoxazole propionic acid (AMPA), and N-methyl-D-aspartic acid (NMDA), among the most selective agonists of receptor subtypes of the same name (Brain Res. in press). In order to determine whether the LH is the actual locus of these effects, a cannula-mapping study was conducted in satiated adult male rats (n=117) to compare the eating responses elicited by LH injection of glutamate, KA, AMPA, and NMDA to those elicited by injections into diencephalic sites bracketing this area. In the LH, food intakes one hour postinjection were markedly and consistently increased in a dose-dependent manner by each agonist (e.g., 5.2 gm for glutamate at 900 nmol; 13.3 gm for KA at 1.0 nmol; 10.9 gm for AMPA at 3.3 nmol; and 13.9 gm for NMDA at 3.3 nmol). In contrast, injections into nearby sites including the anterior and posterior tips of the LH, the perifornical and paraventricular hypothalamus, the amygdala and thalamus only occasionally elicited significant eating. These findings, suggesting that each of these agonists actually act within the LH to elicit eating, argue that multiple subtypes of EAA receptors may exist on LH neurons involved in eating stimulation.

MONOAMINES AND BEHAVIOR: STIMULANTS

509.1

A SINGLE AMPHETAMINE PRETREATMENT RESULTS IN A CALCIUM DEPENDENT ENHANCEMENT IN DOPAMINE RELEASE FOLLOWING AN AMPHETAMINE CHALLENGE. E. C. Warburton[1], S.N. Mitchell, J.A Gray and M.H. Joseph (SPON EBBS).Dept. Psychology, Inst. of Psychiatry, London SE5 8AF, UK. [1]current address Dept. Anatomy, Univ. of Cambridge CB2 3DY, UK

Using *in vivo* microdialysis in a between-subjects design, the effects of one and two administrations of *d*-amphetamine on the release of dopamine (DA) and metabolites from the nucleus accumbens of a freely moving rat, were compared. On the day prior to recording rats were pretreated with either saline or *d*-amphetamine (1mg/kg i.p.) and a microdialysis probe implanted 2.5h later. Twenty-four hours later the subjects were connected for dialysis and were given an amphetamine challenge.

Acute administration of *d*-amphetamine (1mg/kg) produced a 350% increase in DA release 30mins post-injection. The effect of *d*-amphetamine was significantly enhanced by a prior amphetamine pretreatment. In this group, DA levels increased to 650% of baseline 30mins post-injection and remained elevated for 2.5h post-injection. Levels of DOPAC and HVA decreased following acute administration of amphetamine, but were unaffected by the pretreatment. The enhanced increase in accumbens DA was reflected in increased locomotor activity, recorded in a group of non-dialysed animals, but the difference did not reach significance.

The removal of calcium from the perfusate did not affect DA release produced by an acute amphetamine injection but attenuated the enhanced response to a second amphetamine injection, suggesting that the sensitized DA release produced by the single amphetamine pretreatment was calcium-dependent. These data are discussed in terms of possible presynaptic changes relating to autoreceptor down-regulation that may occur following a single amphetamine administration. (Supported by Glaxo Group Research Ltd.)

509.2

A COMPARISON OF STEREOTYPED BEHAVIOR AND NEUROCHEMICAL CHANGES FOLLOWING ACUTE ADMINISTRATION OF METHYLPHENIDATE AND DEXTROAMPHETAMINE IN THE WEANLING RAT. C.J. Truman, H.Y. Lee, and L.A. Raskin*. Dept. Psychology, Neuroscience Program, Amherst College, Amherst, MA 01002.

This experiment compared the stereotyped behaviors elicited by injections of high doses of two psychomotor stimulants, and assessed the neurochemical changes produced by these injections. Animals aged 4-6 weeks were administered acute intraperitoneal injections of 5 mg/kg dextroamphetamine sulfate (DEX), 30 mg/kg methylphenidate hydrochloride (MPH), or saline. Observations were made via videography of 27 motor behaviors. Stimulant-injected animals spent more time engaged in stereotypy. Most interestingly, the two stimulants produced differential motor effects. Animals given DEX spent more time sniffing near walls than both their MPH and saline-injected counterparts. DEX also tended to produce more sniffing at chips than did MPH and saline. In contrast, MPH-injected animals showed a trend towards more gnawing behavior than DEX or saline-injected animals. HPLC analysis of biogenic amines in these animals showed that 5 mg/kg DEX produced elevated 5HIAA levels in the striatum, hippocampus and frontal cortex whereas 30 mg/kg MPH did not. MPH decreased levels of NE in the frontal cortex although DEX did not. These differential motor effects have implications for clinical stimulant use.

509.3

PREEXPOSING RATS TO INTRA-VTA AMPHETAMINE SENSITIZES THEIR LOCOMOTOR RESPONSE TO NUCLEUS ACCUMBENS AMPHETAMINE.
M. Perugini* and P. Vezina. Neurosciences, Loeb Medical Research Institute, Ottawa Civic Hospital, Ottawa, Ontario K1Y 4E9 Canada.

Low doses of amphetamine produce behavioral stimulation in rats that increases after repeated systemic administration. Evidence suggests that the induction of this sensitization to the locomotor effects of amphetamine occurs in the ventral tegmental area (VTA). Repeated microinjections of amphetamine into the VTA, but not the nucleus accumbens (N.Acc.), produces an enhanced locomotor and N.Acc. dopaminergic response when rats are subsequently challenged with systemic amphetamine. These findings suggest that amphetamine acts in the VTA, site of the mesolimbic dopamine cell bodies, to produce behavioral sensitization and that its expression occurs in the terminal region of these cells in the N.Acc. The present experiment more directly evaluated this possibility.

Rats were implanted with chronic bilateral injection cannulae aimed at both the VTA and N.Acc. Following recovery, they received intra-VTA injections of either amphetamine (2.5 µg/0.5 µl/side) or saline (0.5 µl/side), one injection every third day and locomotor activity was measured. Seven to ten days following the last injection, all animals received an intra-N.Acc. injection of amphetamine (1.5-2.5 µg/0.5 µl per side) and locomotor activity was again measured. As previously reported, intra-VTA amphetamine produced no behavioral effects acutely. However, when subsequently tested with intra-N.Acc. amphetamine, animals preexposed to intra-VTA amphetamine dose-dependently showed a significantly greater behavioral response (horizontal locomotion and rearing) than saline preexposed animals. These results together with those above indicate that the induction of behavioral sensitization results from drug-induced changes in the VTA while its expression occurs in the N.Acc.

509.4

INDUCTION OF BEHAVIORAL AND DOPAMINERGIC SENSITIZATION TO SYSTEMIC OR INTRA-VTA AMPHETAMINE IS BLOCKED BY SCH-23390.
P. Vezina.* Neurosciences, Loeb Medical Research Institute, Ottawa Civic Hospital, Ottawa K1Y 4E9 Canada.

The ability of the D-1 dopamine (DA) receptor antagonist, SCH-23390, to block the induction of sensitization to the locomotor and DA-activating effects of amphetamine was assessed in two experiments. In the first, different groups of rats were administered either saline, SCH-23390 (0.2 mg/kg, s.c.) or the D-2 DA receptor antagonist eticlopride (1.0mg/kg, i.p.) prior to each of five injections of saline or amphetamine (1.0mg/kg, i.p.) given one every third day. Both antagonists completely and repeatedly blocked the acute locomotor effects of amphetamine. However, on a subsequent test for sensitization when all animals received saline prior to an injection of amphetamine (0.5mg/kg, i.p.), only SCH-23390 was found to have blocked the induction of behavioral sensitization to the drug. In the second experiment, rats received three intra-VTA injections of either saline, amphetamine (2.5µg/side) or amphetamine+SCH-23390 (0.25-1.0µg/side), one injection every third day. Two weeks following the last injection, *in vivo* microdialysis was used to assess DA neurotransmission in the nucleus accumbens following a challenge injection of amphetamine (1.0mg/kg,i.p.). Animals previously exposed to intra-VTA amphetamine showed significantly greater DA levels in nucleus accumbens in response to challenge than VTA saline preexposed animals. This effect was completely blocked in animals previously exposed to intra-VTA amphetamine+SCH-23390. These findings support the view that D-1 DA receptor activation plays a critical role in the induction of sensitization to amphetamine.

509.5

DIFFERENTIAL EFFECTS OF AMPHETAMINE ON THE ATTACK AND DEFENSE COMPONENTS OF PLAY FIGHTING IN RATS.
Evelyn Field and Sergio M. Pellis*, Dept. Psychology, Univ. Lethbridge, Lethbridge, AB T1K 3M4.

Treatment with d-amphetamine has been shown to cause a decrease in play fighting by juvenile rats. Previous studies, however, did not determine whether all behavioral components of play were equally diminished. In this study the effects of amphetamine on both the attack and defense behavior patterns of play fighting was analysed. Experiment 1 shows that a 0.5 mg/kg dose, injected subcutaneously in the nape, decreases both attack and defense. In contrast, experiment 2 shows that the same dose, injected subcutaneously in the hip, decreases the frequency of playful attack to a similar level, but only mildly decreases defense. This suggests that the 0.5 mg/kg dose of amphetamine is primarily affecting the offensive components of play via its action on the central nervous system. The reduced likelihood of defense for those rats injected in the nape, is likely due to a local anesthetic effect, which numbs the area of the body defended during play fighting. Further doses (0.15 & 1.0 mg/kg), injected in the hip, were also tested. The highest dose decreased both components of play. The lowest dose had no effect on the attack and defense components. Nonetheless, both the treated and non-treated partners showed a disruption to some of their social behavior. This suggests that even a low dose of amphetamine may affect the style, if not the quantity of playful interactions of juvenile rats. The data suggest that the offensive and defensive components of play fighting may be mediated by different neural systems.

509.6

THE ACUTE EFFECTS OF COCAINE ON BEHAVIOR AND EXTRACELLULAR DOPAMINE IN THE NUCLEUS ACCUMBENS OF LEWIS AND FISCHER 344 RATS.
Dianne M. Camp* and Terry E. Robinson. Dept. of Psychology and Neuroscience Program, Univ. of Michigan, Ann Arbor, MI 48109.

Lewis (LEW) and Fischer (F344) rats differ in their response to a variety of drugs of abuse. For example, LEW rats show a greater propensity to self-administer opiates, cocaine and alcohol than F344 rats. In addition, we have reported that both amphetamine-stimulated motor activity and dopamine (DA) release are enhanced in LEW rats compared to F344 rats (Camp et al. *Soc. Neurosci. Abst. 18*, 1992, 719). The purpose of this experiment was to determine whether there are similar strain differences in the ability of cocaine (COC) to elevate the extracellular concentration of DA in the nucleus accumbens by use of in vivo microdialysis. Each rat received saline (i.p.) plus one of 4 doses of COC (5, 10, 15 or 30 mg/kg, i.p.) during the dialysis test. Preliminary analyses indicate that the effect of COC on behavior (crossovers and rears) and extracellular DA are different between the two strains, but in contrast to amphetamine, these differences are dose-dependent. LEW rats generally showed a more pronounced behavioral response than F344 rats at all four doses. However, differences in extracellular DA were seen only at the two lower COC doses, with LEW rats showing a more prolonged increase than F344 rats. As reported previously, the basal extracellular concentrations of DA and the DA metabolites did not differ between the two strains. Experiments are currently in progress to address whether the effects observed are due to pharmacokinetic differences between LEW and F344 rats. Supported by NIDA grant #04294.

509.7

CONTINGENT AMPHETAMINE ADMINISTRATION PRODUCES BEHAVIORAL SENSITIZATION IN RATS.
D. Crippens *, H.S. Crombag[1], T.E. Robinson. Psychology and Neuroscience Program, Univ. of Michigan, Ann Arbor, MI 48104, [1]Experimental Psychology Program, Univ. of Leiden, Leiden, The Netherlands.

Although it is well established that the repeated intermittent administration of many drugs of abuse induces sensitization to their psychomotor activating effects, in nearly all of these studies drug administration was not contingent on the animal's behavior. In studies of drug reinforcement, however, drug administration is usually contingent on an animal's behavior. It has been suggested that sensitization enhances the propensity for drug self-administration, and is critical in the development of addiction. It is important, therefore, to know if *contingent* drug administration also induces sensitization. This was done in the present experiment by use of a task (Ettenberg,1990) in which drug administration was contingent on the animal running down a runway to a holding cage where the experimental group received an i.p. injection of 2.0 mg/kg of *d*-amphetamine (AMPH) and the control group received an injection of saline. Running speed served as a measure of the reinforcing property of the drug. The psychomotor effects of AMPH were determined by monitoring animals inside the holding cages and rating stereotyped behavior using a nine-point scale. Locomotor activity was quantified by the number of cross-overs from one side of the cage to the other. Preliminary results indicate that the psychomotor activating effects of AMPH are progressively enhanced with repeated exposure. These results indicate that AMPH induces sensitization even when the drug treatment is contingent on an animal performing a specific operant.

509.8

CONTEXT-DEPENDENT AMPHETAMINE SENSITIZATION
S. Anagnostaras, A. Badiani and T. E. Robinson*. Psychology and Neuroscience Program, Univ. of Michigan, Ann Arbor, MI 48104

The context in which amphetamine (AMPH) is administered influences subsequent drug effects. We report here on experiments designed to explore context-dependent sensitization in rats given repeated injections of AMPH. The behavioral effect of AMPH was quantified by measuring rotational behavior in rats with unilateral 6-OHDA lesions. Animals were given ten injections of 2-3 mg/kg d-AMPH or saline (daily or every 3-4 days) in a group-specific environment (e.g., home cage, rotometer, alternate environment, or multiple environments). All animals given AMPH showed marked sensitization in the drug-paired environment. However, when given a challenge injection of AMPH in a test environment (rotometer), that was novel to some groups but not to others, only animals that had previously received AMPH in this environment showed evidence of sensitization. Animals for which this was a new environment behaved like saline-pretreated controls, despite showing marked sensitization in their drug-paired environment. However, animals that received injections in multiple environments (but not the rotometer) showed moderate sensitization in the rotometer. These context-specific effects of AMPH were not diminished by a ten trial extinction procedure in which all animals received saline in the challenge environment. In summary, these experiments illustrate that the behavioral sensitization produced by AMPH can be highly context-dependent, even following the repeated administration of relatively high doses. Studies are in progress using microdialysis to assess the neurochemical consequences of context-specific AMPH sensitization on dopamine neurotransmission.

509.9

CHARACTERIZATION OF BEHAVIOR AND EXTRACELLULAR DOPAMINE IN THE NUCLEUS ACCUMBENS DURING AMPHETAMINE WITHDRAWAL. P. E. Paulson* and T. E. Robinson. Dept. of Psych. and Neuroscience Program, Univ. of MI, Ann Arbor, MI 48109

The discontinuation of chronic amphetamine (AMPH) use results in a 'distress syndrome' in humans, including symptoms of depression and dysphoria. In rats, the discontinuation of repeated AMPH treatment produces a 'behavioral depression', including marked nocturnal hypoactivity. In rats, this symptom of AMPH withdrawal persists for 1-2 weeks following the discontinuation of pretreatment with escalating, but non-neurotoxic, doses of AMPH. The present experiment was designed to further characterize the behavioral and neurochemical consequences of this AMPH withdrawal symdrome. To do this, spontaneous locomotor activity and the extracellular concentrations (EC) of DA in the nucleus accumbens were continuously monitored over 20 minute intervals across the entire day-night cycle in AMPH pretreated and saline pretreated control rats at 3, 7 and 28 days after the discontinuation of AMPH pretreatment, using a fully automated *in vivo* microdialysis system. Hypoactivity was evident during the initial 3-7 days of AMPH withdrawal, however, preliminary analyses indicate that there was no effect of AMPH withdrawal on the basal EC of DA in the nucleus accumbens. In addition, there was no effect of AMPH pretreatment during the initial 3-7 days of AMPH withdrawal on AMPH-stimulated DA release. However, a challenge injection of AMPH given 28 days after the discontinuation of AMPH treatment produced enhanced locomotor activity and AMPH-stimulated DA release, relative to saline pretreated controls.

509.10

BEHAVIORAL SENSITIZATION TO AMPHETAMINE IN LEWIS AND FISCHER RAT STRAINS. K. E. Browman*, K. J. Kerwin, and T. E. Robinson. Department of Psychology and Neuroscience Program, The University of Michigan, Ann Arbor, MI 48104.

Lewis (LEW) and Fischer (F344) rats are two inbred strains that represent genetically divergent populations known to differ in their propensity to self-administer a variety of drugs of abuse. LEW rats more readily self-administer opiates, cocaine and ethanol, and also show enhanced amphetamine (AMPH)-stimulated motor activity and AMPH stimulated dopamine (DA) release, relative to F344 rats. The susceptibility to self-administer AMPH has been linked to the susceptibility to sensitization produced by repeated AMPH treatment. The purpose of this experiment, therefore, was to determine if the LEW and F344 rat strains differ in the rate or extent of behavioral sensitization to AMPH. In one experiment, rats were given unilateral 6-hydroxydopamine (6-OHDA) lesions of the nigrostriatal bundle, so the sensitization of rotational behavior could be quantified. In a second experiment, AMPH-induced locomotor activity and stereotyped behavior was quantified. All animals received either 2 mg/kg of AMPH or saline every three to four days for a total of five injections. Preliminary data suggest that genetic differences play a role in an animal's susceptibiltiy to sensitization, in strains known to differ in their propensity to drug self-administration. Results will be presented at the meeting.

Supported by NIDA grant #04294.

509.11

NOVELTY ENHANCES THE DEVELOPMENT OF SENSITIZATION TO THE PSYCHOMOTOR STIMULANT EFFECT OF AMPHETAMINE. A. Badiani *, S. Anagnostaras and T.E. Robinson. Dept. of Psychology and Neuroscience Program, University of Michigan, Ann Arbor, MI 48104, USA.

It has been suggested that the activation of the hypothalamo-pituitary-adrenal (HPA) axis plays an important role in the development of sensitization to the psychomotor activating effects of amphetamine. To explore this hypothesis we compared the rate of sensitization in rats treated repeatedly with amphetamine, either in their home cages or during exposure to a novel test environment, which is known to activate the HPA axis. The animals first received a unilateral 6-OHDA lesion of the nigrostriatal DA system. Following at least two weeks of recovery from surgery, the rats received ten daily IP injections of 2 mg/kg of amphetamine, either in their home cages (group HOME) or in test cages (group TEST), and rotational behavior was quantified. The HOME cages and TEST cages were physically identical. The rats in group TEST, however, lived in stainless steel hanging cages and were transferred to the TEST cages described above for each test session. Group HOME rats lived and received drug injections in these cages. The first injection of amphetamine produced significantly more rotational behavior in group TEST than in group HOME. Furthermore, the rate of sensitization was greater in group TEST ($p < 0.001$). It is possible that the effect of novelty on the initial response to amphetamine was due to differential activation of the HPA axis, and that learning factors magnified this effect with repeated drug pairing. Studies to further examine the role of the HPA axis in this phenomenon are in progress.

509.12

INDIVIDUAL DIFFERENCES IN RESPONSIVENESS TO NOVELTY AND AMPHETAMINE IN RATS P.M. Robinet*, J.K. Rowlett & M.T. Bardo, Dept Psychol, Univ Kentucky, Lexington, KY 40506. Previous work has shown that individual differences in locomotor behavior in an inescapable novel environment predicts the locomotor-stimulant effect of amphetamine. This study assessed if novelty-seeking behavior in a free choice situation also predicts the locomotor-stimulant effect of amphetamine. Rats were first confined to an inescapable novel environment in which activity was recorded. After two habituation days, the animals were allowed free access between this environment (familiar) and a novel environment to assess their place preference. To further assess free choice novelty-seeking behavior a novel object was introduced into the animal's home cage. Approach and contact behaviors with the novel object were recorded for 15 min. All animals then received amphetamine (cumulative dose 0.0, 0.1, 0.4, 1.6 mg/kg) and locomotor activity was recorded. Correlational analyses showed a relationship between locomotor activity in the inescapable novel environment and locomotor response to amphetamine at low doses. However, novelty-seeking behavior, as defined by the place preference and novel object tests, was not correlated with the locomotor response to amphetamine at any dose. This suggests the possibility of different mechanisms mediating novelty-induced reward and novelty-induced locomotor activity.
(Supported by USPHS Grants DA-05312 and DA-06924).

509.13

AMPHETAMINE ANALOGS HAVE DIFFERENTIAL EFFECTS ON BEHAVIOR. J.B. Richards*, K.E. Sabol, K.E. Layton, L.S. Seiden. University of Chicago, Department of Pharm/Phys Sci., Chicago, IL 60637.

The effects of amphetamine (AMPH), methamphetamine (METH), methylenedioxymethamphetamine (MDMA), para-chloroamphetamine (PCA), and fenfluramine (FEN) were determined in rats trained on a differential-reinforcement-of-low-rate-36-second schedule (DRL 36-s). The DRL 36-s schedule requires animals to wait at least 36 seconds between responses in order to gain access to a reinforcer (.05 ml water). Training on the DRL 36-s schedule results in a highly organized pattern of behavior. This behavioral pattern can be visualized by examining the distribution of interresponse times (IRTs). The IRT distributions of rats trained on the DRL 36-s schedule have large well defined peaks near the 36-s requirement for reinforcement. These peaks can be quantitatively characterized by determining the peak area (PkA) and peak location (PkL). AMPH and METH induced large increases in response rate of 298% and 212% respectively (% = percent of control). MDMA and PCA caused smaller increases in response rate 120% and 129% respectively. FEN did not increase response rate. All five drugs decreased response rate at high doses. AMPH, METH, MDMA and PCA shifted the PkL toward shorter intervals (AMPH = METH > PCA > MDMA). FEN did not shift the PkL. All five drugs (including FEN) caused a dose dependent decrease in PkA, indicating a loss of schedule control by the DRL 36-s contingency. The differential effects on behavior are consistent with the different capacities of the amphetamine analogs tested to release dopamine and serotonin. Dopamine release induced by amphetamine analogs may increase response rate, shift the PkL toward shorter IRT durations and decrease PkA. Serotonin release (in the absence of dopamine release) may simply decrease PkA. (Supported by: MH-11191; RSA-10562, L. Seiden)

509.14

CONDITIONED TASTE AVERSION IN RATS INDUCED BY THE α1-ADRENOCEPTOR AGONIST CIRAZOLINE L.R. McMahon, A. Morien, P.J. Wellman* and B. Davies. Dept. of Psych., Texas A&M Univ., College Station, TX 77843.

Recent studies have indicated that α1-adrenoceptor agonists such as phenylpropanolamine (PPA), cirazoline, amidephrine, and SK&F-89748 suppress food intake in rats. These compounds activate α1-adrenoceptors within the paraventricular hypothalamic nucleus (PVN) and may excite efferent fibers which inhibit feeding. Studies of the effects of α1-agonists suggest a specificity for feeding behavior, but no study to date has evaluated whether these agonists may suppress feeding behavior by the induction of malaise. Accordingly, the present experiment compared cirazoline (0, 0.1, 0.2, 0.4 mg/kg, IP) with lithium chloride (32 mg/kg, IP) using the conditioned taste aversion (CTA) paradigm. Systemic administration of 0.1, 0.2 and 0.4 mg/kg cirazoline as well as 32 mg/kg lithium chloride induced significant conditioned aversion to saccharin compared to the vehicle treatment. In contrast to its activity on food intake, cirazoline did not induce dose-dependent aversion to saccharin. This difference in activity suggests that malaise is not a prominent factor in the feeding-suppressive activity of cirazoline. These results may support the use of cirazoline as an effective appetite suppressant.

510.1

ENVIRONMENTAL MODULATION OF BOTH LOCOMOTOR RESPONSE AND LOCOMOTOR SENSITIZATION TO THE DOPAMINE AGONIST QUINPIROLE. H. Einat[1]* and H. Szechtman[1,2] Departments of Biomedical Sciences[1], Psychiatry and Psychology[2] McMaster University, Hamilton, Ontario, Canada L8N 3Z5

The study tested whether differences in locomotor activation during chronic treatment result in differential behavioral sensitization induced by the D2/D3 dopamine agonist quinpirole. One group of rats received repeated injections of quinpirole in their home-cage and another group received this treatment in an alternate environment of similar size. In the home-cage, quinpirole induced less locomotion than in the non-home environment. When tested in activity monitors at end of chronic treatment, the home-cage group showed less sensitized locomotion to quinpirole than the non-home-cage rats. Thus, extent of locomotor sensitization to quinpirole appears related to the amount of locomotion characteristic of the training environment. Such differential sensitization may reflect experiential and environmental factors modulating the hierarchy of expression of quinpirole-enhanced hyperactivity.

510.2

RELEASE OF BEHAVIORAL INHIBITION BY DOPAMINE DEPLETIONS OF THE PREFRONTAL CORTEX IN THE RAT. J.D. Sokolowski*, M.S. Cousins, & J.D. Salamone. Dept. of Psychology, University of Connecticut, Storrs, CT 06269-1020.

Experiments were conducted to characterize the behavioral effects of medial pre-frontal cortex (MPFC) dopamine (DA) depletions. Using an active avoidance paradigm, in which nucleus accumbens DA depletions previously were demonstrated to produce profound impairments in avoidance responding, there was no impairment in MPFC DA-depleted animals. In a passive avoidance punished lever pressing paradigm, MPFC DA depletions resulted in a slight increase in punished responses. MPFC DA depletions also increased amphetamine-induced locomotor activity and stereotypy. Finally, in a study investigating performance on a DRL30 schedule of reinforcement, in which animals must withhold their responses for 30 seconds in order to receive reinforcement, rats receiving DA-depleting lesions in the MPFC exhibited several different impairments. These animals had a higher number of total lever presses, a lower efficiency (reinforcers/lever presses), and showed a bursting pattern of responses as calculated by an inter-response time measure. These results support the notion that MPFC DA is involved in behavioral inhibition.

510.3

LEVELS OF TYROSINE HYDROXYLASE ARE ELEVATED IN THE LOCUS COERULEUS FROM SUICIDE VICTIMS. Gregory A. Ordway* & John W. Haycock. Depts. of Psychiatry & Pharmacology, Case Western Reserve Univ., Cleveland, OH 44109, and Dept. of Biochemistry & Molecular Biology, Louisiana State Univ. Med. Center, New Orleans, LA 70119

Alterations in brain norepinephrine have been implicated in depression, schizophrenia, and anxiety. The locus coeruleus (LC) is the principal source of brain norepinephrine, and biosynthesis of this monoamine is controlled by tyrosine hydroxylase (TH). TH expression in LC neurons can be influenced by psychoactive drugs (e.g. antidepressants) and by environmental stimuli (e.g. stress). The present study investigated whether aberrant levels of TH would be found in the LC from brains of suicide victims. Blot immunolabeling techniques were used to quantitate TH protein levels in tissue sections containing LC from 9 pairs of antidepressant-free suicide victims and age-matched, sudden death controls. More TH protein was present in sections from suicide victims in each of the 9 matched pairs (108 to 172% of controls, $\bar{x}$ = 136%, $p < 0.005$). By contrast, there were no differences in neuron-specific enolase in the same samples (90 to 114% of controls, $\bar{x}$ = 103%) or in the number of cells containing neuromelanin in adjacent sections of LC. Elevated TH in LC of suicide victims may reflect increased expression resulting from exposure to higher levels of stress. Alternatively, higher TH levels in LC may be a primary factor in the pathophysiology leading to suicide. [Supported by NARSAD, and USPHS MH46692, MH00967, and MH46653.]

510.4

BEHAVIORAL AND BIOCHEMICAL RESPONSES TO STRESS IN RATS SELECTIVELY BRED FOR SWIM TEST ACTIVITY. C.H.K. West*, P.M. McCurdy, R.W. Bonsall and J.M. Weiss. Lab of Behavioral Neuroscience & Neuroimmunology, Dept. Psychiatry, Emory Univ. Sch. Med., Atlanta, GA 30306.

Performance in a swim test has been used as the criterion by Weiss and colleagues (Cierpial et al.[1989] Soc. Neurosci. Abstr., 15: 1176) for selectively breeding rats in an attempt to improve animal models of depression. Animals have been selectively bred for high (SwHi) or low (SwLo) activity in the swim test, with SwHi's showing much struggling and little floating and SwLo's showing the reverse. Since previous testing revealed that SwHi's and SwLo's do not differ in their home-cage day or night activity under non-stressful conditions, here we compared them for their motor and corticosterone responses in two additional stressful situations which may be expected to evoke active responses. Male and female SwHi's and SwLo's (13th generation) were tested for (1) activity in the swim test, (2) locomotor activity in a novel environment (LocAct), and (3) immobility in the Porsolt test. Following testing, rats were sacrificed and serum corticosterone levels determined. In the swim test, SwHi's as expected spent much time struggling whereas SwLo's spent most of their time floating. In LocAct tests, SwHi's were more active than SwLo's during the first few min of the 1 hr test, and this difference was more pronounced for females than males. In the Porsolt test, time of immobility differed moderately, but jumping behavior differed dramatically. SwLo's jumped very little (max. = 17) whereas the mean jumps by SwHi's was 75±12 for males and 61±4 for females. Corticosterone levels were elevated by behavioral testing compared to home-cage controls, but levels for SwHi's and SwLo's did not differ significantly. The results indicate that SwHi's and SwLo's, which do not show behavioral differences under non-stressful conditions, also differ little in steroid reactivity to stress, but these lines of selectively-bred animals differ distinctly in their initiation of motor responses to stressful, arousing conditions. Supported by the Stanley Foundation of the National Alliance for the Mentally Ill.

510.5

STRIATAL DOPAMINE AND ITS RELATIONSHIP TO OPEN FIELD MOTOR BEHAVIOR IN NORMAL RATS. L. McCoy, S.B. Schwarzkopf, E.K. Richfield*, and M. Hadjiconstantinou. Depts. of Psychiatry and Neurology, University of Rochester, Rochester, NY 14627 and Depts of Pharmacology and Psychiatry, The Ohio State University, Columbus OH, 43210.

Midbrain dopamine circuits modulate the complex cognitive and sensorimotor activities of the striatum. The dorsal striatum is involved in gross motor control while the ventral striatum may be more critical to motivation-linked motor behavior. Previous data has shown higher striatal dopamine to be associated with more exploratory behavior and less freezing behavior in the normal rat. In the present study, we were interested in the relationship between regional striatal dopamine (DA) measures and open field behavior in adult male Sprague-Dawley rats (N=24). Behavioral measures included global locomotor, exploratory, vertical (rearing) and fine repetitive motor activity. DA measures included DA and its metabolites (DOPAC and HVA), and D1, D2 and dopamine uptake complex (DAUC) densities. Brains were obtained after rapid decapitation (age 120 days), halved through the midline then immediately frozen. One half-brain was used for determining dorsal or caudate/putamen and ventral or nucleus accumbens (NAC) dopamine chemistries using high performance liquid chromatography. The second half-brain was used for measuring DA receptor and reuptake complex densities using quantitative autoradiography. In these animals, specific motor behaviors were found to correlate with regional differences in striatal DA activity. DAUC, or the density of dopaminergic terminals in the striatum, was positively correlated ($r=.62$, $p<.005$) with exploratory behavior. Amount of exploratory behavior also predicted a higher D2 receptor dorsal/ventral gradient (trend). NAC dopamine was associated with rearing ($r=.74$, $p<.01$). These results suggest that normal variation in rat open field motor behavior is associated with central dopaminergic interactions between the dorsal and ventral striatum. *(Supported by MH00859 and MH40381)*

510.6

PHARMACOLOGICAL EFFECTS OF MKC-242: A NOVEL POTENTIAL ANXIOLYTIC AND ANTIDEPRESSANT COMPOUND WITH SELECTIVE 5-HT_{1A} AGONISTIC PROFILE. M.Egawa, M.Abe, R.Tabata, K.-I. Saito*, A.Tobe, T.Matsuda# and A.Baba#. Pharmaceuticals Laboratory I, Research Center, Mitsubishi Kasei Co, Yokohama 227, Japan. #; Dept of Pharmacology, Faculty of Pharmaceutical Science, Osaka University.

The new alkylenedioxybenzene derivative, MKC-242, showed selective and high affinity for 5-HT_{1A} receptors in radioligand binding study in rat brain (K_i=0.11nM). The affinity for other 5-HT receptor subtypes, dopamine D_1 and D_2, $_2$ adrenoceptor, $GABA_A$ and benzodiazepine receptors were low. MKC-242 was found to inhibit forskolin-stimulated adenylate cyclase activity (IC_{50}=2.4nM). MKC-242 (3 mg/kgpo) reduced the 5-HT synthesis in rat midbrain and hippocampus without any influences on dopamine. Head weaving behavior was produced by MKC-242 alone (1mg/kgpo) and MKC-242 antagonized this syndrome induced by 8-OH-DPAT. In behavioral testing, MKC-242 showed a long-lasting anticonflict activity in rats at very low soses (MED=0.0625mg/kgpo). There was no reductionin this effect by repeated treatments of MKC-242. MKC-242 clearly shortened immobility time in forced swimming test (MED=0.25mg/kgip) and its potency was equal to that of amitriptyline (10mg/kgip). In restraint stress model of depression, the ability of MKC-242 to antagonize the decreased locomotion was observed, dose-dependently. These results suggest that MKC-242 may be of therapeutic value in the treatment of anxiety or depressive disorders.

510.7

INFANT RATS' DOPAMINERGIC RESPONSE TO THE STRESS OF ISOLATION IN A NOVEL ENVIRONMENT. P. Kehoe*, H. Chandler, K. Skipsey, & K. Clash. Neuroscience Program, Trinity College, Hartford, CT 06106

Stress in adult rats produces an increase in both dopamine (DA) turnover and levels of synaptic DA. In guinea pig pups, isolation in a novel environment produces an increase in DA turnover. The present study assessed rat pup DA response to isolation with and without familiar cues. Pups were isolated for 5 min in a familiar (cup with bedding) or novel environment (plain box) and compared to siblings remaining in the nest. In Exp.1, following isolation the striatum and septum were assayed by HPLC for DA utilization. In Exp. 2, pups were injected with the radioactive D2 antagonist raclopride following isolation. After 20 min the brain was removed and the striatum, septum and hypothalamus were assessed for radioactivity. In Exp. 3, punches of striatal, septal and hypothalamic tissue were incubated ex vivo with 3H-raclopride. The results showed that DA turnover was significantly higher in striatal and septal tissue for pups isolated in a novel environment. In both binding experiments, radioactivity in all 3 brain parts was significantly less in animals isolated in the novel environment which implies fewer available D2 receptors. Taken together, these data suggest that isolation in the novel environment and not one with familiar cues causes a DA release at the synapse.

510.8

UNILATERAL 6-HYDROXYDOPAMINE LESIONS OF THE MEDIAL PREFRONTAL CORTEX INTERACT WITH INTRINSIC DOPAMINE ASYMMETRY TO DIFFERENTIALLY ALTER NUCLEUS ACCUMBENS DOPAMINE TURNOVER FOLLOWING A FOOTSHOCK STRESSOR. J.N. Carlson*, E.M. Rutledge, K.E. Visker, R.W. Keller, Jr. and S.D. Glick, Dept. of Pharmacology and Toxicology, Albany Medical College, Albany, NY 12208

We have previously shown that a footshock stressor causes lateralized changes in the metabolic activation of dopamine (DA) neurons whose terminals project to the medial prefrontal cortex (PFC) in the rat. The nature of these changes was dependent upon the animal's prior experience with coping with stressors as well as its preexisting turning bias. Animals responding to stress with predominant left or right PFC DA activation displayed effective and ineffective footshock escape behavior, respectively. PFC DA activity has been shown to modulate DA activity in the nucleus accumbens (NAC) and striatum. In the present study we have attempted to assess a specific role for lateralized PFC DA function in the stress response by determining the effects of unilateral lesions on behavior and subcortical DA function. Male Long-Evans rats displaying left or right turning preferences received unilateral PFC 6-hydroxydopamine (6OHDA) lesions and were then tested three weeks later. Both left and right lesions caused a leftward shift in turning behavior of all rats. Lesions on either side caused an increased DOPAC/DA ratio in the NAC of left-biased rats in response to a footshock stressor. However, right-sided lesions in right-biased rats caused a diminished NAC DOPAC/DA ratio as compared to sham lesioned controls. Lesions on either side interacted with turning bias to alter asymmetrically striatal DOPAC levels following footshock. These findings demonstrate a lateralized interaction between cortical and subcortical DA systems during stress and suggest an explanation for the previously observed increased susceptibility of right-biased rats to the aftereffects of uncontrolled stressors. (supported by MH45539)

510.9

THE EFFECTS OF L-DEPRENYL ON MOTORIC CAPACITY AND NOVELTY-INDUCED BEHAVIOR IN THE RAT. **M.P. Murphy, N.W. Milgram and G.O. Ivy*. Univ. of Toronto, Div. of Life Sciences, Scarborough, ONT, Canada, M1C 1A4.**

We have investigated the possibility that l-deprenyl (DEP), an MAO-B inhibitor used in the treatment of Parkinson's Disease, may attenuate the decay of motoric function in the aged rat. Male and female F344 rats (N=20 of each; 13-14 months old) were given either oral DEP (1.0 mg/kg) or vehicle (0.9% saline) 3 times/week for either 4 or 11 months. Animals were evaluated in several tests sensitive to motor function (tilting plane, wire suspension, horizontal bar), and in a novel open-field arena (10 minutes/day for 3 days). DEP had no effect on measures of motor performance, and did not alter locomotor/exploratory activity in the open-field, but was found to increase grooming ($F_{1,32}$= 9.57, $p<0.01$). Since novelty-induced grooming (linked to mild anxiety in the rat) is known to be mediated by dopamine (DA), this effect may be due to increased DA availability. Alternatively, elevated phenylethylamine, a known DA agonist with mild anxiogenic properties, may be responsible. These results raise the possibility that chronic DEP treatment may result in a mildly elevated stress response in the rat. Supported by Deprenyl Animal Health.

510.10

AN INVESTIGATION OF BRAIN STIMULATION-REWARD THRESHOLDS AND SPONTANEOUS LOCOMOTOR ACTIVITY IN FLINDERS SENSITIVE HYPERCHOLINERGIC RATS. B.A. Baldo*[1], K. Matthews[2], A. Markou, [1] O. Lown[1], D.H. Overstreet[3], and G.F. Koob[1]. 1- The Scripps Research Institute, La Jolla, CA, U.S.A. 2- Dept of Mental Health, Univ. of Aberdeen, Scotland, U.K. 3- Univ of North Carolina, Chapel Hill, NC, U.S.A.

The Flinders Sensitive (FSL) and Resistant (FRL) Lines of rats have been bred based on their thermic response to the cholinesterase inhibitor, diisopropyl fluorophosphate. The hypercholinergic FSL rats, which display augmented oxotremorine-induced hypothermia, have been proposed as an animal model of depression. Since anhedonia is one of the core symptoms of depression, an attempt was made to determine whether FSL rats differed from FRL rats and outbred Sprague-Dawleys with regard to brain stimulation-reward (BSR) thresholds. Changes in BSR thresholds were measured in all strains following systemic adminstration of the indirect dopamine agonist cocaine (10 mg/kg), the dopamine D1 receptor antagonist SCH 23390 (0.02 mg/kg), the cholinergic muscarinic antagonist scopolamine (0.1 mg/kg), and the serotonin reuptake blocker fluoxetine (10 mg/kg). Cocaine significantly lowered BSR thresholds, and SCH 23390 and scopolamine significantly elevated BSR thresholds, in all strains. Fluoxetine produced non-significant elevations in BSR thresholds. No statistically reliable between-strain differences were detected in baseline BSR thresholds, or in response to any of the drug challenges. In a second experiment, unconditioned locomotor activity was monitored in all strains. In agreement with previous research, FSL rats were found to show significantly lower levels of spontaneous locomotor activity than FRL rats or outbred Sprague-Dawley control rats. In summary, functional assessment of CNS reward pathways using the BSR technique did not differentiate FSL rats from FRL or control animals. However, it was determined that FSL rats exhibit decreased activity levels, which may reflect alterations in their cholinergic systems.

510.11

AN INVESTIGATION OF STARTLE REACTIVITY, PREPULSE INHIBITION, AND HABITUATION IN FLINDERS SENSITIVE HYPER-CHOLINERGIC, AND FLINDERS RESISTANT RATS. A. Markou,*[1] K. Matthews[2], M.A. Geyer[3], D.H. Overstreet[4], and G.F. Koob[1]. [1]Dept of Neuropharmacology, The Scripps Research Institute, La Jolla, CA, U.S.A. [2]Dept of Mental Health, Univ of Aberdeen, Scotland, U.K. [3]Dept of Psychiatry, Univ of California, San Diego, U.S.A. [4]Univ of North Carolina, Chapel Hill, NC, U.S.A.

The Flinders Sensitive (FSL) and Resistant (FRL) Lines of rats have been selectively bred to differ in their sensitivity to the anticholinesterase diisopropyl fluorophosphate (DFP). The hypercholinergic FSL rats, which exhibit enhanced oxotremorine-induced hypothermia, have been proposed as an animal model of depression based on behavioral, endocrinological, and pharmacological evidence. Because FSL rats have been reported to be "hyper-responsive" to environmental stimuli, the response of the FSL, FRL and outbred Sprague-Dawley control rats to startling acoustic stimuli was investigated. Both FSL and FRL rats showed decreased startle thresholds compared to controls, even though no differences were observed in startle response magnitude at high (120dB) stimulus intensities. Further, all three groups demonstrated robust prepulse inhibition. Interestingly, the FRL showed a sensitization of startle response magnitude across trials, while the FSL rats exhibited habituation of the startle response, similar to that observed in the control animals. Further, the FRL exhibited increased latency to peak startle response compared to both FSL and controls. The decreased startle thresholds exhibited by both FSL and FRL rats further demonstrate the "hyper-sensitivity" of Flinders rats to environmental stimuli, while the sensitization of startle response magnitude exhibited across trials by the FRL provides one of the few indications that the FRL exhibit behavioral differences in relation to control animals.

510.12

EFFECTS OF LESIONS OF THE DORSAL NORADRENERGIC ASCENDING BUNDLE ON SHOCK-INDUCED FREEZING AND VOCALIZATION IN RATS.
Artur H. Swiergiel* and Adrian J. Dunn.
Dept. of Pharmacology, Louisiana State University Medical Center, Shreveport, LA 71130-3932.

Previously, we reported that lesioning of the noradrenergic (NE) neurons in the locus coeruleus (LC) with the neurotoxin DSP-4 potentiated the duration of footshock-induced freezing and increased ultrasonic vocalization. Conditioned freezing and vocalization were not affected. Because peripherally administered DSP-4 lesions both ascending and descending LC projections, we studied 6-OHDA lesions of the dorsal noradrenergic ascending bundle. Footshock-induced freezing and vocalization and conditioned freezing and vocalization were observed 96 and 120 hr, respectively, after the lesions, and in another group of rats 6 weeks after the lesions. There were no significant changes in behavioral patterns in rats observed shortly after the lesions. However, 6 weeks after the lesions, footshock-induced vocalization was potentiated by 292%. The lesions did not alter the responses evoked by re-exposure to the cages in which footshock was administered. Thus vocalization was affected by painful stimuli (footshock), but not by nonpainful (conditioned) stimuli. The LC-NE system is considered to play an important role in mediating behavioral responses observed in animal models of fear and anxiety. These results suggest that LC-NE system may play a role in stress induced vocalization, perhaps by modifying sensitivity to pain during footshock.

Supported by NINDS (NS 27283) and AFOSR (F49620-93-1-0125DEF)

510.13

LOW DOSES OF APOMORPHINE (APO) RESEMBLE HALOPERIDOL (HAL) AND NOT AMPHETAMINE (AMPH) AS REVEALED BY HIGH RESOLUTION ANALYSIS OF MOTOR PERFORMANCE. X. Liu, R.E. Strecker†, and J. Brener*. Depts of Psychology and †Psychiatry, SUNY, Stony Brook, NY.

Sensitive behavioral measures are needed to detect subtle changes in motor and sensorimotor performance in subjects with neurological impairment. Operant cages, equipped with 3 force beams serving as levers, were coupled to custom software allowing high resolution analysis of the kinematics of individual responses, as well as analysis of sequential behavior. As a first step toward addressing the role of dopaminergic mechanisms and the basal ganglia in motor performance, rats were injected with dopaminergic (DA) drugs prior to testing. Low doses of Apo (.03, .1, .3 mg/kg, sc) and Hal (.04,.08, .16 mg/kg, ip) had qualitatively similar effects on kinematic performance that differed from the effects of Amph (.1, .3, 1.0 mg/kg, ip). Apo and Hal produced dose dependent decreases in response rate, peak force and the rate of change of force (dF/dT), and increases in response duration. In contrast, Amph produced no change in response rate or dF/dT, a slight increase in peak force and an increase in response duration. These data indicate that Apo and Hal produced less frequent and weaker beam presses, effects that are consistent with a decrease in dopaminergic tone. Analysis of behavior sequences revealed that all 3 drugs produced dose-dependent suppression of normal response sequences and increases in abnormal sequences. Non-productive repetitive behavioral sequences (stereotyped behavior) were also observed, especially for Apo and Hal. The behavioral effects of selective DA lesions will also be presented. The behavioral effects of DA drugs measured with this high resolution behavioral analysis are consistent with previous reports based on low resolution analysis (ambulation); however, the present method was more sensitive and provided additional information about motor control processes.

510.14

SEROTONERGIC AND NORADRENERGIC LESIONS DO NOT ABOLISH THE BEHAVIORAL EFFECTS OF ANTIDEPRESSANTS IN THE FORCED SWIMMING TEST. **A. Singh*, C.M. Andrews and J. Lucki** Dept of Psychiatry, Univ of Pennsylvania, Philadelphia, PA 19104.

This study examined whether the functional integrity of the serotonergic or the noradrenergic system is necessary for the reductions of immobility time produced by antidepressants that are uptake inhibitors in the forced swimming test (FST). The ability of the serotonin and the norepinephrine selective uptake inhibitors, sertraline and desipramine respectively, to reduce immobility time was assessed following the destruction of serotonergic or noradrenergic neurons.

The FST was conducted as described previously [Wieland and Lucki (1990) Psychopharmacology 101: 497-504]. Serotonin neurons were destroyed by bilateral injections of 5,7-DHT (200 ug, icv), 30 min following pretreatment with desipramine (25 mg/kg,i.p.), to protect NE neurons. Noradrenergic neurons were destroyed using DSP-4 (50 mg/kg,i.p.), 1 h following pretreatment with fluoxetine (5 mg/kg,i.p.) to protect 5-HT neurons. The FST was conducted 10-15 days following neurotoxin administration. Rats received three subcutaneous injections, of sertraline or desipramine at time points corresponding to 0.5, 19 and 23 hours after the 15 min pretest swim session. Behavioral immobility was measured during a 5-min test session, which occurred one hour after the last injection. Doses of sertraline (80 mg/kg,s.c.) and desipramine (10 mg/kg,s.c.) were chosen from preliminary studies and represent doses that produced similar behavioral effects.

Destruction of 5-HT or NE neurons did not disrupt immobility time. Sertraline and desipramine were effective at reducing immobility time following the destruction of 5-HT or NE neurons, suggesting that intact nerve terminals are not necessary for their behavioral effects in the FST. These findings are at variance with our current understanding of the mechanism of therapeutic action of these compounds and suggest that their therapeutic effects *in vivo* may not be related only to their ability to block neurotransmitter uptake *in vitro*.

Supported by USPHS grants MH 36262 and MH 48125.

510.15

EFFECT OF NOVEL STRESSORS ON THE BEHAVIOR AND CENTRAL NORADRENERGIC SYSTEM IN SPRAGUE-DAWLEY (SD) AND WISTAR-KYOTO (WKY) RAT STRAINS. W. P. Pare*[1], J. Yang[2] & S. M. Tejani-Butt[2]. [1]VA Med. Center, Perry Point, MD 21902 and [2]Dept. Psychiatry, Univ. of Penn. Sch. of Med., Phila, PA 19104.

This study compared the effects of repeated novel stressors on "depressive behavior", defined by the forced-swim and open-field tests, in SD and WKY rat strains. Since stress appears to alter brain norepinephrine (NE) activity, this study also investigated the effects of the stressors on ß-adrenoceptors (ßARs) and NE transporter (NET) sites in SD and WKY rats. Stress did not alter ^{125}I-iodopindolol (^{125}I-IPIN) binding to ßARs, nor ^{3}H-nisoxetine (^{3}H-NIS) binding to NET sites in SD rats, compared to non-stressed controls. However, WKY-stressed rats showed a significant reduction ($p<0.05$) in ^{125}I-IPIN binding to $ß_1$ARs in the hippocampus (HIP) and amygdala (AMYG) and to $ß_2$ARs in the AMYG and hypothalamus (HYPO). ^{3}H-NIS binding to NET sites in WKY-stressed rats was also reduced in the cortex (CTX), HIP and AMYG. When control rats from both strains were compared, the most surprising finding was a significantly higher density of NET sites in the CTX, HIP, AMYG and HYPO in WKY rats compared to SD rats. These results indicate that stress not only exacerbates "depressive behavior" in WKY rats, but selectively alters ßARs in the HIP and AMYG and NET sites in the CTX, HIP and AMYG. Thus the WKY strain may serve as an useful animal model of depressive behavior. (Research funds from the VA Medical Research Service and from USPHS grant MH 45472 (S.T-B)).

510.16

SUCROSE CONSUMPTION AND CONTRAST SHIFTS IN SOCIALLY ISOLATED RATS. FS Hall*, T Humby, LS Wilkinson, TW Robbins. Dept. of Exp. Psych., Cambridge University, Cambridge, U.K.

Previous neurochemical and behavioral data suggesting altered mesolimbic dopamine (DA) function in rats reared in social isolation may predict altered response to rewarding stimuli, such as sucrose. In Experiment 1 socially-reared and isolation-reared rats were allowed to consume sucrose for 15 minutes each day in a lick chamber. Five concentrations of sucrose (0.7%, 2.1%, 7.0%, 21.0%, and 34.0%) were given in an ascending order, each for 3 days. There were no differences in consumption between the two rearing groups. In Experiment 2 socially-reared and isolation-reared rats were food and water deprived and allowed to consume sucrose at each of 3 concentrations (0.7%, 7.0%, and 34.0%). Again, there were no differences in consumption between groups. In Experiment 3 isolation-reared rats and socially-reared rats were exposed to 5 sucrose concentrations within the same 3 hour test session - five 5 minute periods of access to sucrose separated by 25 minutes. Subjects received sucrose in either an ascending or descending order of presentation to maximize positive and negative contrast effects respectively. Under these conditions, socially isolated rats consumed more sucrose regardless of the order of presentation. Thus socially isolated rats are more sensitive to positive contrast effect and less sensitive to negative contrast effects. These experiments suggest increased incentive-motivation in isolation-reared rats, perhaps as a consequence of altered mesolimbic DA function.

510.17

TOPOGRAPHIC ANALYSIS OF BRAIN DOPAMINERGIC NEURON SYSTEMS IN RATS SHOWING SELF-MUTILATION BEHAVIOR FOLLOWING NEONATAL 6-HYDROXYDOPAMINE TREATMENT. 1 IMMUNOCYTOCHEMICAL STUDY OF DOPAMINERGIC NEURONS. C.Yokoyama, H.Okamura*, T.Nakajima, Y.Ibata. Depts. of Psych. & Anat., Kyoto Pref. Univ. of Med., Kyoto 602, Japan.

Neonatal intracisternal treatment of 6-hydroxydopamine (6-OHDA) destroys brain dopaminergic (DA) neurons, and induces self-mutilation behavior (SMB) at adult age after loading L-DOPA. Although behavioral and pharmacological studies have made important discoveries, precise mechanism of SMB is still obscured. To localize the brain region important for SMB, in the series of experiments, we analize region-spesific alternation of neurotransmitters and receptors in rats showing SMB. In the first step of this experiment, we investigated the extent of the damaged DA neuronal area in SMB rats by ABC-immunocytochemistry using anti-tyrosine hydroxyrase serum. Desipramine pretreated (20 mg/kg) neonatal Wistar rats were injected with 6-OHDA (100 ug/5 ul with 0.1% ascorbic acid in saline) intracisternally, and for control, the same solution without 6-OHDA was injected. The behavior after Ro-4-4602 (50 mg/kg) and L-DOPA (100 mg/kg) loading was evaluated at 6 weeks of age, and the rats were devided into three groups; neonatal 6-OHDA treated rats showing SMB (SMB(+) group), neonatal 6-OHDA treated rats not showing SMB (SMB(-) group), and neonatal saline treated rats (control group). The DA depleted area of SMB(-) group was restricted in the nigrostriatal DA system, while that of SMB(+) group was observed in both the nigrostriatal and the ventral tegmental area (VTA)-ventral striatal DA systems. The DA neurons and terminals in the hypothalamus, the central nuclei of the ventral midbrain, the cereberal cortex and the insula of Calleja were affected in neither SMB(-) nor SMB(+). The results revealed that the the neuronal damage on brain DA neurons following intracisternal neonatal 6-OHDA treatment shows region-specificity, and the severe destruction of VTA-ventral striatal DA neuron system is particular in SMB(+) group. The destruction of VTA-ventral striatal DA system may altered the neuronal activity in the ventral striatum, and that change may be one of causes of SMB.

510.18

TOPOGRAPHIC ANALYSIS OF BRAIN DOPAMINERGIC NEURON SYSTEMS IN RATS SHOWING SELF-MUTILATION BEHAVIOR FOLLOWING NEONATAL 6-HYDROXYDOPAMINE TREATMENT. 2. RECEPTOR AUTORADIOGRAPHIC STUDY OF DOPAMINE D1 AND D2 RECEPTORS. H.Okamura, C.Yokoyama, F.Kawakami*, Y.Ibata, Department of Anatomy and Psychiatry, Kyoto Prefectural University of Medcine, Kawaramachi-Hirokoji, Kyoto 602, Japan.

The foregoing our immunocytochemical study has demonstrated that the neonatal 6-hydroxydopamine treatment induces region-specific destruction of dopaminergic neurons, and the extensiveness of the damaged area is related to the occurance of self-mutilation behavior (SMB). As for the dopamine D1 and D2 receptors, however, no studies are performed focusing on SMB with particular attension to region specificity. In the present study, we investigated the alternation of D1 and D2 receptor bindings in the 6-OHDA treated rats showing SMB (=SMB(+) group), not showing SMB (=SMB(-) group), and the saline treated rats (=control group), by quantitive in vitro receptor autoradiography using [3H]SCH-23390 for D1 site and [3H]YM-09151-2 for D2 site. Autoradiography was performed in brain sections from rats at age 10-15 weeks, and sections were incubated with 0.5 nM [3H]YM-09151-2 or 1 nM [3H]SCH-23390 at 22 C, for 2 or 3 hours, respectively. Nonspecific bindings were determined in 10 uM (+)-butaclamol. The densities of [3H]SCH-23390 and [3H]YM-09151-2 binding in the caudate-putamen, the nucleus accumbens and the tuberculum olfactrium were not different among three groups. The binding densities of [3H]YM-09151-2 in the substantia nigra pars compacta were decreased both in SMB(+) and SMB(-), while in the ventral tegmental area the binding densities were decreased only in SMB(+) compared with control. The binding densities of [3H]SCH-23390 were increased in the substantia nigra pars reticulata in SMB(+) compared with that of SMB(-). The present results suggest that the induction of SMB following L-DOPA or doapmine D1 receptor agonist loading may be through the activation of upregulated D1 receptor in the substantia nigra pars reticulata.

511.1

IN SITU HYBRIDIZATION ANALYSIS OF NEUROPEPTIDE mRNA LEVELS IN LOCUS COERULEUS NEURONS FOLLOWING MORPHINE WITHDRAWAL IN RATS. P.V. Holmes*, A. de Bartolomeis, V. Koprovica, and I.N. Crawley. Section on Behavioral Neuropharmacology, Experimental Therapeutics Branch, NIMH, Bethesda, MD 20892.

The locus coeruleus (LC) contains galanin-like immunoreactivity or neuropeptide Y-like immunoreactivity coexisting within neurons containing tyrosine hydroxylase-like immunoreactivity. The firing rate of LC neurons is greatly enhanced during withdrawal from morphine. The hypothesis that this phenomenon is associated with altered expression of neuropeptide genes was tested. Rats received incremental doses of morphine or saline for one week. Withdrawal was induced in half of the morphine-treated rats by a single injection of naloxone. The remaining morphine-treated rats received an injection of saline. Characteristic signs of withdrawal, including wet dog shakes, teeth chattering, and diarrhea, were observed in morphine-treated rats injected with naloxone. Levels of mRNA encoding galanin and neuropeptide Y in the LC, and corticotropin-releasing factor and somatostatin in dorsal tegmental areas projecting to the LC, were analyzed by *in situ* hybridization. Neither the chronic morphine treatment nor naloxone-precipitated withdrawal consistently altered the expression of any of the peptide genes studied.

511.2

IMMEDIATE EARLY GENE INDUCTION IN PRECIPITATED OPIATE WITHDRAWAL. Pastor Couceyro* and James O. Douglass. Vollum Institute, Oregon Health Sciences University, Portland, OR.

Physical dependence is perhaps the most detrimental liability of addictive drugs. No where is this better illustrated than in heroin addicts undergoing detoxification. "Withdrawal" is a rebounding homeostatic response to drug induced changes. This response could therefore serve as a model of adaptation and plasticity and hence, gene expression may be involved. To this end we have examined the expression of immediate early genes in the rat brain after precipitated opiate withdrawal. Gene expression for *c-fos*, *zif-268*, *junB* and *fosB*, was determined by slot blot and Northern blot analysis. The severity of dependence was controlled by varying the duration and dosage of opiate administration as well as the dose of naloxone (from 0.01 to 100 mg/kg) utilized to precipitate withdrawal. No alterations in mRNA levels for any of these transcripts was seen in withdrawal precipitated 72 hrs after implantation of a single morphine base pellet (75 mg). Transcriptional changes were noted after longer and larger morphine doses in two other studies. In the first, rats were implanted with one pellet every day for 7 consecutive days and withdrawal precipitated on day 8. In the second study, rats were treated on days 1 (1 pellet), 4 (2 pellets) and 7 (3 pellets) and withdrawal precipitated on day 10. Expression of *c-fos* was dramatically altered in the hypothalamus, cerebellum and striatum 1 hr after precipitated withdrawal in a naloxone dose-depended fashion. Less robust changes were seen in other brain areas. Smaller and more brain region selective changes were noted for *zif-268* but the levels of *junB* and *fosB* mRNA were unaffected. Qualitatively greater changes were seen in the former study. We are in the process of a more detailed analysis using *in situ* hybridization. These observations suggest a role for alterated gene expression in the homeostatic response of opiate withdrawal.

511.3

DOES MK-801 BLOCK NALOXONE-PRECIPITATED OPIATE WITHDRAWAL? K.A. Trujillo* and H. Akil, Mental Health Research Institute, University of Michigan, Ann Arbor, Michigan, 48109-0720.

We previously reported that the non-competitive N-methyl-D-aspartate (NMDA) receptor antagonist MK-801 inhibited the *development* but not the *expression* of physical dependence on morphine. In other words, MK-801 prevented the acquisition of physical dependence when coadministered with morphine, but would not block naloxone-precipitated withdrawal in animals in which dependence had already been acquired. Other laboratories, however, have reported that MK-801 blocks naloxone-precipitated withdrawal in morphine-dependent animals. The present experiment was performed to further examine the effects of MK-801 on naloxone-precipitated opiate withdrawal. Adult, male Sprague-Dawley rats received morphine (10 mg/kg s.c.) twice daily for 10 days. On day 11, animals received morphine, followed 30 minutes later by saline or MK-801 (0.1, 0.5 or 1.0 mg/kg i.p.). Withdrawal was precipitated by administration of naloxone (2 mg/kg s.c.) 30 minutes following the second injection, and animals were observed for signs of withdrawal and gross behavioral effects. MK-801 dose-dependently inhibited signs of naloxone-precipitated withdrawal, including jumping, paw shakes, mouth movements and ptosis, as well as the overall withdrawal rating. The inhibition of withdrawal, however, was accompanied by gross behavioral disturbance in these animals. The 0.1 mg/kg dose of MK-801 produced mild behavioral activation, while the 0.5 and 1.0 mg/kg doses produced effects ranging from ataxia and stereotyped head-weaving to flaccid paralysis. These results lead us to suggest that the effects of MK-801 resulted from the non-specific motor disturbance produced by this drug, rather than from a specific inhibition of the naloxone-precipitated withdrawal syndrome. The results are consistent with our suggestion that non-competitive NMDA receptor antagonists inhibit the *development* rather than the *expression* of opiate tolerance and dependence.

This work was supported by NIDA Grant DA02265 and NIMH Grant MH422251.

511.4

INTRACRANIAL SELF-STIMULATION DURING REPEATED MORPHINE TREATMENT AND MORPHINE WITHDRAWAL: A CURVE-SHIFT ANALYSIS. P. Bauco* and R.A. Wise. Center for Studies in Behavioral Neurobiology and Dept. of Psychology, Concordia Univ., Montréal, Canada, H3G 1M8.

Pothos et al. (Br Res, 1991, 556; 348-350) have reported a naloxone-induced decrease in nucleus accumbens dopamine (DA) levels after termination of a low-dose chronic morphine regimen. We studied the effects on intracranial self-stimulation (ICSS) of the same opiate treatment regimen. Rats were given daily morphine (20 mg/kg i.p.) for 7 days; ICSS was tested prior to and following the morphine on Days 1 and 7, and prior to and following naloxone (20 mg/kg i.p.) on Day 8. On Day 1 the rats failed to respond for the first 2h after injection but then responded for 3h with reduced thresholds (rate-frequency functions shifted 0.3 log units to the left). The same pattern was seen following the 7th morphine injection, except that the animals continued to respond—at slightly elevated thresholds—during the first 2h after injection. On Day 8, thresholds were normal during the pre-injection baseline period but were significantly elevated after naloxone; naloxone shifted rate-frequency functions to the right by 0.3 log units. Tooth-chattering, wet-dog shakes, and diarrhea confirmed that naloxone, but not 24h abstinence, caused opiate withdrawal distress correlated with the observed elevations in reward threshold.

511.5

ATTENUATION OF CONDITIONED OPIATE WITHDRAWAL FOLLOWING EXCITOTOXIC LESIONS OF THE BASOLATERAL AMYGDALA. B. J. Everitt[1], G. Schulteis*[2], and G. F. Koob[2]. [1]Dept. of Anatomy, University of Cambridge, Cambridge, U.K., and [2]Dept. of Neuropharmacology, The Scripps Research Institute, La Jolla, CA 92037.

The basolateral nucleus of the amygdala (BLA) participates in the acquisition of secondary reinforcing properties by stimuli paired with primary positive reinforcers, including drugs of abuse (Robbins & Everitt, Seminars Neurosci. 4:119, 1992). To test whether the BLA participates in conditioning of the aversive consequences of drug withdrawal, male Wistar rats were trained to press for food (FR 15) in daily 30 min sessions. After responding stabilized, half the rats received bilateral quinolinic acid (0.09 M) lesions of the BLA , with the other half receiving SHAM lesions. Following re-establishment of stable responding, all rats received morphine pellets (2 x 75 mg s.c.). Five days later, half of each group received an injection of naloxone (30 µg/kg s.c.) and was exposed to a light/ tone stimulus (CS) during a 20 min operant session, followed 4 hours later by a saline injection in their home cages (conditioned/COND). The other half received a saline injection paired with the CS, and naloxone 4 hours later in their home cages (pseudo-conditioned/PSEUDO). Responding was significantly suppressed during the naloxone/CS pairing in both COND-LESION and COND-SHAM groups. On the following day, when given a saline injection and presented with the CS a second time, the COND-SHAM group showed a significant reduction in responding relative to the COND-LESION group. PSEUDO-SHAM and PSEUDO-LESION animals showed no response suppression during the first or second CS presentation. Thus, conditioned withdrawal may be seen with one pairing of a CS and precipiated withdrawal, and lesions of the BLA significantly attenuate this conditioning process. Supported by DA04043.

511.6

Comparison of butorphanol withdrawal syndromes precipitated by opioid receptor antagonists: nor-binaltorphimine (nor-BNI), naltrindole (NTI) and naloxone (NLX). S.P. Jaw, B. Hoskins and I.K. Ho*. Dept. Pharmacol. and Toxicol., Univ. of Mississippi Medical Center, Jackson, MS 39216.

Male Sprague-Dawley rats (251-300 g) were rendered dependent upon butorphanol by continuous i.c.v. infusion of butorphanol tartrate (26 nmol/h) for 3 days via osmotic minipumps. Control animals received saline infusion (1 µl/h) for the same period of time. Three hours after the termination of butorphanol or saline infusion, rats were randomly divided into different groups and challenged with various doses of nor-BNI, NTI, or NLX (kappa-, delta-selective, or non-specific antagonist) i.c.v. Results indicate that nor-BNI was more potent than NLX in eliciting withdrawal signs, e.g., teeth-chattering (TC), wet shakes (WS), forepaw tremors, yawning (YA), ptosis, and salivation. Both nor-BNI and NLX increased the number of animals exhibiting ejaculation (EJ) at high doses. The potency ratios of ED_{50}s for NLX over nor-BNI in eliciting TC (1.25), YA (2.13) and EJ (0.72) were not significantly different. Nor-BNI was more potent than NLX in causing urination (NLX/nor-BNI: 290). There were no significant differences in ED_{50}s of NTI and NLX in eliciting TC, WS, and YA in butorphanol-dependent rats. These studies appear to indicate that both kappa- and delta-opioid receptors are involved in butorphanol dependence in rats. (Supported by DA 05828)

511.7

MOTIVATIONAL CONSEQUENCES OF NALOXONE-PRECIPITATED OPIATE WITHDRAWAL: A DOSE-RESPONSE ANALYSIS. G. Schulteis, R. Carrera, A. Markou, L. H. Gold*, and G. F. Koob. Dept. of Neuropharmacology, The Scripps Research Institute, La Jolla, CA 92037.

Opiate withdrawal is known to have aversive motivational consequences as measured by disruptions of operant responding for food and conditioned place aversions (Gellert & Sparber, J. Pharmacol Exp Ther 201:44, 1977; Koob et al., Psychopharmacology 98:530, 1989; Stinus et al., Neuroscience, 37:767, 1990). In the current series of studies, male Wistar rats were implanted subcutaneously (SC) with either morphine (2 x 75 mg) or placebo pellets. No sooner than 3 days after implant the rats were given a SC injection of naloxone (0 - 1.0 mg/kg) and tested in one of several behavioral paradigms. In dependent rats, very low (0.01 - 0.03 mg/kg) doses of naloxone produced the following behavioral effects: 1) a reduction in spontaneous locomotor activity, 2) a disruption of schedule-controlled (FR15) operant responding for food, 3) an elevation in intracranial self-stimulation thresholds, and 4) an increase in heroin self-administration (decrease in the inter-injection interval). These doses of naloxone produced no significant effects in non-dependent, placebo-pelleted rats. Importantly, a dose of 0.01 mg/kg naloxone, which in dependent rats produced significant effects on all of the behavioral measures employed, produced only minimal physical signs of withdrawal. The behavioral measures employed in our studies appear to be highly sensitive indices of the aversive motivational consequences of opiate withdrawal and these measures can be used to study the neural substrates contributing to opiate dependence and withdrawal. Supported by grant DA04043.

511.8

LONG-TERM GLUTAMATE DESENSITIZATION IN LOCUS COERULEUS NEURONS: A VOLTAGE CLAMP STUDY IN RAT BRAIN SLICES J.H. Kogan* and G.K. Aghajanian. Depts. of Cellular & Molecular Physiology, Psychiatry, & Pharmacology, Yale Univ. School of Medicine, New Haven, CT 06508.

We have previously demonstrated that long-term glutamate desensitization (LTGD) occurs in locus coeruleus (LC) neurons following both naltrexone-precipitated opiate withdrawal and during a prolonged application of glutamate to a rat LC brain slice. Using extracellular recording techniques in brain slices it was shown that a maximal desensitization of 50-60% can be induced with a 20 min application of glutamate (1mM). The desensitization gradually reversed within 3 hours after wash-out of glutamate.

We now report that LTGD can be induced in LC neurons voltage clamped below firing threshold. The induction and recovery are similar to that observed during the extracellular studies. The glutamate inward current desensitizes about 50% with a 20 min application of glutamate (1mM) and recovery occurs within 3 hours after wash-out. Glutamate inward currents are accompanied by an increase in the apparent membrane conductance. During the development of LTGD as the glutamate current decreases there is a proportional reduction in the membrane conductance suggesting that the reduced response to glutamate is not due to the development of an opposing outward current. No change was seen in the membrane conductance before and after the glutamate applications indicating no change in the passive membrane properties of the cell or an excitotoxic action of glutamate. Cyclothiazide (50μM), which inhibits the acute desensitization of glutamate receptors, results in an initially enhanced response to glutamate but does not block the induction of LTGD indicating that LTGD is distinct from rapid receptor desensitization. These results indicate that LTGD in LC neurons occurs independently of action potential generation or membrane depolarization.

511.9

INCREASED GLUTAMATE RELEASE IN THE LOCUS COERULEUS (LC) DURING OPIATE WITHDRAWAL: A MICRODIALYSIS STUDY G.K. Aghajanian*, J.H. Kogan, and B. Moghaddam. Depts. of Psychiatry, Pharmacology, and Cellular and Molecular Physiology, Yale School of Medicine, New Haven, CT 06508.

Noradrenergic LC neurons in opiate-dependent rats are markedly activated by antagonist-induced withdrawal. This activation may be due in part to an increased release of glutamate in the LC since either glutamate antagonists or lesions of the nucleus paragigantocellularis (a major excitatory amino acid input to the LC; Ennis and Aston-Jones, 1988) reduce substantially the withdrawal response (Rasmussen and Aghajanian, 1989). The present study examined directly by in vivo microdialysis whether an increased release of glutamate can be detected biochemically in the rat LC following opiate withdrawal.

Opiate dependence was induced by the pellet method (one 75 mg morphine pellet (s.c.)/day x 3 days). Two days after the last pellet implantation, microdialysis probes were placed in or near the LC under chloral hydrate anesthesia; glutamate in the perfusate was derivatized and measured by HPLC/fluorescence detection. To improve the accuracy of probe placements, the stereotaxic location of the LC was determined first by unit recording; placements were confirmed histologically. A sustained, 2-3 fold increase in glutamate efflux was found following naltrexone-induced withdrawal (100 mg/kg, s.c.) in rats where the microdialysis probe was in the LC proper; only slight if any increases were seen in pericoerulear sites and no increase was seen in surrounding brainstem areas or in non-dependent rats. These results support the hypothesis that an increased release of glutamate contributes to withdrawal-activation of LC neurons in opiate-dependent rats.

511.10

A ROLE FOR ACCUMBAL D2 RECEPTOR ACTIVITY IN THE INITIATION AND PREVENTION OF THE OPIATE WITHDRAWAL SYNDROME. Glenda C. Harris* and Gary Aston-Jones. Hahnemann University, Department of Mental Health Sciences, Philadelphia, PA 19102.

The nucleus accumbens is well known for its role in mediating the rewarding aspects of opiate use. In the current series of experiments we report that it is also a site involved in opiate withdrawal symptoms. Male Sprague-Dawley rats were made dependent on morphine by daily ip injections (incrementing doses of 10 mg/kg up to 80 mg/kg/day). The non-selective dopamine agonist, apomorphine (300 ug - 2 mg/kg ip) or the more selective D2-agonist, propylnorapomorphine (R-NPA, 50-500 ug/kg ip) significantly reduced the majority of naloxone-precipitated (0.5 mg/kg) somatic withdrawal signs. The D1 receptor agonist, SKF-38393 (2-5 mg/kg) was ineffective. To determine if the effects of these dopamine agonists were mediated by actions within the accumbens, R-NPA (3-10 ug) was microinjected into the accumbens prior to naloxone-precipitated withdrawal. At all doses, R-NPA significantly prevented the occurrence of somatic withdrawal signs when administered into the accumbens but not when administered into the nearby straitum. Dopamine receptor antagonists completely blocked the effects of accumbal R-NPA. SKF-38393 (3 ug) in the accumbens did not prevent the occurrence of withdrawal signs. In addition, the administration of the dopamine antagonists (3 ug) flupentixol (D1/D2) and ecticlopride (D2) alone into the accumbens of morphine dependent rats precipitated several somatic withdrawal signs. These data indicate D2 receptors within the accumbens are important in the manifestation of opiate withdrawal and furthermore, that D2 agonists could be useful in the treatment of opiate addiction. Supported by NIDA grants DA05387 and DA06214.

511.11

SPINAL CORD FOS-LIKE IMMUNOREACTIVITY (FLI) DURING PRECIPITATED ABSTINENCE: COMPARISON WITH PATTERNS EVOKED BY NOXIOUS STIMULATION D.S. Rohde, D.J. Detweiler and A.I. Basbaum*. Depts. Anesthesia, Anatomy and Physiology, UCSF, San Francisco, CA.

The abstinence syndrome that is precipitated in opioid tolerant animals includes hyperalgesia. To identify the population of spinal cord neurons that underlies this state and to compare this with populations of neurons that are activated by noxious stimulation, we monitored the FLI after naltrexone precipitated abstinence and by noxious stimulation in normal and morphine-tolerant male, Sprague Dawley rats. After daily implantation of morphine or placebo pellets (75 mg/kg, 5 days), the rats received an injection of naltrexone, 10 or 100 mg/kg, sc or 5.0% formalin (100μl) into the plantar hindpaw and then behavior was monitored for one hour. Next, the rats were anesthetized and perfused with a 4% paraformaldehyde fixative. Transverse 50μm frozen sections of the spinal cord were immunoreacted with a fos polyclonal antiserum using the ABC method.

Contrary to a previous report (Abbott et al, 1981) formalin did evoke pain behavior in both placebo-treated and morphine-tolerant rats. Although the patterns of fos expression in these groups were comparable (dense labelling in laminae I, IIo and V-VIII), there was enhanced FLI (including increased rostrocaudal and bilateral staining) in the morphine-tolerant rats. Naltrexone-precipitated abstinence in awake rats evoked FLI bilaterally with the most dense staining in laminae I, III, IV, X and in sacral parasympathetic preganglionic neurons. Naltrexone injection in the placebo group did not increase spinal cord FLI. When abstinence was precipitated under Halothane anesthesia, there was a significant decrease in FLI in laminae III and IV, but much less change in lamina I. This suggests that the induction of FLI in lamina I is not secondary to the behavior associated with withdrawal and that spinal cord nociresponsive neurons (mainly in lamina I) are sensitized during the development of tolerance. This is manifested as increased FLI in lamina I with precipitated abstinence and more extensive staining when FLI is induced by a noxious stimulus in morphine-tolerant rats. Supported by DE/NIDA 08973 and NS14627.

511.12

NALOXONE-PRECIPITATED OPIOID WITHDRAWAL FOLLOWING CHRONIC TREATMENT WITH TRIMU-5, A μ2-OPIOID AGONIST. R. M. Eisenberg and D. J. Forbes*. Dept. Pharmacology, Univ. Minn. Duluth, Duluth, MN 55812.

TRIMU-5 is an enkephalin analog with high affinity for both μ1 and μ2-sites, with poor affinity for other opioid receptors. Binding studies have characterized this agent as a μ2 agonist and a μ1 antagonist. The objective of this study was to determine whether opioid dependence could be produced by treatment with this agent by comparing naloxone (NX)-precipitated withdrawal to that following DAMGO. The primary indicator of withdrawal was an increase in plasma corticosterone (CS) following NX. Experiments were conducted on conscious unrestrained male Sprague-Dawley rats with chronic i.v. catheters, and ALZET osmotic pumps or i.c.v. cannula guides allowing for serial blood sampling and drug injection into the right lateral ventricle. During blood sampling, animals remained isolated in sound-attenuated one-way vision boxes. Rats receiving TRIMU-5 (2μg/μl/hr) showed a significant elevation of CS when NX (0.4 mg/kg bwt i.v.) was given approx. 3 d later. Occasionally wet-dog shakes were observed. Similarly, after DAMGO (1μg/μl/hr), CS showed a sustained increase. A variety of characteristic behavioral manifestations were observed (though not scored). Rats given DAMGO, though not TRIMU-5, i.c.v. on an increasing schedule over 3 d showed a significant CS response. Acute dependence did not appear to occur as NX injected 3 hr after a single administration of either TRIMU-5 or DAMGO did not result in a significant CS change. Results indicate that chronic stimulation of the μ2-receptor can produce physical dependence and subsequent NX-precipitated withdrawal. Supported by DA 07186.

511.13

INCREASED DOPAMINE METABOLISM IN THE ROSTRAL VENTRAL MEDULLA (RVM) DURING MORPHINE ANALGESIA, TOLERANCE AND NALOXONE-PRECIPATED WITHDRAWAL. B.K. Taylor* and A.I. Basbaum. Depts. Anatomy and Physiology, UCSF, San Francisco, CA.

Although most studies have emphasized the contribution of medullary serotonergic and noradrenergic mechanisms through which morphine influences nociceptive controls, others have implicated dopaminergic mechanisms. Since we have demonstrated increased *fos* expression in the RVM during abstinence, we used *in vivo* microdialysis to evaluate changes in dopaminergic metabolism (namely, extracellular concentration of homovanillic acid, HVA, in the RVM) during morphine analgesia, tolerance and naloxone-precipitated withdrawal. We instrumented adult male rats with dialysis probes (2.0mm x 200*um*) in the RVM and chronic catheters in the femoral vein. In one group (n=7), we tested the effect of an acute analgesic dose of morphine (3.0 mg/kg iv). In a second group (n=6), we produced tolerance with subcutaneous implantation of morphine pellets (75mg x 5 days), and then precipitated withdrawal with naloxone (0.5mg/kg iv). HPLC with electrochemical detection was used to quantify changes in the concentration of HVA (percent change relative to basal concentrations) in sequential 30-minute samples collected at a perfusion rate of 1.0 *ul*/min. Morphine analgesia was associated with an immediate increase in HVA levels that peaked (218±35%) after 150-180 min, and then gradually returned to basal levels within 240 min. Naloxone-precipitated withdrawal was associated with an almost five-fold increase in HVA levels that peaked (491±156%) after 90-120 min, and then gradually returned to basal levels. These studies provide evidence that dopaminergic systems in the RVM are involved in opiate analgesia and withdrawal mechanisms. Our present studies are directed at measuring changes in dopamine release in the RVM and elucidating the origin of the presumed dopaminergic inputs. Supported by NS-21445 and NS-07265 .

511.14

WITHDRAWAL FROM CHRONIC MORPHINE TREATMENT PRODUCES ALTERATIONS IN THE BETA-ADRENERGIC RECEPTOR-COUPLED ADENYLATE CYCLASE SYSTEM. J.M. Ackerman and H.M. Moises. Dept. of Phys., Univ. of Mich. Sch. of Med., Ann Arbor, MI 48109.

Studies of chronic opiate treatment have demonstrated changes in the ß-adrenergic receptor system and suggest that central noradrenergic mechanisms may play an important role in the mediation of opiate withdrawal. We have previously shown that ß-adrenergic receptor density increased in rat hippocampus after chronic morphine treatment and decreased after morphine withdrawal. These changes were accompanied by corresponding supersensitivity and subsensitivity, respectively, in ß-adrenergic receptor sensitivity assessed electrophysiologically in the hippocampal slice. In the present study, intracellular recordings were obtained from pyramidal neurons in slices from morphine-pellet implanted rats to determine whether changes in the ß-adrenergic receptor response found during morphine dependence and withdrawal might be due in part to alterations occurring beyond the receptor level. The dose-response curve for isoproterenol inhibition of the calcium-activated potassium conductance, I_{AHP}, which occurs in pyramidal neurons following activation of postsynaptic ß-adrenergic receptors, was shifted to the left following chronic morphine treatment. In contrast, the curve was shifted to the right after a 32 to 48h withdrawal period. The dose-response curve for forskolin inhibition was unchanged following treatment but shifted to the right after withdrawal. The results suggest that changes in the ß-adrenergic system in morphine-dependent animals may result from changes at the receptor level, but alterations beyond the level of the receptor may be involved in the mediation of morphine withdrawal.

511.15

TOLERANCE AND WITHDRAWAL AFTER INTRACEREBROVENTRICULAR INFUSION OF THE SELECTIVE μ-OPIOID AGONIST TYR-D-ARG^2-PHE-(NME)GLY^4 (TAPS). B. Gudka, M.O. Adeyemo* and A-L. Sirén. Dept. of Neurology, Uniformed Services University of the Health Sciences, Bethesda, MD 20814.

The development of tolerance and withdrawal after continuous intracerebroventricular (i.c.v.) infusion of Tyr-D-Arg^2-Phe-(NMe)Gly^4 (TAPS), a proposed μ_1-opioid agonist, was compared to that elicited by morphine or D-Ala^2-MePhe^4-Gly-ol^5-enkephalin (DAMGO) in male Sprague-Dawley rats (n=40). The drugs were delivered via an Alzet brain infusion cannula attached to a subcutaneous minipump prefilled with either TAPS (3, 10, or 30 pmol/µl/h), DAMGO (300 or 1000 pmol/µl/h), morphine (10 or 30 nmol/µl/h), or vehicle. Antinociception was measured by the radiant heat tail-flick latency method before minipump implantation and over the next 5 days. On infusion day 5, withdrawal severity was assessed for 60 min following a bolus of naloxone (20mg/kg, i.p.). The opiates produced a dose dependent increase in the tail-flick latency on day 1 with a rank order of potency and efficacy of TAPS > > DAMGO > morphine. Tolerance developed within 2-4 days in all opiate treated rats. The rate of tolerance development as estimated by calculating the exponential decay half-life from the peak effect on day 1 was in order: DAMGO > TAPS = morphine. The order of magnitude of withdrawal was DAMGO = morphine > TAPS. TAPS, 30 pmol/µl/h, resulted in minimal withdrawal which was similar to vehicle while lower doses of TAPS did not significantly differ from DAMGO or morphine. The data indicate that TAPS is a potent antinociceptive agent with less degree of opiate dependence than morphine or DAMGO.

511.16

LASTING CHANGES IN STIMULUS-SECRETION COUPLING IN CENTRAL NERVE TERMINALS UPON MORPHINE WITHDRAWAL. A.N.M.Schoffelmeer*, A.H.Mulder and H.K. G.Tjon Tien Ril. Dept. Pharmacol., Free Univ., Med. Fac., 1081 BT Amsterdam, The Netherlands.

In superfused rat brain slices, depolarization-induced release of noradrenaline (NA, cortex), acetylcholine (ACh, striatum) and dopamine (DA, striatum) is liable to inhibition by presynaptic mu, delta and kappa opioid receptors, respectively. Moreover, morphine enhances in vivo DA release in the striatum, acting on mu receptors in the mesencephalon, which is assumed to be critically involved in opiate reward and reinforcement.
One day after chronic morphine treatment, the electrically evoked release of NA (cortex slices) and that of ACh (striatal slices) was enhanced, whereas that of DA (striatal slices) was reduced. Presynaptic opioid receptor efficacy remained unchanged in brain slices of morphine abstinent rats. Since these adaptive changes in neurotransmitter release in vitro were still observed 3 weeks after opiate withdrawal, i.e. in the absence of physical dependence, persistent changes in the exocytotic release mechanism in central nerve terminals are suggested to play an important role in the maintenance of opiate dependence.

511.17

SUBCUTANEOUS INJECTION OF AN ANALOG OF NEUROPEPTIDE FF PRECIPITATES MORPHINE ABSTINENCE SYNDROME. J.R. Lake*, B. Pal[1], K. Burgess[1], K.R. Arcangeli, K.D. Deshotel, D.D. Hausam, W.E. Witherspoon, V.A. Carter, H.-Y.T. Yang[2] and D.H. Malin. Univ. of Houston-Clear Lake, Houston, TX 77058, [1]Dept. of Chemistry, Texas A&M Univ., College Station, TX 77843 and [2]NIMH, St. Elizabeths, Washington, D.C. 20032.

Neuropeptide FF (NPFF, F8Famide, FMRFamide-like mammalian octapeptide) has been shown to exert various antiopiate actions, including precipitation of opiate abstinence syndrome when injected into the third ventricle of morphine dependent rats (Malin et al. Peptides 11:277, 1990). In the present study, dansyl-Pro-Gly-Arg-Phe amide (dansyl-PQRFa), a lipophilic analog containing the C-terminal fragment of NPFF, was injected subcutaneously into morphine dependent rats and appropriate sham controls. Comparison groups were injected with vehicle alone (30% ethanol in distilled water). In Expt. 1, 14 rats were continuously infused for 7 days with 1.33 mg/kg/hr morphine sulfate prior to receiving either 9 mg/kg dansyl-PQRFa (n=7) or vehicle alone (n=7) s.c. Dansyl-PQRFa precipitated a vigorous opiate abstinence syndrome in the morphine dependent rats; peptide-injected rats had significantly more, p<.01, overall abstinence signs than vehicle-injected rats. In Expt. 2 with 14 sham implanted, non-dependent controls, there was no significant difference between rats injected with dansyl-PQRFa and rats injected with vehicle alone. (NIDA DA06554 and Texas Advanced Technology Program.)

511.18

ENHANCED ANTIOPIATE ACTIVITY AND ENZYME RESISTANCE IN PEPTIDOMIMETICS OF FMRFamide CONTAINING E-2,3-METHANOMETHIONINE. L.S. McDermitt, J.R. Lake, K. Payza[1], T.M. Benson, T.L. Garber, M.L. Waller, T.-A. Luu, R.S. Kelley, D.A. Smith, K.-K. Ho[2], K. Burgess[2] and D.H. Malin*. Univ. of Houston-Clear Lake, Houston, TX 77058, [1]NIMH, St. Elizabeths Washington, D.C. 20032 and [2]Texas A&M Univ., College Station, TX 77843.

FMRFamide is a molluscan peptide which has shown antiopiate activity in a number of mammalian test systems. The current study determined the antiopiate potency of FMRFamide and two conformationally constrained peptidomimetics of FMRFamide containing stereoisomers of E-2,3-methanomethionine. Peptidomimetic 3 contained 2R,3R-E-cyclo-Met, while peptidomimetic 4 contained 2S,3S-E-cyclo-Met. Morphine abstinence signs were observed for 20 mins. after varying doses (0.25 - 25.0 ug) of these substances were injected into the third ventricle of morphine dependent rats. Both peptidomimetics were significantly more potent than FMRFamide itself, and peptidomimetic 3 was significantly more potent than peptidomimetic 4. Although peptidomimetics 3 and 4 bound with lower affinity than FMRFamide to rat spinal cord receptors for NPFF (the mammalian FMRFamide-like peptide), they were 191 and 42 times, respectively, more resistant than FMRFamide to enzymatic degradation by leucine aminopeptidase. Thus, the increased potency of these FMRFamide analogs may be due to increased bioavailability. (NIDA DA06554 and Texas Advanced Technology Program.)

511.19

INHIBITION OF MORPHINE TOLERANCE AND DEPENDENCE BY DIAZEPAM. P.Sribanditmongkol, Ming-Jyh Sheu, G.A.Tejwani*. Department of Pharmacology, Ohio State University, College of Medicine, Columbus, OH 43210-1239

Effect of a benzodiazepine receptor agonist diazepam on the development of morphine tolerance and dependence has been investigated. Male Sprague-Dawley rats were randomized into 8 groups (n=8). Rats in four groups were implanted with pellets containing 75 mg morphine each, and in other four groups were implanted with placebo pellets. Two pellets were implanted subcutaneously on the first day and another four pellets on the second day. Morphine- or placebo-pellet implanted rats were injected with ip saline (1 ml/kg) or diazepam (DZP) 0.025, 0.25 or 2.5 mg/kg after the first day of implantation, then everyday for 5 days. Antinociception was measured by the tail-flick (TF) and hot plate (HP) tests, and sedation by a rotarod test everyday before and one hour after ip injection. On the last day of experiment (day 5), a withdrawal syndrome was induced by injecting naloxone (10 mg/kg sc) and rats were observed for abstinence behaviors for 30 min. Rats were then sacrificed and various brain regions were saved for analysis of met-enkephalin by RIA. We observed that DZP (0.25 and 2.5 mg/kg) inhibited tolerance to morphine antinociception as measured by TF test. Rats implanted with morphine and daily injected with DZP 0.25 mg/kg exhibited less severity of an abstinence syndrome as determined by jumping behavior compared to that in the morphine treated group. DZP did not influence the levels of met-enkephalin in the placebo-implanted rats but antagonized a decrease in the met-enkephalin levels in the hypothalamus, hippocampus, cortex and spinal cord induced by morphine implantation. We conclude from this study that DZP can inhibit morphine tolerance and dependence partly by enhancing the met-enkephalin levels in morphine treated subjects.

DRUGS OF ABUSE: OPIOIDS AND OTHERS—DEVELOPMENTAL EFFECTS

512.1

EFFECTS OF PRENATAL MORPHINE ON BRAIN OPIATE RECEPTORS OF ADULT MALE RATS. Agnes Rimanóczy[1] and Ilona Vathy*. Dept. Psychiatry, Albert Einstein College of Medicine, Bronx, NY 10461. [1]Dept. Neurology and Psychiatry, A. Sz-Györgyi Medical School, Szeged, Hungary.

Saturation binding assays were carried out on membranes of different brain regions of adult male rats exposed to morphine in utero (10 mg/kg twice a day on days 11-18 of gestation). Using the highly selective tritiated ligand D-Ala-Gly-N-Methyl-Phe-Gly-ol (DAMGO) to label the μ opioid receptor subtype the maximal binding capacity (B_{max}) and affinity (K_d) in the frontal cortex, striatum, hypothalamus (HYP), preoptic area (POA), ventral tegmental area and cerebellum were estimated. Nonspecific binding was assessed by 1,000-fold excess of DAMGO. Prenatal morphine treatment increased the B_{max} of DAMGO binding by 30% in the POA and decreased it by 20% in the HYP without altering the binding affinity. In contrast, the binding capacity was not affected by prenatal morphine treatment in any of the other brain regions examined. Thus, it seems that prenatal morphine selectively affects the hypothalamic opioid system as was demonstrated for hypothalamic catecholamine turnover and content (Vathy and Katay, Dev. Brain Res. 68:125-131, 1992). These changes may account for the alterations in adult male sexual behavior previously shown to be induced by prenatal morphine. Supported by DA 05833.

512.2

EFFECTS OF PRENATAL MORPHINE ON CATECHOLAMINE TURNOVER OF ADULT MALE RATS. Eaton, R.*, Rimanóczy, A.[1], Kátay, L.[1] and Vathy, I. Dept. Psych., Albert Einstein Coll. Med., Bronx, NY 10461 and [1]Dept. Neurol. and Psych., A. Sz-Györgyi Med. Sch., Szeged, Hungary.

Norepinephrine (NE) and dopamine (DA) turnover rates were estimated in several brain regions of adult male rats exposed to morphine in utero (10 mg/kg twice a day on days 11-18 of gestation). Animals were injected with the catecholamine synthesis inhibitor α-methylparatyrosine (AMPT) at 0 and 3 hr with 250 and 125 mg/kg, respectively. They were sacrificed at 0, 1.5, 3, 4, 6 or 8 hrs after the AMPT injection and turnover rates of NE and DA measured in the hypothalamus, preoptic area (POA), striatum, cortex and cerebellum. The turnover rate of NE was 40% lower in the hypothalamus ($p<0.05$), but was 42% higher in the POA ($p<0.01$) of morphine-exposed animals when compared to controls. In contrast, the turnover rates of DA were similar in the hypothalamus and POA of saline- and morphine-exposed animals. These results suggest that prenatal morphine exposure selectively targets hypothalamic NE systems, an observation in agreement with our previous findings of elevated hypothalamic NE content in morphine-exposed males (Dev. Brain Res., 68:125-131, 1992). Because our previous work demonstrated reduced hypothalamic NE content in drug-exposed females, studies on NE and DA turnover in prenatally morphine- and saline-exposed female rats are also in progress. Supported by DA 05833.

512.3

PERINATAL EXPOSURE TO THE KAPPA AGONIST U-50,488 ALTERS THE SENSITIVITY OF D2 BUT NOT D1 RECEPTORS IN THE BRAIN OF RAT OFFSPRING. G.-J. Shieh and D.E. Walters*. Div. Pharmacol., Dept. Pharmacal Sci., Auburn Univ., AL 36849.

To determine the effects of chronic perinatal exposure to kappa agonists on the motor development of offspring, 28-day Alzet pumps containing U-50,488, 79 mg/ml, or 0.9% saline vehicle were implanted into Sprague-Dawley rats on G14. The dose of U-50,488 on G14, 20 and P10 was 18, 13.6 and 17.6 mg/kg/day, respectively. On P10, male offspring were injected with the D2 agonist quinpirole, 0.05 mg/kg, or the D1 agonist SKF 38393, 10 mg/kg. Their locomotor activity was then monitored for 1 hr.

Perinatal exposure to U-50,488 significantly decreased the locomotor response to quinpirole but not SKF 38393. There were no significant differences between treated and control mothers in body weight gain during gestation, body weight at P10, or food consumption during gestation or postpartum. Litter sizes were not significantly different between groups; nor were any dead pups born to mothers in either group. Birth weights of offspring exposed to U-50,488 were significantly decreased but there was no significant difference between body weights of experimental and control offspring at P10. The results suggest that chronic perinatal exposure to kappa opioid agonists alters the sensitivity of brain D2, but not D1, receptors mediating locomotor activity. Supported by the AACP and the PMA Foundation.

512.4

PRECIPITATED WITHDRAWAL FROM CHRONIC MORPHINE IN THE WEEK-OLD RAT PUP. K. Jones, S. Wang* and G. A. Barr. Biopsychology Doctoral Program, Dept. of Psychology, Hunter College-CUNY, NY, NY 10021 and New York State Psychiatric Institute, 722 W. 168th Street, NY, NY 10032.

Morphine dependent adult animals manifest a complex withdrawal syndrome but there are few reports on the morphine withdrawal syndrome in infants. In this study, we examine the behavioral effects of precipitated withdrawal in morphine dependent 7 day-old rat pups. Each infant rat was injected with morphine sulfate (3 mg/kg or 10 mg/kg, i.p., b.i.d.) from the first to the seventh day of life at approximately 9:30 AM and 5:30 PM. Controls included saline injected and untreated groups. The last injection was on the morning of the 7th day. All pups in the same litter received the same treatment. One hour after the last morphine treatment, a single pup was injected with one of several doses of naltrexone (0, 0.3, 1.0, 3.0, 10.0, mg/kg) and placed in an observation chamber with the remainder of the litter. The pup was obsreved for a total of 20 minutes and ongoing behaviors recorded every 15 seconds. When the observation period ended, the pup was anesthetized and placed back into the observation chamber with the remainder of the litter. The second pup was then tested similarly with a different dose of naltrexone. We tested five pups in each litter and averaged those scores to obtain a litter score. All observations were scored in a blind manner.

Naltrexone injected pups that had been chronically treated with morphine showed dramatic alterations in their behavior, including increased head movements (rises and sways), rolls, stretches, walking, and wall climbing. They also spent less time with littermates. These results indicate that week-old morphine treated pups experience behavioral changes following opiate antagonist treatment although these behaviors differ from the classical withdrawal syndrome seen in adult animals. (Supported in part by DA-06600)

512.5

ALTERATIONS IN OPIOID PEPTIDES AND THE cAMP SYSTEM IN BRAINS OF MORPHINE ADDICTED NEWBORNS. A. Tempel*, J. Yiang and R. Basheer, Lab of Molecular Pharmacology, Hillside Hospital, Long Island Jewish Medical Ctr./Albert Einstein College of Medicine, Glen Oaks, NY 11004.

The mechanisms involved in the development of morphine tolerance are still unknown. Recently much attention has been directed towards the possible changes in post receptor events. In order to understand the interaction between opioid receptors, G-protein/cAMP system and endogenous opioids, we examined alterations in these systems in brains of morphine exposed newborns. Pregnant dams were implanted on gestation day 16 with either one morphine pellet each (75mg) or with placebo pellets. Rat pups were sacrificed on day of parturition and brains dissected. Total RNA was extracted and hybridized with a ^{32}P-labeled RNA probe for $G_{\alpha s}$ message (1.9kb), PPE message (1.4kb) and the constitutively expressed 1B15 message (1.0kb). cAMP and met-enkephalin levels were measured by radioimmunoassays. Data were analyzed by a MANOVA followed by Post-hoc univariate analysis. Prenatal morphine treatment produced a significant reduction (-16%;$p<.01$) in cAMP levels in striatum of offspring with no significant alterations in $G_{\alpha s}$ mRNA levels. Met-enkephalin levels were decreased by 43% ($p<.05$) while preproenkephalin levels were increased by 37% ($p<.01$) in striatum. These data suggest that prenatal morphine treatment down regulates the endogenous opioid system via the cAMP system and then strives to maintain equilibrium by increasing production of preproenkephalin. (Supported by NIDA-DA-05440 & LIJMC Faculty Research Award.)

DEGENERATIVE DISEASE: ALZHEIMER'S— β -AMYLOID IX

513.1

DECREASED SURVIVAL OF HIPPOCAMPAL NEURONS IN THE PRESENCE OF ß/A_4 AMYLOID PROTEIN IS NOT ALTERED BY CALCIUM CHANNEL BLOCKADE. J. S. Whitson* and S. H. Appel. Dept. of Neurology, Baylor College of Medicine, Houston, TX 77030.

In cortical cultures, ß/A_4 protein increases vulnerability to glutamate insult possibly reflecting the ability of ß/A_4 to destabilize calcium homeostasis [1]. Direct neurotoxicity of ß/A_4 is not observed [1,2]. In hippocampal cultures, we and others find treatment with ß/A_4 protein alone decreases neuronal survival [3], but the mechanism of neurotoxicity is unknown. **Low density, serum-free cultures of hippocampal neurons were used to determine whether the neurotoxicity of ß/A_4 protein *in vitro* could be altered by voltage- or ligand-gated calcium channel antagonists or cyclic nucleotides.**

Cultures from E-18 rat hippocampus were treated with either antagonist (4uM omega-Conotoxin, 1uM Nifedipine, 100nM Diltiazem, 10uM APV, 10uM MK801) or cyclic nucleotide (100uM dibutyryl cAMP, 100uM 8-bromo cAMP, 100uM dibutyryl cGMP, 100uM 8-bromo cGMP). After 20 minutes, synthetic ß/A_4 1-40 was added for 48 hours; then neurons were fixed and counted.

With only one exception, neither calcium channel antagonists nor cyclic nucleotides altered the survival of neurons exposed to 20μM B40. The N-channel antagonist Diltiazem decreased B40 toxicity repeatedly, but slightly, perhaps indicating some involvement by this channel in the molecular cascades preceding neuronal cell death. Nevertheless, the toxicity of beta protein does not appear to be directly altered either by calcium channel blockers or by the addition of cyclic nucleotides. Work supported by NIH AG08664 and AG00183.

1. Mattson *et al.*, *J. Neurosci.* 12:376 (1992) 2. Koh, Yang and Cotman, *Brain Res.* 533:315 (1990) 3. Yankner, *et al.*, *Science* 250:279-82 (1990)

513.2

CULTURED GABA-IMMUNOREACTIVE NEURONS ARE RESISTANT TO β-AMYLOID-INDUCED TOXICITY. C.J. Pike* and C.W. Cotman. Irvine Research Unit in Brain Aging and Department of Psychobiology, *University of California, Irvine, CA 92717 USA.*

Alzheimer's disease (AD) is characterized in part by the loss of particular neuronal populations. Previous studies have shown that cholinergic, glutamatergic, serotonergic, and noradrenergic neuronal populations are selectively vulnerable to degeneration within affected brain regions, whereas neurons containing the neurotransmitter γ-aminobutyric acid (GABA) appear relatively resistant. Although GABAergic neurons in AD generally retain somal integrity, they can exhibit neuritic alterations. Neurodegenerative changes in AD have been widely hypothesized to be mediated in large part by β-amyloid protein (Aβ), which exists in insoluble aggregates as the major component of senile plaques. We have previously demonstrated that aggregated Aβ peptides are directly neurotoxic to cultured neurons. Consistent with the selective degeneration observed in AD, we now report that GABA-immunoreactive cultured neurons exhibit relative resistance to Aβ-induced toxicity. After 10 days *in vitro*, cultures comprised of rat hippocampal neurons plated on a monolayer of astrocytes were treated in serum-free medium with 25 μM Aβ peptide. Thirty-six hours post-treatment, an average of over 75 % of the total neuronal population had degenerated. However, GABAergic neurons (approximately 15 % of the total neuronal population) exhibit only a non-significant 13 % reduction in cell number. Interestingly, viable GABAergic neurons often exhibited neuritic pruning. Determination of the intrinsic neuronal characteristics responsible for Aβ resistance may benefit attempts to both understand the mechanism of Aβ-induced toxicity and delay the progression of AD.

513.3

β-AMYLOID(25-35) INDUCES COMPROMISE OF MITOCHONDRIAL SDH ACTIVITY IN RAT HIPPOCAMPAL NEURONAL COCULTURES AND NEUROBLASTOMA CELLS. J.C. Chisholm*, J.N. Davis, and E.J. Hunnicutt, Jr. Miles Inc., Institute for Dementia Research, West Haven, CT 06516 USA.

The ability to demonstrate neurotoxicity of a β-amyloid protein (βAP), deposited in plaques characteristic of Alzheimer's disease, varies considerably with known and unknown factors, even in vitro. Of the various fragments tested, βAP(25-35) was most potent in some protocols (e.g. Yanker, Science 250:279;'90; Mattson, J. Neurosci. 12:376;92). This 11 amino acid peptide was examined for effects on mitochondrial function (MIT), by measuring the activity of succinate dehydrogenase (SDH) after exposure of cells to the peptide. Formazan production from tetrazolium (MIT) by SDH was decreased by 18-26% in rat hippocampal neuronal cocultures after 24 hr of exposure to 60 μM βAP(25-35), indicating significant MIT compromise. Likewise, in four neuroblastoma cell lines, SDH activity was decreased [e.g. in NIE-115 by 34% (±7%SE n=6)] after 24-48 hr of exposure to 60 μM βAP(25-35), indicating significant MIT compromise in these cell lines also. Correlated measurements of cell viability in the neuroblastoma cells, however, showed no effect of the peptide on esterase-dependent calcein uptake, nor on nuclear staining with ethidium homodimer during MIT compromise. These results indicate that a potentially toxic βAP fragment impairs MIT function in neurotypic cells. In cultured neuroblastoma cells tested, this occurs independently of measurable cell death. This suggests that cell vulnerability to this peptide may be related to the level of cell dependence on aerobic vs. anaerobic energy production.

513.4

THROMBIN STIMULATES PRODUCTION OF BETA-AMYLOID IN CULTURED HIPPOCAMPAL NEURONS, G.J. Brewer* Southern Illinois Univ. Sch. Med., Springfield, IL 62794.

In senile plaques of Alzheimer disease (AD), the presence of thrombin and the overall reduction in levels of its specific inhibitor, protease nexin I, suggests a possible pathologic role for this protease. In considering sources for the altered processing of proteins in AD lesions, we studied the effects of serum from AD patients on cultured rat hippocampal neurons and found increases in β-amyloid immunoreactivity (Brewer & Ashford, J. Neurosci. Res. 33:355 (1992)). Now we find that the purified serum protease thrombin stimulates β-amyloid and Alz-50 immunoreactivities in a dose and time-dependent fashion in these serum-free cultures. Maximum 4G8 anti-βA4 (Kim and Wisniewski) response was observed after a 24 hr. exposure to 27 nM thrombin. As previously reported for PC12 cells, thrombin inhibits sprouting of newly plated hippocampal neurons. In contrast, neurons cultured for 4 or 7 days were not affected morphologically but were stimulated to increase their mean immunofluorescent area by more than 2 fold. Anti-βA4 (4G8) immunoprecipitates of ^{35}S-methionine labeled neuron cell fractions suggest sources of a thrombin-stimulated 4 kD substance.

513.5

BEHAVIORAL AND HISTOLOGICAL ASSESSMENT FOLLOWING BILATERAL INTRAHIPPOCAMPAL INJECTIONS OF ß-AP25-35. David E. Kang, Wayne A. Dornan*, Alex McCampbell, Esther E. Kang. Dept. of Psychology, Illinois Wesleyan University, Bloomington, IL 61702.

Pathologically Alzheimer's disease (AD) is characterized by deposits of neuritic plaques (NP) and neurofibrillary tangles typically found in the cerebral cortex, hippocampus, and basal forebrain. Increasing evidence suggests that the major constituent of NP, a ß-amyloid protein composed of 39-42 amino acids, possesses neurotoxic properties. Recent studies have demonstrated *in vitro* that the putative neurotoxic fragment, ß-AP25-35, disrupts intracellular calcium homeostasis, decreases neuronal survival, and potentiates the toxicity of excitatory amino acids (EAA). Others have shown that the neurotoxic properties of ß-AP25-35 may be dependent on the aggregational state of the peptide. While some evidence supports the direct *in vivo* toxicity of ß-AP, the extent of neuronal damage has not been compared with a standard lesion made by EAAs. Therefore, a comparative behavioral and histological assessment was conducted following bilateral intrahippocampal injections of ß-AP25-35, ibotenic acid (IBO), ß-AP25-35 + IBO, and incubated ß-AP25-35 (1 week at 37°C). A radial arm maze and Morris water maze were utilized for comparative learning and memory assessment. Preliminary results indicate that there is a clear disruption of learning performance in animals coinjected with low doses of ß-AP25-35 (4nmol/ul)) and IBO (1.2 nmol/ul) while identical doses injected separately had no effect. Bilateral injections of the incubated ß-AP25-35 also affected learning when compared to saline-injected controls. In support of the behavioral results, preliminary histological analyses revealed cytotoxic effects in the hippocampus following injections of ß-AP25-35 + IBO or a high dose of IBO. This study suggests that the injection of ß-amyloid into the hippocampus potentiates the toxicity of EAA *in vivo* through a Ca^{2+}-mediated mechanism and disrupts learning/memory in rats.

513.6

JUN AND FOS IMMUNOREACTIVITY IN ALZHEIMER'S BRAIN AND INDUCTION BY ß-AMYLOID IN CULTURED NEURONS. A.J. Anderson*, B.J. Cummings, C.J. Pike, D.L. Loo and C.W. Cotman. IRU in Brain Aging, Dept. of Psychobiology, U. C. Irvine, Irvine, CA 92717.

The protein products of the Jun and Fos immediate early gene (IEG) families are cooperative transcriptional regulatory factors implicated in regulating the expression of many genes. It is now clear that the expression of a variety of proteins such as the amyloid precursor protein and basic fibroblast growth factor are altered in Alzheimer's disease (AD), thus the events regulating these changes are of interest. Both of these genes contain an AP-1 consensus sequence responsive to regulation by the IEG c-Jun. We have examined the distribution of Jun- and Fos-related protein immunoreactivity in control and AD brain, and the correspondence of this immunoreactivity to paired helical filament-1 (PHF-1: a marker for neurofibrillary tangles which recognizes abnormally phosphorylated tau), glial fibrillary acidic protein (GFAP) and thioflavin staining in double-labeling experiments. An intensification of both Jun and Fos immunoreactivity was observed in AD cases; in addition, both Jun and Fos immunoreactivity were co-localized with PHF-1 in some neurons in AD brain. Jun and Fos immunoreactivity were also co-localized with GFAP-positive astrocytes distributed in the cortex of AD and control cases, and surrounding thioflavin-stained plaques in AD brain. We also examined the role of Jun in cultured rat hippocampal neurons treated with ß-amyloid peptide (Aß). Basal levels of Jun expression in these cultures were low; however, after exposure to Aß Jun was detected within 2 hours, peaked in glia at approximately 6 hours, and peaked in neurons at approximately 12 hours. Jun expression was maintained at high levels 24 hours after exposure to Aß. These observations suggest that members of the Jun and Fos IEG families may play a role in cellular response to Aß both *in vitro* and in AD.

513.7

TRANSFORMING GROWTH FACTOR-β PROTECTS HUMAN NEURONS AGAINST β-AMYLOID-INDUCED INJURY. C. C. Chao*, S. Hu, W. R. Anderson, F. H. Kravitz, and P. K. Peterson, Minneapolis Medical Research Foundation and the University of Minnesota Medical School, Minneapolis, MN 55404

Deposition of amyloid fibrils in the brain is the pathologic hallmark of Alzheimer's disease (AD). The principal component of amyloid fibrils is amyloid β-peptide (Aβ), a 40 amino acid protein which has been implicated in the neurodegeneration associated with AD. In the present study, we investigated the protective effect of transforming growth factor (TGF)-β on Aβ-induced neurodegeneration in human fetal cerebral cortical cultures. When these cultures were exposed to $A\beta_{1-40}$, marked ($P<0.01$) neuronal loss (>50%) was observed within 4 days of incubation as assessed by counting surviving neurons and by the uptake of ^{3}H-gamma aminobutyric acid, a functional marker of neuronal viability. Aβ-induced neurodegeneration was dose-dependent. Simultaneous addition of Aβ (40 μM) with TGF-β (20 ng/ml) but not with proinflammatory cytokines tested, reduced ($P<0.01$) by 60% the Aβ-induced neuronal injury. Furthermore, TGF-β dose-dependently protected against Aβ-induced neuronal injury with an ED_{50} of 1 ng/ml. The finding that TGF-β, an anti-inflammatory cytokine, protects against Aβ-induced neurodegeneration supports the hypothesis that AD is an immunologically mediated disease, and suggests that certain immunosuppressive agents may have a role in the treatment of this neurodegenerative disease.

513.8

PROTECTION OF CULTURED HIPPOCAMPAL NEURONS FROM β-AMYLOID TOXICITY BY ANTIOXIDANTS. Raj K. Lartius* and Etsuro Uemura. Neuroscience Program, Iowa State University, Ames, IA 50011.

β-amyloid (βAP) is a peptide that abnormally deposits in diseases such as Alzheimer's disease (AD). Recent evidence has suggested that the active portion of βAP corresponds to amino acids 25 to 35 (β25-35). This peptide has both neurotrophic and neurotoxic effects on cultured neurons. Because amyloid in the mature AD plaque is aggregated and relatively immobile, in a previous study we attempted to simulate effects of an established AD plaque by exposing hippocampal neurons to aggregated β25-35 coated on the culture surface. Neurons had an affinity for β25-35 and β25-35 supported the attachment, growth and survival of cultured hippocampal neurons when grown in the presence of serum. However, β25-35 neurotoxicity occurred when neurons were serum deprived. In the present study, using the same culture system, we have further examined β25-35 toxicity using culture medium supplemented with modified N2 (MN2) or each of its components (ie. progesterone, insulin, rat apotransferrin, putrescine and sodium selenite). When medium was supplemented with MN2, β25-35 supported the attachment, growth and survival of cultured hippocampal neurons. When neurons were exposed to each component of MN2 individually, only rat apotransferrin and sodium selenite significantly protected against β25-35 toxicity. In view of the antioxidant properties of these compounds, vitamin E was also tested. Vitamin E supplemented medium significantly protected against β25-35 neurotoxicity. This survival was comparable to neuronal survival in medium containing MN2. These findings are supportive of the theory that free radical generation may be involved in amyloid related disease such as Alzheimer's and that antioxidants may be useful to prevent further neurodegeneration.

513.9

SECRETED FORMS OF ßAPP PROTECT HIPPOCAMPAL NEURONS AGAINST Aß TOXICITY AND OXIDATIVE INJURY. Y. Goodman[1], R. E. Rydel[2], A. R. Culwell[2] and M. P. Mattson*[1]. [1]Sanders-Brown Center on Aging and Dept. Anatomy & Neurobiology, Univ. of Kentucky, Lexington, KY 40536. [2]Athena Neurosciences Inc., 800 F. Gateway Blvd., South San Francisco, CA 94080.

Alternative processing of ß-amyloid precursor protein (ßAPP) can liberate either secreted forms of ßAPP (APP^s) via secretase action, or amyloid ß-peptide (Aß) by alternate pathways. APP^s can protect neurons against excitotoxic and ischemic insults by a mechanism involving stabilization of intracellular free calcium levels ($[Ca^{2+}]_i$) (*Neuron* 10:242-254; V. L. Smith-Swintosky et al.; Barger et al., this meeting). In contrast, Aß destabilizes neuronal $[Ca^{2+}]_i$ homeostasis and renders neurons vulnerable to excitoxicity (*J. Neurosci.* 12:376-389). Aß, in an aggregated form, was neurotoxic and caused an elevation of $[Ca^{2+}]_i$ which was directly correlated neuronal degeneration; Aß toxicity was prevented by removal of extracellular Ca^{2+}. Confocal laser scanning microscopy and E.M. studies revealed that Aß accumulated in or on the plasma membrane which may be the site at which it disrupts $[Ca^{2+}]_i$ homeostasis. Pretreatment of cultures with APP^s695, APP^s751, or APP^s596 (1-10 nM) protected neurons against Aß toxicity and attenuated the elevation of $[Ca^{2+}]_i$ caused by Aß. Since oxidative damage has been implicated in the pathogenesis of AD, we determined whether APP^ss would modify free-radical-mediated neuronal injury. APP^ss protected neurons against oxidative damage elicited by iron.

513.10

SECRETED FORMS OF ßAPP PROTECT CA1 HIPPOCAMPAL NEURONS AGAINST ISCHEMIC INJURY. V. L. Smith-Swintosky[1]*, L. C. Pettigrew[2], S. D. Craddock[2], R. E. Rydel[3], J. Joseph[1], A. R. Culwell[3] and M. P. Mattson[1]. [1]Sanders-Brown Center on Aging and Department of Anatomy and Neurobiology, [2]Department of Neurology and VAMC, University of Kentucky, Lexington, KY 40536; [3]Athena Neurosciences Inc., S. San Francisco, CA 94080.

Despite the intensive study of the ß-amyloid precursor protein (ßAPP) because of its possible role in Alzheimer's disease, the normal function of ßAPP is unclear. A major processing pathway for ßAPP involves an enzymatic cleavage within the amyloid ß-peptide sequence which liberates secreted forms of ßAPP (APP^s) into the extracellular milieu. We recently reported that both APP^s695 and APP^s751 protect cultured hippocampal neurons against glutamate toxicity and hypoglycemic damage (*Neuron*, 10:243-254 (1993)). The present study extends these experiments to a four-vessel occlusion model of ischemia in rats. Male Wistar rats were divided into four groups: sham, cannula only (n=3); ischemia, saline-treated (n=5); ischemia, APP^s695-treated (n=5); and ischemia, APP^s751-treated (n=5). After 20 min of ischemia, either saline or an APP^s (1 μg/ml) was infused through an intracerebroventricular cannula at the rate of 5 μl for 20 min. Three days later, rats were killed, the brains removed, sectioned and Nissl-stained. Both APP^s695 and APP^s751 significantly protected CA1 neurons from ischemic damage ($p<0.05$ and $p<0.001$, respectively). APP^s treatment led to a 200-300% increase in cell survival compared to controls. The data indicate that APP^ss normally play a neuroprotective role.

513.11

VALPROIC ACID ATTENUATES EXCITOTOXICITY AND NEUROFIBRILLARY TANGLE-LIKE ANTIGENIC CHANGES IN HIPPOCAMPAL NEURONS. R. Mark*, J. W. Ashford, Y. Goodman, and M. P. Mattson. Sanders-Brown Research Center on Aging and Dept. Anatomy & Neurobiology, Univ. of Kentucky, Lexington, KY 40536.

Perhaps the most rational approach to treating Alzheimer's disease (AD) is to prevent or retard the progression of the underlying neuronal degeneration. An excitotoxic mechanism of neuronal injury mediated by increased intracellular calcium levels may underlie the pathogenesis of AD. Previous studies showed that glutamate and calcium influx can elicit antigenic changes in neurons similar to those seen in neurofibrillary tangles, and that amyloid ß-peptide (Aß) can destabilize calcium homeostasis and render neurons vulnerable to excitotoxicity (*J. Neurosci.* 12:376-389). We previously reported that anticonvulsants (e.g., phenytoin, carbamazepine, diazepam) can attenuate glutamate toxicity in hippocampal neurons (*Brain Res.*, 478:337-348). In the present study we examined the neuroprotective potential of valproic acid (VA), using paradigms of neuronal injury relevant to the pathogenesis of AD. Glutamate neurotoxicity was significantly reduced in hippocampal cultures pretreated with VA (100 nM - 10 µM). VA attenuated the glutamate-induced increase in neuronal immunoreactivity with antibodies that recognize epitopes in the neurofibrillary tangles of AD (tau antibodies and anti-ubiquitin). The elevation of $[Ca^{2+}]_i$ induced by glutamate was attenuated by VA suggesting that the mechanism of neuroprotection involves stabilization of neuronal calcium homeostasis. Our data suggest that VA may retard neurodegeneration in AD. (supported by the NIH and the Metropolitan Life Foundation).

513.12

MECHANISTIC STUDIES OF THE DEPRESSION OF INTRANEURONAL CALCIUM CONCENTRATION BY SECRETED FORMS OF β-AMYLOID PRECURSOR PROTEIN. S. W. Barger*§, R. R. Fiscus§, R. E. Rydel¥, A.R. Culwell¥, I. Lieberburg¥, and M. P. Mattson§. §Sanders-Brown Center on Aging, University of Kentucky, Lexington, KY 40536, and ¥Athena Neurosciences Inc., 800F Gateway Blvd., S. San Francisco, CA 94080.

The predominant peptide in amyloid plaques associated with Alzheimer's disease, beta A4, is derived from a larger, transmembrane protein termed the beta amyloid precursor protein (βAPP). Proteolytic processing of βAPP results in secretion of large extracellular domains (APPs) which have been shown to protect neurons from toxicity associated with glutamate, hypoglycemia, iron, and free radicals (Mattson, et al. 1993. Neuron 10: 242-254; and Goodman, et al., this meeting), activities which may involve the ability of APPs to depress intracellular free calcium concentrations ($[Ca^{2+}]_i$). Here we demonstrate a potent elevation of cyclic GMP by APPs695 in cultures from E18 rat hippocampus. The ability of APPs695 to depress $[Ca^{2+}]_i$ in hippocampal neurons was blocked by inhibitors of cGMP-dependent protein kinase, and the abilities of APPs to depress $[Ca^{2+}]_i$ and protect neurons from hypoglycemic damage was mimicked by a cGMP analog (8-bromo-cGMP; 0.1-0.5 mM). At resting levels, $[Ca^{2+}]_i$ could not be depressed by APPs in the presence of thapsigargin, an inhibitor of calcium uptake into intracellular stores. However, removal of extracellular calcium also prevented the $[Ca^{2+}]_i$ depression by APPs, and thapsigargin was ineffective after elevation of $[Ca^{2+}]_i$ by removal of extracellular sodium. These data suggest that cGMP elevated by APPs lowers $[Ca^{2+}]_i$ in hippocampal neurons by at least two mechanisms, dependent or independent of intracellular sequestration.

DEGENERATIVE DISEASE: ALZHEIMER'S—NEUROPHARMACOLOGY AND NEUROTRANSMITTERS IV

514.1

NOVEL, LONG-ACTING AND SELECTIVE PHYSOSTIGMINE ANALOGUES AS POTENTIAL THERAPEUTICS FOR ALZHEIMER'S DISEASE. N.H. Greig, D.K. Ingram, A. Brossi, H.W. Holloway, X-F Pei, S. Asthana*, T.T. Soncrant. Labs. of Neurosciences and of Cellular and Mol. Physiology, NIA, NIH, and Department of Chemistry, Georgetown Univ.

The loss of cortically-projecting basal forebrain cholinergic neurons consistently correlates with the decline in mental abilities in Alzheimer's disease (AD). Previous attempts to augment central cholinergic function in AD probably have failed to dramatically enhance memory as optimal, steady-state brain drug levels were not achieved or maintained during the period of cognition assessment. Most cholinergic agents have a poor pharmacokinetic profile for single administration studies and/or have poor specificity. The alkaloid physostigmine possesses potent anti-cholinesterase (ChE) activity and has effects on memory when administered to humans. However, it has a short plasma half-life and duration of ChE inhibition (18 and 80 min, respectively). Thus, we developed novel physostigmine analogues, with substitutions in the carbamoyl and N(1) positions, to prolong action and increase enzyme specificity. Studies indicate that: (i) our novel agents possess anti-ChE activity similar to or greater than that of physostigmine, but with a dramatically longer duration of action (>8 hr). (ii) Unlike other physostigmine analogues, our agents are highly selective for either AChE or BChE, separately. (iii) Specific agents do not generate active metabolites. Physostigmine and its analogues generate the analgesic metabolite eseroline, equipotent to morphine. Finally, (iv) specific compounds possess a high brain uptake, low toxicity and dramatically attenuate scopolamine-induced learning impairments in rats, a classic model for assessing cognition enhancers. These represent interesting candidate therapeutics for AD treatment.

514.2

CHOLINESTERASE INHIBITORS DIFFERENTIALLY MODIFY RELEASE OF ACETYLCHOLINE AND BIOGENIC AMINES IN RAT CORTEX IN VIVO. G. Cuadra, F. Mori, E. Williams, X-D. Zhu and E. Giacobini*. Dept. Pharmacology, Southern Illinois University Sch. Med., Springfield, IL 62794-9230

Cholinesterase inhibitors (ChEI) are, so far, the only drugs demonstrating clinical efficacy in the treatment of Alzheimer disease (AD). Since the disorder involves a loss of cholinergic as well as monoaminergic synapses in cortex, it is important to determine the effect of ChEI on several neurotransmitters. We have modified the HPLC-ECD method to detect femtomole levels of acetylcholine (ACh) in microdialysis from rat frontal cortex without using an AChEI in the probe to elevate ACh levels (Messamore et al., 1993). We have examined the effect of single and multiple doses of several first generation (physostigmine, THA/tacrine, metrifonate) and second generation (MDL 73,745, heptyl-physostigmine) on ACh, norepinephrine (NE), dopamine and 5-hydroxytryptamine cortical extracellular levels. Our results show that cortical neurotransmitters could exert reciprocal modulatory effects. Cortical ACh and NE in particular, influence each other's extracellular levels. These interactions probably reflect differences in pharmacological effects on cholinergic and adrenergic receptor mechanisms. Further characterization of different pharmacological profiles of these ChEI may improve prediction of therapeutic effects and helps design new drugs to treat AD. [Reference: Messamore E. et al., Neuropharmacology, 32(3):291-296, 1993.] (Supported in part by NIA Core Grant #P30 AG08014; and Mediolanum Farmaceutici, Milan, Italy)

514.3

THA AND PHYSOSTIGMINE ATTENUATE HEMICHOLINIUM-3 INDUCED AMNESIA AND ACh DEPLETION. D.S. Chapin, S.B. Jones, J.A. Nielsen and D. Liston*. Pfizer Central Research, Groton, CT 06340

The most prominent neurochemical and clinical features of Alzheimer's disease (AD) are the loss of cholinergic neurons and memory. Hemicholinium-3 (HC-3), a selective inhibitor of high-affinity choline uptake, can produce decreases in brain ACh stores and memory deficits in rodents, providing an animal model for AD. We have examined the effects of two AChE inhibitors, tetrahydroaminoacridine (THA) and physostigmine (PHY), on HC-3-induced changes in mouse forebrain ACh levels and amnesia in a mouse passive avoidance (PA) model. HC-3 decreased mouse forebrain ACh levels and produced amnesia in a dose- and time-dependent manner. HC-3 (1.78 µg i.c.v.) decreased forebrain ACh levels to 15-25% of control. In PA, the test step-through latency was reduced by 50-60% following pre-treatment with HC-3 two hours prior to the training session. THA (17.8 mg/kg p.o.) or PHY (0.32 mg/kg i.p.) attenuated both HC-3-induced amnesia and forebrain ACh depletion. Both compounds produced statistically significant reversal of amnesia when forebrain ACh was increased to about 40-60% of control ACh levels. Higher doses of THA or PHY produced further increases in brain ACh, but interfered with ability to perform the PA task due to hypolocomotion and tremors. THA possessed a greater therapeutic index (TI) than PHY as measured by a greater separation in doses required to reverse HC-3-induced amnesia and lethal doses. This improved TI may explain, in part, the greater clinical efficacy reported for THA.

514.4

DIFFERENTIAL RESPONSES OF ALZHEIMER'S DISEASE PATIENTS TO TREATMENT WITH PHYSOSTIGMINE AND ARECOLINE; COMPARISON OF OPTIMAL DOSES FOR VERBAL AND VISUO-SPATIAL TASKS. K.C. Raffaele,* A. Berardi, S. Asthana, M.B. Schapiro, J.V. Haxby, and T.T. Soncrant. Laboratory of Neurosciences, National Institute on Aging, Bethesda, MD 20892.

Because of the consistent finding of decreased cholinergic function in Alzheimer's disease (AD), many treatment strategies have focused on enhancing the function of the cholinergic system. During treatment of AD patients with escalating doses of arecoline (a direct muscarinic cholinergic agonist), we found that performance on verbal tasks improved at low doses, while performance on visuo-spatial tasks improved at higher doses. We have now completed a similar treatment protocol with physostigmine (a cholinesterase inhibitor). During inpatient stays, patients received infusions of escalating doses of physostigmine. Performance on 10 neuropsychological tests was measured at baseline and during infusions of 2, 6, 12, 18, and 25 mg/day physostigmine. The dose at which a patient's performance on a particular test was optimal was designated as that patient's 'best dose' for that test. Mean best doses were compared across tests to determine whether performance improved at similar doses across all types of neuropsychological tests or whether performance on different types of tests improved at different physostigmine doses. In contrast to results with arecoline, mean best doses for physostigmine were similar for all task types and were clustered toward the top of the administered dose range. These results reinforce the functional consequences of the differences in mechanisms between the two drugs and may have implications in the development of treatment strategies for AD.

514.5

BIOCHEMICAL AND OVERT BEHAVIORAL EFFECTS OF HUPERZINE IN RODENTS. T.D. Steele*, K.M. Barbieri and K.W. Locke. Interneuron Pharmaceuticals, Inc., Lexington, MA 02173.

Studies were conducted to evaluate the biochemical and behavioral properties of (+)-huperzine, an acetylcholinesterase (AChE) inhibitor that may be useful in the treatment of Alzheimer's disease. Biochemical studies of the inhibition of AChE and butyrylcholinesterase in vitro indicated that HUP was a more specific inhibitor of AChE than either physostigmine (PHYSO) or tacrine (THA). In overt behavioral studies, rats and mice were administered huperzine (0.3-3.0 mg/kg, ip or po) and observed for overt behavioral changes at various times post-injection. The overt effects of huperzine were compared with those of PHYSO (0.1-1.0 mg/kg) and THA (3.0-30 mg/kg). Huperzine produced overt effects characteristic of AChE inhibitors (i.e., SLUD syndrome and muscle fasciculations) at doses greater than 0.3 mg/kg by either route of administration. The duration of these effects was longer with huperzine with either PHYSO or THA. Convulsions were observed only after THA. Relative to the overt behavioral studies, huperzine was active in improving scopolamine-induced amnesia in a one-trial passive avoidance procedure in rats at 30- to 100-fold lower doses (0.003-0.01 mg/kg). Thus, huperzine appears to be an orally-active specific inhibitor of AChE, with a long duration of action and a wide margin of safety.

514.6

MEMORY ENHANCEMENT IN AGED MONKEYS WITH VELNACRINE MALEATE (HP 029). WJ Jackson*[1], JJ Buccafusco[2], AV Terry[2], DK Rush[3]. Depts. of [1]Physiology & Endocrinology, [2]Pharmacology-Toxicology, Medical College of Georgia, Augusta, GA 30912 and [3]Hoechst-Roussel Pharmaceuticals, Inc., Neurosciences Strategic Business Unit, Somerville, NJ 08876.

Velnacrine maleate is a novel orally active acetylcholinesterase inhibitor of the acridine class with a longer duration of activity than physostigmine. Velnacrine has shown efficacy in the treatment of Alzheimer's disease and in improving both normal and experimentally impaired mnemonic function in animals and humans. In the present study, velnacrine was studied for its ability to ameliorate the decline in short-term memory observed in aged nonhuman primates. In the first phase of the study, doses of 1, 2, 4, 6, and 8 mg/kg po (free base corrected) were administered once to each of six aged (25-40 yr) macaques. Memory was assessed 30 min later by a delayed matching-to-sample (DMTS) paradigm. In the second phase of the study, each animals best dose, i.e., the dose at which each animal had exhibited the greatest phase one improvement, was administered an additional three times. In the second phase, velnacrine induced a significant improvement in memory at the longest of three individually titrated delay intervals. Correct Long-delay matching increased from 58.9% during placebo sessions to 66.8% following velnacrine administration. This level of improvement represents 13% of the baseline value. In reference to other compounds tested by this paradigm, orally administered velnacrine appears to induce about the same degree of DMTS enhancement as physostigmine (i.m.) and tacrine (i.m.). The finding that velnacrine improved long-delay DMTS performance in a group of aged memory-impaired primates suggests potential value for treatment of age-related memory dysfunction.

514.7

PERFORMANCE OF AGED RATS IN A 14-UNIT T-MAZE IS IMPROVED FOLLOWING CHRONIC TREATMENT WITH PHENSERINE, A NOVEL ANTICHOLINESTERASE. H. Ikari, N. Greig, E. Spangler, A. Brossi, X-F. Pei, T. Soncrant, D.K. Ingram*, Lab. of Cellular Mol. Physiol. NIA, NIH, Baltimore, MD 21224 and Lab. of Neuroscience, NIA, NIH, Bethesda, MD 20892.

The physostigmine derivative, phenserine, has proven to be a potent and long-acting inhibitor of acetylcholinesterase with a wide therapeutic window for reversing scopolamine-induced learning impairments of young rats in a 14-unit T-maze (Iijima et al. *Psychopharm*, in press). In the current study we examined the effects of chronic phenserine treatment on performance of aged (21 mo) male F-344 rats in the same maze, which has produced evidence of robust age-related performance declines (Ingram, *Neurobiol. Aging*, 9:475,1988). Rats were given phenserine tartrate as daily injections (0, 1, 2, 3 mg/kg i.p.) for Days 1-5. On Day 3 they were pretrained in 1-way active avoidance to a criterion (13/15 avoidances) in a straight runway. On Days 4-5, each rat received 4 trials during 2 daily sessions in the 14-unit T-maze. During each trial, the rat was required to locomote through each of 5 segments within 10 s to avoid footshock (0.8 mA). Performance variables included errors (deviations from correct pathway), runtime from start to goal, shock episodes and duration. Drug injections were administered 30 min prior to training on Days 3-5. Pilot histochemical studies indicated that this chronic phenserine regimen produced marked inhibition of acetylcholinesterase in rat forebrain. Relative to controls, the maze performance of aged phenserine-treated rats was improved as measured by all the variables, with the most effective dose at 2 mg/kg. These results support the potential of phenserine as a cognitive enhancer for further evaluation.

514.8

EEL ACETYLCHOLINESTERASE (AChE) IS NOT A PROTEASE. R.T. Carroll*[1], J. Grassi[2] and M.R. Emmerling[1]. [1]Parke-Davis, Pharmaceutical Research, Division of Warner-Lambert, Ann Arbor, MI, 48106 and [2]Service de Pharmacologie et d'Immunologie, C.E. Saclay, 91191 Gif sur Yvette, France.

Our earlier studies show that, of the AChE studied thus far, only commercially obtained eel AChE has an associated trypsin-like activity. Velocity sedimentation of purified Sigma eel AChE (type V- S) produces two peaks of trypsin-like activity, of which the faster sedimenting 11S peak overlaps with the peak of AChE activity. The protease activity remains associated with AChE upon resedimentation of the gradient fractions containing AChE and after shifting the sedimentation of eel AChE with monoclonal antibody Elec-106. Protease activity is also found in AChE purified from several other commercial preparations of eel enzyme. However, the overlap between the AChE and proteases activities in these preparations is less than the Sigma enzyme. The protease activity can not be attributed solely to contamination by trypsin since Boehringer Mannheim eel AChE has associated protease activity but is solubilized by autolysis. Nondenaturing zymography gel electrophoresis separates the protease activity from the commercial eel AChE. The protease activity runs as multiple bands with molecular weights different from that of AChE. Further characterization of the protease(s) was not possible because of the low abundance. Eel AChE solubilized directly from eel electric organs with 2 M $MgCl_2$ has no apparent intrinsic or associated protease activity, although contaminating protease activity is detected. Contrary to published reports, our results indicate that eel AChE has no detectable intrinsic protease activity. We found an associated protease activity only in commercial eel enzyme. The source of the protease activity is unclear but may result from contamination by endogenous eel proteases during the isolation of the AChE. Based on our results, it seems unlikely that this protease activity has any physiological significance.

514.9

CHEMISTRY OF PYRIFORM CORTEX IN EARLY AND END-STAGE ALZHEIMER'S DISEASE. D. L. Sparks*, T. Landers and C. Hackney. Sanders-Brown Center on Aging, UKMC, Lexington, KY 40536

The activity of cholinergic enzymes (ChAT and AChE) and the levels of aromatic monoamine neurotransmitters and their metabolites were determined in the pyriform cortex of 8 end-stage AD patients, 2 patients dying within 18 months of initial clinical diagnosis of AD, and 14 non-heart disease controls.

The maximum velocity (Vmax) of ChAT activity was decreased 90% in end-stage AD patients compared to control. In contrast, the Vmax of ChAT was doubled in the subjects recently diagnosed with AD compared to control. This same pattern of change was observed for AChE activity; decreased in end-stage AD and increased in early AD - both compared to controls.

Highly significant reductions of 5-HT and 5-HIAA were found in both end-stage and early AD. Dopamine levels were decreased only in the early AD patients ($p < 0.05$). The level of the direct catabolite of dopamine, DOPAC, was equally decreased ($p < 0.05$) in both end-stage and early AD. In early AD HVA levels were double that found in end-stage AD; only the decrease in end-stage disease was significant.

Previous reports indicate the pyriform cortex is affected early, and possibly initially, in the progressive spread of characteristic pathology in AD brain. The presented data may suggest that reduced serotonergic neurotransmission occurs early in the course of AD while cholinergic hyperactivity may precede precipitous decrements. (Supported in part by NIH grants 1-PO1-AG05119 and 1-P50-AG05144)

514.10

CHOLINE ACETYLTRANSFERASE IN RETINAS OF PATIENTS WITH ALZHEIMER'S DISEASE. K. V. Porrello[1]*, B. Knusel[2], F. Hefti[2], C. Spee[1], M. Guidoni[1], S. Schmidt[1] and J. C. Blanks[1,3]. [1]Doheny Eye Institute, [2]Andrus Gerontology Center, U. of So. Calif., and [3]USC School of Medicine, Los Angeles, CA 90033.

Alzheimer's disease (AD) is characterized by a significant loss of cholinergic neurons and a decrease in the biosynthetic enzyme, choline acetyltransferase (ChAT), in the hippocampus and cerebral neocortices of the brain. Some AD patients also exhibit retinal degeneration, which is demonstrated by a loss of neurons in the ganglion cell layer (GCL) and atrophy of the nerve fiber layer. To determine whether retinal degeneration in AD is associated with a cholinergic defect, we have examined the biochemical activity and immunolocalization of ChAT in 9 AD and 12 age-matched control retinas. After dissection, a punch of neural retina was taken from each quadrant, homogenized, and assayed for ChAT activity. Comparable areas were fixed and processed for immunocytochemistry with anti-ChAT antibody. The activity of ChAT was comparable in different retinal quadrants of each eye in both the AD and the control groups. The overall mean ChAT activity for the AD group was higher than that of the control group, and was found to have borderline significance ($p = .053$). ChAT immunoreactivity (ChAT-IR) was localized to the somas and dendritic processes of amacrine cells in the inner nuclear and ganglion cell layers. In some cases, knotty-appearing cells, presumably an amacrine cell sub-type, showed ChAT-IR. The localization of ChAT-IR in retinas of AD eyes was similar to that in normal retinas. The findings based on biochemical and immunochemical analyses suggest that ChAT has a uniform regional distribution in human retinas from normal and AD eyes. Further studies are in progress to determine if elevated ChAT activity is a significant feature in AD retinas that can be substantiated biochemically and histochemically. Nevertheless, our findings provide evidence that retinal degeneration in AD, unlike that of the brain, is not accompanied by a cholinergic deficit.

514.11

OVARIECTOMY REDUCES ChAT ACTIVITY AND NGF mRNA LEVELS IN THE FRONTAL CORTEX AND HIPPOCAMPUS OF THE FEMALE SPRAGUE-DAWLEY RAT. M. Singh*, E.M. Meyer, F.S. Huang, W.J. Millard, and J.W. Simpkins. Center for the Neurobiol. of Aging. Univ. of Fla., Gainesville, Fl. 32610.

It has been postulated that a decrease in target-derived trophic support may contribute to the neuropathology seen in Alzheimer's Disease (AD). Based on recent reports that suggest an interaction between estrogens and nerve growth factor (NGF), we hypothesized that estradiol (E2) may serve a neurotrophomodulatory role on basal forebrain cholinergic neurons. We assessed choline acetyl transferase (ChAT) activity and both NGF protein and mRNA levels in the frontal cortex (CTX) and hippocampus (HIPP) of 3 groups of animals: the ovary-intact animals (INTACT), ovariectomized (OVX) and E2-replaced (E2) animals. Neurochemical analysis was done at 5 weeks and 28 weeks post-ovariectomy while NGF levels were assessed at 3 months post-ovariectomy. Previous studies from our laboratory have demonstrated that OVX animals perform relatively poorly in a 2-way active avoidance paradigm. (Neurosci. Abst. # 518.19. pp 1246, 1992). Also we demonstrated that OVX reduced and E2 replacement increased high affinity choline uptake (Endocrine Soc. Abst. 1993). In the present study, we found that 5 weeks of ovariectomy was sufficient to reduce ChAT activity in the HIPP while levels in the CTX were relatively unaltered. E2 replacement reversed this effect. Interestingly, there was a 56% reduction in ChAT levels between the 5- and 28-week time points in the CTX of OVX animals while reduction in ChAT activity in E2 animals was less severe (16%), suggesting a cytoprotective effect of E2 on basal forebrain cholinergic neurons. We also addressed the possibility that E2 may mediate its 'trophic' actions through a classical neurotrophic factor, NGF. While 2-site ELISA failed to reveal any significant treatment-related differences in NGF protein levels, dot blot analysis revealed a 45% reduction in NGF mRNA in the CTX of 3 month OVX animals. E2 replacement resulted in a partial recovery of mRNA levels. Collectively these data suggest that estrogens may play a vital role in the normal function of basal forebrain cholinergic neurons and offer promise as therapeutic agents in neurodegenerative disorders such as AD. (Supported by AG 10485 and 1T32AG00196)

DEGENERATIVE DISEASE: ALZHEIMER'S—OTHER VI

515.1

GENERATION OF MONOSPECIFIC ANTIBODIES AGAINST THE PRECURSOR PROTEIN OF NOVEL AMYLOID COMPONENT (NAC) IN ALZHEIMER'S DISEASE . A.Iwai*, K.Uéda, M.Yoshimoto, D.Otero, and T.Saitoh.
Dept. of Neurosciences, University of California at San Diego, La Jolla, CA 92093-0624, USA

Recently we have identified a novel amyloid component (NAC) as a new constituent of Alzheimer's disease (AD) amyloid and cloned a cDNA for its precursor protein (NACP) composed of 140 amino acids. From the identified NACP sequence, new rabbit antisera were raised against N-terminal 9 aa and C-terminal 10 aa of NACP, and a portion of NAC (15 aa). In Western blots of both the human cerebral cortex cytosolic fraction and the bacteria made NACP from its cDNA, the NACP antibodies stained a protein of relative molecular mass of 19,000 (Mr19K). They showed 2.5 - 10 times higher sensitivity and better linearity than the previous anti-NAC antibodies that stained amyloid well in brain sections, suggesting that anti-NAC antibodies have higher affinity for amyloid form of NAC than its precursor, NACP. These data suggest that these antibodies should be useful to study the processing of NACP to NAC/amyloid with a potential for diagnostic use. (Supported by American Health Assistance Foundation and Yamanouchi Pharmaceutical Co., Ltd.)

515.2

NOVEL AMYLOID COMPONENT (NAC) DIFFERENTIATES ALZHEIMER'S DISEASE FROM NORMAL AGING PLAQUES

K. Uéda*, E. Masliah, Y. Xia, A. Iwai, M. Yoshimoto, and T. Saitoh.
Dept. of Neurosciences, UC San Diego, La Jolla, CA 92093

Recently, we have identified a novel amyloid component (NAC) from Alzheimer's disease (AD) brain, and cloned the corresponding cDNA. NAC is derived from the precursor protein (NACP) composed of 140 amino acids with a calculated *Mr.* of 14.5 kDa. Hydropathy analysis of the predicted amino acids sequence showed NAC to be most hydrophobic, possibly a membrane-binding domain of NACP. Secondary structure predictions show that the NAC peptide has a strong tendency to form β-structures, like amyloid β/A4 protein. Antibodies to NAC peptides detected a 19 kDa protein in Western blot of brain homogenate. Quantitative immunohistochemical analyses showed that 30% of the diffused plaques and 55% of the immature and mature plaques as well as vascular amyloid are strongly stained in AD, while the plaques of the normal aged cases are not. This could imply that a sub population of the plaques as found in normal aged cases represents physiological products that are not stained with the anti-NAC antibody, whereas the plaques with extensive neuritic changes and amyloid condensation represent AD specific plaques that are positive with anti-NAC antibody. We conclude that NAC might be a critical component in the pathogenesis of the AD plaque.

515.3

35kDa PROTEIN IN SENILE PLAQUE AMYLOID IN ALZHEIMER'S DISEASE. Y. Takamaru, R. Fukatsu[2], K. Tsuzuki[3], H. Chiba[1], K. Kobayashi[1], Y. Aizawa[2], N. Takahata[2], T. Ishikane* and T. Ueno Dept. of Psychiatry and Neurology, [1]Dept. of Laboratory Medicine, Hokkaido Univ. Sch. of Med., [2]Dept. of Neuropsychiatry, [3]Dept. of Microbiology, Sapporo Medical Univ. Sch. of Med., Sapporo 060, Japan

To understand the components of amyloid and the process of amyloid formation in Alzheimer's disease (AD) brain, we established monoclonal antibodies recognizing senile plaque amyloid.

Native amyloid and brain homogenate were used for immunogen. Monoclonal antibodies immunohistochemically reacted with senile plaque were selected. Among these mcAb's, four mcAb's recognized 35kDa antigen in brain homogenate, cerebrospinal fluid or plasma proteins. Reactive antigen was purified from human plasma using PEG fractionation and some chromatographies. Amino-terminus amino acid sequence was determined, and the results showed that 35kDa antigen was identical to apolipoprotein E (apo E).

The mcAb's also reacted with approximately 35kDa band in soluble fraction prepared from brain homogenate of AD similar to plasma protein. Immunohistochemical study showed that various type of senile plaque and vascular amyloid were stained by the mcAb's reacted with apo E, and fibrous background structure was also stained diffusely.

Our data show that apo E seems to be expressed in AD brain and play an important role in amyloid plaque formation.

515.4

THE REGULATION OF AMYLOID PRECURSOR PROTEIN PROCESSING BY cAMP AND NITRIC OXIDE IN OLFACTORY NEUROBLASTS FROM ALZHEIMER DONORS. B.L. Wolozin*, J. Basaric-Keys, R.M. Pluta, R.S. Lebovics and T. Sunderland. Laboratory of Clinical Science, NIMH, Bethesda, MD 20892

Previous studies of olfactory neuroblasts (ON) have demonstrated a 7-fold increase in the levels of amyloid precursor protein C-terminal derivative (CTD) seen on immunoblots of Alzheimer vs. control ON subjected to treatment with the lysosomal inhibitor, chloroquine. Further investigations have shown that the amount of CTD detected can be regulated by agents that influence second messenger systems. One axis of regulation appears to be cAMP. Treatment of ON with 0.5 mM dibutyril-cAMP and 0.5 mM IBMX elicits a 92% decrease in CTD levels. We now show that other agents that modulate cAMP levels, such as pertussis toxin, isoproterenol or theophylline also elicit decreases in CTD levels. A second axis of regulation may be mediated through nitric oxide, which is known to regulate cGMP metabolism. Treatment of ON with nitroprusside shows a bimodal response of CTDs seen on immunoblot. At 0.1-1 μM nitroprusside, CTD levels are reduced, whereas at 100 μM nitroprusside there is a small increase in CTDs. Studies using free radical scavengers, which capture endogenously produced free radicals, suggest that nitric oxide or a related free radical is produced endogenously. Treatment of ON with oxyhemoglobin or free radical scavengers, such as ascorbic acid, N-acetylcysteine or dimethylthiourea, all elicit increased CTDs. This also suggests that the decrease in CTD is the most physiologically relevent effect. We are currently using nitric oxide synthase inhibitors to determine whether this mechanism involves nitric oxide synthase directly. These data suggest that second messengers are capable of regulating APP, and raise the possibility that abnormalities in signal transduction pathways can influence APP metabolism.

515.5

APP MEDIATES CELL ADHESION AND CELL-MATRIX INTERACTIONS. H. Mechler *, U. Mönning, C. L. Masters § and K. Beyreuther. Center for Molecular Biology Heidelberg, University of Heidelberg, D-6900 Heidelberg, Germany. § Department of Pathology, University of Melbourne, Parkville, Victoria 3052, Australia.

We have analysed cellular and cell-matrix interactions of F98 rat glioma cells mediated by the amyloid precursor protein (APP). The cells were transfected with the cDNAs coding for APP695, 751 and 770 under the control of the strong human CMV promoter. Stable cell lines, termed F695, F751 and F770, were established by G418 selection. In a cocultivation assay, we used F695, F751 and F770 cells as a culture substrate for nontransfected F98 cells seeded on microporous membranes. We observed accelerated adhesion of F98 cells in response to all three overexpressed secretory APP isoforms. Compared to APP751 and APP770, secreted APP695 induced a higher increase of bound cells, indicating different mechanisms involved in the regulation of adhesion-promoting activity. To study further biological effects of APP695, 751 and 770, transfected and nontransfected F98 cells were grown in a collagen matrix to mimic a three-dimensional tissue-like situation. While wild type F98 cells formed smooth spherical cysts in the gel, F695 cells grew in large aggregates extending long branching cords radially into the surrounding matrix. In contrast, F751 and F770 cells showed a random distribution of individual cells throughout the collagen gel. These results show that the APP isoforms share an activity promoting cell adhesion and specifically regulate cellular outgrowth in an extracellular matrix.

Acknowledgements: Supported by the DFG (SFB 258 and 317), BMFT, Metropolitan Life Foundation and by the NHC and MRC of Australia and the Victorian Health Promotion Foundation.

515.6

THE AMYLOID PRECURSOR PROTEIN (APP) IN NEURO-IMMUNE REACTIONS U. Mönning[1], R. Banati[2]*, C. Masters[3] and K. Beyreuther[1] [1]Center for Molecular Biology (ZMBH), University of Heidelberg , Im Neuenheimer Feld 282, D-6900 Heidelberg F.R.G. [2]Department of Neuromorphology, Max-Planck-Institute for Psychiatry, Martinsried, F.R.G. [3]Department of Pathology, University of Melbourne, Parkville, Victoria 3052, Australia

The mechanism of proteolytic breakdown of the ßA4-amyloid precursor (APP) has attracted much attention because of its relevance for Alzheimer's disease. Apart from the role of APP during amyloidogenesis, many efforts have been made to identify the functional significance of this widely expressed protein in various biological processes. Employing biochemical techniques, we demonstrated that APP under pathological conditions might have characteristics of an an acute phase protein.

Since the presence of brain macrophages (activated microglia) is closely associated with various neuropathological states including neurodegenerative and inflammatory diseases in human brain, we studied the biosynthetic behavior of APP in cultured microglial cells upon immunological activation We also compared their APP-specific biosynthesis to that of immunocompetent cell of the peripheral immune system. From these studies we conclude that APP is associated with early response mechanisms in the central nervous system involving the brains intrinsic immune system. The results support that microglia may play a critical role in plaque formation in Alzheimer's disease.

515.7

ISOLATION OF THE FAMILIAL ALZHEIMER'S DISEASE GENE ON CHROMOSOME 14 R. E. Tanzi, S. M. Gaston, W. Wasco, D. M. Romano, J. Peppercorn, A. Crowley, M. Paradis, K. B. Sims*, A. I. Bush, J. Haines, J.F. Gusella and P. St George-Hyslop. Laboratory of Genetics and Aging and Molecular Neurogenetics Unit, Mass. General Hospital, Boston, MA 02129 and Tanz Neuroscience Inst., University of Toronto, Ontario.

Familial Alzheimers disease (FAD) is a genetically heterogeneous disorder. While a small subset of families (2-3%) contain mutations in the gene encoding the amyloid β-protein precursor (APP) the vast majority of early-onset (65 years) FAD is caused by a mutation in a gene on the long arm of human chromosome 14. The greatest evidence for genetic linkage to FAD is observed with DNA markers in the region 14q24.3. We will present the results of our efforts to isolate the FAD gene defect for chromosome 14. We have genetically determined a minimal candidate region of approximately 1-2 megabases by recombination analysis and have cloned this region in the form of yeast artificial chromosome (YAC) and cosmid clones. These clones are being used to generate polymorphisms to search for linkage disequilibrium to further narrow down the minimal candidate region and to isolate expressed sequences from this region by either exon trapping or direct cDNA screening. Candidate genes obtained in these experiments, along with known candidate genes mapping to 14q24.3 (HSP70 and FOS) are being assessed for potentially pathogenic mutations by single stranded conformational polymorphism analysis and by direct sequencing. While the coding region of FOS has been found to contain no FAD associated mutations, we are still examining the 5 prime and 3 prime untranslated regions. We are also attempting to determine whether the HSP70 gene resides in the minimal candidate region. Assessment of known and novel genes found to reside in the minimal candidate region will be discussed.

515.8

GENETIC AND CLINICAL LINKS BETWEEN TRISOMY 21, DOWN SYNDROME, AND ALZHEIMER'S DISEASE. L.N. Geller,* @L.F.M. Scinto, M.B. Benjamin, @K.R. Daffner, @S. Weintraub, @M.M. Mesulam, D.Dressler, and H. Potter. Dept. Neurobiology, Harvard Medical School and @Beth Israel Hospital, Boston, MA 02115.

A connection between Alzheimer's disease (AD) and Down syndrome (trisomy 21) is indicated by the fact that Down syndrome individuals develop AD neuropathology by the third or fourth decade of life. We have also found that AD, like Down's patients, are hypersensitive to the effect of the cholinergic antagonist, tropicamide, on pupil dilation. One explanation for this connection would be that the overexpression of a gene or genes on chromosome 21 results in Alzheimer's disease. However, mutations in the amyloid precursor protein gene on chromosome 21 have been found to be associated with only a very small percentage of familial AD cases. Instead, some Alzheimer's disease cases may be caused by sporadic trisomy of chromosome 21, resulting from mutations or toxins that cause chromosome nondisjunction. Quantitative fluorescence *in situ* hybridization has been used to determine the number of trisomy chromosome 21 cells in cultured fibroblasts from AD and unaffected individuals. Results suggest that there is a small, but significant increase in the number trisomy 21 in cells from AD individuals. To explore the possibility that a defect in the mitotic spindle apparatus may underlie this aneuploidy, cultured lymphoblasts from AD and unaffected individuals were briefly exposed to the microtubule-disrupting agent colchicine. As assayed by the subsequent appearance of metaphase chromosomes showing centromere separation, cells from AD patients were significantly more sensitive to colchicine treatment compared to cells from unaffected individuals.

515.9

CLONING OF THE cDNA FOR A CANDIDATE GENE FOR ALZHEIMER'S DISEASE. G. Ali, X.-G. Cai, P. Szabo and J.P. Blass.* Burke Medical Research Institute and Division of Geriatrics, Cornell Universtiy Medical College, White Plains, NY & NY, NY. 10605

Because of confirmed evidence of a deficit of activity of the α-ketoglutarate dehydrogenase complex (KGDHC) in Alzheimer disease (AD) brain (15-50% of normal) and evidence of a decrease in KGDHC activity in cultured AD fibroblasts (55% of normal), we obtained a full-length human cDNA clone for the E2k component of KGDHC. A 1.4 kb cDNA probe obtained by PCR amplification from human fetal fibroblast mRNA, with primers chosen from the published rat E2k sequence, had 95% homology with the coding region of rat E2k cDNA. This probe was used to screen a human fetal brain library in λ-ZAP II (Stratagene). The full-length cDNA which was obtained and sequenced was 3.0 kb, in good agreement with the size of the human E2k message. We localized the human E2k gene to chromosome 14 (14q24); no other component of KGDHC is on C14. Studies are underway to determine if the human E2k gene is in the precise region of C14 associated with AD. We have found genetic heterogeneity in E2k in human populations, but further studies are needed to determine whether mutations of this gene associate with the occurence of clinical AD in specific kindreds. E2k is a candidate gene for the C14-linked form of AD.

515.10

ALTERED SENSITIVITY TO NGF OF DORSAL ROOT GANGLION CELLS CULTURED FROM MOUSE TRISOMY 16, A MODEL OF DOWN SYNDROME. R.J. Pearce*, A. Balbo, Z. Galdzicki & S.I. Rapoport. LNS, NIA, NIH, Bethesda, MD 20892.

Nerve growth factor (NGF) is an endogenously derived protein necessary for survival of certain populations of embryonic neurons. In culture, NGF significantly enhances survival of sensory neurons. We compared the sensitivity to NGF of primary cultures of dorsal root ganglion (DRG) neurons from trisomy 16 (Ts16) mouse fetuses with normal diploid neurons. Mouse Ts16 is a model for human trisomy 21, which gives rise to Down syndrome (DS). All DS subjects develop Alzheimer disease neuropathology after the age of 35 years. Dorsal root ganglia from E15-E17 fetuses were dissociated into a cell suspension by trituration and then plated onto 35mm laminin-coated dishes (50-150,000 cells/dish). Plating media consisted of Minimum Essential Medium supplemented with 10% horse serum, 10% fetal calf serum (FCS) and 7S-NGF (1-160ng/ml). Cells were maintained for 3-4 weeks and media were exchanged every 3-4 days (media same as above with FCS excluded).

In both Ts16 and normal preparations, the proportion of DRG neurons plated which adhered to the laminin coated culture dishes depended on the amount of 7S-NGF included in the media ($p<0.001$). Neuronal survival was assessed as adhesion to the substratum with the extension of neurites. We previously showed that, at 40ng/ml NGF, only 20% of Ts16 DRG neurons seen 24hr after plating survive the first week in culture, as compared to 50% for normal diploid neurons (Orozco *et al*, 1987). This has now been repeated for a range of NGF levels. We found that survival in culture was not only NGF dependent for both populations ($p<0.001$), but also that the degree of survival across this range was significantly different between trisomic and diploid neurons ($p=0.002$). Also, the initial survival of the normal DRG neurons over the first 5 days correlated significantly with the density of the cells 24hr after plating ($r=0.661;p=0.001$), whereas the same was not the case for the trisomic neurons ($r=0.077;p=0.803$).

515.11

ELECTROPHYSIOLOGICAL PROPERTIES OF SEPTAL NEURONS CULTURED FROM FETAL TRISOMY 16 MOUSE. L.D. Acevedo*, Z. Galdzicki, R.J. Pearce, and S.I. Rapoport. Laboratory of Neuroscience, National Institute on Aging, NIH, Bethesda, MD 20892.

Mouse trisomy 16 is a model for human trisomy 21, which gives rise to Down's syndrome (DS). All DS subjects eventually develop Alzheimer disease (AD). To better understand these disorders, we examined the electrophysiological properties of septal and hippocampal neurons from the trisomy 16 mouse fetus, as in humans, homologous regions develop AD pathology. Fetal mouse trisomy 16 hippocampal neurons in culture have abnormal electrical characteristics [Galdzicki et al., 1993, Brain Res 604:69-78].

Septal brain regions from trisomic and diploid fetuses at 16 days gestation were triturated to yield dissociated neurons which then were cultured on poly-L-lysine-coated plastic dishes. The cultures were grown in MEM supplemented with 5% horse serum and 40 ng/ml 7S-NGF. Under these conditions, we obtained neurons of diverse morphology having soma diameters ranging from 5 to 30 μm and surviving 3 weeks or longer.

We first examined whole-cell voltage clamp records from septal neurons at 9-16 DIV. The passive properties and current/voltage relations of trisomic neurons were compared to those of septal neurons derived from normal diploid fetuses. For this comparison we chose neurons having a capacitance between 7 and 9 pF, and included records from 6 normal and 13 trisomic neurons. We found the two groups did not differ significantly in their passive properties ($P<0.05$), including input resistance and resting potential. The maximum inward current, maximum inward conductance, and maximum outward conductance, when normalized by capacitance, also were not different between the groups. The ratio of inward to outward conductance also was not significantly different. Based on this preliminary analysis, we find no difference between the electrophysiological properties of trisomy 16 and normal diploid septal neurons.

515.12

LONG-TERM EXPRESSION OF LAC-Z GENE FROM LATENT HSV IN RAT HIPPOCAMPAL NEURONS. N.T. Maidment*, D.C. Bloom, A. Tan, L.T. Feldman and J.G. Stevens. Dept of Psychiatry and Dept of Microbiology and Immunology, UCLA School of Medicine, Los Angeles, CA 90024.

A major hurdle in the development of the Herpes Simplex Virus (HSV) approach for gene transfer in the CNS has been the need for a promoter that facilitates long-term expression during latency. Using the LAT promoter to drive the lacZ gene, we were able to see B-galactosidase (B-gal) activity in mouse sensory ganglia for over 2 months. However, no B-gal mRNA could be detected at this time-point, either by in situ hybridization or PCR. This suggested that the enzyme is very stable and highlighted the need for transcript analysis in the evaluation of latent gene expression.

Using an ICP4- construct (8117/43) with the Mo-MuLV LTR driving the lacZ gene we have previously demonstrated transcripts in mouse dorsal root ganglia. We now report on the utility of this construct for long-term expression in the rat brain. 8117/43 or KD6 (an ICP4-, LAT+ control virus) was stereotaxically injected into the hippocampus (1×10^6 p.f.u.) and the animals subsequently sacrificed at various intervals up to 2 months. PCR analysis of viral DNA at 2 months demonstrated that both viruses establish a latent infection in this brain region. Using both x-gal staining and in situ hybridization we were able to demonstrate that 8117/43 expresses B-gal for at least 2 months in hippocampal neurons whereas LAT expression is minimal at this time. Staining for x-gal was also apparent in cells of the medial septum suggesting retrograde transport of the construct within septo-hippocampal neurons. These studies indicate that the LTR promoter affords long-term expression of foreign genes in the septo-hippocampal system.

515.13

DISTRIBUTION OF HSV-1 DNA IN SELECTED AREAS OF NORMAL AND ALZHEIMER'S DISEASE BRAINS : A PCR STUDY. Guillaume D.*, Bertrand P., Hellauer K., Dea D., Lindsay J., Kogan S., Gauthier S., Poirier J. Douglas Hospital Research Center, the McGill Center for Studies in Aging, Montreal and the Laboratory for Disease Control, Ottawa, CANADA, H4H 1R3.

The possibility exists that reactivated Herpes Simplex (HSV) viruses travelling by centripetal force from the trigeminal ganglia affect the CNS areas subject to degeneration in Alzheimer's disease (AD). We addressed whether the HSV-1 genome was present more often in AD brains than in those of controls (C), and whether it was detected preferentially in those brain areas where the AD pathological changes occur. We used formalin-fixed brains of 57 C and 98 AD, preparing genomic DNAs from 5 areas (hippocampus, frontal cortex, occipital cortex, cerebellum and striatum). The DNA was PCR amplified for the presence of a 138bp piece from the glycoprotein D gene of HSV-1. Results show that elderly often exhibit HSV-1 DNA in their brain genome: 72% of C and 75% of AD had at least one HSV-1 infected region, while 46% of C and 43% of AD had all 5 regions infected. The prevalence of HSV-1 DNA within each brain area was similar between the two groups and within the C and AD groups: the 5 regions which were examined, were affected in about 60% of all C and AD individuals. Thus, end-stage AD does not correlate with a preferential HSV-1 gradient through the CNS. Nevertheless, chronological links between AD and HSV-1 infection need to be determined. (Supported by the Center for Disease Control, Canada).

516

SYMPOSIUM. GABA AS A DEVELOPMENTAL SIGNAL. J.L.Barker, NINDS, NIH (Chairperson); A.J. Tobin, UCLA; N.C. Spitzer, UCSD; P.R. Gordon-Weeks, King's College London; D.A. Redburn, Univ. of Texas Health Science Center, Houston.

Recent evidence indicates that γ-aminobutyric acid (GABA) is expressed during CNS development in all vertebrate species. Tobin, who has studied the molecular biology of glutamate decarboxylase (GAD), the rate-limiting enzyme in GABA synthesis, will review data that shows GAD is derived from two different genes each encoding proteins of similar molecular weight but different intracellular distributions. Both enzymes become co-expressed in subsets of GABAergic neurons throughout the well-differentiated CNS. Spitzer has studied the signal transduction cascade in amphibian spinal neurons that is associated with the initial appearance of GABA. He will relate fluctuations in intracellular Ca^{2+} levels to the control of transcriptional events. Gordon-Weeks has investigated the mechanisms underlying GABA release from the growth cones of developing neurons. Regulated release of GABA suggests that GABA plays extracellular autocrine and paracrine roles in neurite outgrowth and synaptogenesis. Redburn has examined the role of GABA in retinal development. Her observations in the rabbit indicate that GABA is a critical signal in the very establishment of the primary visual pathway. Barker will review studies in the rat that indicate GABAergic cells and signals are co-expressed during the last half of embryonic development throughout most of the CNS. All of these results in different vertebrate species support the idea that GABA is a developmental signal involved in the morphogenesis of the CNS before it transmits fast transients at synapses.

517

SYMPOSIUM. NEURONAL FUNCTIONS OF CALMODULIN-DEPENDENT PROTEIN KINASE II. T.R. Soderling, Vollum Institute (Chairperson); H. Schulman, Stanford Univ.; H. Hidaka, Nagoya Univ.; A. Silva, Cold Spring Harbor.

Multifunctional CaM-kinase II (CaM-K II) is the most abundant protein kinase in brain where it regulates both presynaptic and postsynaptic functions. This symposium will focus on recently discovered functions of CaM-kinase II with an emphasis on its involvement in cellular learning and memory. CaM-K II is of particular interest as it constitutes the major postsynaptic density protein at excitatory synapses, and it becomes constitutively active after autophosphorylation. Dr. Schulman will describe another function of autophosphorylation which allows the kinase to act as a unique Ca^{2+} spike frequency detector. He will also describe cellular mechanisms of "cross-talk" between CaM-K II and PKC. Dr. Hidaka will discuss the use of recently developed, cell-permeable inhibitors of CaM-K II (e.g., KN-62) to probe neuronal functions such as tyrosine hydroxylase activation, immediate early gene expression and long term potentiation. Dr. Soderling will focus on the activation of CaM-K II in cultured hippocampal cells by NMDA receptor stimulation and the resultant phosphorylation and regulation of non-NMDA glutamate receptor ion channels by CaM-K II. The role of CaM-K II in learning and memory will be further explored by Dr. Silva who will describe behavioral studies on mice lacking the major alpha subunit isoform of CaM-K II.

519.1

Morphology of the Blue-ON ganglion cell type in macaque retina. D.M. Dacey*. Dept. of Biol. Structure, Univ. of Washington, Seattle, WA 98195.

We have been studying the physiological properties of identified ganglion cell types by combining intracellular recording and cell filling in an in vitro preparation of the macaque retina. The intact retina, choroid and pigment epithelium are dissected from the eyecup and placed in a superfusion chamber on the stage of a light microscope; oxygenated culture medium is continuously superfused over the retinal surface and temperature is maintained at 37°C. The cell bodies of midget, parasol and other ganglion cell types were selected for recording under direct microscopic control after staining with a vital fluorescent dye. Ganglion cell responses to chromatic and luminance flicker were studied with a light-emitting diode-based stimulator (Smith et al., J. Physiol. 1992, 458:191). A two degree stimulus field was projected onto the retinal surface via the camera port of the microscope. Using the LED stimulus, red-green, blue-ON and phasic non-opponent types have been identified. The dendritic morphology of the recorded cells was shown by intracellular injection of Neurobiotin. Results confirm the expectations that parasol cells are phasic, non-opponent cells and midget ganglion cells give sustained, color-opponent responses. However, all midget cells recorded from thus far showed only red-green opponency. To date we have also recorded from 12 cells classified as Blue-ON; all of these cells displayed a distinctive non-midget, bistratified dendritic morphology. Dendritic field size and coverage of these bistratified cells suggest that they make up ~6-8% of the ganglion cells in the retinal periphery. Dendritic stratification suggests direct input from blue-cone bipolar cells near the inner border of the inner plexiform layer. Thus, the red-green and blue-yellow opponent pathways of the macaque visual system arise in parallel from morphologically distinct ganglion cell mosaics.

519.2

SOME SUPPRESSIVE ROD-CONE INTERACTIONS INVOLVE CENTRAL VISUAL PATHWAYS. G. Lange, M. Schütte, N. Denny, and T. E. Frumkes*. Dept. Psychol., Queens College CUNY, Flushing, New York 11367.

Dark adapted rods of vertebrate retina exert tonic suppression upon cone-mediated vision. We have discerned two classes of mechanisms. (1). Sensitivity to rapid flicker progressively decreases during rod-dark adaptation, and increases if rods are adapted to increasing intensity backgrounds. Neurophysiological, pharmacological, and psychophysical data indicate that this "flicker effect" is consistent with a distal retinal mechanism involving a prominent role for horizontal cells. (2). Sensitivity to high spatial frequency gratings similarly decreases during rod-dark adaptation, and increases if rods are adapted to increasing intensity backgrounds. However, this "grating effect" is as prominent with interocular adapting fields as with one-eyed stimulation, and psychophysical data are inconsistent with an electrical coupling model accounting for the flicker effect. We suggest that this spatial effect could be mediated by a monoaminergic, hypothalamo-retinal pathway which we have recently described in mammals. However, we cannot rule out involvement of some intrinsic cerebral mechanism.

Supported by grants from NSF and CUNY

519.3

COLOR OPPONENT NEURONS IN MOUSE RETINA
S. Yamamoto, P. Gouras*
Harkness Eye Institute, Columbia University, New York, NY 10032

We have been examining the activity of single retinal ganglion cells to full field spectrally defined stimuli in normal and dystrophic rats and mice. We have examined 122 single units in 23 normal mice. All units had spontaneous activity but only 91% were driveable by light. 82/111 (74%) had broad-band action spectra; 8/82 (10%) of these had a uniquely high sensitivity to short wavelengths which we consider a short wavelength sensitive (S) cone input. 21/82 (26%) showed color opponency between an S-cone and a middle wavelength mechanism. The S-cone input was either excitatory or inhibitory. Color opponent units were found throughout the retina although twice as frequent in the lower half than the upper half. Since recent anatomical studies indicate that the lower half of the mouse retina contains only S-cones (Szel, et al., J Comp Neurol 325:327, 1992) the color opponency in the lower retina may occur between rods and S-cones. The existence of color opponent units in mouse retina implies that mice have color vision.

519.4

DIFFERENT EFFECTS ON CYTOCHROME OXIDASE ACTIVITY AND AXOPLASMIC TRANSPORTATION OF RETINAL GANGLION CELLS FOLLOWING INT RAOCULAR HYPERTENSION IN RAT.Yaping Chu, Zhonghao Liu and Xuegang Luo*. Dept. of Neurobiology, Hunan Medical University 410078,Changsha,Hunan,P.R.China

The retrograde horseradish peroxidase(HRP) tracing method combined with cytochrome oxidase(C.O.) histochemistry was used to investigate the effects of intraocular hypertension on oxidative metabolism and axoplasmic transportation of the retinal ganglion cells(RGCs) in rats.Isotonic saline was perfused into the anterior chamber of the left eye, as experiment, with intraocular arterial perfusion pressure for 1 hr and then the intraocular hypertension was decreased to normal.The anterior chamber of the right eye was only pierced by needle (no injection),as control, for 1 hr. During the intraocular hypertension, 0.3ul 30% HRP(Sigma VI) was injected into each of 8 sites which were distributed symmetrically in the superficial layer of bilateral superior colliculi and 4 sites in bilateral dorsal lateral genicular nuclei. After the rats were survived for 48 hrs, the retinal whole-mounts were prepared in the fixative and then incubated with DAB method for HRP reaction followed by C.O.-cobalt chloride procedure for C.O. reaction. For a quantitative analysis, the numerical density of double labeled (C.O./HRP) RGCs in experimental group was 607/mm^2,2175/mm^2 in control group; while the single C.O. positive RGCs in experimental group was 1145/mm^2, 775/mm^2 in the control group. The double labeled RGCs in the experimental group decreased significantly ($P<0.01$) but the single C.O. labeled RGCs increased relatively ($P<0.05$) as compared with the control group. According to the early experiment, the restoration of C.O. activity in various degrees followed by prolonging survival time (X. Luo et al., Acta Anatomica Sinica. 19 (1988) 85). It was presumed that the restoration of the C.O. activity was earlier than those of axoplasmic transportation.
Supported by NSFC 3870621 and Research Grant from HMU.

519.5

NEW LAGOMORPHIC PERSPECTIVE ON X- AND Y-GANGLION CELLS IN MAMMALIAN RETINA. E. V. Famiglietti*
Dept. Anat. & Lions' Sight Ctr., Univ. Calgary, Calgary AB Canada T2N 4N1.

X and Y retinal ganglion cells (GCs) were characterized physiologically in cat retina, where their morphological counterparts are beta/class II and alpha/class I cells, respectively. In primate retina, a bipartite physiological subdivision of GCs with "brisk" characteristics is problematical. Yet the well-established distinction between "midget" and "parasol" GCs suggests strong parallels with beta/class II and alpha/class I cells. X and Y cells, collectively called "brisk", have also been identified in rabbit. Unlike those of cat, they are similar in (large) receptive field size and in (rapid) axonal conduction velocity. "Alpha" but no "beta" GCs were identified in one study of rabbit retina (Peichl et al.,'87), but in an earlier brief report we identified class I and class II cells (Famiglietti and Siegfried, '79), the analysis of which is now complete. Class I cells have the largest GC bodies and axons; those of class II cells are slightly smaller on average. Dendritic field sizes are similar in the visual streak, but class I cells are larger in the periphery. Branching patterns are similar, but class I cells are "radiate" and class II cells are commonly "tufted", by the criterion of m_p (Famiglietti, '92). Dendritic stratification of class II cells in cat is broad, overlapping that of class I cells, but in rabbit it is narrow, and similar to that of midget ganglion cells, in that two sets of class II cells (IIa1, IIb2/blue ON-centre?) lie nearer the boundaries of the inner plexiform layer (IPL), flanking the two principal sets of class I GCs (Ia2, Ib2). Additional sets of class I/II GCs (Ia1, Ib1, IIb1) may include "large field" units. Rabbit class I and class II GCs appear to reflect an early phylogenetic stage in the structural and functional separation of mammalian X and Y cells.

Supported by the AHFMR and the MRC of Canada.

519.6

DENSITY OF MIDGET AND NON-MIDGET GANGLION CELLS IN MACAQUE FOVEA D.J. Calkins*, S.J. Schein[1], Y. Tsukamoto[2], P. Sterling. U. of Penn., Phila., Pa 19104, [1]UCLA, 90024, [2]Hyogo Col. Med., 663 Japan.

The number of ganglion cells per cone in primate fovea has been estimated at 2-4. The fraction of the total ganglion cell population that are midget ganglion cells has been estimated at 80-95%. We identified in electronmicrographs of serial sections every ganglion cell and cone pedicle in a small foveal patch and found 150 ganglion cells (non-displaced) and 55 pedicles, for a ganglion cell/cone ratio of 2.7. Of the ganglion cells 110 (73%) were midget and 40 (27%) were non-midget. Of the non-midget cells, 9 were bistratified (also parvocellular[1]) and 31 were monostratified. The 55 cone inner segments corresponding to the pedicles occupied 735 μm^2 at an eccentricity of 1.1 - 1.25° nasal. The densities (cells/mm^2) corresponding to this retinal locus were: cones and midget ganglion cell pairs, 74,830; bistratified ganglion cells, 12,245; monostratified cells, 42,177. The resolution limit (Nyquist) for the array of midget ganglion cell pairs is 32 c/deg, and for the array of bistratified ganglion cells, it is 13 c/deg. Thus, enough non-midget ganglion cells are present in foveal retina to support several subcomponents of the "M" and "P" pathways.

[1] Rodieck, R.W. in *From Pigments to Perception*, Plenum Press, New York, 1991.

519.7

SPATIO-TEMPORAL RECEPTVE FIELD (STRF) OF RETINAL GANGLION CELLS. J.L. Wang and Ken-ichi Naka*, Dept of Ophthalmol., NYU Medical Center, New York, NY 10016

The neuron network in the vertebrate retina processes information in the joint domains of time and space. The neurons's receptive field, including that of ganglion cells, must be defined in time and space. A ganglion cell's STRF can be defined by cross-correlating spatio-temporal white-noise input against a train of spike discharges (transformed into a unitary pulses) evoked by such a stimulus. Our past studies have shown that spatial or color information is carried largely by the linear component in a spike train. The resulting correlograms, the linear part of a STRF, are, therefore, an adequate description of the ganglion cell's receptive field.

A spatio-temporal white-noise stimulus, a checker matrix of 16x16 elements modulated by M-sequence, was produced by VSG2/2 visual stimulus generator (Cambridge Research Systems, UK) and spike discharges were recorded extracellularly by means of a tungsten electrode. We used two preparations, the retinas of a channel catfish, *Ictalurus punctatus*, and of kissing gourami, *Helostoma temmincki*. All measurements were made under photopic condition. There is only one class of cone in the catfish retina and there are tow classes of cone, red and green, in the gourami retina. We will cross-reference results from these retinas to analyze how signals are processed in time and space in the retinal neuron network.

Supported by NIH grants EY07738 and NS30772 and Research to Prevent Blindness Inc. professorship to KIN.

519.8

AMINO ACID RECEPTORS OF PRIMATE MIDGET AND PARASOL CELLS IN A RETINAL SLICE. Z.J. Zhou*[1], D.W. Marshak[2] and G.L.Fain[1]. [1]Jules Stein Eye Inst., UCLA Sch. of Med., Los Angeles, CA; [2]Dept. Neurobiol. & Anatomy, U. of Texas Med. Sch., Houston,TX.

Primate parasol and midget ganglion cells are known to have different response properties, but we do not know if these functional differences are due to differences in synaptic receptors and/or voltage-gated channels or to differences in synaptic inputs. We have studied the effects of bath-applied amino acid transmitters on both parasol and midget ganglion cells of baboon and macaque monkey retina in a retinal slice preparation. Parasol and midget cells were whole-cell voltage-clamped at -70 mV using Lucifer yellow-filled electrodes. In Ringer containing 1mM Cd^{+2} to block synaptic transmission, both the excitatory and inhibitory amino acid agonists kainate (20μM), AMPA (60μM), NMDA (200μM), GABA (200μM), and glycine (200μM) elicited inward currents in both on- and off- parasol and midget cells. The kainate- and AMPA-induced currents reversed near the equilibrium potential for cations and were antagonized by DNQX (10μM). Responses to NMDA recorded in Cd^{2+}-free Ringer were reversibly blocked by D-AP7 (200μM) and by Mg^{2+} (1mM) in a voltage-dependent manner. Responses to GABA and glycine had reversal potentials close to the Cl^- equilibrium potential and were antagonized by bicuculline (10μM) and strychnine (4μM), respectively. We conclude that primate midget and parasol cells have similar types of excitatory and inhibitory amino acid receptors.

519.9

EXTRA RECEPTIVE FIELD FACILITATION IN RABBIT'S RETINAL DIRECTIONAL SELECTIVITY. **N. M. Grzywacz*, F. R. Amthor and D. K. Merwine. Smith-Kettlewell Inst., 2232 Webster St., San Francisco, CA 94115 and Univ. Alabama at Birmingham.**

Amthor et al. (1989), and Yang and Masland (1992) showed that the sizes of dendritic trees and excitatory receptive field centers (RF) of On-Off directionally selective (DS) ganglion cells of rabbit are similar. This led to the suggestion that directional selectivity models based on asymmetric, excitatory amacrine synapses onto DS cells cannot be correct. These models might predict an RF displaced asymmetrically relative to the DS cells' dendritic tree. However, another possible prediction is that the RF might coincide with the ganglion cells' dendrites due to bipolar-cell inputs and that facilitation for preferred-direction motion might be transmitted asymmetrically to ganglion cells. To test this alternative, we recorded extracellular responses of DS cells to preferred- and null-direction apparent motions beginning outside the RF and ending just inside it. We also recorded responses to motions beginning just outside the RF and ending in various positions in it.

The data show that stimuli from outside RF positions can facilitate responses to stimuli inside the RF during preferred-direction motions. In contrast, null-direction motions only elicit inhibition. Positions that elicit facilitation can be at least 180 μm outside the RF. Moreover, stimuli near the border of the RF can cause facilitation even if the motions terminate in or past the middle of the RF. For the majority, *but not all* cells, the strongest facilitation occurs for the shortest distance tested, that is, 60 μm. Bell-shaped facilitation distance dependence argues that extra RF facilitation is not due to ganglion cell threshold. In conclusion, asymmetric extra RF facilitation supports an excitatory asymmetric amacrine model for directional selectivity and suggests that traditional RF, which might be due to bipolar-cell inputs to ganglion cells, may be different from the region of motion facilitation.

Supported by NEI (EY08921 and EY05070) and ONR (N0014-91-J-1280).

519.10

RELATION BETWEEN PREFERRED DIRECTION AND DENDRITIC ORGANIZATION OF IDENTIFIED, CONTACTING ON-OFF DS GANGLION CELLS AND ACH. AMACRINE CELLS IN RABBIT RETINA.
F.R. Amthor* and C.W. Oyster. Depts. of Psychology & Physiological Optics, University of Alabama at Birmingham 35294.

We have perfected a visualized recording system for isolated rabbit retina in which multiple ganglion and amacrine cells in a given field may be recorded and stained to determine their dendritic inter-relationships. We report that On-Off directionally selective (DS) ganglion cells of the same preferred direction form exclusive, tiling-like domains in which dendrites of same-direction neighbors terminate at close appositions at their first point of contact. This tiling is independent for the inner and outer dendritic ramifications, similar to that of Neurobiotin coupled cells shown by Vaney and collegues. In contrast, dendrites of On-Off DS ganglion cells with different preferred directions overlap extensively and fasciculate together frequently, which almost never occurs for dendrites of same-direction neighbors. We thus, by these findings, demonstrate a separate tiling coverage of the rabbit retina by each of the four directional subtypes of On-Off DS ganglion cells.

We have also intracellularly stained DAPI-accumulating (presumed Ach.) amacrines surrounding physiologically identified and intracellularly stained On-Off DS ganglion cells. Our initial results indicate many more close appositions, and therefore opportunities for synaptic contact, between processes of DAPI amacrines on the preferred than on the null side of On-Off DS ganglion cells. This finding, if confirmed on further analysis to be done shortly, has strong implications for models of directional selectivity such as proposed by Vaney and collegues, and by Borg-Graham, Grzywacz and Amthor.

Supported by NEI EY05070 and ONR N00014-91-J-1280.

519.11

A MODEL WITH COUPLED NONLINEAR OSCILLATORS CAN DESCRIBE SPIKE TRAINS OF CAT RETINAL GANGLION CELLS. A. W. Przybyszewski*, M. J. M. Lankheet, and W. A. van de Grind Dept. Physiol., Freie Universität Berlin, Germany and Utrecht Biophysics Research Institute, Dept. Comp. Physiol., Utrecht University, The Netherlands.

Ganglion cell activity was recorded intracellularly in the optically intact *in vivo* eye of the cat. Using a wavelet method, the recorded intracellular responses were separated into a "slow" G-potential and the corresponding spike train. We had shown previously (Przybyszewski et al., Vis. Res., 33 (1993): 861- 875) that the G-potential responds to sinusoidally modulated light spots with at least two kinds of oscillations and only the slower one is always locked to the stimulus. The G-potential oscillations can be simulated with the modified van der Pol oscillator. On the basis of the patch clamp data (Lipton & Tauck, J. Physiol., 385 (1987): 361-391) it appears that local membrane properties can be simulated by another nonlinear oscillator. The two nonlinear oscillators were coupled to model spike trains obtained experimentally. Irregularities appear in the experimental and the simulated data when the temporal stimulus frequency is increased. Changing the coupling strength causes changes in irregularities, which are suggestive of a noise-reduction mechanism. If the coupling coefficient depends on the synapse position in the dendritic tree, the membrane could filter psp's in a position - dependent manner. Such a mechanism might generate signals that look more or less noisy, whereas in fact there is no noise but rather the complex dynamic interaction of the two nonlinear oscillators.

519.12

THE DECODING OF MULTI-NEURONAL SIGNALS FROM THE RETINA.
D. K. Warland and M. Meister*. CDB Department, Harvard University, Cambridge, MA 02138.

How is information about a visual scene represented in the optic nerve? How could higher processing centers extract this information in real time using known neuronal mechanisms? To unravel the rules by which the eye encodes a visual scene, we have attempted to reconstruct the visual input directly from the simultaneous spike trains of many ganglion cells. A small randomly flickering spot of light was imaged on the photoreceptor layer of a salamander retina, while the action potentials from about 50 ganglion cells were recorded extracellularly using a microelectrode array. We then constructed a decoding algorithm that estimates the instantaneous light intensity of the spot from the recorded patterns of ganglion cell spikes. The parameters of this retinal decoder, which is based on linear filtering, were adjusted to optimize the correspondence between the estimated and the actual stimulus. This simple method of interpreting neural spike trains appears to extract much of the information transmitted by the retinal ganglion cells. Performance of the decoder degrades significantly for flicker frequencies above 2 Hz, consistent with the poor flicker response of cone photoreceptors. Signals from ON and OFF retinal ganglion cells complement each other; by contrast, the information from two cells of the same type is often highly redundant. Our results indicate that retinal ganglion cells cannot be considered as independent information channels; rather, the visual scene is at least in part encoded via concerted firing patterns involving many neurons.

Supported by a Lucille P. Markey grant, ONR Grant N00014-92-J-4072, and The Whitaker Foundation.

519.13

EVIDENCE FOR AN INHIBITOR OF GANGLION CELL DIFFERENTIATION IN THE EARLY EMBRYONIC CHICK RETINA. C.P. Austin and C.L. Cepko. Dept. of Genetics, Harvard Medical School, Boston, MA 02115.

The factors affecting cell type determination and differentiation in the vertebrate retina are largely unknown. Ganglion cells (GC) are the first cell type to be produced in the chicken retina, and we have used an in vitro system to study possible factors influencing their development. Though the percentage of retinal cells which are GC at any time during development is never more than 15%, when retinas from E4 chick retinas were dissociated into single cells and cultured in collagen gel or monolayer conditions at low density, the percentage of cells expressing GC-specific markers increased to 70%. Time-course experiments showed that most of this increase occurred rapidly, between 6 and 10 hours after the cells were placed in vitro. When cells were cultured at progressively higher cell densities, the percentage of cells which turned on GC-specific markers incrementally declined; when cells were spun into a pellet before culturing, the percentage of GC after 24 hours was the same as in a retina left in vivo. This phenomenon is developmentally variant: the most GC-marker positive cells were produced in vitro when the starting population was from the period of maximal GC generation in vivo. Experiments to test the commitment state of the cells differentiating as GC in vitro suggested that only a subset of the marker positive cells irreversibly committed to the GC phenotype under these conditions, and that a majority of the cells differentiated as GC without being committed to this pathway. These experiments suggest the possibility that at an early stage in chick retinal histogenesis, many more cells than normally become GC are competent to commit to and/or differentiate along this pathway, but are prevented from doing so by an inhibitor that is diluted out by the low density culture conditions. *Supported by K11 EY 00321 (C.P.A.) and RO1 EY 09676 (C.L.C.) from the National Eye Institute.*

GENE STRUCTURE AND FUNCTION V

520.1

MUSCARINIC ACTIVATION OF PNMT GENE EXPRESSION MAPS TO UPSTREAM PROMOTER REGIONS. L.M. Hemmick* and M.J. Evinger, Dept. of Pediatrics, SUNY Stony Brook, Stony Brook, NY, 11794.

Molecular mechanisms underlying neural regulation of phenylethanolamine N-methyltransferase (PNMT) gene expression are elucidated by expressing PNMT promoter-reporter gene constructs in primary cultures of bovine adrenal chromaffin cells. Our previous studies show that the cholinergic agonist muscarine stimulates expression of the PNMT gene by (a) increasing the rate of PNMT gene transcription in run-on assays, (b) stimulating steady-state levels of PNMT mRNA, and (c) enhancing expression of transfected reporter genes by the rat PNMT promoter. Transfected constructs containing nested size deletions of the rat PNMT gene were expressed in primary bovine adrenal chromaffin cells. Luciferase activity was measured in lysates of cells treated with muscarine (100 uM) 16-24 hr. Muscarine stimulates reporter gene expression by 1.5 to 2-fold for constructs bearing promoter regions from -900 to -440 bp but not downstream sequences (proximal 440 bp). To resolve whether muscarine induces production of specific nuclear protein factors in chromaffin cells, gel retardation analyses have been performed using a -765 to -440 bp fragment of the PNMT promoter. Muscarine treatment for 16 hr induces a protein(s) which alters the migration of the PNMT fragment. This shift also occurs with nuclear extracts from bovine adrenal medulla and adrenal cortex, but not from bovine liver or cytoplasmic extracts of these tissues. DNase I footprinting analysis of this fragment reveals a region spanning -480 to -460 bp of the promoter protected by nuclear proteins from muscarine-treated cells. Thus, muscarine appears to stimulate PNMT gene transcription in chromaffin cells by inducing specific nuclear proteins which interact positively with upstream PNMT promoter elements.

520.2

THE NEUROPEPTIDE PACAP REGULATES PNMT GENE EXPRESSION IN BOVINE CHROMAFFIN CELLS. E.C.Tonshoff, L.M.Hemmick, T.H.Joh# and M.J.Evinger*, Dept. of Pediatrics, SUNY, Stony Brook, NY,11794 and #Cornell Univ. Med. Col., NY, NY, 10021.

Nicotinic and muscarinic components mediate the cholinergic response of the gene for the epinephrine synthesizing enzyme phenylethanolamine N-methyltransferase (PNMT) in the adrenal medulla. Moreover, the pituitary adenylate cyclase activating polypeptide (PACAP) stimulates via non-cholinergic mechanisms catecholamine release and tyrosine hydroxylase mRNA levels in the adrenal medulla (rev. by Arimura, 1991).

We sought to determine whether PACAP influences PNMT mRNA production. Northern analyses of total RNA from primary bovine adrenal chromaffin cell cultures reveal that PACAP increases steady state levels of PNMT mRNA in a dose-dependent manner. 10^{-9} M PACAP elicits maximal response (equivalent to that of depolarization by 50 mM K^+) and its effects are additive with those of K^+-depolarization and nicotine, consistent with action through separate intracellular mechanisms. Transient transfections using constructs containing 5' upstream regions of the PNMT gene linked to luciferase reporters were performed in chromaffin cell cultures. After 24 hr treatment with PACAP, -500 to -900 bp constructs on heterologous promoters showed slight (20-30%) stimulation. No stimulation of reporter gene activity was observed with 300 or 3000 bp constructs. This information in conjunction with absence of canonical cAMP responsive elements on the PNMT gene, indicates that the primary mode of PACAP stimulation of PNMT mRNA is not transcriptional. Transcriptional and stabilization analyses are in progress to investigate further mechanisms for PACAP-mediated regulation of PNMT expression.

520.3

THE ROLES OF DYAD SYMMETRY AND GC-RICH ELEMENTS IN THE EXPRESSION OF BOVINE TYROSINE HYDROXYLASE GENE. E. Kim, S. Maltchenko, M.K. Stachowiak*, Barrow Neurological Inst., Phoenix, AZ, 85013

Computer analysis has revealed several dyad symmetry elements (DSEs) within proximal 430 bp promoter region of the bovine, human, and rat TH genes. Those DSEs do not share sequence homology with known protein binding sites. Their length and localization are conserved in the TH genes from different species. Bovine and human TH gene promoters contain also GC-rich sequence of similar length and relative position. To examine the roles of DSEs and GC-elements we have constructed a set of bovine TH promoter mutants in which individual elements were deleted. The wild type TH promoter (-430/+25) and its deletion mutants were linked to luciferase gene in pGL2 plasmid. pGL2TH plasmids were transiently expressed in cultured catecholaminergic cells - bovine adrenal medullary (BAMC) and human neuroblastoma SH5YSY. Deletion of the longest DSE4, positioned close to the TATA box (-105/-75bp), led to an increase in luciferase expression in SH5YSY and BAMC. Point mutation that disrupted dyad symmetry of DSE4 had an opposite effect on promoter activity. Deletion of DSE0 or DSE1 in the distal region of TH promoter (-430/-302), decreased promoter activity in SH5YSY and in BAMC. In cells that do not express endogenous TH gene (TE671) the same deletion increased expression of TH-Luciferase construct. Thus, DSE0 and DSE1 could contribute to a cell-specific regulation of the TH gene. Deletion of GC-rich element led to a 3-4-fold increase in promoter activity in SH5YSY and in BAMC. We conclude that the novel dyad symmetry and GC-rich elements found in TH gene may play a role in the regulation of basal promoter activity. Together with classical regulatory elements (AP1, CRE, etc.) they could confer regulation specific for the TH gene. The mechanisms by which they act are now under investigation.

520.4

In Vivo Deletion Analysis of the Rat Preproenkephalin Promoter Using a Defective Herpes Simplex Viral Vector M.G. Kaplitt[1,*], J. Yin[1], A.D. Kwong[2], S.P. Kleopoulos[3], C.V. Mobbs[3], D.W. Pfaff[1]
[1]Laboratory of Neurobiology and Behavior, The Rockefeller University, NY, NY, 10021; [2]Schering-Plough Research, Bloomfield, NJ, 07003; [3]Fishberg Center for Neurobiology, Mount Sinai School of Medicine, NY, NY, 10029.

We have previously reported that a 2.7kb fragment of the rat preproenkephalin (PPE) promoter can yield region-specific expression of the bacterial lacZ gene within the rat brain from a herpes simplex virus (HSV) defective viral vector. In the current study, PPE promoter deletions were generated in the amplicon pHENK by restriction digestion and re-ligation of the plasmid. The resulting defective HSV vectors were infused into the amygdala, piriform cortex and caudate nucleus. Three days following infusion, animals were sacrificed and brain sections were histochemically processed to detect β-galactosidase expression. While substantial expression was demonstrated in these regions with the 2.7kb PPE promoter, confirming earlier observations, there was a large decrease in the number of lacZ-positive cells in these regions when 1.5kb, 0.8kb or 0.5kb of the PPE promoter was employed. This suggests that there is an element between 2.7kb and 1.5kb which positively influences PPE expression in these brain regions. Finally, we continue to see a complete absence of expression in the dorsolateral neocortex from the 2.7kb promoter. Interestingly, however, some cells were observed with the 1.5kb promoter, but again no positive cells were detected with the 0.8kb or 0.5kb promoter fragment.

520.5

CHARACTERIZATION OF THY-1 REGULATORY ELEMENTS IN TRANSGENIC MICE. K.A. Kelley*, A. Sonshine, Y. Hu, J. Li, J. Lax, V.L. Friedrich Jr. Fishberg Research Center for Neurobiology, and Brookdale Center for Molecular Biology, The Mount Sinai School of Medicine, New York, NY 10029.

Three Thy-1.2/*lacZ* hybrid genes were constructed and used for the production of transgenic mice. Two of the hybrid genes consist of the Thy-1.2 promoter (up to the ATG translational start site within the second exon) fused to the *lacZ* coding region with either an SV40 or Thy-1.2 polyadenylation signal. The third construct consists of the entire Thy-1.2 gene with the *lacZ* coding region inserted into the second exon such that the only protein encoded by this fusion gene is the bacterial reporter. Although a Thy-1.2 transgene (modified by the addition of a unique oligonucleotide sequence to the 3' untranslated region) was expressed in a position-independent manner as expected, the Thy-1/*lacZ* hybrid genes were only weakly expressed in a position-dependent manner in the CNS of some of the transgenic mice examined. There is wide variability in the cell-type specific expression patterns observed by ß-galactosidase histochemistry in the Thy-1 *lacZ* mice. Finally, mice produced from a Thy-1.2 hybrid gene containing the promoter, a segment of exons 2 and 3 in an antisense orientation and the polyadenylation signal expressed this transgene in a position-independent manner at levels which are very similar to those of the endogenous gene. These results suggest that either the Thy-1.2 sequences which are necessary for appropriate neuron-specific expression are not contained solely within the proposed CNS enhancer in the first intron, or that the placement of the *lacZ* coding region within the second exon may disrupt normal Thy-1 regulatory signals.

Supported by the NSF (BNS-9021155).

520.6

c-JUN SYNERGISTICALLY ACTIVATES NEUROTENSIN GENE EXPRESSION IN COMBINATION WITH GLUCOCORTICOIDS AND ADENYLATE CYCLASE ACTIVATORS IN PC12 CELLS. P. R. Dobner*, R. H. Harrison and G. P. McNeil. Dept. of Mol. Genetics and Micro., Univ. of Mass. Med. Cent., Worcester, MA 01655.

Neurotensin (NT) gene expression is synergistically regulated in PC12 cells by combinations of nerve growth factor, dexamethasone, and adenylate cyclase activators. A consensus AP-1 site located within a 200 bp region flanking the start site of transcription of the rat gene is a critical *cis*-regulatory element required for full induction, but functions only in the context of adjacent inducible sites resulting in the integration of multiple signals at the level of the promoter. To determine whether specific AP-1 complexes are involved in mediating NT gene activation, various AP-1 expression plasmids were co-transfected by electroporation into PC12 cells and the cells were grown either in the presence or absence of dexamethasone (1 µM) and forskolin (1 µM). Co-transfection of individual AP-1 expression plasmids revealed that c-Jun transactivated reporter gene expression most effectively and acted synergistically with dexamethasone and forskolin to increase reporter gene expression by up to 2,500-fold. Co-transfection of pairs of AP-1 expression plasmids revealed that both c-Fos and Fos B potentiated the synergistic effect of c-Jun, while Fra-1 or Fra-2 in combination with c-Jun resulted in negative regulation by glucocorticoids and forskolin. Surprisingly, c-Fos potentiated synergistic interactions between c-Jun and glucocorticoids over a wide range of input c-Fos expression plasmid concentrations. This contrasts sharply with the ability of c-Fos to reverse the direction of interaction between c-Jun and glucocorticoids in the regulation of the proliferin composite GRE. These results indicate that specific AP-1 factors, namely c-Jun homodimers and either c-Jun/c-Fos or c-Jun/Fos B heterodimers, selectively activate the NT promoter due to functional interactions with the glucocorticoid and cAMP signalling pathways.

520.7

ANTISENSE OLIGONUCLEOTIDE OF c-myc IN PROBING THE DIFFERENTIAL REGULATION OF METALLOTHIONEIN BY ZINC. A. Takeda, J.S. Norris, J.E. Mata, P.L. Iversen, and M. Ebadi*. Dept. of Pharmacology, Univ. Nebr. Coll. Med., Omaha, Nebraska 68198-6260.

The metallothionein II genes, whose structure is highly conserved throughout the animal kingdom is composed of three exons and two introns. By using synthetic antisense oligonucleotides with sequences complementary to the mRNA coding for human metallothionein II, we have shown that A) inhibition of metallothionein synthesis causes cells to die from metal toxicity; B) metallothionein possesses an essential gene; and C) modification in oligonucleotide structures exhibit specificity in inhibiting metallothionein synthesis. Furthermore, we have prepared a synthetic antisense oligodeoxyribonucleotide with sequence complementary to the mRNA specific for human c-myc and tested its potential to regulate metallothionein synthesis in cells in culture. The results of this study revealed that the c-myc antisense oligodeoxyribonucleotide lead to induction of zinc promoted but not cadmium- or dexamethasone initiated induction of metallothionein. The results of these studies are interpreted to suggest that c-myc acts as a repressor of at least one of the six human metallothionein isoforms and demonstrates a unique regulation capable of discriminating between zinc- and dexamethasone-induced synthesis of metallothionein. (Supported in part by grants from USPHS ES 03949 and Lynx Therapeutics, Inc.)

520.8

BOTH BASAL AND cAMP-INDUCIBLE TRANSCRIPTION OF THE DOPAMINE-ß-HYDROXYLASE GENE REQUIRE cAMP-DEPENDENT PROTEIN KINASE (PKA). K.S. Kim, H. Ishiguro, D.H. Park, J.A. Wagner and T.H. Joh*. Cornell Univ. Med. Coll. at the Burke Med. Res. Inst., White Plains, NY 10605.

The human DBH gene has a cAMP response element (CRE)-like sequence motif (5'-TGACGTCC-3') at -181 to -174 bp upstream of the transcription initiation site. Site-directed mutation of this element not only diminished >95% of basal transcription activity, but also significantly reduced its responsiveness to elevation of intracellular cAMP, thus defining this CRE as an essential cis-acting element for both basal and cAMP-inducible transcription of the human DBH gene (Hiroshi et al., Soc. Neuro. Abst. 18, 243, 1992). Based on this result, it is tempting to speculate that PKA regulates both basal and cAMP-inducible transcription of the human DBH gene via the CRE. To test this hypothesis, we transiently transfected plasmids coding for either catalytic subunit of PKA (PKA_c) or a specific inhibitor of PKA (PKI), and assessed the effect of each on expression of a co-transfected reporter gene construct, DBH-CAT. Expression of PKI, but not an inactive mutant form of PKI, specifically blocked transcriptional induction following treatment with forskolin. Moreover, co-transfection of PKA_c robustly stimulated transcriptional activity of the DBH promoter, but not that of the Rous Sarcoma Virus (RSV) promoter, in a dose-dependent manner. Primer extension analysis demonstrated that reporter gene activity represented correctly initiated transcripts. Furthermore, we analyzed several PC12 cell lines rendered PKA-deficient by either genetic manipulation or chemical mutagenesis. Notably, DBH gene expression and regulation were substantially altered in all cell lines tested. This study strongly indicates that PKA regulates both basal and inducible expression of the human DBH gene at the transcriptional level. Supported by MH48866 (KSK) and MH24285 (THJ).

520.9

GROWTH HORMONE RELEASING FACTOR INDUCES SOMATOSTATIN MRNA LEVELS IN THE RAT PERIVENTRICULAR NUCLEUS BY ACTIVATING A GUANYLATE CYCLASE VIA NITRIC OXIDE. M.C. Aguila*. Dept. of Physiology, UT Southwestern Med. Ctr., Dallas, TX 75235.

Growth hormone releasing factor (GRF) stimulates somatostatin (SRIF) release and cGMP accumulation from median eminences in vitro by activating calmodulin. GMP analogues can increase SRIF mRNA levels and its release from periventricular nucleus (PeN). Thus, this study was undertaken to evaluate the effect of GRF and the possible role of cGMP on SRIF mRNA levels and its release in the PeN. of male rats incubated in vitro. PeN explants were incubated in Waymouth's medium with GRF (10^{-9}M), methylene blue (10 µM, a guanylate cyclase inhibitor) and Rp8BrcGMPS [1 µM, a protein kinase G (PKG)inhibitor]. Levels of SRIF mRNA were determined by S_1 nuclease protection assay using a ^{32}[P]labelled rat SRIF riboprobe. SRIF release and cGMP formation were measured at 30 min and 6 hr by RIA. GRF (10^{-9}M) increased SRIF mRNA levels ($P<0.001$). SRIF release and cGMP formation were also stimulated by GRF (10^{-9}M) at 30 min and 6 hr. The stimulating effect of GRF was abolished by N^G-monomethyl-L-arginine (10^{-5}M,L-NMMA), but not by D-NMMA. The GRF evoked SRIF mRNA levels and release was abolished by methylene blue and Rp8BrcGMPS. By themselves they did not modify basal values. These results indicate that GRF-induced cGMP formation from the PeN via nitric oxide. The cGMP generated activates PKG and increases SRIF mRNA levels and SRIF release. (NIH grant NS26821).

520.10

DIFFERENTIAL INDUCTION OF EARLY GENES IN THE CNS BY TYPICAL AND ATYPICAL NEUROLEPTICS. P. J. Rogue*, G. Vincendon and A. N. Malviya. Centre de Neurochimie, 5 rue Blaise Pascal, Strasbourg, 67084 France.

Dopamine D_2 receptors regulate the expression of a specific set of immediate early genes (IEG) in the rat striatum. A single injection I.P. of haloperidol (2 mg/kg) or sulpiride (100 mg/kg) produces a rapid and transient increase in c-*fos*, c-*jun*, *jun* B and zif268 mRNA, but has no influence on the expression of ETR1 or *jun* D (Brain Res Bull 29, 469). These inductions are specifically blocked by pretreatment with a D_2 agonist (1 mg/kg quinelorane). We further studied the effect of clozapine and dopamine D_2 receptor antagonists on IEG expression in different regions of the CNS by northern analysis and ISH. Both clozapine (20 mg/kg) and haloperidol (2 mg/kg) induce zif268, c-*fos*, and *jun* B in the nucleus accumbens. However, only haloperidol induces all of these proto-oncogenes in the striatum, whereas in the frontal cortex clozapine induces c-fos but not zif268. The effects of the prolonged administration of these compounds will also be presented. The significance of these specific IEG activation patterns will be discussed with respect to the mechanism of action of antipsychotics and to the expression of dopamine D_2 receptor gene upon prolonged treatment with antagonists (European J Pharmacol 207, 165).

520.11

DIFFERENT ELECTRICAL ACTIVITY PATTERNS SELECTIVELY REGULATE GENE EXPRESSION A. Buonanno*, R. Beers, and S. Basu, Laboratory of Developmental Neurobiology, NIH, Bethesda, MD 20892

We have analyzed how motoneuron innervation selectively regulates gene expression in skeletal muscle. The selective expression of the two troponin I isoforms in fast- or slow-muscle is controlled by innervation. We found that TnIs (slow) and TnIf (fast) mRNA levels were dramatically reduced 2 days after denervation. However, if the rat soleus (composed of slow-twitch myofibers) was denervated and immediately stimulated with extracellular electrodes using a "slow" muscle pattern (20 Hz,10s every 30s), expression of the TnIs mRNAs was maintained at the same levels of innervated muscle. The response to the activity pattern was selective, because stimulation with a pattern typical of fast-twitch muscle (100 Hz,1s every 100 sec) could not prevent the down-regulation of the TnIs gene after denervation. Interestingly, stimulation using 100 Hz activated transcription of the TnIf gene in this slow-type muscle. The TnIs gene was cloned and sequences required for its transcriptional regulation were studied in transfected cells and transgenic mice. We found that TnIs sequences upstream of the CAP site, as well as in the second intron, were necessary for high level CAT reporter expression in transfected C2C12 myotubes. Deletion analysis of the TnI 5'-flanking sequence demonstrated that 200 bp are sufficient to confer tissue- and differentiation-specific expression in transfected cultured cells. Interestingly, the MyoD-related factors were found to transactivate the TnI-CAT constructs differentially in transfected NIH-3T3 fibroblasts. In transgenic mice, TnIs upstream sequences conferred specific skeletal muscle expression, as well as confined transcription to slow-type muscle. Recent experiments suggest that the MyoD-related factors do not underlie the confined expression to muscle-types. In summary, activity patterns selectively activate different programs of gene expression underlying the plasticity of muscle phenotype.

ION CHANNEL MODULATION AND REGULATION II

521.1

OPENING OF LARGE-CONDUCTANCE CALCIUM-ACTIVATED POTASSIUM CHANNELS AND DEPRESSION OF SYNAPTIC TRANSMISSION BY THE SUBSTITUTED BENZIMIDAZOLONE, NS004. V. K. Gribkoff[1]*, M.C. McKay[1], S.I. Dworetzky[1], N.A. Meanwell[1], C.G. Boissard[1], J.T. Lum-Ragan[1], P.H. Reinhart[2], I.B. Levitan[3], J.P. Adelman[4], and S.P. Olesen[5]. [1]Bristol-Myers Squibb Co., Wallingford CT 06492, [2]Dept.Neurobiology, Duke Univ. Med. Cntr., Durham NC 27710, [3]Grad. Dept. Biochem. & Cntr. For Complex Systems, Brandeis Univ., Waltham MA 02254, [4]Vollum Institute, Oregon Hlth. Sci. Univ., Portland ORE 97201, [5]NeuroSearch, A/S, Glostrup Denmark DK-2600.

The ability of 5-trifluoromethyl-1-(5-chloro-2-hydroxyphenyl)-1,3-dihydro-2*H*-benzimidazole-2-one, NS004, to open large-conductance Ca^{2+}-activated K^+ (maxi-K, BK) channels was examined with channels in membrane patches from a clonal pituitary cell line (GH_3), with channels from rat brain plasma membrane preparations reconstituted into planar lipid bilayers, and with the recently-described *Slo* BK clone from *Drosophila* expressed in *Xenopus laevis* oocytes. In GH_3 cells and in oocytes expressing the *Slo* gene product, NS004 produced an increase in an iberiotoxin or TEA-sensitivewhole-cell outward current, and produced a significant increase in the activity of single BK channels characterized by an increase in channel mean open time, a decrease in interburst interval, and an apparent increase in channel Ca^{2+}/voltage sensitivity. In hippocampal slices, NS004 produced a concentration- dependent depression of population synaptic potentials that was largely blocked by iberiotoxin. NS004 reduced orthodromically -driven population spikes to a significantly greater extent when compared to its effects on antidromically driven responses. These data indicate that NS004 is a useful tool for investigating the properties of BK channels, and for delineating the roles of BK channels in neuronal function.

521.2

ACTIVATION OF Ca^{2+}-DEPENDENT K^+ CHANNELS BY THE BENZIMIDAZOLONES NS 004 AND NS 1619. S.-P. Olesen, E. Munch, P. Moldt & J. Drejer*. NeuroSearch A/S, 26B Smedeland, DK-2600 Glostrup, Denmark.

The ability of novel benzimidazolone derivatives to activate large-conductance Ca^{2+}-dependent K^+ channels (BK channels) was studied in cultured cerebellar granule cells by the patch-clamp technique.

In nystatin-perforated whole-cell recordings the granule cells expressed an outward BK current, which was strongly increased by the compounds NS 004 and NS 1619. The BK channel openers shifted the activation curve towards more negative membrane potentials, and the average effects of the most potent compound, NS 1619, at 10 and 30 μM concentrations were shifts of -26 and -55 mV, respectively. The effects were fully reversible and were antagonized by TEA^+ (0.3-1 mM). The compounds did not significantly modulate the Na^+ and K_V currents, which were also recorded in the granule cells. NS 1619 (30 μM) hyperpolarized the cells by an average -13 mV. In single channel recordings the compounds increased the channel open probability in a dose-dependent way by up to 24 fold (30 μM NS 004) and 92 fold (30 μM NS 1619). On inside-out patches the compounds increased the channel activity at a wide range of free Ca^{2+} concentrations (10-300 nM), and the activation did not require internal ATP.

The compounds also activated BK channels in cultured cortical neurones, PC12 cells and smooth muscle cells from calf aorta.

We have developed novel activators of BK channels in neurons and smooth muscle, which may significantly modulate cell excitability.

521.3

INHIBITION BY ATP OF A Ca^{2+} CURRENT AND Ca^{2+}-ACTIVATED K^+ CHANNEL IN ISOLATED NEUROHYPOPHYSIAL TERMINALS. G. Wang* and J.R. Lemos, Worcester Found. Exp. Biol., Shrewsbury, MA 01545.

Since ATP is known to act as a neurotransmitter and is co-released with neuropeptides from neurohypophysial terminals, we examined the effects of externally applied ATP on terminal activity. Elicited action potentials exhibited a slower second phase of repolarization in the presence of 0.5 mM external ATP. 1 mM ATP had no effect on the fast, transient K^+ or A current, which is involved in the first phase of repolarization, nor on the Na^+ current of these terminals.

In contrast, externally applied ATP did block the Ca^{2+}-activated K^+ current, which appears to be responsible for the latter phase of repolarization, with an IC_{50} of *ca.* 0.5 mM. At mM concentrations ATP could also inhibit the transient, but not the long-lasting, component of the terminal Ca^{2+}-current. It thus seemed possible that ATP's inhibition of the K^+ current was solely due to a reduction in Ca^{2+} entry. When ATP was directly applied to outside-out patches, however, the open probability and apparent amplitude of the type II Ca^{2+}-activated K^+ channel were greatly reduced. The inhibition by ATP was unaffected by H-7, indicating that it was not mediated by activation of a kinase. We are determining if these are direct effects of ATP or if they are mediated by a purinergic receptor. (Supported by grants from NIH and NSF).

521.4

MODULATION OF *SLO* AND LARGE-CONDUCTANCE CA^{2+}-ACTIVATED K^+ CHANNELS FROM RAT CORTEX. J. Krause, F.M. Schmalz, and P.H. Reinhart*, Dept. of Neurobiology, Duke University Medical Center, P.O. Box 3209 Durham, NC 27710.

We have characterized the modulation of two large-conductance (240 pS) Ca^{2+}-activated K^+ channels from rat cortex vesicles, and the modulation of *SLO*, a 120 pS Ca^{2+}-activated K^+ channel from *Drosophila*. All three channels are modulated by protein kinases and protein phosphatases. The modulation of *SLO* is similar to that of Type-II Ca^{2+}-activated K^+ channels from rat cortex in that the addition of Mg-ATP (in the absence of any exogenously added protein kinase) can up-modulate this channel in inside-out patches from *Xenopus* oocytes. The effect can be enhanced by the addition of phosphatase inhibitors and partially reversed by the addition of protein phosphatases to the cytoplasmic side of the channel. The hydrolyzable ATP analog ATP-γ-S can mimic this effect but the non-hydrolyzable analog AMP-PCP cannot indicating that a channel-associated protein kinase is mediating this effect of ATP. Mg-ATP has a second effect on both rat cortex Type-II and on *SLO* channels. This effect is to induce both sub-conductance states, and long-lived closed states. The non-hydrolyzable ATP analog AMP-PCP can mimic this effect, indicating that it is due to ligand binding rather than phosphorylation. We are currently examining these modulatory phenomena in more defined conditions by reconstituting *in vitro* translated *SLO* channels into planar lipid bilayers. [Supported by NIH grant NS31253-01 to PHR]

521.5

HALOTHANE BLOCKS BK CHANNEL ACTIVITY BY ALTERING ITS STATE OF PHOSPHORYLATION R.A. Pearce* and K. Bielefeldt Departments of Physiology, Anatomy, and Anesthesiology, Univ. Wisconsin, Madison, WI 53706.

Many channels that are affected by volatile anesthetics are also modulated by phosphorylation. To test the hypothesis that volatile anesthetics affect channel activity by altering channel phosphorylation state we examined the effect of halothane on calcium activated potassium channels (BK) from posterior pituitary nerve terminals. These channels are modulated by a protein kinase and a protein phosphatase that remain associated with the channel and functional even upon excision of membrane patches containing single channels. Excised inside-out patches were exposed to symmetrical solutions containing (mM) KCl 150, $MgCl_2$ 2, $CaCl_2$ 0.1-0.5, EGTA 0.5, HEPES 10 at pH 7.2. Halothane 2% (gas phase concentration) was blown across the recording chamber, reaching a concentration of 0.22 mM.

In the presence of MgATP (1 mM), halothane significantly reduced single channel activity by shifting the voltage of half-activation by approximately +40 mV (n=6). This effect was independent of the concentration of free calcium, was identical to the effect of dephosphorylation, and was reversible upon washing out halothane. Single channel conductance was not altered, and halothane had no effect on the unphosphorylated channel in the absence of MgATP.

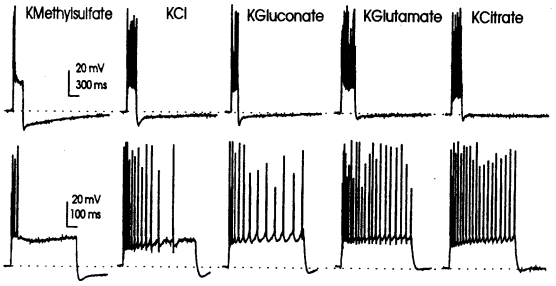
To test whether halothane altered channel activity by altering its state of phosphorylation, channel activity was recorded in the presence of MgATP and the phosphatase inhibitor okadaic acid (50 nM). By preventing channel dephosphoryation, okadaic acid blocked the effect of halothane (n=5). This effect appeared to be due to inhibition of the protein kinase rather than activation of the phosphatase, since even in the presence of okadaic acid halothane prevented recovery of channel activity upon addition of MgATP.

Supported by NIH grants NS30016 (M.B. Jackson) and NS01548 (R.A.Pearce).

521.6

EFFECTS OF INTERNAL ANIONS AND Ca BUFFERS ON Ca^{2+}-DEPENDENT I_{AHP} IN HIPPOCAMPAL CA1 NEURONS L. Zhang* & P.L. Carlen, Playfair Neurosci. Unit, MRC group "Nerve Cell and Synapse", Bloorview Epilepsy Prog., Univ. of Toronto, Canada M5T 2S8.

We studied the slow AHP and underlying Ca^{2+}-dependent K^+ current (I_{AHP}) in mature rat hippocampal neurons using whole-cell recordings in brain slices with internal solutions containing different potassium salts (see below). The AHP and strong firing adaptation could only be reliably recorded with KMethylsulfate-containing electrodes. The I_{AHP} was not affected by ryanodine or dantrolene, but was blocked by BAPTA (K_d = 160 nM). However, it is greatly prolonged by the low-affinity Ca^{2+} chelator difluoro BAPTA (K_d = 4.6 μM), but not by APTRA (K_d = 25 μM). We conclude that cytoplasmic Ca^{2+} homeostasis is greatly influenced by internally applied anions and Ca^{2+} buffers.



521.7

CALCINEURIN INVOLVEMENT IN 5-HT STIMULATION-PRODUCED REDUCTIONS IN MBK1 CHANNEL ACTIVITY. J. Farley*, J. Hoger, N. Davison and H. Lester. Program in Neural Science, Indiana University, Bloomington, IN 47405 and Division of Biology, California Institute of Technology, Pasadena, CA 91125.

Stimulation of Xenopus oocytes expressing serotonin type 1c (5-HT1c) receptors and KV1 type potassium channels from mouse brain (MBK1) with 5-HT results in a calcium-dependent suppression of MBK1 channel activity which is unaffected by the general kinase inhibitor H-7 (Hoger et al., Neuron, 1991). We have examined the possibility that the calcium-activated phosphatase PP2B (calcineurin) may participate in this modulation by testing the effects of 5-HT upon MBK1 channels following exposure of oocytes to several potent phosphatase inhibitors. Exposure of oocytes to cyclosporine A -- a specific inhibitor of PP2B -- greatly attenuated 5-HT-produced reduction of MBK1 channels despite the absence of any large or consistent effect upon the 5-HT-induced Cl^- current. In contrast, neither Calyculin A nor okadaic acid, potent inhibitors of PP1 and PP2A, prevented 5-HT-mediated reduction of MBK1 channels when bath-applied at concentrations 10-100 times greater than their reported IC_{50}-values. As judged by their effects on several of the currents endogenous to the oocyte, both compounds appeared to enter the cell. In preliminary experiments, injection of pre-activated PP2B into oocytes resulted in a clear decrease in MBK1 activity. Collectively, our results suggest that calcium-dependent dephosphorylation regulates the functional activity of MBK1 channels.

521.8

ANALYSIS OF ION CURRENTS MEDIATING MODULATION OF CONTRACTIONS OF THE ARC MUSCLE OF *APLYSIA* BY SIMULTANEOUS ON-LINE LENGTH MEASUREMENT AND CURRENT/VOLTAGE CLAMP. V. Brezina* and K. R. Weiss. Dept. of Physiology and Biophysics, Mt. Sinai School of Medicine, CUNY, New York, NY.

In response to behavioral demands, ACh-induced contractions of the ARC muscle of *Aplysia* are modulated by serotonin (5-HT) and a variety of peptide cotransmitters released from the muscle's own motorneurons, among them the small cardioactive peptides (SCPs), myomodulins (MMs) and FMRFamide-related peptides (FRFs). The SCPs, 5-HT and MM_B predominantly potentiate the contractions, the FRFs depress them, and MM_A both potentiates and depresses. Previously, working with single dissociated ARC muscle fibers, we found that this pattern of effects on contractions is reproduced in the pattern of the modulators' effects on two ion currents: the potentiating modulators enhance the 'L'-type Ca current, while the depressing modulators activate a large K current. We have now combined current and voltage clamp (CC, VC) with on-line video measurement of contractions of the single fibers to obtain the following direct evidence that these actions on ion currents indeed underlie the modulation of contractions: (1) Ca^{2+} entry through the 'L'-type Ca channels is essential for normal contraction. Depolarization beyond the activation threshold of the Ca current is both necessary and sufficient for contraction, equally with ACh application and CC/VC steps in the absence of ACh. All such contractions cease in Ca^{2+}-free solution or when the Ca channels are blocked with nifedipine. (2) All contractions involving activation of the Ca current, whether due to ACh or CC/VC steps, are equally potentiated by the potentiating modulators, whereas contractions that bypass its activation, e.g. induced by the Ca^{2+}-ionophore A23187 or caffeine, are not. (3) Depression of ACh or CC contractions is associated with a reduction in the depolarization attained, and thus the degree to which the Ca current is activated. This reduction is both sufficient and necessary (VC contractions are not depressed). Both the reduction in depolarization and the depression of contractions are blocked by low-micromolar 4-AP, which selectively blocks the modulator-activated K current. It thus appears likely that the enhancement of the 'L'-type Ca current is in large part responsible for the potentiation, and activation of the 4-AP-sensitive K current for the depression, of ARC-muscle contractions by the SCPs, MMs, FRFs and 5-HT.

521.9

HETEROTYPIC GAP JUNCTION CHANNELS FORMED OF HUMAN CONNEXINS 43 AND 45 IN MAMMALIAN CELLS CONSERVE THE PROPERTIES OF HEMICHANNELS. Alonso P. Moreno*, Glenn. I. Fishman, Eric.C. Beyer and David C.Spray. Dept of Neuroscience, A. Einstein College of Medicine, Bronx N.Y. 10461 and Dept of Medicine, Washington University, St Louis MO. 63110.

Some cells express multiple connexins (Cx), raising the possibility of heterotypic channels formed by homomeric hemichannels. Studies in exogenous expression systems (*Xenopus* oocytes or mammalian cell lines) have revealed that conductance of homotypic mammalian gap junctions is reduced by transjunctional voltage of either polarity. Homotypic human Cx43 (hCx43) expressed in SKHep1 cells forms channels with unitary conductance (γ_j) of ≈60 and ≈90 pS with modest voltage dependence (V_o=61 mV, A=.085 and gmin=0.37), whereas hCx45 forms more voltage dependent (Vo=14 mV, A=0.115 and gmin=.06) channels of 30 pS . Cells expressing hCx45 were loaded with a red fluorescent dye (λ=680 nM) and cells expressing hCx45 were loaded with a green fluorescent dye (λ=520 nm); subsequently they were split and plated together. Pairs of bi-color cells exhibited junctional properties expected for the sum of the corresponding hemichannels. Gates of both hemichannels closed for relatively negative potentials. Total junctional conductance in these pairs was 5 times higher than for hCx45 pairs, suggesting that hCx43-hCx45 interactions are favored over homotypic hCx45 coupling. γ_j values ranged between 30 and 50 pS, corresponding to the Ohmic sum of conductances from each connexon. Dye coupling was also connexon-specific; homotypic hCx43 pairs were Lucifer yellow permeant, but homotypic hCx45 (n=50) and hCx45-hCx43 pairs (n=16) were not. All pairs were permeable to Neurobiotin. Thus, heterotypic channels conserved the voltage dependence, permeability and unitary conductance properties, characteristic of the constituent hemichannels.

521.10

PHOTORELEASE OF ATP REVERSES THE EFFECTS OF 2-DEOXYGLUCOSE ON Ca^{2+}-ACTIVATED CHLORIDE CURRENTS RECORDED FROM CULTURED DRG NEURONES S.R.Stapleton, J.F.Wootton+ and R.H.Scott*. Dept.Physiology. St.George's Hospital Medical School London SW17 ORE.UK and +Dept.Physiology. Cornell University. Ithaca. N.Y.

In this study we have used whole cell Ca^{2+}-activated Cl^- currents ($I_{Cl(Ca)}$) recorded from cultured dorsal root ganglion neurones as a physiological index of intracellular Ca^{2+} homeostasis. $I_{Cl(Ca)}$ was activated as a tail current following Ca^{2+} current evoked by 500ms steps to 0mV or by intracellular photorelease of Ca^{2+} from DM nitrophen (4mM with 2mM Ca^{2+}). Under control conditions $I_{Cl(Ca)}$ tail currents deactivated by 63% (t63%) in 2990±660ms (n=7) and this did not change when 4 currents were activated at 1 min^{-1}. However, with no ATP in the patch pipette solution and substitution of glucose with 2-deoxyglucose (2DG) (5mM) the $I_{Cl(Ca)}$ was greatly prolonged. The t63% under these conditions were 4000 ±800ms and 7350±2080ms (n=11) for the 1st and 4th currents respectively. The deactivation of $I_{Cl(Ca)}$ evoked by photorelease of Ca^{2+} was also slowed with 2DG compared with controls. The effects of 2DG were prevented by including fructose 1,6-diphosphate (500μM) in the patch pipette solution or by intracellular photorelease of about 300μM ATP from a caged precursor. Our results suggest that efficiency of intracellular Ca^{2+} handling is reversibly attenuated by metabolic stress as reflected by prolongation of $I_{Cl(Ca)}$.

521.11

THE GTP-ANALOGUE 2',3'-DIALDEHYDE GTP ABOLISHES THE MODULATION OF NEURONAL CALCIUM CURRENTS BY VARIOUS NEUROTRANSMITTERS. S.Boehm, C.Nanoff, M.Hohenegger, M.Freissmuth, S.Huck. (SPON: European Neuroscience Ass.) Depts. of Neuropharmacology and Pharmacology, Univ. of Vienna, Waehringerstrasse 13a, A-1090 Vienna, Austria.

Neurotransmitter modulation of calcium currents (I_{Ca}) is mediated by pertussis toxin (PTX)-sensitive G proteins in many neuronal cell types. In order to study the regulation of ion channels by G proteins, we have synthetized 2',3'-dialdehyde GTP (oGTP), a ligand which is predicted to irreversibly inactivate G proteins via Schiff's base formation; this assumption was verified as follows: (i) oGTP inhibited the binding of [^{35}S]GTPγS to the recombinant stimulatory G protein α-subunit ($rG_{s\alpha}$) with an affinity comparable to that of GTP; (ii) in contrast to GTP, oGTP was bound in a quasi-irreversible manner, since dissociation could not be achieved by an excess of GTPγS; (iii) following borhydride reduction, oGTP was covalently incorporated into $rG_{s\alpha}$; (iv) in the S49 cyc$^-$ reconstitution assay, oGTP supported a single cycle of activation as determined by using a GTPase-deficient mutant of rGsα, R187E, but oGTP prevented the GTPγS-dependent activation of adenylyl cyclase by $rG_{s\alpha}$, since it trapped the G protein in the inactive conformation.

Using the whole-cell patch-clamp technique, we applied the $GABA_B$ agonist baclofen in chick sensory neurons, somatostatin in chick sympathetic neurons, the muscarinic agonist oxotremorine in rat sympathetic neurons, and the α_2-agonist bromoxidine in all these neurons. With 2mM GTP included in the recording pipette the inhibitory effect of bromoxidine (30%-45%) was smaller than the action of baclofen (48%), somatostatin (55%), or oxotremorine (45%) (n=4). After a 24h treatment with 100ng/ml PTX inhibition of I_{Ca}'s in sympathetic neurons was <4%, and with intracellular GDPβS (100μM) the I_{Ca}'s of sensory neurons were reduced by <5% (n≥3), indicating an involvement of G proteins. Inclusion of 10μM oGTP in the recording pipette progressively reduced the inhibitory action of all agonists (when recordings were started 90s after formation of the whole-cell configuration), and agonist effects were entirely abolished within ≤15min (<5% inhibition, n.s. vs controls, n=4). To confirm the site of action we added increasing concentrations of GTPγS to the internal solution containing 10μM oGTP: 100μM GTPγS suppressed I_{Ca}, as determined by the reversal through large depolarizing prepulses, and prevented an additional inhibition by external agonists.

These results indicate that oGTP inactivates G proteins involved in the modulation of I_{Ca} and render the drug a versatile tool for patch-clamp experiments, particularly for delineation of signalling pathways via PTX-insensitive G proteins.

521.12

TACHYKININS, METABOTROPIC GLUTAMATE AGONISTS, AND NEUROTENSIN EXCITE NEURONS FROM THE RAT VENTRAL TEGMENTAL AREA. R.H. Farkas[1], S.Nakajima[2] and Y.Nakajima[1]* Depts. of Anat. and Cell Biol.[1] and Pharmacol.[2], Univ. of Illinois, College of Med. at Chicago, IL, 60612.

The slow depolarization caused by many excitatory neurotransmitters results from the simultaneous induction of nonselective ionic current and suppression of potassium conductance. Using whole cell patch clamp recording, we found that neurokinin B, the metabotropic glutamate agonist 1S3R-ACPD, and neurotensin excited, by such dual ionic mechanisms, neurons cultured from the rat ventral tegmental area. Each induced an inward current and suppressed inwardly rectifying potassium conductance elicited by the D2 agonist quinpirole. The inward current could be greatly reduced by lowering external sodium. Lowering intracellular Ca^{2+} by loading neurons with 5.5 mM BAPTA, did not reduce the inward current, indicating that it was not due to calcium activated non-specific cation (CAN) channels. When the neurokinin B or 1S3R-ACPD responses were desensitized by continuous drug application, the neurotensin response remained, suggesting receptor level desensitization. The neurotensin antagonist SR48692 reversibly and selectively blocked the neurotensin response. In neurons loaded with GTPγS, neurotensin irreversibly suppressed inwardly rectifying potassium conductance but failed to irreversibly induce the nonselective conductance, suggesting that the potassium conductance is gated by a G protein while the nonselective conductance is G protein-independent. Supported by AG06093, NS24711, and F30MH10167.

INGESTIVE BEHAVIORS V

522.1

ROLE OF GASTRIC AFFERENT VAGUS IN THE MEDIATION OF CCK SATIETY. T.H. Moran*, C.F. Salorio, I. Tominack, P.R. McHugh and G.J. Schwartz. Department of Psychiatry, Johns Hopkins University School of Medicine, Baltimore, MD 21205

Previous work has shown that either sectioning of both gastric vagal branches or subdiaphragmatic vagal deafferentation were sufficient to eliminate the feeding inhibitory actions of CCK. Together these results suggested that gastric vagal afferents were the necessary vagal componets for CCK satiety. The present work directly assessed this hypothesis. Five surgical groups were prepared. To replicate the results of prior studues, we included animals with bilateral gastric branch vagotomy, unilateral afferent or efferent rootlet transections combined with contralateral subdiaphragmatic vagotomy and animals receiving sham surgery. To specifically address the present question, we also prepared a group with unilateral afferent rootlet transection combined with contralateral gastric branch vagotomy. Following surgery animals were maintained on a liquid diet (Ensure) and the responses to CCK (1-16 μg/kg) were assessed in 1 hr glucose consumption tests following daytime deprivation. The results demonstrated that either bilateral gastric branch vagotomy or subdiapharagmatic vagal deafferentation were sufficient to eliminate CCK satiety. However, neither subdiaphragmatic vagal deefferentation nor specific gastric vagal deafferentation significantly affected the ability of CCK to inhibit intake. These results demonstrate that gastric vagal afferents are not the necessary component for CCK satiety and suggest that CCK may interact with multiple vagal components. (DK19302).

522.2

PUSH-PULL PERFUSION REVEALS MEAL-DEPENDENT CHANGES IN THE RELEASE OF BOMBESIN (BN)-LIKE PEPTIDES. H. Plamondon[1]* & Z. Merali[1,2]. [1]Sch. of psychology & [2]Dept. of Pharmacology, University of Ottawa, Ontario, K1N 6N5.

Post-prandial increases in central BN-like immunoreactivity (BLI) have recently been reported. Many factors may contribute to this increase including changes in the synthesis, release and/or uptake of BN-like peptides. The present study was designed to assess the physiological release profile of BN-like peptides in relation to food intake. Male Sprague-Dawley rats were implanted with push-pull cannulae aimed at the paraventricular nucleus of the hypothalamus (PVN). Our data revealed significant differences between pre-prandial, prandial and post-prandial extracellular BLI, with an approximate 3-fold diminution during meal as compared to before and after a meal. Prior to the first spontaneous meal, the BLI levels were 2-fold higher than those detected during the meal. Post-prandially, the levels shot up by 3-fold. In the present study we also simultaneously monitored levels of biogenic amines and metabolites, using HPLC. The PVN perfusate contained detectable levels of NE, EPI, HVA, 5-HIAA and DOPAC, none of which changed in a clear meal-related pattern. These results strongly support a physiological role for BN-like peptides in the control of food intake and suggest that the state of satiety is maintained by elevated interstitial levels of BLI at the PVN and that a drop in the peptide levels may trigger and/or maintain a meal.

522.3

SUPPRESSION OF FEEDING BY BOMBESIN (BUT NOT CCK) IS MEDIATED BY CENTRAL HISTAMINE. Z. MERALI[1]*, K. BANKS, P. KENT and G.D. PRELL[2]. [1]Psychology & Pharmacology, Univ. of Ottawa, Ottawa, Ont. Canada, K1N 9A9. [2]Dept. Pharmacology, Mt. Sinai Sch Med., 1 Gustave Levy Pl, N.Y. 10029.

Systemically administered bombesin (BN) suppresses food intake in most mammals. The mechanism(s) by which this satiety-like state is achieved is not known. Blockade of central histamine receptors has been reported to induce feeding in *ad libitum* fed rats. We hypothesised that BN may mediate its satiety effect through release of histamine. Male Sprague-Dawley rats were trained to consume the daily ration during a 4 hr food access period in the light phase. BN (6 μg/kg; i.p.) suppressed (by about 50%) food intake for up to 2 hr. α-methyl histamine (α-MH), a histamine H_3 autoreceptor agonist that prevents the release of histamine, blocked BN's satiety effect. This effect was specific as α-MH failed to alter food intake on its own and also failed to alter the satiety effect of CCK. Since α-MH can bind to heteroautoreceptors, the specificity of the observed effects were further validated. Binding of BN to its receptors (assessed autoradiographically) was not inhibited by α-MH. Furthermore, direct blockade of histamine postsynaptic receptors by mepyramine (H_1 antagonist) and famotidine (H_2 antagonist) administered centrally, also blocked the satiety effects of systemically administered BN. We thus believe that BN may mediate satiety through the release of central histamine.

522.4

THE EFFECT OF BOMBESIN AND ANTAGONISM OF BOMBESIN BY BW2258U89 ON SALT APPETITE. S.P. Frankmann*, J.H. Dokko and J. Gibbs. Bourne Lab, Cornell Med. Ctr, White Plains, NY 10605.

Little is known of the signals for the satiation of salt appetite. Peripheral injection of the peptide, bombesin (BN), elicits satiety for liquid food in overnight food-deprived rats. The present study asked whether BN would decrease the salt intake elicited by sodium depletion and whether a selective BN antagonist, Burroughs Welcome 2258U89 (BW), would reverse this effect.

Male, Sprague Dawley rats (n = 10) were sodium depleted by injection of the natriuretic/diuretic drug, Lasix (furosemide, 10 mg, sc), and overnight sodium deficient diet and water. Twenty-four hours later, 0.3M NaCl and water intakes were recorded for 1 h in a salt appetite test. Rats were injected with BW (50 μg/kg) or vehicle (V) at -15 min and BN (4 μg/kg) or V at -5 min before the start of the appetite test. Thus the rats were tested three times (1 test/week) under the following conditions: 1) V-V, 2) V-BN and 3) BW-BN. As can be seen below, BN reliably suppressed NaCl intake and the BW partially reversed this suppression. There were no differences in water intake among the groups.

0.3M NaCl Intake, ml (mean ± SEM)

Condition	5 min	10 min	15 min	30 min	60 min
V-V	4.1 ±0.7	6.9 ±0.6	9.6 ±0.6	12.9 ±0.6	16.4 ±1.0
V-BN	3.3 ±0.7	3.7 ±0.6*	3.8 ±0.5*	4.8 ±1.1*	8.7 ±1.3*
BW-BN	5.4 ±0.8	7.0 ±0.#	7.0 ±1.0#	9.4 ±1.0*#	12.2 ±1.5*#

* = p < .05 vs V-V, # = p < .05 vs V-BN

These data demonstrate that BN decreases salt intake after salt depletion and this decrease is partially reversed by the BN antagonist, BW2258U89. BN-like peptides may be endogenous signals for the satiation of salt intake.

Supported by NIH DK39180 (SPF) and DK33248 (JG). BW2258U89 was generously supplied by the Burroughs Wellcome Company.

522.5

THE HINDBRAIN, FEEDING, AND NEUROPEPTIDE Y-RELATED PEPTIDES: EVIDENCE FOR COMPLEX RECEPTOR INTERACTIONS. E.S. Corp,* C. Wahlestedt[1] and J.A. McQuade. Bourne Lab and [1]Division of Neurobiology, Cornell University Medical College, White Plains, NY 10605

Injected into the hypothalamus, NPY_{2-36} is a more potent stimulus of feeding than NPY. Its potency in the hindbrain, another sensitive site of action for NPY in feeding, is not known. Thus, we examined the dose-response effect of NPY_{2-36} on food intake following fourth ventricular administration in comparison with the effects of related NPY-receptor agonists including: NPY; peptide YY (PYY); the Y1 receptor agonist [Leu^{31},Pro^{34}]NPY (LP-NPY); and the Y2 agonist, NPY_{13-36}. Food intake was measured in non-deprived rats two hours after peptide administration. Data are presented below. MAX = maximal intake (g) above baseline in the dose-range tested. ED_{50} = dose (pmole) eliciting the half-maximal response for the given peptide.

PEPTIDE	PYY	NPY	NPY_{2-36}	LP-NPY	NPY_{13-36}
MAX	6.9 ± 1.2	3.7 ± 0.6	2.5 ± 0.6	2.1 ± 0.6	0.9 ± 0.2
ED_{50}	84 ± 2.0	304 ± 1.2	184 ± 1.7	947 ± 2.3	370 ± 1.2

NPY_{2-36} displayed relatively low efficacy compared with NPY and PYY. At low doses, however, NPY_{2-36} was more potent than NPY suggesting that the full response in feeding involves multiple NPY-receptor components. A high affinity component recognizes NPY_{2-36} (and PYY) in preference to NPY, while NPY_{2-36} is relatively inactive at the low-affinity component. Alternatively, NPY, unlike NPY_{2-36} and PYY, may interact with an additional population of receptors functionally-linked to inhibition of feeding. The relatively low potency of the Y1 agonist LP-NPY suggests that typical Y1 receptors are only marginally involved in feeding. The Y2 agonist NPY_{13-36} had the lowest efficacy; however, this peptide initiated feeding at lower doses than LP-NPY suggesting some orexigenic activity associated with Y2 receptors, or the interaction of this fragment with another receptor (other than Y1). These results are consistant with multiple NPY-receptor interactions in feeding.

Supported by The Whitehall Foundation

522.6

IMMUNONEUTRALIZATION OF NEUROPEPTIDE Y (NPY) SUPPRESSES NIGHTTIME FEEDING: A PHYSIOLOGICAL ROLE. M.G. Dube*, B. Xu, P.S. Kalra and S.P. Kalra, Dept. Obstet. & Gynecol., Univ. Florida, Gainesville, FL 32610

The potent orexigenic effects of NPY are well-recognized. Continuous intracerebroventricular (ivt) infusion of NPY elicits insatiable appetite and feeding occurs in episodes resembling the pattern seen during the night when rats normally eat. Further, NPY levels and release are augmented selectively in the hypothalamic paraventricular nucleus in association with increased appetite. We tested the hypothesis that NPY secretion is responsible for normal nighttime food intake in rats. Adult male rats, maintained on a controlled light-dark cycle (lights off 1900-0500 h), were implanted with permanent cannulae in the third cerebroventricle. One week later food and water intake were monitored for 24 h to ensure recovery from surgical stress. Rats were then implanted s.c. with osmotic mini-pumps (Alza, Corp.) attached to the ivt cannula to deliver either NPY antiserum (NPY-Ab) or normal rabbit serum (both diluted 1:1 with artificial cerebrospinal fluid) at a rate of 9 μl/h. Infusion was started at 1500 h and continued for 24 h. NPY-Ab infusion significantly suppressed 24 h cumulative food and water intake: food intake: 3.7 ± 0.9 g vs 10.5 ± 2.6 g for controls ($p < 0.03$, n = 9/group); water intake: 10.6 ± 1.5 ml vs 26.6 ± 5.3 ml for controls ($p < 0.02$). Rats receiving NPY-Ab also showed a significantly greater body weight loss than controls ($p < 0.02$). Since passive immunoneutralization of endogenous NPY suppressed food intake, these results support the hypothesis that NPY is a physiological orexigenic signal that is responsible for normal nighttime feeding. (Supported by NIH DK 37273).

522.7

HYPOTHALAMIC NEUROPEPTIDE Y (NPY) REGULATION MAY BE INDEPENDENT OF ENERGY INTAKE IN LONG-EVANS RATS. Beck* B., Stricker-Krongrad A., Burlet A., Nicolas J.P., Burlet C. - INSERM U.308 - MRCA - 38, rue Lionnois, 54000 NANCY (France)

Neuropeptide Y (NPY) is the most powerful orexigenic peptide actually known. Overeating in the obese Zucker rat is associated with elevated concentrations of NPY in the hypothalamus. In the normal rat, overeating can be induced by diet manipulation i.e. by feeding the animals on high caloric density diets. Furthermore, we have recently shown that diet composition influences hypothalamic NPY levels. So, the aim of the present experiment was to determine if there was a relationship between diet-induced overeating and hypothalamic NPY. For this purpose, 3 groups of 21 day-old male LE rats were fed during 14 weeks on either a well-balanced control diet, a high carbohydrate (starch + 25% sucrose solution) diet (HC) or high fat diet (HF). Food intake and body weight were recorded and NPY was measured in several microdissected nuclei involved in the regulation of eating behavior. Final body weight of the HF rats was significantly greater than that of the HC rats (+13.6%; $p<0.006$). During the last week of the experiment, energy intake of the control rats was significantly smaller than that of the HC rats (-14.7%; $p<0.02$) and significantly greater than that of the HF rats (+14.8%; $p<0.001$). However, both variarions of food intake were specifically associated with diminished NPY concentrations in the arcuate nucleus (-22.2% (HC vs control); $p<0.02$ and -35.3% (HF vs control); $p<0.001$) as well as in the parvocellular part of paraventricular nucleus and more particularly in its for the HF group only (-32.8%; $p<0.001$). These variations in the functional pathway of regulation of feeding behavior by NPY can be explained by either a relative independence towards energy intake or by the existence of 2 distinct sensor systems specific of each macronutrient and that may differentially regulate NPY concentrations.

522.8

SPECIFIC INHIBITION OF ENDOGENOUS NEUROPEPTIDE Y SYNTHESIS IN THE ARCUATE NUCLEUS (ARC) BY ANTISENSE OLIGONUCLEOTIDES SUPPRESSES FEEDING BEHAVIOR AND INSULIN SECRETION. A. Akabayashi*[1], C. Wahlestedt[2], J.T. Alexander[1], W.K. Cheung[1], I. Silva[1], and S.F. Leibowitz[1]. The Rockefeller University[1] and Cornell University Med. College[2], New York, NY 10021.

Neuropeptide Y (NPY), which is synthesized in neurons of the ARC, is known to have potent effects on eating behavior and hormone secretion. To test the hypothesis that endogenous NPY is essential for the normal expression of these responses, the present study used two unmodified antisense oligodeoxynucleotides (ODNs) to disrupt the synthesis of NPY in the ARC and to examine the impact of this disturbance on nutrient intake, as well as on circulating hormone levels. Brain-cannulated rats on macronutrient diets were given daily, bilateral injections, over a 4-day period, of NPY antisense ODNs, sense ODNs or saline into the ARC. The NPY antisense ODNs significantly reduced (-33% relative to sense ODNs and -40% relative to saline, $p<0.05$) NPY levels in this nucleus examined by RIA. In association with this reduction in the NPY, the antisense-treated animals exhibited a significant decrease in feeding behavior measured during the first 90 minutes of the natural feeding cycle, as well as over the 24-hour period. In the 90-minute interval, both carbohydrate and fat intake were suppressed by 65-70% ($p<0.05$, relative to both saline and sense ODNs). In addition, circulating insulin levels were significantly reduced by 50-55% ($p<0.05$ relative to both saline and sense ODNs). These findings provide the first evidence for physiological disturbances that may result from an inhibition of endogenous NPY production within neurons of the ARC.

522.9

CNS INSULIN DEFICIENCY CONTRIBUTES TO OVEREXPRESSION OF HYPOTHALAMIC NPY mRNA AND HYPERPHAGIA IN DIABETIC RATS. A. J. Sipols*, D. G. Baskin, S. C. Woods and M. W. Schwartz. Seattle VA Med. Ctr. and Depts. of Psych., Medicine and Bio. Str., U. of Washington, Seattle, WA 98195.

Untreated insulin-deficient diabetes mellitus is characterized by low plasma insulin levels and significant weight loss despite marked hyperphagia and polydipsia. We hypothesized that a deficiency of CNS insulin contributes in part to diabetic hyperphia by increasing neuropeptide Y (NPY) biosynthesis, since (a) diabetes is associated with elevated production and release of hypothalamic NPY, and (b) insulin infused into the 3rd cerebral ventricle of fasted rats decreases arcuate nucleus NPY mRNA levels and reduces food intake during refeeding. To test this hypothesis, adult male Wistar rats were made diabetic with streptozotocin (55-65 mg/kg i.v.), and 10 days later received continuous infusions of either insulin (3 mU/24μl/day; n=11) or saline (24 μl/day; n=10) into the 3rd ventricle (ivt) for 6 days. Compared to ivt vehicle-treated, nondiabetic controls (n=15), food intake was increased 2-fold in diabetic rats receiving ivt saline (25.3 ± 0.7 vs 48.8 ± 1.3 g/day, $p<0.001$). Treatment of diabetic rats with ivt insulin reversed this hyperphagia by 64% (33.8 ± 1.4 g/day, $p<0.05$ vs ivt vehicle-treated diabetic and nondiabetic rats). This reduction in food intake was associated with a 69% greater loss of body weight in insulin- vs. vehicle-treated diabetics (32.4 ± 2.3 vs 19.2 ± 3.5 g, $p<0.05$). NPY mRNA was detected by in situ hybridization and measured by computer densitometry. Preliminary analysis indicates a strong correlation between levels of NPY mRNA in the hypothalamic arcuate nucleus and food intake ($r=.75$, $p<0.01$) across all animals. In contrast to its effect on food intake, ivt insulin did not alter either hyperglycemia (blood glucose: 667 ± 27 vs 622 ± 32 mg/dl) or polydipsia (water intake: 224 ± 10 vs 233 ± 11 ml/day) in diabetic animals. In summary, (a) insulin infused into the CNS results in a marked reduction of the hyperphagia of diabetic rats, and (b) a significant correlation exists between arcuate nucleus NPY gene expression and food intake. We conclude that a relative insulin deficiency in hypothalamic nuclei associated with NPY-stimulated food intake contributes to diabetic hyperphagia.

522.10

INTRAVENTRICULAR (IVT) INSULIN DECREASES NET mRNA IN DIABETIC RAT LOCUS COERULEUS. D. Figlewicz Lattemann*, P. Szot, A.J. Sipols, D.G. Baskin, and S.C. Woods. Dept. of Cell Biol. and Anat., Oregon Health Sciences U., Portland OR 97201, Depts. of Psychiat., Med., Biol. Struct., and Psychol., U. of Washington, Seattle WA 98195, and the VA Medical Center, Seattle WA 98108.

We have reported that insulin (INS) increases endogenous noradrenergic activity in rat CNS, and one potential mechanism is the inhibition of norepinephrine (NE) re-uptake secondary to decreased membrane concentrations and synthesis of the NE re-uptake transporter (NET). CNS NE transmission or activity may be decreased in diabetes; reported changes are corrected by peripheral INS. We tested whether insulin can alter one aspect of NE transmission--synthetic capacity of locus coeruleus (LC) neurons for NET--directly within the diabetic CNS. Streptozotocin-diabetic Wistar rats (65 mg/kg iv) received IVT vehicle (STZ) or 3 mU/day INS (STZ-INS) for 6 days. NET mRNA was assayed in LC with a 226 bp riboprobe labelled with ^{35}S-UTP (2 pmol/ml). Hybridization was determined on emulsion-coated slides after 2 wk exposure. IVT IRI significantly suppressed NET mRNA levels (STZ=4337±1143 pixels, n=4; STZ-IRI=1113±294 pixels, n=5; $p<0.05$, ANOVA). We conclude that the ability of insulin to decrease NET mRNA in STZ rats is a direct CNS action, and that INS may directly increase CNS aminergic activity independent of any effects on metabolic substrates. (Studies were supported by NIH R01 DK40963 and the Veterans Administration. The NET clone was kindly provided by Dr. Susan Amara.)

522.11

EXOGENOUS INSULIN INCREASES THE RATE OF GASTRIC EMPTYING AFTER BUT NOT DURING INGESTION. W. Siemers, J.M. Kaplan*, and H.J. Grill. University of Pennsylvania, Philadelphia, PA 19104.

The administration of insulin has many metabolic and physiological consequences, including the acceleration of gastric emptying. We have recently shown that the gastric emptying of glucose solutions is described by different rules during and after stomach fill. It is therefore necessary to determine whether insulin affects both phases of emptying equivalently. On each test day, 5 male rats consumed 12 ml of an intraorally infused glucose solution (12.5%) 3 hours following the injection of either insulin (5U/kg) or saline. Stomach contents were removed either during or after the infusion and the glucose concentration determined. Insulin significantly increased gastric emptying after the termination of the infusion, however, it did not affect the rate of emptying during ingestion The results suggest that insulin does not effect the distribution of glucose solutions during meal-taking; but may influence nutrient distribution between meals.

GABA RECEPTORS: FUNCTION—IN VIVO STUDIES

523.1

GABAERGIC INHIBITION IS ABSOLUTELY LIMITED IN THE RAT NEOCORTEX. D.S.F. Ling*[1] and L.S. Benardo[1,2]. Depts. of Pharmacology[1] and Neurology[2], SUNY-HSCB, Brooklyn, NY 11203.

Neuronal excitability in the neocortex is gated by inhibitory mechanisms. Several physiological and pathological conditions occur as a consequence of the failure of GABAergic inhibition to limit glutamatergic excitation. The specific mechanisms underlying such behavior are unknown. These issues were addressed by detailed examination of excitation-inhibition interplay in isolated, coronally sectioned slices (400μm) of somatosensory cortex. Layer V neurons were recorded in whole-cell patch clamp configuration to gain information about synaptic strength. We examined stimulus-response characteristics under physiological conditions, and in the presence of magnesium-free solution. Recordings were obtained using cesium-gluconate-containing electrodes. EPSCs were recorded at holding potentials of -70 to -80 mV. Isolated $GABA_A$-IPSCs (slow $GABA_B$-IPSCs blocked) were recorded at the EPSC reversal potential (~0 mV). Under physiological conditions, EPSC amplitude increased steadily as stimulus intensity was increased, with no apparent maximum reached. By contrast, the fast IPSC reached a maximum magnitude at a stimulus strength of approximately 1.5-2X the threshold stimulus. When slices were exposed to magnesium-free solutions, IPSCs reached their maximum at lower stimulus strengths, but there was no change in the magnitude of the absolute maximal current. These results suggest that $GABA_A$-inhibition is limited in the neocortex regardless of the level of excitation. (Supported by NS 01386)

523.2

PAIRED PULSE DEPRESSION IN CULTURED HIPPOCAMPAL NEURONS IS DUE TO A PRESYNAPTIC MECHANISM INDEPENDENT OF $GABA_B$ AUTORECEPTOR ACTIVATION.
K.S.Wilcox*[1] & M.A.Dichter[1,2] Departments of Neurology[1] and Pharmacology2, University of Pennsylvania, School of Medicine, and Graduate Hospital, Philadelphia, PA 19104

GABAergic neurotransmission exhibits frequency-dependent modulation and sequential inhibitory postsynaptic currents (IPSCs) evoked with interstimulus intervals between 25 msec and 4 seconds routinely result in the attenuation of the amplitude of the second IPSC. This synaptic plasticity is known as paired pulse depression (PPD). These experiments were designed to directly determine the location of the mechanism of PPD in hippocampal neurons maintained in low density tissue culture. Evoked IPSCs were recorded between isolated pairs of neurons that were patch clamped simultaneously. Miniature IPSCs (mIPSCS) originating from the same synapses that were being stimulated during evoked release were measured. PPD occurred routinely, but the amplitudes of mIPSCS following IPSCs were unchanged; thus, a presynaptic mechanism mediates PPD. The inability of $GABA_B$ receptor antagonists (2-OH-saclofen and phaclofen) to block PPD indicated that this plasticity was not due to autoinhibition of transmitter release via activation of presynaptic $GABA_B$ receptors. Manipulations which lowered the probability of release of neurotransmitter during the first action potential of a trial, (low calcium & baclofen), prevented the development of PPD. These results indicate that inhibitory synaptic vesicles have a relatively high probability of release following a single action potential, but the probability rapidly falls such that subsequent action potentials release much less transmitter for periods as long as 4 seconds. Supported by NS24260 (M.A.D.).

523.3

KINETICS OF ULTRAFAST ACTIVATION OF GABA-A RECEPTOR CHANNELS IN EXCISED PATCHES OF RAT CORTICAL NEURONS.
R.E. TWYMAN*. Departments of Neurology, Pharmacology and Physiology, University of Utah, Salt Lake City, UT, USA 84112.

GABA-A receptor channels in outside-out patches of fetal rat cortical neurons in culture were exposed to GABA (0.5μM-10mM) using a piezoelectric ultrafast ligand delivery system (100μs exchange time). Receptor activation and closing kinetics were studied using two paradigms: a) brief, repetitive pulsed applications (800μs duration, 1-5hz, 25 applications) to simulate serial quantal synaptic events and, b) step application for continuous GABA exposure. Patches contained 1-30 channels and were voltage clamped at -75mV in symmetrical Cl^- solutions; rapid kinetic data were digitized at 125kHz, 5-10kHz bandwidth; responses were analyzed individually and as ensemble averages for each patch. In pulsed paradigms, τ onset of averaged currents (fit to steep phase) decreased with increased concentration up to 1mM (e.g. 3.5-4ms at 100μM; 0.3-0.35ms at 1 and 10mM). At 1-10mM, currents decayed with τs ranging 20-35ms and average burst durations from patches with <3 channels were ~30ms, indicating that current decay depended on channels exiting intrinsic bursting states rather than on duration of GABA exposure. Initial current onset was sigmoidal in shape, indicating >1 GABA molecule participated in activation. In step paradigms, onset shape and rates were similar. In pulse paradigms, peak currents decreased with time and was faster at higher pulse frequencies; this was slower compared to continuous exposure, which at 10mM, initial rapid decrement (τ ~8ms) was followed by a slower phase (τ ~150ms). These data indicate that for GABA-A receptors: a) activation is slower than ACh receptors, and b) rapidly entered desensitized states exist. Rates of current rise (10-90% time ~1ms) and decay at saturating GABA concentrations are similar to that reported elsewhere for miniature inhibitory currents and suggests that synaptic concentration of GABA is at least 1mM.

523.4

MODULATORY EFFECT OF ANTIDEPRESSANTS ON PERIPHERAL-TYPE BENZODIAZEPINE RECEPTORS. M. Gavish[1]*, R. Burgin[1] and R. Weizman[2]. [1]Dept. of Pharmacology, Bruce Rappaport Fac. of Med., Technion-Israel Inst. of Technology, 31096 Haifa, and [2]Tel Aviv Community Mental Health Center and Sackler Fac. of Med., Tel Aviv Univ., Tel Aviv, Israel.

The effect of 21 days of imipramine hydrochloride (10 mg/kg) and phenelzine sulfate (10 mg/kg) administration followed by a 7-day period of withdrawal on peripheral-type benzodiazepine receptors (PBR) was studied in male Sprague-Dawley rats. Both imipramine and phenelzine down-regulated adrenal PBR (-18% $P<0.05$; and -32%, $P<0.01$, respectively) and up-regulated hepatic PBR (+27%, $P<0.05$; and +76%, $P<0.0001$, respectively), while no alteration was observed in the kidney. Adrenal PBR decreased further (-30% vs. controls) after the withdrawal from imipramine, while a full restoration to normal values occurred following phenelzine withdrawal. [^{3}H]PK 11195 binding to the liver did not differ significantly from the control values after withdrawal from both drugs. The observed changes can be explained as adaptive responses to antidepressant-induced hormonal and cellular alterations.

523.5

IMIDAZENIL: A NEW PARTIAL POSITIVE ALLOSTERIC MODULATOR OF GABA ACTION AT $GABA_A$ RECEPTORS. A. Guidotti, P. Giusti°~, R. Arban°, G. Puia, I. Ducic, E. Costa. Fidia-Georgetown Institute for the Neurosciences, Georgetown Univ Med Sch, Washington, DC 20007 USA and Department of Pharmacology°, University of Padua, Italy.

When tested on a broad spectrum of native or recombinant $GABA_A$ receptor subtypes, imidazenil positively modulates the GABA-elicited Cl^- current with a potency 5 to 10 fold higher than that of diazepam. But it has an efficacy 30 to 50% lower than that of diazepam and it antagonizes the effect of diazepam. Imidazenil also possesses a marked anticonvulsant action in the rat and is more potent than bretazenil, diazepam, or alprazolam in antagonizing bicuculline or pentylenetetrazol (PTZ) induced seizures. Unlike diazepam and alprazolam, imidazenil does not produce ataxia, sedation nor potentiates the effect of ethanol or barbiturates in rats and monkeys. Moreover, imidazenil, unlike diazepam and alprazolam, fails to produce tolerance in the antagonism of bicuculline-induced convulsions even after 5 months of continuous treatment. PTZ, in subconvulsant doses, has been reported to induce a panic-like syndrome in human and animals. Thus, the potential antipanic action of imidazenil was evaluated in the PTZ facilitation of punishment suppressed behavior in the proconflict Vogel test paradigm (J. Pharmacol. Exp. Ther. 257:1062, 1991). The antiproconflict potency of imidazenil (EC_{50} 0.06μmol/kg, i.v.) was 50 fold higher than that of diazepam and 5 to 10 fold higher than that of other known antipanic drugs (i.e., alprazolam, bretazenil, clonazepam). Thus, imidazenil is a partial allosteric modulator at $GABA_A$ receptors. It possesses a potent anticonvulsant and antipanic action and is devoid of side effects such as ataxia, sedation, ethanol potentiation, and tolerance liability.

Supported in part by grant RO1 MH49486-01.

523.6

IMIDAZENIL ATTENUATES THE DISRUPTIVE EFFECTS OF ALPRAZOLAM ON COMPLEX LEARNING AND PERFORMANCE IN MONKEYS. D. Thompson^, A. Guidotti*, E. Costa. Department of Pharmacology^ and Fidia-Georgetown Institute for the Neurosciences, Georgetown Univ Med Sch, Washington, DC 20007.

Imidazenil is a partial allosteric modulator of GABA action at $GABA_A$ receptors, whereas alprazolam is a full allosteric modulator at these receptors. To determine whether this difference is reflected in their behavioral effects, we studied these two drugs, alone and in combination, in monkeys working in a complex behavioral task. In one component of a multiple schedule (repeated acquisition or "learning"), patas monkeys acquired a different four-response chain each session by responding sequentially on three keys in the presence of four geometric forms. In the other component (performance), the four-response chain was the same each session. The response chain in each component was maintained by food presentation under a fixed-ratio schedule. When alprazolam (0.01 - 0.32 mg/kg, po) was administered alone, the overall response rate in both learning and performance decreased and the percent errors in both components increased with increasing doses. Learning, however, was more sensitive than performance; i.e., error-increasing effects were seen in learning at doses that had no effect on performance accuracy. When imidazenil, at a dose having little or no effect when given alone (1 mg/kg, po), was administered 60 min before alprazolam (0.1 or 0.32 mg/kg, po), the large disruptive effects of alprazolam on learning and performance were greatly attenuated. The results indicate that the partial allosteric modulator imidazenil can partially antagonize the disruptive behavioral effects of a full allosteric modulator at $GABA_A$ receptors.

523.7

COMPARISON OF TOLERANCE PRODUCING EFFECTS OF α-ETHYL, α-METHYL THIOBUTYROLACTONE AND CLONAZEPAM. J.A. Ferrendelli, A.C. McKeon, K. Xu, J.W. Miller*, D.F. Covey. Washington University School of Medicine, St. Louis, MO 63110.

Benzodiazepines and butyrolactones act at benzodiazepine and pictoxin receptors, respectively, on the GABA receptor/chloride ionophore, but both can augment chloride conductances and have similar acute behavioral effects. We now compare some effects of chronic treatment of mice with α-ethyl, α-methyl thiobutyrolactone (α-EMTBL) or clonazepam (CZP) in chronically treated animals to determine if α-EMTBL produces tolerance similar to benzodiazepines. Mice (CF-1) were treated for 10 or 14 days with a TD_{90} dose of either α-EMTBL or clonazepam. All animals treated with CZP (n=109) became tolerant to the toxic effects of a TD_{90} dose of this drug whereas 91% of the animals treated with α-EMTBL (n=69) continued to be toxic after treatment with a TD_{90} dose of this drug. Brain membranes from animals chronically treated with CPZ had no change in benzodiazepine receptor binding affinity (Kd), but had decreased number of benzodiazepine receptors (Bmax). Chronic treatment with α-EMTBL did not change the number or affinity of benzodiazepine or picrotoxin receptors. CPZ treatment did not affect picrotoxin receptors and α-EMTBL did not alter benzoodiazepine receptors. These results demonstrate that α-EMTBL, unlike clonazepam, does not produce tolerance after chronic administration and leads to the conclusion that α-EMTBL and perhaps other butyrolactones would be better therapeutic agents than benzodiazepines in patients requiring prolonged treatment.

Supported, in part, by NIH Grant NS14834

523.8

DIAZEPAM, BUT NOT ZOLPIDEM, IMPAIRS MOTOR REFLEX IN THE MUTANT ANT RATS. E.R. Korpi* and M. Sarviharju. Biomedical Research Center, Alko Ltd, POB 350, SF-00101 Helsinki, Finland.

ANT (Alcohol Non-Tolerant) rats are abnormally sensitive to motor-impairing effects of benzodiazepine agonists. These rats have a point mutation in their cerebellar $GABA_A$ receptor α6 subunit, which alters the resulting receptor subtype from normal diazepam insensitive to diazepam sensitive (Korpi et al. Nature 361: 356-359, 1993). In the present autoradiographic study, we found that zolpidem, a potent benzodiazepine receptor agonist, failed to displace "diazepam-insensitive" component of the [^{3}H]Ro 15-4513 binding in cerebellar sections of the ANT rats, whereas diazepam at micromolar concentrations fully displaced it. Correspondingly, in behavioral experiments with ANT rats, zolpidem failed to produce any motor impairment in the tilting plane test 30 min after IP injection (1-10 mg/kg), whereas diazepam significantly impaired the performance (10-20 mg/kg, IP). The results support the hypothesis that the abnormal benzodiazepine sensitivity of the ANT rats is caused by the altered drug sensitivity of their mutant cerebellar granule cell-specific $GABA_A$ receptors.

523.9

ACCUMULATION OF CIRCULATING ENDOZEPINES AS PRECIPITATING FACTOR OF ENCEPHALOPATHY IN CIRRHOTIC PATIENTS. R. Avallone, I. Venturini°, M.L. Zeneroli°, A. Monzani, M. Di Bella, M. Baraldi*, Dept. of Pharmaceutical Sciences and Chair of Clinical Methodolgy°, Modena University,41100 Modena, Italy.

During the last ten years evidence have been provided that an increased sensitivity of the GABAergic neurotransmission affects the level of consciousness in animal models and in human with hepatic encephalopathy (HE) due to acute or chronic liver diseases. Several pathogenetic agents, such as ammonia, seem to be responsible together with metabolic alterations (hypoglycemia, hypoxia) of glutamate-related neurotoxicity leading to gliosis and neuronal degeneration which in turn could explain the denervation supersensitivity of the GABAergic system. The demonstration of endogenous benzodiazepine-like compounds (EBz) in mammalian tissues, mainly of alimentary origin, prompted several groups to study the levels of these EBz in animal and patients with HE. Indeed few reports on small groups of patients with HE have been shown to have an increased level of EBz in serum and CSF. Herein we report an extensive study on the levels of EBz in the serum of 156 patients comprising 53 controls and 89 liver cirrhosis (LC) patients free of exogenous Bz and 14 Bz consumers. The levels of EBz in LC were about 8 fold higher than in controls (mean ± SE: 2.81 ± 0.33 vs 0.44 ± 0.07 pmol DE/ml: $p < 0.0001$). The levels of EBz were found progressively increased in LC with the severity of the liver disease: in compensated LC the values were 0.50 ± 0.11 ; in decompensated LC were 2.51 ± 0.47 ($p < 0.001$ vs compensated); in severely decompensated were 4.80 ± 0.57 pmol DE/ml ($p < 0.0001$ vs decompensated). The HE was present in these last two groups of patients severely . From these data we can surmise that the increase of EBz in LC might be a contributing factor in precipitating HE, but only in the presence of an increased sensitivity of the cerebral GABAergic system. In fact LC patients with HE never reach the levels found in the 14 normal subjects assuming modest amount of exogenous Bz (9.21 ± 1.6 pmol DE/ml) which were free of any consciousness disturbance.

523.10

CORRELATION OF NEUROACTIVE STEROID MODULATION OF [^{35}S]TBPS AND [^{3}H]FLUNITRAZEPAM BINDING AND $GABA_A$ RECEPTOR FUNCTION. J. E. Hawkinson*, C.L. Kimbrough, R.H. Purdy, and N.C. Lan. CoCensys, Inc., Irvine, CA, 92718.

Neuroactive steroids, including both progesterone metabolites and their derivatives, are positive allosteric modulators of the $GABA_A$ receptor-chloride ionophore complex (GBRC). They potentiate GABA-activated chloride currents and GABA- and muscimol-stimulated $^{36}Cl^-$ uptake, enhance the binding of [^{3}H]flunitrazepam ([^{3}H]FLU), and inhibit the binding of [^{35}S]TBPS. However, the relationship between neuroactive steroid modulation of binding and effect on receptor function has not been demonstrated.

We examined the abilities of a series of 31 neuroactive steroids to modulate [^{35}S]TBPS and [^{3}H]FLU binding in rat brain cortical preparations and correlated these two measurements with each other and with potentiation of chloride uptake as an indicator of receptor mediated function. There is a good correlation ($r > 0.9$) between [^{35}S]TBPS IC_{50} and [^{3}H]FLU EC_{50} indicating that the allosteric modulation of [^{35}S]TBPS and [^{3}H]FLU binding by neurosteroids occurs via similar neurosteroid binding sites. However, there is a lower correlation ($r \sim 0.7$) between the efficacies in the two binding assays. Furthermore, a similar correlation ($r = 0.7$-0.8) was observed between [^{35}S]TBPS or [^{3}H]FLU binding and the abilities of these steroids to potentiate muscimol-stimulated $^{36}Cl^-$ uptake in rat cortical synaptoneurosomes (Purdy *et al.*, 1990, *J. Med. Chem.* 33:1572-1581). These data indicate differential coupling efficacies among neuroactive steroids for various functional sites on the GBRC which may in turn suggest receptor heterogeneity for neurosteroid action.

523.11

THE BIOCHEMICAL AND BEHAVIORAL PHARMACOLOGICAL EFFECTS ON THE INTRODUCTION OF A DOUBLE BOND AT THE 16,17 POSITION OF NEUROACTIVE STEROIDS. S. Wieland*, J. Hawkinson, H. Xia, R. Upasani, M. B. Bolger[1], N.C. Lan. CoCensys, Inc., Irvine, California 92718.[1]University of Southern California, School of Pharmacy, Los Angeles, California 90033

Neuroactive pregnane steroids have been shown to be potent anticonvulsants, anxiolytics and hypnotic agents. Previous studies have shown that introduction of a double bond at the 16,17 position of the sedative/anesthetic compound, alphaxalone (3α-hydroxy-5α-pregnane-11,20-dione), completely eliminated its anesthetic properties. It is of particular interest to examine whether such a structural modification selectively alters the sedative action, but maintains the potent anticonvulsant activity of neurosteroids. We have studied the biochemical and behavioral effects of introducing a 16,17 double bond into the naturally occurring steroids 3α-hydroxy-5α-pregnan-20-one (3α,5α-P) and 3α-hydroxy-5β-pregnan-20-one (3α,5β-P).

The 16-ene analog of 3α,5α-P was 8-fold less potent in inhibiting [^{35}S] TBPS binding in rat cortical membranes, whereas the 16-ene analog of 3α,5β-P was 10-fold less potent than the parent compound. In behavioral activity measurements, the 16-ene analog of 3α,5α-P and 3α,5β-P had reduced potency against metrazol-induced seizures in mice by 10-20 fold and 15-fold, respectively, compared to their parent compounds. Similarly, the sedative potency was reduced by 25- and 7-fold for the 16-ene analogs of 3α,5α-P and 3α,5β-P, respectively.

Taken together, the data suggests that although introduction of a 16,17 double bond decreased the sedative potency of 3α,5α-P and 3α,5β-P, it also reduced their anticonvulsant potency, thereby limiting the potential clinical usefulness of this structural modification.

523.12

THREE MECHANISMS FOR INCREASED GABAERGIC INHIBITION AFTER KINDLING-INDUCED EPILEPSY. T.S. Otis*[1] and I. Mody[2]. [1]Dept. Neurology & Neurological Sci., Stanford Univ., Stanford, CA, and [2]Depts. of Anesthesiology & Neurology, UT Southwestern Med. Ctr., Dallas, TX.

The hypothesis that a reduction in GABAergic inhibition necessarily takes place in chronic epilepsies is controversial. We studied synaptic inhibition of granule cells (GCs) from slices of control and kindled dentate gyrus (DG) using whole-cell recordings to examine $GABA_A$-mediated spontaneous inhibitory postsynaptic currents (sIPSCs), and to measure stimulus-evoked monosynaptic GABAergic input to GCs.

The frequency of sIPSCs impinging on a given GC was measured under three successive conditions: (1) in control solutions, (2) in the presence of 10 μM CNQX and 40 μM D-AP5, and (3) in CNQX/D-AP5 and 1 μM tetrodotoxin (TTX). In all conditions, sIPSCs frequencies in kindled GCs were equal to or higher than in controls. On average, CNQX/D-AP5 reduced the frequency of sIPSCs in kindled GCs by 22%, but had no effect on the frequency in control cells (2% increase), indicating the emergence of a tonic excitation of GABAergic neurons during kindling. Addition of TTX reduced sIPSC frequency more in control (47%) than in kindled slices (19%). Finally, the mean conductance, but not the kinetics, of miniature IPSCs was significantly larger (76% increase) in kindled neurons, suggesting an increased quantal size.

Stimulus-evoked monosynaptic $GABA_A$ or $GABA_B$ currents were comparable in control and epileptic preparations, but presynaptic inhibition of GABA release was significantly decreased after kindling. Thus, GABAergic inhibition of DG GCs is not impaired in kindling-induced epilepsy; on the contrary, a "compensation" for the hyperexcitability appears to be accomplished in the following ways: (1) by increasing excitatory input onto GABAergic neurons, (2) by increasing the IPSC quantal size, and (3) by reducing presynaptic autoinhibition of GABA release.

Supported by NINDS grants NS-12151, NS-30549, and the Klingenstein Foundation (I.M.), and a Howard Hughes Predoctoral Fellowship (T.S.O.)

BRAIN METABOLISM AND BLOOD FLOW I

524.1

SUITABILITY OF α-CHLORALOSE ANESTHESIA PLUS HALOTHANE INDUCTION FOR CEREBROVASCULAR RESEARCH. G. Bonvento, P. Lacombe, R. Charbonné, J. Benavides*$ and J. Seylaz. CNRS UA641, Université Paris VII, 75010 Paris, France; $Department of Biology, Synthélabo Recherche, 92220 Bagneux, France.

Alpha-chloralose is a widely used anesthetic in neurophysiological studies including the cerebrovascular field since it both maintains cardiovascular reflexes and potentiates the generation of evoked responses. However, the use of α-chloralose has been ethically questioned mainly because it induces an EEG that cannot readily be interpreted (J. Physiol., 445, pp. v-xvi, 1992) and a cataleptic behavior, two facts that preclude a proper assessement of the adequacy of anesthesia. In this study, we sought to determine whether α-chloralose when associated with halothane (for the initial surgical step) could be a suitable anesthetic regimen. Using laser-Doppler flowmetry to measure cortical blood flow in either chronically instrumented rats (n=5) or in an acute preparation (n=7), we have shown that halothane (1.5%) given during the first 30-45 min of α-chloralose anesthesia (40mg/kg s.c.) without curare leads to stable cardiovascular parameters and immobility of ventilated rats placed in ear bars for at least 3h without any sign of discomfort, and that halothane delays the appearance of desynchronized EEG activity (stage I). With regards to the cerebrovascular reactivity, a nearly total inhibition of the vascular response to CO2 is observed (-85%, $p<0.01$), but this inhibition is reversible since a CO2 reactivity similar to that observed in the awake rat is restored 2.5-3h after α-chloralose injection and before the appearance of desynchronized activity. Interestingly, halothane (1.5%) given alone continuously induces a marked and sustained decrease in the CO2 reactivity (-75%, $p<0.01$) and a consistent hypotension. These results provide evidence that α-chloralose used in conjonction with a short initial period of halothane prevents any discomfort for the animals and that this ethical anesthetic regimen displays a temporal window of normal cerebrovascular reactivity.

524.2

NITRIC OXIDE MEDIATES NMDA-INDUCED INCREASES IN CEREBRAL BLOOD FLOW IN NEWBORN LAMBS. F.J. Northington, J.R. Tobin*, R.C. Koehler, R.J. Traystman. Depts. of Pediatrics and Anesthesiology/CCM, Johns Hopkins Medical Institutions, Baltimore, MD 21287

Several studies suggest that increases in cortical activity are coupled to increases in local cerebral blood flow (CBF) via a nitric oxide(NO)-dependent mechanism. We adapted the ^{14}C-arginine to ^{14}C-citrulline conversion assay, developed for *in vitro* NO measurement, for use with microdialysis to measure *in vivo* NO production in brain. This study examines the effect of NMDA on local CBF and assesses the contribution of NO to CBF changes. Newborn lambs were anesthetized with halothane and bilateral superficial parietal cortex microdialysis cannulas implanted. Cannulas were perfused at 1 μl/min with either: mock CSF(n=10), CSF with 1mM NMDA(n=10), or 1mM NMDA + 1mM L-nitroarginine methyl ester (L-NAME) (n=5). Infusions also contained 3μM ^{14}C-arginine. Microdialysis effluent was collected at hourly intervals for 4 hours, and CBF measured by H_2 clearance. CBF was unchanged throughout the 4 hr period in the mock CSF group (53± 6.9, 55.6± 6, 46± 2.9 and 48± 3.6 ml/min/100g) and NO production was unchanged until 4 hours of perfusion (6.7± 0.7, 18.9± 3.6, 33.5± 9.5, 55.2± 10.1* fm/min). NMDA caused significant increases in CBF (88± 7.5*, 99.8± 7.9*, 114± 9.5*, and 116± 10.5* ml/min/100g) at all times, and NO production (36.9± 11.9, 75.5± 17.2*, 103.4± 18.1*, and 141.9± 20.3* fm/min) at 2, 3, and 4 hours compared to control. When 1mM L-NAME was added to NMDA, neither CBF nor NO production was different than control (37.5± 2.6, 53± 7.6, 45.5± 4.3, 43.3± 4.4ml/min/100g) and (6.1± 3.1, 12.0± 4.9, 21.9± 3.8, 59.3± 14.0 fm/min). These results demonstrate that NMDA-induced changes in CBF are correlated with NO production, and that inhibition of NO synthesis completely inhibits NMDA-induced increases in CBF. Microdialysis is capable of measuring *in vivo* NO production. Supported by NIH Grant: NS20020.

524.3

THE ROLE OF L-ARGININE-NITRIC OXIDE SYSTEM IN THE MEDIATION OF REGIONAL CEREBROVASCULAR CO_2 RESPONSIVENESS OF THE CAT. P. Sandor, K. Komjati, M. Reivich*, I. Nyary. Cerebrovascular Res. Ctr., Univ. of Pennsylvania, Philadelphia, PA 19104 USA and Semmelweis Univ. Med. Sch., Budapest, 1082 Hungary

The role of nitric oxide (NO) in the mediation of cerebrovascular CO_2-responsiveness was studied in ten distinct brain and spinal cord regions of anesthetized, ventilated, temperature controlled normoxic cats. Regional cerebral blood flow was measured with 15 μm radiolabeled microspheres in hypocapnic, normocapnic and hypercapnic conditions. CO_2-responsiveness of each region was determined from the equation of the best fit regression lines to the obtained flow values. The effect of decreased endothelial and / or neuronal NO synthesis on CO_2-responsiveness was studied following either selective blockade of the NO-synthase enzyme by L-Name (Nω-nitro-L-arginine methyl ester, 3 or 30 mg/kg i.v.) or following combined administration of 3 mg/kg L-Name and a large dose of the NO-precursor L-Arginine (30 mg/kg, i.v.). Blockade of NO synthesis by 30 mg/kg L-Name resulted in a significant reduction of the steady state regional blood flow values, and in an almost complete abolition of the CO_2-sensitivity in each region studied. Changes of the basal flow values as well as the reduction of the regional CO_2-sensitivity were dose dependent. Hypothalamic, sensory motor cortical and cerebellar regions were the areas most sensitive to the NO blockade. Impaired CO_2-responsiveness following NO-synthase inhibition, however, was reversed in these these regions by simultaneous administration of a large dose of i.v. injected L-Arginine. These findings suggest a major role of nitric oxide in the mediation of regional cerebrovascular CO_2-responsiveness in cats. (Supported by NIH NS 10939, OTKA-1371 and ETT 4-280 Grants)

524.4

NITRIC OXIDE-DEPENDENT AND INDEPENDENT COMPONENTS OF CEREBROVASODILATION ELICITED BY HYPERCAPNIA C. Iadecola*, F. Zhang, and J. Li, Dept. of Neurology, Univ. of Minnesota, Minneapolis, MN 55455.

Inhibitors of nitric oxide (NO) synthase (NOS) attenuate, but do not abolish, the increase in cerebral blood flow (CBF) elicited by hypercapnia (PNAS 89:3913, 1992), suggesting that part of the vasodilation is independent of NO synthesis. Whether this NO-independent component of the response is more pronounced at higher levels of hypercapnia remains to be established. We, therefore, studied the effect of nitro-L-arginine methyl ester (L-NAME), a NOS inhibitor, on the increases in CBF elicited by graded elevations in arterial pCO_2 up to 140 mmHg. Rats were anesthetized with halothane and ventilated. CBF was monitored over the parietal cortex using a laser-Doppler flowmeter. Increasing levels of hypercapnia elicited graded increases in CBF that reached a plateau at pCO_2=82±1 (CBF:+215±25%). L-NAME (40 mg/kg, i.v.; n=8), but not D-NAME (n=8), reduced resting CBF by 42±4% ($p<0.05$) and attenuated the increase in CBF elicited by hypercapnia. The attenuation occurred only at pCO_2 40-80 (e.g., pCO_2:54-55, before:+134±21%; after L-NAME:+33±10%; $p<0.001$) and not at pCO_2 130-140 (before:+233±19%; after:+271±35%; $p>0.05$). To determine whether the effect of L-NAME was due to the vasoconstriction induced by this agent, the CO_2 response was tested before and after reducing resting CBF with the anesthetic chloralose. In halothane-anesthetized rats, chloralose administration (20-30 mg/kg i.v.; n=5) reduced resting CBF (-31±9%; $p>0.05$ from L-NAME) but did not affect the CBF response to CO_2 (pCO_2:43-137 mmHg; $p>0.05$). We conclude that L-NAME does not attenuate the CBF response to CO_2 uniformly at all levels of hypercapnia. Rather, significant attenuation occurs only at pCO_2 40-80. Thus, the cerebrovascular response to CO_2 may have NO-dependent and NO-independent components: During moderate hypercapnia NO mediates most of the vasodilation, whereas in extreme hypercapnia other vasodilating agents are responsible for the CBF increase. (Supported by the L. Sklarow fund and the American Heart Association)

524.5

SOURCES OF NITRIC OXIDE OF IMPORTANCE FOR HYPERCAPNIC RISE OF CEREBRAL BLOOD FLOW. M. Lauritzen* & M. Fabricius. Depts. of Medical Physiology & Clinical Neurophysiology, Univ. of Copenhagen, DK-2200 Copenhagen, Denmark.

We examined sources of nitric oxide (NO) modulating the hypercapnic rise of cerebral blood flow (CBF) by laser-Doppler flowmetry in halothane anesthetized rats. NOS inhibition was achieved by i.v. (30 mg/kg) and/or topical application (1 mM) of N^G-nitro-L-arginine (NOLAG). Activity in cortical perivascular nerves was inhibited by topical application of TTX (20 µM). Parietal CBF changed by 2.89% ± 0.18% and cerebellar CBF by 3.41±0.17%/mmHg change of $PaCO_2$ during control conditions. I.v. NOLAG decreased basal CBF by 8±4% and inhibited the hypercapnic rise of CBF already at 10 minutes after infusion to 0.72±0.17% in parietal cortex and 1.19±0.09% in cerebellum. When NOLAG was infused after 10 min of sustained hypercapnia, CBF rapidly decreased. L-arginine partially prevented the effect of NOLAG. Topical NOLAG (1 mM) reduced the CO_2 reactivity to 2.04±0.17% in parietal cortex, and 2.21±0.15% in cerebellum within 45 min. When NOLAG was applied after 10 min of sustained hypercapnia CBF remained constant. TTX decreased basal CBF by 23±3% in parietal cortex and by 9±3% in cerebellum, while CO_2 reactivity increased to 3.21±0.20% and 4.74±0.66% respectively. Blood-borne NOLAG was far more effective as blocker of the CO_2 response than topical NOLAG. Endothelial cells, not neurons or perivascular nerves, appear to be the source of NO regulating hypercapnic CBF.

524.6

A HIGH RESOLUTION TIME-OF-FLIGHT POSITRON EMISSION DETECTION PROBE SYSTEM: PHYSICAL CHARACTERIZATION AND HUMAN STUDIES. K.J. Jeffries*, C.A. Tamminga, H.L. Loats, R.R. Conley, and A.C. Lahti. Maryland Psychiatric Research Center, P.O. Box 21247, Baltimore, MD 21228.

A high resolution time-of-flight positron emission detection probe system has been developed and applied to the study of drug pharmacokinetics in the human brain. The probe system in its simplest form consists of a pair of barium fluoride (BaF_2) radiation detectors which are placed on opposite sides of the head. Time-of-flight detection circuitry has been developed which provides spatial resolution between the detectors of at least 6 mm. For circular detectors, the probe provides resolution elements in the form of disks with diameter roughly equal to 1/2 that of the detectors and width of no more than 6 mm. The probe system provides high count efficiency relative to PET allowing for lower radiation dose. This provides the ability to perform significantly more studies in a single subject (i.e., 20 - 40 studies per year). Further phantom studies demonstrating the spatial resolution and count efficiency of the system will be presented. Results with human studies using multiple time points after neuroleptic drug administration to show occupancy kinetics with ^{11}C-N-methyl-spiperone will also be presented. The time-of-flight positron emission detection probe system represents a new technology for the study of *in-vivo* brain chemistry. The system provides the ability to measure the distribution of positron-emitting radionuclide labeled compounds with both high spatial (i.e., 6 mm) and temporal (i.e., 5 studies per day) resolution.

524.7

THE EFFECTS OF (+)SK&F 10047 AND MK-801 ON CORTICAL SPREADING DEPRESSION. R.N. Willette*, P.G. Lysko and C.F. Sauermelch. Division of Pharmacology, SmithKline Beecham Pharmaceuticals, King of Prussia, PA 19406.

NMDA-receptor mediated neurotransmission is essential for the initiation and/or propagation of cortical spreading depression (CSD). The demonstration of CSD in migraine patients has lead to the suggestion that antagonism of NMDA-receptor mediated mechanisms may represent a novel therapeutic approach to the treatment of migraine. In the present study, the effects of (+)SK&F 10047 (N-allylnormetazocine) and MK-801 on CSD (evoked with KCl) were compared. MK-801 is an effective NMDA-receptor channel blocker which binds with high affinity (Kd = 6 nM) in a use/voltage dependent manner. (+)SK&F 10047 is an equieffective inhibitor of the NMDA-receptor channel, but it binds with much lower affinity (Kd= 475 nM) to the MK-801 site. MK-801 and (+)SK&F 10047 caused a dose-related inhibition of the EEG suppression and cortical hyperemia associated with CSD and reduced the CSD propagation rate; ED_{50} = 1 mg/kg, iv and 20 mg/kg, iv, respectively. MK-801 had a delayed onset of action (inversely related to dose) and a prolonged duration of action at all doses (>2h). In contrast, (+)SK&F 10047 had a rapid onset of action (< 30 min) and a predictable dose-related duration of action. These results suggest that an efficacious compound acting with low affinity at the NMDA-receptor channel, eg (+)SK&F 10047, may possess a time-course and toxicity profile which is more suitable than agents interacting with high affinity.

524.8

COMPARISON OF THERMAL AND AUTORADIOGRAPHIC MEASUREMENTS OF CBF. Datong Wei., Gerald M. Saidel, Alejandro D. Perez-Trepichio, Carol R. Radinsky and Stephen C. Jones*. Cerebrovascular Research Lab., Cleveland Clinic Foundation, Cleveland, OH 44195 and Dept. of Biomed. Eng., Case Western Reserve Univ., Cleveland, OH 44106.

A thermal system was developed to quantify cerebral blood flow (CBF) in absolute units (mL/100g-min) as well as continuous CBF changes. From the dynamic heat transfer model, CBF and other parameters in the model can be obtained by non-linear least-squares estimation. The thermal CBF estimates were compared to the measurements from the autoradiographic technique.

Thirteen Sprague-Dawley rats were anesthetized with halothane and artificially ventilated. Changes in blood flow were produced by modifying inspired CO_2 or respiration rate ($PaCO_2$ 15–85 mm Hg). Two cranial windows (7 mm diameter) were created for thermistors (0.65 mm diameter) encased in styrofoam cylinders. The flow probe was a self-heated thermistor placed on the tissue surface. Thermal CBF (CBF_T) was averaged from twelve flow estimates obtained from corresponding transient temperature responses to step heating power changes. Tissue equilibration or indicator fractionation of ^{14}C-IAP and autoradiography were used to determine the mean autoradiographic CBF (CBF_A) in the areas under the thermal flow probe. Linear regression showed that $CBF_T = 56 + 0.7 \bullet CBF_A$, with $r = 0.75$. The slope and intercept were significantly different from zero (*t*-test, $p<0.01$). The coefficient (mean ± SD) between CBF and temperature changes linearized at steady-state perfusion levels was 0.8 ± 0.2 mL/100g-min-m°C.

By statistical analysis, the mean thermal CBF was found to be linearly related to the mean autoradiographic CBF. The advantage of the thermal method is that it can follow and quantify continuous changes in CBF.

Supported by NSF grant BNS-9022190.

524.9

MEASUREMENT OF THE PENTOSE PHOSPHATE PATHWAY (PPP) IN BRAIN AND GLIOMA *IN VIVO* BY MICRODIALYSIS. B.D. Ross*, O. Ben-Yoseph, D.M. Camp‡ and T.E. Robinson‡. University of Michigan, Departments of Radiology, Biological Chemistry and ‡Psychology, Ann Arbor, MI 48109.

The activity of the PPP has traditionally been assayed *in vitro* by comparing the relative rates of $^{14}CO_2$ production from [1-^{14}C]glucose and [6-^{14}C]glucose. Here, we report the use of a novel isotope, (1,6-$^{13}C_2$,6,6-$^2H_2$)glucose (1), in combination with intracerebral microdialysis and gas chromatography/mass spectrometry (GC/MS) for measuring the relative activities of the PPP and glycolysis in the forebrain and intracerebral 9L glioma in the conscious rat. Metabolism of (1,6-$^{13}C_2$,6,6-$^2H_2$)glucose through glycolysis produces (3-^{13}C)lactate (m + 1) and (3-^{13}C,3,3-$^2H_2$)lactate (m + 3), whereas metabolism through the PPP produces (3-^{13}C,3,3-$^2H_2$)lactate (m + 3). GC/MS analysis of extracellular lactate, collected in dialysate over 50 minute intervals, revealed a basal PPP activity of 3.5 ± 0.4% and 6.4 ± 0.5% (n=4) of glucose metabolized to lactate in rat brain and glioma, respectively. Addition of 50µM phenazine methosulfate (PMS) to the perfusate in brain and glioma resulted in an increased PPP activity to 18.7 ± 1.9% and 8.4 ± 1.1%, respectively, followed by a rapid return to basal levels upon removal of PMS. The higher basal PPP activity in glioma compared to brain may be due to the increased NADPH and ribose 5-phosphate requirements for lipid and DNA synthesis of proliferating cells. This technique provides a unique opportunity to measure the PPP in localized regions of the brain (and glioma) in a conscious rat. Sample collection using microdialysis together with high GC/MS sensitivity allow for dynamic changes in PPP activity to be followed in a single animal with a time resolution of 5 min or less. Measurements of PPP activity have also proven successful in tissue cultures. As the PPP is coupled to the glutathione pathway, this technique will provide the ability to monitor oxidative stress known to be associated with various neuropathological conditions.

1. B.D. Ross and P.B. Kingsley, J. Biol. Chem., 1993 (submitted).

Support: ACS grant #BE-149 and NIH grants 1P20NS31114-01 & DA04294.

524.10

NONLINEAR ENHANCEMENT OF CAROTID ARTERIAL OCCLUSION EFFICIENCY IN THE RAT BY ROSE BENGAL-MEDIATED PHOTOTHROMBOSIS CONDUCTED AT 562 NM. B.D. Watson*, R. Prado, W.D. Dietrich, CVD Research Center and Dept. of Neurology, Univ. of Miami Sch. of Med., Miami, FL 33101.

Occlusion of cerebral arteries by an intravascular photochemical interaction sensitized by an appropriate laser has been used to induce experimental stroke, and been proposed for occlusion of arteriovenous malformations. With rose bengal (RB) as photosensitizer, we measured occlusion time (OT) for the rat common carotid artery (CCA) with 1 W laser irradiation at 514.5 nm (standard argon line) and at 562 nm (tunable argon/dye laser) - the RB absorbance (A) maximum in tissue. The right CCA of male Wistar rats (300-390 g) was exposed and covered with saline with rats supine. After 40 mg/kg RB injection the CCA was irradiated at an intensity of ca. 150 W/cm^2 until occlusion. OT at 562 nm was 23 ± 17 sec (S.D., n=4), but OT at 514.5 nm was 287 ± 87 sec (n=3). Since the number of photons (T) generating occlusion is proportional to OT*A, and $A_{562}/A_{514.5}$ = 2.6, the relative occlusion efficiency is $T_{514.5}/T_{562}$ = 20%. The reaction lacks reciprocity and is thus nonlinear. Further, 562 nm-induced thrombi consist only of agglutinated platelets; those at 514.5 nm are mixtures of platelets, RBCs and coagulum (J Neurosurg 66:748-754, 1987). Irradiation at 562 nm therefore accentuates the intrinsic nonlinear platelet response, and greatly enhances photothrombotic occlusion efficiency.

524.11

PREOPERATIVE CORTICAL FUNCTIONAL MAPPING FOR PATIENTS WITH INTRACEREBRAL GLIOMA. T. Nariai[1)2)], M. Senda[1)], K. Ishii[1)], T. Maehara[2)], K. Ishiwata[1)], H. Toyama[1)], K. Oda[1)], T. Murakoshi[2)*], K. Hirakawa[2)]. Tokyo Metropolitan Inst. of Gerontology[1)], Tokyo Med. & Dent. Univ.[2)], Tokyo, Japan.

In order to remove the intracerebral glioma in the maximum extent without damaging the cortical function, we have developed a protocol to visualize the spatial relationship between the tumor and the eloquent cerebral cortex using 3D registration of PET and MRI. The results were compared to the intraoperative cortical mapping. Informed consent was obtained from each patient. Four patients bearing glioma adjacent to the motor or language cortex underwent the MRI scan of 3 mm thickness and PET scans using ^{11}C-methionine to visualize active tumor area and $H_2{}^{15}O$ activation technique to locate eloquent cortex. The hand and oral movement task and the words repetition task was used to map the motor and the language cortex respectively. The PET images were re-sliced at the MRI image planes using 3D registration program on the workstation Stellar GS2000. The area with high ^{11}C-methionine uptake and the area activated by the task was mapped on the MRI images. The registered ^{11}C-methionine image was superior to Gd-enhanced MRI in locating cortical gyri with tumor infiltration. Intraoperative localization of the somatosensory cortex (by the cortical recording of SEP) and the language cortex (by the electrical stimulation of cortex under local anesthesia) confirmed the accuracy of our 3D registration technique in locating the eloquent gyrus, although the mass effect of tumor shifted the cortical structures. Using this protocol, successful resection of tumor was performed without damaging the remaining cortical function.

524.12

MAPPING OF CEREBRAL CORTEX METABOLISM IN SOMAN INDUCED SEIZURES. O.U. Scremin*, T.-M. Shih and M.G. Li., West LA VAMC/UCLA, Los Angeles, CA 90073 and USAMRICD, APG, MD 21010.

This investigation was undertaken to map in detail the pattern of metabolic activation of the cerebral cortex during seizures induced by soman, a highly toxic cholinesterase inhibitor. Twelve rats were injected with 100 μg/kg soman, s.c., after pretreatment with 26 μg/kg pyridostigmine, i.m., to decrease lethality. Seven animals developed seizures (S), and 5 did not (NS). Five additional rats were injected with saline (C). Glucose utilization (CGU) of cortical regions and brain stem was determined with the quantitative, autoradiographic technique. Values of CGU cortex/CGU brain stem (nCGU) (Mean ± SE, p vs C) were calculated for 96 regions in 9 coronal slices between 1.7 mm rostral and 8.3 mm caudal to bregma. Group S showed statistically significant increases with regard to C in 33 regions, 27 of which were in a single cluster, with the piriform cortex (C=1.08±0.06; S=2.53 ±0.37, P<0.01) at its center. Perirhinal cortex of bregma planes -1.8 to -5.3, piriform cortex of bregma planes -0.8 to -5.3 and insular cortex of planes 1.7 to -1.3, showed also significantly higher nCGU in S than in C. Other foci of elevated nCGU were found in the frontal (C=1.37±0.06; S=2.07±0.25, P<0.05) and parietal (C=1.36±0.08; S=2.20±0.29, P<0.05) regions at bregma -3.3. In the non-seizing animals, the only region with a significantly higher nCGU than controls was piriform cortex at bregma -3.3 (C=1.08±0.06; NS=1.31±0.06, P<0.05). In conclusion, the presence of seizures and not soman determined the pattern of nCGU cortical activation. The piriform cortex showed nCGU increase prior to seizures and became the center of the most important focus of activation during seizures.

Supported by a VA-DOD Merit Review, Department of Veterans Affairs.

PEPTIDES: RECEPTOR PHYSIOLOGY

525.1

NEUROTENSIN INHIBITS GROWTH AND STIMULATES INTRACELLULAR CALCIUM RELEASE IN THE HUMAN PROSTATIC CARCINOMA CELL LINE LNCaP. C.A. Mayr*, G. Powis and T.P. Davis. Dept. of Pharmacology and Toxicology, University of Arizona College of Medicine, Tucson, AZ 85724 USA.

Neurotensin (NT) has been postulated to act as a modulatory agent in the central nervous system. Besides its presence in brain, a NT-like immunoreactivity is produced and secreted in the prostate cancer cell line LNCaP. High affinity binding of NT in these cells has also been previously shown, leading to the suggestion that NT may function as a regulatory peptide in prostate cancer, acting in an autocrine fashion. Using a soft agar in vitro growth assay, we report that exogenous NT (0.1nM to 1.0 μM) significantly ($p<0.01$) decreased colony formation of LNCaP cells. Exposure of the LNCaP cells to NT (3.3nM to 3.3μM) in an aequorin protein intracellular calcium assay, increased intracellular calcium content. The increase in intracellular calcium is evidence that the LNCaP cell line expresses the NT receptor, and that the inhibition of growth may be via interactions at the NT receptor. In conclusion, NT causes decreased growth by a mechanism which may involve NT acting at receptors leading to the activation of a second messenger system and subsequent intracellular calcium release.

Supported by NIH grants DK36289, MH42600, and DA06284.

525.2

GHRP-6 RAISES INTRACELLULAR CALCIUM IN RAT PITUITARY CELLS BY TWO DIFFERENT MECHANISMS. J. Herrington, A. Naumov, M.E. Freeman* and B. Hille. Dept. of Physiology and Biophysics, Univ. of Washington, Seattle, WA 98195.

Cellular mechanisms mediating stimulation of growth hormone release by the enkephalin-derived synthetic hexapeptide, GHRP-6, are not known. We have studied the changes in intracellular calcium concentration ($[Ca^{2+}]_i$) produced by GHRP-6 in single pituitary cells isolated from 100-150 g male rats. $[Ca^{2+}]_i$ was monitored by measuring fluorescence intensity of indo-1 (loaded into cells as the acetoxymethylester) at two wavelengths, 405 nm and 500 nm. Increases in the 405/500 emission intensity ratio (R) reflect increases in $[Ca^{2+}]_i$.

Presumptive somatotropes were chosen based on cell size, presence of spontaneous transients in $[Ca^{2+}]_i$ and ability of 100 nM somatostatin (SOM) to inhibit the transients. Applicaton of 500 nM GHRP-6 increased $[Ca^{2+}]_i$ in 32 out of 56 cells studied; in 17 cells R increased from 0.66 (±0.09 SD) to 1.03 (±0.12). Long duration applications (100 sec) produced a relatively sustained elevation in $[Ca^{2+}]_i$ that was blocked 79% (±3; n=4) by lowering extracellular Ca^{2+} to 50 nM, 91% (±4; n=3) by removal of extracellular Na^+ and 84% (±6, n=5) by 100 nM SOM. Application of GHRP-6 for 20-25 sec following prexposure of cells to low Ca^{2+}, 0 Na^+, SOM or 1 μM nitrendipine resulted in only a transient elevation in $[Ca^{2+}]_i$ that decayed to pre-agonist levels with a τ = 13.8 sec (±9.7; n=22) (resting R= 0.61 ±0.06; peak R= 0.96 ± 0.18; n= 4 each for 0 Ca^{2+}, 0 Na^+, NIT and n=10 for SOM). Long (50-80 sec) applications under these conditions did not produce further rises in $[Ca^{2+}]_i$ (n=6). These results suggest that GHRP-6 elevates $[Ca^{2+}]_i$ in somatotropes by at least two mechanisms, a rapid release of Ca^{2+} from intracellular stores followed by enhanced influx of Ca^{2+} through Ca^{2+} channels probably requiring action potentials.

Supported by NS08174, NS07332, HD12629, AR17803 and McKnight Found.

525.3

INSULIN ELEVATES INTRACELLULAR CALCIUM AND CAUSES OSCILLATIONS IN CALCIUM LEVELS IN THE BAG CELL NEURONS OF *APLYSIA*. E. A. Jonas*, R. J. Knox, J. A. Connor, L. K. Kaczmarek. Dept. Pharmacology, Yale Univ. Sch. Med., New Haven, CT 06510, and Dept. Neurosciences, Roche Institute of Molec. Biol., Nutley, NJ 07110.

Insulin may produce changes in the properties of cells by activating signalling pathways linked to tyrosine kinases. Previous work by Solomon et. al., Jonas et. al., (Soc. Neurosci. Abstr., 1992) has shown that the bag cell neurons of *Aplysia* express insulin receptors and that insulin produces enhancement of calcium action potentials and voltage dependent calcium current. We have now used imaging techniques to study the actions of insulin on intracellular calcium levels in isolated bag cell neurons. Cells were injected with the calcium indicator fura-2, or with dextran-conjugated fura-2, a large molecule less subject to transport into intracellular compartments. Control cells demonstrated stable Ca^{2+} concentrations of 200-300nM. After addition of insulin to the bathing medium, Ca^{2+} levels were found to increase in the somata over a period of at least one hour. Levels in the neurites remained relatively unchanged. In 4 cells, Ca^{2+} levels were seen to oscillate with periods of 5-10 seconds. In cells injected with fura-2, but not in cells injected with dextran fura-2, insulin induced "hot spots" of elevated Ca^{2+} levels in the somata, suggesting that insulin may also induce changes in Ca^{2+} levels within certain intracellular organelles. Elevations of Ca^{2+} levels by insulin did not occur in cells placed in media lacking Ca^{2+}. In insulin-treated cells, injection of heparin did not reverse the effects of insulin. Our studies suggest that a change in intracellular Ca^{2+} homeostasis may be one component of long-term changes in the excitability of bag cell neurons induced by insulin.

525.4

REGULATION OF DARPP-32 PHOSPHORYLATION BY CHOLECYSTOKININ OCTAPEPTIDE (CCK-8S) IN STRIATAL SLICES. G.L. Snyder*, G. Fisone, P. Morino[1], V. Gundersen[2], O.-P. Ottersen[2], T. Hökfelt[1], and P. Greengard. Lab. of Molec. & Cell. Neurosci., The Rockefeller University, NY, NY 10021; [1] Dept. of Histology & Neurobiology, Karolinska Institute, S-10401 Stockholm, Sweden, and [2] Dept. of Anatomy, University of Oslo, Blindern, N-0317 Oslo, Norway.

DARPP-32, a dopamine and cAMP-regulated phosphoprotein, Mr 32,000, that is highly enriched in medium-sized spiny neurons comprising the striatonigral pathway, is converted to a potent inhibitor of protein phosphatase-1 upon phosphorylation on threonine[34], *in vitro*. In the present study, COOH-terminal sulfated octapeptide of cholecystokinin, CCK-8S, inhibited the forskolin-induced phosphorylation of DARPP-32 by one-half in striatal slices. The inhibition of DARPP-32 phosphorylation by CCK-8S was probably mediated through activation of a CCK-B receptor, since the effect was prevented by pretreatment of slices with CI-988, a potent and selective antagonist of this receptor. NMDA-type glutamate receptors also were apparently involved, since the inhibition of DARPP-32 phosphorylation by CCK-8S was blocked by MK-801, an NMDA antagonist and since the inhibitory effects of NMDA and CCK-8S on DARPP-32 phosphorylation were not additive. Immunohistochemical analysis revealed the presence of CCK- and glutamate-immunoreactive nerve endings in the striatum, presumably with a cortical origin and aspartate-containing interneurons as possible sites for the action of CCK-8S. These data provide further evidence for the hypothesis that DARPP-32 is a final common pathway which integrates the actions of numerous first and second messengers released from nigro-striatal and cortico-striatal neurons, and perhaps interneurons, on the activity of striatonigral neurons. (Supported by MH-40899 to PG and The Swedish Medical Research Council #04-2887).

525.5

MEDULLARY SITES OF CENTRAL ACTION OF TRH TO INDUCE VAGAL MEDIATED GASTRIC CYTOPROTECTION IN URETHANE ANESTHETIZED RATS.

K. Kato, H. Yang, I. Murai* and Y. Taché. CURE/VA Wadsworth Medical Center and Dept. of Medicine and Brain Research Institute, UCLA, Los Angeles, CA 90073 and Dept. of Biochemistry, Nihon University School of Medicine, Tokyo, JAPAN*.

The vagus nerve plays an important role in gastric cytoprotection as well as gastric lesion formation. Our previous report (Gastroenterolgy 102:1568, 1992) indicates that intracisternal injection of TRH at low doses is cytoprotective against ethanol lesions in conscious rats. The role of the dorsal motor nucleus of the vagus (DMN) in providing gastric cytoprotection was further investigated.

Method: Under urethane anesthesia (1.25 g/kg, ip), fasted rats were microinjected with saline (50 nl) or TRH analog RX 77368 (1.5, 3.0, 5.0, 10, 15, 30 ng/50 nl) into the DMN 30 min before intragastric adminstration of 60% ethanol (5 ml/kg). Percentage of corpus mucosa containing lesions was determined by computerized image analizer one hour after ethanol. Atropine sulfate (2 mg/kg, sc, -30 min) and indomethacin (5 mg/kg, ip, -60 min) was given before microinjection of RX 77368 (3 ng).

Results: RX 77368 (3, 5, 10 ng, into DMN) significantly decreased gastric damage (lesions: 3 ng: 2.5 ± 0.6%, 5 ng: 3.8 ± 0.4%, 10 ng: 4.6 ± 0.6%, compared with saline :10.2 ± 1.4%, $P<0.01$) The other doses had no cytoprotective effect as well as peptide injection (3 - 10 ng) into the hypoglossal nucleus, or medullary reticular field. The cytoprotective effect of RX 77368 (3ng, into DMN) was completely blocked by atropine and significantly inhibited by indomethacin.

Conclusion: These results suggest a possible role of TRH in the DMN in the control of gastric mucosal integrity through vagal cholinergic and prostaglandin dependent pathways.

525.6

DIFFERENTIAL REGULATION OF GALANIN ANTAGONISTS M15 AND M35 ON ACETYLCHOLINE RELEASE IN THE RAT STRIATUM.

A. Pramanik*†, S.O. Ögren†, T. Land‡ and Ü. Langel‡. †Dept. of Histology and Neurobiology, Karolinska Institute, Box 60 400, S-104 01 Stockholm, Sweden; ‡Dept. of Neurochemistry and Neurotoxicology, Arrhenius Laboratories, Stockholm University, S-106 91 Stockholm, Sweden.

Galanin (GAL), a 29-amino acid peptide with numerous biological functions (Rökaeus (1987), Trends Neurosci. 10, 158-164), has a widespread distribution in the CNS and the PNS. In view of the multiple actions of exogenous GAL it is of interest to develop GAL agonists or antagonists in order to characterize the functional role of endogenous GAL. Recently, the putative GAL antagonists M15 [(GAL-(1-12)-Pro-substance P-(5-11)-amide] and M35 [GAL-(1-12)-Pro-bradykinin-(2-9)-amide] have been developed (Bartfai et al. (1992), Trends Pharmacol. Sci. 131, 312-317).

The effects of M15 and M35 on the basal and the GAL-evoked release of acetylcholine (ACh) in the striatum were studied in male Sprague-Dawley enflurane-anaesthetized rats (body wt. 270-300 g) using in vivo microdialysis and HPLC techniques. M15 and M35 were able to produce differential effects on both the basal and the GAL-evoked ACh release in vivo in the striatum. Thus, the GAL-evoked ACh release was completely blocked by M15 but partially by M35 when they were coinfused with GAL (300 µM or 3 nmol/10 µl, intrastriatal infusion). Moreover, M35 elevated the basal ACh release whereas M15 failed to do so. These results suggest that M15 is a full antagonist of the striatal GAL receptor modulating ACh release while M35 behaves as a mixed agonist-antagonist. In addition, M15 and M35 recognized two types of galanin binding sites in striatal membranes of the rat with equal affinities but with different relative occupancies (Ögren et al. (1993), Eur. J. Pharmacol., submitted). Thus, different relative receptor occupancies may be one of the possible explanations for the difference between M15 and M35. These findings may be of significance for the analysis of multiple GAL receptors in the central nervous system.

525.7

BIOLOGICAL ACTIVITY OF A POTENT BOMBESIN RECEPTOR ANTAGONIST.

M. Knight,* K. Takahashi, B. Chandrasekhar, R.T. Jensen, D.B. Strader and T.W. Moody. Peptide Technologies Corp., 8401 Helgerman Court, Gaithersburg MD 20877, National Institutes of Health, Bethesda MD 20892 and George Washington University, Washington DC 20037.

Structural modifications of the known inhibitory heptapeptide sequence of the tetradecapeptide bombesin (BBN), a neuropeptide, has produced a potent inhibitor. [N-chlorambucil, D-Ala"] BBN(7-13)ethyl ester, (PTC 821) inhibits the effect of BBN to raise cytosolic Ca^{++} levels in small cell lung carcinoma cells (SCLC), a neuroendocrine tumor. The peptide displays specific binding to SCLC BBN receptors with an $I.C._{50}$ of 8 nM. The peptide with one more amino acid in its sequence was found to prevent colony formation of gastrin releasing peptide-containing human tumor cell lines. PTC 821 has also been studied for its effects on pancreatic acinar cells which also have BBN receptors and are stimulated by BBN to secrete the digestive enzymes. The peptide is a specific BBN antagonist with an $I.C_{50}$ of 4.9 nM. The chlorambucil is a large aromatic structure with N-alkyl chlorides that can alkylate certain reactive groups in the receptor protein. Complete irreversible inhibition was not observed, but the biologic inhibition remained potent and long lasting in washing experiments on the cells without any toxic effects. Whether the potency of this modification is due, not only to the aromaticity of the derivative but also to the alkyl halide groups, remains to be studied. Nevertheless, this is a useful structure for further development of a chemotherapeutic for neuroendocrine tumors. (Supported by SBIR NIH grant no. CA55468)

525.8

BOMBESIN BINDING SITES IN THE NUCLEUS OF THE SOLITARY TRACT ARE NOT OF VAGAL ORIGIN.

E.E. Ladenheim*, R.T. Jensen, S.A. Mantey, K.A. Moore and T.H. Moran. Dept. Psychiatry, Johns Hopkins Univ. Sch. Med., Baltimore, MD 21205 and NIDDKD, NIH, Bethesda, MD 20892.

High affinity binding sites for bombesin (BN)-related peptides have been identified in the nucleus of the solitary tract (NTS), the primary site of termination for vagal afferent fibers. We evaluated the possibility that BN binding sites in this region are of vagal origin in two ways. In the first experiment, rats were pretreated intraperitoneally with either 145 mg/kg of capsaicin, a neurotoxin that destroys small diameter vagal sensory neurons, or the injection vehicle. Binding of ^{125}I-(Tyr^4)BN was examined in the medial NTS by in vitro receptor autoradiography. In the second experiment, peripheral transport of BN binding sites was evaluated in rats receiving unilateral ligation of the left cervical vagus nerve distal to the nodose ganglia. Accumulation of BN binding sites in the ligated nerve segment was examined 24 h after ligation by receptor autoradiography. Our results indicate that capsaicin treatment had no effect on ^{125}I-(Tyr^4)BN binding in the NTS compared with controls. Additionally, there was no accumulation of BN binding sites in the vagus nerve proximal to the ligation. Together, these results suggest that BN binding sites in the NTS are not of vagal origin but are most likely postsynaptic to vagal afferent input.

525.9

A NEW GALANIN RECEPTOR SUBTYPE IS INVOLVED IN THE REGULATION OF 5-HT_{1A} RECEPTORS IN THE RAT BRAIN.

P. B. Hedlund*, N. Yanaihara[1] and K. Fuxe. Dept. of Histology and Neurobiology, Karolinska Institutet, Box 60400, S-10401 Stockholm, Sweden and [1]School of Pharmaceutical Sciences, University of Shizouka, 395 Yada, Shizouka, Japan.

Galanin is a peptide with 29 amino acid residues widely distributed in the brain and associated with several biological effects. The N-terminal part of the molecule has been shown to be critical for the retention of biological activity. Recently the existence of specific binding sites for [^{125}I]galanin-(1-15) with a unique distribution in the brain was demonstrated. In line with the previously demonstrated reciprocal interactions between galanin and 5-hydroxytryptamine$_{1A}$ (5-HT_{1A}) receptors we have in the present study evaluated whether galanin-(1-29) and galanin-(1-15) can modulate the 5-HT_{1A} receptors using [^{3}H]8-OH-2-(di-n-propylamino)-tetralin ([^{3}H]8-OH-DPAT) as a radioligand in membrane preparations from the ventral limbic cortex, an area having both porcine [^{125}I]galanin-(1-29) and [^{125}I]galanin-(1-15) binding sites, and from the dorsal hippocampus, an area having only the [^{125}I]galanin-(1-15) fragment binding sites. In the dorsal hippocampus galanin-(1-15) produced a concentration dependent increase in the K_D value of [^{3}H]8-OH-DPAT with a maximal effect of approximately 65 % at 3 nM of galanin-(1-15), whereas galanin-(1-29) had no effect. In the ventral limbic cortex galanin-(1-15) also produced a concentration dependent increase in the K_D value of [^{3}H]8-OH-DPAT. The effect of galanin-(1-15) (3 nM) on the K_D value of [^{3}H]8-OH-DPAT binding in the dorsal hippocampus could be completely counteracted by the putative galanin antagonist M35 (1 nM). The B_{max} values were not affected in any experiment. These results give further evidence for the existence of a galanin receptor subtype mainly recognizing N-terminal galanin fragments and for the first time provide evidence that also this putative galanin fragment receptor can reduce the affinity of 5-HT_{1A} receptors.

525.10

BW-2258: A NEW GASTRIN RELEASING PEPTIDE RECEPTOR ANTAGONIST

T.W. Moody*, F. Zia, J.J. Leban, J.D. McDermed and M. Fagarason. Dept. Biochemistry and Molecular Biology, The George Washington University Med. Sch., Washington, D.C. 20037, Organic Chemistry Div., Burroughs Wellcome Co., Research Triangle Park, NC 27709

Bombesin/Gastrin releasing peptide (BN/GRP) may function as a growth factor in certain tumor cells. Previously, GRP receptors were identified on human glioblastoma (U-138) and neuroendocrine small cell lung cancer (NCI-H345) cells. Here the effects of synthetic antagonists which have a reduced peptide bond between the 26 and 27 position of GRP were investigated.

Specific (^{125}I-Tyr^4)BN to U-138 or H345 cells was inhibited with high affinity (IC_{50} = 5-10 nM) using BW462, 1023 or 2123. Also, ^{125}I-BW1023 bound with high affinity (Kd = 5 nM) to a single class of sites (Bmax = 20,000/cell). Specific (^{125}I-Tyr^4)BN but not ^{125}I-BW1023 binding was internalized at 37°C. BN (10 nM) elevated the cytosolic Ca^{2+}. In contrast, BW462, 1023 or 2123 had no effect on the cytosolic Ca^{2+} but inhibited the increase caused by 10 nM BN. Also, BN caused a transient increase in c-fos mRNA and the increase in c-fos mRNA was reversed by 10 µM BW2258. BN (10 nM) stimulated colony formation and BW2258 inhibited proliferation. These data suggest that BW2258 may be a useful peptide to antagonize GRP receptors. Supported in part by NCI-grant CA-53477.

525.11

Alterations in hypothalamic-brain stem angiotensin II (AII) receptor binding in captopril (CAP) treated spontaneously hypertensive rats (SHR)

Kathleen H. Berecek* and Jian -Nan Wu
Hypertension Program, University of Alabama at Birmingham, Birmingham AL 35294

The purpose of this study was to determine the effects of the angiotensin converting enzyme inhibitor CAP on hypothalamic AII receptor binding. We performed radioligand binding studies (^{125}I - [Sar^1, Ile^8] AII) with hypothalamic-brain stem membranes from control (CON) and CAP treated Wistar Kyoto rats (WKY) and SHR at 1, 8 and 120 days post birth. We also estimated the percentage (%) of AII receptors subtypes (AT_1 and AT_2) by generating competition curves using losarthan and CGP42112 to block AT_1 vs AT_2 receptors, respectively. SHRCON at one days of age showed a greater number of AII receptors (Bmax) 43.1±2.8 fmol/mg protein) than the other rat groups. CAP treatment of SHR but not WKY lowered the number of AII receptors (34.6±2) to levels not different from WKYCON (31.7±4) and WKYCAP (33.3±0.6). The same pattern occurred at 120 days of age although all rats showed a decrease in the number of AII receptors. At 1 day of age 30% of the AII receptors were AT_1; whereas at later ages the % of AT_1 fell to 20%. The proportion of subtypes was not different between CON and CAP treated SHR and WKY. Our data suggest that part of the antihypertensive action of CAP may be due to a decrease in AII binding in central cardiovascular areas.

525.12

EFFECTS OF DESOXYCORTICOSTERONE ACETATE ADMINISTRATION ON PREPROTACHYKININ-A GENE EXPRESSION IN DISCRETE RAT BRAIN REGIONS : AN *IN SITU* HYBRIDIZATION ANALYSIS.

P. Pompei, F. Riftina* and B. McEwen, Lab. of Neuroendocrinology, the Rockefeller University, New York, NY 10021.

It is well-known that doses of the mineralocorticoid desoxycorticosterone acetate (DOCA) in excess of about 1mg/kg/day exhibit sharp increases in salt intake. Male Sprague-Dawley rats (n=6) received subcutaneous (sc) daily DOCA injection (5mg/kg/day), whereas control (CON) animals were injected sc with isotonic saline solution. On day 8 they were given access to 2% saline to drink for 2 hr and on day 11 were sacrificed by decapitation. This study describes the presence and abundance of Preprotachykinin-A gene (PPT-A) mRNA transcripts in the bed nucleus of the stria terminalis (BNST), in the caudate-putamen (CP) and in the medial preoptic area (mPOA) of DOCA treated and CON animals. *In situ* hybridization with synthetic oligodeoxyribonucleotide probes specific for PPT-A gene was performed. Quantitative analysis revealed a sharp decrease by 50% in the level of PPT-A mRNAs in BNST and CP of DOCA treated when compared to CON rats. The intensity of silver grains in the ventral CP was greater than in the dorsal CP. Moreover, the expression of PPT-A mRNAs in the mPOA of DOCA treated rats was decreased by 25% when compared to CON animals.

These findings show changes in the PPT-A gene expression under DOCA administration, further suggesting a possible interaction between tachykinins and mineralocorticoids in the regulation of sodium appetite. (Supported by MH 43487).

FORMATION AND SPECIFICITY OF SYNAPSES

526.1

IMAGING TRANSFECTED MUSCLE FIBERS AND THEIR SYNAPSES OVER TIME IN LIVING MICE.

P. van Mier*, M.J. Donoghue, J.R. Sanes, and J.W. Lichtman. Dept. Anatomy & Neurobiol., Washington University Sch. Med., St. Louis, MO 63110

In order to better understand the regulation of synapse formation and maintenance we have begun to study transfected muscle fibers in the sternomastoid muscle of living mice. Muscle fibers were transfected in situ (see Wolff et al., 1990, Science 247:1465-68) by placing pellets of DNA on the muscle surface near the endplate band and gently pressing the DNA into the muscle with a needle. The DNA encoded bacterial β-galactosidase (*lacZ*) driven by RSV (viral) regulatory elements; subsequent staining for the presence of *lacZ* in fixed muscle revealed 5-10 transfected muscle fibers per sternomastoid, of which an average of about ¼ was found on the surface of the muscle. Transfected muscle fibers were observed as early as 2 days, and for at least 2 months after DNA application. In living mice, single transfected muscle fibers were located using FDG (Molecular Probes, Eugene, OR), a caged fluorescein that is released in the presence of *lacZ*. Fluorescent muscle fibers and their rhodamine-bungarotoxin labeled acetylcholine receptors were imaged using standard epifluorescence optics in conjunction with a SIT camera connected to a digital imaging system. Once located with FDG staining, transfected muscle fibers could be restained on multiple occasions and some were followed for up to 4 weeks. In later views transfected muscle fibers and their neuromuscular junctions appeared unchanged suggesting that transfection with a reporter gene and repeated staining did not affect the muscle fibers. We have also used a recombinant adenovirus vector to deliver *lacZ* to sternomastoid muscle fibers. Currently we are trying to transfect or infect with vectors that encode both *lacZ* and synaptically relevant protein molecules that affect synaptic interactions.

526.2

LOCAL CONTROL OF ACh RECEPTOR STABILITY AND LOCATION WITHIN NEUROMUSCULAR JUNCTIONS.

R.J. Balice-Gordon*, C. Garriga, and J.W. Lichtman Dept. Anatomy & Neurobiology, Washington Univ. School Medicine, St. Louis, MO 63110.

After motor nerve terminals are paralyzed by exposure to botulinum toxin, functional recovery is accompanied by loss of some original synaptic sites and the concurrent formation of new ones. We studied how the distribution of postsynaptic AChRs changed as activity resumed in botulinum toxin poisoned junctions in the mouse sternomastoid muscle using *in vivo* imaging as previously described. Fluorescently tagged αBTX was used to label the original distribution of AChRs at the first view; at intervals of 1-2 weeks, the distribution of remaining originally labeled AChRs and the location of newly inserted AChRs was determined by comparing the location of AChRs before and after restaining.

There were striking differences in the distribution of AChRs 3-4 weeks after botulinum poisoning compared to that present at the first view. After restaining at the second view, some areas in the AChR distribution that were present at the first view had disappeared entirely. The remaining areas were patchy in that the density of AChRs varied widely from one small area to another. These differences suggested highly localized control of AChR stability within recovering junctions. The local variability in AChR density could be due either to local differences in AChR turnover or to a redistribution of AChRs within the junction. Consistent with the latter possibility, we found at the second view (before restaining) that some of the AChRs which were labeled with αBTX at the first view had been relocated to new synaptic sites which were not present at the first view. Thus AChRs may migrate away from postsynaptic areas underlying inactive motor nerve terminals still paralyzed by botulinum toxin and cluster beneath new, active terminal sprouts. The local differences in AChR stability we observed may be one consequence of 'competition' between motor nerve terminal branches that resume activity at different times.

526.3

WEAKENING OF SYNAPTIC INPUTS DUE TO LOW QUANTAL CONTENT AND EFFICACY AT DEVELOPING NEUROMUSCULAR JUNCTIONS

H. Colman* and J.W. Lichtman, Dept. of Anatomy & Neurobiology, Washington University School of Medicine, St. Louis, MO 63110

During the process of synapse elimination at developing neuromuscular junctions, all but one of the axons initially innervating each junction are eliminated. The mechanism underlying this synaptic loss is not well understood. Changes in synaptic efficacy are associated with synapse elimination (*Soc. Neurosci. Abst.* 15:165, 1989), indicating that the synaptic strengths of the competing axons are altered during the process. To determine what causes this alteration in synaptic strength, we recorded intracellularly from mouse trapezius muscle fibers and separately stimulated each axon converging on multiply innervated fibers. By bathing the muscle in a high magnesium Ringer's solution, we could simultaneously assay the quantal content (the number of vesicles released in response to stimulation) and quantal efficacy (the postsynaptic response to the release of a single vesicle of ACh) for each axon innervating a junction.

Measurement of relative quantal contents of inputs to the same muscle fiber demonstrated that the synaptic strengths of the competing inputs becomes increasingly different over the first two postnatal weeks. At early ages, the quantal contents of the axons innervating a single muscle fiber were usually similar, while at later ages, one axon was likely to have a substantially larger quantal content than its competitor.

Analysis of the amplitudes of single quanta evoked from each innervating axon indicated that changes in quantal efficacy also occur during synapse elimination. We found many cases in which the postsynaptic responses from one axon contained a subset of unusually small events. These were a discrete population of quantal events that were smaller than other single quantal events recorded from either axon. In one extreme case in which we recorded 3000 responses to stimuli of each axon, approximately 70% of the quantal responses from one axon were small. Observation of small quantal responses was correlated with multiple innervation; singly innervated junctions at the same ages rarely showed small quanta. This evidence suggests that a physiological correlate of imminent synapse elimination is the appearance of small quantal events. Because the weaker axon often had both a low quantal content and small evoked quantal events, these phenomena may be two features of synapses at risk for elimination.

526.4

MUTATIONAL ANALYSIS OF THE CELL ADHESION MOLECULE FASCICLIN I IN THE DEVELOPMENT OF SYNAPTIC FUNCTION IN *DROSOPHILA*.

Y. Zhong*, Neuroscience Center Cold Spring Harbor Lab, P.O. Box 100, Cold Spring Harbor, NY 11724.

5-HT stimulated down regulation of *Aplysia* cell adhesion molecules has been suggested as a mechanism of learning-related synapse formation (Bailey et al., Science 256: 654). In this study, I examined mutations of the *Drosophila fasciclin I* (*fas I*) gene, which encodes a membrane-associated glycoprotein capable of homophilic cell adhesion (Elkins et al., 1990, Cell 60:565), for their effects on nerve terminal arborization and synaptic transmission during development of larval neuromuscular junctions. As compared to normal larvae by anti-HRP staining, the numbers of nerve terminal branches and varicosities were increased in the *fas* I^{TE} mutant which lacks the *fas I* protein. However, evoked excitatory junctional currents (ejcs) were significantly smaller in *fas* I^{TE} recorded by voltage clamp at 0.1 to 0.4 mM external Ca^{2+} concentrations. Conversely, duplication of the *fas* I^{+} gene in *Dp(fas* I^{+}*)* or additional *fas* I^{+} introduced by a P element insertion in *P(fas* I^{+}*)* reduced the numbers of branches and varicosities, but enhanced transmitter release. Such an inverse relation of less varicosities or synapses with stronger synaptic transmission led me to further examine the eletrophysiological properties of these neuromuscular junctions. After a 10 to 30 second tetanic stimulation (10 Hz), spontaneous discharges or multiple discharges in response to a single stimulus could be induced in *Dp (fas* I^{+}*)* and *P(fas* I^{+}*)*, a phenomenon which was not observed in normal or *fas* I^{TE} larvae. It suggests that the membrane excitability is enhanced in nerve terminals with more *fas I* proteins, which may account for the larger ejcs observed. In addition, the duration of post-tetanic potentiation induced by 5 or 8 Hz stimulation is much shorter or even missing in both *fas* I^{TE} and *Dp (fas* I^{+}*)* or *P(fas* I^{+}*)*. This result demonstrates for the first time *in vivo* that changes in the number of a cell adhesion molecule can influence not only the number of synapses formed, but also the strength of synaptic transmission and capability of synaptic plasticity.

526.5

Development of CNS structure and connections in the NMDA receptor deficient mouse. Yuqing Li[1], Elizabeth Messersmith[2], Carla Shatz[2*], Susumu Tonegawa[1]. [1]Center for Cancer Research, Massachusettes Institute of Technology, Cambridge, MA 02193, [2]Department of Molecular & Cell Biology, University of California, Berkeley, CA 94720

Development of precise neural connections in the vertebrate central nervous system (CNS) occurs by two distinct phases; one that occurs early and acts via molecular cues, and one that subsequently requires neuronal activity. Recently, evidence has suggested that precise connections in the visual system are achieved via neural activity acting through NMDA receptors. To study the role of NMDA receptors during formation of CNS connections, we have created, using the embryonic stem cell technology, knockout mice lacking the NMDAR1 subunit, which renders the NMDA receptor nonfunctional. We have examined the brains of newborn NMDA receptor deficient mice for abnormalities in gross anatomy, neurogenesis, cell migration, and formation of neuronal pathways. We find that the brains of newborn NMDA receptor deficient mice do not differ in gross morphological appearance in comparison to normal mice. Birthdating studies with 3H-thymidine indicate that there is no difference in neurogenesis. DiI labeling of retinal ganglion cells (RGC) projections indicate that RGC axons do not grow to alternative target sites in the absence of NMDA receptor activity, but that they grow to their appropriate target within the thalamus. These observations suppport the hypothesis that initial pathfinding and target selection by growing axons is not dependent upon NMDA receptors and leaves open the possibility that later fine tuning to the precise adult pattern does require activation of NMDA receptors. Funded by Howard Hughes Medical Institute (ST) and NSF IBN 92-12640, March of Dimes (CJS).

526.6

***Passover* in *Drosophila* and *unc-7* in *Caenorhabditis* both disrupt neural connectivity and code for proteins that are homologous. R.J. Wyman, G.P. Swain, E. Frei, Y.A. Sun* and S.N. Krishnan.**

Department of Biology, Yale University, New Haven, CT 06511.

Mutations at the *Passover* locus disrupt transmission through the Giant Fiber pathway which commands the light-off escape jump in *Drosophila*. The block is at the output from the Giant Fiber to its post-synaptic targets, the Peripherally Synapsing Interneuron and/or the Tergotrochanteral motoneuron which drive the middle leg jump muscle. The synapses from the GF to these cells are dual electrical/chemical. The fast electrical pathway is the effective one in the startle response.

Mutations of the *C. elegans* gene *unc-7* (Starich et al., Genetics **133**:527-541, 1993) cause miswiring. Instead of moving in a sinuous pattern during forward locomotion, the body 'kinks'. Electron micrographic reconstruction of a mutant nerve cord shows that the AVA interneuron, which normally forms synapses only with motoneurons for backward locomotion, now makes gap junctions with motoneurons for forward locomotion. If the interneuron elicited forward and backward locomotion at the same time, the opposite running sinusoids would interfere, causing kinks. The neurons observed to connect abnormally in *unc-7* form gap junctions as do those disrupted by *Pas*. Pas and unc-7 amino acids are 33% identical + 15% conserved (over 293 aa). Both are transmembrane with similar genomic and protein structure. Since nematodes are evolutionarily quite distant from insects, it is likely that homologous molecules exist in other animals, perhaps defining a molecular family involved in connectivity of the nervous system.

526.7

THE ROLE OF HEPARIN-BINDING GROWTH-ASSOCIATED MOLECULE (HB-GAM) IN POSTSYNAPTIC INDUCTION IN CULTURED MUSCLE CELLS. H. Benjamin Peng ‡, Zhengshan Dai‡, Erkki Raulo¶ and Heikki Rauvala¶. ‡Dept. of Cell Biol. and Anatomy and Curr. in Neurobiology, Univ. of North Carolina, Chapel Hill, NC, USA and ¶Inst. of Biotechnology, Univ. of Helsinki, Helsinki, Finland.

HB-GAM (pleiotrophin, p18) is a novel heparin-binding growth factor (HBGF). Unlike several other HBGFs, its transcript possesses a signal sequence and its secretion has been shown. It is widely expressed in the brain and spinal cord and its transcripts have also been detected in skeletal muscle. We examined the function of HB-GAM in inducing the formation of acetylcholine receptor (AChR) clusters in cultured muscle cells. Using an antibody against recombinant rat brain HB-GAM, we found that this protein is present prominently on the surface of cultured *Xenopus* myotomal muscle cells by immunocytochemistry. It is associated with heparan-sulfate proteoglycans because heparin and heparinase treatment greatly diminished the antibody labeling. HB-GAM is concentrated at a fraction of the spontaneously formed AChR clusters (hot spots). To assess its function in synaptic induction, we applied recombinant HB-GAM-coated beads to cultured muscle cells to effect focal presentation. Over 70% of the beads induced the formation of AChR clustering as shown by fluorescent α-bungarotoxin labeling. This induction can be blocked by the polyanion suramin (100μM), suggesting the stimulation of a cell surface receptor. It is also blocked by a tyrosine kinase inhibitor (tyrphostin RG-50864) with a half maximum inhibitory dosage of 50μM, suggesting the role of tyrosine phosphorylation in this induction. This work indicates that HB-GAM is an endogenous muscle-derived factor that may play an important role in postsynaptic induction. (Supported by NIH and MDA)

526.8

AGRIN ISOFORMS IN DEVELOPING CHICK MOTOR NEURONS. L. S. Honig*, D. Stone#, K. Nikolics#, and U. J. McMahan. Dept. of Neurobiology, Stanford University School of Medicine, Stanford, CA 94305; and #Genentech, Inc., S.S.F., CA 94080.

Agrin is thought to mediate the motor neuron-induced aggregation of AChRs in muscle fibers at their neuromuscular junctions. The nervous system expresses several alternatively spliced isoforms of agrin; isoforms active in inducing aggregation of AChRs in cultured chick myotubes must have sequence inserts at two C-terminal sites called A (4 amino acids) and B (8, 11, or 19 (8+11) amino acids). We have examined the expression of agrin isoforms in the chick lumbar spinal cord using *in situ* hybridization with oligonucleotides complementary to the specific isoforms. We find that at embryonic days 4-10 when motor neurons begin to form neuromuscular junctions, B0 mRNA is concentrated in the mitotic ventricular zone (VZ). In contrast, B11 and B19 mRNA are concentrated in the postmitotic mantle, including the motor neuron column. B19 mRNA is far more abundant in motor neurons than other cells. In later embryos (≥10d), B8 mRNA appears in motor neurons; B8 persists postnatally (0-2wk) while the amounts of B11 and B19 mRNA decrease below detectability. At early stages, A0 mRNA predominates in the VZ, while A4 mRNA is concentrated in the postmitotic mantle. A4 persists in motor neurons to maturity. We conclude that motor neuron progenitor cells express A0/B0 agrin, but by the time differentiated motor neurons are positioned in the ventral horn they express isoforms having both A and B inserts. Developing motor neurons express isoforms having each of the B inserts, but during neuromuscular junction formation, isoforms having the B19, and later the B8, insert are most abundant.

LSH supported by Dana, and Walter V. & Idun Berry Fellowships.

526.9

PRODUCTION OF MONOCLONAL ANTIBODIES TO THE AGRIN RECEPTOR. M.A. Bowe*, K.A. Deyst and J.R. Fallon. Worcester Foundation for Experimental Biology, Shrewsbury, MA 01545.

Agrin is a synapse organizing molecule crucial for the formation and regeneration of the neuromuscular junction. Agrin induces the clustering of acetylcholine receptors in the postsynaptic muscle cell membrane. The cell surface receptor for agrin has not been identified. However, our laboratory has previously characterized a putative agrin receptor, based on both cell biological and biochemical criteria (Nastuk et al., 1991 Neuron 7:807).

Here, we have used immunological methods to further our studies of the agrin receptor. We developed a screen to detect the immunoprecipitation of agrin binding proteins from solubilized membrane preparations. Mice were immunized with postsynaptic-enriched membranes from the electric organ of *Torpedo*, and hybridomas were produced. Hybridomas secreting antibodies that immunoprecipitate the agrin receptor were cloned. We have characterized one MAB in detail and found that it binds an integral membrane glycoprotein in postsynaptic membranes. This MAB immunoprecipitates the agrin receptor but not the acetylcholine receptor. We are currently using this antibody to identify and purify the agrin receptor.

Supported by NIH and MDA.

526.10

NEURONAL SPECIFICITY IN THE EXTERNALIZATION OF AGRIN. M.W. Cohen* and E.W. Godfrey. Dept. of Physiol., McGill Univ., Montreal, Que. and Dept. of Cellular Biol. & Anat., Medical College of Wisconsin, Milwaukee, WI.

Agrin mRNA transcripts are present in the ventrolateral spinal cord (SC) as well as in dorsal root ganglia (DRG) of rat and chick. Previously we found that a subpopulation of Xenopus SC neurons externalize agrin along their neuritic outgrowth in culture. In this study we examined whether Xenopus DRG neurons also externalize agrin. Agrin immunofluorescence was rarely detected in the DRG cultures, and the few positive examples were faint and limited to regions of DRG somata. In addition the DRG neuritic pathways, unlike SC neuritic pathways, failed to cause acetylcholine receptors (AChRs) on test muscle cells to accumulate at sites where they contacted the pathways. The DRG neurons may have failed to synthesize significant amounts of agrin, the factors regulating externalization of agrin may differ in SC and DRG neurons, or the binding properties of SC agrin and DRG agrin may be different. The correlation that SC and DRG neurites differ not only in their deposition of agrin but also in their capacity to influence AChR distribution on muscle cells supports the conclusion that agrin is the primary neural agent responsible for the accumulation of AChRs at newly forming nerve-muscle synapses. [Supported by MRC (M.W.C.) and NIH (E.W.G.)].

526.11

EXPRESSION OF THE POSTSYNAPTIC 43K PROTEIN IN *XENOPUS* EMBRYONIC CELLS AND ITS ASSOCIATION WITH PHOSPHOTYROSINE. Zhengshan Dai‡, Paula B. Scotland¶, Stanley C. Froehner*¶ and H. Benjamin Peng‡, Departments of ‡Cell Biology and Anatomy and ¶Physiology, and ‡¶the Curriculum in Neurobiology, University of North Carolina, Chapel Hill, NC 27599.

The postsynaptic membrane of cholinergic synapses in skeletal muscle and electric organ is enriched in 43K protein. Expression of this protein alone in *Xenopus* oocytes or in fibroblasts results in the formation of membrane-associated 43K micropatches and coexpression with AChRs results in the microclustering of the receptors. In this study, we expressed the 43K protein with or without AChRs in *Xenopus* embryos by mRNA injection. Blastomeres at 8-cell stage were injected with mRNAs and the animal caps were dissected out prior to gastrulation, dissociated and cultured. When AChR and 43K were coexpressed, AChR and 43K clusters were observed. When the 43K was expressed alone, 43K microclusters with a diameter of 0.5 to 3μm were observed in both cultured cells and whole-mount animal caps. Most of the clusters were associated with the substratum-facing membrane in cultured cells and with cell-cell contacts in whole mounts. No clusters were observed in cells that expressed AChRs alone. In 43K-expressing cells, double-labeling with a polyclonal 43K antibody and a monoclonal phosphotyrosine (PY) antibody revealed a colocalization of PY-containing proteins with the 43K clusters in the absence of AChRs. Although they were colocalized, the 43K and PY patches were often not exactly congruent. Since there is no evidence that the 43K protein is a substrate for tyrosine kinase, these data suggest other PY-containing proteins are co-clustered with 43K in these cells. This embryonic cell culture should offer a convenient system for understanding the function of postsynaptic proteins in synaptogenesis. (Supported by NIH and MDA)

526.12

DEVELOPMENTAL EXPRESSION OF ARIA mRNA IN THE CHICK: AN *IN SITU* HYBRIDIZATION STUDY G. Corfas*, K.M. Rosen, H. Aratake and G.D. Fischbach. Dept. of Neurobiology, Harvard Medical School, Boston, MA 02115 and Asahi Chemical Co., Shizuoka, Japan.

ARIA is a protein purified from chick brain that promotes an increase in synthesis of acetylcholine receptors. It may play a role in the formation and maturation of the neuromuscular junction. ARIA has been recently cloned and its sequence shown to be homologous to Heregulin and NDF, two putative ligands for the receptor tyrosine kinase HER2/neu, and to glial growth factor. In order to study the role of ARIA in the development of the nervous system we have studied the expression of ARIA mRNA in the chick by *in situ* hybridization. ^{35}S-labelled riboprobe coding for the 5' most 337 bp of the pro-ARIA1 clone was used.

A clear signal above background is evident in the neural tube at embryonic day 4 (E4), the earliest time examined. On E5, grains are concentrated over spinal cord motorneurons. This distribution is maintained throughout embryonic development and on post-hatched day 1 (P1).

ARIA mRNA is not uniformly distributed in the P1 brain. Rather, the label is concentrated in brain stem motor nuclei, the isthmo-opticus nucleus, the optic tectum, in cerebellar granule cells and the hippocampus. At earlier times the label is somewhat more widely distributed.

The gene is also expressed outside the nervous system. Significantly the message can be detected with the same probe in skeletal muscle. The probe used here is directed to regions of ARIA that are expected to be present in many different spliced forms of the molecule. Different isoforms may exert different biological actions, and their expression may be developmentally regulated. This could be analyzed using isoform-specific probes.

TRANSMITTERS IN INVERTEBRATES

527.1

PHARMACOLOGICAL PROPERTIES OF A SEROTONIN-ACTIVATED SODIUM CURRENT IN AN IDENTIFIED NEURON FROM *HELISOMA TRIVOLVIS*. C. J. Price* and J. I. Goldberg. Dept. of Zoology, Univ. of Alberta, Edmonton, Alberta, Canada T6G 2E9.

Serotonin (5-HT) activates a cyclic AMP-dependent sodium current in neuron B19 from *Helisoma trivolvis*. To determine the pharmacological characteristics of the receptor mediating this response, tight-seal whole cell recordings were obtained from isolated neuron B19s and the agonist and antagonist activities of various compounds were tested. Dose-response experiments indicated a relatively high affinity for 5-HT, with an EC50 in the range of 10 to 100 nM. To obtain a pharmacological profile of the response, the abilities of various indoles to mimic 5-HT's action were examined. 5-carboxyamidotryptamine and tryptamine both activated the response with an EC50 around 1 μM, while 5-methoxytryptamine and α-methyl serotonin had EC50s close to 10 μM. Methysergide displayed weak agonist activity with relatively high affinity (EC50 between 10 and 100 nM). Several compounds were tested for specific antagonist activity. Two compounds, mianserin and cyproheptadine, showed apparent antagonistic actions. However, at the high concentrations (>1 μM) needed for antagonism, these drugs also reversibly blocked voltage-gated sodium and potassium currents. Therefore, it remains possible that the apparent antagonism of the B19 5-HT receptor was actually due to nonspecific blockade of the ion channels mediating the 5-HT-activated sodium current.

This research was supported by NSERC Canada.

527.2

SEROTONERGIC SELF-RE-EXCITATION IN THE LOCOMOTORY PEDAL NEURONS VIA CYCLIC AMP-GATED Na^+ CURRENT IN *Pleurobranchaea californica*. L.C. Sudlow* & R. Gillette. Dept. of Physiology and Biophysics, U. of Ill., Urbana Il 61801.

Serotonin (5-HT) induces a cAMP-gated Na^+ current ($I_{(Na,cAMP)}$) in 5-HT immunoreactive pedal neurons (G neurons) involved in locomotion. Using two different measures we have characterized the interactions of the $I_{(Na,cAMP)}$ and 5-HT in these neurons. Two-electrode voltage clamp experiments (one electrode for voltage sensing, one double barrel electrode for both current injections and cAMP iontophoresis) were used to measure responses to cAMP injections. Occlusion of the cAMP response during 5-HT treatment or steady-state cAMP injections was indicative of saturation of the cAMP cascade. Depolarization-dependent inactivation of 5-HT-induced current was similar to $I_{(Na,cAMP)}$ inactivation. Measures of phosphodiesterase (PDE) rate constants indicated that 5-HT application caused no changes in the activity of PDE. 5-HT applied to the G neurons of the pedal ganglion activated the cAMP cascade resulting in an increase in $I_{(Na,cAMP)}$ and the net excitation of these neurons. Self-excitation induced by release of 5-HT in the neuropil by the G neurons may be used to establish motor patterns in this population of motoneurons that innervate the foot musculature. This research was supported by NIH RO1 NS26838 to RG.

527.3

DIFFERENT PATTERNS OF APPEARANCE OF OCTOPAMINE- AND SEROTONIN-IMMUNOREACTIVE NEURONS IN THE DEVELOPING LOBSTER CNS *H. Schneider[1]*, E. Peckol[2], P. Budhiraja[1], B.S. Beltz[2], & E.A. Kravitz[1]; [1]Neurobiology Dept., Harvard Medical School, Boston, MA 02115, and [2]Biology Dept., Wellesley College, Wellesley, MA 02181.*

In the lobster (*Homarus americanus*), octopamine and serotonin have opposing effects on postural regulation. Amine neurons likely to be involved in that regulation have been identified by immunocytochemical and biochemical methods. In the present study we describe cell-type specific patterns of appearance of octopamine-immunoreactive neurons throughout embryonic and early larval life. At E43% octopamine-immunoreactivity is seen in brain neurons and in descending interneurons in the subesophgeal ganglion (SEG). Later, neurosecretory cells in the SEG and the second thoracic ganglion show staining (E69%). The last group of cells to stain are ascending interneurons in the SEG (E82%). The adult complement of cells is not fully stained until early larval life. By contrast, serotonin-immunoreactive cells appear early in embryonic development with the adult complement labeled by midembryonic life. The observed differences suggest that regulation of the onset of octopamine and serotonin synthesis in each neuron type is distinct. Behavioral or other factors involved in that regulation are under investigation. Supported by NIH and DFG.

527.4

MOLECULAR CHARACTERIZATION OF THE DROSOPHILA HISTIDINE DECARBOXYLASE GENE REQUIRED FOR PHOTORECEPTOR SYNAPTIC TRANSMISSION. M.G. Burg*, J. Liu#, and W.L. Pak, Dept. of Biol. Sci., Purdue Univ., West Lafayette, IN and #Dept. of Biology, Univ. of Science and Technology, Hefei, China.

We have previously identified and characterized the *Drosophila* cDNA which encodes histidine decarboxylase (HDC) involved in the synthesis of histamine, used as transmitter in photoreceptors and a subset of neurons in the brain (*EMBO J 12*:911, 1993). Immunocytochemical analysis of mutants isolated in this gene (*hdc*) suggests that *hdc* expression may be differentially regulated between photoreceptors and neurons in the brain. To study the transcriptional regulation of this gene, we isolated 12 overlapping genomic clones, covering ~ 30 kb, by high stringency screening of a *Drosophila* genomic library. The *hdc* transcription unit was identified within the cloned genomic region by restriction analysis and Southern blotting of the genomic clones. A 9.4 kb XbaI fragment, containing the transcriptional unit and a 4.4 kb upstream sequence was used to rescue an *hdc* mutant by P-element mediated germline transformation. Deletion analysis of the 5' noncoding region is in progress with a view toward identifying the regulatory regions required for normal *hdc* expression and the regions responsible for differential expression between photoreceptors and brain neurons. These results will also be useful in the molecular analysis of *hdc* mutants that are presently available for study. Supported by EY06214 and EY00033.

527.5

GABA IS AN EXCITATORY AMINO ACID ACTING ON SPECIFIC IDENTIFIED FEEDING MOTONEURONS IN THE SNAIL, HELISOMA. A.D. Murphy*. Dept. Biol. Sci., University of Illinois at Chicago, Chicago, IL 60607.

The neural pattern underlying feeding in Helisoma is triphasic, with each of 3 sets of interneurons driving a corresponding set of motoneurons. We previously showed that the primary neurotransmitter of phase 2 interneurons is glutamate, which has both excitatory and inhibitory effects on subsets of motoneurons. GABA, like glutamate, has both inhibitory and excitatory effects in Helisoma. GABA inhibits some motoneurons via a $GABA_A$ mechanism. However, GABA excites identified phase 1 motoneurons, apparently also via a $GABA_A$-like mechanism. These cells are depolarized by GABA or muscimol and the depolarization is picrotoxin sensitive. The excitatory effects are seen in the presence of 10mM $CoCl_2$, and thus appear to be direct and not due to disinhibition. These data suggests that phase 1 motoneurons may have a "reversed" chloride gradient leading to $GABA_A$ excitation. Finally, immunocytochemistry suggests that some phase 1 interneurons are GABAergic. (Supported by NSF IBN-9121374).

527.6

INHIBITION OF THE CRAYFISH SWIMMERET RHYTHM BY GABA AND GLUTAMATE. C.M. Sherff* and B. Mulloney. Zoology, University of California, Davis, CA 95616.

While swimming, the crayfish beats its swimmerets with alternating power-strokes and return-strokes. This pattern is generated in the CNS and transmitted to the swimmeret muscles by excitatory and inhibitory motor axons. At neuromuscular junctions, the excitors release glutamate, while the inhibitors release GABA. The motor neurons in the swimmeret system also make inhibitory chemical synapses in the CNS (Heitler,1978). The difference in peripheral and central responses to neurotransmitter, especially in the case of glutamate, which excites muscle cells but inhibits swimmeret neurons, suggests that either these central synapses are not monosynaptic or that different postsynaptic mechanisms are used at the muscle and in the CNS. We studied the effects of these neurotransmitters on the swimmeret system by pressure-injecting GABA and glutamate from a multibarreled micropipet into the lateral neuropil, while recording the swimmeret motor pattern from peripheral nerves and the responses of individual swimmeret neurons with microelectrodes.

GABA and glutamate (0.1-1.0 mM) independently inhibited the production of the swimmeret motor pattern in the hemiganglion in which they were applied, but did not alter the ongoing rhythm in the contralateral hemiganglion or in other ganglia. Activity in swimmeret interneurons and motor neurons was also inhibited by GABA and glutamate. The cellular responses consisted of a decrease in input resistance and, usually, a hyperpolarization of the membrane potential. These neurotransmitter effects on the swimmeret neurons were direct since they can be elicited when the nerve cord is perfused with saline containing elevated Mg^{2+} and reduced Ca^{2+} concentrations. These results suggest that the motor neuron transmitters, GABA and glutamate, have different functions in peripheral and central synapses, and that these different functions are mediated by the different postsynaptic receptors located at the muscles and in the CNS.

527.7

ISOLATION AND SEQUENCING OF TWO NEW MEMBERS OF THE *MANDUCA SEXTA* FLRFAMIDE FAMILY OF PEPTIDES. J.L. Witten*[1], T. Kingan[2], J. Shabonowitz[3] and D.F. Hunt[3]. [1]Dept. Biol. Sci., UW-Milwaukee, [2]ARS, USDA, Beltsville, MD and [3]Dept. Chem., U. Virginia.

FMRFamide-like immunoreactivity (FLI) is present in the nervous and neuroendocrine systems of the tobacco hornworm, *Manduca sexta*. The FLI in motoneurons, however, is developmentally regulated and is found only in larvae (Witten and Truman, 1990, Soc. Neuro. Abstr.). As a first step towards understanding the physiological significance of this developmental plasticity, we isolated and sequenced the immunoreactive material in the larval thoracic and abdominal ganglia.

The rising ecdysteroid titers at the onset of metamorphosis regulate the decline in FLI in the motoneurons (Witten and Truman, 1990). Thus, by manipulating the ecdysteroid levels to prolong the larval stage, we obtained a ten-fold increase in FLI which facilitated the sequencing. Using a two-step HPLC purification combined with a competitive ELISA, two immunoreactive fractions were detected. Tandem mass spectroscopic analysis yielded the following amino acid sequences: DPSFXRF-NH_2 and GNSFXRF-NH_2 with X being either a L/I. These heptapeptides are structurally related to the larger, myoactive FLRF peptide, pEDVVHSFLRF-NH_2, isolated from the neuroendocrine system of adults (Kingan et al., 1990, Peptides 11: 849-856). They differ, h000owever, in one striking aspect. Only the larger, neuroendocrine derived peptide is protected from degradation at both termini consistent with its proposed role as a circulating factor. This suggests that the structural differences may reflect distinct functional roles for the members of this peptide family. Preliminary results showing a differential tissue distribution of the FLRFamide peptides supports this hypothesis.

527.8

LOCUST VPLI NEURONS CONTAIN THREE VASOPRESSIN-RELATED PEPTIDES; VPLI ACTIVITY REDUCES CYCLIC AMP LEVELS IN THE CNS. KSJ Thompson, RA Baines, RC Rayne, JR Thorpe, A Alef and JP Bacon* Sussex Centre for Neuroscience, Sussex University, Brighton BN1 9QG, UK.

The pair of vasopressin-like immunoreactive (VPLI) neurons of locusts have remarkable arborisations which extend to every ganglion of the CNS. Their immunoreactivity was reportedly due to expression of a vasopressin-related peptide and its antiparallel dimer. We have used HPLC separation and radioimmunoassay to detect vasopressin-like peptides in an extract of 200 electrophysiologically identified VPLI neurons. Three vasopressin-related peptides were found: the nonamer CLITNCPRGamide (1.2 pg/cell), and its antiparallel (1.5 pg/cell) and parallel (1.6 pg/cell) dimers. None of these peptides has any diuretic effect on locust Malpighian tubules[1], despite published reports to the contrary.

In a survey of 17 grasshopper species, we found two forms of VPLI morphology: that typified by *Locusta migratoria* has processes in peripheral nerves; the other, typified by *Schistocerca gregaria*, has no such processes[2]. Using immunogold labelling in *Locusta*, we find that most peripheral VPLI processes lie deep within the nerve, unlike superficial neurohaemal endings. This suggests VPLI peptides are released centrally. We have searched for a central effect by measuring second messenger levels in the CNS. Stimulation of a VPLI neuron causes a significant cyclic AMP reduction (38.9 ±16 pmol/mg protein, n=25; approximately 12% of control) compared with the inactive contralateral side. This is an unusual, possibly unique, example of a neurotransmitter reducing cyclic AMP levels in an invertebrate. (Supported by SERC grant GR/G52524)

1. Coast et al. (1993) J Exp Biol 175:1-14.
2. Tyrer et al. (1993) J Comp Neurol 329:385-401.

527.9

EXPRESSION OF THE NEUROPEPTIDE GENE FMRFamide AND MYOMODULIN IN *LYMNAEA*. J.F. Burke*, N. Santama, E. Kellett, S. Saunders, M. Yeoman and P.R. Benjamin Sussex Centre for Neuroscience, Biological Sciences, University of Sussex, Brighton, E Sussex BN1 9QG (UK)

The FMRFamide gene in *Lymnaea* consists of at least six exons. These are alternatively spliced to produce two major transcripts whose expression is mutually exclusive (Saunders *et al.* (1992) J Neurosci). The FMRFamide peptide is produced in 250 cells including the heart motor neurons, whereas the GDP/SDPFLRFamide peptides are produced in fewer cells, including a major interneuron (Bright *et al.* (1993) J Neurosci; Skingsley *et al.* (1993) J Neurophys). A novel peptide (abbreviated to SEEPLY) encoded by the FMRFamide precursor protein has been identified on the basis of immunocytochemistry and protein sequencing (Santama *et al.* (1993) Eur J Neurosci). This peptide exhibits physiological activity in association with FMRFamide. The myomodulin peptides from *Lymnaea* are encoded in a similar manner to those of *Aplysia*. By *in situ* hybridization it appears that this gene is expressed in approximately 200 neurons. Some of these cells appear to be associated with a previously described neural network regulating feeding behaviour (Elliott and Benjamin (1985) J Neurophys).

527.10

LOCALIZATION OF TRANSCRIPTS ENCODING FMRFAMIDE-LIKE PEPTIDES IN *C. ELEGANS* AND COMPARISON OF TWO *CAENORHABDITIS* GENES ENCODING FMRFAMIDE-LIKE PEPTIDES.

K. Schinkmann and C. Li*. Dept. of Biology, Boston University, Boston, Ma 02215.

To examine the cellular and molecular mechanisms of transmitter expression, we are studying the regulation of the family of FMRFamide(Phe-Met-Arg-Phe-NH_2)-like neuropeptides in *C. elegans*. We have shown previously that one of the functions of FMRFamide-like peptides in *C. elegans* is to potentiate the actions of serotonin in egg-laying.

A gene, *flp-1*, produces two transcripts encoding FMRFamide-like peptides in *C. elegans*. *In situ* hybridization experiments on whole mount animals suggest that most of the FMRFamide-like immunoreactivity can be accounted for by *flp-1* expression and indicate differential expression of the two transcripts.

To identify possible regulatory elements in the *flp-1* gene, we cloned and characterized a FMRFamide-like gene, *cvflp-1*, from the closely related nematode *C. vulgarensis*. Genomic Southern blotting experiments indicate the presence of one FMRFamide-like gene in *C. elegans*, but possibly more than one related gene in *C. vulgarensis*. The genomic organization of the *flp-1* and the *cvflp-1* genes is very similar. Comparison of the regions upstream of the start site of transcription revealed several areas of homology, the functional significance of which remains to be elucidated by promoter deletion studies.

527.11

STORAGE AND RELEASE OF PEPTIDE COTRANSMITTERS FROM B16 NEUROMUSCULAR JUNCTIONS OF *APLYSIA*. F.S. Vilim*[1], E.C. Cropper[2], D.A. Price[3], I. Kupfermann[1], and K.R. Weiss[2]. [1]Cntr. Neurobiol. & Behav., Columbia Univ., NYS Psych Inst., NY, NY, [2]Dept. Physiol. & Biophys., Fishberg Res. Ctr. in Neurobiol., Mt. Sinai School of Med., NY, NY, and [3]Whitney Labs St Aug. FL.

The accessory radula closer muscle (ARC) and its innervation provide a model system for studying the behavioral role of neuromodulation and cotransmission. The ARC is innervated by two cholinergic motorneurons, B15 and B16, and a serotonergic modulatory neuron, the MCC. The motorneurons also contain modulatory peptide co-transmitters falling into 3 families. B15 contains the SCPs and buccalins (BUCs), and B16 contains the BUCs and myomodulins (MMs). The SCPs and MMs act postsynaptically to increase contraction amplitude and relaxation rate. The BUCs act presynaptically to decrease contraction amplitude by decreasing ACh release. We have developed a method using RIA to directly measure release of these peptide cotransmitters in the ARC following intracellular stimulation of a single motorneuron. We previously showed that SCPs and BUCs are co-stored in the same dense core vesicles (DCV's), and are co-released in response to physiologically relevant patterns of B15 stimulation. Here, we report that the BUCs and MMs are co-stored in the same DCV's, and they are co-released in response to physiologically relevant patterns of B16 stimulation. Ultrastructural immunogold double labeling was used to demonstrate that the two peptide families were co-stored in DCV's of B16 motor terminals in the ARC. The kinetics of the release of MMs and BUCs in the ARC were identical, consistent with co-storage and co-release from the same DCV's. BUC and MM release was dependent on extracellular calcium and independent of the contraction of the muscle indicating that release of these peptide cotransmitters was not an artifact of stimulation or contraction of the muscle. These results provide evidence that these peptides are co-released *in vivo*, and suggest that they play a role in feeding behavior.

527.12

CHARACTERIZATION AND MODULATION OF THE ACCESSORY RADULA OPENER NEUROMUSCULAR SYSTEM OF *APLYSIA*. C.G.Evans*, O. Harish, F.S. Vilim, E.C. Cropper, I. Kupfermann and K.R. Weiss. Mt. Sinai Schl. Med., N.Y., N.Y., 10029.

Feeding behavior in *Aplysia* requires that the radula opens and closes. Based on extensive studies of the neuromuscular system that produces radula closing it has been suggested that when animals feed quickly, neuromodulators decrease the duration of closer contractions, preventing coactivation with the opener muscles. In order to test this hypothesis opener muscles must be characterized. Towards this end we are now describing a set of muscles which when stimulated electrically in a dissected prep, will open the radula. Recordings from these muscles during feeding suggest that their contractions contribute to radula opening. The muscles are innervated by a cholinergic buccal motoneuron which preliminary HPLC data indicates contains myomodulin and FMRFamide. Additionally, the muscles show 5HT and FRF-like immunoreactivity. Action potentials in the motoneuron are 1:1 with hexamethonium sensitive EJPs in the fibers, and summation of EJPs can lead to a spike. A denervated muscle will contract rhythmically when subjected to a constant stretch. Contractions are accompanied by spikes in the fibers. These spikes are not blocked by hexamethonium. Both motoneuron and stretch-induced contractions are modulated by MMa, MMb, 5HT and FMRFamide. MM, 5HT, and physiological motor neuron stimulation increases cAMP levels in the muscles. These effects are mimicked by forskolin. Thus, it appears that contractions of the radula opener can be induced (a) by the radula opener motor neuron, and (b) by stretch produced by contraction of the radula closer muscle. Muscle contractions elicited in either manner are modulated. Comparisons of the modulation of these two forms of contraction as well as comparisons of the modulation of the opener muscle with the nonspiking ARC muscle may provide new insights into the behavioral role of neuromodulation.

DEGENERATIVE DISEASE: ALZHEIMER'S—β -AMYLOID X

528.1

NEUROTOXICITY OF AMYLOID β PROTEIN IN CULTURED CORTICAL AND CEREBELLAR GRANULE CELLS: IMPORTANCE OF NEURON/GLIA INTERACTIONS. M. D. Ikonomovic*, M. Grekova, S. Pshenichkin, A. Berkovich, B. C. Wise, and D. M. Armstrong. Fidia-Georgetown Institute for the Neurosciences, Georgetown Univ Med Sch, Washington, DC 20007.

At present the effect of amyloid-beta protein (Aβ) on neurons grown in culture remains controversial. These controversies may in part reflect certain technical aspects such as the peptide employed including its aggregation state as well as the culture system and/or the culture conditions under study. In our investigations neonatal cortical neurons exhibited a significant decrease in viability when exposed to Aβ 1-40 22 μM or 25-35 90μM. Importantly, the neurotoxic effect of Aβ in cortical neuronal cultures was inhibited when cultures were treated with an allosteric antagonist of the NMDA-selective glutamate receptor (eg. MK-801) thus reinforcing the notion that glutamate participates in the Aβ-mediated neurotoxicity. In contrast to the above findings, cultured cerebellar granule cells failed to exhibit any neurotoxicity when exposed to Aβ 1-40 or 25-35 although they do exhibit a clear dose-dependent excitotoxicity to glutamate in the absence of Aβ. Importantly, a fundamental difference between the two culture systems is the presence of numerous glial cells in the cortical culture, whereas cerebellar granule cultures are relatively free of glia. However, when cerebellar granule cells are grown under culture conditions which promote glial cell proliferation the neurons exhibit a significant Aβ-mediated neurotoxicity similar to cortical neurons. These data suggest that the interactions between neurons and glia may be an important factor in determining Aβ-mediated neurotoxicity. In addition, studies using pure glial cultures demonstrated a mitogenic effect of Aβ upon these cells.

Supported by NIH AG08206 & The American Health Assistance Foundation.

528.2

Ca^{2+} ELEVATIONS INDUCED BY β-AMYLOID PEPTIDE 25-35 IN CULTURED NEURONS DEPEND ON GLUTAMATERGIC SYNAPTIC ACTIVITY. J.R. Brorson*[1], J.C. Chisholm[3], C.J. Marcuccilli[2], V.P. Bindokas[2], and R.J. Miller[2]. Departments of [1]Neurology, [2]Pharmacological and Physiological Sciences, University of Chicago, Chicago IL 60637, and [3]Miles Inc., West Haven, CT, 06516-4175.

The β-amyloid peptide fragment 25-35 (βAP25-35) has been found to enhance the toxicity of glutamate in neuronal cultures over prolonged exposures. The mechanism is unknown. We examined the effects of βAP25-35 (10-60μM) on cultured rat hippocampal neurons by microfluorimetric measurements of the free intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) and with whole-cell patch clamp recordings. In whole-cell current clamp recordings βAP25-35 produced reversible increases in excitatory activity, with bursts of action potentials or EPSPs, in a fraction of neurons. In keeping with the electrophysiological findings, fura-2 microfluorimetry revealed elevations in the $[Ca^{2+}]_i$ during βAP25-35 exposure in 61 of 98 cells examined. These responses were enhanced in Mg^{2+}-free medium but were blocked completely by tetrodotoxin (0.5μM) or by CNQX (10μM), and incompletely by D-AP5 (25-50μM). Fluorescence imaging revealed concurrent $[Ca^{2+}]_i$ spikes among synaptically connected neurons. Neither the βAP1-11 fragment nor solvent alone duplicated these effects. Responses were not observed in cultures of cerebellar neurons, in which >80% of the neurons are inhibitory. These results suggest an effect of βAP25-35 which depends on endogenous excitatory synaptic activity in networks of cultured neurons which is mediated by glutamatergic synapses. This may relate to the *in vitro* toxicity of βAP25-35.

528.3

INDUCTION OF β-APP RESULTS IN INCREASED SECRETION OF SOLUBLE β-APP INTO CSF OF SUBCORTICALLY LESIONED RATS. V. Haroutunian*, I. Lieberburg, K.L. Davis, E. Fiber, R. Gluck, and W.C. Wallace Lab. Biochem. Genetics, NIMH, Washington D.C. Dept. Psychiatry, Mount Sinai School of Medicine, NY, NY,and Athena Neurosciences, South San Francisco, CA.

Lesions of various subcortical inputs to the rat cortex result in a rapid (within 1hr) and persistent (at least 6 wks) induction of β-APP in the cortical projection areas. The induction was observed as both elevated β-APP mRNA (northern blots) and increased synthesis of β-APP (polysome run-off assays). This induction occured in response to neurotransmitter deficits and was independent of physical injury. We examined cortical homogenates by immunoblots using various antibodies directed at β-APP and observed that the levels of mature β-APP in the transmitter deficient cortical tissues did not exhibit any increase from 1 hour to 6 weeks post subcortical lesioning. The elevated synthesis of β-APP in the absence of accumulation is consistent with an increased turnover of β-APP in the transmitter deficient cortex. To test this possibility we examined CSF of lesioned and naive rats. CSF from one week post lesion animals exhibited significantly elevated levels of β-APP (2.3-fold n=6/group). This β-APP species reacted with α5 antibody (directed to an extra cellular domain), but not α6 (directed to an intracellular C-terminal domain) indicating that the species was the secreted form of β-APP. No such elevation was found 1 hour post lesion. These results indicate that upon loss of subcortical innervation the cortex responds with induction of β-APP and increased secretion of the soluble form β-APP. These results taken together with evidence for secreted β-protein in the CSF of humans indicate that the subcortically lesioned rat represents a relevant in situ model for studying the physiological roles of β-APP and the factors which lead to the deposition of β-amyloid protein.

528.4

ß-Amyloid and its Precursor (APP) in AD Fibroblasts. G. M. Cole*[1], K. Mak[1], J. Wu[1] and S.A. Frautschy[2] Neurosciences[1], UCSD, La Jolla, Ca. 92093 and Dept.of Cellular and Molecular Biology[2], Whittier Institute, 9894 Genesee, La Jolla, Ca.92037

ß-flanking APP mutations have been linked to <1% of familial Alzheimer's disease(AD); one results in ~6 fold more secreted ß-protein per mole APP in non-neuronal cells. If increased ß production accelerates amyloidosis and AD to the fifth decade, then other, earlier, onset familial AD cases could also have greatly increased APP and ß-protein or ß as a % of APP in non-neuronal cells. Accordingly, we studied levels of APP and ß-protein in fibroblasts from 3 families with AD linked to chromosome 14 using 12 confirmed AD and 10 probable escapees. Detergent lysates and media from duplicate, 16 hour labeled, confluent 75 cm^2 flasks were examined by immunoprecipitation. No significant differences in C-terminal APP in lysates or APP or ß-protein in conditioned media could be found. Because heat shock (HS) elements and AP-1 sites are found in the APP promoter, HSP70 and c-fos genes near linkage markers on chromosome 14 are candidate AD genes. We tested effects of heat shock and serum stimulation of c-fos, but again found no differential effect on APP production. Studies of ß-protein and APP in AD and control CSF also indicate no increase in ß or APP in AD. Therefore, we propose that other factors accelerate ß-amyloidosis in the majority of AD cases and we will present experimental evidence for a novel example of one of them.(Supported by Calif. Dept. Health Service,(GMC), AG09009, (GMC) and AG10685 (S.A.F.))

528.5

β-AMYLOID PRECURSOR PROTEIN IN AXOLEMMA AND PERIAXOLEMMAL-MYELIN. V. Sapirstein, M.L. Berg, R. Durrie & N. Marks*
Div. of Neurobiology, N.S. Kline Inst. Orangburg NY 10962.
The β amyloid precursor protein (BAPP) is widely distributed within the CNS. BAPP is transported down the axon and has been found in synaptic endings. We have utilized techniques to isolate axolemma (AX) and periaxolemmal-myelin (PAM) and have found BAPPs, Mr 100-110Kd, to be major constituents of these membrane. Isolation of AX and PAM and compact myelin show that BAPP represents 1% AX and 0.6% PAM protein but is absent from compact myelin. These results indicate that BAPP transported down the axon is deposited at sites in the axolemma as well as at the synapse and that within the oligodendrocyte BAPP, is targeted to the periaxolemmal domain. These results were replicated in membranes isolated from bovine brain. Bovine studies were extended to analysis of white matter clathrin coated vesicles; these data show that coated vesicles isolated from white matter under con- ditions that yield largely endocytic vesicles, contain levels of BAPP comparable to that found in AX and PAM. Studies carried out on human autopsy material, frozen 2 hours post-mortem, showed nearly identical results. In addition, both rat and human axolemma contained a C terminal 10.5Kd BAPP peptide suggesting this membrane is a site for endopeptidase cleavage of BAPP. Incubation of axolemma with metallo-endopeptidase did not detectably degrade BAPP. However, incubation of axolemma with cathepsin B at pH 6.0 caused a rapid loss of BAPP when analyzed with antibodies to BAPP 672-695 but not when analyzed with antibodies reacting with sites more distal to the C terminus. Thus, cathepsin B selectively cleaves membrane bound BAPP at a site close to the C terminus and may be a valuable tool for altering C terminal BAPP function. Supported by NYS-OMH.

528.6

EXPRESSION OF AMYLOID PRECURSOR PROTEIN IN THE ISCHEMIC RAT HIPPOCAMPAL SLICE PREPARATION

R.N. Kalaria* and A.B. Pax. Departments of Neurology & Neurosciences, CWRU Sch. of Medicine, Cleveland, OH 44106.

It is now established that the amyloid precursor protein (APP) of Alzheimer's disease (AD) is rapidly induced during brain tissue injury. We recently described the expression of APP in experimental ischemic brain injury and chronic hypoperfusion (*Neuroreport* **4**, 211-214, 1993). Here, we studied the regulation of APP after experimental manipulations in *in vitro* hippocampal slice preparations from rat brain. Tissue slices were incubated and maintained in oxygenated culture medium containing radiolabelled ^{35}S-methionine for incorporation into newly synthesized proteins and the effects of ischemic/anoxic insults were examined. We observed that brief periods (20-40 mins) of anoxia affected the incorporation of ^{35}S-methionine in the tissue proteins including APP. Cell extracts of slices assessed for APP showed that antibodies to C-terminal domains of APP and A4/β protein immunoprecipitated full-length APP forms and smaller amyloidogenic fragments. Our findings emphasize that ischemic or oligemic events, which presumably trigger release of cytokines or related factors and heat shock proteins, may mediate APP accumulation and metabolism that is germane to β/A4 amyloid deposition in Alzheimer's disease.

Supported by grants from the USPHS (NIA and ADRDA.

528.7

AMYLOID β-PROTEIN (Aβ) IS PRODUCED BY I-CELLS EXPRESSING SEVERE LYSOSOMAL DEFICIENCY. M. Podlisny*, C. Haass, C. Miller, T. Oltersdorf[1], I. Lieberburg[1], and D. Selkoe.
Harvard Med. Sch., Boston & [1]Athena Neurosciences, South San Fran.

Insoluble cerebral aggregates of Aβ are deposited in AD and to a lesser extent during aging. Soluble Aβ is released from cells into the media during normal metabolism in vitro. It has previously been postulated that Aβ could be generated from larger (~10-22 kD) C-terminal fragments of βAPP that are found in lysosomes. These potentially "amyloidogenic" fragments are enhanced in amount by inhibition of certain lysosomal proteases by leupeptin. To investigate whether lysosomal processing of βAPP is necessary for Aβ production, we examined βAPP processing in skin fibroblasts from normals and in I-cell fibroblasts derived from patients with mucolipidosis II, a fatal disease caused by severe deficiency of lysosomal enzymes due to failure of mannose-6-phosphate addition. Immunoprecipitation of cell lysates revealed markedly enhanced levels of ~10-22 kD C-terminal βAPP fragments in I-cells compared to normal fibroblasts treated with or without leupeptin. Immunoluorescent labeling of I-cells localized these C-terminal fragments to endosomes and lysosomes. Precipitation of conditioned media with R1280 (to $A\beta_{1-40}$) revealed Aβ production by both normal and I-cell fibroblasts. These data support the notion that the proteolytic cleavages generating both Aβ and C-terminal fragments largely occur in vesicles prior to lysosomes. Similar techniques for studying βAPP processing and Aβ production are being applied to skin fibroblasts derived from FAD patients and (in collaboration with V. Askanas) to cultured myoblasts containing intracellular Aβ from patients with inclusion body myositis.

528.8

AMYLOID PRECURSOR PROTEIN-ENRICHED MEDIUM STIMULATES MAP KINASE AND PHOSPHORYLATION OF TAU. S.M. Greenberg, E.H. Koo, W.Q. Qiu, A.W. Sandrock*, K.S. Kosik. Center for Neurologic Diseases, Brigham and Women's Hospital and Harvard Medical School, Boston, MA, 02115.

The microtubule-associated protein tau is a major constituent of paired helical filaments (PHF) in Alzheimer's disease (AD). PHF tau is abnormally phosphorylated at multiple sites, many of which are phosphorylated *in vitro* by the mitogen-activated protein (MAP) kinases. The stimulus for abnormal phosphorylation of tau in AD is unknown. Potential candidates include amyloid ß-peptide (Aß) and its progenitor the ß-amyloid precursor protein (ß-APP).

We report that medium from CHO cells stably transfected with ß-APP activates MAP kinase in PC-12 cells. The 44 kD form of MAP kinase was stimulated approximately 25-fold by 10 min exposure to medium from cells transfected with ß-APP_{965} and to a comparable extent with cells expressing ß-APP_{770}. This activity was largely blocked by prior immunoprecipitation of conditioned medium with the antibody anti-B5, which recognizes sequence in APP outside the Aß region. Purified Aß had no effect. Induction of an inhibitory form of the ras protein blocked the activation of MAP kinase, suggesting that the ß-APP-enriched medium stimulates a ras-mediated pathway. Following incubation in [^{32}P] phosphate, 15 min exposure to conditioned medium increased intracellular phosphorylation of tau by approximately 2-fold. These findings suggest a potential mechanism by which ß-APP may stimulate phosphorylation of tau.

528.9

REGULATION OF ß-AMYLOID PRECURSOR PROTEIN PROCESSING INTO NON-AMYLOIDOGENIC AND AMYLOIDOGENIC DERIVATIVES BY PROTEIN KINASE C. Albert Y. Hung, T.L. Munsat*, and Dennis J. Selkoe.
Center for Neurologic Diseases, Harvard Medical School and Brigham and Women's Hospital, and *Tufts N.E., Medical Center, Boston, MA.

The 4 kDa amyloid ß-protein (Aß), the major component of cerebral and cerebrovascular plaques in Alzheimer's disease, is derived from proteolytic cleavage of a larger membrane-bound precursor, the ß-amyloid precursor protein (ßAPP). In addition to Aß, processing of ßAPP gives rise to a large, secreted molecule (APP_s) whose generation precludes the formation and deposition of amyloid. The cellular mechanisms which regulate ßAPP metabolism are largely unknown. However, protein phosphorylation has been proposed to play a role. Addition of the protein kinase C (PKC) activator phorbol 12,13-dibutyrate (PDBu) to cells expressing ßAPP resulted in a dramatic inhibition of Aß release, in parallel with an increase in APP_s secretion. This effect was enhanced by the protein phosphatase inhibitor okadaic acid and blocked by staurosporine, a kinase inhibitor. To determine if this regulation involves direct precursor phosphorylation, we examined the phosphorylation state of ßAPP in ßAPP-transfected 293 cells. ßAPP is phosphorylated only on serine residues. Surprisingly, phosphoserine was detected only within the ectodomain, resulting in the secretion of phosphorylated APP_s. Addition of PDBu did not enhance the phosphorylation of ßAPP. Furthermore, mutation of the two cytoplasmic serine residues did not abolish the increase in APP_s release and the decrease in Aß production observed upon PKC activation. Truncation of the ßAPP cytoplasmic domain similarly did not abolish the effect of PDBu on APP_s upregulation and Aß inhibition. These results indicate that PKC plays a critical role in the control of ßAPP processing into non-amyloidogenic and amyloidogenic derivatives. However, in contrast to current postulates, this regulation appears to be independent of direct precursor phosphorylation and is thus likely to involve an additional kinase substrate, perhaps APP secretase.

528.10

INHIBITION OF BETA AMYLOID PRODUCTION BY ACTIVATION OF PROTEIN KINASE C. J. Busciglio*, D. Gabuzda and B.A. Yankner. Dept. of Neurology, Harvard Medical School, Children's Hospital and Dana Farber Cancer Institute, Boston, MA 02115.

The β amyloid protein is secreted by many different cell types in culture. Protein kinase C (PKC) has previously been shown to increase constitutive secretion of the amyloid precursor protein (APP). We examined the effect of PKC activation and inhibition on the secretion of β amyloid in transfected COS cells and a non-transfected human glioma cell line. Activation of PKC by the phorbol ester PMA inhibited secretion of the 4 kD β amyloid peptide by 60-90%. The inactive phorbol ester analog 4α-PDD was ineffective suggesting that the inhibitory effect of PMA is due to activation of PKC. PMA increased secretion of the 3 kD truncated derivative of β amyloid and secretion of APP_s. Immunoprecipitation of cellular and secreted APP from ^{32}P-labeled cells showed that both APP and APP_s were phosphorylated on serine residues. The 4 kD β amyloid protein was not phosphorylated. PMA did not increase ^{32}P incorporation into APP or APP_s suggesting that PKC does not directly phosphorylate APP. These results suggest that PKC modulates the proteolytic processing of APP indirectly by phosphorylation of another protein. The level of β amyloid secretion may be regulated by neurotransmitters and growth factors that act through the PKC signal transduction system.

528.11

β-AMYLOID REGULATES S100β GENE EXPRESSION IN C6 GLIOMA CELLS AND PRIMARY ASTROCYTE CULTURES.
L.A. Peña*, C.W. Brecher, and D.R. Marshak. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York 11724-2220.

S100β, a calcium-binding protein expressed by CNS astrocytes, has trophic effects *in vitro* (neurite extension and glial proliferation). In Alzheimer's disease and Down's syndrome, severely afflicted brain regions exhibit up to 20-fold higher levels of S100β protein, and astrocytes surrounding neuritic plaques exhibit highly elevated levels of S100β immunostaining. A major constituent of plaques, β-amyloid, has been reported to have neurotoxic and neurotrophic effects *in vitro*.

In our study we examined the responses of CNS glia to β-amyloid. C6 glioma cells and primary rat astrocyte cultures were synchronized and treated with βA(1-40) peptide at doses up to 1μM. Weak mitogenic activity, measured by [^{3}H]-thymidine incorporation, was observed. Northern blot analysis revealed increases of S100β mRNA within 24 hours in a dose-dependent manner. Nuclear run-off transcription assays showed that βA(1-40) specifically induced new synthesis of S100β mRNA in cells maintained in serum, but under serum-free conditions, there was a general elevation of several mRNA species. Corresponding increases of S100β protein synthesis were observed by immunoprecipitation of [^{35}S]-labeled cellular proteins. The hypotheses that β-amyloid activity is mediated via neurokinin receptors or by calcium fluxes were evaluated. These data suggest that in neuropathological conditions, β-amyloid itself is an agent which may provoke gliosis and the production of trophic substances by astrocytes.

528.12

THE β-AMYLOID PRECURSOR PROTEIN REGULATES CYTOKINE PRODUCTON IN VITRO. Russell E. Rydel*, Alan R. Culwell, Elizabeth F. Brigham, and Ivan Lieberburg. Athena Neurosciences, Inc., South San Francisco, CA 94080.

Alzheimer's disease (AD) is a neurodegenerative disorder characterized by the extracellular accumulation of senile plaques in the brains of affected individuals. Senile plaques are primarily composed of a 40-42 amino acid amyloid-β peptide that is derived from a much larger (largely extracellular) membrane-spanning β-amyloid precursor protein (βAPP). Recent studies have demonstrated that several mediators of inflammation are also associated with senile plaques. These include cytokines (eg., IL-1, IL-6, TNFα), acute phase proteins (eg., α1-antichymotrypsin), brain macrophages (ie., activated microglia), and classical pathway complement proteins. We have found that secreted forms of βAPP (APP^{S}s, nM) and the amyloid-β peptide (Aβ, μM) are potent inducers of cytokine release in human cortical mixed brain cultures. These activities are not due to endotoxin contamination, are dose-dependent, and occur in forms of APP^{S}s that both contain (APP$^S_{751}$) and lack (APP$^S_{695}$) the Kunitz-type protease inhibitor domain. These results extend the known activities of βAPP to include the regulation of cytokines, and suggest that alternative processing of βAPP allows for differential regulation of cytokines by APP^{S}s and Aβ. These studies further suggest the βAPP may play a primary role in the inflammatory responses shown to occur in the brains of patients with Alzheimer's disease.

528.13

ß-AMYLOID (Aß 1-40) MODULATES NIGROSTRIATAL (A9) DOPAMINERGIC NEURONS IN VIVO: NEUROCHEMICAL EVIDENCE FOR A FUNCTIONAL NEUROMODULATORY ROLE IN THE NORMAL ADULT RAT BRAIN. S. Iyengar*, R. M. Simmons and D. Li. CNS Research, Eli Lilly and Company, Indianapolis, In 46285.

Aberrant processing of the Amyloid Precursor Protein, APP, leading to the generation of ß amyloid (Aß), has been implicated in the pathology of Alzheimer's disease. Recent findings suggest that APP and some of its product peptides may interact with Ca++ mediated neuronal mechanisms (Mattson et al., 1990, J. Neuroscience, 12:376; Nitsch et al., 1992, Science, 258:304). Although neuronal degeneration in the Alzheimer's brain appears in specific forebrain pathways, APP appears to be present in brain regions other than forebrain structures as well. These observations raise the possibility that APP or its product peptides may have a functional role in normal brain. The current study was initiated to evaluate Aß (1-40) in defined brain regions for potential neurochemical effects in normal adult rat brain. Aß (1-40) (Bachem, California, lot WJ 209) was injected intracerebroventricularly (i.c.v.) at various doses, into awake, freely-moving rats (Sprague Dawley, Charles River, 225-250 g) that had been implanted with PE20 cannulas 7 days prior to the experiment. Aß caused dose dependent increases in striatal dopamine metabolism and release 60 minutes after injection, as was evident from the levels of dopamine and its metabolites, DOPAC, HVA and 3 -MT, which were measured by GC/MS. This neurochemical observation appears to be the first evidence for the ability of Aß (or other APP products) to modulate neuronal function in a defined neuronal circuit (A9 nigrostriatal dopaminergic pathway), in vivo, in the normal rat brain. This data further raises the possibilty that APP and its product peptides may modulate neuronal function.

CEREBELLUM IV

529.1

CEREBELLAR AGENESIS. M. Glickstein*. Department of Anatomy and Developmental Biology, Unversity College London, Gower Street, London WC1 England

The importance of the cerebellum for motor control has sometimes been questioned because of an alleged absence of obvious motor deficits in people born with partial or compete agenesis of the cerebellum. A few such cases have been described in the literature as having been discovered only at autopsy. Some reviewers have concluded that other brain structures therefore must compensate for the absence of the cerebellum. The original case reports, however, point to quite a different conclusion. The myth of normal functioning in cases of agenesis tends to reinforce itself. I report here on one such case. A brain in the Department of Anatomy at Cambridge University has been on dsplay for about forty years with the label "The man without a cerebellum". Verbal reports suggested that in life, the man was either a skilled carpenter or a high scaffolding worker. The evidence is, however, that the story was elaborated over time by oral tradition. The brain was in fact from an unclaimed body, with the designation of "building worker" on the death certificate. Moreover, a sagittal MRI scan of this brain revealed that agenesis was not complete. A very small cerebellum was present, rather than complete agenesis. Combined with a sceptical review of the literature, this case re-affirms the major importance of the cerebellum for normal motor control.

529.2

A PET COMPARISON OF CEREBRAL ACTIVATION ASSOCIATED WITH ESSENTIAL AND WRITING TREMOR. A.J.Wills, I.H.Jenkins, P.D.Thompson, L.J.Findley and D.J.Brooks*. MRC Cyclotron Unit, Hammersmith Hospital, Du Cane Road, London W12 and Institute of Neurology, Queen Square, London WC1, UK.

There has been debate about whether writing tremor (WT) is a variant of essential tremor (ET) or a separate entity (dystonia). The aim of our study was to compare the abnormal patterns of cerebral activation associated with these two conditions. Six ET patients had rCBF measured with $H_2{}^{15}O$ PET during postural tremor of the right arm and at rest. The field of view ranged from medulla to thalamus. Abnormal bilateral cerebellar, red nuclear and contralateral pontine activation was seen. Six WT patients had rCBF measured during writing tremor and again whilst holding a pen in the same hand but without tremor. Bilateral cerebellar overactivity was also demonstrated. No olivary activity was apparent in either condition.

We have previously demonstrated similar findings in patients with "rubral" and neuropathic tremors. This suggests a common abnormality of cerebellar networks in all these tremulous disorders.

529.3

CEREBELLAR ACTIVATION DURING WRIST FLEXION AND EXTENSION MOVEMENTS INVESTIGATED USING MAGNETIC RESONANCE IMAGING. D. Flament*, J. Ellermann, S.G. Kim, Q.-G. Fu, H. Merkle, K. Ugurbil and T.J. Ebner. Depts. of Neurosurgery and Radiology, University of Minnesota, Minneapolis, MN 55455.

Using multi-slice functional imaging at high field strength (4T Seimens/SISCO whole body system) we studied cerebellar activation in 8 subjects making a series of wrist flexions against constant inertial loads and in 6 subjects also making alternating wrist flexions/extensions in a finger-to-nose pointing task. Typically, 10 control, 10-20 task and 10 recovery T_2^* weighted images of each slice were obtained.

Cerebellar activation was predominantly bilateral, but stronger ipsilateral to the moving limb. The pattern of activation varied considerably, ranging from highly localized, discrete parasagittal strips in the intermediate zone to a general mosaic-like activation of the entire hemisphere. Some subjects had activity localized in a medio-lateral band that extended from the midline to the lateral aspect of the cerebellum, following the contour of individual folia.

Time-course plots of T_2^* measurements confirmed that activation began after the onset of a series of movements and persisted throughout the series. Activation intensity ranged from 2.6% to 11.5% above baseline. The finger-to-nose pointing task was also an effective activator but produced slightly lower activation of the cerebellum than the inertial load task. Our results show that in humans cerebellar activation is strongest ipsilateral to the moving limb, but that bilateral activation is the norm.

Supported in part by NIH grant RO1-NS-18338.

529.4

DIFFERENTIAL EFFECTS OF LOCALIZED INACTIVATION OF DEEP CEREBELLAR NUCLEI ON REACHING IN THE CAT. S.E.Cooper*, J.H. Martin, C.Ghez. Columbia U., Ctr for Neurobio. and Behav., NY,NY 10032

In this study, we have explored the deep cerebellar nuclei with small muscimol injections in cats trained to reach into a baited food well. We analyzed changes produced by inactivation in the animal's ability to aim movements and adapt them to variations in the vertical and horizontal target position and to obstacles in the movement path.

Normal animals reach for targets at different heights by scaling the amplitude and velocity of elbow flexion. Motions at other joints are coordinated with the elbow to control paw path. Movements are adapted to horizontal position of the target and to obstacles by changes in movements of shoulder and wrist, rather than elbow. Elbow muscles acted in a simple way to accelerate or decelerate the elbow. In addition to such simple actions, shoulder and wrist muscles interacted in a more complex ways with torques due to motion of other joints (e.g., elbow).

Neither accuracy nor adaptation were substantially altered by inactivation within *dentate*, or *fastigial* nuclei although the fastigial injections produced severe postural defects. *Posterior interpositus* injections produced overshot reaching (Milak et al., 1992) and 'goose-stepping' gait.

Inactivation of *anterior interpositus* (AIP) severely degraded accuracy as well as adaptation to changes in horizontal target position and to obstacles. Errors consisted of systematic biases in which the paw reached below the target ('undershot'). Scaling of elbow flexion with variations in target height was unimpaired. However, shoulder flexion and wrist dorsiflexion occurred prematurely; moreover, movements of these joints no longer varied with the relevant task parameters. We propose that the AIP is essential for coordinating the action of muscles acting at a given joint with the motion of other joints that produce interaction torques. Supported by NS31391.

529.5

EFFECTS OF RED NUCLEUS STIMULATION ON FORELIMB EMG ACTIVITY DURING REACHING IN THE CAT. K. M. Horn*, P. L. E. Van Kan, and A. R. Gibson. Barrow Neurological Institute, Phoenix, AZ., 85013.

The magnocellular red nucleus (RNm) receives input from the cerebellar interpositus nucleus and projects to interneurons of laminae V - VII throughout the spinal cord. RNm neurons terminate directly on motoneurons that innervate distal forelimb muscles, suggesting that the RNm preferentially controls movements of the hand and fingers. This hypothesis was tested in cats trained to reach to grasp a lever, a task that involves the entire forelimb.

We stimulated (monopolar pulses, 0.2 msec, 10 Hz) at 15 sites within the RNm of 2 cats and constructed stimulus triggered averages of rectified EMG activity of 4 distal and 3 proximal forelimb muscles. For each muscle, EMG activity was averaged during phases of the reach to grasp in which the muscle was active.

RNm stimulation resulted in post stimulus facilitation at latencies that ranged from 5 to 8 msec. Although both distal and proximal muscles showed facilitation, at threshold stimulus strength (average threshold: 12 uA) 12 of the 15 sites produced facilitation of digit or wrist muscles and not elbow or shoulder muscles. These results support further a preferential involvement of RNm in the control of the distal forelimb during reaching to grasp.

529.6

PROGRESSIVE CHANGES IN THE MODULATION OF CEREBELLAR NUCLEAR NEURONS DURING THE LEARNING OF A COMPLEX FORELIMB MOVEMENT. J.R. Bloedel*, V. Bracha, M.S. Milak. Barrow Neurological Institute, Phoenix, AZ 85013.

Experiments were performed using a chronic multiple single unit recording technique to examine the changes in the modulation of cerebellar nuclear neurons during the acquisition of a complex, sequential forelimb movement in cats. On cue each animal was required to reach for a vertical bar and to move it to a target zone through a template by learning the orientation of 2-3 straight segments. A 24 microwire, adjustable electrode system was used to record activity in each cerebellar nucleus as the animal learned and practiced the task. The kinematics of the ipsilateral forelimb movements and the EMG of specific ipsilateral and contralateral forelimb muscles also were recorded. After sorting trials into groups based on specific stages of task acquisition, the amplitude of event-related responses in each group's perievent histogram was plotted as a function of task acquisition. Ninety-two percent of the measured responses changed in a strikingly similar manner during learning: their modulation was maximal during the first successful, smoothly executed trials and then decreased progressively as the task was practiced and performance improved. These observations are consistent with the notion that the cerebellum participates in the synthesis of movements required to learn new motor skills, possibly by contributing to the specification of the optimal strategy with which a new goal-directed movement is executed.

NIH Grants NS21958 and NS30013.

529.7

THE LAMELLAR BODY: A NEW NEURONAL ORGANELLE ASSOCIATED WITH DENDRODENDRITIC GAP JUNCTIONS. C.I. De Zeeuw*, E. Hertzberg, and E. Mugnaini. Dept. of Anat., Erasmus University Rotterdam, 3000 DR, Rotterdam, The Netherlands.

Gap junctions, the site of electrotonic coupling, occur between certain types of neurons and are ubiquitous between glial cells. Recently, sequence-specific antibodies to various regions of gap junction proteins, termed connexins (Cxs), have been used to map the distribution of these gap junctions in the brain. No antibodies yet developed, however, bind specifically to neuronal gap junctions. We have found that an antibody, designated α12, to the first extracellular loop of rat Cx43 (amino acids 49-60, highly conserved among known Cxs) provides punctate labeling in a very restricted number of brain regions in the rat. The highest density of labeling occurs in the inferior olive (IO) where the puncta are ubiquitously distributed in its subnuclei. Robust immunoreactivity was also observed in areas CA1 and CA3 of the hippocampus, the dentate gyrus, the olfactory bulb, and the pyriform cortex. Ultrastructural analysis demonstrates that in all these areas dendrodendritic gap junctions do exist, yet the immunoreactive puncta do not directly indicate neuronal gap junctions. Rather, they correspond to a special lamellar organelle that is exclusively located in dendritic appendages. Another antibody against Cx43, which stains astrocytic gap junctions did not label any neuronal structures, suggesting that another member of the Cx family may be responsible for forming dendrodendritic gap junctions. In the adult IO, the density of puncta is highest in the rostral medial accessory olive, the subnucleus where electrotonic coupling is most prominent. Preliminary data indicate that immunoreactivity in the IO appears between P9 and P15, which coincides with the appearance of gap junctions in this nucleus. The present study shows that antiserum α12B specifically detects a new neuronal organelle that may be related to dendrodendritic gap junctions. In addition, it suggests that dendrodendritic coupling in the IO is more prominent than in other brain regions. Supported by PHS grant GM 30667 and PHS grant NS 09904.

529.8

TEMPORAL RELATIONS OF THE ACTIVITY OF PURKINJE CELL PAIRS IN THE RABBIT NODULUS. D.R. Wylie*, C.I. De Zeeuw, D. Wang and J.I. Simpson. Physiology & Biophysics, NYU Medical Center, New York NY, 10016.

Gap junctions between neurons in the inferior olive (IO) are thought to be important for synchronization of the complex spike (CS) activity of cerebellar Purkinje cells (P-cells) within the same parasagittal zone (e.g. Llinás and Sasaki, 1989). The ventral nodulus can be divided into at least four parasagittal zones, based on CS responses to optokinetic stimulation (OKS) as well as the differential inputs from the IO to each zone. In the most medial zone cells are non-responsive (NR) and receive climbing fibre (CF) input from the beta-subnucleus of the IO. Most P-cells in the adjacent zone and the most lateral zone respond best to OKS about the vertical axis (VA). Between these two VA zones are P-cells that respond best to OKS about an horizontal axis perpendicular to the ipsilateral anterior canal. The three visual zones receive CF input from specific regions of the dorsal cap and ventrolateral outgrowth. To estimate the degree of synchrony we recorded CS activity of P-cell pairs in the ventral nodulus of ketamine-anesthetized pigmented rabbits. The two electrodes were independently manipulable, and spaced 200-600μm apart rostrocaudally and $<250\mu$m mediolaterally. Recordings were obtained from 37 pairs of the same functional type (like-pairs, e.g. VA/VA), and 13 unlike-pairs (e.g. VA/NR). CS activity occurring spontaneously and during OKS produced by a rotating planetarium projector was obtained. We found that the CS activity of 14 like-pairs was synchronous as determined from peaks in the cross-correlograms binned at 1 msec intervals (cross-correlation coefficients ≥ 0.01, as high as 0.25). The degree of synchrony was unaffected by OKS. For unlike-pairs, synchronous firing within 1 msec was rare, and cross-correlation coefficients did not exceed 0.007. These data demonstrate that synchronous firing is most prominent between cells of the same functional type, and that the degree of CS synchrony is not influenced by optokinetic stimulation that modulates their firing rates.

529.9

THE MICROSTRUCTURE OF COHERENCE IN THE OLIVOCEREBELLAR SYSTEM DURING RHYTHMIC MOVEMENT IN NORMAL AND DEAFFERENTED RATS. J.P.Welsh,* E.J. Lang, and R. Llinás, Dept. of Physiology & Biophysics, NYU Medical Center, NY, NY 10016.

We have demonstrated strong temporal relationships between coherent activity within the olivocerebellar system and rhythmic oro-facial movements. We sought to determine a) whether these rhythmic and coherent olivocerebellar activities are causally related to movement; b) whether they are driven by sensory consequences of the movement; and c) the spatial distribution of coherent activity across the cerebellar cortex during movement. Ten rats were trained to perform large and repetitive 7-Hz protrusions of the tongue in response to a tone before glass microelectrodes were implanted into 2-mm^2 of Crus IIa to record the complex spike (CS) activity of 18-33 Purkinje cells (PCs). Prior to recording, the faces of 6 rats were deafferented by bilateral resection of the supralabial and mental branches of the V nerve alone or in combination with bilateral resection of the external nasal branches of the V nerve. Deafferentation did not alter the tone's ability to reset olivary rhythmicity and entrain rhythmic (5-11 Hz) CS firing of individual PCs or impair the rhythm of the movement. However, deafferentation impaired movement initiation and thereby degraded the temporal relation between rhythmic CS activity and movement. In normal and deafferented rats, groups of 3-7 PCs fired CSs coherently (within 1 ms) during movement. These groups exhibited diverse spatial organizations that included distinct parasagittal, oblique, and fractured architectures. We conclude that rhythmic olivocerebellar activity is not in itself sufficient to generate rhythmic tongue protrusions, but neither is it driven by the sensory consequences of the movement. Yet, olivary neurons, uncoupled during states of quiescence, become coupled during movement to induce complex patterns of coherent activity in the cerebellar cortex. Support by NS31224 and NS13742.

529.10

SPATIO-TEMPORAL STRUCTURE IN ARRAY RECORDING OF THE BEHAVING RAT INFERIOR OLIVE.

A. Sivaramakrishnan*, J. P. Welsh, E. J. Lang & R. Llinás, Department of Physiology and Biophysics, New York University Medical Center, 550 First Avenue, New York NY 10016

We apply recently developed statistical techniques to microelectrode array recordings of Purkinje cell complex spikes from the surface of the conditioned rat cerebellar cortex while it responds to stimuli. The stimuli and trained responses were continuously monitored during the experiment. Such data are known to show 5-11Hz oscillations and various spatial patterns including rostro-caudal banding of Purkinje cell climbing fiber activation. These patterns are indicative of the intrinsic neuronal organization of the inferior olivary nucleus, the site of origin of the climbing fibers. We investigate the patterns of coherent activity on the cortical surface on timescales of tenths of a second to a second during stimulus and response in order to determine the degree of spatial organization of inferior olivary firing – known to reflect electrical coupling – and the relation of such patterns to movement. Signals indicating the presence of trains of complex spikes with interspike intervals between 80 to 200ms are cross-correlated. A significant increase in correlated activity is found both following stimulus-evoked movement as well as when the animal initiates movement spontaneously. Protracted movement is accompanied by a decrease in correlated activity in the olive when compared with the initiation of the movement. (ONR N00014-93-0225)

529.11

HIGH-RESOLUTION IMAGING OF CLIMBING FIBER RESPONSES IN THIN RAT CEREBELLAR SLICES USING A VOLTAGE-SENSITIVE DYE

M. Ichikawa, I Vranesic, T. Knöpfel, G. Matsumoto* and T,Iijima
Electrotecnical Laboratory, 1-1-4 Umezono, Tsukuba, Ibaraki 305, Japan and Pharmaceuticals Division, R&D, ciba, CH-4002 Basel, Switzerland

Until recently, the applicability of voltage-sensitive dyes for imaging the propagation of electrical signals along the membranes of single cells or within neuronal circuits was limited mainly due to the lack of photodetector system for accurate measurements of optical signals with both high spatial and temporal resolution. We report here the application of a novel low-noise imaging system (frame rate 1.7kHz, 64 x 64 pixels) to recordings of the spatio-temporal properties of climbing fiber responses in thin slices of rat cerebellum.

Thin rat cerebellar slices from the vermis were stained with the voltage-sensitive absorption dye RH-155. Electrical stimulation in the granule cell layer induced all-or-nothing climbing fiber responses in the adjacent Purkinje cells. These climbing fiber responses first emerged at a level close to the Purkinje cell bodies and subsequently, at the level of distal dendrites. Lowering the external calcium concentration from 2 to 0.2 mM abolished the long-lasting plateau component, but left an initial fast rising spike.

Supported by the Human Frontier Science Program (HFSP). I.V. is a recipient of Science and Technology Agency (STA) Fellowship (Japan).

529.12

CONVERGENCE ON SINGLE CEREBELLAR FOLIA OF NEURONS LOCATED IN LAMELLA-SHAPED SUBSPACES OF THE BASILAR PONTINE GREY

J.G.Bjaalie*, A.M.Nikundiwe and P.Brodal Dept. Anatomy, Oslo University, P.O.Box 1105 Blindern, N-0317 Oslo, Norway

The aim of the study was to search for general rules determining the mutual arrangement of pontocerebellar neurons according to cerebellar target region. The paraflocculus of the cat was chosen as a model. Individual folia, adjacent or separated, were injected with either of the fluorescent tracers Rhodamine-B-isothiocyanate, Fluoro-Gold, or Fast Blue. Three-dimensional computer reconstructions of the distributions of the retrogradely labeled pontine neurons were made. At a large scale, we found that the majority of the cells of each labeled population was confined to a lamella-shaped tissue volume. Each lamella extended from medial to lateral, and accordingly, followed the curving of the pontine grey around the corticospinal and corticobulbar fiber tracts. At a smaller scale, i.e., within each lamellar subspace, the neurons belonging to one labeled population were distributed in aggregates of various shapes. To enable further analysis of the shapes of the intralaminar aggregates, we developed a computer program for unfolding of the lamellae, based on cubic B-spline approximation. The flattened reconstructions were three-dimensional polygonal windows, circumscribing the large majority of the labeled cell swarm (usually 70 - 80 % of the total number of labeled cells in one population).

The present findings, taken together with previous data on a gradual, rather than disjunctive, shift of pontocerebellar neuronal position in relation to a gradual shift of target region (Bjaalie et al., Anat.Rec. 231:510-523, 1991), suggest that the cerebro-ponto-cerebellar system may be organized according to a set of fairly simple, topographic rules.

529.13

3D VISUALIZATION OF CEREBELLAR GROSS ANATOMY IN THE RAT

E.M.Santori* and A.W.Toga
Laboratory of Neuro Imaging, Dept of Neurology, UCLA Los Angeles, CA 90024

Radial organization of lobules in the cerebellum makes comprehension of its gross anatomy difficult. Through the application of three-dimensional (3D) digital reconstruction techniques we have sought a better representation of this anatomic division in the rat brain.

Representations were developed from a 3D stereotaxic map of the rat brain. This map is a digital volume made up of cryosectional images from a single subject which based on anatomic correspondence is representative of a cohort of six specimens.

Closely spaced coronal, sagittal, and horizontal serial images reconstructed from the map reveal the evolution of structural features across the cerebellum. Furthermore, as these orthogonal images are from the same subject, their shared spatial framework facilitates the localization of structural features in 3-space.

Surface-based models of lobules and nuclei were created. Rendered displays of these models effectively convey the shape and structural divisions of the cerebellum. Interactive display of these models is especially helpful in illustrating the relationships of folia in the vermis and hemispheres.

These digital representations provide more information about the configuration of cerebellar lobules than has previously been possible. Resampled cutplanes provide an almost seamless view of the neuroanatomy and 3D rendering of surface models provide a powerful means for understanding spatial relationships in the cerebellum.

This work was supported by NSF (DIR 89-08174) and NIH (RR05956-01).

530.1

TRANSGENIC RATS AS MODEL SYSTEMS TO STUDY VASOPRESSIN GENE EXPRESSION

O. Zeng, J. Funkhouser, D. A. Carter , D. Murphy*. Neuropeptide Laboratory, Institute of Molecular and Biology, National University of Singapore, Singapore 0511, Republic of Singapore.

We are studying the molecular mechanism of the vasopressin (VP) gene expression in specific groups of neurons in rat hypothalamus and its regulation by physiological stimuli, including dehydration. By observing normal rats, we have shown that there is an increase in VP gene transcription and a dramatic increase in the poly(A) tail length of the VP transcript in the supraoptic nucleus in response to the physiological stimulus of water deprivation. In order to define the cis-acting elements responsible for these effects, we have generated transgenic rats bearing rat VP-CAT (chloramphenical acetyl transferase) constructs. The transgenic rats are successfully expressing the transgene appropriately in the hypothalamus. The transgenes are regulated by physiological stimuli in parallel with the endogenous rat VP gene. Indeed, the magnitude of the increase in transgene transcripts is 5-10 fold greater than the increase in the level of the endogenous VP mRNA. These transgenic rats will provide us with more information about how VP gene expression is confined to the hypothalamus and how the VP gene in the hypothalamus responds transcriptionally and posttranscriptionally to physiological stimuli.

530.2

REGULATION OF VASOPRESSIN (AVP) GENE EXPRESSION IN THE HYPOTHALAMUS OF THE OSMOTICALLY-CHALLENGED RAT BY TESTOSTERONE AND ITS METABOLITES. J.A. O'Keefe*, R.S. Crowley, N.B. Kim, J.A. Amico, University of Pittsburgh School of Medicine and VA Medical Center, Pittsburgh, PA 15261

Chronic osmotic stimulation enhances AVP expression in the hypothalamus of the intact rat. We reported (Soc. N.S. Abs. 392.12: 930, 1992) that gonadectomy (GDX) prior to osmotic challenge prevented enhanced accumulation of AVP mRNA in the hypothalamus of male and female rats. The present study was done to determine the contribution of testosterone (T) or its metabolites, dihydrotestosterone (DHT) or estradiol (E_2), upon the AVP gene in the hypothalamus of the osmotically-stimulated male rat. To determine the effect of T, one-week (wk) GDX, sham GDX (intact), and GDX-T-implanted male rats received 2% NaCl solution x 5 days and matched cohorts received tap H_20 x 5 days. All NaCl groups lost comparable amounts of weight, developed hypernatremia, and had significantly lower pituitary stores of AVP compared to tap H_20 controls ($p < .05$, Scheffé F-test). Intact and GDX-T-replaced rats, but not GDX rats, receiving 2% NaCl increased hypothalamic AVP mRNA accumulation compared to respective tap H_20 controls ($p < .05$, Scheffé F-test). To determine the contributions of T metabolites, one-wk GDX osmotically-challenged male rats received T, DHT, E_2, DHT + E_2, or sham implants at GDX. NaCl groups developed comparable hypernatremia and depletion of pituitary AVP stores. GDX osmotically-stimulated animals with T, DHT, or DHT + E_2, but not E_2 or sham implants increased AVP mRNA accumulation compared to GDX males receiving tap H_20. The data suggest that induction of AVP mRNA by T is the result of the action of DHT in the hypothalamus of the osmotically-challenged rat.

530.3

C-FOS AND C-JUN EXPRESSION IN HYPOTHALAMIC MAGNOCELLULAR NEURONS AFTER PITUITARY STALK INJURY. J. Dohanics*, G.E. Hoffman and J.G. Verbalis. Univ. of Pittsburgh, Pittsburgh, PA 15261

Compression of the pituitary stalk (SC) in rats causes disappearance of vasopressinergic (AVP) and oxytocinergic (OT) axons from the posterior pituitary, but hypothalamic AVP neurons are much more susceptible than OT neurons to retrograde degeneration following this injury. In view of this finding, we evaluated the effect of SC on basal and stimulated expression of the proto-oncogene products c-Fos and c-Jun by magnocellular AVP and OT neurons. Adult male rats were perfused 21 d after either SC or sham surgery. Rats were given 2 ml of either 150 mM NaCl (NS) or 2M NaCl (HS) iv 75 min before perfusion. Serial hypothalamic sections were immunocytochemically stained for c-Fos or c-Jun protein and AVP or OT immunoreactivities. In both SC and sham rats neither AVP nor OT neurons expressed c-Fos following NS treatment, but both expressed abundant c-Fos in response to HS treatment. Only faint c-Jun staining was present in normoosmotic sham rats, and was predominantly localized in OT rather than AVP neurons. In normoosmotic SC rats c-Jun expression was very significantly increased, but predominantly in OT neurons. Osmotic stimulation also increased c-Jun expression in both SC and sham rats, but again more prominently and consistently in OT neurons. These results indicate that: 1) the activity of AVP and OT neurons as reflected by c-Fos expression is not basally elevated after SC; 2) magnocellular responsiveness to osmotic stimulation is not impaired by SC; and 3) magnocellular injury causes a prolonged expression of c-Jun that is much more marked in OT neurons. The last finding supports the possibility that c-Jun may play an important role in regenerative and reparative responses following axonal injury, and if so this might account for the much greater resistance of OT neurons to cell death following axonal injury.

530.4

CHOLECYSTOKININ ACTIVATES HYPOTHALAMIC C-FOS EXPRESSION IN A SPECIES-SPECIFIC PATTERN. J.G. Verbalis*, D.A. Schreihofer, J.L. Cameron, W.H. Kaye, E.M. Stricker and G.E.Hoffman. University of Pittsburgh, Pittsburgh, PA 15261.

Previous studies from our laboratories have shown a marked species variability in cholecystokinin (CCK)-stimulated neurohypophyseal secretion. In rats systemic administration of CCK causes a dose-related increase in plasma oxytocin (OT) levels but no increase in plasma vasopressin (AVP) levels, whereas in both monkeys and humans CCK stimulates pituitary AVP but not OT secretion. Using c-Fos as a marker of neuronal activation, we have shown that in rats CCK activates magnocellular OT, but not AVP, neurons projecting to the pituitary and also parvocellular OT neurons projecting centrally within the brain. To determine whether CCK activates hypothalamic AVP and/or OT neurons in primates, we analyzed c-Fos expression in the hypothalamus of adult male rhesus monkeys treated with CCK 15 µg/kg iv 75 min before perfusion. Serial brain sections were stained immunocytochemically for c-Fos protein (Oncogene Science) and either AVP or OT. In the supraoptic nucleus (SON) the pattern of c-Fos expression paralleled pituitary secretion: there was abundant c-Fos expression in virtually all AVP neurons but in very few (<1%) OT neurons. In the paraventricular nucleus (PVN) similar results were found for AVP, but in addition many OT neurons in the caudal and medial regions of the PVN also expressed c-Fos. These results show that c-Fos expression in magnocellular neurons accurately reflects the differential pituitary hormone secretion found in monkeys and rats, but despite a reversed pattern of pituitary peptide secretion, CCK activates parvocellular OT neurons in both species. Thus, CCK activation of central OT neurons appears to be conserved across species, which suggests that brain OT secretion likely contributes to central effects produced by CCK in primates as well as in rats.

530.5

FOS PRODUCTION IN RETROGRADELY - LABELED NEURONS OF THE LAMINA TERMINALIS FOLLOWING INTRAVENOUS INFUSION OF EITHER HYPERTONIC SALINE OR ANGIOTENSIN II. B.J. Oldfield*, D.K. Hards, M.J. McKinley. Howard Florey Institute of Experimental Physiology and Medicine, University of Melbourne, Parkville, Victoria, 3052, Australia.

Neurons in the lamina terminalis (LT) have been shown previously to be activated by intravenous infusions of hypertonic solutions or angiotensin II (ANG II). The present studies have been designed to determine the extent to which there is differential activation of those neurons within the LT which project to the supraoptic nucleus (SON). Cholera toxin-gold (CTB-Au) was injected bilaterally into the SON of rats anaesthetised with sodium amobarbital. After at least 1 week intravenous infusions of hypertonic saline, n = 4 (1.5 ml of 1.5M NaCl) or ANG II, n = 4 (1mg/ml/hr) were made and after 90-120 mins. rats were perfusion fixed. Antibodies raised against Fos were applied to brain sections and located using avidin-biotin-peroxidase procedures. In four cases, HRP was injected intravenously 3 mins prior to killing rats in order to delimit the boundaries of structures in the LT which lack a blood brain barrier. This material was prepared for EM examination. Neurons within the component regions of the LT which contained both retrogradely-transported CT B-Au and Fos-positive nuclei were counted. The mean percentage of double-labeled neurons following iv hypertonic saline was by far the highest in the organum vasculosum lamina terminalis (OVLT) and decreased in the median preoptic nucleus (MnPO) and subfornical organ (SFO). Conversely neurons in the subfornical organ projecting to the SON were more often activated following ANG II infusion. The results show firstly, that different populations of neurons in the LT are activated following the two stimuli, and secondly, that osmotically activated neurons, which project directly to the SON, are concentrated in the dorsal aspect of the OVLT.

Supported by grants from the NHMRC Australia.

530.6

FOS-LIKE IMMUNOREACTIVITY IN ARCUATE NUCLEUS AFTER INTRACEREBROVENTRICULAR HYPERTONIC NaCl. L.P. Solano-Flores,* M.P. Rosas-Arellano and J. Ciriello. Department of Physiology, University of Western Ontario, London, Canada N6A 5C1.

Intracerebroventricular infusions of hypertonic NaCl solutions have been shown to elicit drinking behaviour, natriuresis and cardiovascular responses. The arcuate nucleus of the hypothalamus (Arc), a periventricular structure, has been suggested to be a possible site of action of the hypertonic stimulus. However, direct evidence in support of this suggestion is not available. This study was done to investigate whether neurons in Arc alter their activity to infusions of NaCl solutions into the rostral part of the third ventricule in the awake rat. The immunohistochemical detection of c-fos was used as an indicator of neuronal activation. Infusions of either isotonic (143 mM) or slightly hypertonic (172 mM) NaCl solutions resulted in the c-fos labelling of very few Arc neurons. On the other hand, infusions of hypertonic (339 mM) NaCl solution resulted in a large number of c-fos labelled neurons within Arc. In particular, the very rostral pole of Arc contained a conspicuous cluster of c-fos labelled cells. These data demonstrate that changes in the environment of the intraventricular fluid after infusion of hypertonic NaCl activates Arc cells and support the suggestion that Arc functions as a component of central neural and humoral mechanisms in the control of body fluid and circulatory homeostasis (Supported by MRC of Canada and HSFO; LPSF and MPRA are fellows from UNAM México).

530.7

EFFECT OF ARCUATE NUCLEUS STIMULATION ON THE RESPONSE OF SUBFORNICAL ORGAN NEURONS TO SYSTEMIC CHANGES IN ANGIOTENSIN II OR PLASMA OSMOLALITY. M.P. Rosas-Arellano*, L.P. Solano-Flores and J. Ciriello. Department of Physiology, University of Western Ontario, London, Canada, N6A 5C1.

We have previously shown the existence of a pathway from the arcuate nucleus of the hypothalamus (Arc) to the subfornical organ (SFO) in the rat. In addition, we have shown that the discharge rate of SFO neurons is altered during Arc stimulation. This study was done to investigate the effect of Arc activation on the discharge rate of SFO neurons during systemic changes in Angiotensin II (Ang II) or plasma osmolality in the urethane anesthetized rat. Extracellular recordings were made from 44 SFO neurons. Of these, 24 neurons responded to stimulation of Arc. Ten of these neurons were also excited by intracarotid infusion of hypertonic saline (0.5 M in 0.1 ml) and 5 were also excited by intracarotid infusion of Ang II (0.5 μg in 0.1 ml). Of the 10 units excited by hypertonic saline or Arc stimulation, the response of 9 of the neurons to hypertonic saline was potentiated after Arc stimulation. Similarly, in two units excited by Ang II or Arc stimulation, the response of the units to Ang II was potentiated after Arc stimulation. In the three units excited by Ang II or inhibited by Arc stimulation, the excitatory response to Ang II injection was attenuated in two units and not alterd in one unit after Arc stimulation. These data suggest that Arc functions to modulate the response of SFO neurons to systemic changes in circulatory levels of Ang II and to hypernatremia. (Supported by MRC of Canada and HSFO; MPRA and LPSF are fellows from UNAM México).

530.8

ALTERATIONS IN PLASMA OSMOLALITY ELICITS INCREASES IN NEURONAL DISCHARGE OF NUCLEUS TRACTUS SOLITARIUS NEURONS. S. L. Hochstenbach* and J. Ciriello. Department of Physiology, University of Western Ontario, London, Ontario, N6A 5C1.

Recently, we have demonstrated that increases in plasma osmolality results in c-fos expression in neurons of nucleus tractus solitarius (NTS) in the rat. In addition, microinjections of hypertonic saline into NTS elicits depressor and bradycardic responses. This study was done to investigate the effect of changes in plasma osmolality and baroreceptor activation on the discharge rate of NTS neurons. NTS was systematically explored for spontaneously active single units that altered their discharge rate to intracarotid infusions of hypertonic saline (0.5 M; 0.1-0.2 ml) in the chloralose-anesthetized, paralyzed and artificially ventilated rat. Responsive units were then assessed for their response to reflex activation of baroreceptors following pressor doses of phenylephrine (2-10 μg/kg, i.v.). Of 70 spontaneously active cells recorded extracellularly in NTS, 21 (30%) responded to plasma hypernatremia with an increase in firing frequency. Sixteen of these cells were also tested for baroreceptor input: 5 cells (31%) were excited, 3 cells (19%) were inhibited and 8 (50%) did not respond. These data suggest that within NTS there exists a pool of neurons that are sensitive to plasma osmolality changes and that these neurons may also function in cardiovascular regulation. (Supported by HSFO and MRC of Canada.)

530.9

BRAINSTEM VASOPRESSIN (VP) RECEPTORS MEDIATE THE PRESSOR RESPONSE TO OSMOTIC STIMULATION OF THE SUPRAOPTIC NUCLEUS (SON). M.F. Callahan*, M. Ludwig, L.J. Sim, K.P. Tsai and M. Morris. Dept. of Phys. and Pharm., Wake Forest Univ. Medical Center, Winston Salem, NC 27157 and Dept. Biosci., Univ. Leipzig, Leipzig, Germany.

Osmotic stimulation of the SON results in an increase in arterial pressure and heart rate. We have shown that blockade of vascular VP receptors has little effect on this response. Blockade of brainstem VP receptors blocks the pressor response to Angiotensin II or hypertonic saline (HS) administered intracerebroventricular (ICV). We examined whether this blockade would affect pressor responses to direct osmotic stimulation of the SON.

Male S-D rats received an arterial catheter, fourth ventricular cannula and bilateral microdialysis probes directed at the SON. HS was administered via the microdialysis probe (90μl/30min), followed by administration of either artificial CSF or d(CH_2),Tyr(Me)AVP (9μg/30μl/30 min). The HS was then readministered. ICV artificial CSF had little effect on the peak pressor response to hypertonic saline (1M) delivered by microdialysis probe (20.5 ± 2 mmHg before -v- 17 ± 8). ICV VP antagonist inhibited the pressor response (20 ± 5 before -v- 3.5 ± 0.5 after the antagonist).

The results of this study suggest that there may be an important cardiovascular pathway from the SON to brainstem regulatory centers. Alternatively, this may indicate that direct osmotic stimulation of the SON causes activation of previously demonstrated pathways from the PVN to brainstem. (Supported by HL 43178).

530.10

GABA-ERGIC GRANULE CELLS ARE THE POST-SYNAPTIC TARGETS OF THE CCK-ERGIC INTRABULBAR ASSOCIATION SYSTEM IN THE RAT OLFACTORY BULB

W-L. Liu* and M. T. Shipley.
Dept. Anat. & Cell Biology, University of Cincinnati Col. of Med., Cincinnati, OH 45267

The intrabulbar association system (IAS) interconnects opposite sides of the olfactory bulb. Using combined retrograde tracing (WGAapoHRP-Au) and CCK immunocytochemistry we have demonstrated that this system is composed exclusively of CCK-containing axons that originate from the superficial middle and external tufted cells. Using biocytin anterograde tracing and CCK immunocytochemistry we further show that the IAS termeinates densley and focally in the internal plexiform layer (IPL) on the opposite side of the olfactory bulb. The strict location of IAS terminals to the IPL suggests that this system synaptically targets neural elements present in the IPL. We hypothesized that the most likely post-synaptic targets of the terminals of this system are the dendrites of the granule cells coursing through the IPL to the EPL.

To test this hypothesis, biocytin was injected into the superficial part of the EPL of the olfactory bulb; 60 μm thick sections were cut on a vibratome and reacted by avidin-biotin complex (ABC)-DAB. Sections containing biocytin labeled axons in the IPL were processed for EM immunocytochemistry using post-embedding immunogold staining for GABA. Biocytin labeled axons and terminals in the IPL were easily observed as dark profiles at the EM level. The biocytin-labeled terminals were apposed to dendritic profiles. Biocytin labeled boutons made synaptic contacts with dendrites that had a high density of immunogold particles for GABA. As the granule cells are GABAergic and their apical dendrites comprise the majority of dendrites in the IPL, we conclude that the post-synaptic targets of the terminals of the IAS are the GABAergic granule cells.

In other neural systems, CCK consistently produces postsynaptic depolarizing responses. If CCK has a similar action in the IAS, then it is reasonable to hypothesize that IAS functions to depolarize granule cell dendrites. This could increase GABA release from granule cell synapses thus inhibiting a discrete population of mitral/tufted cells on the same and opposite sides of the bulb. Alternatively, CCK could shunt conduction in the granule cell dendrites causing a decrease in GABA release, thus exciting discrete populations of mitral/tufted cells.

Supported by NIH DC00347, NS29218 and US Army DAMD 17-91-C1071.

530.11

A NEW DIRECT AFFERENT TO THE HYPOTHALAMUS: THE VOMERONASAL ORGAN. J.Larriva-Sahd, H. Orozco, R. Aguilar-Roblero*, A. Rondán and M.R. Sánchez-Robles. Laboratory of Experimental Pathology, Instituto Nacional de la Nutrición, México City, Tlalpan 14,000. México City, MEXICO.

In terrestrial mammals, the vomeronasal organ (VO) allows the recognition of conspecifics in terms of specie. In rodents, pheromones contained by urine, are uptaken by the VO, influencing neuroendocrine responses, particularly LH secretion. It has been established that the VO neuroepithelium projects to the accessory olfactory bulb, from which afferent fibers reach the `vomeronasal amygdala´(VNA). More centrally, fibers from the later proceed to the bed nucleus of the stria terminalis(BNST). The VNA also projects to the BNST and medial preoptic (MP) area via the ventral amygdaloid path. Both fiber systems project to the ventral medial hypothalamic nucleus (VMHN). In this study the vomeronasal nerves (VN)(but not terminal nerves) of six young albino rats from each sex were cut by stereotaxical surgery though the palate. Six animals (three from each sex) were sham lesioned. 72 h.s. later animals were killed by transcardial perfusion with Karnovsky´s fixative and their brains processed for electron microscopy. Only in VN lesioned animals, orthograde degeneration was found in dendrites and neuronal somata of (in order of frequency): the MP nucleus (MPN), VMHN, BNST, and arcuate. It is concluded that 1. neurosensory VO cells provide a direct innervation to the MPN and hypothalamus; 2 neurosensory cells of the VO may exert direct, perhaps faster, hypothalamic control on sex behavior and LHRH secretion via this neural path.

Supported by a grant from CONACYT.

530.12

PERFORMANCE DEFICITS IN CONDITIONED TASTE AVERSION AND SALT DISCRIMINATION CAUSED BY LESIONS IN THE PARABRACHIAL NUCLEI. A.C. Spector* and S.J. St. John. Dept. of Psychology, Univ. of Florida, Gainesville, FL 32611.

Rats with lesions in the gustatory zone of the parabrachial nuclei (PBN) are severely impaired in their ability to form conditioned taste aversions (CTA). Accordingly, we used this behavioral paradigm as a functional assay of the PBN lesion and then tested rats for their ability to discriminate NaCl from KCl in a conditioned shock avoidance task. Rats with electrophysiologically-guided lesions (ibotenic acid, 4 ug/0.2 ul, pH = 7.4) centered in the gustatory zone of the PBN (PBNX; n = 12) and vehicle-treated controls (CON; n = 7) received 15 min presentations of 0.1 M sucrose immediately followed by LiCl injection (2.0 mEq/kg, ip) on 3 separate occasions. On the fourth presentation all control rats ingested virtually no sucrose, whereas PBNX rats drank between 10.0 and 33.0 ml. In a specially-designed gustometer, half the rats were then trained to suppress spout licking in response to various concentrations (0.05 - 0.2 M) of NaCl and to maintain licking to the same concentrations of KCl in brief access trials. The other half were trained in the opposite manner. Six PBNX rats learned to discriminate NaCl from KCl and final performance was not substantially different from CON rats. Five PBNX rats showed no sign of competence in the NaCl vs. KCl discrimination and one rat was partially impaired. Rats with the most complete lesions were the most impaired on both behavioral tasks, although partial lesions appeared to have a greater effect on CTA than on conditioned salt discrimination performance. This suggests that the pre-PBN circuitry is not capable of supporting performance on either behavioral task.

530.13

CLASSIFICATION OF TASTE RESPONSES IN BRAIN STEM: MEMBERSHIP IN MULTIPLE GROUPS. Robert P. Erickson,* Patricia M. Di Lorenzo and Max A. Woodbury. Department of Psychology, Duke University, Durham, N.C. 27706.

Classification schemes for gustatory neurons have taken several forms. For example, neurons have been grouped according to the stimuli to which they are most responsive, by their proximities in multidimensional spaces, and by their temporal patterns of response. With each of these methods, the neurons encompassed by a group are quite heterogeneous in their response characteristics. An alternative method is presented which allows "grades of membership" for each neuron in several categories. This method also provides classification of stimuli. A neuron is given ratings which represent membership in several categories, and each stimulus is also rated in these same categories; i.e. instead of being in one category each, each neuron and stimulus belongs in various degrees to several categories. These few categories might be related to real physiological variables, such as receptor and/or other mechanisms. With three stimulus/neuron categories, a good fit is found to the responses of neurons in the rat nucleus of the solitary tract and pontine taste area. The idiosyncratic temporal pattern of response of each neuron to each stimulus is also accounted for by a few subdivisions of the stimulus part of the three stimulus/neuron categories. Supported by Duke University, the Whitehall Foundation and NIA/NIA.

530.14

ASCENDING AND DESCENDING EFFERENT NEURONS IN THE NUCLEI OF THE SOLITARY TRACT COMPRISE TWO DISTINCT POPULATIONS. C.B. Halsell*, S.P. Travers and J.B. Travers, Dept. of Oral Biology, The Ohio Stat Univ., Columbus, OH 43210.

The rostral division of the nucleus of the solitary tract (rNST) is the site of the first central synapse for primary taste afferents. In rodents, rNST efferent neurons send ascending projections to specific areas of the parabrachial nucleus and descending projections to local medullary regions, including the parvocellular and intermediate reticular nuclei (RF) and specific subdivisions of the caudal division of the NST. The descending pathway is not as well studied as the ascending pathway and the relationship between rNST neurons comprising these two pathways is unknown. The present study directly compared the distribution and morphological characteristics of the rNST neurons contributing to these two efferent pathways, using dual injections of different fluorescent retrograde tracers. The tissue was processed in the conventional manner. The majority of ascending and descending efferent neurons were separate. Ascending efferents were located in the medial half of the rostral-central NST as a tight cluster that contained few neurons with descending connections. Descending efferents, on the other hand, were scattered throughout the rostral-lateral and ventral NST, and subjacent RF. Although some ascending efferent neurons were intermingled with the descending efferents ventrally, the proportion of double-labelled cells was small. Most efferent neurons with connections to the RF terminated in close proximity to the NST, with fewer projections to caudal and deep ventral RF levels. In contrast, more neurons with intra-NST connections had longer projections, extending to caudal levels of the NST. These findings support a segregation of direct projection neurons ascending to higher brain regions and descending to orofacial pre-motor regions.

Supported by NIH grants DC00416, DC00417 and OSU Coll. of Dent. Grant.

VISUAL CORTEX: EXTRASTRIATE—MOTION PROCESSING

531.1

INTEGRATION OF LOCAL MOTION SIGNALS IN AREA MT. M.N. Shadlen*, W.T. Newsome, E. Zohary, & K.H. Britten. Depts. of Neurobiology and Neurology, Stanford Univ., Stanford, CA 94305

MT neurons receive direction selective afferents from V1 and other extrastriate areas. In principle, this input can account for direction selective responses within small subregions of the MT receptive field, but not for broader interactions. We wish to assess the relative contribution of local and broad range spatiotemporal interactions to MT direction selectivity. Therefore we devised an apparent motion (AM) stimulus that permits independent manipulation of local and broad range motion signals. The stimulus consists of a brief localized motion which is sequentially repeated at one deg displacements across the receptive field. It may be approximated as a strobe-sampled AM display, convolved with the impulse response of a linear motion filter. Mechanisms that confer sensitivity to local motion signals should be unaffected by the direction of AM, while broad range interactions should be reflected by a difference in response to opposing directions of AM despite identical local motion cues. We have recently reported that MT neurons characteristically respond to the local motion in these displays (ARVO, 1993). We found no evidence for direction selective mechanisms operating over spatial displacements exceeding one deg for speeds of 7-8 deg/sec. One possibility is that such interactions only occur at faster speeds and shorter temporal intervals. We therefore examined the direction selectivity of MT neurons in the alert macaque monkey to these stimuli over a range of speeds from 6 to 20 deg/sec. Although the response to local motion signals varies as a function of speed, we find no evidence for spatiotemporal interactions over one deg displacements (approx. 1/10 RF diameter). This is puzzling in light of the sophisticated processing attributed to many MT neurons. Yet for simple translation, our findings suggest that MT merely summates direction selective input over a broader spatial scale. [National Eye Institute (EY05603) and HHMI.]

531.2

THE RELATIONSHIP BETWEEN DIRECTIONAL TUNING AND MOTION STRENGTH IN AREA MT. K.H. Britten* and W.T. Newsome. Center for Neuroscience, UC Davis, Davis, CA 95616 and Dept. of Neurobiology, Stanford University, Stanford CA, 94305.

We have attempted to model threshold psychophysical performance from the responses of MT neurons to variable strength motion stimuli. This effort requires estimation of the profile of activity within a population of MT neurons, which in turn depends on cell tuning properties. However, previous tuning measurements employed stimuli of maximum strength (100% correlation) rather than the near-threshold strengths relevant to our modelling efforts. We therefore measured direction tuning curves for motion stimuli of variable strengths.

We measured direction tuning curves over a range of correlation levels in 40 cells from one monkey. For these cells, stimulus strength did not strongly influence tuning bandwidth. Although response rate fell with decreasing stimulus correlation, the normalized tuning curves were superimposed in most cases, indicating linear scaling of the tuning curve with stimulus correlation. Bandwidth was estimated for each level by fitting Gaussian functions, and the dependence of σ on stimulus correlation was explored with linear regression. Only one cell showed a significant relationship, and the distribution of regression slopes was shifted slightly, but not significantly, towards negative values (decreasing bandwidth with increasing correlation). Thus, bandwidth appears to be roughly invariant with stimulus strength.

We found that neuronal thresholds (ROC analysis) for discriminating opposed directions of motion were elevated only modestly (roughly 70% on average) for motion axes up to 30° from the peak of the tuning curve. For any single axis of discrimination, then, perhaps 1/6 of MT neurons at the appropriate topographic location could provide reliable motion signals. Thus the number of sensitive neurons available during direction discrimination performance is quite large indeed. (Supported EY-05603).

531.3

SHORT TERM PLASTICITY OF NEURONAL AND PSYCHOPHYSICAL THRESHOLDS DURING REPEATED VISUAL STIMULATION. E Zohary*, S Celebrini KH Britten & WT Newsome. Dept of Neurobiology, Stanford University, Stanford,CA 94305.

We recorded from 274 neurons in areas MT and MST of four macaque monkeys while the animals performed a near-threshold direction discrimination task. Psychophysical thresholds improved by 20% on average during the first 300-500 trials of a given experiment. Remarkably, the sensitivities of single cortical neurons also improved during this time interval. Moreover, a similar improvement in neuronal sensitivity occured in a naive monkey which simply maintained fixation during the stimulus presentation intervals. We measured the time course and magnitude of this effect by averaging the sensitivities of all cells for successive time intervals during an experiment. The average discrimination threshold for the neurons improved roughly linearly as the experiment progressed, saturating after about 400 trials. Neuronal thresholds were, on average, 14% lower during the last 200 trials as compared to the first 200 trials. The gain in sensitivity was highly significant for both neuronal and psychophysical performance. To determine whether the plastic effect occurs at the level of whole receptive fields or at the level of subunits, we presented an initial series of 100 stimuli in a subregion of the receptive field, S1, and then presented "test" stimuli in S1 and in an adjacent subregion, S2. Preliminary results indicate that the initial sequence of visual stimulation in S1 leads to improved sensitivity in both S1 and S2, suggesting that the observed plasticity depends upon mechanisms in MT and MST as opposed to earlier levels of the motion pathway. (Supported by NEI (05603), the McDonnel-Pew Program in Cognitive Neuroscience, and by the CNRS of France).

531.4

USING BURSTS OF ACTION POTENTIALS AS EVENTS FOR NEURAL SIGNALING W. Bair, C. Koch* Computation and Neural Systems, California Institute of Technology, Pasadena, CA 91125.

In a previous study of neurons in area MT (Newsome, Britten & Movshon, 1989), direction discrimination thresholds for an ideal observer (based on ROC analysis) counting the total number of spikes fired by the cell during a 2 sec stimulus are found to be similar to psychophysical thresholds measured simultaneously in alert monkeys.

Because about 2/3 of the 212 MT cells studied often fire action potentials in bursts, i.e. more clustered than expected from a Poisson process with refractory period, and because the number of spikes per burst is not tuned for the stimulus direction or coherence in the majority of these cells, we replaced spike count with "event" count as the information available to the ideal observer. Events are defined as the longest trains of consecutive spikes with interspike intervals < 4 msec and therefore may be bursts or single isolated spikes. We found that for the 41 most bursty cells, the neuronal threshold lowered (improved) by 8% on average, and for 3 cells, thresholds were roughly cut in half.

This result is consistent with a simple stochastic model in which (1) the number of events per stimulus period is Poisson distributed with mean rate depending on the stimulus coherence and direction and (2) the number of spikes in an event is a random variable independent of both the stimulus coherence and the number of spikes in any other event.

We have tried other schemes in which events are weighted as a function of their number of spikes (i.e., relative weightings of bursts to isolated spikes and weighting events by the square or square root of their spike count) but find the greatest improvement when events are weighted equally regardless of spike count. We stress that neither the code used to carry motion information nor whether post-synaptic cells differentiate between bursts and single spikes is known.

531.5

SUMMATION PROPERTIES OF MACAQUE MT AND MST NEURONS. S. Raiguel*, V. Marcar, D.-K. Xiao, H. Maes and G.A. Orban, Lab. Neuro- en Psychofysiologie, Medical School, KULeuven, B-3000 Leuven, Belgium.

We compared the summation properties of MT and dorsal MST cells for translating moving dot fields after centering the stimuli on the center of mass of 2-D spatial sensitivity profiles. Three series of stimuli were presented in interleaved fashion: a series in which the diameter of the moving dot field increased in 8 steps (summation series), a series in which a central disc of increasing diameter was removed (inverse summation) and a series of annuli corresponding to the successive steps in the first two series. In MT the summation series indicated that about half the cells had an antagonistic surround. In all cases the response decreased steadily in the inverse summation test. In MST the results of the summation series were similar, but the inverse summation series revealed cells with properties not seen in MT. In a number of cells without surround, the response in the inverse summation series was flat. These cells consistently responded better to the large annuli than to the small ones. Other MST cells showed reversals in preferred direction in the inverse summation series but not in the summation series. Some of these cells preferred opposite directions for an annulus and a disc. These results indicate that MST receptive fields are adapted to analyze wide field motion, even in cluttered environments.

531.6

MT NEURONAL RESPONSES TO 1ST- AND 2ND-ORDER MOTION.

L. P. O'Keefe*, M. Carandini, J. M. H. Beusmans and J. A. Movshon†
Center for Neural Science and †Howard Hughes Medical Institute, New York University, New York 10003.

Extrastriate cortical area MT is thought to process behaviorally important visual motion signals. Psychophysical studies suggest that there may be two motion systems. The 1st-order system responds to conventional motion targets, and the 2nd-order motion system can be probed through use of "drift-balanced" stimuli, whose motion is visible to human observers, but invisible to 1st-order motion sensors.

We wished to know if area MT is involved in the analysis of 2nd-order motion. We measured responses to 1st- and 2nd-order gratings of single neurons in the central visual field representation of area MT in anesthetized, paralyzed macaque monkeys. For each neuron, we measured directional and spatio-temporal tuning with luminance gratings and with drift-balanced gratings created by spatial modulation of the contrast of a random texture.

Cells whose receptive fields lay near the fovea often gave vigorous direction-selective responses to both luminance and drift-balanced gratings. However, cells whose receptive fields were further than a few degrees from the fovea usually responded weakly and with less direction selectivity to drift-balanced targets. This may explain the psychophysical observation that 2nd-order motion is easily detectable foveally, but weak or absent in the periphery. The results suggest that the very neurons in foveal MT that are thought to signal 1st-order motion are also capable of extracting 2nd-order motion, and that both kinds of motion may be signalled by a single system.

531.7

CHROMINANCE VS. LUMINANCE AS TOKENS FOR DIRECTIONAL SELECTIVITY IN MACAQUE VISUAL AREA MT. KR Dobkins* and TD Albright. The Salk Institute for Biological Studies, La Jolla, CA 92037.

Although MT neurons do not exhibit traditional chromatic selectivity, many continue to signal direction of motion when stimulated with moving patterns defined solely by chromatic contrast. MT neurons are also highly sensitive to moving patterns defined solely by luminance contrast. To directly assess the relative virtues of chrominance and luminance as motion correspondence tokens, we have now compared responses of individual MT neurons elicited by heterochromatic vs. achromatic moving patterns that contained identical levels of luminance contrast. In doing so, we sought to determine whether chromatic contrast confers any benefit to motion processing when luminance contrast is also present in a moving stimulus.

We used apparent motion stimuli consisting of achromatic (yellow/black) and heterochromatic (red/green) sine-wave gratings repeatedly undergoing 1/4 cycle phase displacements (see Dobkins & Albright, 1993, *Vision Res, 33*: 1019). Luminance contrast of achromatic patterns was varied from 1.3% to 10%. For heterochromatic stimuli, luminance contrast was varied over a small range (10%) centered on each neuron's "isoluminant point". Indices of directional selectivity (DIs) were calculated using responses elicited by movement in preferred and anti-preferred directions.

Upon comparison of DIs elicited by heterochromatic vs. achromatic stimuli possessing identical luminance modulation, we found that at and near isoluminance, the addition of chromatic contrast markedly improved direction discrimination by MT neurons. At larger luminance contrasts (greater than ~4%), however, heterochromatic and achromatic DIs were not significantly different, suggesting that color provides little benefit when luminance contrast is sufficiently large. Furthermore, the relative strength of heterochromatic vs. achromatic responses did not vary as a function of receptive field eccentricity. Therefore, the central visual field representation in MT does not appear relatively more specialized for color than for luminance processing.

531.8

REPRESENTATION OF DEPTH FROM MOTION AND STEREO CUES IN MACAQUE CORTICAL VISUAL AREA MT. G.J. Carman* and T.D. Albright, Salk Institute Vision Center, San Diego, CA 92186.

Under natural viewing conditions, motion parallax (MP) and stereo parallax (SP) each provide cues to the distance (D) of surfaces relative to the fixation distance (FD). In order to determine how these cues support the representation of depth, we recorded from neurons in the superior temporal sulcus (STS) of alert *Macaca mulatta* viewing surfaces presenting gaussian distance profiles. These gaussian stimuli were delivered as dynamic, stereoscopic pairs of images rendered using random dots of constant density and limited life, and could be manipulated so as to present MP and/or SP cues. For each neuron, we determined the classical receptive field, direction selectivity, and disparity selectivity using conventional bar and planar random dot stimuli. We then recorded responses to gaussian stimuli for which D was varied relative to FD (e.g. 51 < D < 63 cm at 57 cm FD). The majority of MT neurons responded either to near surfaces (D < FD) or to far surfaces (D > FD), and exhibited a monotonic increase in response to greater magnitude of relative distance (RD = D-FD) for stimuli presenting MP and SP cues. Some neurons exhibit strikingly linear (r ~ 0.95) response as a function of RD, with the slope of this response function being modulated by FD. Our results suggest that some neurons in area MT provide a linear representation of MP cues to relative distance, and use SP cues to disambiguate MP and to provide the variable gain necessary for perceptual depth constancy.

(NIH EYO6179 to GJC and NIH EY07605 to TDA.)

531.9

MSTd NEURONAL RESPONSES TO THE CENTER-OF-MOTION IN OPTIC FLOW FIELDS. C. J. Duffy* and R. H. Wurtz, Lab. of Sensorimotor Research, National Eye Institute, Bethesda, MD 20892.

A prominent feature of optic flow fields is the focus of expansion in radial patterns or the center of rotation in circular patterns. Radial and circular flow fields can be shifted to displace the center-of-motion (COM) while preserving the global pattern. Such stimuli simulate the distribution of directional motion in optic flow fields seen when the observer's direction of self-movement does not match the direction of gaze. We studied the effect of a shift in the COM on the responses of neurons in the dorsal medial region of the medial superior temporal area (MSTd) of Rhesus monkey extrastriate cortex.

Stimuli consisted of white dots in patterned motion on a 100° x 100° projection screen. The COM in a radial or circular pattern was positioned at selected points on the screen while the monkey maintained centered visual fixation. MSTd neurons were found which responded specifically to each of the COM positions tested throughout the visual field, with increasing numbers of neurons responding to COMs closer to the fixation point. Some MSTd neurons responded best to stimuli in which the COM was directly over the fixation point with little response to stimuli with COMs shifted off that point. Other MSTd neurons responded best to stimuli in which the COM was displaced off of the fixation point. These neurons had a response preference for shifts a specific distance away from the fixation point and in a specific direction relative to the fixation point.

Thus, each neuron was activated by stimuli with a COM in any part of the visual field, limited with respect to distance and direction from the fixation point. The selectivity of these responses support the hypothesis that MSTd neurons might contribute to a neural representation of optic flow.

531.10

PURSUIT RELATED ACTIVITY IN MACAQUE VISUAL CORTICAL AREAS MST AND LIP IS MODULATED BY EYE POSITION

F.Bremmer* and K.-P.Hoffmann, Dept.Zoology & Neurobiology, Ruhr University Bochum, D-4630 BOCHUM, Germany

Saccade- and stimulus-related responses of neurons in monkey prestriate Lateral Intra Parietal Area (LIP) and area 7A (Andersen et al., 1990) as well as areas V3A and V6 (Galletti et al., 1989, 1991) are modulated by the position of the eye in the orbit.

Pursuit related responses have been shown for neurons in the Medial Superior Temporal area (MST), which is reciprocially connected to area LIP. In our study we tried to reveal whether pursuit related neurons exist in area LIP also and whether pursuit related activity of MST as well as LIP neurons is modulated by eye position.

Single unit recordings were done in 4 hemispheres of three monkeys. In a first step neurons were tested while the monkey pursued a target moving into different directions on a tangential translucent screen. 67 out of 112 LIP neurons and 81 out of 177 MST neurons tested showed a clear pursuit related activity. In order to test modulation of responsiveness of these neurons by the position of the eye in the orbit the monkey had to pursue the same target which started moving with the same velocity from different locations on the screen. For most of the neurons, 72% in area MST as well as 88% in area LIP, the pursuit related response was depending on the pursuit starting position. Response strength as a function of horizontal and vertical eye position could be fitted highly significantly by a two-dimensional linear regression plane.

Supported by the F.Ebert Stiftung and ESPRIT INSIGHT I.

531.11

DEFICITS OF NON-FOURIER MOTION PERCEPTION IN A PATIENT WITH NORMAL PERFORMANCE ON SHORT-RANGE MOTION TASKS. L.M. Vaina[1,2*] M.LeMay[2] N.M. Grzywacz[3]
Intelligent Systems Lab. & Neurology Dept.-Boston University[1], Health Sciences and Technology and Neurology Dept, .M.I.T. and Harvard Med. School[2] Boston Ma 02215, Smith Kettlewell Institute of Visual Sciences, San Francisco, Ca. 94115 [3].

This study provides experimental evidence for the hypothesis that Fourier and long-range, non-Fourier, motion are carried out at different stages of processing in the human brain.

Chubb and Sperling introduced non-Fourier motion stimuli in which the expected power of any Fourier component moving in a given direction and speed is equal to the power of the same component moving in the opposite direction with the same speed. To measure subjects' sensitivity to non-Fourier motion we devised a stimulus consisting of a dense random-dot pattern and an imaginary square-wave grating drifting over it. The imaginary grating defined the probability that the contrast polarity of each dot would switch from frame to frame. The subjects' task was to determine in a 2AFC the direction of motion.

We show data from control subjects and a stroke patient, **F.D.**, with a left posterior parietal-temporal lesion, who presented with severe deficits on this task, yet his perception on a large class of Fourier-type short range motion stimuli was normal, as were his contrast and temporal frequency discrimination. We will discuss possible anatomical correlates of this dissociation. *Supported by NIH-NEI grant RO1 EY07861*

HUMAN COGNITION: ATTENTION AND MEMORY

532.1

MEMORY FOR TEMPORAL AND SPATIAL DISTANCES FOR NEW AND PREVIOUSLY LEARNED GEOGRAPHICAL INFORMATION IN HYPOXIC SUBJECTS. R.O. Hopkins* and R.P. Kesner, Department of Psychology, University of Utah, Salt Lake City, Utah 84112.

Hypoxic subjects and age matched control subjects were tested for memory for temporal and spatial distances for data-based (new information) and knowledge-based (prior knowledge) information. Memory for data-based spatial and temporal distance information was assessed using a map of New Brunswick. In the study phase subjects were presented with 8 cities, on a map of New Brunswick, one at a time, for 5 seconds each. In the test phase subjects were presented with the names of 2 cities and were asked to choose the city that occurred earlier in the sequence. Temporal distance was determined by the number of items that occurred in the study phase between the two test items. Temporal distances of 0, 2, 4 and 6 were assessed. In the spatial distance task the study phase was identical to that of the temporal distance task. In the test phase subjects were presented with the names of 2 cities and were asked to remember the city that was located further to the north, south, east or west. There were equal numbers of trials for each direction. Spatial distance was determined by the number of cities that were located between the two test cities, in the chosen direction. The knowledge-based tasks were identical to those of the data-based tasks, except that maps and cities in the United States were used. Both temporal and spatial distances were assessed. Results indicate that hypoxic subjects, relative to control subjects, were impaired across all distances on the data-based tasks, for both temporal and spatial distance information. Hypoxic subjects were impaired relative to control subjects for knowledge-based temporal distance memory. However, hypoxic subjects performed similar to control subjects for all distances in the knowledge-based spatial distance task. Thus, a relatively permanent representation of a spatial cognitive map does not appear to reside in the hippocampus.

532.2

LATERALIZATION OF FRONTAL LOBE ACTIVITY ASSOCIATED WITH WORKING MEMORY FOR FACES CHANGES WITH RETENTION INTERVAL: A PARAMETRIC PET-RCBF STUDY. JV Haxby*[1], B Horwitz[1], LG Ungerleider[2], J Maisog[1], DG Allen[1], M Kurkjian[1], MB Schapiro[1], SI Rapoport[1], CL Grady[1] Lab. Neurosci., NIA[1] & Lab. Neuropsych., NIMH[2], NIH, Bethesda, MD 20892.

Regional brain activity associated with working memory for faces, defined as the retention of a face over brief, nondistracting intervals, was studied in 5 healthy, right-handed, young men. Changes in regional cerebral blood flow (rCBF) were measured with positron emission tomography (PET) using $H_2{}^{15}O$, while subjects performed face working memory and control tasks. Five working memory scans were obtained with different retention interval lengths (1, 6, 11, 16, or 21 sec). Control tasks were a sensorimotor control and a nonmemory perceptual face matching control. All working memory tasks and the perceptual matching task were two alternative, forced choice, match-to-sample tasks. Bilateral foci in inferior prefrontal cortex (Brodmann 45) showed increased rCBF during working memory as compared to control tasks. With lengthening retention intervals, right frontal activity increased to a maximum at 11 sec and decreased to nonsignificant changes at longer intervals. By contrast, left frontal activity continued to increase with longer intervals. Direct comparison of short and long intervals showed significantly greater right frontal rCBF at short as compared to long intervals and significantly greater left frontal rCBF at long as compared to short intervals ($p < 0.001$, one-tailed, uncorrected). Longer retention intervals were also associated with rCBF decreases in ventral occipitotemporal cortex (Brodmann 37), probably due to decreased perceptual activity, and in left thalamus, and with rCBF increases in left precuneus, left motor cortex, and left inferior temporal cortex. These results suggest, in concert with subject reports of difficulty maintaining a clear mental image over the longer intervals, that the neural representation of working memory for a face may shift from a visual image (right frontal) to a verbally or analytically mediated representation (left frontal) with increasing retention interval.

532.3

ACCESS TO STORED BIOGRAPHIC INFORMATION FROM FACES AND PROPER NAMES: A PET STUDY. J. Sergent*, B. MacDonald, and E. Zuck. Cognitive Neuroscience Lab., Dept. of Neurology & Neurosurgery, McGill Univ., Montreal, Canada.

The functional organization of the brain is designed to accommodate a variety of factors and constraints which determine the locations and relations of the many processing structures that underlie cognition. To examine some of the principles governing this organization, normal adults were subjected to a series of cognitive tasks, on either faces or proper names, that made the same processing requirements and differed only by the nature of the incoming information to be processed. Variations in cerebral blood flow were measured with positron emission tomography during the performance of these tasks, and the pattern of cortical activation associated with the cognitive operations specific to each task was calculated with the subtraction method. As expected, the results showed that different cerebral hemispheres were engaged in the processing of faces and of names. Less expected was the asymmetric organization of the network underlying similar cognitive functions, as accessing biographic information from faces engaged the ventro-medial cortex of the right hemisphere whereas the lateral cortex of the left hemisphere was engaged with names. In addition, there was no overlap of cerebral activation in the face and the name semantic tasks, suggesting that the same biographic information may be accessed and stored in distinct regions of the brain.

532.4

OPTICAL IMAGING OF HIGHER COGNITIVE ACTIVITY FROM HUMAN RIGHT AND LEFT TEMPORAL LOBES. M. M. Haglund*, G. A. Ojemann, and D. W. Hochman. Department of Neurological Surgery, University of Washington, Seattle, WA 98195.

Awake neurosurgical procedures that expose eloquent cortex provide a unique opportunity to study the cortical organization of human cognitive functions. Previously, these localization studies could only be accomplished by cortical stimulation mapping (Ojemann, J Neurosci 1991); however, more recently, through the use of optical imaging of the intrinsic signal, cortical maps of language and sensory cortex have been obtained (Haglund et al., Nature,1992).

In patients undergoing awake surgery for intractable epilepsy, a CCD camera was attached to the operating microscope and the exposed cortex illuminated with far red light (>690 nm). Optical images of the reflected light were obtained during a baseline task and then during the cognitive task of interest; and percentage difference maps calculated based on the difference between these images. A warping algorithm was used to compensate for small amounts of patient motion.

In nine patients, optical imaging was accomplished while the patient performed a series of tasks involving identification of facial expressions and matching of faces or complex figures. Cortical maps of optical changes in the right temporal lobe showed unique patterns of optical changes during face matching and facial expressions. In a separate series of twelve patients, optical changes occurred in the left temporal lobe at specific sites with object naming and the initial retrieval from memory. Optical imaging of the human cortex has the potential to provide new insights into the cortical organization of higher cognitive functions. [MMH supported by Klingenstein and American Association of Neurological Surgeons Research Foundation Fellowships; GAO supported by NIH NS21724,17111, and 20482]

532.5

ACTIVATION OF DORSOLATERAL PREFRONTAL CORTEX IN HUMANS DURING A WORKING MEMORY TASK USING FUNCTIONAL MRI.
J. D. Cohen, S. Forman, B. J. Casey, D. Servan-Schreiber, D. C. Noll & D.A. Lewis*. Clinical Cognitive Neuroscience Lab, The University of Pittsburgh and Carnegie Mellon University, Pittsburgh, PA 15213.

Six subjects were studied while alternating between two versions of the continuous performance test (CPT). In both tasks, single letter stimuli appeared one at a time in a visual display. In the control task, subjects responded to any occurrence of the letter "X". In the experimental task, subjects responded whenever a letter reappeared separated by a different one (e.g., A-F-A, but not A-A or A-Q-G-A). Presentation rate, target frequency, and frequency of repeated letters were identical in the two tasks. Both the control and experimental tasks required encoding and evaluation of each letter, and a response to targets by pressing a button. However, the experimental task also required that the two previous letters be kept in mind in order to identify targets. Each task was performed for 60s blocks, which were alternated 10 times. During each block, T2*-weighted coronal images were acquired from 6 contiguous locations in prefrontal cortex using a spiral scan sequence on a standard 1.5T GE scanner. No shimming procedures nor any specialized hardware was used. Sets were acquired every 6s, for a total of 100 scans per location per task. Activation images were generated by performing pixel-wise t-tests between images acquired during each task, separately for the first and second halves of the data (split-halves method). Signal intensity was also plotted against time, in order to verify that periods of activation corresponded appropriately to the tasks. 5 out of 6 subjects showed significant, reliable areas of activation along the middle frontal gyrus (area 46 in the Tailarach atlas). This was the most significant area of activation in all 5 subjects. Signal intensity varied consistently as a function of task in this area. The one subject who did not show activation was the subject who performed most poorly on the task. Activation also correlated significantly with performance within subjects. One subject was studied a second time using the same paradigm, and showed identical areas and amount of activation as in the first study.

532.6

ACTIVATION OF THE ANTERIOR CINGULATE DURING THE STROOP CONFLICT PARADIGM USING FUNCTIONAL MRI.
B.J. Casey*, J.D. Cohen, D.C. Noll, S. Forman, and J.L. Rapoport. Child Psychiatry Branch, NIMH, Bethesda, MD 20892.

The role of the anterior cingulate in the Stroop attentional conflict paradigm was examined using functional magnetic resonance imaging. Scanning was performed on a standard 1.5 T GE Signa magnet, using two 5" surface coils mounted on each side of the head. 7mm scans were obtained at six contiguous locations anterior to the anterior comissure. Each set of coronal images was acquired using a 10 interleave T2* sensitive spiral scan sequence with a TR of 600ms, TE of 35 ms, flip angle of 45 degrees and a field of view of 24mm. No shimming procedures or specialized hardware was used. Subjects were asked to name the color of 3 different stimulus types: color words (e.g., the word "red" printed in "green"), neutral words (e.g., the word "table" printed in yellow"), and a series of X's (XXXXX). Each condition was run for 60 seconds, during which 10 sets of scans were acquired. Each of the conditions was repeated 5 times in an alternating sequence. Pixel-wise t-tests (split-halves method) revealed reliable activation in the right anterior cingulate during both the color and neutral word conditions compared to the XXXXX condition. The comparison of the color and neutral word conditions revealed no differences in activation. The results are consistent with recent PET findings and provide further support for a proposed anterior attention system.

532.7

MAPPING THE AUDITORY SELECTIVE ATTENTION GENERATORS USING A COMBINED BRAIN EVOKED POTENTIALS AND PET ACTIVATION STUDY.
N. Tzourio*, F. El Massioui, L. Raynaud, B. Mazoyer, B. Renault, Groupe d'Imagerie Neurofonctionnelle, S.H.F.J., C.E.A., Orsay, 91406, C.H.U. Bichat, Université Paris 7, and L.E.N.A., C.N.R.S., La Salpétrière, Paris, France.

We measured relative regional cerebral blood flow (RCBF) using PET and [15O]-water in 6 young right-handed healthy volunteers, during 2 runs of 3 different conditions : 1- silent rest, 2 - passive dichotic listening of rare (20%) high (1790 Hz) and frequent low (750 Hz) tones randomly delivered to each ear, 3 - attending to and detecting right ear high tones. Simultaneous ERPs were recorded from five of these subjects during both condition 2 and 3 using a coronal montage of 5 electrodes. RCBF images were aligned with individual magnetic resonance images (MRI), and RCBF variations between pairs of measurements (N = 12) were computed in regions of interest with anatomical boundaries that were defined using a three dimensional reconstruction of each subject MRI data.

During the passive condition, we found a bilateral activation of the superior temporal gyrus (Right ST: 3.8 ± 2.9, mean ± SD in %, p = .0009, Left ST: 3.1 ± 2.5, p = .001). During selective attention, in addition to a bilateral activation of the superior temporal gyrus (RST: 3.6 ± 3.6, p = .005, LST: 3.9 ± 2.4, p = .0002), we observed activations of the right anterior cingulate (RAC: 7.8 ± 8.2, p = .007) and right supplementary motor area (RSMA: 6.5 ± 5.7, p = .002). RSMA activation was significantly correlated with the 50 to 150 msec processing negativity (PN) amplitude of the right side electrodes, while RAC activation was significantly correlated with the 150 to 250 msec PN amplitude of bilateral frontal and temporal electrodes (Spearman r < - 0.89, p = .01).
(Supported by a grant from C.N.R.S./Cogniseine)

532.8

COMBINED PET AND ERP STUDIES OF VISUAL SPATIAL SELECTIVE ATTENTION IN HUMANS.
G.R. Mangun[¥], H.J. Heinze[†], W. Burchert[†], H. Hinrichs[†], M. Scholz[†], T.F. Münte[†], A. Gös[†], H. Hundeshagen[†], M.S. Gazzaniga[¥]& S.A. Hillyard[*§], [¥]UC Davis, [†]Medizinische Hochschule Hannover, FRG, [§]UC San Diego

The brain mechanisms of visual selective attention were investigated in normal human subjects using a combined electrophysiologic (ERP) and positron emission tomographic (PET) approach. Stimuli consisted of bilateral arrays of symbols (two in the left and two in the right hemifields) that were briefly flashed on a video monitor: subjects continuously maintained fixation on a central point. In separate sessions, ERPs and PET measures of cerebral blood flow ($H_2{}^{15}O$) were obtained in two attention conditions (attend left and attend right): A passive viewing condition was also included. ERPs in the latency range of 90-140 msec post-stimulus were greater in amplitude over the lateral occipital scalp sites contralateral to the attended hemifield; topographic mapping of the voltage current density fields on the scalp indicated this ERP attention effect to be maximal over the lateral extrastriate cortex. In the PET session, significant increases were observed in cerebral blood flow (averaged images N=11) in lateral extrastriate cortex (area 19) contralateral to the attended hemifield for subtraction images (attend - passive, & att left - att right). No PET activation was obtained in striate cortex as a function of attention, however, activation was obtained in the anterior cingulate cortex. These data provide combined temporal (ERP) and functional-anatomical (PET) localization of modulations of sensory-cortical processing as a function of spatial selective attention. In humans, spatially focused visual attention alters the processing of incoming sensory stimuli by 90 msec post-stimulus in extrastriate area 19 as one mechanism for the early selection of inputs.

532.9

THE NEUROPHYSIOLOGICAL BASIS OF SELECTIVE ATTENTION BASED ON THE TEMPORAL STRUCTURE OF NEURAL SIGNALS
E. Niebur* & C. Koch, Computation and Neural Systems Program, Caltech 216-76, Pasadena, CA 91125.

We propose a model for the neuronal implementation of selective visual attention based on the temporal structure of neuronal activity. In particular, we set out to explain the electrophysiological data from areas V4 and IT in monkey cortex of Moran and Desimone (1985) using the "temporal tagging" hypothesis of Crick and Koch (1990). In our model, neurons in primary visual cortex respond to visual stimuli with a Poisson distributed spike train with an appropriate, stimulus-dependent mean firing rate. The firing rate is constant for neurons outside the "focus of attention" but it is temporally modulated for neurons whose receptive fields overlap with the focus of attention. This modulation is detected by inhibitory interneurons in V4 and is used to suppress the response of V4 cells associated with non-attended visual stimuli. We implement this tagging by either weakly modulating the Poisson spike rate using a 40 Hz modulatory signal or by influencing the temporal synchronization among V1 cells within the focus of attention. Both forms of tagging exhibit the experimentally observed suppression of unattended responses in V4 and the shrinking of receptive fields around the attended stimulus (Moran and Desimone, 1985). Pyramidal cells in V4 also exhibit the same form of temporal tagging (oscillations or synchronized activity) and can therefore transmit the "tagged" or "attended" signal to IT, the next stage of processing.

532.10

TEMPORAL CONSTRAINTS OF COGNITION: FURTHER EVIDENCE OF AUTOMATIC TEMPORAL INTEGRATION.
N. v. Steinbüchel, E. Szelag, M. Reiser and E. Pöppel* Inst. f. Med. Psychol., LMU, Munich and Forschungszentrum Jülich, F.R.G.

On the basis of neuropsychological and psychophysical evidence it can be concluded that sensory information is not processed continuously, but in a discrete fashion. On a high-frequency level atemporal system states with a duration of approx. 30 ms are conceived of as providing a logistical basis for "primordial" events. On a hierarchically distinct level, successive system states are automatically linked together. This temporal integration appears to be limited to intervals up to approx. 3 seconds. Experimental evidence for this kind of presemantic integration comes from a number of different paradigms. For instance in sensorimotor synchronization subjects can anticipate stimulus occurence up to approx. 3 seconds, but not beyond. Here we report experimental evidence on temporal constraints of information processing in brain-injured patients and control subjects using two different paradigms, i.e. temporal reproduction and spontaneous alternation rates of ambiguous figures. Either visual or auditory reversable stimuli were chosen; within both modalities a reversal could result in a percept of the same o a different semantic category (like the 2 perspectives of the Necker cube or vase vs. faces). On average the reversal rate for visual stimuli was higher than for auditory stimuli, and there was a tendency for the different semantic categories to be reversed faster.

532.11

PREFRONTAL CORTEX CONTRIBUTION TO THE CONTINGENT NEGATIVE VARIATION
S. K. Rosahl & R. T. Knight*; Dept. of Neurology and Center for Neuroscience, University of California at Davis, VAMC, 150 Muir Road, Martinez, CA 94553.

The Contingent Negative Variation (CNV) is generated during the interval between a warning cue and an imperative stimulus. It is comprised of at least two components including an early phase with frontal predominance and a late phase maximal over fronto-central sites. The potential has been associated with anticipation, attention and response preparation (McCallum, 1988; Rockstroh et al., 1989).

We recorded the CNV in a classical auditory S1-S2 paradigm with a three second interstimulus interval. Subjects pressed a button upon detection of an acoustically cued imperative tone (S2, 1000 Hz, Go). Responses were withheld if the warning tone (S1, 1500 Hz in Go trials) was lower in frequency (500 Hz, NoGo). Subjects were eight neurological patients with damage centered in Brodmann areas 9, 44, 45 and 46 of the dorsolateral prefrontal cortex (PFCx) and age matched controls. All lesions were unilateral and due to infarction in the precentral branch of the middle cerebral artery. The average lesion volume was 51.8 cm^3.

Patients responded more slowly (672 msec vs. 491 msec; F=7.77; p<0.009). Two averaging windows were set for statistical analysis of the early (500-700 msec post-S1) and late (200-0 msec pre-S2) CNV component. PFCx lesions resulted in focal reduction of the late phase of the CNV in electrodes located over areas of cortical damage (F7=3.4 μV, F8=-10.3 μV; t=3.6; p<0.003; F3=-7.1 μV; F4=-15.2 μV; t=3.57 p<0.003). Conversely, the early CNV was not reduced, but rather enhanced in amplitude over lesioned cortex (F7=-10.7 μV, F8=-6.1, t=2.3, p<0.04).

Persistence of the early post-S1 negativity over areas of extensive cortical damage supports involvement of subcortical structures in this potential (Skinner & Yingling, 1977; Steriade, 1981). The current results indicate that functionally intact cortex is crucial for generation of the late component of the CNV.

Supported by NINDS NS21135

532.12

Shifting attention: Comparison of autistic and cerebellar patients.
E. Courchesne, N.A. Akshoomoff*, J.P. Townsend, and O. Saitoh, Neuropsychology Research Laboratory, Children's Hospital Research Center, 8001 Frost Street, San Diego, CA 92123

MR and autopsy studies on autistic patients find evidence of early maldevelopment of the cerebellum These findings raise the question of how cerebellar maldevelopment can contribute to the cognitive and social communication deficits seen in infantile autism.

Shifting of attentional focus is an essential operation in joint social attention, a milestone in the normal infant's development of social communication skills, and in the exploration and apprehension of the non-social environment. We had autistic patients perform tasks that required them to selectively, accurately and rapidly shift their focus of attention back and forth between visual and auditory stimuli. We previously reported that patients with acquired cerebellar lesions were impaired in this task. As compared to normal controls, autistic and cerebellar patients were similarly impaired at coordinating accurate and rapid mental shifts of attention, but given sufficient time, were able to correctly execute shifts. For more than 3 seconds following a command to shift attention from the current sensory modality, the autistic patients, like cerebellar patients, failed to fully inhibit attention to stimuli in that modality, and failed to detect a large percentage of stimulus information in the new modality of attention.

These findings are consistent with our proposal that the human cerebellum is involved in the voluntary coordination of selective, accurate, and rapid shifts of attention in a fashion analogous to its long-established analogous role in the coordination of movement. We suggest that cerebellar maldevelopment in autism may underlie the inability to control voluntary shifts of attention, undermining the normal operation of joint social attention, with the downstream consequence of impairing social and cognitive development.

GENESIS OF NEURONS AND GLIA III

533.1

GENERATION OF OLFACTORY RECEPTOR NEURONS IN VITRO: CHARACTERIZATION OF NEURONAL PROGENITOR CELLS AND THEIR MITOGENS.
A.L. Calof*[1], M. DeHamer[1], M. Adusumalli[1], J. Guevara[1], A. Lopez[2]., and B. Olwin[2]. [1]Dept. of Biological Sciences, U. of Iowa, Iowa City, IA 52242 and [2]Dept. of Biochemistry, U. of Wisconsin, Madison, WI 53706.

The ability to generate new neurons is a unique property of the olfactory epithelium (OE) of adult mammals, and neurogenesis is robust in OE cultures as well. In explant cultures of OE purified from mouse embryos, the majority of ^{3}H-TdR-incorporating, migratory cells divide once to generate 2 daughter cells in serum-free, defined culture medium; the daughter cells are immature olfactory receptor neurons, which express the neuronal cell adhesion molecule, NCAM, approximately 12 hrs after their precursors' terminal S phase. These precursor cells have been termed INPs, for Immediate Neuronal Precursors of olfactory receptor neurons. To understand further the cellular stages in this neurogenic pathway, we have characterized mitotically active cells in OE cultures and identified factors that affect their proliferation. Mitogenic effects on INPs are assessed by determining the number of migratory cells incorporating ^{3}H-TdR from 24-48 hrs in culture, a period when neurogenesis has virtually ceased in the absence of growth factor stimulation. FGF1 and 2 (acidic and basic FGF, respectively) cause large increases (>2 fold) in this number, whereas PDGFaa, NGF, BDNF, and NT3 appear not to have a mitogenic effect. Pulse-chase experiments show that 85-90% of migratory cells incorporating ^{3}H-TdR in FGF2-treated cultures also acquire NCAM immunoreactivity within 24 hrs, indicating that this population is composed primarily of INPs. Analysis of RNA prepared from OE indicates that tyrosine kinase FGF receptors FR1, 2, and 3 are expressed, providing a likely basis for observed effects of FGFs. Interestingly, a small subpopulation (3-4%) of migratory cells in OE cultures reacts with an antibody to the transcription factor MASH1, but these form only a fraction of mitotically-active cells. These MASH1-expressing cells may be a distinct subpopulation of OE progenitor cells, at a different stage of differentiation than the INPs. *Supported by a seed grant from the Diabetes & Endocrinology Research Center of the University of Iowa.*

533.2

CHARACTERIZATION OF CULTURED NOESs (NEWBORN OLFACTORY EPITHELIAL SPHERES): AN IN VITRO SYSTEM OF MAMMALIAN NEUROGENESIS.
S.K. Pixley*, M. Bage, V.M. Lee, D. Miller and M.L. Miller, Depts. of Anatomy and Cell Biology*, Environmental Health, Univ. of Cincinnati, Cincinnati, OH 45267-0521.

To obtain neurogenic olfactory cell cultures (Pixley, 1992 Neuron 8:1191), newborn Spraque Dawley rat nasal and olfactory cells were plated onto neocortical rat glial cells. Neurogenesis occurred predominantly in cellular aggregates that began to coalesce at 4 days and formed compact Newborn Olfactory Epithelial Spheres (NOESs) by 10-15 days after plating. These contained immature olfactory neurons (neuron specific tubulin-positive, olfactory marker protein-negative (OMP-)) and mature, OMP+, neurons. Here we show NOESs also contained dividing cells and four non-neuronal olfactory cell types. To examine internal organization, immunostained cultured cells were encapsulated in gelatin, plastic-embedded and sectioned (2 μm). Most NOESs contained central cavities with multicellular walls. Neurons were primarily in outer 1-2 layers and sent dendritic processes centrally (confirmed by confocal microscopy). Cavity-lining cells were sustentacular (supporting), basal and Bowman's gland cells. Dividing cells were between neurons and non-neurons. NOESs also contained networks of glial cells and processes. Thus, olfactory cells in vitro re-created epithelial relationships similar, but not identical, to those in vivo. Double immunostaining showed that while almost all NOESs contained dividing cells and immature neurons, only the approximately 50% with OMP+ neurons contained the olfactory non-neurons. This suggests that olfactory non-neurons support neuronal maturation, but play little or no role in neurogenesis. [Support: NIH DC00347 to S.K.P.]

533.3

NEURONAL STEM CELLS IN THE VOMERONASAL EPITHELIUM OF GARTER SNAKES ARE IDENTIFIED BY AN ANTIBODY FOR THE PSTAIRE REGION OF CELL DIVISION CYCLE KINASES.
D.A. Holtzman* & C.L. Clarke, Neuroscience & Biopsychology Program, Oberlin College, Oberlin, OH 44074.

As in the olfactory epithelium of all vertebrates examined, the hypertrophied vomeronasal epithelium (VNE) of adult snakes is capable of generating new neurons as a result of normal turnover. Previous studies have shown that neuronal stem cells are located at the base of the VNE, incorporate 3H-thymidine (3H-T), and migrate apically through tall columns of bipolar, receptor cells as the cells age. An antibody was used which recognizes an evolutionarily conserved peptide sequence (PSTAIRE) within a family of cell division cycle homologous kinases (CDCHs). CDCHs have been shown previously to participate in the regulation of progression through the cell cycle in all cell types examined. In the present study, neonatal snakes were examined for anti-PSTAIRE immunoreactivity (IR) in relation to 3H-T labeling. After survival times of 1 h, 1 day, 1 week, 1 mo, and 2 mo post-3H-T injection, migration of 3H-T-labeled cells is observed from the base of the VNE (1h and 1 day) into the receptor cell columns (1 week-2 mo). After 2 mo post-3H-T injection, labeled cells are seen in the base of the VNE, which have not been seen previously in adults. Anti-PSTAIRE IR is only seen at the base of the VNE for all animals. Incubation of antiserum pre-absorbed with $p34^{cdc2}$ results in an absence of IR. In sections double-labeled for 3H-T and anti-PSTAIRE, all double-labeled cells are found at the base of the VNE, and the number of double-labeled cells/mm^2 decreases with increasing survival time. 15% of all 3H-T-labeled cells are not anti-PSTAIRE IR at 1 h post-3H-T injection, suggesting that only a subset of the total stem cell population is anti-PSTAIRE IR. The number of anti-PSTAIRE IR cells/mm^2 is significantly less in 5-6 mo old snakes (2 mo survival group) than in 2-4 mo old snakes (1h-1mo survival groups, p<0.05). We are currently examining both the number of anti-PSTAIRE IR cells in the adult snake VNE and the ratio of 3H-T-labeled cells to anti-PSTAIRE IR cells.

533.4

MAMMALIAN HOMOLOGS OF *DROSOPHILA* PRONEURAL AND NEUROGENIC GENES ARE EXPRESSED DURING RAT RETINAL DEVELOPMENT.
I. Ahmad* and C.J. Barnstable, Dept. of Ophthalmology & Visual Science and Section of Neurobiology, Yale University School of Medicine, New Haven, CT.

An approach to understand the mechanism of the mammalian neuronal differentiation is to identify and study the expression of the mammalian homologs of *Drosophila* genes that are involved in neurogenesis. We have begun to identify and analyze the retinal expression of mammalian homologs of *Drosophila* proneural genes of *achaete-scute* complex and neurogenic gene, *Notch*. We have cloned a rat homolog of *achaete-scute* gene, bHLH*eye*1 which is expressed in the developing eye. bHLH*eye*1 gene is expressed in early embryo (E12) and postnatal stages. The presence of a functional bHLH transcription factor in retina was suggested by gel shift assays using PN1-retinal nuclear extract and an E-box element from the muscle creatine kinase (MCK) gene. The assay detected the formation of a complex which could be competitively inhibited by an excess of unlabeled E-box sequence, suggesting a role for bHLH transcription factor(s) in the regulation of retinal genes that may contain E-box element in their promoter. The homolog of *Drosophila Notch* which plays an important role in the determination of binary cell fate is expressed during retinal development. We have cloned *Notch* cDNA from rat E14 retina. The expression of *Notch*, which is observed as early as E12 in the developing eye vesicle is not detected in the postnatal retina. Preliminary immunocytochemical analysis using antibody raised against human *Notch* labels cells in the ventricular zone in E15 retina suggesting a role for *Notch* during the differentiation process in retina. (Supported by NIH)

533.5

PROLIFERATION ZONES IN THE BRAIN OF ADULT GYMNOTIFORM FISH: A QUANTITATIVE MAPPING STUDY. G.K.H. Zupanc* and J. Horschke. Department of Physical Biology, Max Planck Institute for Developmental Biology, 72011 Tübingen, Federal Republic of Germany.

In contrast to mammals, in fish the capability for the production of new neurons and glial cells in the brain during adulthood is very pronounced. In the present study, mitotic activity in the brain of *Apteronotus leptorhynchus*, a gymnotiform fish, was measured through the incorporation of 5-bromo-2'-deoxyuridine (BrdU) into DNA. Proliferation zones were visualized by employing an anti-BrdU antibody to frozen sections. In 5 fish analyzed quantitatively, the total number of cells generated in the brain within 2 hours varied between 66,000 and 128,000. For the following characterization of mitotic activity, the percentage of labelling found in the different cell groups was calculated relative to the total number of BrdU-labelled cells in the brain and averaged over the 5 individuals.

In the *telencephalon* and *diencephalon*, cell proliferation was low and totalled only 5 % in each of these two portions of the brain. Seven percent of all cells generated in the brain were localized in the *mesencephalon*. Proliferation was highest in the dorsal subdivision of the torus semicircularis (3 %) and in the optic tectum (2 %). In the *rhombencephalon*, the percentage of newborn cells totalled 14 %. The highest proliferative activity was found in the electrosensory lateral line lobe (4 %) and in the reticular formation (4 %). By far the greatest number of newborn cells, namely 70 %, were found in the different parts of the *cerebellum*. Cell proliferation was most pronounced in the molecular layers of the corpus cerebelli/eminentia granularis pars posterior (19 %) and of the valvula cerebelli (11 %) as well as in the eminentia granularis pars medialis (28 %). In contrast to the high proliferative activity in the molecular layers of the corpus cerebelli/eminentia granularis pars posterior and of the valvula cerebelli, the number of newborn cells was low in the respective granule cell layers (4 % and 2 %).

533.6

DEVELOPMENT OF NADPH DIAPHORASE IN THE CNS OF RAINBOW CICHLID FISH. Anne C. Rusoff*. Dept. of Biology, Montana State University, Bozeman, MT 59717.

Although Hope and Vincent (1989) did not find NADPH-diaphorase activity in the brain of any of the species of fish that they examined, this activity is plentiful in the brain of the rainbow cichlid, a perciform fish. Many clusters of cells and processes that are NADPH diaphorase + are present and clearly differentiated from neighboring non-positive cells and neuropil. The diaphorase activity first appears very early in development; 24 hours post-fertilization, when the eye is just visible, a cluster of diaphorase + cells is present on either side of the midline in the diencephalon. Additional clusters of diaphorase + cells gradually appear in a rostral to caudal gradient. The next cells appear in the hindbrain just rostral to the otic vesicle; before the embryos hatch, 3 clusters of diaphorase + cells are present in the hindbrain in the region of the otic vesicle and individual cells are seen in the rostral spinal cord. Gradually individual cells appear more caudally in the spinal cord--both near its dorsal and ventral margin. In the 3 days between hatching and swimming, many more diaphorase + cells are found in each of these areas, as well as in other regions, including other parts of the brainstem, the telencephalon, and the retina where some amacrine cells also become positive for NADPH diaphorase. (Sponsored by MONTS.)

533.7

BIRTHDATE AND IDENTIFICATION OF SEROTONERGIC CELLS IN EMBRYONIC AND LARVAL *APLYSIA*. R. Marois*, P. Hofstadter, & T.J. Carew. Interdepart. Neurosci. Progr., Depts. of Biol. and Psychol., Yale Univ., New Haven, CT 06520.

We previously described the emergence of five serotonergic (5HT) cells - an unpaired median (UM) and 2 bilateral pairs of cells - during the embryonic and larval development of *Aplysia* (Marois & Carew, 1990). Since 5HT is implicated in synaptic plasticity and growth cone motility, we wished to determine the functions of these cells in neuronal development. Here we focus on their birthdate, identity, fate, and central connections.

Based on whole-mount immunocytochemistry (ICC), we previously suggested that the 5HT cells were part of the cerebral ganglia. However, our ultrastructural studies now indicate that these cells are located in a hitherto unrecognized structure in *Aplysia* (but previously identified in other marine invertebrates: Bonar, 1978), the cephalic sensory organ. This structure is closely associated with the dorsal surface of the cerebral ganglia and its commissure. By hatching, this organ contains about 20 cells of various types, with the 5HT cells interspersed between 4 cells bearing sensory cilia that protrude to the animal's surface.

To trace the birth and fate of the five 5HT cells, we have exposed *Aplysia* to BrdU for 24 hr on each of the 11 days of embryonic development. Double-labeling ICC for both BrdU and 5HT showed that the UM cell is born on day 3 of embryogenesis, while the bilateral cells are generated between day 4 and 6. Although 5HT ICC suggests that the UM cell is resorbed at metamorphosis, we are currently assessing the fate of the other 5HT cells by looking for double-labeled cells in juvenile *Aplysia*.

We recently described the widespread projections of the 5HT cells at hatching (Marois et al., 1992). We now show that the 5HT profiles in the central ganglia are located both in the neuropil and on the surface of ganglion cells. This observation now enables us to examine the nature and function of these profiles during the development of the CNS of *Aplysia*.

533.8

GROWTH OF NEURONAL POPULATIONS DURING POSTEMBRYONIC DEVELOPMENT IN *HELISOMA TRIVOLVIS*. J. I. Goldberg* and K. J. Cavers. Dept. of Zoology, Univ. of Alberta, Edmonton, Alberta, Canada T6G 2E9.

In the CNS of various gastropod molluscs, the number of serotonin-immunoreactive neurons increases during specific periods of postembryonic development. These increases may result from the generation and differentiation of new neurons, the differentiation of extant precursor cells or the switching of neurotransmitter phenotypes. As a first step in distinguishing between these developmental pathways, we carried out neuronal cell counts on *Helisoma* ganglia at two stages of postembryonic development. Neurons were selectively stained with methylene blue and counted in wholemount preparations of the buccal, left parietal and visceral ganglia. In each of these ganglia, there was a significant increase in the neuronal population between postembryonic stages P3 and P10. Furthermore, the increase in neuron number was well beyond the corresponding increase in serotonin-immunoreactive neurons for each ganglion. Ganglia were treated with 5-bromodeoxyuridine (BrDU) and then processed for anti-BrDU immunofluorescence to test for intraganglionic cell divisions. Pairs of stained cells were observed in some ganglia. Moreover, the size of the stained cells was often larger than that observed for non-neuronal cells, indicating that at least some of the cell divisions generate neurons. These data suggest that neuron addition, possibly through intraganglionic cell division and differentiation, is a normal characteristic of postembryonic development in gastropod molluscs.

This research was supported by NSERC of Canada.

533.9

EXPRESSION OF NEURON-SPECIFIC ANTIGENS DURING EMBRYONIC DEVELOPMENT OF THE LOBSTER. V. Garzino*, S. Therianos and H. Reichert. Department of Zoology, University of Basel, CH-4051 Basel, Switzerland.

To investigate the developmental processes that generate the crustacean nervous system, we have produced several monoclonal antibodies that recognize specific neural epitopes in the developing embryonic nervous system of the lobster, *Homarus gammarus*. One of these antibodies recognizes an antigen expressed by all parts of the central and peripheral nervous system including the stomatogastric nervous system. This antigen, a 60 kD glycoprotein, is membrane associated and is localized either in the extracellular matrix or on glia that surrounds the cell bodies and processes of all neurons. The two other antibodies label different subpopulations of neurons as well as their neurites. The biochemical characterization of the antigens recognized by these two antibodies is in progress. The fact that all of these antigens are expressed with high specificity on developing neuronal cells suggests that they might play an important role in the formation of the lobster nervous system. Moreover, their specific expression during the embryonic development of the lobster nervous system makes them excellent cellular markers for investigations of neurogenesis and axogenesis in the crustaceans. (Supported by the Human Frontiers Science Program).

533.10

PERIPHERAL INDUCTION OF SEGMENT SPECIFIC NEURONS IN THE LEECH MEDIATED BY IDENTIFIABLE CENTRAL NEURONS. T. Becker*, A.J. Berliner and E.R. Macagno Dept. of Biological Sciences, Columbia University, New York, NY 10027 USA

Midbody ganglia (MG) 5 and 6 of the leech *Hirudo medicinalis* contain several hundred small neurons not found in other ganglia, the peripherally induced central (PIC) neurons. PIC neuron birth is induced by the male sexual organ during a critical period from embryonic day (E) 13 to 16 of leech embryogenesis. The mitogenic signal is conveyed by the so-called sex nerves that connect MG5 and MG6 to the male sexual organ. To test whether the signal is conveyed by either afferent fibers or efferent fibers we have undertaken a cellular analysis of the sex nerves in segments 5 and 6.

By staining with neuron-specific monoclonal antibodies we have determined that peripheral neurons in the male sex organ develop at about E15, but their axons do not reach the CNS before E20 and hence are unlikely to be involved in the induction.

By injection of the dyes DiI and DiO into the primordia of the male sex organ, we have mapped central neurons that are connected to this organ during the critical period. Both MG5 and MG6 contain a complement of 10 pairs of neurons, and MG6 an additional pair, that innervate the male genitalia. Single and pairwise ablations have shown that at least two of these neurons are involved in the induction in that their ablation interferes with subsequent PIC neuron birth in MG5 and MG6. Hence, a retrograde interaction of central neurons with the periphery leads to segment-specific neuron birth.

533.11

BRAIN NEUROBLASTS IN THE GRASSHOPPER EMBRYO: CELLULAR AND MOLECULAR CHARACTERIZATION.
H. Reichert*, L. Williams, D. Zacharias and T. Meier. Department of Zoology, University of Basel, CH-4051 Basel, Switzerland.

The brain neuroblasts in the embryonic grasshopper were studied by BUdR incorporation and immunocytochemistry and were mapped by reconstruction of serial sections. An array of large dividing neuroblasts is observed by the 25% stage. Each of these neuroblasts divides asymmetrically to produce a chain of ganglion mother cells, and each ganglion mother cell divides symmetrically to produce a pair of neurons. A set of 130 mitotically active, large neuroblasts are found in each brain hemisphere at the 30-45% stages. Through morphogenetic movements that occur between the 30-35% stages these neuroblasts become located in positions which are characteristic of the major regions of the mature brain. Many of the brain neuroblasts can be identified as individuals based on their stereotyped position in the neurogenic array. Immunocytochemical experiments with antibodies against, *engrailed*, fasciclin I and TERM-1 show that brain neuroblasts can also be characterized by their expression of cell-specific molecular labels. These studies indicate that many features of the insect brain derive from a surprisingly simple and stereotyped set of neuronal precursor cells. Thus, concepts and methods that have been used to study neurogenesis in the simpler segmental ganglia will also be applicable to the insect brain. (Supported by SNSF).

533.12

MUTATIONS IN *TROL* AFFECT NEUROBLAST DIVISION IN THE LARVAL CNS OF *DROSOPHILA MELANOGASTER*.
S. Datta*. Dept. of Biochemistry and Biophysics and Center for Advanced Invertebrate Molecular Sciences, Texas A & M University, College Station, Texas 77843-2475

We are interested in the molecular mechanisms regulating neuroblast proliferation and differentiation in the CNS. We have previously shown that a hypomorphic mutation at the *trol* locus causes a 90% drop in the dividing cell population of the larval CNS (1). To investigate this further, we have used bromodeoxyuridine incorporation studies to determine if *trol* is required for the proliferation of specific neuronal lineages within the larval CNS or for the differentiation of a specific stage of development. Bromodeoxyuridine was applied topically or fed to staged mutant and control larvae. Incorporation patterns were visualized with primary anti-bromodeoxyuridine antibodies and secondary antibodies conjugated with horse radish peroxidase. Studies of the hypomorphic allele $trol^{sd}$ suggest that low levels of *trol* expression are sufficient for the proliferation of some lineages in the larval lobes, but not for most lineages in the thoracic portion of the ventral ganglion. Studies with a null allele of *trol*, $trol^{s4m}$, are underway.

1. Datta, S. and Kankel, D. R. (1992). Genetics 130, 523-537.

533.13

***pollux* , A Novel Membrane Protein , Is Concentrated In A Subset Of Axon Fascicles Within The Drosophila CNS.**
S. D. Zhang [1] , J . A . Kassis [2], B. A. Olde [1] ,D . M. Mellerick [3] , A. J . Nazarali * [3] and W. F .Odenwald [1] 1) Lab. of Neurochemistry, NINDS, NIH, Bethesda, MD 20892 , 2) Ctr. for Biologics Evaluation and Research , FDA , Bethesda , MD20892 , 3) Lab. of Biochemical Genetics , NHLBI , NIH , Bethesda MD 20892

Enhancer-detection screening in flies has led to the discovery of a gene , *pollux* , which encodes a 77KD protein that contains an N-terminally-located RGD cell attachment sequence and a centrally-located transmembrane domain . The putative transmembrane protein also contains a leucine zipper motif and shares 43% idenity (66% similarity) over 74 aa with the human oncogene tre-1 . The siginificance of this high homology is currently unknown . Immunolocalization studies carried out with polyclonal antibodies raised against a full-length recombinant protein indicate that within the CNS , *pollux* is selectively concentrated in the axon fascicles which reside in the longitudinal connectives and the supraoesophageal commissure . Little or no protein is detected in axon fascicles which makeup other commissures . *pollux* message and protein are also detected in the trachea . Over expression in cultured Drosophila S2 cells does not induce cell-cell aggregation , indicating that *pollux* is not a homophilic adhesion molecule . We are currently testing the hypothesis that *pollux* may play a role in cell adhesion via its RGD motif .

CELL LINEAGE AND DETERMINATION: VISUAL SYSTEM

534.1

RETINOIC ACID INFLUENCES THE DIFFERENTIATION OF PHOTORECEPTOR CELLS IN EMBRYONIC RAT RETINA *IN VITRO*.
M.W. Kelley* and T. A. Reh. Department of Biological Structure, University of Washington, Seattle, WA 98195.

The differentiation of specific cell types in the developing mammalian retina is influenced by environmental cues. However, the specific biochemical factors that determine individual cell types have not been identified. Retinoic acid has been demonstrated to effect determination of individual cell types during vertebrate development and is present in the embryonic retina.

The effects of retinoic acid on the differentiation of specific retinal cell types were examined by establishing embryonic retinal cells from different time points during development as high density dissociated cell cultures. Different concentrations of all-trans retinoic acid or of the retinoic acid vehicle (DMSO) were added to the culture media. After 6 or 8 days *in vitro*, differentiation of specific cell types was determined using immunocytochemistry. Based on immunolabelling with two different photoreceptor markers (Rho 4D2 and Recoverin), the application of retinoic acid caused a significant and dose dependent increase in the number of photoreceptor cells in each culture. This effect was observed in cultures established on E15, E18 and P4. The overall cell number did not change with retinoic acid treatment, and there was no increase in the number of amacrine cells or cone bipolar neurons.

In order to determine whether the effect of retinoic acid was to increase the rate of differentiation of already committed cells or to influence the commitment of uncommitted progenitor cells, whole E18 retinas were incubated in BrdU prior to establishment of dissociated cell cultures. Preliminary results indicate a significant increase in the number of double labelled rod cells in retinoic acid treated cultures.

The results of these experiments suggest that retinoic acid may play a controlling role in the determination of retinal progenitor cells as photoreceptor cells.

Supported by funds from NINDS and NEI.

534.2

REGENERATION OF PHOTORECEPTORS IN GOLDFISH RETINA FOLLOWING LASER LESIONS.
J.E. Braisted* & P.A. Raymond. Dept. Anat. & Cell Bio., Univ. Mich., Ann Arbor, 48109.

Adult goldfish were lentectomized and 2-4 lesions were placed in ventral retina with an argon laser. Cones were selectively killed in a ~220μm diameter patch after 130mw lesions, and both rods and cones in a ~300μm patch after 160mw lesions. To monitor cell proliferation, eyes were injected with bromodeoxyuridine (BUdR) at 3d and examined at 4d. Radial cryosections were double-labeled with anti-BUdR and RET1, specific for a nuclear antigen in some retinal cells in goldfish, or FGP2, specific for goldfish GFAP (from M. Schwartz). Within the lesions, most BUdR+ nuclei were in the photoreceptor (PR) layer. Some, which labeled with RET1 and FGP2, were Müller glia. To monitor regeneration, retinas were examined at 3-5 wks after multiple injections of BUdR. Regenerated cones (RET1+/BUdR+) were found in all lesions, and regenerated rods (RET1-/BUdR+) in 160mw lesions. To determine whether dopaminergic (DA) neurons regenerated if they were selectively killed in addition to PRs, eyes were injected with 6-hydroxydopamine, then lesioned (160mw). At 7-9 wks after muliple injections of BUdR, retinal wholemounts were triple-labeled with anti-tyrosine hydroxylase (for DA neurons), RET1, and anti-BUdR. Although PRs regenerated within the lesions, DA neurons did not. Together with our previous data, this suggests that PRs can regenerate if they are selectively lost, but regeneration of DA neurons requires substantial loss of both PRs and inner retinal neurons.

Supported by EY04318 and MH10220.

534.3

REGENERATION OF NEURAL RETINA FROM RETINAL PIGMENTED EPITHELIUM (RPE) IN ADULT GOLDFISH. J.K. Knight* and P.A. Raymond, Neuroscience Program and Dept. Anat. & Cell Biology, University of Michigan, Ann Arbor, MI 48109.

In some species, under conditions such as addition of growth factors, differentiated RPE can transdifferentiate into neural retina. Here we present evidence that the RPE in adult goldfish can transdifferentiate into retina without exogenous growth factors.

The neural retinas of goldfish were removed by vacuum suction. To examine the time course of regeneration, bromodeoxyuridine (BrdU) was injected intraocularly at 4d to 18d, and retinas were examined 1d to 10d later. By 5d after retinal removal, RPE cells had begun to lose melanin granules and to incorporate BrdU. By 11d, a neuroepithelium had formed, which differentiated into a laminated but disorganized retina by 30 d. Regenerated retinas had three distinguising characteristics: 1) They were labelled across their full extent by an anti-goldfish-vimentin antibody (from M. Schwartz) which in the intact eye labelled only the proliferative germinal zone of the retina and the RPE. 2) Some cones, inner nuclear layer neurons and ganglion cells in regenerated retinas were double-labelled with BrdU and a retina-specific antibody, RET1. 3) In all cases, a monolayer of RPE was present, and retinas were oriented correctly, with ganglion cells on the vitreal side.

Work by others has implicated basic fibroblast growth factor (FGF) in RPE transdifferentiation. We are currently using both antibodies and *in situ* hybridization to assay for endogenous changes in FGF and FGF receptor expression. Supported by NIH EY04318.

534.4

ENVIRONMENTAL FACTORS THAT INFLUENCE DETERMINATION OF THE GANGLION CELL PHENOTYPE IN DEVELOPING RETINA. **D.K. Waid*, S.C. McLoon, Dept. of Cell Biol. and Neuroanatomy, Univ. of Minnesota, Minneapolis, MN 55455.**

The mature retina consists of seven major cell types. Previous studies showed that all cell types can arise from common precursor cells. This suggests that cell fate is not determined solely by lineage dependent mechanisms. Moreover, other studies suggest that local environmental factors are important in the determination of cell fate. Ganglion cells are the first cell type to develop in the retina, and few ganglion cells are born late in development. It could be that the environment of the older retina is not conducive to ganglion cell determination. To test this, retinal cells from young (E4) chick embryos, an age prior to the genesis of most ganglion cells, were cultured in the presence of retinal cells from E4 or older (E9) embryos, an age by which most ganglion cells have been generated. The two cell populations were separated by a membrane that prevented direct cell-cell contact between the two populations but that allowed the passage of soluble molecules. After one or two days in culture, the cells were fixed, and immunohistochemistry with retinal ganglion cell specific antibodies was used to identify and quantify the number of ganglion cells in the test population. E4 retinal cells cultured in the presence of other E4 cells produced approximately twice as many ganglion cells as E4 cells cultured in the presence of E9 cells. This result suggests that the cells in the older developing retina produce some factor that actively inhibits development of the ganglion cell phenotype and/or promotes development of other cell types. Furthermore, it suggests that the factor responsible for this activity is diffusable. (Supported by EY07133 and EY09537.)

534.5

DIVERSITY OF IDENTIFIABLE RETINAL NEURONS DIFFERENTIATING IN MONOLAYER CULTURES. H.-D. Hofmann* and V. Möckel, Institute of Anatomy, University of Freiburg, D-7800 Freiburg, Germany

On the basis of morphological, immunocytochemical and autoradiographic citeria we have characterized the phenotypes expressed by immature retinal neurons differentiating in monolayer cultures prepared from early postnatal rabbits. Survival of Thy 1-positive ganglion cells (1-2% of all cells) depended on the presence of neurotrophic factors. A morphologically heterogenous cell population which showed GABA-IR and high affinity uptake of 3H-GABA was classified as GABAergic amacrine cells (6.5%). Different subpopulations could be distinguished according to their responsiveness to the glutamate receptor agonists NMDA, kainate and quisqualate as demonstrated autoradiographically. As in situ presumed dopaminergic neurons in vitro were low in number, had large dendritic fields and showed colocalization of tyrosine hydroxylase-IR and GABAergic markers. Presumptive glycinergic amacrine cells (18.5%) were rather uniform in size and released their transmitter in response to kainate and quisqualate, but not to NMDA. Cultured bipolar cells (4%) consisting of glutamatergic and glycinergic subpopulations were identifiable by specific monoclonal antibodies and by their characteristic bipolar cell-like morphology. Horizontal cells (approximately 1%) of both the A- and B-type were highly differentiated with respect to morphology, transmitter phenotype and expression of characteristic antigens (neurofilaments, calbindin-28kD). Incompletely differentiated photoreceptors (73%) took up 3H-glutamate and were recognized by antibodies to opsin.

These results demonstrate that in the artificial and largely homogenous environment of monolayer cultures a variety of retinal cell types are capable of expressing many of their specific phenotypic properties, apparently under the control of intrinsic programs.

Supported by Deutsche Forschungsgemeinschaft / SFB 325.

534.6

DEVELOPMENT OF NEUROPEPTIDE Y AND TYROSINE HYDROXYLASE AMACRINE CELLS IN TADPOLE RETINA: CELL LINEAGE AND SPATIAL DOMAINS. **S. Huang* and S. A. Moody, Dept. Anatomy and Neuroscience Program, The George Washington University, Washington, D.C. 20037**

The blastomere origin and the regional location of two amacrine neurotransmitter phenotypes in the tadpole retina were investigated to understand how neurotransmitter choices are made during development. Amacrine cells first become immunoreactive to Neuropeptide Y (NPY) and tyrosine hydroxylase (TH) shortly after the cells are generated (stage 39-40). By labeling retinal progenitors with a fluorescent dye, we show that the ancestral origin of these two neurotransmitter phenotypes is similar; both NPY and TH amacrine cells are proportionally derived from the same group of animal pole blastomeres that produces other retinal cell types (Huang and Moody, J. Neurosci., 1993). By mapping the location of these two amacrine neurotransmitter phenotypes, we demonstrate that the earliest detectable TH and NPY amacrine cells are scattered throughout the developing retina. There is no boundary between the two neurotransmitter phenotypes, but more TH amacrine cells are in the periphery, whereas more NPY amacrine cells are in the center. At later stages, when the TH and NPY cell numbers increase, the amacrine cells with same neurotransmitter phenotype tend to segregate as small cell clusters. The early scattered NPY and TH cells are joined by others with the same neurotransmitter phenotype. These data demonstrate that the neurotransmitter phenotype of amacrine cells is not determined by their ancestral origin but is under the influence of local cell interaction. Supported by NIH-EY10096.

534.7

EGF AND TGFα CONTROL CELL FATE DETERMINATION IN DEVELOPING RAT RETINA R.M. Anchan* and T. A. Reh, Department of Biological Structure, University of Washington, Seattle, WA. 98195.

The mechanisms that control the choice of cell phenotype during retinal development are not well characterized. We previously reported that EGF and TGFα are mitogens for retinal progenitor cells (Anchan et al, 1991, Neuron). We now report that EGF and TGFα also selectively control the differentiation of particular retinal cell types. Using Bromo-deoxyUridine (BrdU) pulse labelling studies of embryonic day 13 , 15, 17 and postnatal day 3 rat retinas grown for up to 17 days *in vitro* , we were able to identify dividing progenitor cells in our cultures. By coupling BrdU incorporation with the expression of specific neuronal antigens as determined by immunostaining, we followed the fate of these cells. Immunolabelling for rod photoreceptors revealed that the number of rods generated *in vitro* is concomitantly reduced in EGF treated embryonic cultures as evidenced by decreased BrdU-Recoverin, BrdU-Rho-4D2 immunostained cells. These populations were 50 and 10 percent of control cultures respectively. In contrast the number of double labelled C1+BrdU immunoreactive cells increased in the growth factor treated cultures nearly three fold that of controls. These results demonstrate that for embryonic cultures EGF or TGFα inhibit the production of Rho-4D2 and Recoverin immunoreactive cells while enhancing the generation of C1 immunoreactive cells. These data indicate that EGF and TGFα control differentiation of neuroepithelial cells into specific retinal phenotypes by influencing cell commitment during rat retinal development. NIH 2RO1 NS28308-04

534.8

INFLUENCE OF NATURALLY OCCURING CELL DEATH ON APPARENT CLONE COMPOSITION IN RETINAL LINEAGE ANALYSIS. James T. Voyvodic* and Martin C. Raff. Biol.Dept, Univ.Coll.Lond., London WC1E 6BT, UK.

Cell lineage studies in the nervous system provide information about the developmental history of different cell types. Identifying branch points within a lineage helps to indicate the stages during development at which cell-identity decisions are made. Accurately determining such branch points, however, requires that all cells within a clone be identified. To what extent, therefore, might naturally occuring cell death confuse the analysis of lineage relationships by making the surviving members of a clone appear more closely related than they actually are?

A computer model of 1000 identical 8-cell "clones" was tested, varying the probability of cell death from 1% to 75%. With 20% death, 0.2% of clones had only 2 surviving cells, whereas at 50% death, 14% of 8-cell clones had 2 survivors. In these cases the surviving pair of cells were more likely to be distant cousins than siblings.

In the rat retina, we and others estimate normal death within some cell types to be over 60%. The model therefore predicts that analysis of retinal clone composition after the cell death period will indicate more lineage branching variability than may actually be the case.

535.1

THE βAMYLOID PRECURSOR PROTEIN FUNCTIONS IN NEURITE OUTGROWTH AND BINDS TO THE IKVAV ACTIVE SITE OF LAMININ. M.C. Kibbey, M. Jucker, B.S. Weeks, R.L. Neve, W.E. Van Nostrand, and H.K. Kleinman*. LDB, Natl. Inst. Dental Research, NIH, Bethesda MD 20892; Swiss Fed. Inst. Tech., Zurich Switzerland; McLean Hospital, Belmont MA 02178; UC@Irvine CA 92717.

We previously described a 110 Kd binding protein (LBP110) from mouse neonate brain for the IKVAV containing site of the laminin A chain. Due to similarities in localization of both LBP110 and the βamyloid precursor protein (APP) under both normal conditions and following injury, we investigated if LBP110 was a member of the APP family. Affinity-purified LBP110 was recognized by 4 different APP antisera and 2 different sources of APP were recognized by LBP110 antiserum. By ligand blot, APP specifically bound IKVAV-containing peptide. NGF-treatment of PC12 cells upregulated an LBP110-immunoreactive protein similar to that which has been reported for APP. PC12 cells transfected with APP antisense had decreased LBP110 and APP and reduced ability to form neurites on both laminin and IKVAV peptide. These studies suggest a normal function for APP in neurite outgrowth and binding to the IKVAV site of laminin.

535.2

ANTI-IDIOTYPIC ANTIBODIES TO GM1 ACT AS PROBES TO IDENTIFY GANGLIOSIDE BINDING PROTEINS AND ENHANCE CELL ADHESION. M.J. Riggott* and W.D. Matthew. Dept. of Neurobiology, Duke University Medical Center, Durham, NC 27710

Axon outgrowth during development and regeneration is accompanied by changes in ganglioside composition. Exogenously added gangliosides are potent stimulators of neuritogenesis and sprouting and facilitate cell adhesion. Gangliosides may act by direct interaction of their carbohydrate moieties with membrane proteins. The study of ganglioside-protein interactions has been hampered by the lack of probes that recognize ganglioside binding proteins. We have used a novel immunological method to generate putative anti-idiotypic antibodies that bind the functional sites of GM1 binding proteins. These antibodies bind the beta subunit of cholera toxin, a GM1 binding protein. These antibodies facilitate cell adhesion of dissociated embryonic hippocampal neurons in *in vitro* bioassays. The antibodies recognize antigens on the cell surface. In Western blots, these antibodies bind proteins at 150, 66, 57 and 45 kD. The 150 kD protein is recognized only in homogenates of neural tissue. Cultured embryonic hippocampal cells treated with the antibody respond by protein tyrosine phosphorylation of proteins at 75 and 45 kD. The identity of the proteins recognized by the antibodies and the substrate of protein tyrosine phosphorylation will indicate whether anti-idiotypic antibodies can be used to determine the mechanism for ganglioside-protein interaction in cell adhesion and neurite outgrowth in the nervous system.

535.3

CHARACTERISTICS OF GANGLIOSIDES INCLUDING *O*-ACETYLATED SPECIES IN GROWTH CONES. [1]M. Igarashi*, [1]S. Saito, [1]Y. Komiya, [2]H. Waki, and [2]S. Ando. [1] Department of Molecular and Cellular Neurobiology, Gunma University School of Medicine, Maebashi, Gunma 371, and [2] Department of Membrane Biochemistry, Tokyo Metropolitan Institute of Gerontology, Itabashi, Tokyo 173, Japan.

Growth cones are thought to achieve accurate synaptogenesis through special molecular recognition systems, although most of these systems have not been characterized. Gangliosides, or sialic acid-containing glycolipids, are enriched in neural tissues and are believed to play roles in modulating cell functions. Certain specific gangliosides are expressed only at early developmental stages in nervous systems. *O*-acetyl-GD3, in particular, has been proposed to be involved in mammalian CNS development. To investigate the characteristics of gangliosides, including the *O*-acetylated species, we isolated the gangliosides from growth cone membranes (GCM) of rat brain at several developmental stages, without alkali saponification, and quantitatively analyzed them. At several stages, GCM contained significantly larger amounts of gangliosides than the other membrane subfractions. The absolute ganglioside content of GCM increased in amount with development, while the relative amount of GD3 gradually decreased, and that of GD1a dramatically increased. GCM had the higher ratio of GD1a to GM3 plus GD3 than the perinuclear fraction. There were three *O*-acetylated gangliosides in GCM: *O*-acetyl-GD3, *O*-acetyl-GT1b, and *O*-acetyl-GQ1b. The molar ratio of *O*-acetyl-GD3 decreased in GCM at later stages (5% of the total gangliosides at embryonic day 17 to 1% at postnatal day 5), however, the other *O*-acetylated species were almost constant (approximately 1.5% of the total). Our results show that there are significant differences in ganglioside content and composition between the membrane subfraction of growth cones and the perinuclear fraction, suggesting that several species of gangliosides, including *O*-acetyl-GD3, play a role in growth cone function.

535.4

THE WSPWS MOTIF OF THROMBOSPONDIN PROMOTES NEURITE OUTGROWTH FROM EMBRYONIC NEURONS D.J. Osterhout*, V.M. Dixit, and K.S. O'Shea+, Departments of Pathology, and Anatomy and Cell Biology+, University of Michigan, Ann Arbor, MI 48109

Thrombospondin (TSP) is a trimeric glycoprotein which is enriched in the extracellular matrix during the development of the nervous system. The ability of TSP to modulate cell adhesion, migration and neurite outgrowth appears to be mediated by certain functional domains within the molecule. The VTCG and WSPWS sequences, for example, found in the type I repeats of TSP, support adhesion of various non-neuronal cells. Since A4.1, a monoclonal antibody directed to the type I repeat, can inhibit neurite outgrowth, both sequences were tested for their ability to promote neurite outgrowth.

When dissociated rat embryonic neurons were plated onto various synthetic peptides or TSP adsorbed to nitrocellulose, the WSPWS peptide stimulated neurite outgrowth in a dose dependent manner. Neurites formed on WSPWS were morphologically similar to those observed on intact TSP. Soluble WSPWS inhibited neurite outgrowth on TSP in a dose dependent manner; the VTCG peptide had no effect on process extension. Addition of soluble heparin was partially effective in inhibiting process extension on the WSPWS peptide, suggesting that this activity may be mediated by binding to a heparin - like moiety.

535.5

INHIBITION OF AXONAL GROWTH BY SNAP-25 ANTISENSE OLIGONUCLEOTIDES IN VITRO AND IN VIVO

Julie K. Staple, Astrid Osen-Sand, Marina Catsicas#, Kenneth A. Jones, Guidon Ayala, Jonathan Knowles, G. Grenningloh and Stefan Catsicas*

Glaxo Institute for Molecular Biology, Geneva; and Institute of Anatomy#, University of Lausanne; Switzerland.

Axonal elongation and the transformation of growth cones to synaptic terminals are major steps of brain development and the molecular mechanisms involved form the basis of the correct wiring of the nervous system. The same mechanisms may also contribute to the remodelling of nerve terminals that occurs in the adult brain, as a morphological substrate to memory and learning. Here we have investigated the function of the nerve terminal protein SNAP-25 during development. We show that SNAP-25 is expressed in axonal growth cones during late stages of elongation and that selective inhibition of SNAP-25 expression prevents neurite elongation by rat cortical neurones and PC-12 cells in vitro and by amacrine cells of the developing chick retina in vivo. These results demonstrate that SNAP-25 plays a key role in axonal growth. They also suggest that high levels of SNAP-25 expression in specific areas of the adult brain may contribute to nerve terminal plasticity.

535.6

CHANGES IN MOLECULAR EXPRESSION OF LAMP ON LAMINA II NEURONS IN RAT SPINAL CORD AFTER DORSAL ROOT DEAFFERENTATION. B. Zhang*, P. Levitt and M. Murray. Department of Anatomy and Neurobiology, Medical College of Pennsylvania, Philadelphia, Pa 19129.

Limbic System Associated Membrane Protein (LAMP) is a 64 kd membrane protein expressed by neurons in limbic system or limbic system associated structures. In developing systems, LAMP is expressed on axons and on their target neurons. In adults, LAMP expression is confirmed to the post synaptic membranes. Changes in molecular expression of LAMP in lamina II neurons of adult rat spinal cord after deafferentation have been studied using immunocytochemical techniques. Dorsal roots were sectioned and dorsal root ganglia removed from L1 to S2 in 17 rats. Four unoperated rats and two sham operated rats served as controls. At 3, 10 or 60 days after deafferentation, rats were perfused with 4% paraformaldehyde, and the L4-5 segments of the spinal cord were removed and prepared for light and electron immunocytochemistry (LM-ICC and EM-ICC). LM-ICC demonstrated that the density of LAMP immunoreactivity in lamina II appeared normal compared with controls at 3 days postoperatively, but started to decrease at 10 days and had decreased still more by 60 days after deafferentation. LAMP is expressed on post-synaptic membranes in control, but is expressed on both pre- and post-synaptic membranes at 10 days after deafferentation. The decrease of LAMP immunoreactivity density indicates that molecular expression on the second order neurons of lamina II may be regulated by afferent information or changes in the pre-synaptic complement. The reexpression of LAMP on axonal profiles after deafferentation may identify axons that sprout in response to deafferentation.

Supported by NIH grant No. NS24707 and MH45507.

535.7

LAMP: A NOVEL GPI-LINKED PROTEIN INVOLVED IN CELL-TO-SUBSTRATUM ADHESION BY SPECIFIC NEURONAL POPULATIONS.
V.Zhukareva and P.Levitt*Department of Anatomy and Neurobiology, The Medical College of Pennsylvania, Philadelphia, PA 19129

We have recently shown that the limbic system-associated protein (LAMP) is a phosphatidylinositol (PI)-linked protein. Members of this family in the nervous system have been postulated to participate in a variety of recognition events, including cell adhesion. LAMP exhibits characteristics, such as an early and restricted pattern of expression and disruption of fiber-target interactions by anti-LAMP (Keller, et al, 1989), that are indicative of a developmentally significant protein. PI-specific phospholipase C (PI-PLC) can be used to release LAMP from membranes for subsequent affinity purification. To test the ability of LAMP to facilitate substrate adhesion and growth, we utilized a system developed by Lagenaur and Lemmon (1987). Methanol-solubilized nitrocellulose-coated dishes were dotted with affinity purified LAMP or poly-D-lysine (PDL) and embryonic brain cells from limbic and non-limbic areas were plated for 9 - 24 h. All population of neurons bind to and extend processes on the PDL. Perirhinal limbic cortical neurons bind specifically to substrate-bound LAMP, forming small cell aggregates with neuritic processes. Monoclonal anti-LAMP significantly reduces such binding and initiating of process growth. Almost all non-limbic fetal neurons from somatosensory cortex and olfactory bulb failed to bind, and those that bound did not extend neurites to any significant extent. The present results provide direct evidence that LAMP can specifically regulate adhesion and outgrowth of select neurons during development. Supported by NIMH grant MH45507.

535.8

EXPRESSION OF THE GROWTH-RELATED NEURAL CELL ADHESION MOLECULE TAG-1/AXONIN-1 IN THE ADULT MAMMALIAN BRAIN
D.P. Wolfer*, A. Henchan-Beatty, R. Giger, P. Sonderegger and H.P. Lipp
Departments of Anatomy and Biochemistry, University of Zurich, Switzerland

TAG-1/axonin-1, a member of the Ig-FNIII-superfamily of neural cell adhesion molecules, occurs in two forms, one axonally secreted, the other phosphoinositol-anchored to the cell membrane. It is expressed by neurons extending axons *in vitro* and, as an immobilised substratum, stimulates axonal growth. Soluble TAG-1/axonin-1 affects neurite fasciculation in culture. *In vivo*, in the rodent nervous system, axonin-1-like immunoreactivity is associated with the development of spinal and cranial nerves, with the formation of several, mostly long projecting, fibre tracts, and with the precerebellar neuronal migratory streams. cDNAs have been cloned for the homologues of TAG-1/axonin-1 in chick, mouse, rat and man.

A polyclonal antiserum against TAG-1/axonin-1 shows staining in the molecular layer of adult rodent and human cerebellum, if tissue is fixed with glutaraldehyde. Correspondingly, mRNA for TAG-1/axonin-1 can be visualised in cerebellar granule cells of 4 month old mice by *in situ* hybridisation using digoxigenin-labelled cRNA probes. Moreover, in the adult mouse brain, TAG-1/axonin-1 mRNA is detected in dentate granule cells, in pyramidal cells of hippocampal areas CA1 and CA3, as well as within scattered cells in some regions of the cerebral cortex and basal forebrain. Since no immunoreactvity is found in their axons, these cells may synthesise TAG-1/axonin-1 mostly or exclusively in its secreted form. In certain regions of the adult brain, expression of other growth-associated proteins, such as GAP-43, has been related to persistent neural plasticity or regenerative capacity. Further studies will clarify whether the expression of the growth-associated neuronal protein TAG-1/axonin-1 by specific neurons of the adult brain serves neural plasticity or regenerative capacity as well. *(Supp. Swiss National Foundation for Scientific Research 31-27737.89)*

535.9

REMOVAL OF POLYSIALIC ACID AFFECTS ARBORIZATION OF OPTIC AXONS DURING DEVELOPMENT IN XENOPUS LAEVIS TADPOLES.
J.K. Ivins*, M.J. Li, U. Rutishauser and S.E. Fraser, Caltech and Case Western Reserve University.

The neural cell adhesion molecule NCAM is often coupled to long chains of α2,8 linked sialic acid residues known as polysialic acid (PSA). NCAM-PSA has been implicated as a regulator of neurite outgrowth and branching both *in vitro* and *in vivo* by virtue of its ability to interfere with cell-cell interactions. We have determined the distribution of NCAM and NCAM-PSA during visual system development in *Xenopus laevis* tadpoles and have examined the effects of enzymatically removing PSA on the patterns of arborization of optic axons in living tadpoles. Immunohistochemical localization of NCAM and PSA in tadpoles showed that NCAM is present on both retinal ganglion cell axons and tectal neurons, while PSA is present only on tectal neurons. To examine the role of PSA in visual system development, tadpoles were injected intraventricularly between stages 45 and 47 with a highly specific endoneuraminidase, endo N, which cleaves PSA from NCAM. Individual retinal ganglion cells were labeled with dil and their axons visualized by low light-level video microscopy. We observed that the retinal ganglion cells in endo-N treated tadpoles sprouted consistently longer, typically unbranched terminal arbors than control animals. These observations are most consistent with a model in which PSA on the tectal neurons promotes fasciculation between optic fibers. Removal of PSA from the tectum may facilitate cell surface interactions between afferent fibers and the tectal neuropil, resulting in increased arbor growth.

535.10

ANALYSIS OF THE EFFECT OF THE STRUCTURE OF A PATTERNED GRID SUBSTRATE ON CELL DISTRIBUTION IN CULTURE.
E. M. Powell and H. M. Buettner*. Dept. of Chem. and Biochem. Engg, Rutgers Univ., Piscataway, NJ 08855.

To investigate growth cone guidance at a laminin boundary, 8 day embryonic chick dorsal root ganglion (DRG) neurons were cultured on a patterned grid substrate of square (laminin) and lane (bovine serum albumin) regions, originally developed by Hammarback and Letourneau (Dev. Biol. 117:655 (1986)). The substrate was characterized with respect to laminin surface concentrations. The ratio of laminin concentrations in the square to the lane regions was determined by an indirect immunochemical staining technique using colloidal gold and was found to be in the range of 0.95 to 2.20, corresponding to applied laminin concentrations of 30.0 μg/ml to 100.0 μg/ml. Neurite lengths were measured and found to be beta distributed with a mean of 47.5 μm. The locations of cells on the substrate were analyzed using statistical mechanics techniques, and the results suggest the lane regions form three-dimensional boundaries between adjacent squares, with an approximate height of 2.1 μm as determined by optical measurements. Further analysis will elucidate the effects of the physical structure on cell behavior.

Supported by the Whitaker Foundation, and by a pre-doctoral fellowship to EMP through the NIH/Rutgers/UMDNJ Biotechnology Training Program.

535.11

SEVERAL CHEMICALLY DEFINED SURFACES ARE CONDUCIVE TO THE ADHESION, SURVIVAL AND DIRECTED GROWTH OF EMBRYONIC HIPPOCAMPAL AND SPINAL CORD NEURONS IN VITRO.
A.E. Schaffner*[1], K. Foster[2], J.J. Hickman[2], D.A. Stenger[3] and J.L. Barker[1], Laboratory of Neurophysiology, NINDS, NIH, Bethesda, MD 20892[1], Science Applications International Corp., Mclean, VA 22102[2] and Naval Research Laboratory, Washington, D.C. 20735[3].

Several trimethoxysilane derivatives were covalently attached to glass coverslips and compared to poly-d-lysine for their ability to support the adhesion and growth of embryonic neurons. Tissue from 19 day embryonic rat hippocampus and 15 day embryonic cervical, ventral spinal cord was enzymatically or mechanically dissociated and plated at a density of 350 cells/mm^2 on glass coverslips in serum-containing or serum-free medium. Surfaces were assayed qualitatively for neuronal survival and neurite outgrowth over a 2-week period. In general, cells did better after mechanical dissociation in serum-free medium and spinal cord neurons grew on a greater number of different chemically modified surfaces than did hippocampal neurons. Appropriate surfaces were later used to construct simple geometric patterns (such as parallel lines ~ 10-100 μm apart). We were able to achieve directed growth of nerve cell processes on the patterned substrates. We are presently devising patterns and conditions that will allow us to manipulate synaptic interactions between defined neuronal phenotypes in vitro.

535.12

CELL ALIGNMENT ON CHEMICALLY-PATTERNED SURFACES REVEALS A HIERARCHY OF PREFERENCES
B. Lom*, K.E. Healy† and P.E. Hockberger# Institute for Neuroscience, †Biomaterials and Biomedical Engineering Depts., and #Physiology Dept., Northwestern Univ. Medical and Dental Schools, Chicago, IL 60611.

A convenient method for evaluating the relative adhesivity of cells to different chemicals is to observe their distribution on chemically-patterned surfaces. While several patterning techniques have been described in the literature, each is limited in the number and types of molecules that can be patterned as well as in its precision. We have developed a versatile technique which allows the patterning of a wide variety of chemicals with submicron precision (Lom et al., *Soc. Neurosci. Abstr.* 18: 39, 1992). This technique combines silane coupling chemistry, photolithography, and protein adsorption to create well-defined, patterned surfaces. We tested the adhesivity of mouse neuroblastoma cells (N1E-115) on glass surfaces patterned with amines (ethylenediamine-propylsilane, EDA-PS), alkanes (dimethylsilane, DMS; octadecyl-dimethylsilane, OD-DMS), and proteins (collagen IV; fibronectin; laminin; bovine serum albumin, BSA) in the following combinations: alkane-glass, protein-glass, alkane-alkane, amine-alkane, amine-protein (other combinations have not been tested yet). From observations of cell distribution 24 hours after plating the following hierarchy of adhesive preference was established: collagen IV, fibronectin, laminin > EDA-PS, glass > DMS, OD-DMS, BSA. The low preference of neuroblastoma cells for BSA indicates that preference for extracellular matrix proteins was due to specific properties of these proteins. The preference for matrix proteins over EDA-PS indicated that this specificity was not simply due to positively-charged moieties on the matrix proteins. In the accompanying abstract (Soekarno et al.) we show that preference was correlated with greater overall attachment to surfaces even though parts of cells attached equally well to the non-preferred surface.

Supported by MH-10217, NS-17489 and The Whitaker Foundation.

535.13

EVIDENCE FOR PREFERENTIAL ADHESION AS A MECHANISM FOR NEURONAL PATHFINDING IN CULTURE

A. Soekarno*, B. Lom, and P. E. Hockberger# Inst. for Neuroscience and #Dept. of Physiology, Northwestern Univ. Med. Sch, Chicago, IL, 60611.

Pathfinding is a fundamental behavior that guides growth cones to their targets. One plausible pathfinding mechanism is growth cone attachment to and guidance along adhesive surfaces. Conversely, growth cones may be guided by and advance faster along less adhesive surfaces. An alternative to these "differential adhesion" models is guidance by a signal transduction mechanism. In this model the inherent adhesivity of materials is less important than the specific molecules that activate the signal transduction cascade. Since growth cone movement presumably requires adhesion, guidance would occur by "preferential adhesion" to surfaces with the appropriate signals. Here we present evidence in culture that supports the preferential adhesion model. We used interference reflection microscopy (IRM) to directly monitor the attachment of mouse neuroblastoma cells (N1E-115) to chemically-patterned surfaces. The patterns consisted of amine lines (5-15 μm-wide by 1 mm long) separated by either alkanes or laminin lines (see Lom et al., *Soc. Neurosci. Abstr.* 18: 39, 1992 for patterning technique). On amine-alkane patterns, cells and growth cones attached to and displayed similar adhesion profiles (focal and close contacts) on both surfaces, even though they were guided by the aminated pathways. Similarly, on amine-laminin patterns, most cells and growth cones (80%) adhered equally well to both regions though some (20%) adhered more tightly to laminin surfaces. On this pattern all cells and growth cones were guided by the laminin. Thus we were unable to find a consistent correlation between the pathway of choice and the degree of attachment. These results are inconsistent with both models of differential adhesion and instead support the model of preferential adhesion.

Supported by grants from NIH (MH-10217, NS-17489) and The Whitaker Foundation.

535.14

PATTERNED MICROCIRCUITS OF HIPPOCAMPAL NEURONS: COMPARISONS OF PHOTORESIST VERSUS LASER ABLATION PROCESSES, B.C. Wheeler*[1], J.M. Corey[1], and G.J. Brewer[2]. [1]Neuroscience Program, Univ. Illinois, Urbana IL 61801 and [2]Southern Illinois Univ. Sch. Med., Springfield IL 62794.

Study of CNS neuron growth, development and synaptic specificity would be facilitated by limiting the number and direction of growing axons and dendrites. Although selective photoablation of polylysine coated substrates has been successful for us, the short shelf-life and suspected uneven coating led us to use more stable aminosilanes. Here we compare two techniques for patterning adhesive substrates with aminosilanes on glass coverslips: 1) UV laser photoablation through a quartz mask, and 2) photoresist patterning for selective deposition. Rat embryonic hippocampal neurons were dissociated and grown on these substrates in serum-free medium, GIBCO B27/Neurobasal. Variations in the laser process important for a well-defined adhesive path include laser energy density at 190 nm and the number of 30 ns pulses. In photoresist processing variation in the density of bonding of the aminosilane causes poor compliance of cells to paths less than 10 mm wide. An alternative approach of application of aminosilane, followed by selective photoresist development, appears more promising. Other considerations include equipment costs and processing time. These findings will aid applications to localize neurons over substrate electrodes and studies of linear process extension and synaptogenesis. Supported by NIH R03 RR06870 and SIUSM CRC funds.

535.15

THE FORMATION OF HIPPOCAMPAL CA3 NETWORKS IN EXPLANT CULTURES OF NEONATAL RAT. K.L. Smith and J.W. Swann*. Cain Foundation Labs, Department of Pediatrics and Division of Neuroscience, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030.

The extent to which CA3 hippocampal pyramidal cells form local excitatory connections with each other has only recently been appreciated. Recurrent excitatory synapses likely play an important, but yet undetermined, role in normal hippocampal functioning. However recent results show these connections participate in the generation of epileptiform discharges. Moreover, recordings from immature rats have shown that these synapses are present early in postnatal life and likely contribute to the enhanced seizure susceptibility of neonatal rats. Studies were undertaken, to understand those factors that regulate the formation of CA3 recurrent excitatory axon and synapses. Slices from 5-day-old rat hippocampus were grown in culture in a defined media on Millipore millicell membranes. After only 24 hours in culture explants were capable of generating large and prolonged electrographic seizures when exposed to picrotoxin (10 μM). These were abolished by CNQX. This was surprising, since slices taken acutely from rat pups produce these discharges no earlier than postnatal day 8. Thus it appears that the formation of CA_3 recurrent collaterals is accelerated in culture compared to that occurring *in vivo*. In a second experiment, explants were grown in media containing 3 mM kynurenic acid (KYN). When KYN was washed from the recording media, explants were also capable of producing picrotoxin-induced seizure-like discharges. Thus KYN does not appear to prevent the formation of recurrent collaterals and synapses in hippocampal area CA_3. Indeed, in a number of instances electrographic seizures occurred spontaneously upon KYN washout and before picrotoxin application. Thus blockade of excitatory amino acid receptors may accelerate the proliferation of local circuit recurrent collaterals. (Supported by NIH grant NS18309).

535.16

TARGET-DERIVED INFLUENCES ON AXON GROWTH PATTERNS IN EXPLANT CO-CULTURES OF TRIGEMINAL GANGLIA. R. Erzurumlu*, S. Jhaveri and R. McKay. Dept. Brain & Cog. Sci. M.I.T., Cambridge, MA, 02139

Co-cultures of embryonic trigeminal ganglia with "peripheral" and "central" targets provide an *in vitro* model for studying axon-target interactions. When E15 rat trigeminal ganglion explants are placed between peripheral (vibrissa pad) and CNS targets, peripheral processes of ganglion cells invade the vibrissa pad explants and form a circumfollicular pattern. Central processes of ganglion cells invade many isochronic CNS explants and form a fasciculated tract without any arbors (Erzurumlu et al., PNAS, 1993). We have examined the growth of E15 and E20 trigeminal axons in heterotypic and heterochronic co-cultures with various cutaneous (skin from the back, forepaw and vibrissa pad explants) and CNS (brainstem, parietal cortex, olfactory bulb) targets by DiI labeling after 5-7 days in culture.

Peripheral axons from E15 ganglia are patterned differentially in all three cutaneous fields: they are organized around the follicles in vibrissa pads, course in thick fascicles between the digits in forepaw and are distributed diffusely in dorsal skin. Central axons invade various CNS targets and either elongate to form a tract, or arborize, depending on target maturity. In contrast to the unbranched elongation seen *in vivo* as well as in E15 brainstems, E15 trigeminal axons form collateral branches and arbors in more mature brainstem, neocortex or olfactory bulb slices.

On the other hand, central processes from E20 trigeminal ganglia (which normally arborize in the brainstem trigeminal nuclei) revert to an elongation mode of axon growth when challenged with younger (E15) brainstem explants. Their peripheral processes also invade E15 vibrissa pads and become arranged around the follicles, but in a more diffuse pattern than that seen with E15 ganglia. These observations suggest that growth pattern of trigeminal axons is strongly influenced by the target tissue.
(Supp. by N.I.H. grants NS27678 and NS21991.)

535.17

SELECTIVE NEURITE OUTGROWTH OF CULTURED CORTICAL NEURONS ON SPECIFIC REGIONS OF BRAIN CRYOSTAT SECTIONS. M.C. Halloran* and K. Kalil. Neuroscience Training Program and Dept. of Anatomy, University of Wisconsin, Madison, WI 53706.

During neural development axons of the mammalian CNS show a high degree of selectivity in the territories into which they extend. To begin to address the mechanisms underlying this regional selectivity we tested the ability of developing cortical neurons to extend neurites onto specific regions of cryostat brain sections from early postnatal hamster. The goal of this work was to grow neurons on a natural brain substratum which could then be manipulated biochemically. Small cortical explants from newborn hamster were placed onto various regions of cryostat sections from 2-10 day postnatal brains. Neurite outgrowth was visualized 48 hours later with the fluorescent vital dye 5-(and-6)-carboxyfluorescein diacetate applied to the explants shortly before fluorescence microscopy. Results showed that cortical neurites avoided cerebellar regions of the section. These neurites were stunted and preferred to fasciculate with each other rather than grow onto the cerebellum. Preliminary results suggest that the inhibitory effects of the cerebellum increase with the age of the sections. Olfactory bulb also had an inhibitory effect on cortical neurite outgrowth. In contrast, explants plated onto the cerebral cortex as well as regions of the forebrain, midbrain, and brainstem extended many long neurites that were relatively unfasciculated. Results also suggest that neurites extending onto cortical regions showed a preference for growth in the radial orientation, perhaps following the processes of radial glia. These results show that cortical neurites respond to cell surface cues by growing upon regions of the sections permissive for their outgrowth *in vivo* but avoid regions that are not normally invaded by cortical axons. We are currently treating the sections with enzymes that remove specific glycoprotein moieties in order to classify cell surface molecules that determine specificity of cortical neurite outgrowth. Supported by NIH Grant NS14428 to K.K.

535.18

GROWTH CONES IN THE PRIMARY VISUAL PATHWAY OF THE CHICK EMBRYO. S.A. Dunlop, [1]P.G. Nelson, W.M. Ross & M.M. Stewart. Dept. of Psychology, University of Western Australia 6009, [1] National Institute of Child Health and Human Development, NIH, Bethesda, MD 20892.

We have examined the morphology of retinal ganglion cell growth cones labelled with horseradish peroxidase in the chick embryo using Nomarski optics. At E3-4 growth cones arise from first-born ganglion cells and pioneer their way through a virgin pathway. In the optic stalk, growth cones are elongate and have a few short filopodia extending in front of the growth cone body, whereas in the presumptive chiasm they are spread and have large lamellipodia. Within the tract, growth cones are again elongate but have many filopodia extending from along their length; ipsilaterally projecting growth cones are common. At E10, growth cones arise from amongst the last-born ganglion cells and navigate along an established pathway towards the tectum. In the nerve, growth cones are elongate and have many long filopodia extending in front of the growth cone body. Growth cones in the chiasm and tract appear similar to the pioneer growth cones although ipsilaterally projecting growth cones are rare. As in the mouse (1) there are pronounced differences in growth cone morphology between regions of the developing visual pathway but, in contrast to the mouse, pioneer and later-born growth cones tend to have a similar morphology.
1. Bovolenta P. & Mason C.A. 1987. J Neurosci. 7,1447-1460.

536.1

DEVELOPMENT OF A BIOSYNTHETIC 3-D EXTRACELLULAR MATRIX EQUIVALENT FOR NEURITE OUTGROWTH. R. Bellamkonda*, J. Ranieri and P. Aebischer. Surgical Research Division, CHUV, Lausanne University, Switzerland.

The extracellular matrix (ECM) has been shown to play an important role in neuronal cell attachment, maintainence and differentiation. Our aim is to develop a simple, well defined, biosynthetic hydrogel based system capable of supporting neuronal cell attachment and differentiation in three dimensions. The particular suitability of a hydrogel as a biomaterial stems from the similarity of their physical properties to those of living tissues vis-a-vis their high water content, soft rubbery consistency and low interfacial tension. Cell attachment and differentiation promoting oligopeptides like GRGD, YIGSR and IKVAV, derived from ECM proteins like laminin and fibronectin, were covalently linked to transparent, thermoreversible agarose hydrogels using 1'1 Carbonyldiimidazole chemistry. Embryonic (E14) rat striatal cells were three dimensionally suspended inside the gels. Striatal cells were viable upto 10 days in culture as determined by a fluorosceindiacetate assay. At time points 24, 48 and 72 hrs, 42%, 52% and 55% of the cells extended neurites in three dimensions inside agarose gel. This was comparable to the striatal cell neuritic extension in 100% Matrigel™, a commercially available basement membrane preparation extracted from the EHS mouse sarcomma. E8 chick ciliary ganglions also extended processes when suspended in three dimensions inside the agarose gel. Thus agarose and derivatized agarose gels are capable of supporting significant neuritic extension from anchorage dependant primary neuronal cultures in three dimensions *in vitro*. This system may serve to enhance nerve regeneration when introduced into the various animal models. *In vivo* studies are currently in progress.

536.2

Electric Field-Guided N1E-115 Neurite Growth: Is Calcium Influx Necessary And Sufficient? R.S. Bedlack Jr*, P. Amarante, M.-d Wei and L.M. Loew. Univ. of Conn. Health Ctr., Farmington, CT 06030.

D.C. electric fields evoke localized membrane depolarizations, calcium influx and growth cone filopodial protrusions in N1E-115 cells; such actions might underlie electric field-directed neurite initiation, elongation, sparing from retraction and turning, which are also seen in these cells (Neuron 9, 393-403 (1992)). Temporal and spatial correlations between the described events support this hypothesis, but we are attempting to gain further proof. Here we report that prevention of field-induced calcium influx (by extracellular calcium removal or inorganic or organic calcium channel blockers) eliminates field-biased filopodial protrusions and field-directed growth in N1E-115 cells; however, this also causes diminished filopodial activity, growth cone collapse and neurite retraction in non-field treated cells. Conversely, agents which cause non-localized calcium influx (elevation of extracellular KCl or addition of calcium ionophores) are known to elicit non-localized increases in N1E-115 growth cone filopodia. We now demonstrate that these agents also evoke non-localized increases in N1E-115 neurite initiation, elongation and stability. Our results suggest that calcium influx is necessary for normal and field-directed N1E-115 neurite growth. Further, the observation that non-localized influx can create non-localized increases in growth suggests that localized calcium influx may be sufficient for explaining field-directed N1E-115 neurite initiation, elongation and sparing from retraction. (Supported by USPHS Grant ES05973).

536.3

GROWTH CONE DYNAMICS AND NERVE ORIENTATION IN A SMALL ELECTRIC FIELD ARE SUBSTRATE DEPENDENT. C.D. McCaig, L. Erskine & M.N. Wallace* Department of Biomedical Sciences, University of Aberdeen, Aberdeen, Scotland. AB9 1AS.

Embryonic *Xenopus* neurites grown on tissue culture plastic turn cathodally in steady fields above 10mVmm^{-1}. The direction of orientation is substrate dependent; neurites grown on poly-l-lysine coated plastic selectively grew anodally (Rajnicek et al, Soc. Neurosci. Abstr.15,(2):1036). These observations have been repeated and extended by studying growth cones at high resolution, with video enhanced contrast-differential interference contrast microscopy (VEC-DIC). *Xenopus* nerves grown on glass or on laminin-coated glass (10ugml^{-1}) and exposed to a small electric field (70 - 190mVmm^{-1} for up to 3h), oriented cathodally; those on poly-l-lysine anodally. On all three substrates, filopodia were distributed asymmetrically, being both longer and more numerous on cathodal-facing than on anodal-facing sides of growth cones. Rates of extension towards either cathode or anode of sheets of growth cone lammelipodia did not differ on glass or on polylysine. On laminin however, lammelipodia from the cathodal-facing side of a growth cone extended at rates 19% faster than those extending from the anodal-facing side ($p<0.001$). It is concluded: 1. that galvanic nerve orientation is substrate dependent, 2. that filopodial asymmetry at the growth cone need not mandate the subsequent direction of nerve orientation and 3. that an electric field can induce asymmetric cytoskeletal polymerisation and/or membrane addition at the growth cone.

Supported by The Wellcome Trust and The Carnegie Trust.

536.4

CHONDROITIN SULFATE C ENHANCES GALVANOTROPIC ORIENTATION OF CULTURED FROG NERVES. L. Erskine and C.D. McCaig*. Department of Biomedical Sciences, University of Aberdeen, Aberdeen, Scotland. AB9 1AS.

In d.c. electric fields of 50 - 200 mVmm^{-1} dissociated neurons from the neural tube of stage 18 to 20 *Xenopus* embryos orient cathodally on tissue culture plastic. *In vivo* the direction and number of neurites orienting may depend on interactions between endogenous fields and extracellular matrix components. Proteoglycans, components of the extracellular matrix of embryos, are highly negatively charged molecules that have been implicated in regulating nerve growth. The effect of the glycosaminoglycan chondroitin sulphate C (CSc) on field induced neurite orientation was therefore examined. In normal medium 62% (n=103) of neurites turned to grow cathodally in applied fields of 50 - 133 mVmm^{-1}. At higher field strengths (143 - 200 mVmm^{-1}) >80% oriented. A neurite was categorised as having turned if over a 5 hour observation period it showed a sustained deviation of >15 deg from its original direction of growth as measured from hourly photographs. Addition of 10 µgml^{-1} CSc to culture medium 30 min after plating significantly enhanced the orientation response. At low field strengths (56 - 120 mVmm^{-1}) 85% (n=66) of neurites turned cathodally, a significant increase. A two fold increase in the mean total angle turned was also observed. At higher field strengths (166 - 175 mVmm^{-1}) the rate of turning was significantly faster than that observed in normal medium. Bioelectric fields and matrix components may therefore be interactive cues for neurite guidance *in vivo*.

Supported by The Carnegie Trust and The Wellcome Trust.

536.5

DEVELOPMENTAL EXPRESSION OF UROKINASE AND TISSUE PLASMINOGEN ACTIVATORS IN RAT NERVOUS SYSTEM. Y. Sumi*, R.J. Morris and M.A.R. Dent. Norman and Sadie Lee Centre, Laboratory of Neurobiology, National Institute for Medical Research, Mill Hill, London NW7 1AA, U.K.

The expression of urokinase and tissue plasminogen activators has been studied in developing cerebellum, hippocampus, cerebral cortex, olfactory bulb and olfactory mucosa of the rat by *in situ* hybridisation. All identifiable neurons express urokinase mRNA from an early stage in their development, and this expression appears to coincide with the onset of axogenesis. Glial cells generally do not express urokinase message, except for transient expression by oligodendrocytes in developing fibre tracts during the period of myelination. In contrast, although high levels of tissue plasminogen activator are found in the embryonic floor plate, in postnatal brain it is expressed only by ventricular ependymal cells.

536.6

TARGETING OF GONADOTROPIN-RELEASING HORMONE (GnRH) AXONS TO THE MEDIAN EMINENCE (ME): AN IN VITRO STUDY. T.J. Wu*, M.C. Rogers, M.J. Gibson and A.-J. Silverman. Dept. of Anatomy & Cell Biology, Columbia Univ., New York, NY 10032, and Dept. of Medicine, Mt Sinai School of Medicine, New York, NY 10029.

In mice, GnRH neurons complete their migration from the olfactory placode into the forebrain by E15/16 and are concentrated in the septal-preoptic area (POA). Upon entering the CNS, GnRH axons elongate and project along stereotyped paths to their target in the ME. A similar precision in targeting is observed following implantation of normal fetal POA into the third ventricle of adult hypogonadal (hpg) mice. Experiments involving cografts of fetal POA and ME in hpg mice suggested that the ME might exert a positive influence on GnRH axonal growth. To test the hypothesis that the ME produces a diffusible signal that alters the amount or direction of GnRH outgrowth, we have cocultured explants of POA and ME (which includes the mediobasal hypothalamus) within three-dimensional collagen matrices. In preliminary experiments, explants of E15 mouse POA were cultured alone (n=18) or cocultured (n=30) with an ME for 48-120 h. The ME was positioned near the rostral aspect of the POA at 1.0-1.5 mm. GnRH neurons (up to 300) were detected immunocytochemically in 85% of the POA explants and axonal growth into the collagen matrix (of both GnRH and unknown phenotype) was observed. In some cases, the GnRH axons reached the ME. To document the overall innervation of the ME by the POA explant, DiI crystals were placed in the ME after fixation and allowed to diffuse at 37 C for 10-14 days. Retrogradely labeled cells were found in the POA after 120-h of culture but not at 48-h. Placement of DiI on the POA revealed robust outgrowth after 120 h and only minimal outgrowth after 48 h. This culture system will be useful in demonstrating the presence of ME-derived agents responsible for the targeting of GnRH axons. NS220335 (MJG), T32 NS07062 (TJW).

536.7

RAT VOMERONASAL ORGAN AND OLFACTORY BULB COCULTURE.
T. Osada, M. Ichikawa, P.P.C. Graziadei and D.M. Easton*.
Department of Biological Science, Florida State University Tallahassee FL 32306

The vomeronasal organ (VNO) and the olfactory bulb (OB) of rat were cocultured from E17 littermates on collagen in Dulbecco' Modified Eagle's Medium containing 5% fetal calf serum and 5% horse serum.

After 48 hrs, isolated fibres, presumably sensory, together with some large fasicles, were seen originating from the VNO. Their direction was random and did not appear to be affected by the OB presence. After 7 to 8 days, however, only the large bundles, mostly those connected to the OB, survived. Vicia Villosa agglutinin (VVA) when added to the culture medium at the start of the incubation inhibited the formation of large fasicles originated from the VNO.

We conclude that the establishment of connections between the VNO and the OB in coculture favors the survival of the large nerve fasicles, however, the presence of the OB does not determine the initial orientation of the fibres and fasicles from the VNO explant.

FORMATION AND SPECIFICITY OF SYNAPSES: RECEPTOR LOCALIZATION

537.1

MOLECULAR CHARACTERIZATION OF ZEBRAFISH ACETYLCHOLINE RECEPTOR MUTATION, *nic1*.
Diane S. Sepich* and Monte Westerfield, Institute of Neuroscience, University of Oregon, Eugene, Oregon.

We are studying neuromuscular synapse assembly by characterizing a zebrafish mutant, *nic1*[b107] which lacks functional muscle acetylcholine receptors (AChRs). In previous studies, we showed that *nic1* mutant embryos are paralyzed and that the *nic1* mutation acts autonomously within muscle cells to block AChR synthesis or clustering.

To learn whether the *nic1* mutation alters the genes encoding AChR subunits, we isolated the zebrafish AChR α subunit cDNA and performed Southern analysis. Restriction fragment length polymorphisms in this gene are linked to the *nic1* phenotype. We observed either smaller or fewer restriction fragments in *nic1* than in wild-type DNA, a pattern consistent with a deletion.

To test whether the *nic1* mutation is a deletion in the AChR α subunit gene and to learn how it might act, we isolated a genomic region spanning the suspected deletion in *nic1* and the corresponding region from the wild-type gene. The differences between the *nic1* and wild-type genes reside in an intron. Because preliminary Northern analysis shows that *nic1* AChR α subunit mRNA is present and larger than in wild-type embryos, we suggest that the mutation blocks intron excision.

These results suggest that the molecular defect of the *nic1* mutation is a deletion within the AChR α subunit gene. We suggest that the mutation acts by causing the mRNA to retain an intron which leads to a subunit protein that fails to form a functional receptor. Supported by NIH NS21132 and HD22486.

537.2

REDISTRIBUTION OF ACETYLCHOLINE RECEPTORS AT INNERVATION SITES IN SHORT-TERM MAMMALIAN VENTRAL HORN-MUSCLE CULTURES.
E. K. Dutton*, A.E. Schaffner, S. C. Fitzgerald and M. P. Daniels, Labs Biochem. Genetics, Neurophys., Dev. Neurobiol., NIH, Bethesda, MD 20892.

The mechanisms that regulate postsynaptic differentiation have been studied extensively in vitro. Mammalian nerve-muscle culture systems, however, have yielded little information about early events in neuromuscular synapse formation. We are investigating the redistribution of acetylcholine receptors (AChR) and the assembly of synaptic components at newly formed innervation sites using a novel mammalian coculture system. Ventral horn neurons were plated on myotube cultures containing few non-muscle cells (Daniels, J. Cell Science, 1991). Standard fluorescence labeling methods were used to visualize the distribution of AChR at nerve-muscle contact sites. Sixteen hr after neuron plating, substantial neurite outgrowth was seen as well as AChR redistribution. Neurite-induced AChR redistribution was maximal 24 to 48 hr after neuron plating and occurred predominantly on the ventral or substrate-apposed surface. AChR redistribution appeared in two patterns, both of which could be induced by the same neurite: 1) When neurites crossed pre-existing ventral AChR patches, the AChR density on the muscle surface directly apposed to the neurite was sharply reduced as if the neurite had "etched" the AChR patch, but the density within one μm from the neurite edge was often higher than that of the surrounding ventral patch. 2) Newly aggregated AChR formed a 5-10 μm wide swath along the neurite path in a bottle brush-like arrangement of AChR enriched domains, and, as seen with ventral patches, the AChR density was reduced directly over the neurite but increased within one μm from the neurite edge. Neurons that induced AChR redistribution had both thick (dendrite-like) and thin (axon-like) neurites. AChR redistribution, however, was generally induced by thin neurites. The distribution of muscle cytoskeletal and extracellular matrix proteins was analyzed at neurite-induced aggregate sites. The 43 and 58 kd proteins and dystrophin were concentrated in neurite-induced AChR-enriched domains and reduced in concentration where neurites had "etched" pre-existing aggregates. Laminin was concentrated in some neurite-induced aggregates, in pre-existing aggregates, and it was "etched" from the neurite path.

537.3

CHANGES IN THE DISTRIBUTION OF 43K PROTEIN DURING SYNAPSE ELIMINATION AT THE NEUROMUSCULAR JUNCTION.
S.M.Culican*, J.B.Cohen, and J.W.Lichtman, Dept. Anatomy and Neurobiology, Washington Univ. Sch. of Med, St.Louis, MO 63110 and Dept. Neurobiol., Harvard University, Boston, MA 02115.

During naturally occuring synapse elimination at the developing neuromuscular junction (NMJ) and also following reinnervation in adults, nerve terminals associated with one axon and nearby post-synaptic acetylcholine receptors (AChRs) are removed from synaptic sites, with AChR loss preceeding the removal of overlying nerve terminals (J. Neurosci. 9:1781-805,1989; 13:834-55,1993). To study the mechanism that leads to AChR loss we have examined the distribution of the receptor associated 43K protein in areas undergoing elimination in reinnervated mouse NMJs. AChRs at NMJs of reinnervated muscle fibers in the adult sternomastoid were labelled in a whole mount with rhodaminated alpha-bungarotoxin. A monoclonal antibody followed by a fluorescein labelled secondary antibody was used to label the 43K protein. Areas of the endplate undergoing elimination had a lower density of AChRs relative to the rest of the endplate. At every site where AChRs were faintly stained, the density of 43K was also diminished as compared to the 43K staining in the rest of the junctional area. To determine whether 43K loss occurred before or after AChR loss, the relative intensity of faint and bright areas for both AChRs and 43K was compared. While the absolute intensity of the two labels varied from one endplate to another, at any one endplate the loss of staining in the faint areas was similar for each molecule, indicating that both are disappearing from the post-synaptic membrane at virtually the same rate. This result indicates that the loss of 43K and AChRs is closely coupled. Other molecules associated with the post-synaptic apparatus are currently under investigation.

537.4

DEVELOPMENTAL CHANGES IN EXPRESSION OF AGRIN mRNA SEQUENCE VARIANTS IN THE CHICK EMBRYO NERVOUS SYSTEM.
E. Ma, R. Morgan and E.W. Godfrey*. Department of Cellular Biology and Anatomy, Medical College of Wisconsin, Milwaukee, WI 53226.

Agrin is a synapse-organizing protein externalized by motoneurons that aggregates acetylcholine receptors and other postsynaptic components found at the neuromuscular junction. In the nervous system, we found agrin mRNA most concentrated in motoneurons of spinal cord (SC), dorsal root ganglia (DRG), cerebellar Purkinje cells and retinal ganglion cells (Ma, Morgan and Godfrey, submitted). The agrin mRNA sequence varies by alternative splicing at two positions, A and B, near the 3' end of the coding region (Ruegg et al., Neuron 8:691, 1992). Most transcripts contain an insert at the A position, but we find four variants at position B, encoding no insert (B_0) or 8-, 11- and 19-amino acid inserts (B_8, B_{11}, B_{19}). Expression of partial cDNAs indicates that chick agrin containing the B_{11} insert has synapse-organizing activity, while the B_0 isoform may not (Ruegg et al., 1992). Here we report developmental changes in agrin mRNA sequence variants in SC and DRG, and changes in the pattern of expression of agrin mRNAs in developing chick embryo cerebellum.

Using polymerase chain reaction (PCR), we detected only B_0 in E2 (Stage 15) SC, but by E4 (St 23), B_{11} and B_{19} transcripts were also expressed, correlating with a large increase in agrin mRNA in presumptive motoneurons seen by *in situ* hybridization. The B_8 variant appeared in SC by E14, while B_{11} levels decreased markedly from E14 to E20. In DRG, B_0, B_{11} and B_{19} variants were present at E6 and E9, but decreased by E14. Thus, alternative splicing of agrin mRNA is developmentally regulated in embryonic spinal cord and DRG.

In cerebellum, agrin mRNA was seen by *in situ* hybridization as early as E9. Both external granule and Purkinje cell layers were labeled at E10. The signal gradually decreased in the external layer as it became thinner and increased in the cytoplasm of Purkinje cells, peaking at about E18, but it was still intense at 3 days after hatching. [Supported by NIH grants NS27218 and HD20743.]

537.5

SINGLE NEURONS EXPRESS MULTIPLE AGRIN MRNA ISOFORMS. M.A. Smith*[1], C.Tran[1,2], and D.K. O'Dowd[1,2]. Depts. of Anatomy and Neurobiology[1]; Developmental and Cell Biology[2], UC Irvine, Irvine, CA 92717

Alternative splicing of an 8 and an 11 amino acid exon results in the expression of four distinct agrin transcripts designated agrin$_0$, agrin, agrin$_{11}$, and agrin$_{19}$. While the results of a number of studies suggest that agrin mRNA is present in both neurons and non-neuronal cells, the use of pan-specific probes in these studies has precluded analysis of the cellular localization of specific agrin isoforms. To address this issue we have used single cell PCR to determine the agrin mRNA profile of individual chick ciliary ganglion cells. Whole-cell recording pipets were used to obtain voltage-clamp and current-clamp recordings from isolated cells acutely dissociated from embryonic ciliary ganglia. Following electrophysiological measurements the contents of the cell were aspirated into the pipet, agrin mRNA reverse transcribed, and subjected to two rounds of amplification using nested primers flanking the region of alternative splicing. Analysis of cells dissociated from E14 ganglia reveals that while some neurons express a single agrin isoform, the majority of neurons express agrin$_{19}$ at high abundance together with one or more of the other agrin isoforms. In contrast, non-neuronal cells, identified by their small size and absence of voltage-gated currents, expressed only agrin$_0$. Based on these results we conclude that alternative splicing of agrin mRNA is regulated in a cell specific manner. Supported by a grants from the Plum Foundation and NS-27563 to M.A.S., and NS27501 to D.K.O'D.

537.6

REGULATION OF AGRIN GENE EXPRESSION BY SEIZURE ACTIVITY IN THE ADULT RAT CNS. L.T. O'Connor*, J.C. Lauterborn, C.M. Gall, and M.A. Smith. Dept. Anatomy and Neurobiology, University of California, Irvine, CA 92717.

We have recently shown that agrin mRNA is expressed by neurons in the adult rat brain suggesting a role for agrin in formation and maintenance of synapses in the CNS. The expression of a number of proteins, including receptors for neurotransmitters, is regulated by synaptically mediated activity. We sought to determine whether agrin gene expression might be under similar control using a limbic seizure paradigm. In these studies, placement of an electrolytic lesion in the hilus of the dentate gyrus induces an episode (8-10 hrs) of recurrent limbic seizures. *In situ* hybridization studies reveal that levels of agrin mRNA in the CNS are altered both temporally and spatially following seizure activity. In comparison to paired controls, hybridization was increased in hippocampus, caudate/putamen, septum and intermediate layers of neocortex but markedly reduced in superficial layers of neocortex and piriform cortex. Within the olfactory tubercle and granule cell layer of hippocampus, increases in agrin mRNA were apparent 24 hrs postlesion and had not returned to control levels after 4 days. By contrast, decreases in agrin mRNA within piriform cortex were also observed 24 hrs after lesion placement but returned to control levels by 48 hrs. We have previously shown that four alternatively spliced agrin mRNAs are expressed in the adult rat brain. However, seizure activity does not appear to influence the relative levels of the different isoforms at 24 hrs postlesion. We conclude that the level of agrin mRNA but not alternative splicing may be regulated by synaptically mediated activity in the rat CNS. Supported by a grants from the Plum Foundation and NS-27563 to M.A.S., NS-07351 to L.T.O., and NS-26748 to C.M.G.

537.7

CELL-CELL INTERACTIONS INFLUENCE AGRIN GENE EXPRESSION IN THE CHICK CILIARY GANGLION. W.S. Thomas*[1], M.H. Jacob[3], D.K. O'Dowd[1,2], M.A. Smith[1], Dept. Anat. and Neurobiol.[1]; Dept. Dev. and Cell Biol.[2], UC Irvine, CA 92717; Worcester Fdn. for Exp. Biol.[3], Shrewsbury, MA 01545.

We have previously demonstrated that in the chick ciliary ganglion (CG) both the levels of agrin mRNA and the pattern of alternative splicing are regulated during normal development. Expression of acetylcholine receptors and α-bungarotoxin binding sites on CG neurons have been shown to be influenced by signals derived from presynaptic inputs and target muscle tissues. In order to determine whether similar cell-cell interactions modulate agrin gene expression in the ganglion, we have used *in situ* hybridization to examine the effects of axotomy and denervation on levels of agrin mRNA. Surgery was performed on newly hatched chicks and ganglia examined 9 days after denervation and 5 days after axotomy. Our results demonstrate that denervation of the ganglion results in a two-fold increase ($p<0.001$) in density of labelling over neuron cell bodies relative to neurons in contralateral unoperated control ganglia. In contrast, after ganglionic axotomy, agrin gene expression in neurons decreases to two-thirds of that in controls. The results indicate that preganglionic influences modulate agrin mRNA levels in the CG and suggest that target derived signals may have an effect as well. Supported by the Pfeiffer Foundation (M.H.J.), Plum Foundation (M.A.S.), NIH NS21725 (M.H.J.), NS27563 (M.A.S.), NS27501 (D.K.O.), and T32 NS07351 (W.S.T.).

NEUROTROPHIC FACTORS: RECEPTORS AND CELLULAR MECHANISMS I

538.1

DEPOLARIZING STIMULI INCREASE *trk* B PROTEIN IN EMBRYONIC RAT HIPPOCAMPAL NEURONS. J.A. O'Keefe*, Y. Goodman, and M.P. Mattson Sanders Brown Center on Aging and Department of Clinical Sciences, University of Kentucky, Lexington, KY 40536.

Neurotrophic factors modulate neuronal growth, differentiation and survival and play a role in neural connectivity by binding to tyrosine protein kinase receptors. Complex interactions between trophic factors, their receptors, and neuronal activity have been hypothesized to account for mechanisms of neuroarchitecture establishment and learning and memory formation. *Trk* B ($p145^{trkb}$) is the functional high affinity receptor for brain derived neurotrophic factor (BDNF), a member of the neurotrophin family which is found in relatively high concentrations in the hippocampus. We employed cultured embryonic rat hippocampal neurons as a model system to test the hypothesis that *trk* B is regulated by neuronal activity. A depolarizing stimulus in the form of high extracellular K^+ concentration [50 mM] elicited an approximately two-fold increase in the *trk* B protein in hippocampal dissociated cell cultures as determined by Western blot analysis using an affinity purified rabbit polyclonal antibody (Santa Cruz Biotech, Inc). Time course studies revealed increased protein production above control values by 6.5 hrs post K^+ treatment with maximum levels of *trk* B evident by 24 hrs. These elevations in *trk* B protein levels were maintained through 48 hrs. Similar results were obtained in dissociated neocortical cultures. Current studies are directed towards investigating the effects of neuronal activity on *trk* B mRNA as well as underlying mechanisms for activity driven induction of this neurotrophin receptor. Our findings suggest that impulse activity may modulate the expression of the *trk* B protein, suggesting a complex paracrine or autocrine interaction between neurotrophic factors, their high affinity receptors and neurotransmitter molecules in activity dependent processes in the hippocampus.

538.2

BIDIRECTIONAL AXONAL TRANSPORT OF $p140^{trk}$ HIGH AFFINITY NGF RECEPTOR. R. Loy*[1] Dorota K. Poluha[2] and A.H. Ross[2]. [1]Dept. Neurology, Univ. Rochester, Rochester, NY 14620, Canandaigua VAMC and [2]Worcester Foundation for Experimental Biology, Shrewsbury, MA 01545.

The major biological effects of nerve growth factor (NGF) are thought to be mediated by binding to high affinity cell surface receptors and retrograde transport to the cell body. We have used two polyclonal antibodies specific to $p140^{trk}$ to look for build-up of immunorective antigen proximal and distal to a transection of the fimbria/fornix. Antibodies 679 and 683 were prepared against $p140^{trk}$ peptide residues 160-176 and 379-394, respectively. These antisera react with $p140^{trk}$ but not with $p140^{trkB}$ or $p140^{trkC}$ and both detect the *trk*-encoded protein in *trk*-virus-infected Sf9 cells but not in control cells. Male Sprague-Dawley rats received knife cuts of the fimbria/fornix between the hippocampus and medial septal nucleus and of the overlying cortex. Eight to 12 hours later, rats were perfused and the brains removed. 40 μm frozen sections were cut and stained using antibodies 679 and 683. Diagonal band, medial septal and nucleus basalis cell bodies are $p140^{trk}$-immunoreactive, although few processes are stained. Both distally and proximally to the transection sites, there appear beaded and varicose $p140^{trk}$-immunoreactive fibers about 50-100 μm in length. These look identical to transected fibers in adjacent sections stained for low-affinity NGF receptor using IgG192 and to axons ligated in the sciatic nerve in previous studies, indicating both anterograde and retrograde transport of $p140^{trk}$. Supported by AG 09231.

538.3

QUANTITATIVE ANALYSIS OF p140^{trk} mRNA EXPRESSION IN THE DEVELOPING RAT BASAL FOREBRAIN AND HIPPOCAMPUS.

F. Cirulli*[1], S. Levine[1], and E. M. Shooter[2]. [1] Department of Psychiatry, and [2] Department of Neurobiology, Stanford University School of Medicine, Stanford, CA 94305.

In the central nervous system (CNS) Nerve Growth Factor (NGF) acts as a target-derived growth factor for developing cholinergic neurons of the basal forebrain. It has been shown that p140^{trk}, a receptor tyrosine kinase, mediates NGF biological activity. We have studied the mRNA expression of p140^{trk} in the developing septo-hippocampal system using a sensitive ribonuclease protection assay. Basal forebrain and hippocampus were dissected from rats at postnatal day (PND) 0, 4, 12, 16, 21 and 60. Total mRNA was extracted and hybridized with an antisense riboprobe. A standard curve was obtained hybridizing increasing amounts of synthetically transcribed sense mRNA. Our results show that mRNA for p140 ^{trk} was barely detectable at birth in both basal forebrain and hippocampus. A significant increase in mRNA occurred around PND 16 and levels increased steadily until adulthood (PND 60). These data indicate that the expression of p140^{trk} mRNA is developmentally regulated in both basal forebrain and hippocampus. The developmental increase in p140^{trk} mRNA parallels the maturation of the septo-hippocampal cholinergic system, suggesting that this receptor contributes to its functional organization.

538.4

NERVE GROWTH FACTOR RECEPTOR (NGFR) IMMUNOREACTIVITY IN THE DEVELOPING HUMAN BASAL FOREBRAIN.

D.Klisovic (1), I.Delalle (1), M.Herlyn (2) and I.Kostovic*(1), (1) Croat. Inst. Brain Res., Sch. of Med., Univ. Zagreb, 41000 Zagreb Croatia, (2) Wistar Inst., Philadelphia, PA19104-4268, USA

Nerve growth factor (NGF) is a trophic factor for the cholinergic neurons in the human basal forebrain (Allen et al. '89). We applied NGFR-immunocytochemistry and Nissl staining on basal forebrain (BF) of 14 postmortem human brains ranging from 15 weeks of gestation (w.g.) to 25 years of life. From 15 to 25 w.g. Nissl stained sections revealed increase in perikaryal size, progressive somal hyperchromasia and the differentiation of all cell groups (Ch1-4) within BF. After 25 w.g. only minor perikaryal enlargement was observed. At the age of 15 w.g. NGFR immunoreactivity (NGFR-IR) was present in pars compacta of the nucleus basalis (Ch4). Positive fibers were noticed in the external capsule. In the subsequent ages increasing amounts of NGFR-IR within perikarya and dendrites of magnocellular somata in all BF subdivisions were found. Axonal pathways were noticed from the age of 25 w.g. onward in the external capsule, intramedullary laminae of globus pallidus, lateral portion of anterior commissure, longitudinal striae and fornices. Our results show a correlation between the morphological and trophic factor's activity maturation in the developing human BF. It is also evident that biochemical maturation of cholinergic neurons in BF is an early prenatal developmental event.

538.5

EXPRESSION OF NERVE GROWTH FACTOR AND ITS HIGH AFFINITY RECEPTOR (*trk*) IN THE RAT AND MONKEY CORTEX.

A.F. Pitts* and M.W. Miller. Depts. of Psychiatry & Pharmacology, Univ. of Iowa Coll. of Med., Iowa City IA 52242 and Dept. of Psychiatry and Res. Serv., V.A.M.C., Iowa City IA 52246.

Nerve growth factor (NGF) is required for the survival of neurons which project axons from the basal forebrain to neocortex. We used immunohistochemical procedures to identify the distribution of NGF and the NGF receptor, *trk*, both in the basal forebrain and neocortex. The procedures relied on polyclonal antibodies to NGF (courtesy of E.M. Johnson) and to *trk* (Oncogene). Some sections were stained with cresyl violet for cytoarchitectonic analyses. NGF-immunoreactive neurons in rat and monkey cortex were distributed in all layers. About one third of the neurons in a particular layer were NGF-positive. The staining was somatic and occasionally extended into the base of the apical dendrite. *trk*-immunoreaction was also identified in all layers of cortex, however, the staining was arranged in pericellular rings. Like NGF, *trk*-immunostaining was associated with about one third of the neurons. In the rat basal forebrain *trk*-immunostaining was cytoplasmic. Most of the cell bodies of basal forebrain neurons were *trk*-positive, and in contrast, only a fraction were NGF-positive. These data are consistent with the model that NGF is produced by cortical neurons which retrogradely maintain the basal forebrain projection neurons.

Funded by the Department of Veterans Affairs and NIH grants DE 07734, AA 06916, and AA 07568.

538.6

DEVELOPMENTAL PATTERN OF TRKB IMMUNOREACTIVITY IN THE MAMMALIAN CEREBRAL CORTEX.

R.J. Cabelli, K.L. Allendoerfer, M.J. Radeke#*, S.C. Feinstein#, and C.J. Shatz. Dept. of Mol. Cell Biol., UC Berkeley, CA 94720, and #Neuroscience Research Institute, UC Santa Barbara, CA 93106.

TrkB, a tyrosine kinase that serves as a receptor for the neurotrophin BDNF, has been shown to be present in the developing mammalian brain. We have recently demonstrated the developmental regulation of trkB protein expression in ferret visual system, using cross-linking of iodinated neurotrophins to receptors (Allendoerfer et al., Soc. Neurosci. Abs, 1992). To determine the specific cellular localization of trkB, an immunohistochemical analysis was performed at ages from E24 to adult, using 2 anti-peptide antibodies directed against distinct epitopes in the extracellular domain of trkB. At prenatal ages, immunostained fibers were observed in the internal capsule and intermediate zone. Within the cerebral hemispheres two distinct fascicles, one deep and one superficial, are present. Fiber staining declined with increasing age, while staining of cell bodies increased with development. At E30 some cells in the preplate were stained. By P10, two populations of cortical neurons were stained: subplate cells and layer 5 pyramidal cells. By P45, the staining for trkB had extended to neurons in all cortical layers, although the intensity of the staining may have been somewhat reduced from that seen at P10. Radially oriented processes, shown by double-labeling with anti-vimentin at P10 to correspond to radial glia, were stained as early as E30. At P10 and, to a lesser extent, P24, we observed cellular staining in the subventricular zone, a mitotic zone thought to be the site of generation of glial cells. Staining of mature astrocytes was observed at P24 and later ages when higher concentrations of antibody were used. Staining of hippocampal and basal forebrain neurons was observed in postnatal animals, as expected. Both the fiber staining seen early in development and the cellular staining of neurons and glia seen later were completely blocked by preincubation of the affinity-purified antibodies with the peptides against which they were made. These data suggest that different subsets of cortical neurons, and both neurons and glia, may bind and respond to neurotrophins such as BDNF at discrete developmental times. [Supported by EY06327 (RJC), ACS grant S-68-91 (MJR), NSF grant IBN-9120836 (SCF), and EY02858 and the Alzheimer's Assn. (CJS)].

538.7

ANTIBODIES RAISED AGAINST HUMAN p140 ^{trkA} PEPTIDE SEQUENCES STAIN A DISTINCT SET OF NEURONS IN THE RAT PERIPHERAL AND CENTRAL NERVOUS SYSTEM.

M Hu-Tsai[1], CJ Woolf[1], G Evan[2], J Winter[3]*. [1]Dept. Anatomy, University College London, London WC1E 6BT, [2]Imperial Cancer Research Fund, Lincoln Inns Flds, London WC2, [3]Sandoz Institute for Medical Research, London WC1E 6BN, UK.

In order to study the distribution of the high affinity NGF receptor, we have raised antibodies in rabbits to 2 distinct 25aa peptide sequences (A_1 & A_2) of the human gp 140^{trkA} with no homology to trkB or trkC. These have partial homology with the corresponding rat gp 140^{trkA} sequences. The antibodies immunoprecipitate a 130-140k protein from lysates of PC-12 cells and neonatal rat DRG. Affinity purification of the antibodies was performed on CNBr-activated sepharose columns using the peptides. No cross reactivity was detected between A_1 serum and A_2 peptide. The flow through of A_1/A_2 serum through A_1/A_2 peptide bound columns lacked antipeptide activity and were used for immunohistochemical controls.

A similar immunohistochemical staining pattern was found for both antibodies. The majority of adult DRG, nodose and SCG neurons were immunoreactive. In the CNS high levels of staining were found in specific motoneuron pools, preganglionic neurons, the pontine nuclei, some hypothalamic nuclei, the geniculate body, particular reticular nuclei and hippocampal cells. The most immunoreactive cells were Purkinje cells in the cerebellum.

This distribution is not fully congruent with the distribution of NGF responsive cells, NGF high affinity binding sites or *in-situ* hybridization for gp140^{trkA} mRNA. Work is in progress to identify which rat trk these antibodies are recognizing.

538.8

NEUROTROPHIN RECEPTORS IN THE DORSAL COLUMN NUCLEI: IMMUNOCYTOCHEMICAL LOCALIZATION AND HIGH AFFINITY BINDING ASSAYS.

D.R. Foschini*, D.P. Crockett and M.D. Egger. Dept. of Neuroscience and Cell Biology, UMDNJ-Robert Wood Johnson Med. Sch., Piscataway, NJ 08854-5635.

Neurotrophin receptors have been immunocytochemically localized in the dorsal column nuclei (DCN) in the brainstem of the rat, suggesting that the DCN may be neurotrophin responsive. The DCN, which consist of the cuneate nucleus (CN) and the gracile nucleus (GN), possess immunoreactivity for *1)* p75^{NGFR}, the common low affinity binding site for all neurotrophins, *2)* trk A, the high affinity nerve growth factor (NGF) receptor, and *3)* trk B, the high affinity brain derived neurotrophic factor (BDNF) receptor. In the CN, p75^{NGFR} immunoreactivity is confined to an intense blotchy pattern in the *middle region.* Trk A and trk B immunoreactivity is more disperse: trk A immunoreactivity is localizable to neuronal-like cell bodies in the CN and fibers in the cuneate fasciculus; trk B immunoreactivity is associated with fibers, astrocyte-like cells and neuron-like somata in the CN and external CN. Immunoreactivity for all three neurotrophin receptors is also present in the GN.

A high affinity NGF binding assay was performed on adult rat brainstem tissue. The middle region of the CN binds ^{125}I-NGF specifically.

538.9

NEURONAL DEFICIENCIES IN MICE LACKING THE NEUROTROPHIN RECEPTOR GENES *trk*, *trk*B AND *trk*C. A.M. Fagan*[1], R. Klein[1,2] S.A. Lira[1], J.D. Wallace[1], S. Bryant[1], L. Long[1], W. Wurst[3], A.L. Joyner[3], M. Barbacid[1], R.J. Smeyne[1]. [1]Department of Molecular Biology, Bristol-Myers Squibb Pharmaceutical Research Institute, Princeton, NJ 08543, [2]EMBL Differentiation Program, G900 Heidelberg, Germany, [3]Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto, Canada

The Trk family of tyrosine kinase receptors are the functional receptors for the NGF family of neurotrophins. The product of the *trk* proto-oncogene serves as the high affinity receptor for NGF, the *trk*B for BDNF and *trk*C for NT-3. Using homologous recombination techniques in ES cells, we generated mice that lack either the Trk, TrkB or TrkC kinase receptors. Heterozygous animals of all three mutant lines show no apparent defects when compared to wild-type animals. However, homozygous *trk*B (-/-) mice die within the first 48 hours, apparently of starvation/dehydration, since no milk is found in their stomachs. Examination of the neural pathways involved in feeding showed significant defects in the trigeminal ganglion and facial motor nucleus. Deficits were also present in two targets that have been shown to be responsive to BDNF, the dorsal root ganglia and motor neurons of the spinal cord. The abnormalities observed in the *trk*B (-/-) mice will be compared to those found in the *trk* (-/-) and *trk*C (-/-) mice.

538.10

DIFFERENTIAL DISTRIBUTION OF mRNAS ENCODING FOR TRKB VARIANTS IN DEVELOPING AND ADULT NERVOUS SYSTEM M. P. Armanini*, H. S. Phillips, E. J. Mufson, D. L. Shelton. Dept. Neuroscience, Genentech, Inc. S. S. F., CA 94080 and Dept. Neurological Sciences, Rush Presbyterian St. Lukes Med. Ctr., Chicago, Ill 60612.

trkB binds both BDNF and NT4/5 with high affinity and binding of these ligands leads to tyrosine phosphorylation and subsequent activation of the receptor. In the rat, there are two described truncated versions of trkB, t1 and t2, which do not contain tyrosine kinase domains. Although the function of these truncated receptors remains obscure, the high degree of homology reported here for mouse and human cDNA clones encoding t1 and t2 suggests a conserved biological role. In situ hybridization using probes specific for the tyrosine kinase containing (TK) or truncated forms (t1, t2) of trkB was used to study the distribution trkB mRNAs.

During embryonic development, both t1 and t2 were prominently expressed throughout the ventricular zone of the neural tube, while expression of TK was confined to regions of postmitotic maturing neurons. In the adult brain, TK expression remained confined to regions of neuronal perkarya, t1 was primarily expressed in choroid plexus and ependyma with lower expression in neurons, and t2 was limited to expression in neurons. Unexpectedly, although TK hybridization displayed a typical pattern of diffuse labeling throughout neuronal cell bodies, hybridization to t2 mRNA displayed a striking perinuclear localization. In a rodent model, age-related decreases of hybridization for t2, but not TK,were observed in hippocampal neurons, suggesting that probes specific for human trkB variants may be useful tools to study human pathological states.

538.11

DEVELOPMENTAL CHANGES IN FULL-LENGTH AND TRUNCATED *TRKB* RECEPTORS IN THE RAT CNS. R.H. Fryer,* D.R. Kaplan,[1] S.J. Rabin,[1] and L.F. Kromer. Dept. of Anatomy & Cell Biol., Georgetown Univ. Medical Center, Washington, DC 20007 and [1]the ABL-BRP, NCI-FCRDC, Frederick, MD 21702

The neurotrophins BDNF and NT-4/5 presumably mediate their trophic effects through full-length *trkB* receptors which contain an intracellular signal transducing tyrosine kinase domain. A truncated form of the *trkB* receptor which lacks the intracellular kinase domain also has been identified in the adult CNS. This receptor appears to be highly expressed in the ependyma and choroid plexus, however, its function is unknown. Thus, the purpose of the present study was to further examine the distribution of truncated and full-length *trkB* receptors in various CNS regions and to compare the relative levels of these receptors during CNS development and maturation. Beginning at embryonic day 14 various regions of the CNS were dissected, proteins were separated by SDS-PAGE, and both types of *trkB* receptors were identified with an antibody against the extracellular domain of the receptor. All brain regions analyzed (ventral mesencephalon, neostriatum, septum, and retina) contain both full-length and truncated receptors. At early developmental time points the full-length receptor predominates. However, as development progresses there is a steady increase in the level of the truncated receptor so that beginning at about postnatal day 5-10, the truncated receptor predominates in most regions. The wide spread distribution of truncated *trkB* indicates that this receptor is expressed in either neurons or glia in addition to the ependyma. Moreover, developmental changes in the relative amounts of full-length and truncated *trkB* receptors suggest that both forms of the receptor may be important for the maturation and normal function of the adult CNS. Supported by NIH grant NS-31445.

538.12

Low Affinity Nerve Growth Factor Receptor (LNGFR) Immunoreactivity in the Dorsal Lateral Geniculate Nucleus (dLGN) of the Adult Cat. E.C. Foley* and H.E. Pearson. Department of Anatomy and Cell Biology, Temple University School of Medicine, Philadelphia, PA 19140.

The LNGFR is one of the components involved in the biological actions of the neurotrophins. Therefore, the localization of the LNGFR within retinal ganglion cell (RGC) target areas is an important step in determining if RGCs can respond to any of the neurotrophins. In this study, we have used immunocytochemistry to localize the LNGFR within target regions of adult cat RGCs. 3 adult cats were perfused with 4% paraformaldehyde, and the dLGN and superior colliculus (S.C.) were sectioned on a vibratome at 40μm. Sections were incubated in BSA and triton in PBS, followed by incubation with a monoclonal antibody to LNGFR. The primary antibody was omitted in control sections. Sections were processed according to the Vectastain ABC immunoperoxidase method, mounted onto gelatin coated slides, dehydrated, and coverslipped. The density of LNGFR-like immunoreactivity in the dLGN and S.C. was determined, and statistical differences between LNGFR-like immunostaining and background staining in the optic tract were calculated. The density of LNGFR-like immunostaining within the dLGN was significantly different from background staining, but there were no differences between LNGFR-like immunostaining and background staining in the S.C. These results provide evidence that one of the components necessary in the actions of neurotrophins is located within the retinogeniculate pathway of the cat.

538.13

EXPRESSION OF EXTRACELLULAR DOMAINS OF $p140^{trkA}$ AND $p145^{trkB}$ IN INSECT CELLS.

M. Ashcroft, D. Dawbarn, S.H. MacGowan, S.J. Allen, R. Feeney, S.M. Colebrook*. Molecular Neurobiology Group, Dept. Med. (Care of the Elderly), Bristol Univ., Bristol, BS2 8HW.

Neurotrophic factors bind with high affinity (Kd of 10^{-11} M) to specific protein tyrosine kinase receptors. These receptors have been designated $p140^{trkA}$, $p145^{trkB}$, $p145^{trkC}$, on the basis of their apparent molecular weight and their specificity for binding NGF, BDNF, and NT-3 respectively. PCR methodologies were used to generate the extracellular regions for the human $p140^{trkA}$ and the rat $p145^{trkB}$ receptors with stop codons added at the 3' end. These were subcloned into the baculovirus transfer vectors, pEVmXIV and pBlueBacHis and used to cotransfect Sf21 cells with BaculoGold™ linearized DNA. Positive plaques were identified and used to generate high titre recombinant viral stocks which were used to infect suspension cultures of insect cells for the production of recombinant secreted (pEVmXIV) and non-secreted (pBlueBacHis) proteins. The 3-dimensional structure of these proteins will be determined by X-ray crystallography.

538.14

CLONING OF FGF RECEPTORS mRNA FROM RAT BRAIN. C.Andersson* and F.Eckenstein.Dept. of Cell Bio. & Anat.,Ore.Health Sci.Univ.,Portland,OR 97210

Five fibroblast growth factor receptors (FGFR) genes are known. Structural features shared by the FGFR family include glycosylated immuno-globin-like (Ig) loops in the extracellular domain and a conserved transmembrane tyrosine kinase domain. Several of the receptors in this family also exhibit alternate splice forms. However, the receptors have only been sequenced from human, mouse, Xenopus, Drophilia, and chick. In order to perform nuclease protec-tion assays in rat, mRNA was isolated from postnatal and adult rat brain, reverse transcribed, and PCR amplified using three degenerate oligo-nucleotides primers. The first primer was from the conserved extra-cellular region; the second and third primers were from the conserved tyrosine kinase one and two regions, respectively. The third primer was used in conjunction with primer one or two. The resulting PCR products yielded two fragments at 500 and 1100 bp, which were subcloned, analyzed by restriction digests and sequenced. Restriction digests revealed a number of possible subtypes for FGFR1 and FGFR2. Sequence analysis reveals a considerable amount of sequence homology between rat FGFRs and FGFRs from other species.

538.15

DIFFERENTIAL EXPRESSION AND ALTERNATIVE SPLICING OF FIBROBLAST GROWTH FACTOR RECEPTORS IN THE CNS. F. Yamaguchi, H. Saya, J. Bruner, W. McKeehan and R.S. Morrison*. Dept. of Neurosurgery, M.D. Anderson Cancer Center, Houston, TX 77030.

Basic fibroblast growth factor (FGF) influences multiple cell types in the CNS. The underlying basis for the diversity of FGF actions on CNS cells is not understood, but may be related to cell type-specific expression of FGF receptors (FGFR). In the present study we evaluated FGFR expression in normal and neoplastic human brain using RT-PCR southern blotting and immunocytochemistry. FGFR1 mRNA expression was abundant in normal cortex (grey matter), but was expressed at low to non-detectable levels in normal white matter. Conversely, white matter expressed abundant levels of FGFR2. Consistent with its expression in white matter, benign astrocyte tumors also contained abundant levels of FGFR2 mRNA. However, FGFR2 expression diminished in astrocytic tumors in relation to their loss of differentiation. FGFR1 antibodies strongly labeled neurons in frozen sections of adult human brain but only stained short, delicate cell processes in normal white matter. Malignant astrocytes stained intensely for FGFR1. Normal cortex expressed an alternatively spliced form of FGFR1 mRNA containing three immunoglobulin (Ig) domains while malignant astrocytes expressed a form containing two Ig domains. The results of the present study suggest that 1) glial cells may preferentially express FGFR2 while, FGFR1 expression is principally restricted to neurons, 2) FGFR2 expression may be required for astrocyte differentiation and 3) transformed astrocytes express an alternatively spliced form of FGFR1 that may provide them with an increased growth advantage.

538.16

***In Situ* Hybridization of *trk*B in the Developing Chick Visual System and Brain** J.E. Johnson*[1], S.W. Wang[1], A. Garner[2], K. Baeshore,[2] T. Large[2], S. McKay[1], R.W. Oppenheim[1]. [1] Dept. of Neurobiology and Anatomy, Bowman Gray School of Medicine of Wake Forest University, Winston-Salem, N.C. 27157 [2]Dept. Neurosci., Case Western Reserve Univ. Sch. of Medicine, Cleveland, OH 44106

Results from previous studies have indicated that brain-derived neurotrophic factor (BDNF) can support the survival of isolated retinal ganglion cells *in vitro* (J. Neurosci. 6(10): 3031-3038). *TRK*B, a member of the *trk* family of tyrosine kinase receptors, appears to transduce the cell survival activity of BDNF. Recent observations from Garner and Large have revealed alternate 5' and 3' splice variants for both *trk*B and *trk*C in the chick. These include kinaseless variants similar to those reported in both rat and mouse.

In situ hybridization was performed with riboprobes prepared against the extracellular domain of *trk*B on the developing chick retina and brain to localize the production of the BDNF receptor during normal chick visual system development. Larger cells within the ganglion cell layer of the E18 chick retina showed specific labeling when compared with sense controls. In addition, a few cells on the inner margin of the inner nuclear layer were also specifically labeled. Interestingly, specific labeling was observed in the stratum griseum centrale of the optic tectum at a variety of ages. Labeling was also observed in a variety of other brain nuclei including midbrain nuclei associated with the visual system as well as non-visual areas. Results from these localization studies are consistent with the hypothesis that BDNF may play a role in the development of the chick visual system as well as other regions of the avian brain. Experiments are in progress to further map the expression of splice variants for both *trk*B and *trk*C during normal development in the chick visual system and brain.

538.17

TRKB IMMUNOREACTIVITY BUT NOT TRKB mRNA IS MODULATED BY MONOCULAR DEPRIVATION DURING THE CRITICAL PERIOD. A. Cellerino, Y. Bozzi, R. Siciliano, L. Domenici* and L. Maffei. Neurophysiol. Inst. (CNR) and Scuola Normale Superiore, Pisa, Italy.

Recently it has been reported that an exogenous supply of NGF prevents the effects of monocular deprivation (MD) suggesting a role for NGF and possibly other related neurotrophins in visual cortical plasticity. Here we investigated whether MD modulates neurotrophins receptors at the level of visual cortex. Ten hooded rats (Long Evans) were monocularly deprived under chloral hydrate anaesthesia at different ages of postnatal development (N=6 rats at P15, N=2 at P23 and N=2 at P30) for a period of two weeks. MD effects were evaluated using immunohistochemical (policlonal antibody to TrkB, Santa Cruz, USA) and in situ hybridization techniques on tissue fixed slices. Policlonal antibodies and riboprobes were directed versus the intracellular domain and both recognize the full length form of TrkB.

We found that TrkB like immunoreactivity is reduced in the visual cortex contralateral to deprived eye; this effect was present in the groups of animals deprived at P15 and P23 but not in animals deprived at P30. On the contrary TrkB mRNA was normal in MD animals. We conclude that MD modulates TrkB probably acting at a posttrasductional level. This modulation is restricted to a time window of postnatal development overlapping the rat critical period for MD effects.

538.18

LEVELS AND RATIO OF RGNTF AND ITS RECEPTOR CONTRIBUTE IN THE DETERMINATION OF GROWTH AND APOPTOSIS OF NEONATAL RAT RETINAL GANGLION CELLS. **F Ren* and RMW Chau, Department of Anatomy, Univ. of Hong Kong, Hong Kong.**

Survival of only 30% RGCs in neonatal rat retina depended on the supply of a retinal ganglion neuronotrophic factor (RGNTF) from the superior colliculus. The apoptosis of RGCs may be due to their own turn-off expression of RGNTF. RGNTF monoclonal antibody (MAb) was raised and used to raise anti-idiotypic antibody (Id Ab). These MAb and Id Ab were used to localize the RGNTF and its receptor in the retinas of rats aged E16 through PO to adult. Results revealed that the RGNTF immunoreactivity in RGCs is highest at E16 about 3X of that at PO or 4X of P12, while that for its receptor is highest at E18 about 16% higher than that at E16 or 2.6X of PO. Ratios of the immunoreactivities of RGNTF and its receptor is 3:1 at E16, and 2:1 at E18-PO. These results indicated that at E16 RGCs divide rapidly with a factor/receptor ratio at 3:1, while from E18 to PO these RGCs undergo processes of migration, differentiation and maturation with the ratio at 2 to 1. It seems that besides other intrinsic or extrinsic factors, the ratio of RGNTF and its receptor contributes in the early proliferation and differentiation of RGCs while the apoptosis of neonatal RGCs is induced by the reduced expression of RGNTF and its receptor in embryonic RGCs as well as the reduced supply of RGNTF from the superior colliculus.

538.19

NEUROTROPHIN RECEPTORS DURING DEVELOPMENT OF THE CHICK AND RAT RETINA. Nestor G. Carri* [1,2], Reg Williams [2], Anders Bäckström [2], Klas Kullander [2], Finn Hallböök and Ted Ebendal [2]. Department of Developmental Biology [2], Biomedical Center, Uppsala University, Box 587, S-751 23 Uppsala, Sweden, and IMBICE [1], La Plata, Argentina.

The neurotrophin family is a group of distinct molecules which enhances neuronal survival and differentiation, including neurite outgrowth. However, little is known of the effects of these neurotrophins on the subpopulations of neurons within the embryonic retina, nor of the high-affinity receptors of the TRK family of tyrosine kinases that these cells are expressing during development. We used molecular methods to produce recombinant neurotrophins in order to analyse the effects of NGF, BDNF, NT-3 and NT-4 on fibre outgrowth from chick retinal explants at E6 and rat retinal explants at E16 put on collagen gels. The resulting neurite outgrowth was measured and dose-dependent responses to the neurotrophins were obtained. In particular, the retina responded well to BDNF and NT-3. To study the expression of TRKs we cloned and sequenced cDNAs encoding trk, trkB and trkC from the chick retina. The cellular expression of TRKs were then analysed by in situ hybridization using corresponding specific antisense oligonucleotides. The results revealed differential expression of the neurotrophin receptors in different cell layers of the retina at different stages of development, providing a base for the observed growth responses to the different neurotrophins seen in culture. It is concluded that different retinal neurons are the targets for several members of neurotrophin family thus likely to have developmental functions in the establishment of the visual pathways. (CONICET-PID-BID 1431-91, Swedish NSF and MRC).

538.20

ISOLATION OF HIGH-AFFINITY TRK RECEPTORS, FROM THE CHICKEN AND THEIR EXPRESSION IN EMBRYONIC SENSORY GANGLIA. A. Bäckström, R. Williams, F. Hallböök and T. Ebendal*, Dept. of Developmental Biology, Biomedical Center, Uppsala University, Box 587, S-751 23, Uppsala, SWEDEN.

The neurotrophin family consists of four members, NGF, BDNF, NT-3 and NT-4/NT-5. All neurotrophins are able bind to the low-affinity NGF receptor p75 , with similiar affinity. However this receptor is unable to mediate a cellular response and hence its function is not clear. The functional response to the neurotrophins is mediated by high-affinity receptors, of which three receptors are currently known; trk, trkB and trkC which belong to the TRK family of tyrosine kinase receptors. Recent studies have also indicated the existence of different isoforms of trkB and trkC which are able to bind neurotrophins at high-affinity and yet, because these isoforms lack an intracellular kinase domain there is no signal transduction. Using degenerate oligonucleotides and PCR we have isolated DNA fragments for the different trk:s in chick. These fragments have then been used to screen cDNA libraries from different embryonic organs to get full-length sequences. From these sequences, it has been possible to design specific oligonucleotides for the different trk:s and their isoforms, for use in *in situ* hybridization. In this way we have looked at the mRNA expression in the sensory ganglia at embryonic day 9 in the chick. 5´primer extension reactions allowed for cloning of full-length sequences, genomic clones to allow for examination of promoter regions are now in progress.

538.21

mRNA EXPRESSSION FOR THE HIGH-AFFINITY NEUROTROPHIN RECEPTORS DURING CHICK EMBRYONIC DEVELOPMENT. R. Williams*, A. Bäckström and T. Ebendal. Department of Developmental Biology, Uppsala University, Biomedical Centre, S-751 23 Uppsala, SWEDEN.

Antisense oligonucleotides specific for the high-affinity neurotrophin receptors trk, trkB and trkC were used to examine the expression of mRNA for these receptors during chick embryonic development. Special emphasis was placed on the high-affinity receptor expression in the developing sensory nervous system, on which an examination was performed from stage 15 through to E18. TrkC mRNA expression was observed in the newly forming trigeminal ganglion at stage 15, in the cells of the trigeminal placode and in the cells migrating from this placode. TrkB and trk mRNA expression within the sensory ganglia were first evident at consecutively later stages of development and in complementary neurons within this, and other ganglia. At all stages subsequent to the initial expression of the mRNA for each of these receptors, the continued expression was evident. In each of the sensory ganglia examined there existed a typical pattern of expression for each receptor mRNA which became increasingly evident as development progressed. At E18, the neurons expressing mRNA for each of the high-affinity receptors have been characterized in respect to the relative percentages and distribution of each within the ganglia and their neuronal diameter.

538.22

Murine cell lines of septal origin are responsive to CNTF

P. Rajan, A. J. Symes*, B. H. Wainer and J. S. Fink
Molecular Neurobiology Laboratory, Massachusetts General Hospital and Department of Neurology, Harvard Medical School, Boston, MA 02114; Department of Pathology, Albert Einstein College of Medicine, Bronx, New York.

Ciliary neurotrophic factor (CNTF) influences the survival and development of motor and sensory neurons, the differentiation of sympathetic neurons and the survival of some central nervous system (CNS) neurons after injury. We have previously shown that a 180 base pair cytokine responsive element (CyRE) mediates transcriptional activation of the vasoactive intestinal peptide (VIP) gene in response to CNTF in a human neuroblastoma cell line NBFL. To begin to elucidate the molecular mechanism of action of CNTF on cells derived from the CNS, we have employed cell lines (SN48 and SN56) derived by fusing N18TG2 neuroblastoma cells with murine septum. As CNTF has been shown to produce survival of rat septal neurons after transection of the fimbria-fornix, we reasoned that septal-derived cell lines may be CNTF-responsive. We tested whether the CyRE of the VIP gene (linked to luciferase) would mediate transcriptional activation in response to CNTF in SN48 and SN56 cell lines. CNTF treatment of SN48 and SN56 cells transfected with the CyRE-luciferase gene reporter increased luciferase activity 5 and 3 fold respectively. Our results indicate that the cell lines of septal origin are responsive to CNTF and suggest that common molecular mechanisms may mediate CNTF responsiveness in cell lines of CNS and PNS origin.

538.23

EVIDENCE THAT THE EFFECTS OF CILIARY NEUROTROPHIC FACTOR (CNTF) IN THE CENTRAL NERVOUS SYSTEM (CNS) ARE MEDIATED VIA ASTROCYTES. R.E. Clatterbuck*, D.L. Price and V.E. Koliatsos. The Johns Hopkins Univ. Sch. of Med., Balto., MD 21205.

The neural cytokine CNTF has effects on broad classes of neurons in both the CNS and peripheral nervous system as well as some glial cells *in vitro*. CNTF has also been shown to exert effects on various populations of neurons *in vivo*. In the CNS, the expression of both CNTF and its signal transduction proteins is widespread. To examine the possible role that astrocytes might play roles in CNTF signalling cascades, we have studied the model of anterior thalamic degeneration following cingulectomy. In this model, CNTF prevents the retrograde death of thalamic neurons [Clatterbuck et al., *Proc. Natl. Acad. Sci USA.* **90**:2222, 1993]. Using immunocytochemistry for glial fibrillary acid protein (GFAP), we have demonstrated a profound transformation of astrocytic morphology following the rescue of anterior thalamic neurons with CNTF. These astrocytes are "gemistocytic" in appearance as compared to the reactive, fibrous astrocytes seen in CNS lesions. In addition, we have shown that the injection of CNTF into the undamaged CNS induces the expression of GFAP and the alteration of astrocytic morphology well beyond that seen in control injections. Using reverse transcriptase-polymerase chain reaction, we have also demonstrated the expression of mRNA for CNTF and for CNTF receptor α in the optic nerve, a pure population of CNS glia. Given that CNTF in the CNS is probably produced by astrocytes, we propose an autocrine role for this peptide in response of the CNS to neuronal injury.

538.24

RETROGRADE TRANSPORT OF NEUROTROPHIN RECEPTORS. M.D. Ehlers[1], D.R. Kaplan[2] and V.E. Koliatsos*[1]. The Johns Hopkins Univ. Sch. of Med., Balto., MD 21205 and National Cancer Institute, Frederick, MD 21702.

The neurotrophin family of growth factors (of which nerve growth factor [NGF] is the prototype) consists of a group of small polypeptides that are synthesized by target tissues and that exert their influence on neurons at the nerve terminal. Although much is known about the local molecular signal transduction events mediated by neurotrophins, very little is known about the nature of the retrogradely transported neurotrophin signals sent from nerve terminals to cell bodies. The neurotrophins, as well as the low-affinity NGF receptor p75, are retrogradely transported. It is possible that, upon NGF binding, the NGF/NGF receptor complex is itself internalized and transported, representing the primary trophic signal. We have examined this hypothesis by studying the retrograde transport of the high-affinity NGF receptor, trkA, in the adult rat sciatic nerve. Following sciatic nerve ligation (18 hours), trkA protein accumulates distally to the ligation site, as determined by Western blot analysis. This accumulation is enhanced by injecting NGF into the rat footpad and is abolished by the injection of blocking NGF antibody. In addition, by immunoprecipitation and phosphotyrosine Western blot, we observe that trk species, which accumulates distally but not proximally to the ligation site, is tyrosine phosphorylated. NGF injection increases the accumulation of this tyrosine phosphorylated species, and blocking NGF antibodies abolish the accumulation. Taken together, our results provide *in vivo* evidence for the retrograde transport of trkA in a tyrosine phosphorylated state. These data suggest that active trkA tyrosine kinase could be the primary retrograde neurotrophin signal.

538.25

NEUROTROPHINS BINDING TO trkB ARE TROPHIC FACTORS FOR MAMMALIAN MOTOR NEURONS. M.H. Cayouette, R.E. Clatterbuck, L.R. Berkemeier, J.W. Winslow[1], A. Rosenthal, G.K. Gouras*[2], D.L. Price, and V.E. Koliatsos. The Johns Hopkins Univ. Sch. of Med., Balto., MD 21205 and [1]Genentech, South San Francisco, CA 94080, [2]The Harvard Med. Sch., Boston, MA 02115.

Although earlier studies indicate that neurotrophins do not influence α-motor neurons, it appears that a subset of this family of peptides transduced via trkB (brain derived neurotrophic factor [BDNF] and neurotrophin-4/5 [NT-4/5]) are potent trophic factors for motor neurons. First, both BDNF and NT-4/5 are expressed in striated muscles during the period of developmental death of motor neurons and are regulated by injury (muscle denervation). Second, α-motor neurons express a catalytic isoform of trkB, the high-affinity receptor for BDNF and NT-4/5, throughout their postnatal life. Third, NT-4/5 and BDNF are transported retrogradely to α-motor neurons in a saturable manner; retrograde transport is not blocked by an excess of heterologous neurotrophins. Finally, BDNF and NT-4/5 promote the survival of α-motor neurons following axotomy in neonatal animals. The patterns of expression and effects of BDNF and NT-4/5 on α-motor neurons raise the possibility that some neurotrophins may be useful in treating patients with motor neuron disease.

538.26

DIFFERENTIAL EXPRESSION OF *trk* and *trkB* IN ENTERIC AND ENDOCRINE CELLS OF THE RAT DIGESTIVE SYSTEM. C. Sternini, D. W. Rickman, R. De Giorgio* and N. C. Brecha. CURE and Depts. of Medicine and Anatomy & Cell Biology, UCLA and VAMC-West Los Angeles, LA, CA 90073.

We reported the presence of immunoreactivity (IR) for the neurotrophin high-affinity receptors, *trk* and *trkB*, in the developing enteric nervous system (ENS) of the rat, suggesting a role of neurotrophins in the developmental regulation of the rodent gut. We further investigated the sites of expression of *trk* and *trkB* in the fetal and adult rat digestive tract with single and double-label immunohistochemistry. *trk*- or *trkB*-IR is present in the developing myenteric plexus at low levels at embryonic day (ED) 14, and by ED 18-20 it is strong in both enteric plexuses, and in fibers in the gut wall, vasculature and pancreas. *trk*- and *trkB*-IR fibers and cell bodies are immunolabeled with neurofilament antibodies confirming their neuronal nature. The density of *trkB*-IR in the ENS decreases postnatally, whereas *trk*-IR remains abundant throughout adulthood. Interestingly, *trkB*-IR is also detected in gut and pancreatic endocrine cells from prenatal ages to adulthood. Pancreatic *trkB*-IR islet cells are distinct from somatostatin, insulin or pancreatic polypeptide cells, and are likely to be glucagon cells. *trkB*-IR in endocrine cells is not blocked by an excess of pancreatic hormones. *trk*- or *trkB*-IR is blocked by the homologous antigen. This study suggests that neurotrophins play a broader role than simply being target-derived neurotrophic molecules and that each neurotrophin has distinct functions in the gut.

Supported by NIH grant DK37852 and VAMC Merit Review Funds.

538.27

TRKB mRNA EXPRESSION IN THE CHICK EMBRYO: CORRELATION WITH BDNF RESPONSIVENESS AND BINDING.

S. Biffo*, G. Dechant, H. Okazawa and Y.-A. Barde. Max-Planck-Institute for Psychiatry, Dept. of Neurobiochemistry, D-82152 Planegg-Martinsried FRG..

Brain-derived neurotrophic factor (BDNF) allows the survival of a variety of embryonic neurons, as shown, for the most part, with cells isolated from chick embryos. In mammals, the tyrosine kinase trkB has been shown to be a receptor for BDNF. In order to examine the extent to which the cellular expression of trkB correlates with known targets of BDNF, trkB-encoding c-DNAs were isolated. In situ hybridisation studies were performed using sections of E3-E14 chicks and a riboprobe recognising a 9 kb transcript encoding for a full-length receptor. In all ganglia from the peripheral nervous system, a good correlation was found between BDNF responsiveness and trkB expression (e.g., trigeminal, acoustic, nodose, spinal sensory ganglia were positive, whereas sympathetic and ciliary ganglia were negative). In the CNS, low levels of expression were detected over the area of the spinal cord containing motoneurons (known to respond to BDNF in vivo). So far however, we were unable to detect trkB expression in developing retinal ganglion cells, which is surprising as these cells are known to respond in vitro to BDNF. Using a riboprobe detecting also truncated forms of trkB, expression was found (as in rodents) in non-neuronal cells such as ependymal and mesenchymal cells. In the developing acoustic system, the expression of truncated trkB was seen in cells forming a ring surrounding the sites of expression of both BDNF mRNA (developing cochlea) and of full-length trkB mRNA (acoustic ganglion). By receptor autoradiography on frozen sections with ^{125}I-BDNF (used at $3x10^{-11}$ M), not only neurons expressing full-length trkB, but also the cells expressing truncated form of trkB were found to be labelled. We are currently investigating the extent to which binding of labelled BDNF matches with the expression of trkB, as investigated by in situ hybridisation.

538.28

trk EXPRESSION IN ADULT PRIMARY SENSORY NEURONES PROJECTING TO DIFFERENT PERIPHERAL TARGETS

S. B. McMahon*[1], M. P. Armanini[2], L. H. Ling [2], H. S. Phillips[2], [1]DeptPhysiology, UMDS, London SE1 7EH, and [2]Dept Neuroscience, Genentech, South San Francisco, CA 94080.

Primary sensory neurones projecting to skin, muscle, and visceral targets were identified by applying flurogold to the cut saphenous, gastrocnemius or pelvic nerves, respectively, in adult rats under pentobarbitone anaesthesia with sterile precautions. 48 hrs later the appropriate dorsal root ganglia (DRG) were fixed and removed. 14 micron cryostat sections of the ganglia were hybridized in situ with S^{35}-labelled cRNA riboprobes against trkA, or tyrosine kinase containing forms of trkB or C. After 3-4 weeks of emulsion autoradiography, fluorogold-containing cells expressing a particular trk could be clearly identified by the high concentration of silver grains distributed over the cell soma. Afferents innervating different targets exhibited distinct patterns of trk expression, as follows:

	cutaneous	visceral	muscle
trkA	35%	92%	20%
trkB	24%	91%	50%
trkC	16%	0%	73%

Clearly, some trks must be co-expressed in some afferents. Conversely, some afferents, notably at least about 30% of those innervating skin, do not appear to contain any known trk. This last conclusion was further supported by hybridizing some sections of L4/5 DRG with a 'cocktail' of antisense trk probes. 30% of neurones(small diameter) exhibited no labelling above background levels.

538.29

NEUROTROPHIN RECEPTOR GENE EXPRESSION IN DEVELOPING DRGs.

X. Mu, I. Silos-Santiago, R. Gerfen, W. D. Snider*. Dept. Neurology, Washington Univ., Med. Sch., St. Louis, MO 63110.

Expression of the neurotrophin receptors, *trk*A, *trk*B, *trk*C, and p75 is thought to determine the neurotrophin (NT) responsiveness of different populations of central and peripheral neurons. In order to better understand the functions of these receptors during development, we have studied their expression in developing rat DRGs by *in situ* hybridization. Expression of *trk*s is readily apparent by E12, only 24 hours after neurogenesis begins in DRGs. mRNA for these receptors appears to be diffusely expressed at E12. However, even at this early age, none of the *trk*s is expressed by all DRG cells. By E15, distinct patterns of receptor gene expression in DRGs are apparent in that different *trk*s are expressed by neurons whose soma areas show differing size-frequency profiles. These distinct patterns persist into adulthood. p75 expression is also readily apparent by E12. Interestingly, p75 was not expressed by all DRG neurons at any developmental stage. Comparisons of percentages of p75 and *trk* expressing DRG neurons suggest that p75 is not invariably co-localized with a member of the *trk* family. Surprisingly, at PN21, size-frequency histograms of p75-expressing neurons are skewed toward larger cell diameters. We have also examined expression of one truncated form of *trk*B neurotrophin receptor, $gp95^{trkB}$. Expression of $gp95^{trkB}$ was observed in ependymal cells around the central canal. However, we did not detect expression of $gp95^{trkB}$ in spinal cord or DRG neurons at any developmental stage.

The expression of *trk*s and p75 in DRGs by E12 suggests that DRG cells are competent to respond to target-derived and locally expressed NTs from the earliest stages of DRG development. By E15, *trk*s are expressed by DRG cells with differing morphologies suggesting responsiveness to specific NTs for different classes of DRG cells at later developmental stages. Finally, the pattern of expression of p75 suggests that *trk*s are not co-localized with p75 in all DRG cells.

538.30

IMMUNOCYTOCHEMICAL LOCALIZATION OF TRKA IN RAT DORSAL ROOT GANGLIA AND SPINAL CORD.

D.C. Molliver*, M. J. Radeke, Q. Yan, S.C. Feinstein, W.D. Snider, UC Santa Barbara, Amgen, and Dept. Neurology, Washington Univ. Sch. Med., St. Louis, MO 63110.

One impediment to the study of neurotrophin actions has been the lack of immunocytochemical markers for the trk family of high-affinity receptors, which would allow the localization of receptor proteins within diverse populations of neurons. We used a recently generated antibody to the high-affinity nerve growth factor (NGF) receptor, trkA, to examine the distribution of the protein in dorsal root ganglia (DRG) and spinal cord of the rat. A polyclonal antibody to the intracellular domain of trkA, designated antitrkIn, has been generated against recombinant trkA intracellular domain and affinity-purified against full-length rat trkA . We tested the selectivity of antitrkIn by examining the pattern of trkA-like immunoreactivity (trkA-LI) in superior cervical (SCG) and nodose ganglia, two ganglia known to contain very different percentages of trkA mRNA-expressing neurons. Staining conformed to the results of in situ hybridization studies reported by Richardson et al (1992, J. Neurosci., 12:4011-4022): virtually all SCG neurons were labelled, whereas only a very few cells were immunoreactive in the nodose ganglia. In contrast to labelling in nodose and SCG, trkA-LI in the DRG was restricted to a population of small and medium-size neurons, and was evident in embryonic ganglia by E-15. Most, but not all small cells were immunopositive. Size-frequency histograms of selected DRG sections indicated a mean soma area for immunoreactive cells of 318 um^2, as opposed to 615 um^2 for negative cells. Consistent with the small size of labelled cells, immunoreactive fibers entering the spinal cord were restricted to lamina I and II of the dorsal horn, the target fields of Aδ and C primary afferent fibers.

Colocalization of trkA-LI with other immunocytochemical markers should make it possible to characterize functional classes of DRG neurons that express trkA and are thus responsive to NGF.

Supported by ACS CA div. S68-91, NSF IBN-9120836, NIH NS25936.

538.31

NEUROTROPHIN RECEPTOR (*TRK*A AND *TRK*C) mRNA EXPRESSION IN EARLY EMBRYONIC QUAIL TISSUES AND DORSAL ROOT GANGLIA (DRG).

D. Zhang*, L. Yao, and P. Bernd. Department of Anatomy and Cell Biology, State University of New York, Health Science Center, Brooklyn, NY 11203.

In situ hybridization was used to determine whether mRNA for the neurotrophin receptors *trk*A and *trk*C was expressed in neural crest cells and DRG of quail embryos. Examination of cryostat sections following *in situ* hybridization with ^{35}S labelled riboprobes (kindly provided by Dr. L. Parada) and radioautography revealed the presence of *trk*C mRNA in migrating neural crest cells (E2.5-3; stages 18-20), as well as in DRG throughout development. Control sections were hybridized with a labelled sense probe. The presence of trkC mRNA was also demonstrated by RT-PCR to be present in the neural tube of E2 (stages 12-14) quail, an age at which the neural crest is still adherent to the neural tube. The cellular localization of *trk*C mRNA in DRG varied during development. At early ages (E3-4.5; stages 22-25), *trk*C mRNA was homogenously expressed on most cells in the DRG. These are small phase-dark cells which may represent neuroblasts and/or support cell precursors. At older ages (E5, 6.5, 7, 10; stages 27, 30, 32, 36), *trk*C mRNA is expressed in a subpopulation of large phase-bright neuron-like cells in the ventral part of the DRG. In contrast, *trk*A mRNA is homogeneously distributed in DRG throughout development. Furthermore, *trk*A mRNA appears to be less abundantly expressed at earlier stages (i.e. E3-3.5; stages 21-23). These results suggest that NT-3, the preferred ligand for *trk*C, may play an important role in neural crest cell migration and differentiation. Our accompanying abstract (Yao et al.) demonstrates the presence of NT-3 mRNA in early embryonic tissues. Supported by a grant from the Dysautonomia Foundation.

538.32

DIFFERENTIAL DISTRIBUTION OF *trk*C-POSITIVE SENSORY NEURONS IN SPINAL GANGLIA.

R. A. Oakley*, A.S. Garner, T. H. Large and E. Frank. Dept. of Neurobiology, University of Pittsburgh School of Medicine, Pittsburgh, PA 15261 and Dept. of Neuroscience, Case Western Reserve University, Cleveland, OH 44106.

In a previous study, neurotrophin-3 (NT-3) was found to support the survival of more chick sensory neurons from ganglia at limb levels than from thoracic ganglia. Moreover, identified muscle afferents survived best when cultured in NT-3 as opposed to other neurotrophins (Hory-Lee et al., PNAS 90:2613). These results suggest that NT-3 may be a selective neurotrophin for muscle afferents and that segmental differences in NT-3 responsiveness are due to the presence of more of these neurons in limb-level ganglia.

If NT-3 is a selective neurotrophin for muscle afferents, these neurons should express *trk*C, a receptor for NT-3. As a first step to determine if muscle afferents express *trk*C, we used in situ hybridization to compare the message levels for this receptor at limb vs. thoracic level dorsal root ganglia (DRG) in stage 36 chick embryos. The probes were complementary to the unique extracellular domain of *trk*C from chick.

Although *trk*C-positive neurons populate both lumbar and thoracic DRG, they comprise a greater proportion of DRG cells at lumbar levels. More than 8% of the cells in lumbar ganglia were *trk*C-positive. In contrast, fewer than 3% of the cells in thoracic ganglia were *trk*C-positive. Since muscle afferents populate both thoracic and lumbar DRG but comprise a larger proportion of the neurons at lumbar levels, our findings are consistent with the hypothesis that muscle afferents express *trk*C. Moreover, virtually all of the *trk*C-positive cells we observed at both axial levels were large cells, consistent with the idea that they are muscle spindle afferents. To confirm the identity of these *trk*C-positive neurons we are using in situ hybridization in combination with retrograde labeling from either muscle or cutaneous nerves.

Supported by grants from the NIH.

538.33

MOLECULAR CLONING AND EXPRESSION OF HUMAN trk, trkB, AND trkC. D. L. Shelton*, J. Sutherland, K. Carroll, and S. Broz. Depts. of Neuroscience and Cell Genetics, Genentech, Inc. South San Francisco, CA. 94080.

In order to better understand their normal function and possible roles in neurodegenerative disease, human cDNA clones were isolated for each of the currently known members of the trk family of tyrosine kinase receptors. The sequence of these clones was determined and found to be very similar to that of previously isolated clones from other species. In addition to the tyrosine kinase containing forms of the receptors, clones encoding two forms of truncated trkB and one form of truncated trkC were obtained. In clones encoding the extracellular domains, two different sequences were obtained for both trk and trkC, presumably due to alternate splicing.

Protein was expressed for each of these members of the trk family by transfecting cDNA encoding the entire receptor into mammalian cells. Expression of the protein was confirmed by metabolic labeling, binding of neurotrophin and ligand induced or overexpression induced phosphorylation. In all cases, the appropriate size protein was synthesized, expressed at the cell surface, bound ligand and had tyrosine kinase activity. In separate experiments, fusion protein was produced which consisted of the extracellular domain of a trk fused to the fc tail of a human IgG. These were secreted into media, and demonstrated high affinity ligand binding.

538.34

GENERATION OF ANTI-HUMAN NEUROTROPHIN-4/5 SPECIFIC MONOCLONAL ANTIBODIES. J.S. Hongo, A. Rosenthal* and B.M. Fendly. Departments of Medicinal and Analytical Chemistry and Neuroscience, Genentech, Inc., South San Francisco, CA 94080.

A panel of nine monoclonal antibodies (MAbs) specific to human neurotrophin-4/5 (hNT-4/5) have been developed and characterized. Mature hNT-4/5 has about 50-65% homology with nerve growth factor, brain-derived neurotrophin factor, neurotrophin-3 and Xenopus neurotrophin-4, and mediates its action through the trkB tyrosine kinase receptor. hNT-4/5 is expressed in many peripheral organs and its tissue distribution correlates with its putative role as a target-derived survival factor for peripheral neurons.

Conventional techniques were used to develop and characterize the MAbs, which included the analysis of purity and specificity, functional pairing, epitope specificity, affinity ranking and the ability of the MAbs to block hNT-4/5 binding to the trkB receptor.

Eight MAbs are capable of binding non-reduced and reduced protein. The MAbs have demonstrated the ability to pair in ELISA, appear to be directed against at least two distinct epitopes, and have demonstrated a wide range of relative affinities. Four MAbs appear to block binding of hNT-4/5 to the trkB receptor. Novel, specific MAbs to hNT-4/5 should be useful for studying the *in vivo* and *in vitro* expression of hNT-4/5.

538.35

EXPRESSION OF RECEPTOR TYROSINE PHOSPHATASE-σ (RPTP-σ) IN THE RAT NERVOUS SYSTEM. H. Wang*, H. Yan, O. Silvennoinen, A. Grossman, J. Schlessinger, J.M. Musacchio. Dept. of Pharmacology, N.Y.U. Med. Ctr., New York, NY 10016.

RPTP-σ, cloned from a rat brain stem cDNA library, has the typical structure of a type II receptor tyrosine phosphatase. The extracellular domain is composed of 3 immunoglobulin and 5 fibronectin type III-like repeats. The cytoplasmic portion has two phosphatase domains. A probe complementary to the cDNA of RPTP-σ from nucleotide 2918 to 2967 was synthesized, purified and 3'-end labeled with [α-^{35}S]dATP. Northern blot and *in situ* hybridization studies indicated that RPTP-σ is expressed primarily in the CNS, where it can be detected at E12. RPTP-σ is expressed throughout development in most of the CNS, including the spinal cord and retina. The mRNA is also abundant in the trigeminal and dorsal root ganglia. The mRNA levels decrease progressively after birth. During the postnatal maturation of the cerebellum, the mRNA expression follows the migrating pattern of the granule cells. In the adult rat brain, the expression is seen in the hippocampus, cerebellum, olfactory bulb and cortex. Within the hippocampus, it is present in the pyramidal cell layer, and in the granular cell layer of the dentate gyrus. Labeling was also observed in several thalamic, pontine and oculomotor nuclei. The temporal pattern of RPTP-σ mRNA expression indicates that this phosphatase may play an important role in the development of the nervous system.

538.36

CLONING OF RECEPTOR TYROSINE PHOSPHATASE SIGMA THAT IS HIGHLY EXPRESSED IN THE NERVOUS SYSTEM. H. Yan, A. Grossman, H. Wang, P. D'Eustachio+, K. Mossie# J. M. Musacchio, O. Silvennoinen, R. Kris*, and J. Schlessinger. Departments of Pharmacology and Biochemistry+, NYU Medical Center, New York, NY 10016; Sugen#, 515 Galveston Drive, Redwood City, CA 94063-4720

RPTP Sigma (RPTP-σ), a novel transmembrane receptor protein tyrosine phosphatase, was cloned from a rat brain stem cDNA library. The extracellular segment of RPTP-σ contains 824 amino acids and is composed of 3 Ig-like and 5 fibronectin type III (FNIII)-like repeats. The 627 amino acid cytoplasmic region of RPTP-σ consists of two catalytic domains oriented in tandem. RPTP-σ is most homologous to rat LAR, with 70% overall identity. It is a new member of the type II receptor tyrosine phosphatases. Northern blot analyses indicate that RPTP-σ is highly expressed in the brain as a 5.7 kb transcript. RPTP-σ is also expressed in the lung and intestine as a 6.9 kb transcript but at significantly lower level. *In situ* hybridization studies confirm that RPTP-σ is localized predominately in the nervous system and can be detected in the rat as early as embryonic day 12 (E12). In adult rat brain, expression is mostly seen in the olfactory bulbs, the cerebellum, and hippocampus. Transfection of RPTP-σ cDNA into human embryonic kidney 293 cells results in the synthesis of a protein with an apparent molecular weight of 200 kDa as detected by immunoprecipitation and immunoblot analyses using polyclonal antibodies against the FNIII-like repeats present in the extracellular domain of RPTP-s. RPTP-σ, like LAR and PTP-κ, undergoes proteolytic processing. The gene for RPTP-σ has been mapped to distal chromosome 17 in the mouse.

538.37

THE EXPRESSION OF RECEPTOR TYPE TYROSINE PHOSPHATASE-β (RPTP-β): EVIDENCE FOR A ROLE IN MORPHOGENESIS AND DEVELOPMENT OF THE NERVOUS SYSTEM. P.D. Canoll, G. Barnea, J.B. Levy, J. Sap, O. Silvennoinen, M. Ehrlich, J. Schlessinger, J.M. Musacchio*. Dept. of Pharmacology, N.Y.U. Medical Center, New York, NY 10016.

The recently cloned RPTP-β (Levy et al. Mol Cell Biol 13:1497, 1993) has a molecular structure indicative that it may function as a cell surface receptor. It has a large extracellular domain with a sequence strikingly homologous to carbonic anhydrase, and a fibronectin type III repeat. The extracellular domain is connected through a single transmembrane segment to a cytoplasmic portion that has two tandem catalytic phosphatase domains. *In situ* hybridization and immunocytochemical studies showed that RPTP-β is expressed predominantly in the developing nervous system, specifically in radial glia and in other forms of glial cells that play an important role as guides during neuronal migration and axonal elongation. High levels of RPTP-β are also seen in nerve fiber tracts throughout the central and peripheral nervous system during periods of axonal outgrowth. In the adult, low levels of RPTP-β are seen throughout most of the CNS. However, high levels of RPTP-β are seen in regions where there is continued neurogenesis and neurite outgrowth. The spatial and temporal pattern of RPTP-β expression suggest that it plays an important role in the morphogenesis and plasticity of the nervous system.

538.38

RETINOIC ACID-INDUCED DIFFERENTIATION OF IMMATURE CHICK SYMPATHETIC NEURONS.
A. v. Holst, A. Rodriguez-Tébar# and H. Rohrer* Max-Planck-Institut für Hirnforschung, Abt. Neurochemie, Deutschordenstr. 46, D-60496 Frankfurt/M., Germany; #Instituto Cajal de Neurobiología, Doctor Arce 37, E-28002 Madrid, Spain

The onset of survival response and dependence on nerve growth factor (NGF) is a critical step in the development of sympathetic neurons. Immature sympathetic neurons from embryonic day 6.5 (E 6.5) do not depend on NGF for survival, are unable to acquire NGF-responsiveness with time in culture, and die after 5 days in vitro even in the presence of NGF. However, addition of all-trans retinoic acid (RA) induces NGF-dependent survival and high-affinity NGF-receptors in E 6.5 cultures. This effect of RA on neuronal differentiation, leading to a more mature state, is observed at nanomolar concentrations of RA. The maturation effect of RA is also reflected by the inhibition of neuronal proliferation. The number of dividing sympathetic neurons is reduced when RA is added in a similar concentration range. A variety of differentiation markers analysed (e.g. tyrosine hydroxylase, N-CAM, F11 and neurofilament) were not affected by RA, suggesting a specific effect on sympathetic neuron development.

To understand the mechanism(s) of action of RA, we tested different RA analogues for their effects on proliferation and long-term survival. These analogues bind to the known retinoic acid receptor subtypes with different affinities, which leads to a selective activation of RA-responsive genes. We found that the antiproliferative effects are in part mediated by different analogues specific for retinoic acid receptors alpha (RARα) and beta (RARβ) but they could not substitute for RA. However, the onset of NGF responsiveness is induced by RA analogues specific for RARα, but not by agonists specific for RARβ or RARγ. As an antagonist for RARα inhibits the RA effect it is concluded that the induction of NGF responsiveness is mediated by RARα.

538.39

Retinoic Acid and NGF in Embryonic Chick Sympathetic Neuronal Development. L.A. Plum[1], P. Tsoulfas[2], L.F. Parada[2] & M. Clagett-Dame[1]*. [1]School of Pharmacy & Interdepart. Grad. Prog. Nutr. Sci., Univ. of Wisconsin-Madison, WI 53706, & [2]NCI-Frederick Cancer Res. Ctr., Frederick, MD 21702.

Studies were initiated to investigate the role of retinoic acid (RA) in promoting NGF-dependent neurite outgrowth and survival of chick embryonic sympathetic neurons. Sympathetic ganglia were isolated from chick embryos at days 6.5-7, 7.5-8, 8.5-9, 11 and 15 and used for *in vitro* culture experiments or to isolate RNA. Ganglia were dissociated and neurons were cultured in the presence of NGF (20 ng/ml), all-*trans* RA (5 nM), NGF+RA or vehicle. Neurons from 6.5-7 day embryos exhibited no outgrowth of neuritic processes and died within 4-5 days in the presence of NGF and/or RA. In contrast, neurons from chicks at embryonic days 7.5-8 and 8.5-9 showed extensive neurite outgrowth and survived for long periods (10-30 days) when exposed to NGF+RA. Neither NGF nor RA alone was able to promote neurite outgrowth or long-term survival of neurons.

To begin to delineate the mechanism of action of RA, chick RA receptor (RARβ and RXRγ) mRNAs were examined in ganglia by Northern blotting. The transcript for p140^{trk} was also studied. The steady-state level of RXRγ mRNA (2.2 kb) was highest at day 6.5-7 and declined 2.5-fold by days 11 and 15. Transcripts for RARβ (3.1 and 2.7 kb) and p140^{trk} (2.8 kb) increased 3- to 4-fold from embryonic day 6.5-7 to 8.5-9 and declined thereafter. It is noteworthy that both RARβ and p140^{trk} mRNAs are highest at the time when the most dramatic response to RA+NGF is apparent in cultured neurons (embryonic day 8.5-9). Elevation of RARβ mRNA may suggest that endogenous retinoid is released by the embryo at this stage of development and could be involved in the development of neuronal responsiveness to NGF. Studies are underway to determine if RA regulates the expression of p140^{trk} mRNA in cultured embyronic sympathetic neurons.

538.40

EXPRESSION OF TYPE II ACTIVIN RECEPTOR mRNA IN RODENT BRAINSTEM, HYPOTHALAMUS AND PITUITARY. V.L. Trudeau*[,1], L. Pope[1], W.J. Giffin[1], J.P.deWinter[2], M.M. Matzuk[3], R.J.G. Haché[1] and L.P. Renaud[1]. [1]Loeb Research Inst., University of Ottawa, Canada, [2]Dept. Endocr. and Reprod., Erasmus University, Rotterdam, Netherlands and [3]Dept. Pathol., Baylor College of Med., Houston, U.S.A.

Activin (Act; β_A/β_B-subunit dimers), a member of the TGF-β molecular family produced in brain, has been reported to promote neuronal survival, stimulate hypothalamic (HYP) corticotropin-releasing factor and oxytocin release, and modulate adenohypophyseal hormone release. A membrane-associated receptor serine/threonine kinase is believed to mediate the diverse actions of Act. The present study evaluated the production of receptor in brain. Total RNA (10-40 µg) extracted from selected tissues was hybridized with a 260-base ^{32}P-labelled riboprobe for type II rat Act-receptor (ActRII). After digestion with S1-nuclease and separation by PAGE, a single 180-base protected fragment was visualized autoradiographically. ActRII mRNA was detected in Long-Evans rat brainstem (including nucleus tractus solitarius), HYP and pituitary. Similar levels of ActRII mRNA were found in HYP of young virgin, 7-day weaned and 15-day lactating female rats. Prepubertally castrated male rats had 2.5-fold higher HYP ActRII mRNA levels than 4-month old age-matched sham controls. Two week treatment of castrates with estradiol (200 ng/g, i.p. every 2 days) reduced HYP ActRII mRNA expression to control levels; the same dose of testosterone (T) had no effect. Castration increased brainstem ActRII mRNA levels 1.5-fold and T reduced ActRII mRNA expression. Comparable ActRII mRNA levels were detected in HYP of wild-type and α-inhibin gene-deleted mice with sex cord-stromal tumours (Nature 360: 313). These results demonstrate that ActRII mRNA is produced in rodent brain and suggest that the ActRII gene may be steroid-regulated. (Supported by AHFMR and MRC).

538.41

PLATELET-DERIVED GROWTH FACTOR RECEPTOR EXPRESSION IN *PATCH* MUTANT MICE AND NORMAL LITTERMATES. X. Zhang*[†] and J.B. Hutchins[†§]. Depts. of [†]Anatomy and [§]Neurology, University of Mississippi Medical Center, Jackson, MS 39216.

Platelet-derived growth factor (PDGF) has been shown to control differentiation and cell division of a number of mesenchymal cell types. Our laboratory and others have more recently provided evidence strongly indicating that PDGF plays a role in CNS development as well. PDGF is contained within developing neurons and the β receptor subunit is found on neurons both *in vitro* and in tissue sections. However, the role (if any) played by the α receptor in nervous system development is not clear. Other investigators have shown that oligodendrocytes, and possibly the glia-like sustentacular cells of olfactory epithelium, express this receptor subunit. In the heterozygous *Patch* mutant mouse, one copy of the PDGF receptor α subunit (PDGF-Rα) gene is deleted. *Patch* homozygotes die *in utero*. *Patch* heterozygotes can be distinguished from their normally-pigmented, C57Bl littermates at postnatal day 2.5 (P2.5). Brain homogenates were prepared from C57Bl and *Patch* littermates at a range of postnatal ages. Blots of brain homogenates separated by SDS-PAGE were probed using antibodies selective for PDGF-Rα and PDGF-Rβ. No apparent difference in PDGF-Rα protein levels are seen between *Patch* mice and normal littermates. This suggests that the translation efficiency and/or stability of PDGF-Rα transcript may be adjusted to equalize levels of receptor protein expressed. Based on the work cited above and further preliminary data, our working hypothesis is that, in addition to oligodendrocytes, astroglia also express PDGF-Rα; ongoing studies to support or refute this idea are in progress. Supported by NIH EY08228 (to J.B.H.).

NEUROTROPHIC FACTORS: RECEPTORS AND CELLULAR MECHANISMS II

539.1

BINDING AND FUNCTIONAL PROPERTIES OF CNTF RECEPTORS ON A HUMAN NEURAL CELL LINE. G. Lawrance, S. Gupta, P. Richardson, R. Dunn and R. Riopelle*. Apps Research Centre of Kingston General Hospital, Queen's University, Kingston CAN. K7L 2V7 (GL,RR), and Centre for Research in Neuroscience, McGill University, Montreal CAN. H3G 1A4.

A human neural cell line (LAN-2) was used to analyze the binding and functional interactions of CNTF with its receptors. Recombinant CNTF interacted with two classes of binding sites on the cell line; a high-affinity interaction (Kd_1 pM) was shared with the cytokine LIF while a low-affinity interaction (Kd_2 nM) was unique. Affinity cross-linking of [^{125}I]-CNTF revealed two putative receptors with approximate molecular masses of 70 and 150 kDa.

In serum-free conditions CNTF, LIF, and IL-6 had no influence on cell survival for periods as long as 16 days. During this time CNTF and LIF, but not IL-6 induced neurite growth and cholinergic differentiation that were half-maximally saturated at concentrations of ligand consistent with high-affinity receptor interactions.

The LAN-2 system may prove useful for study of CNTF regulation of intracellular mechanisms promoting cholinergic differentiation. The present results are consistent with previous indications that CNTF and LIF may have common receptor components.

Supported by the Canadian Federal Centres of Excellence Program in Neural Regeneration and Functional Recovery.

539.2

PROPERTIES OF INTERACTIONS OF TRKA, NGFR, AND NGF ON PC12 CELLS. G.M. Ross* and R.J. Riopelle. Apps Research Centre of Kingston General Hospital, Queen's University, Kingston CAN. K7L 2V7.

Nerve Growth Factor (NGF) interacts with distinct receptor proteins (trkA and NGFR) on PC12 pheochromocytoma cells. Radioligand binding studies with NGF demonstrate two discrete binding affinities on these cells; a high affinity state (Kd_1 pM), and a lower affinity state (Kd_2 nM). Both trkA and NGFR form homodimeric complexes with NGF suggesting that multiple affinity states might be due to receptor oligomerization. We have utilized a variety of chemical cross-linking reagents and immunoprecipitation with specific antireceptor antibodies to examine NGF binding to monomers and oligomers of both trkA and NGFR. Competition studies with unlabelled NGF reveal that both trkA monomers and homodimers display heterogeneous binding of NGF with Kd's in the pM and nM ranges, while the binding of NGF to NGFR monomers and homodimers is homogeneous with Kd in the nM range. These studies confirm that NGF participates in homodimer interactions of trkA and NGFR and indicate that receptor homodimerization is not a determinant of NGF binding affinity. The observations are consistent with recent observations on trkA and NGFR expression and co-expression in non-neural cells.

The observation that low levels of trkA-NGFR heterodimerization can be detected using novel cross-linking conditions raises the possibility that specificity of neurotrophin signalling is mediated by direct extracellular interactions between trk family members and NGFR.

Supported by the Canadian Federal Networks of Centres of Excellence Program in Neural Regeneration and Functional Recovery.

539.3

SILENCING OF gp130 GENE EXPRESSION BY ANTISENSE RIBOZYME TECHNOLOGY BLOCKS CNTF SIGNAL TRANSDUCTION IN EMBRYONAL CARCINOMA CELLS. S.K. Gupta, S. Carbonetto, P.M. Richardson and R.J. Dunn*. Montreal General Hospital and McGill University, Montreal.

CNTF supports survival and neuronal differentiation of P19 murine embryonal carcinoma cells that otherwise die in the absence of serum. One signal-transducing subunit of the CNTF receptor complex has been tentatively identified as gp130, a component of the interleukin-6 receptor complex. To silence expression of the gp130 gene, a 22 base pair ribozyme (rz) motif was inserted into the 5'-end of murine gp130 cDNA: antisense transcripts bearing the rz motif were shown to cleave sense gp 130 transcripts. Sense and antisense gp130 constructs bearing the rz motif were subcloned into an expression vector with the phosphoglycerokinase gene promoter and stable transfectants were obtained in P19 cells. The survival-promoting activities of CNTF were abrogated in cells transfected with the antisense-rz construct but were not diminished in cells with the sense-rz construct. The findings indicate that gp130 is required in P19 cells to mediate the trophic actions of CNTF.

539.4

STRUCTURE AND FUNCTION OF THE CNTF RECEPTOR. N. Stahl, T. Boulton, S. Davis, N. Y. Ip, and G. D. Yancopoulos*. Regeneron Pharmaceuticals, 777 Old Saw Mill River Rd. Tarrytown, NY 10591.

We have defined a three-component structure for the ciliary neurotrophic factor (CNTF) receptor, consisting of a specificity component (CNTFRα), and two 'β' subunits (gp130 and LIFRβ). Interleukin-6 (IL6) also uses gp130 and an IL6Rα, while leukemia inhibitory factor (LIF) and oncostatin M (OM) use gp130 and LIFRβ, but apparently do not require an 'α' component. Thus the broad expression of gp130 and LIFRβ account for the widespread actions of LIF and OSM, while the restricted distribution of CNTFRα limits CNTF action and can be used to define novel targets for CNTF. We have found that the components of the tripartite CNTF receptor complex are initially unassociated, and come together in an ordered fashion following ligand exposure. The last step in complex formation - heterodimerization between gp130 and LIFRβ - initiates intracellular signaling; LIF and OM also heterodimerize gp130 and LIFRβ, while IL6 instead homodimerizes gp130. Hetero- or homodimerization of β subunits activates a cytoplasmic tyrosine kinase associated with the β subunits, and results in tyrosine phosphorylation of an apparently identical set of >10 proteins (called CLIPs) in a wide variety of cell types. Although some of the CLIPs (eg. the ERKs) may be activated by other classes of factors (such as NGF, EGF or FGF which utilize receptor tyrosine kinases), ERK activation by CNTF and its cytokine relatives occurs much later and through a different pathway. Interestingly, we have shown that the CNTF signaling pathway shows synergy with those of FGF and EGF. We find that CNTFRα is also one of the only known receptors that can function in soluble form, endowing CNTF-responsiveness to cells normally responsive only to LIF; this mechanism may be physiologically relevant since soluble CNTFRα is found in the CNS and is released from muscle after denervation.

539.5

ACETYL-L-CARNITINE ARGININE AMIDE IS A POSSIBLE AGONIST FOR THE HIGH-AFFINITY NGF BINDING SITE. G. Taglialatela *, D. Navarra, A. Olivi, M.T. Ramacci, J.R. Perez-Polo[1] and L. Angelucci [2]. Inst. for Res. on Senescence Sigma Tau, 00040 Pomezia, Italy; [1] Dept. of HBC&G, UTMB, Galveston TX, USA; [2] Inst. of Pharmacology II, School of Medicine, "La Sapienza" Univ. of Rome, Italy

Senescence of the central nervous system is characterized by a progressive loss of neurons which may be use to lack of central neuronotrophic activity. Thus, a proper pharmacological approach to these phenomena would be to support degenerating neurons with trophic factors. In the present paper we report that the arginine amide derivative of Acetyl-L-Carnitine (ST 857) stimulates differentiation and neurite outgrowth in the rat pheochromocytoma PC12 cells in a manner similar to NGF. The neuritogenic action of ST 857 requires *de novo* synthesis of mRNA, although it does not activate *c-fos* mediated nuclear pathways, and is independent from the presence of any other trophic substance, as it is exerted also in a serum-free environment. The integrity of the ST 857 molecular structure is essential for its action, as its moieties have no such effects on the cells. Also, slight chemical modifications of ST 857 abolish its neuritogenic effect. Lastly, ST 857 competes with the high affinity NGF binding in a dose dependent fashion.

These results suggest a possible role for ST 857 in those pharmacological strategies aiming to increase the trophic support to the nervous system.

539.6

INTERACTIONS OF THE NEUROTROPHINS WITH ALTERNATIVE FORMS OF TRK RECEPTORS IN NEURONAL AND NON-NEURONAL CELLS. N. Y. Ip*, T. N. Stitt, P. Hanzopoulos, D. Valenzuela, D. J. Glass and G. D. Yancopoulos. Regeneron Pharmaceuticals, Inc., 777 Old Saw Mill River Road, Tarrytown, New York 10591.

We have used a battery of approaches to explore the specificity of the three Trk receptors for the neurotrophins. In fibroblasts, NGF is a primary ligand for TrkA, NT-3 is a primary ligand for Trk C, and BDNF and NT-4/5 are primary ligands, and NT-3 is a secondary ligand, for TrkB. In neuronal populations, however, the specificity of the Trks is apparently altered. While in some neurons NT-3 may still act as a secondary ligand for TrkB, on other neurons it is incapable of activating the TrkB receptor; thus, the neuronal context can in some cases restrict the ability of TrkB to interact with its non-preferred ligand. Similarly, while both BDNF and NT-4/5 are equipotent in activating TrkB in some neurons, there are other neurons in which they have differing effects.

We have found that the specificity of the Trks for the neurotrophins may be modulated by accessory molecules found on neurons, such as the LNGFR. In addition, we have defined naturally-occurring alternate forms of the Trk receptors that, in some cases, may allow for altered responses to the neurotrophins. These include truncated versions of TrkC lacking the kinase domain or full-length forms of TrkC with different-sized inserts in their kinase domains. The naturally-occurring truncated Trks can act as "dominant negatives" to modulate the activation of a full length Trk (e.g. TrkB) by a non-preferred ligand (e.g. NT-3). The insert-containing forms of TrkC retain the ability to autophosphorylate in response to NT-3, but they can no longer mediate proliferation in fibroblasts or neuronal differentiation in PC12 cells.

539.7

BINDING SITES AND RETROGRADE TRANSPORT OF NEUROTROPHINS IN THE CHICK EMBRYO NERVOUS SYSTEM. S. Homma,* D. M. Prevette, Q. Yan and R. W. Oppenheim. Dept. of Neurobiol. and Anat., Bowman Gray Sch. of Med., Winston-Salem, NC 27157-1010, and Neurobiol. Program, Amgen Inc., Thousand Oaks, CA 91320.

Members of the neurotrophin family can promote the survival as well as differentiation of various neuronal cell populations. We have studied the binding sites and retrograde transport of NGF, BDNF and NT-3 in the chick embryo using ^{125}I-labeled neurotrohins. In the cervical spinal cord on E4, systemically injected neurotrohins bound to cells in the intermediate layer, including motoneurons, as well as to peripheral nerve and somite. On E7-8 all three neurotrohins were retrogradely transported to the motoneurons and dorsal root ganglion (DRG) following limb injections. Within the DRG, the ventrolateral cell population preferentially transported BDNF and NT-3, whereas the mediodorsal cell population preferentially transported NGF. We are currently studying the binding sites of neurotrophins in the brain during the period of naturally occurring cell death of responsive neuronal populations. The retrograde transport and binding sites of other putative trophic factors (e.g. IGF, PDGF, TGF) are also being studied.

539.8

MUTAGENESIS DEFINES N-TERMINAL RESIDUES OF HUMAN NGF NECESSARY FOR TRKA RECEPTOR BINDING AND BIOLOGICAL ACTIVITY. J. W. Winslow*, G.R. Laramee, Ai Shih, C. Schmelzer, D.L. Shelton, L.E.Burton. Genentech, Inc., South San Francisco, CA 94080

In contrast to the reported interactions of NT3 with the trkA-NGF receptor kinase, we find that human NGF binds 500-fold more efficiently to trkA than does human NT3. Human NGF and NT3 differ in 52/120 amino acids; 44/52 (82%) of the differences are located within 7 regions representing the N- and C-termini, 4 beta-turns and one beta sheet. To determine the structural basis for specific hNGF and hNT3 receptor interactions, homolog- and alanine-scanning mutagenesis was performed on hNGF and analyzed by trkA, p75, and p75-trkA receptor binding (PC12 cells), trk autophosphorylation, and PC12 cell differentiation. Twelve chimeric mutants, replacing these divergent regions within NGF with the corresponding NT3 sequences, were generated by oligonucleotide-directed mutagenesis and expressed in mammalian cells. Single or double regional chimeras in most cases display nearly normal trkA and p75 binding and activation of autophosphorylation. Significant loss of trkA binding and function resulted from N-terminal chimera between NGF and NT3 or BDNF. A single Ala or Asp replacement of His4 of hNGF also reduces trkA binding by 1000-fold while p75 binding is unaffected. N-terminally truncated forms (6-118) and (10-118) hNGF result in 10- and 300-fold reduction in trkA binding; p75 binding is reduced by 10-fold only in the (10-118) hNGF. The potency of trkA autophosphorylation and PC12 differentiation elicted by the N-terminally modified hNGF forms were consistent with trkA binding profiles. These results indicate that the specific N-terminal sequence of hNGF contributes to structural interactions neccessary for trkA receptor binding and ligand-induced signalling. The absence of major functional losses by most of the chimera suggests that the multiple regions of sequence divergence between hNGF and hNT3 may all contribute to receptor binding specificity by determining the tertiary structure and geometry of contact residues that are possibly conserved among the neurotrophins.

539.9

BIOLOGICAL ACTIVITY AND INTERMOLECULAR TRANSPHOSPHORYLATION OF TNF-Trk CHIMERIC RECEPTORS.

Marco Canossa, Giorgio Rovelli and Eric M. Shooter*. Department of Neurobiology, Stanford University School of Medicine, Stanford, CA 94305-5401

To explore the signal transduction mechanism of the Trk kinase receptors, chimeric molecules containing the extracellular domain of the tumor necrosis factor (TNF) receptor (p70) and the transmembrane and cytoplasmic domains of the neurotrophin receptors TrkA (p70-TrkA), TrkB (p70-TrkB) and TrkC (p70-TrkC) were produced. Transfected PC12 cells expressing the chimeric receptors showed TNF-dependent neurite outgrowth and cell survival. Chimeras containing a K547 mutation in the ATP binding site of the tyrosine kinase domain failed to show TNF-dependent effects. Together, these results suggest that ligand-mediated activation of the Trk kinase domain is both necessary and sufficient for induction of a neuronal phenotype in PC12 cells. It is known that the ligand-activation of Trk kinase receptors induces tyrosine autophosphorylation. COS7 cells were used to investigate the ligand-induced phosphorylation of the chimeras. When p70-Trk(A, B or C) were coexpressed with wild-type Trk(A, B or C), increased tyrosine phosphorylation was observed in both receptors in response to either TNF or NGF. Thus, the Trk kinase domain appears capable of Trk transphosphorylation activity irrespective of the extracellular domain present on the receptor, indicating that homologous cytoplasmic domains can mediate Trk receptor transphosphorylation.

539.10

CHARACTERIZATION OF A NOVEL NGF HETERODIMER

J.I.S. Treanor, C. Schmelzer and L.E.Burton*
Genentech, Inc., South San Francisco, CA 94080

Current evidence suggests that NGF mediates its biological activity through a high affinity tyrosine kinase receptor (trkA). To investigate this further a novel NGF heterodimer (118:110-118rhNGF) has been produced. This group has previously shown that the amino terminal region of NGF is extremely important in the trkA receptor binding and ligand-induced signalling. Baseline separation of the heterodimer was achieved using HPLC cation exchange chromatography and it's stability monitored at 4°C (2 weeks), 22°C and 37°C (>24 hours). The heterodimer was stable under under these conditions when analyzed by HPLC.

Primarily, characterization of the heterodimer was carried out using trkA and p75-trkA (PC12 cells) receptor binding and autophosphorylation. A 2-fold loss in EC_{50} of 125I-NGF displacement was seen in PC12 cell binding with the heterodimer, whilst a 10-fold loss was seen with trkA binding when compared to homodimeric 118:118rhNGF. Homodimeric 110-118:110-118rhNGF showed a 20-fold loss in PC12 cell binding and a 500-fold loss in trkA binding. Autophosphorylation of trkA expressing fibroblasts indicated a 10-fold loss with the heterodimer and a 500-fold loss with homodimeric 110-118:110-118rhNGF which paralleled the trkA binding data.

Further biological characterization data will be presented.

539.11

Reciprocal regulation of estrogen and NGF receptor systems by their ligands in PC12 cells.

Farida Sohrabji[1]*, Lloyd A. Greene[2], Rajesh C. Miranda[1] and C. Dominique Toran-Allerand[1]. Columbia University, Depts of Anatomy and Cell Biology[1] and Pathology[2], NYC, NY 10032.

Both estrogen and the neurotrophins influence neural development. We questioned whether some developmental actions of estrogen and the neurotrophins result from interaction of these factors, by examining the potential for reciprocal regulation of their receptor systems by these ligands. PC12 cells were grown in the presence(+) (2 weeks) or absence of NGF. NGF+ cells were then exposed to estrogen (E) (10^{-9}M) for 0-7 days. While specific nuclear estrogen-binding sites were present in both NGF+ and untreated PC12 cells, NGF elicited a 6-fold increase in estrogen-binding sites. Estrogen regulation of NGF receptor genes appeared to be time- and receptor-specific. In NGF+ cells, we found transient down-regulation of $p75^{NGFR}$ mRNA by 3d of E-treatment, and a return to basal levels by 7 days. *trkA* mRNA, in contrast, increased 2-fold following 7 days of E-treatment. Regulation of estrogen and NGF receptor systems by their cognate ligand indicated that E-treatment of NGF+ cells down-regulated estrogen-binding, while NGF up-regulated $p75^{NGFR}$ mRNA, but did not up-regulate *trkA* mRNA consistently. These data indicate that NGF may increase neuronal sensitivity to estrogen, and that estrogen, by differentially regulating $p75^{NGFR}$ and *trkA* mRNA, may alter the ratio of the two NGF receptors, and, consequently, neurotrophin responsivity. Given the widespread co-localization of estrogen and neurotrophin receptor systems in the developing CNS, the reciprocal regulation of these receptor systems by NGF and estrogen, seen in PC12 cells, may have important implications for processes governing neural maturation and the maintainance of neural functioning. (Supported by NIH(NIA), NSF and NIMH RSA (CDT-A)

539.12

INCREASED PRODUCTION OF TRUNCATED NGF RECEPTOR FOLLOWING FORNIX LESIONS.

D.D. Gordon*, M.D. Lindner, Y.S. Choe and R. Loy. Dept. of Neurology, Univ. Rochester School of Med., Rochester, NY 14620 and Canandaigua VAMC.

Peripheral nerve injury results in upregulation of both cellular and truncated forms of the low-affinity nerve growth factor (NGF) receptor, but it is not known if similar upregulation occurs after CNS injury. NGF-dependent basal forebrain cholinergic neurons atrophy in Alzheimer's disease, and we have found that in early stages of the disease the level of truncated receptor in urine is increased over controls. To see if degenerating central neurons could be contributing to this increase, we performed fornix transections on 19-month-old, female Spraque-Dawley rats to induce retrograde degeneration in septal neurons. We collected urine before surgery to gather a baseline and at seven other timepoints after transection to measure levels of the truncated form of the NGF receptor by ELISA. The urine levels of truncated receptor are increased three-fold at days seven and fourteen following transection. This provides support for the argument that the increase in truncated NGF receptor in early stages of AD can be influenced by basal forebrain neuron degeneration. Truncation may occur with increased receptor turnover or due to metabolism of cellular receptors in initial stages of neuron degeneration. Supported by AG09231.

539.13

LOCALIZATION OF NEUROTROPHIN RECEPTORS IN NORMAL AND DEGENERATING CAT RETINA.

M.J. Radeke, G.P. Lewis, D. Blumberg, S.K. Fisher*, and S.C. Feinstein. Neuroscience Research Institute and Department of Biological Sciences, University of California, Santa Barbara, CA 93106.

Retinal detachment, as well as many other diseases of the eye, is marked by a rapid loss of photoreceptor outer segments and a proliferation of non-neuronal cells, in particular retinal pigment epithelia (RPE) and Müller cells. Recent evidence (LaVail et al., 1992, PNAS 89: 11249) obtained using light damage as a model for retinal degeneration has suggested possible roles for the neurotrophins BDNF and/or NT-3 for photoreceptor survival. In order to help determine which neurotrophins might be required for maintenance and repair of the retina, we have sought to ascertain the pattern of expression of the *trk* family of neurotrophin receptors in normal and detached cat retinas using a panel of anti-*trk* antibodies. In the normal retina, antibodies directed against either the extracellular or intracellular domain of *trk*A stain cones, Müller cells, ganglion cell bodies and the RPE. Antibodies specific for two different *trk*B extracellular peptides both label Müller cells, cones and rods, ganglion cell bodies and axons, astrocytes and RPE. Anti-peptide antibodies specific for full length *trk*B detect ganglion cell bodies and axons, whereas antibodies directed against two truncated forms of *trk*B stain photoreceptor outer segments, Müller cells, astrocytes and RPE. An antibody which detects full length forms of *trk*C identified photoreceptor outer segments and Müller cells. Following retinal detachment there is an apparent increase in labelling of truncated *trk*B associated with Müller cell hypertrophy and proliferation. In addition, localization of full length *trk*B in ganglion cell axons appears to decrease following chronic detachment. Our data suggest a role for neurotrophins in the maintenance and response to injury throughout the retina. Perhaps most interestingly, when taken together with the ligand binding specificities of the *trks* and the effect of BDNF and NT-3 on damaged retinas, it suggests a possible role for truncated *trk*B in the maintenance of photoreceptors. Supported by NSF IBN-9120836, ACS-CA Division S68-91 and NIH EY00888.

539.14

LOW-AFFINITY NERVE GROWTH FACTOR RECEPTOR REEXPRESSION IN AXOTOMIZED FACIAL AND SCIATIC MOTOR NEURONS: DIFFERENCES BETWEEN NORMAL AND OLA MUTANT MICE

P. Burnham*, T. Hagg, S. Varon, M.A. Bisby# Dept. Biology, Univ. California San Diego, La Jolla; # Dept. Physiology, Queen's Univ., Kingston, Canada

The C57BL/Ola mutant mouse is characterized by delayed and slow Wallerian degeneration of lesioned distal peripheral and central nerves, apparently resulting from delayed axonal breakdown and lack of macrophage recruitment. As a result, motor and sensory regeneration is impaired. Spinal motor neurons express low-affinity NGF receptor (LNGFR) during development but not in the adult. After peripheral nerve injury, rat motor neurons temporarily reexpress LNGFR, probably during the process of axonal outgrowth. We compared in normal C57BL/6J and C57BL/Ola mice, the responses of sciatic motor neurons to nerve crush or transection and of facial neurons to crush injury. Several observations include: i) mice respond like rats, ii) LNGFR expression by Ola motor neurons is less robust (lower number, less intense staining) than normal, iii) LNGFR expression after a crush is maintained one week longer in sciatic motor neurons of Ola mice, consistent with slower axon regeneration, iv) facial neurons respond as rapidly as sciatic neurons, v) Ola facial neurons show a second phase of increase in LNGFR at 28 days, while normal ones show no LNGFR at this time, vi) LNGFR changes correlate in time with somal size changes in these neurons, suggesting a relation between LNGFR and a state of neuronal "growth". These findings are consistent with the idea that LNGFR expression is regulated by two different mechanisms, one related to the initial response to the injury and another related to the outgrowth phase of the neuron/axon. We wish to thank L. Reichardt and G. Weskamp for their LNGFR antibodies. Support: NINCDS grant NS16349, 27047 (SV); MRC Canada MT5198 (MAB)

539.15

DIFFERENTIAL EXPRESSION OF $p140^{trk}$, $p75^{NGFR}$ AND GAP-43 GENES IN NEURONS OF NUCLEUS BASALIS MAGNOCELLULARIS FOLLOWING CORTICAL DEVASCULARIZATION. B.C. Figueiredo*, W. Tetzlaff†, M. Skup, L. Garofalo & A.C. Cuello, Dept.of Pharmacology and Therapeutics, McGill University, Montreal, and Dept. of Physiology†, University of Ottawa, Ottawa, Canada.

A loss of target-derived neurotrophic factors is hypothesized to be one of the major determinants of CNS neuronal vulnerability to degeneration. In order to gain more insight of early neuronal responses to injury, lesion-induced alterations in high or low-affinity nerve growth factor (NGF) receptors, as well as growth-associated protein (GAP-43) gene expression, in NGF-responsive nucleus basalis magnocellularis (NBM) neurons were studied.

For this purpose, adult rats were operated by unilateral cortical devascularization and received, i.c.v. via minipump, either vehicle or NGF (12 μg/day), and were sacrificed at 1, 3 and 7 post-lesion days. In situ hybridization studies, using ^{35}S-labeled oligonucleotide probes for $p75^{NGFR}$, $p140^{trk}$ and GAP-43, revealed that these genes were differentially regulated following the lesion. $p140^{trk}$ gene expression was shown to be decreased while $p75^{NGFR}$ and GAP-43 mRNA levels were increased after lesion. Moreover, GAP-43 mRNA levels, but not $p75^{NGFR}$ or $p140^{trk}$, were largely increased in pyramidal neurons located in the remaining adjacent cortex of all decorticated animals. NGF treatment recovers levels of $p140^{trk}$ mRNA in the lesioned NBM to values observed in the contralateral NBM. These findings suggest that $p140^{trk}$ mRNA levels in NBM neurons of naive animals depend upon NGF.

Supported by MRC (Canada) and the Canadian Network of Centres of Excellence for Neural Repair and Functional Recovery. B.C.F. is a recipient of a CNPq studentship (Brazil) and M.S. and L.G. are recipients of NCE fellowships.

539.16

EXPRESSION OF THE LOW AFFINITY, NEUROTROPHIN RECEPTOR, $p75^{NGFR}$, IN THE DEVELOPING AND LESIONED OLFACTORY SYSTEM. Christopher P. Turner* and J. Regino Perez-Polo, Dept. Human Biological Chemistry & Genetics, Univ. Texas Med. Branch, Galveston, TX 77555-0652.

Using the monoclonal antibody, MAb192, we studied the distribution of $p75^{NGFR}$-immunoreactivity (p75-ir) in the main olfactory bulb (MOB) of rat during development and under lesioned conditions. *Developmentally*, we observed an increase in intensity and organization in p75-ir in the glomerular layer (GL), which we interpret to be due to a developmental increase in peripheral input to the MOB. *Lesion 1* was a chemically induced, peripheral deafferentation of the MOB, employing a TX100, nasal irrigation procedure. Saline was used as a control. The distribution of p75-ir was monitored 1-16 weeks after the lesion. In the saline controls, p75-ir was abundant in the GL and relatively sparse in the olfactory nerve layer (ONL). After 1 and 2 weeks postlesion, TX100 produced a decrease in p75-ir in the GL and an induction of p75-ir in the ONL. After 8-16 weeks postlesion, the distribution of p75-ir later returned to that observed with saline controls, coinciding with the regenerative capacity of the peripheral input. *Lesion 2* was a unilateral bulbectomy (OBX; influencing both centrifugal and centripetal pathways of the MOB). Sham-lesioned animals served as controls. The distribution of p75-ir was monitored 1-16 weeks after the lesion. After 1-4 weeks postlesion, OBX animals showed no change in p75-ir in the remaining MOB. An increase in p75-ir in the GL was observed 8 and 16 weeks postlesion, as compared to sham controls. Elevated p75-ir was also observed in the ONL in the 16 week postlesion group. Thus p75-ir in the MOB is regulated under conditions of 1) increased peripheral input, 2) regeneration of peripheral input and/or 3) central pathway reorganization. Supported by NINDS grant NS18708.

539.17

DISTRIBUTION OF EXOGENOUS NEUROTROPHINS IN THE RAT CNS FOLLOWING ICV INJECTION. C.R. Matheson*, J. Sun, M.J. Radeke¶, S.C. Feinstein¶, J. Miller and Q. Yan, Neurobiol. Dept., Amgen, Inc., Thousand Oaks, CA 91328; ¶Neurosci. Res. Inst. and Dept. of Biol. Sciences, Univ. of California, Santa Barbara, CA 93106.

To assess the potential effectiveness by which injected neurotrophins can diffuse throughout the CNS, we used autoradiographic and immunohistochemical techniques to examine the distributions of NGF, BDNF and NT-3 after a single injection into the lateral cerebral ventricle (ICV). As described previously, ^{125}I-NGF labeled cholinergic neurons in the basal forebrain Only light labeling was detected in the brain parenchyma surrounding the lateral ventricle. In contrast, injection of ^{125}I-BDNF resulted in few or no labeled neurons in the basal forebrain or in the substantia nigra. However, a gradient of very intense labeling extended from the ventricular surface into the parenchyma for a distance of approximately 0.5 mm. ^{125}I-NT-3 distribution was intermediate between that of NGF and BDNF. No significant differences were detected in neonate versus adult animals. Complementary immunohistochemical studies utilizing anti-NGF and anti-BDNF antisera revealed that unlabeled neurotrophins injected into the ICV distributed in precisely the same manner as their iodinated counterparts. Consistent with the intense BDNF labeling of the ventricular surface, *in situ* hybridization analysis has confirmed previous studies showing that mRNA encoding the BDNF receptor (trkB) is highly expressed by ependymal cells lining the ventricular surface as well as by many neurons and glia. On the other hand, expression of the high affinity NGF receptor (trkA) is much more restricted. In addition, antisera specific for the trkA or trkB receptors demonstrated that their expression pattern closely reflects their mRNA distribution. These data suggest that the presence of the trkB receptor protein on the ependymal layer and its expression throughout the subependymal zone and brain parenchyma represents a significant impediment to the adequate diffusion of ICV injected BDNF into the brain for delivery to target neurons.

NEUROTROPHIC FACTORS: RECEPTORS AND CELLULAR MECHANISMS III

540.1

NGF-INDUCED TYROSINE PHOSPHORYLATIONS OF $p140^{trk}$ AND ERK IN COMPARTMENTED CULTURES OF RAT SYMPATHETIC NEURONS. D.L. Senger* and R.B. Campenot, Dept. of Anatomy and Cell Biology, University of Alberta, Edmonton, Alberta, T6G 2H7.

The first step in intracellular signaling that mediates all NGF responses is believed to be tyrosine phosphorylations by the NGF receptor, $p140^{trk}$ (*trk*). Since *trk* is itself a substrate for its own tyrosine kinase activity, we used immunoprecipitation with a *trk* specific antibody and then western blot analyses using a phosphotyrosine antibody to assess *trk* tyrosine kinase activity. We found that after short-term NGF deprivation, increasing concentrations of 2.5S NGF (10-200 ng/ml) caused a concentration-dependent increase of tyrosine phosphorylation of *trk* in mass cultures of sympathetic neurons occuring within 10 minutes. Also, in compartmented cultures we found that *trk* was present in the distal neurites and that application of 200 ng/ml NGF to distal neurites caused a local increase in *trk* phosphorylation in the distal neurites within 10 minutes. Furthermore, we found that 500 nM K-252a and 100 nM staurosporine (both shown by others to inhibit *trk* phosphorylations in PC12 cells) inhibited the elongation of distal neurites when locally applied to them in compartmented cultures. However, neither K-252a nor staurosporine inhibited distal neurite elongation when these drugs were locally applied to cell bodies and proximal neurites. These data suggest that phosphorylations by *trk* play a role in mediating neurite elongation at or near the site of NGF interaction with the cell surface, but that *trk* phosphorylations proximal to the source of NGF may not be involved in mediating the neurite growth response. We have begun similar investigations into the role of ERKs (extracellular receptor mediated kinases, also known as MAP kinases) which so far indicate that ERKs are rapidly tyrosine phosphorylated in rat sympathetic neurons in response to NGF and are present in abundance in distal neurites.

540.2

NEURONAL RESPONSES TO NGF ARE SPATIALLY-REGULATED: NGF EXPOSURE AT TERMINALS VERSUS CELL BODIES DIFFERENTIALLY ALTERS NEURONAL GENE EXPRESSION. J.G. Toma*, D. Rogers, R.B. Campenot, and F.D. Miller, Dept. Anat. & Cell Biol., University of Alberta, Edmonton, Canada T6G 2H7.

We have previously demonstrated that NGF supplied to neuronal terminals *in vivo* can regulate gene expression in mature sympathetic neurons, and have documented NGF-induced increases in both p75 NGF receptor and tyrosine hydroxylase (TH) mRNAs in mass cultures of sympathetic neurons. To determine how an NGF receptor:ligand interaction at neuronal terminals can distally regulate gene expression in the cell body, we have turned to compartmented cultures of sympathetic neurons. In these experiments, neurons were established and maintained in 10 ng/ml NGF for 7 days, and were then "induced" with 200 ng/ml NGF a) in side compartments, which contain only neurites (distal addition), or b) in both center and side compartments (global addition). When 200 ng/ml NGF is added globally, both p75 NGF receptor and TH mRNAs increase significantly within 24 hours, and continue to rise until at least 48 hours postaddition, as previously observed in mass cultures. In contrast, levels of *trk*A mRNA do not change. However, when NGF is added distally, the pattern of response differs. Neither p75 NGF receptor nor TH mRNAs are altered 24 hours following increased NGF distally. By 2, 3, and 7 days postaddition, p75 NGF receptor mRNA levels are increased over controls, but remain significantly lower than those observed in sister cultures exposed to increased NGF globally. This differential regulation is even more striking for TH: increased terminal NGF elicits only a small increase in this mRNA versus a greater than 5-fold increase elicited by globally increasing the NGF. Thus, neuronal gene expression is regulated by NGF as a function of the spatial location of the activated receptor:ligand complexes. These data suggest that, *in vivo*, neuronal responses to NGF may differ as a consequence of the neurons spatial relationship with the cellular source of the neurotrophin.

540.3

LEUKEMIA INHIBITORY FACTOR (LIF), CILIARY NEUROTROPHIC FACTOR (CNTF) AND ONCOSTATIN M (OSM) ACTIVATE P21^{ras} IN A NEUROBLASTOMA CELL LINE. S.E. Lewis*, M.A. Schwarzschild, W.T. Dauer, L.K. Hamill, A.J. Symes, J.S. Fink and S.E. Hyman. Molecular and Developmental Neuroscience Lab, Mass. General Hospital, Charlestown, MA 02129.

The cytokines LIF and CNTF regulate survival and differentiation of many types of neurons. The receptors for LIF and CNTF associate with the same signal transducing subunit utilized by the IL-6 receptor, gp130. The receptor for OSM has not yet been identified, however, gp-130 binds OSM and appears to be necessary for its action. GP-130 has no known catalytic activity and the second messenger systems activated by this family of receptors are unknown. One putative intracellular effector, the product of the proto-oncogene *ras*, has been implicated in the signal transduction pathways of other growth factors and cytokines. We therefore investigated a role for Ras in LIF, CNTF and OSM responses in the neuroblastoma cell line NBFL.

In NBFL cells, these cytokines increase levels of activated Ras in a rapid, transient, and dose-dependent fashion. In addition, all three increase the tyrosine phosphorylation of several proteins. Similar phosphotyrosine bands and activation of Ras are induced by LIF and CNTF in cultures of rat sympathetic neurons. One effect of LIF, CNTF and OSM in the NBFL cells is induction of VIP mRNA. The protein kinase inhibitors staurosporine and K252a inhibit the cytokine-induced tyrosine phosphorylations, p21-Ras activation, and increase in VIP mRNA in NBFL cells. Our data suggest that Ras may be involved in the regulation of VIP gene expression by LIF, CNTF and OSM in this cell line.

540.4

RAS-INDEPENDENT INDUCTION OF TYPE II NA CHANNEL EXPRESSION IN NGF-TREATED PC12 CELLS. G. R. Fanger* and R.A. Maue. Depts of Physiology and Biochemistry, Dartmouth Medical School, Hanover, NH 03755-3833.

The molecular mechanisms underlying the neuronal-specific responses to nerve growth factor (NGF) are not well understood, especially those responsible for the effects of NGF on neuronal ion channel expression. To determine if the activity of p21*ras*, which has been implicated in the signal transduction pathways activated by NGF, is necessary for the NGF-mediated induction of sodium (Na) channel expression in rat pheochromocytoma (PC12) cells, we have analyzed Na channel expression in PC12 sublines stably overexpressing the dominant inhibitory mutant c-Ha-*ras*(Asn-17) (MM17-2, MM17-26; Szeberenyi et al., Mol. Cell. Biol. 10(1990): 5324). Northern blot analysis of total RNA from control and NGF-treated cells revealed 3- to 5-fold increases in the steady state level of Na channel mRNA in both PC12 and *ras*-deficient MM17-2 and MM17-26 cells. RNAse protection assays using probes specific for type I, type II, or type III Na channel mRNA were also used to analyze Na channel mRNA in these cells. A probe directed against the 3' untranslated region of type II Na channel mRNA detected type II Na channel mRNA in control and NGF-treated PC12, MM17-2, and MM17-26 cells, with the NGF-mediated induction of type II Na channel mRNA comparable to that observed in the Northen blots. Similar results were obtained using a probe directed against the 5' untranslated region of the type II mRNA. In contrast, type I and type III Na channel mRNA were not detected in either control or NGF-treated PC12, MM17-2, and MM17-26 cells. Whole-cell patch clamp recordings verified that the NGF-mediated increases in Na channel mRNA in the MM17-2 and MM17-26 cells resulted in an increase in functional Na current density. Despite NGF-induced increases in the size of the MM17-2 and MM17-26 cells, as estimated by cell membrane capacitance, there were 3- to 5-fold increases in Na current density in the *ras*-deficient cells, comparable to that occuring in PC12 cells. The results suggest that *ras* activity is not necessary for the NGF induction of type II Na channel expression in PC12 cells, and provide further support for the existence of both *ras*-dependent and *ras*-independent mechanisms underlying neuronal differentiation. Supported by the Alfred P. Sloan Foundation and NIH NS28767.

540.5

ROLE OF *C-FOS* AND *C-JUN* IN INSULIN-MEDIATED STIMULATION OF RETINA NEURONAL DIFFERENTIATION. R.E. Hausman*, Y. Ren, Y. Ben-Shaul#, and B.H. Shah. Dept. of Biology, Boston University, Boston, MA 02215, USA; #Dept. of Immunology and Cell Biology, Tel-Aviv University, Tel-Aviv, Israel.

In chick embryo retina, insulin stimulates cholinergic and GABAergic differentiation. However, it does not affect succinic dehydrogenase or lactic dehydrogenase activity. These effects are stage-specific suggesting a neurogenic role for insulin in retina development. We investigated the mechanism of signal transduction. An early response was transcription of *c-jun* mRNA and its translation detected by Northern and Western blotting within minutes after insulin exposure. A rise in *c-fos* transcription and translation followed. These increases were transient and protein levels returned to normal by 45 min. Late gene expression, seen as an increase in glutamic acid decarboxylase (GAD) and choline acetyltransferase (ChAT) protein, occurred within 60 minutes. The increase in jun protein can be detected by immunocytochemistry in the ganglion cell and inner nuclear layers within 15 min after insulin addition and the increase in fos protein later. GAD and ChAT activity later increase in the ganglion, amacrine and displaced amacrine cells. Since these cells express neuronal insulin receptors, the above results suggest that fos and jun proteins, acting as an AP1 transcription complex, are causally involved in neurogenic differentiation of embryonic chick ganglion and amacrine cells. Experiments with phosphorothioate antisense oligonucleotides for *c-jun* and blockers of signal transduction suggest a causal but not necessarily direct role for *c-fos* and *c-jun* in the induction of cholinergic and GABAergic differentiation by insulin.

540.6

THE PHOSPHORYLATION AND DNA BINDING OF THE DNA-BINDING DOMAIN OF THE TRANSCRIPTION FACTOR NGFI-B. Y. Hirata*, K. Kiuchi, H.-C. Chen, J. Milbrandt[1] and G. Guroff. Section on Growth Factors, NICHD, NIH, Bethesda, MD 20892 and [1]Department of Pathology and Internal Medicine, Washington University School of Medicine, St. Louis, MO 63110.

NGFI-B is an orphan member of the nuclear receptor superfamily encoded by an immediate-early gene. It is rapidly synthesized and phosphorylated in PC12 cells in response to nerve growth factor (NGF) and other agents and is differentially phosphorylated dependent upon the inducing stimulus. The DNA-binding domain (DBD) of NGFI-B has been expressed in bacteria and purified. It is phosphorylated by protein kinase A or by extracts from NGF-treated PC12 cells. The phosphorylated residues within the DBD have been identified as Ser340 and Ser350. The use of mutants in which either or both of these residues were replaced with alanines revealed that phosphorylation of Ser350, located within the "A box", a motif necessary for DNA binding by NGFI-B, resulted in a decrease in binding to the NGFI-B response element (NBRE), while phosphorylation of Ser340 had little or no effect. These findings demonstrate that phosphorylation of a nuclear receptor DBD results in a change in DNA binding and provides another potential mechanism for regulating NGFI-B activity.

540.7

CNTF, LIF AND ONCOSTATIN M ACTIVATE VIP GENE EXPRESSION THROUGH A COMPLEX CYTOKINE RESPONSE ELEMENT
Aviva Symes, Prithi Rajan, Lisa Corpus, E.M.Adler* and J.Stephen Fink
Molecular Neurobiology Laboratory, Massachusetts General Hospital and Department of Neurology, Harvard Medical School, Boston, MA 02114

The cytokines ciliary neurotrophic factor.(CNTF), leukemia inhibitory factor (LIF) and oncostatin M (OM) are structurally and functionally related through similar protein structure, shared receptor subunits and overlapping functions. We have previously shown that a 180 bp cytokine reponse element (CyRE) mediates the transcriptional activation of the vasoactive intestinal peptide (VIP) gene to these cytokines in a human neuroblastoma cell line, NBFL.

To characterize further the molecular mechanisms of cytokine-mediated transcriptional activation we have identified functional domains within the CyRE and examined nuclear proteins in NBFL cells which bind to the CyRE both before and after cytokine treatment. One candidate transcription factor is c/ebpß (NF-IL6) as this is induced in response to IL-6, a related cytokine, in the hepatic acute phase response. Through gel shift and footprint analyses we show that members of the c/ebp family can bind to the 180 bp CyRE, and cotransfection experiments indicate that c/ebp family members are able to transactivate the CyRE. Transfections of CyRE deletion mutants linked to a luciferase reporter revealed that there are multiple functional regions within the180 bp which contribute to the cytokine response in NBFL cells. Using these functional domains within the CyRE as probes, gel shift analysis showed that there are distinct DNA binding proteins which are induced after cytokine treatment. These results suggest that CNTF, LIF and OM induce VIP transcription in NBFL cells through a complex set of interacting DNA binding sites and transcription factors.

540.8

REGULATION OF NEU EXPRESSION IN SCHWANN CELL-DORSAL ROOT GANGLION CO-CULTURES. P. Mason*, T. Neuberger, B. Attema, G.H. De Vries
Dept. of Biochemistry and Molecular Biophysics, Med. Coll. Va., Richmond, VA 23298.

The physiological significance of the proto-oncogene Neu is not known. Schwann cell (SC)-dorsal root ganglion (DRG) co-cultures provide a unique opportunity to investigate Neu expression, since the culture conditions can be manipulated to allow SC proliferation only (serum free cultures) or to allow SC differentiation (serum plus ascorbic acid). SC free-DRG cultures were obtained from E16 d rat ganglia. SC cultures were isolated from 2 d rat sciatic nerves. Co-cultures were prepared by adding SC to DRG cultures, followed by maintenance for 3 wks in either serum free media or media containing serum plus 5 µg/ml ascorbic acid. Immunofluorescent localization of Neu showed cytoplasmic Neu immunoreactivity in neurites at 14, 26 and 35 days of culture of SC-free DRG. SC cultured alone showed strong immunoreactivity for both Neu and S100 protein. Neu could not be immunologically detected in S100 positive cells in contact with DRG neurites cultured under serum free conditions; however, cytoplasmic Neu immunoreactivity was still present in neurites. In SC-DRG cultures some SC at the periphery of the culture were not in contact with neurites. These SC continued to express strong Neu immunoreactivity. In myelinating media, a subpopulation of SC that are in contact with DRG neurites appeared to reexpress Neu antigen. These studies show that Neu is expressed in DRG neurites in both serum-free and serum-containing media. Our results suggest that Neu expression may be involved in SC differentiation and that axonal contact controls Neu expression in SC. (Supported by NS15408).

540.9

INDUCTION OF NFκB TRANSCRIPTION FACTORS IN RAT HIPPOCAMPAL ASTROCYTES BY INTERLEUKIN-1. W.J. Friedman[1*], S. Thakur[2], and A. Rabson[2]. [1]Dept of Neuroscience and Cell Biology and [2]CABM, UMDNJ-Robert Wood Johnson Medical School, 675 Hoes Lane, Piscataway, N.J. 08854

Astrocytes in the central nervous system become activated in response to stimulation by cytokines such as interleukin-1 (Il-1) and tumor necrosis factor α (TNFα) . Astrocyte activation results in increased proliferative activity and production of a variety of growth and trophic factors involving induction of gene expression. As an early event in the glial response to cytokine stimulation, we have examined expression and induction of the NFκB transcription factors in hippocampal astrocytes. The NFκB family of transcription factors consists of at least five proteins which dimerize to interact with specific NFκB binding sites in the promoter region of responsive genes. We have identified the specific NFκB mRNA's which are expressed and/or induced in hippocampal astrocytes by Il-1. Moreover, we have shown that upon Il-1 stimulation, nuclear extracts from hippocampal astrocytes contain proteins which specifically bind NFκB oligonucleotides, indicating the presence of functional NFκB proteins. These data suggest that an early event in the activation of astrocytes by cytokine stimulation may involve activation of NFκB transcription factors. Supported by NIH grant NS 31357-01.

540.10

Nerve growth factor induces CREB phosphorylation and cyclic AMP reponse element-dependent transcription of *c-fos*. Azad Bonni, David D. Ginty, Anirvan Ghosh*, and Michael E. Greenberg. Dept. of Microbiology and Molecular Genetics, Harvard Medical School, Boston, MA 02115.

Nerve growth factor (NGF) promotes the survival and differentiation of specific populations of neurons in the peripheral and central nervous system. The biological actions of NGF result from intraneuronal biochemical cascades that are set in motion by NGF receptor activation. A major outcome of these signaling cascades is the transcriptional induction of the immediate early genes, the prototype of which is the proto-oncogene c-*fos*. The mechanisms by which NGF regulates the transcription of c-*fos* are addressed in this study. Previous studies have suggested that the serum response element (SRE), located at -300 nucleotides upstream of the c-*fos* transcriptional start site, is required for NGF-induced c-*fos* transcription. We now demonstrate that, in addition to the SRE, the cyclic AMP repsonse elements (CREs) within the c-fos promoter are also required for NGF-induced transcription of c-*fos*. Using an RNAse protection assay, we determined that in PC12 cells NGF efficiently induced transcription of CAT from a c-*fos*-CAT fusion construct (-356*fos*CAT) that contained 356 base pairs of the c-*fos* promoter. Transcription of a -356*fos*CAT construct lacking intact CREs was only poorly induced in response to NGF. In addition, transcription of a -356*fos*CAT construct lacking an intact SRE was reduced but not completely abolished. Previous studies have demonstrated that agents that increase intracellular levels of Ca^{+2} or cAMP, which activate c-*fos* transcription through the CRE induce the phosphorylation of the transcription factor, cyclic AMP response element binding protein (CREB). Phosphorylation of serine 133 on CREB is required for CREB-mediated c-*fos* transcriptional induction. Using antibodies that recognize specifically serine 133-phosphorylated CREB, we have discovered that, in addition to agents that increase intracellular levels of cAMP or Ca^{2+}, NGF induces phosphorylation of CREB ser^{133}. In contrast to cAMP-induced CREB phosphorylation, NGF-induced CREB phosphorylation is dependent on activation of the small GTP binding protein Ras and occurs in PC12 cells deficient in the activity of cAMP-dependent protein kinase. These results demonstrate that CREB ser^{133} is a target of an NGF-regulated protein kinase/phosphatase in neuronal cells and CREB phosphorylation may be critical for transcriptional activation of c-*fos* and other NGF-responsive genes,

540.11

LIGAND CONTROL OF NEUROTROPHIN RECEPTOR RESPONSIVENESS IN BRAIN CELLS. B. Knüsel*, F. Hefti, and D.R. Kaplan. University of Southern California, Los Angeles, CA 90089 and NCI-Cancer Research and Development Center, Frederick, MD 21701.

We recently observed pronounced developmental changes of the neurotrophin responsiveness of rat brain. In adult tissue only minimal Trk type receptor tyrosine phosphorylation is induced by the TrkB ligands BDNF, NT-3 and NT-4/5. Speculating that chronic stimulation by endogenous neurotrophins might down-regulate the TrkB response, we chronically treated cultures of embryonic rat cortex with neurotrophins for at least 3 days. After a recovery period, in absence of neurotrophin, the cultures were acutely treated for 4 min and abundance and tyrosine phosphorylation of Trk type proteins were tested by western blotting. Chronic treatment resulted in very low levels of Trk tyrosine phosphorylation. With BDNF or NT-5, no recovery of the effect of acute treatment with the same factor was observed up to 6 hrs after termination of the chronic treatment and after 1 day the acute response was 10-20% of the maximal response. In contrast, recovery after chronic NT-3 started immediately and after 1d was approx. 50%. Wheat germ lectin precipitation and specific TrkB probing showed pronounced reduction of the level of full length TrkB but no change in truncated TrkB protein with chronic treatment. mRNA for both forms was not changed. Since in adult brain TrkB protein is not reduced, the observed down-regulation of neurotrophin response in cortical cultures does not explain the virtual lack of receptor phosphorylation induced by TrkB ligands in adult brain. Our protein results also contrast with the reported increase of TrkA mRNA after NGF treatment in adult rats (Holtzman et al., Neuron 9:465-478, 1992). Present data suggest that the neurotrophins regulate the function of their receptors at transcriptional, translational and posttranslational levels.

540.12

PHOSPHOTYROSINE (p-Tyr)-IMMUNOREACTIVITY (IR) IN THE DEVELOPING RAT RETINA. Dennis W. Rickman* and Nicholas C. Brecha. Department of Anatomy and Cell Biology, UCLA and VAMC Los Angeles, CA. 90073

Previously, we described the expression of the *trk* oncogene family of high-affinity, tyrosine kinase, neurotrophin receptors in ganglion cells of the developing rat retina. To better understand the role of these and other tyrosine kinases (e.g. pp^{60src}) in retinal development, we have examined the pattern of expression of p-Tyr in the early postnatal rat retina using a mouse monoclonal antibody against p-Tyr and standard immunohistochemical methods. Staining specificity was determined by preabsorption of the antibody with excess O-phospho-L-tyrosine. On the day of birth (postnatal day-0, PND-0), specific p-Tyr-IR was present in sparsely distributed, medium to large neurons in the ganglion cell layer (GCL). These cells were densest at the peripheral retinal margin. At PND-2, in addition to cells in the GCL, many smaller, lightly-stained p-Tyr-IR cells were present at various strata of the inner nuclear layer (INL) and, occasionally, in the outer retina. At PND-5 and 10 heavy p-Tyr immunostaining was present in cells in the GCL and in many cells at different levels of the INL. No staining was observed in the nerve fiber layer, the optic nerve or the plexiform layers at any time studied. Also, no staining was seen in either normal or colchicine-treated adult retinas. This pattern of p-Tyr expression further suggests a role for tyrosine kinase receptors, and perhaps neurotrophins, during the period of cellular differentiation and synapse formation in the retina.

Supported by EY04067 and VA Medical Research Funds

540.13

BDNF RECEPTORS (TRKB) AND SIGNAL TRANSDUCTION IN PRIMARY CULTURES OF RAT CORTICAL ASTROCYTES. J. D. Roback*, H. N. Marsh, M. Downen, H. C. Palfrey and B. H. Wainer. Depts. Pathology and Pharmacological and Physiological Sciences, Univ. Chicago, Chicago, IL 60637.

BDNF has a widespread distribution in the nervous system and has trophic effects on several neuronal populations. Three types of BDNF receptor that are members of the TrkB family have been identified in rat: a full-length form possessing a tyrosine kinase domain (TrkB-TK^+) and two truncated forms lacking the kinase domain (TrkB-TK^-). These receptors have different distributions in the CNS (Klein et al.. Cell 61, 647, 1990). We investigated TrkB receptors in cultured primary rat cortical astroglia and assessed the ability of BDNF to transmit signals in these cells. Immunoblot and immunocytochemical analysis of glial cultures with specific antibodies revealed the presence of abundant TrkB-TK^- protein in a majority of cells, but no detectable TrkB-TK^+ receptors. Similarly, only *trk*B-TK^- transcripts could be detected by RNA blot analysis; the levels of this receptor were sensitive to culture conditions and increased markedly if cells were growth-arrested in low serum. However, *trk*B-TK^+ transcripts were detected by RT-PCR, suggesting that astroglia may express low levels of the full-length receptor. BDNF treatment resulted in rapid increases in protein tyrosine phosphorylation, intracellular [Ca], MAP kinase activity and c-*fos* mRNA expression. NGF and NT-3 were without effect, but EGF had a large effect on protein tyrosine phosphorylation, MAP kinase activation and c-*fos* expression. Pretreatment of cultures with K252a (100 nM) abolished the BDNF effect, but not that of EGF, on MAP kinase activity. This result suggests that the BDNF response was mediated by TrkB-TK^+ receptors. These data demonstrate that astoglia respond to BDNF and suggest that BDNF-initiated signal transduction is transduced via a small number of TrkB-TK^+ receptors. However, a role for the much more abundant TrkB-TK^- receptors in BDNF signalling cannot be ruled out.

540.14

MULTIPLE INTRACELLULAR TYROSINE RESIDUES INVOLVED IN trk A-MEDIATED NEURITE ELONGATION IN PC 12 CELLS. Naoyuki Inagaki*, Hans Thoenen, and Dan Lindholm, Department of Neurochemistry, Max-Planck-Institute for Psychiatry, 8033 Planegg-Martinsried, Germany

Trk-A, a tyrosine kinase receptor, acts as a receptor for NGF. In this type of receptors, specific tyrosines are phosphorylated after activation and they switch on the downstream signal events. However, little is known about the tyrosines involved in trk A-mediated signal transduction. Trk A, B, and C have 10 conserved tyrosine residues in the intracellular region. To identify those involved in trk-A induced neuronal differentiation, we made rat trk A (1) mutants in which these tyrosines are changed to phenylalanine, namely; Y499F, Y594F, Y643F, Y679F, Y683F, Y684F, Y704F, Y726F, Y760F, Y794F and analyzed the activity for neurite elongation using PC12nnr5, a PC12 cell mutant lacking trk A (2). All 10 mutants showed the same activity as the wild-type trk A. This suggests that multiple tyrosine residues are involved in trk A signal transduction. We are now making mutants in which multiple tyrosines are changed.

1) Meakin,S.O. et al., PNAS 89 (1992) 2374.
2) Leb,D. et al., Cell 66 (1991) 961.

540.15

NOVEL RAT NERVOUS SYSTEM PROTEIN TYROSINE PHOSPHATASES.
J. Li, S. E. Snyder, and S. R. J. Salton*.
Fishberg Research Center for Neurobiology, Mt. Sinai School of Medicine, New York, NY, 10029.

Tyrosine phosphorylation, an important regulator of protein function, is controlled by balancing the activities of specific protein tyrosine kinases with those of protein tyrosine phosphatases (PTP). Several transmembrane PTPs have been shown to contain cell adhesion domains, potentially allowing coupling of extracellular axonal guidance clues to intracellular signal transduction pathways. We have used PCR and differential library screening to isolate rat tyrosine phosphatase clones encoding mRNAs that are developmentally regulated and expressed with relative abundance in the developing nervous system. Sequence analysis and database comparison revealed that several of these cDNAs were most closely related to the transmembrane PTP rat LAR. RNase protection analysis revealed that the encoded RNAs were most abundant in the embryonic and postnatal brain, with detectable levels in adrenal, spleen, lung and testis. RNAs encoded by two additional clones were detected exclusively in nervous tissues. One of these is highly homologous to PTP-ζ, a human transmembrane tyrosine phosphatase containing a carbonic anhydrase-like extracellular domain. The mRNA is found most abundantly in cells of glial lineage *in vitro*. *In situ* hybridization indicates very high relative levels in the ventricular zones of embryonic spinal cord and brain, and in dorsal root ganglia, cranial ganglia, olfactory epithelium, and eye.

540.16

LAR TYROSINE PHOSPHATASE RECEPTOR: ALTERNATIVE SPLICING OF A NOVEL 12 BASE PAIR CASSETTE EXON. J.S. Zhang and F.M. Longo* Dept Neurology, UCSF, San Francisco, CA 94143

The Leukocyte Common Antigen-Related (LAR) gene codes for a tyrosine-phosphatase receptor with extracellular domain sequence similarity to N-CAM. Tyrosine phosphatase receptors may influence neural growth or differentiation by modulating effects of growth factors acting through tyrosine kinase receptors. LAR is expressed by neurons and PC12 cells (Longo et al; Le Beau and Longo; Zhang and Longo, Soc Neurosci Absts; 17:762,1991; 18:949,1992). Screening an adult rat hippocampus cDNA library for additional LAR clones yielded several clones with sequence identical to LAR except for a 12 bp insert (designated LASE-b, LAR Alternatively Spliced Exon-b) 5 bases downstream from the transmembrane domain. LASE-b codes for amino acids SKQE. Sequence of flanking introns showed only 7/12 pyrimidines at the upstream intron splice-acceptor site, instead of the more typical consensus acceptor of 11-12/12 pyrimidines. RT-PCR and southern blot analysis of PCR products with LASE-b probes demonstrated that the majority of LAR transcripts do not contain LASE-b and that LASE-b splicing increased in the cortex and cerebellum during development. LASE-b splicing increased during NGF-induced PC12 differentiation and upon 3T3 fibroblasts reaching confluency. LASE-b was detected in muscle but not in other peripheral tissues. These observations indicate that both *cis*- and *trans*-acting factors control LASE-b splicing and provide a model for understanding nervous system specific regulation of alternative splicing in the context of a gene likely to regulate cell growth and development. (Supported by United Cerebral Palsy, NIH AG09873 and the VA).

540.17

MC192 TREATMENT OF PC12 CELLS RESULTS IN REDUCED NGF-MEDIATED TRK AUTOPHOSPHORYLATION. P.A. Barker* and E.M. Shooter. Department of Neurobiology. Stanford University. Stanford, CA. 94305.

NGF binds to two distinct receptors, trkA and LNGFR, on the surface of most responsive cells. The trkA receptor is a tyrosine kinase that appears to play a crucial role in transducing functional signals in response to NGF but the role of the LNGFR in mediating NGF signal transduction remains unclear. MC192, a monoclonal antibody directed against the LNGFR extracellular domain, reduces the ability of primed PC12 cells to respond to NGF (Chandler et al. JBC. 259. 6882) and attenuates the NGF-mediated c-fos response in PC12 cells (Millbrandt. PNAS. 83. 1950). We have undertaken a series of experiments to determine the mechansism by which MC192 affects NGF signal transduction.

PC12 cells were treated first with MC192 (8 ug/ml) for 30 minutes and then with MC192 and NGF (5 ng/ml) for 40 minutes. RNA was isolated from the treated cells and analyzed by Northern blot. As previously reported, the pretreatment of PC12 cells with MC192 strongly attentuates the c-fos mRNA induction. This affect is not specific to the c-fos gene; other early genes (NGFIA and VGF) were similarly affected by the MC192 treatment. EGF-dependent induction of immediate early (IE) genes was not affected by MC192 treatment, indicating that the MC192 effect is specific to the NGF pathway.

Ligand-mediated activation of many tyrosine kinase receptors results in IE gene induction. We therefore performed experiments to determine if the presence of MC192 affected NGF-induced trkA autophosphorylation in PC12 cells. In cells pretreated with MC192, the NGF-mediated (5 ng/ml) trk autophosphorylation response observed five minutes after NGF addition was sharply reduced as compared to controls. This effect of MC192 was reduced at 50 ng/ml NGF and not observed at 250 ng/ml NGF.

These results indicate that the effect of MC192 on NGF-mediated IE gene induction may be ascribed at least in part to a reduction in trkA activity.

NEUROTROPHIC FACTORS: RECEPTORS AND CELLULAR MECHANISMS IV

541.1

INHIBITION OF PROTEIN TYROSINE KINASES AFFECTS SURVIVAL AND NEURITE OUTGROWTH OF EMBRYONIC SENSORY NEURONS IN CULTURE H.J.L. Fryer*, M. Sahin and S. Hockfield. Sect. of Neurobiology, Yale Univ. Sch. of Med., New Haven, CT 06510

Growing evidence suggests that tyrosine phosphorylation plays a key role in neural development. The state of protein tyrosine phosphorylation is determined by the opposing actions of protein tyrosine kinases (PTKs) and protein tyrosine phosphatases.We have begun to examine the role of tyrosine phosphorylation in developing sensory neurons by culturing E15 rat dorsal root ganglion neurons in the presence of the PTK inhibitor, genistein, and its inactive analog, daidzein.

Genistein affects neuron survival in a concentration-dependent manner. At >50 μM, genistein is toxic to DRG neurons in a 24-hour culture period. Because the toxicity of genistein can be overcome by incubating DRG neurons with high concentrations of NGF, genistein neuro-toxicity may be due to its functional inhibition of the trk PTK through which NGF exerts its effects. Between 3-50 μM and below 0.3 μM, genistein has no observable effect on neuron survival. Between 1-3 μM, DRG neuron survival is increased even in the absence of NGF. Daidzein has no effect on neuron survival.

Genistein also affects neurite outgrowth of DRG neurons. Neurite outgrowth of chick ciliary ganglion neurons (Bixby and Jhabvala, 1992) is enhanced with genistein. Similarly, we find that, in a 4-hour culture assay, concentrations of genistein >30 μM increase the number of rat DRG cells with neurites in a concentration-dependent manner. The neurite outgrowth promoting effect of genistein is enhanced in the presence of NGF. Daidzein has no effect on neurite outgrowth.

These results suggest that tyrosine phosphorylation is involved in the survival, death and neurite outgrowth of developing sensory neurons. Supported by NS22807.

541.2

Kinase inhibitors and the staurosporine paradoxical effect on neurite outgrowth in PC12 cells. X. Z. Campbell, L. Gollapudi, and K. E. Neet*. Dept. of Biological Chemistry, UHS/ The Chicago Medical School, North Chicago, IL 60064.

Staurosporine (STSP), a potent kinase inhibitor, inhibits nerve growth factor (NGF)-induced neurite outgrowth and stimulates fibroblast growth factor (FGF)-induced neurite outgrowth at low concentration but induces neurite extension by itself at higher concentration. In order to understand the action of STSP in both induction and inhibitory modes, we examined these PC12 cell bioassays with different protein kinase inhibitors. Phorbol 12-myristate 13-acetate (PMA) chronic treatment had no effect on NGF-, FGF- or STSP-induced neurite outgrowth. Bryostatin (BRYO) chronic treatment inhibited NGF- and FGF-induced neurites but not the higher concentration STSP-induced neurite outgrowth. Both sphingosine and 6-thioguanine inhibited NGF, FGF, and the higher concentration STSP-induced neurite outgrowth. K252a, which inhibits NGF stimulation of PC12 cells, did not affect FGF or STSP-induced neurite outgrowth. Thus, NGF, FGF, and the higher concentration STSP-induced neurite outgrowth go through different signal pathways, but may share certain later steps. Low concentrations of PMA or BRYO enhanced NGF- or STSP-induced neurite extension by shifting the dose response curve to lower concentrations. STSP inhibited PC12 cell DNA replication during neuritogenic differentiation, as does NGF. These data indicate that STSP acts downstream in the signal pathway as well as at the Trk receptor level. This downstream step(s) must be past the bryostatin-sensitive step but before the sphingosine and 6-thioguanine sensitive steps. (Supported by NIH grant NS24380)

541.3

MONOAMINE-ACTIVATED α_2-MACROGLOBULIN IS A SPECIFIC INHIBITOR FOR NGF-STIMULATED *TRK*-a AUTOPHOSPHORYLATION AND SIGNAL TRANSDUCTION. W.-S. Qiu and P.H. Koo*. Department of Microbiology and Immunology, Northeastern Ohio Universities College of Medicine, Rootstown, OH 44272.

Monoamine-activated α_2-macroglobulin (α_2M) has been shown to inhibit β-nerve growth factor (NGF)-promoted neurite outgrowth and other functions of embryonic neurons and pheochromocytoma PC12 cells, whereas normal α_2M has little or no such activity. The objective of this study is to elucidate the mechanism and specificity of inhibition by monoamine-activated α_2M. The binding of methylamine activated α_2M (MA-α_2M) to *trk*, which is a part of high-affinity NGF receptor, was studied with PC12(6-24) cells and NIH-3T3 fibroblasts over-expressing *trk* (*trk*-3T3). In each case MA-α_2M readily forms stable complexes with *trk in vivo*, whereas normal α_2M does not. As determined by the Western blottings and [γ-^{32}P]-ATP incorporation assays, both 5-hydroxytryptamine-activated α_2M (5HT-α_2M) and MA-α_2M also dose-dependently blocks NGF-promoted autophosphorylation of *trk in vivo*, whereas normal α_2M is much less effective. Neither MA-α_2M, 5HT-α_2M nor normal α_2M, however, blocks either platelet-derived growth factor-stimulated or epidermal growth factor-stimulated tyrosine phosphorylation of the respective receptors, nor does MA-α_2M block fibronectin activation of focal adhesion kinase (FAK) via integrin receptors. NGF-promoted tyrosine phosphorylation of phospholipase C-γ1 (PLC-γ1) and extracellular signal-regulated kinase-2 (ERK-2) was also dose-dependently blocked by MA-α_2M, whereas NGF-independent phosphorylation of FAK was unaffected. We conclude that monoamine-activated α_2M may block neurite outgrowth and neuronal survival by its specific binding to NGF receptors, and its specific blocking of the NGF-promoted activation of intracellular second messenger pathways.

541.4

INVESTIGATION OF SIGNAL TRANSDUCTION EVENTS UNDERLYING THE RESPONSE OF PC12 CELLS TO NERVE GROWTH FACTOR USING THE CYTOSENSOR™ MICROPHYSIOMETER. S. Pitchford* & B.S. Glaeser, Molecular Devices Corp., Menlo Park, CA 94025.

Using the Cytosensor Microphysiometer we have demonstrated that PC12 cells respond to a 12 minute incubation with nerve growth factor (NGF) by increasing the cellular acidification rate of a low-buffered RPMI medium bathing the cells in an apparent dose-dependent fashion (Soc Neurosci. Abst. Vol. 18. Part 1, p614). This increase in acidification rate was reduced in a dose-dependent manner (1-50μg/ml) by pre-incubation of the cells with genistein, an inhibitor of some tyrosine kinases and by incubation with the protein kinase C inhibitor staurosporine at 10 & 100nM. Pre-incubation of PC12 cells with 10μM Rp cAMPS, an inhibitor of protein kinase A, however, did not effect the response of cells to NGF. In addition, activation of cells by NGF in a glucose-free medium resulted in a transient increase in acidification rates (compared to the longer lasting response in glucose-containing medium). This transient peak was blocked by 10μM methylisobutyl amiloride (MIA), an inhibitor of the Na^+/H^+ antiporter, suggesting the involvement of this exchanger in the response. These data suggest that the early response of PC12 cells to NGF involves the interplay of a number of cellular and membrane bound events.

541.5

MIMICKING THE NEUROPROTECTIVE MECHANISM OF GROWTH FACTOR ACTION WITH LOW MOLECULAR WEIGHT AGENTS. M. P. Mattson, B. Cheng, Y. Goodman, V. L. Smith-Swintosky, J. W. Ashford* and J. Oeltgen. Sanders-Brown Center on Aging and Dept. of Anatomy & Neurobiol., Univ. of Kentucky, Lexington, KY 40536.

Traditional and newly identified neurotrophic factors have recently been shown to protect various neuronal populations against excitotoxic, metabolic and free radical-mediated injury (*Sem. Neurosci.*, Fall, 1993 issue). Work in this laboratory indicates that in many cases the mechanism of action of the neuroprotective factors involves stablization of intracellular free calcium levels ($[Ca^{2+}]_i$) and enhancement of free radical-neutralizing systems. For example, neurotrophins (NGF, BDNF and NT-3), bFGF, IGFs, TNF, and secreted forms of ß-amyloid precursor protein protect cultured hippocampal neurons against hypoglycemic damage by preventing the late, NMDA receptor-mediated elevation of $[Ca^{2+}]_i$ (see: Cheng et al.; Barger et al.; Smith-Swintosky et al., this meeting). We now report that staurosporine, K-252a and K-252b (low M.W. compounds of microbial origin initially characterized as protein kinase inhibitors) can, at very low concentrations (100 fM -100 pM), protect cultured hippocampal, septal and cortical neurons against a variety of insults including hypoglycemia and oxidative damage (initiated by exposure to iron or H_2O_2). We show that at neuroprotective concentrations these compounds stimulate tyrosine phosphorylation of multiple proteins and stabilize $[Ca^{2+}]_i$. The data suggest that the mechanism of action of these compounds may be similar to that of endogenous neuroprotective factors. (NIH, Metropolitan Life Foundation, and French Foundation for Alzheimer's Research support).

541.6

TNF-ß AND TNF-α PROTECT AGAINST CALCIUM-MEDIATED NEURONAL INJURY IN HIPPOCAMPAL AND SEPTAL CULTURES. B. Cheng*[1], S. Christakos[2] and M. P. Mattson[1]. [1]Sanders-Brown Center on Aging and Dept. of Anatomy and Neurobiology, Univ. of Kentucky, Lexington, KY 40536. [2]Dept. Biochem. and Molec. Biol., UMD-New Jersey, Newark, NJ 07103.

Increasing data indicate that cytokines serve a variety of roles in the nervous system. Since several growth factors including neurotrophins promote the survival of CNS neurons, and because the low affinity NGF receptor (p75) is homologous to tumor necrosis factor (TNF) receptor, we sought to determine whether TNFs influence the survival of CNS neurons. TNF-ß and TNF-α (1 - 1000 ng/ml) protected cultured embryonic rat hippocampal and septal neurons against hypoglycemic injury. Calcium influx was involved in hypoglycemic injury since hypoglycemia caused an elevation of $[Ca^{2+}]_i$ and neurons were protected by incubation in calcium-deficient medium. The TNFs attenuated the hypoglycemia-induced elevation of $[Ca^{2+}]_i$. Our recent findings indicate that the mechanism of action of some excitoprotective growth factors may involve regulation of the expression of proteins involved in $[Ca^{2+}]_i$ homeostasis. In the present study TNF-ß, TNF-α, and NT-3 caused a 5- to 8-fold increase in the number of neurons expressing calbindin D28k in hippocampal cultures. The data suggest roles for TNFs in development and neurodegenerative conditions. (supported by NIH, Metropolitan Life Foundation and French Foundation for Alzheimer Research).

541.7

DOWN-REGULATION OF trk AND TYROSINE HYDROXYLASE IN PC12 CELLS BY ANTISENSE B-raf. M. Oshima*, U. R. Rapp, and G. Guroff. Section on Growth Factors, National Institute of Child Health and Human Development, Bethesda, MD 20892 and Viral Pathology Section, National Cancer Institute, Frederick Cancer Research and Development Center, Frederick, MD 21702-1201.

B-raf, the gene for one of the raf family of ser/thr kinases is expressed primarily in neuronal tissues. Previous work from these laboratories showed that PC12 cells contain a 95KDa B-raf protein kinase that is activated by treatment with nerve growth factor. In order to study the role of B-raf in the action of nerve growth factor, PC12 cells were transfected with antisense B-raf. Several clones of B-raf-negative cells were obtained and all showed, in addition, a marked decrease or a total absence of both $p140^{trk}$, the high affinity nerve growth factor receptor, and of tyrosine hydroxylase. Normal levels of $p75^{NGFR}$, the low affinity nerve growth factor receptor, choline acetyltransferase, and the epidermal growth factor receptor were present. Antisense B-raf did not alter the expression of either raf-1 or A-raf. The changes were reversible upon transient transfection. These results suggest that B-raf is involved in the expression of certain proteins that are crucial for neuronal development.

541.8

PROTEIN KINASE C ISOFORMS IN NGF-SIGNALLING. M.W. Wooten*, G. Zhou, M.L. Seibenhener, and E.S. Coleman Dept. of Zoology, Auburn University, AL 36849

Activation of cPKC compared to nPKC family members in response to PMA or NGF treatment of PC12 cells was examined. cPKC, δ and ε-nPKC isoforms were predominantly cytoplasmic (70-90%) while ζ-PKC was distributed in the nucleus (11%), cytoplasm (38%) and membrane (51%) fraction. PMA (40nM) translocated all PKC isoforms to the membrane fraction, while NGF translocated cPKC and ε-PKC, with no change in δ-PKC, whereas ζ-PKC translocated to the cytoplasm and was inhibited by sphingosine (2.5μM). Down-regulation of PKC (1 μM PMA for 48hrs) resulted in depletion of all PKC isoforms except ζ-PKC and failed to inhibit NGF-stimulated increase in cytoplasmic ζ-PKC. PMA down-regulation did not inhibit NGF- induced neurite outgrowth whereas sphingosine (2.5μM) was a potent inhibitor. Immunocytochemistry revealed ζ-PKC localization in the cell body as well as neurite branch points. Findings will be presented: 1) addressing the role of Ca^{2+} in the redistribution of ζ-PKC and whether translocation reflects the activation state of the kinase and 2) the effect of ζ-PKC 'knock-out' on NGF-induced neurite outgrowth. Taken together, our findings clarify the role of PKC isoforms in NGF signalling and suggest a novel role for ζ-PKC upstream of p21*ras*.

541.9

REQUIREMENT FOR PROTEIN KINASE C DURING LEUKEMIA INHIBITORY FACTOR MEDIATED ACTIVATION OF THE MAP KINASE CASCADE. W.P. Schiemann*, and N.M. Nathanson. Department of Pharmacology, Univ. of Washington, Seattle, WA 98195.

Leukemia inhibitory factor (LIF) is a member of the rapidly expanding family of multifunctional cytokines capable of stimulating a variety of physiological responses both in hematopoietic and non-hematopoietic cells, including neurons, myoblasts, hepatocytes, and adipocytes. While the identity of the cellular mediators involved during LIF signal transduction remains largely unknown, recent evidence implicates the activation of protein tyrosine kinases (PTKs) as possible initiators of LIF receptor (LIFR) signalling. Since stimulation of PTKs results in activation of the MAP kinase cascade in response to numerous growth factors and neurotrophic agents, we investigated if stimulation of LIFRs could similarly activate this cascade. We find that treatment of 3T3 L1 cells with LIF resulted in a 4-6-fold stimulation in cell extracts of several components of the MAP kinase cascade, including MAPKK, MAPK, and an S6K activity. Half-maximal activation of these kinases occurred at ~15 ng/ml. In response to saturating concentrations (100 ng/ml), kinase activities peaked at 15 min and declined rapidly to basal during the subsequent 15 min. Protein kinase C (PKC) activity was down-regulated in order to test its role in LIFR action. Chronic treatment of 3T3 L1 cells with PMA, but not the inactive phorbol ester PDD, attenuated LIF stimulated activation of MAPKK, MAPK, and S6K activities. Our results suggest that at least some of the cellular responses of LIF maybe mediated by stimulation of the MAP kinase cascade, and that this process appears dependent upon the presence of PKC.

541.10

THE ROLE OF NGF/PROTEIN KINASE C INTERACTIONS IN DIFFERENTIATION VERSUS MAINTENANCE OF PC12 CELLS. E. J. Martin* and E. M. Meyer, Department of Pharmacology and Therapeutics, College of Medicine, Univ. of Florida, Gainesville, FL 32610-0267

Protein kinase C (PKC) is a phospholipid-dependent, calcium- and diacylglycerol-activated kinase that preferentially phosphorylates serine and threonine residues. This enzyme has been implicated in the second messenger pathways underlying trophic events in the brain, including those triggered by nerve growth factor (NGF) administration. However, to what extent PKC is involved in the events that take place secondary to NGF binding its receptor remains unclear. We therefore designed experiments to study the effects of NGF and phorbol ester (TPA) applied at different concentrations and for various periods of time. PC12 cells are a commonly accepted model system for studying the complex biological transduction processes initiated by NGF. Cultures of both undifferentiated and differentiated PC12 cells were assayed for PKC activity by permeabilization with digitonin. By permeabilizing the cells, it is possible to introduce a specific substrate peptide and phosphate source [(32P) ATP] into the cells. These components interact with the intracellular environment in such a way that PKC-phosphorylated substrate can be collected on phosphocellulose filters and quantified. We have demonstrated in undifferentiated PC12 cells that 10 nM TPA was able to stimulate PKC activity within 15 minutes. This stimulation was attenuated with PKC inhibitor peptide [PKC (19-36)]. NGF (100 ng/ml) was also able to stimulate enzyme activity. However, this response was slower than that seen with TPA. These studies were repeated in serum-free cultures of differentiated PC12 cells. Together, the results of these enzyme activity assays in combination with assessment of cell viability indicate that PKC is partially involved in the differentiating effects of NGF and that protein kinase A may be involved in the neurite maintenance effects of NGF in PC12 cells.

541.11

MODULATION OF NEUROTROPHIN- AND EGF-INDUCED PC12 CELL DIFFERENTIATION BY K-252 COMPOUNDS; ROLE OF PROTEIN KINASE C. F. Isono, F. Ohsawa*, F. Hefti and B. Knüsel, Andrus Gerontology Center, U.S.C., Los Angeles, CA 90089;

Effects of K-252a and b on differentiation-inducing activity of neurotrophins and EGF were examined in PC12 cells. K-252a and b inhibit the differentiation of PC12 cells induced by NGF and, at lower concentrations potentiate NT-3 induced neurite outgrowth. NT-3 alone resulted in less than 3% neurite bearing cells. In simultaneous presence with 10nM K-252a or 30 nM K-252b, up to 40% of neurite bearing cells were counted. BDNF and NT-5 did not induce neurite outgrowth in PC12 cells in absence or presence of K-252a or b. The effect of sub-optimal concentrations of NGF was not potentiated.

EGF in PC12 cells activates various second messengers similar to NGF, without inducing neurite outgrowth. Co-treatment with 100 - 1000nM K-252a, but not K-252b, induced neurite outgrowth in up to 80% of cells. A similar effect was obtained with PDBu or TPA, consistent with an involvement of protein kinase C in EGF potentiation by K-252a. The fact that K-252b was inactive, suggests a different mechanism for potentiation of EGF than NT-3.

Since K-252a, but not K-252b, penetrates into the cytosol, our findings suggest that some K-252 compounds can exert a neurotrophin potentiating effect by interacting with the extracellular or transmembrane portion of the trk molecule while other actions of these compounds might involve inhibition of intracellular kinases.

541.12

GUANOSINE STIMULATES ACCUMULATION OF INTRACELLULAR CGMP IN PC12 CELLS. S. Hindley*, P.J. Middlemiss and M.P. Rathbone. Depts. Biomed. Sci. and Medicine, McMaster University Medical Centre, Hamilton, Ontario L8N 3Z5.

Rat pheochromocytoma cells (PC12) respond to nerve growth factor (NGF) by extending neurites and differentiating - assuming characteristics of sympathetic neurons. Extracellular guanosine also stimulates neurite outgrowth and synergistically enhances NGF stimulated neurite outgrowth in PC12 cells (Gysbers and Rathbone, NeuroReport 3:997, 1992). The signalling mechanism by which guanosine produces its effects is unknown. Recently guanosine was reported to increase intracellular cGMP in vascular smooth muscle (Vuorinen et al., Br. J. Pharma. 105:279, 1992). Therefore, we investigated whether guanosine stimulated intracellular accumulation of cGMP in PC12 cells. Papaverine, but not Zaprinast (an inhibitor of cGMP dependent PDE-MB 22948) inhibited PDE activity in PC12 cells. This is in agreement with Whalin et al. (Mol. Pharm. 39:711, 1991) who reported that only isoquinolines inhibit PDE activity in PC12 cells. Extracellular guanosine or vehicle control was added to the cultures for various periods (1-10 min). Basal cGMP levels were 0.59 pmol/mg protein. Addition of guanosine induced a rapid concentration-dependent increase in cGMP to 1.13 ± 0.04 pmol/mg protein after 2 minutes. cGMP levels then slowly decreased over the next 8 min. Guanosine (3.3-330 μM) increased intracellular cGMP, more so at higher concentrations. We are investigating whether increases in intracellular cGMP are associated with stimulation of neurite formation by guanosine. Support: Hospital for Sick Children Foundation and Ontario Heart and Stroke Foundation.

541.13

Molecular mechanisms of guanosine and GTP induced neurite outgrowth. J.W. Gysbers[1], D.P. Lamb[1], M.K. O'Banion[2,3], M.R. Martzen[3] and M.P. Rathbone[1] [1]Depts. of Biomed. Sci. and Med., McMaster Univ. Health Sci. Centre, Hamilton, Ont., Canada L8N 3Z5 and [2]Depts. of Neurol. and [3]Neurobiol. and Anat., Univ. of Rochester Med. Center, Rochester, NY 14642.

Guanosine (NeuroReport 3, 997-1000, 1992) or GTP enhances neurite outgrowth in NGF-treated rat pheochromocytoma (PC12) cells. Stimulation of neurite outgrowth by these purine compounds occurs even in the presence of the nucleoside transport inhibitors, dipyridamole or NBTI (5-(*p*-nitrobenzyl)-6-thioinosine). This suggests that the effects of guanosine and GTP may be mediated by extracellular receptors. These receptors have not been definitively identified.

We are comparing the intracellular molecular responses of PC12 cells treated with guanosine and GTP to those treated with NGF. Giant 2-D gel electrophoresis of phosphorylated proteins indicates that guanosine and GTP induce different phosphorylation patterns from those induced by NGF alone. We are assaying expression of several NGF-responsive genes and their corresponding proteins which are involved in neurite outgrowth, such as c-fos, Ras, MAP2 and GAP-43. Preliminary results from assays of GAP-43 mRNA and protein expression suggest that GAP-43 may be modulated by the actions of guanosine and GTP. In addition, the ratio of activated Ras to inactive Ras was found to increase upon exposure of PC12 cells to extracellular guanosine or GTP, as it is with NGF. This indicates that GTP or guanosine may act via a similar second messenger pathway as one of those stimulated by NGF. Immunocytochemical studies indicate that NGF increases high molecular weight neurofilament proteins. In contrast, guanosine and GTP added alone to the cultures did not induce high molecular weight neurofilaments.

Support: Hospital for Sick Children Foundation (Toronto) and Ontario Heart and Stroke Foundation (MPR). JWG is a Post-Doctoral Fellow of the Neuro-Oncology Foundation.

541.14

EXPRESSION OF THE DUAL SPECIFICITY CLK KINASE INDUCES THE DIFFERENTIATION OF PC12 CELLS. M. Myers, M. Murphy and G. Landreth*. Alzheimer Laboratory, Depts. of Neurology and Neuroscience, Case Western Reserve University, Cleveland, OH 44106.

The discovery of a novel class of dual specificity kinases which phosphorylates serine/threonine and tyrosine residues has lead to a substantial reevaluation of the functional properties of protein kinases. The identification of dual specificity kinases has resulted in the appreciation that these enzymes are central components of signal transduction pathways, operating immediately down stream of receptor-tyrosine kinases. We tested whether the founding member of this class, CLK (*click*; cdc2-like kinase), could intervene in NGF signal transduction cascades. The CLK gene was stably expressed in PC12 cells (PC-CLK4) under the control of the heavy metal-inducible promoter, MT1. PC12 cells do not normally express this gene. Incubation of PC12-CLK4 cells with Zn++ CLK mRNA levels resulted in the induction of CLK mRNA to maximal levels within 3 hrs, the mRNA levels then declined over the next several hours. CLK kinase activity was detected by the appearance of a 55 kDa phosphoprotein and the enhanced phosphorylation of the exogenous CLK substrate, myelin basic protein.

Sustained exposure of PC-CLK4 cells to Zn++ resulted in the morphological differentiation of the cells with the development of neurites, similar to the effect of NGF.

Treatment of the cells with NGF initiates a cascade of signal transduction events and the serial activation of protein kinases. Similarly, induction of CLK expression resulted in the activation of the MAP kinases and two S-6 kinases, p85rsk and S6PK. However, the activity of two other NGF regulated kinases, p70s6k and Fos kinase, were not affected. These data indicate that CLK can selectively regulate the activity of protein kinases involved in NGF-stimulated signal transduction and mediate the morphological differentiation of the cells.

541.15

SPECIFICITY OF NGF•*trk* ACTION: EFFECTS ON PI-3 KINASE and PLCγ. D. Blumberg*, M.J. Radeke, and S.C. Feinstein. Neuroscience Research Institute and Dept. of Biological Sciences, Univ. of California, Santa Barbara, CA 93106.

Growth factor receptor tyrosine kinases (RTK) have emerged as a key element in the regulation of cell growth and differentiation. However, the specificity of RTK action remains poorly understood. Toward addressing RTK specificity within a single cellular context, we have used PC12 cells to examine tyrosine phosphorylation of early signal transduction molecules in response to NGF, EGF, and FGF, which induce neuronal differentiation, mitogenesis and partial differentiation, respectively. Toward addressing cell-type specific responses to a single ligand•RTK complex in different cell types, we have examined tyrosine phosphorylation in response to NGF activation of its *trk* receptor in PC12 cells and in NIH-3T3 cells stably transfected with *trk* (where NGF induces mitogenesis).

Several recent reports have approached these issues. However, considerable controversy remains. Our PC12 observations are (1) NGF and EGF induce distinct patterns of PI-3 kinase tyrosine phosphorylation; FGF induces little to none, (2) we find no evidence for a direct association between *trk* and PI-3 kinase following NGF treatment, (3) we find little to no change in the overall amount of PI-3 kinase activity resulting from NGF treatment and (4) NGF induces marked tyrosine phosphorylation of PLCγ, whereas FGF appears to induce none. Lastly, the qualitative pattern of NGF•*trk* induced PI-3 kinase phosphorylation is markedly different between PC12 cells and *trk* transfected NIH-3T3 cells. Taken together, these data are consistent with a model in which different biological outcomes of a given RTK activation event can be orchestrated both by unique interactions with downstream signal transduction elements and by interactions between particular RTK's and cell type specific elements. In addition, our NGF/*trk* data are consistent with a model in which the main effect of ligand binding to its receptor with regard to PI-3 kinase activity is not to modulate the overall level of the activity but rather to influence its sub-cellular localization by virtue of SH2 interactions. Supported by NIH 1-F32-NS09350-01, ACS-CA Division S68-91 and NSF IBN-9120836.

541.16

MODULATION OF INTRACELLULAR Ca^{2+} IN CULTURED PURKINJE CELLS BY INSULIN-LIKE GROWTH FACTOR I. M. Villalba, J. Satrústegui, and I. Torres-Aleman[1]. Molecular Biology Center, and [1]Cajal Institute, CSIC. Madrid. Spain.

A prolonged inhibition of glutamate-stimulated GABA release by Purkinje cells is found when IGF-I is co-administered with glutamate in vivo. This protracted inhibition is reminiscent of the functionally plastic phenomenon of long-term depression (LTD) known to occur in the cerebellar cortex. Since elevated intracellular Ca^{2+} levels ($[Ca^{2+}]_i$) are known to be necessary in LTD, we now investigated whether IGF-I modulates $[Ca^{2+}]_i$ fluxes in cultured Purkinje cells. We found that the addition of IGF-I elicits a dramatic, prolonged increase in $[Ca^{2+}]_i$ as measured by Fura-2 microfluorimetry. Other types of cells such as small neurons and astrocytes did not respond to IGF-I. Ca^{2+} entry from extracellular sources was not essential since in the absence of extracellular Ca^{2+} the increase in $[Ca^{2+}]_i$ after IGF-I, although somewhat reduced, was still present. Furthermore, $[Ca^{2+}]_i$ increases in response to 10 nM glutamate pulses were potentiated by co-administration of IGF-I. Since LTD is known to require an increase in $[Ca^{2+}]_i$ in Purkinje cells, the present results further reinforce the notion that IGF-I is the climbing fiber messenger involved in LTD.

541.17

INSULIN (INS) AND INSULIN-LIKE GROWTH FACTOR I (IGF-I) ACTIVATE p21RAS IN CULTURED FETAL NEURONS. K.A. Heidenreich*, L. J. Robinson, J.W. Leitner, and B. Draznin. Depts. of Pharmacol. and Med., Univ. of Colorado Hlth. Sci. Ctr., and VAMC, Denver, CO 80262

We have previously shown that INS and IGF-I support the growth and differentiation of chick forebrain neurons and activate c-fos transcription by a PKC-mediated pathway in these cells. To explore other potential steps in the signalling pathway, we examined the ability of INS and IGF-I to activate p21ras by measuring increases in the amount of GTP bound to p21ras. Neurons prelabeled with 32p-orthophosphate were incubated with and without growth factor and cell lysates were immunoprecipitated with anti-ras antibodies (Y13-259, Oncogene). Pelleted 32p-labeled guanine nucleotides were separated by TLC. GDP and GTP spots were visualized by autoradiography and quantitated by liquid scintillation spectroscopy. INS and IGF-I increased GTP binding to p21ras by 21.2 +/- 4.7 and 22.6 +/- 3.3 percent, respectively. Ras activation by each peptide reached a maximum by 5 min. In the presence of INS, ras activation was sustained for 3 hr; whereas, in the presence of IGF-I, the activation was more transient. Maximal stimulation occurred at 10 ng/ml of each peptide indicating that both INS and IGF-I receptors mediate p21ras activation in these cells. The results suggest that p21ras activation represents an early step in INS and IGF-I signalling in neurons.

HORMONES AND DEVELOPMENT: NEUROANATOMICAL FINDING

542.1

DEVELOPMENTAL REGULATION OF ACTIVATIONAL HORMONE EFFECTS: AXOTOMY PREVENTS THE NORMAL DEVELOPMENT OF STEROID-SENSITIVITY IN MOTONEURONS. J.L. Lubischer* & A.P. Arnold, UCLA Brain Research Inst. and Dept. Psychol., Los Angeles, CA 90024-1563

Like other motoneurons, those in the spinal nucleus of the bulbocavernosus (SNB) recover poorly after axonal injury in development. Only 42% of SNB motoneurons survive axotomy on postnatal day 14 (P14). After axotomy at later ages (P21-adult), there is no significant SNB cell death. We asked whether motoneurons that survive axotomy at P14 show lasting effects not seen in motoneurons axotomized at later ages.

Six weeks after unilateral axotomy of SNB motoneurons, rats were castrated and given testosterone (T) or blank implants, then perfused 30 days later. As expected, the somata of SNB motoneurons contralateral to the axotomy were larger in rats given T implants ($p < .0001$). In contrast, motoneurons axotomized on P14 did not show a T-induced increase in soma size ($p > .5$). Target muscles on both sides were larger in T-treated rats (p's $< .05$). We then lengthened the time between P14 axotomy and hormone treatment, castrating and implanting rats at 5 months of age. In addition, a group of rats was axotomized on P21, then castrated and implanted at 5 months of age. Motoneurons axotomized on P21 responded to T treatment, as did their contralateral counterparts (p's $< .05$). Motoneurons axotomized on P14, however, did not show a significant response to T treatment, even at 5 months of age ($p > .05$).

SNB motoneurons axotomized (and thereby separated from their targets) early in development failed to develop normal adult sensitivity to T. Preliminary immunohistochemical evidence suggests that androgen receptors, initially down-regulated by axotomy, have recovered by the time of T-treatment in these studies. Thus, the mechanisms underlying the failure to develop T sensitivity after developmental axotomy remain unknown. Supported by NIH grant HD15021 and an NSF graduate fellowship.

542.2

DORSAL RHIZOTOMY DOES NOT ALTER DEVELOPMENT OF MOTONEURON MORPHOLOGY IN THE SPINAL NUCLEUS OF THE BULBOCAVERNOSUS. L.A. Goldstein*, A.C. Mills, and D.R. Sengelaub. Program in Neural Science, Indiana University, Bloomington, IN 47405.

Dendritic development of motoneurons in the spinal nucleus of the bulbocavernosus (SNB) in rats is biphasic--SNB dendrites grow exuberantly through four weeks of age and then retract to mature lengths by seven weeks of age. In several neural systems, afferents have been implicated in the development and modulation of dendritic morphology. To determine if dorsal root afferents might be involved in the development of SNB dendrites, we attempted to manipulate afferent availability using dorsal rhizotomy.

Dendritic development was examined in intact males and in males who received a unilateral dorsal rhizotomy (L5-S1) on postnatal day (P)7. SNB motoneurons were retrogradely labeled with cholera toxin-HRP at P28, when dendritic length is normally maximal (mean= 7034.5 μm), and at P49, when dendritic lengths are mature (mean= 4266.5 μm). Rhizotomy was confirmed using dorsal horn area measures and TMPase histochemistry. Dendritic length and soma size of SNB motoneurons were assessed with a computer-based morphometry system. Following rhizotomy, dendritic length of SNB motoneurons developed normally, reaching typical exuberant lengths at P28 (mean= 6028.3 μm) and retracting to mature lengths by P49 (mean= 3910.2 μm). Similarly, no differences in SNB soma size between intact (P28 mean= 818.5 sq μm; P49 mean= 886.9 sq μm) and rhizotomized animals (means= 920.3 and 972.4 sq μm, respectively) were observed. These results suggest that dorsal root afferents to the SNB do not influence the development of SNB motoneuron morphology.

542.3

DORSAL RHIZOTOMY DOES NOT BLOCK ESTROGEN SUPPORT OF DENDRITIC DEVELOPMENT IN A SEXUALLY DIMORPHIC RAT SPINAL NUCLEUS. T.C. Hays, A.C. Mills, and D.R. Sengelaub*. Program in Neural Science, Indiana University, Bloomington, IN 47405.

The lumbar spinal cord of the rat contains two sexually dimorphic motor nuclei, the spinal nucleus of the bulbocavernosus (SNB) and the dorsolateral nucleus (DLN). Dendrites of motoneurons in the SNB and DLN grow postnatally and establish their adult lengths by seven weeks of age. This growth is hormone-dependent, and after castration SNB and DLN dendrites fail to grow, but growth is supported in castrates treated with testosterone. In the SNB, initial dendritic growth is also supported in castrates treated with either of testosterone's metabolites, estrogen or dihydrotestosterone. To determine if DLN dendritic growth could be regulated by the conversion of testosterone to estrogen, male rats were castrated on postnatal day (P)7 and given daily injections of estrogen. Developing DLN motoneurons were retrogradely labeled with cholera toxin-HRP and their morphology was assessed in three dimensions (Eutectics NTS). As in the SNB, estrogen treatment supported initial DLN dendritic growth, and at P28 DLN dendritic length in estrogen-treated castrates did not differ from that of intact males at the same age.

Because the dorsal root ganglia which innervate the SNB/DLN target musculature (L6/S1) appear to be estrogen sensitive (Mills and Sengelaub, '93), we attempted to block the estrogenic support of DLN dendritic growth by performing unilateral dorsal rhizotomy at P7 in estrogen-treated castrates. No differences in DLN dendritic length were observed at P28 (three weeks post-rhizotomy). Thus, while estrogen can support initial SNB and DLN dendritic development, this effect is likely not mediated through the dorsal root ganglia.

542.4

EFFECTS OF TESTOSTERONE METABOLITES ON NEURON NUMBER IN SEXUALLY DIMORPHIC RAT DORSAL ROOT GANGLIA. A.C. Mills* and D.R. Sengelaub. Program in Neural Science, Indiana University, Bloomington, IN 47405.

Penile reflexes in the rat are controlled by a sexually dimorphic neuromuscular system in which males have larger perineal muscles and more numerous motor and sensory neurons innervating those muscles than do females. These dimorphisms arise through hormonal action during development. For example, females treated with testosterone during a critical perinatal period retain the perineal muscles and have masculine numbers of motoneurons in the spinal nucleus of the bulbocavernosus (SNB) and in the L6/S1 dorsal root ganglia (DRG). The sex difference in motor and sensory neuron number is a result of hormone-regulated normally occurring neuron death. In the developing SNB, motoneurons are preserved by androgen but not estrogen; females treated with estrogen (E) during the perinatal period have feminine numbers of motoneurons. To determine if the critical hormone in DRG neuron preservation is testosterone or one of its androgenic or estrogenic metabolites, we treated female rats with either dihydrotestosterone (DHT, prenatal only) or E (pre- and postnatal), and counted DRG neurons over the period in which adult neuron numbers are established (embryonic day 22 through postnatal day 10). Preliminary results indicate that by postnatal day 10, DRG neuron number is fully masculinized in females treated with E, but is unaffected by DHT treatment. These results suggest that unlike SNB motoneuron number, L6/S1 DRG neuron number can be regulated by estrogens. (Supported by NIH NS08917 to AM)

542.5

TESTOSTERONE REVERSES AGE-RELATED REGRESSIVE CHANGES IN AN ANDROGEN-SENSITIVE RAT SPINAL NUCLEUS. M.C. Clark*, M.W. Harty, and D.R. Sengelaub. Program in Neural Science, Indiana University, Bloomington, IN 47405.

Motoneurons in the rat spinal nucleus of the bulbocavernosus (SNB) are sensitive to androgens. Castration of young adults significantly reduces SNB dendritic length, soma size, and target muscle weight, and these changes are reversed with androgen treatment. Androgen titers decline with normal aging in male rats, and we have previously reported concomitant regressive changes in the morphology of the SNB system. To determine if the changes were due to age or declining androgen, we examined the response of two aging neuromuscular systems to acute or chronic testosterone treatment.

SNB motoneuron morphology and target muscle weight were assessed at 22 months of age in normal males, and males castrated at either 16 or 20.5 months and implanted with testosterone-filled Silastic capsules. SNB motoneurons were retrogradely labeled with cholera toxin-HRP and their morphology was assessed in three dimensions (Eutectics NTS). Degree of labeling was comparable across groups, and no differences in arbor area or maximal radial dendritic extent were found. However, both chronic or acute testosterone treatment restored SNB dendritic length, soma size, and target muscle weight to young adult levels. In contrast, cholera toxin-HRP labeled motoneurons in the retrodorsolateral nucleus (RDLN) showed no changes in soma size with age or androgen treatment, and no changes in target muscle weight were observed. Thus, the regressive changes seen in SNB motoneurons and their target muscles are not common to all aging neuromuscular systems. Furthermore, the age-related regressive changes in the SNB are likely due to declining androgen levels, and can be reversed with androgen treatment. (Supported by NIH AG09309)

542.6

VARIATION IN AXON DENSITY IN THE SPLENIUM OF THE RAT CORPUS CALLOSUM: POTENTIAL INFLUENCES ON SEX DIFFERENCES IN AXON NUMBER **J. H. Y. Kim* and J. M. Juraska. Neuroscience Program and Department of Psychology, University of Illinois at Urbana-Champaign, Champaign, IL 61820.**

Previous research from our laboratory demonstrated a sex difference in axon number (female > male) in the splenium of adult rats raised from weaning in either the complex (EC) or the isolated (IC) environments (Juraska and Kopcik, 1988). We recently reported that the sex difference is present by weaning age (postnatal day 25) (Kim and Juraska, 1990).

In the present electron microscopy study, we extend these results to socially housed (SC). 60 day old animals and note large variation in axon density within the splenium (posterior tenth). There was a significant variation in axon density rostro-caudally and dorso-ventrally in this region. Unmyelinated axon density was highest ventrally and lowest dorsally; conversely, myelinated axon density was lowest ventrally and highest dorsally. Axon density was higher in the caudal-most portion of the splenium. Axon density was multiplied by the area of the posterior tenth to get axon number. Females had more unmyelinated axons than males while males had more myelinated axons. Because unmyelinated axons outnumbered myelinated axons by 7:1, total axon number was significantly greater in females. Thus, the sex difference in axon number in adult rats is present whether the animals are raised in EC, IC or SC. Also, if the splenium is not systematically sampled, the observed variation in axon density could potentially influence the calculation of sex differences in axon number.

542.7

ONTOGENY OF SEX DIFFERENCES AMONG HVC PROJECTION NEURONS. M.J. Burek*, K.W. Nordeen, and E.J. Nordeen. Dept. of Psych. and Neurosci. Program, U. of Rochester, Rochester, NY, 14627.

Only male zebra finches sing and song-control nuclei exhibit estrogen (E2)-dependent sex differences in neuron number. One nucleus, the Higher Vocal Center (HVC) projects to two other sexually dimorphic regions, RA and Area X. Thus, while HVC contains some E2 target cells, it is not known if sex differences in HVC stem from direct hormone action, or rather arise indirectly through interactions with its sexually dimorphic targets. To explore this issue we determined when sex differences first develop among HVC projection neurons in relation to their interaction with RA and Area X.

We have shown previously that the number of HVC neurons labeled by ^{3}[H]-thymidine injections on days 15 and 16 posthatch is greater in males than in females by 25 days posthatch (i.e. 9 days after their birth). By combining thymidine autoradiography with retrograde-labeling we also determined that many of these same neurons project to RA in adult males. Yet, we report here that these HVC neuronal cohorts are not labeled by Fluorogold (Flg) injections into RA in 25 day old males. Thus, sex differences in the number of HVC-RA projection neurons emerge before these cells innervate RA.

Anterograde tracing using Di-I shows that HVC axons first invade Area X-LPO about 12 days posthatch. Therefore, we studied how sex differences develop among HVC-Area X neurons by injecting Flg into the LPO of males and females at 12, 20, and 50 days posthatch. After a 5 day survival time there are far more Flg-labeled HVC neurons in males than in females, even at the earliest age examined (d17) when Area X is first apparent in males. This sex difference enlarges with age because the number of HVC-Area X neurons increases in males, but not females. Our results suggest that sex differences in the number of HVC projection neurons precede, and likely are independent of, interactions with their sexually dimorphic targets.

542.8

EVIDENCE FOR POST-PUBERTAL ONSET OF THE VOLUMETRIC SEXUAL DIMORPHISM AND POST-PUBERTAL GROWTH OF THE ANTEROVENTRAL PERIVENTRICULAR NUCLEUS OF THE RAT HYPOTHALAMUS. E.C. Davis, N. Elihu, J.E. Shryne, and R.A. Gorski*, Department of Anatomy and Cell Biology and Brain Research Institute, UCLA School of Medicine, Los Angeles, CA 90024.

This lab has reported that a sex difference in the volume of the anteroventral periventricular nucleus (AVPv) was apparent on day 90 (females larger than males), but not on day 15 or 30. A possible role for gonadal steroids at puberty was postulated. In this study, we determined more specifically when the sexual dimorphism of the AVPv develops. Intact male and female Sprague-Dawley rats were sacrificed at days 30, 40, 60, and 80. We observed a main effect of both sex ($p<0.001$) and age ($p<0.025$) on the volume of the AVPv. There was no sexual dimorphism at day 30. On days 40, 60, and 80, the AVPv volumes of males and females were significantly different (for day 40, $p<0.05$; for days 60 and 80, $p<0.01$). The volume of the AVPv in males does not change significantly with age; however, in females, the volume increases significantly (a 1.35-fold increase; $p<0.01$) from day 30 to 60 and appears to level off between days 60 and 80. These findings indicate that the sex difference in AVPv volume does not appear until after puberty has occurred in the female and that the sex difference develops because the AVPv continues to grow after day 30 in females but not in males. Studies in progress examine the contributions of perinatal, pubertal, and postpubertal gonadal steroids to the sex difference in AVPv volume as well as the nature of the increase in AVPv volume with age in females. Supported by NIH grant HD-01182 and training grant HD-07228.

542.9

TIMING OF CELL BIRTH IN TARGET AREAS OF THE SEXUALLY DIMORPHIC VASOPRESSIN INNERVATION OF THE RAT BRAIN A.M. Klein, H.A. Al-Shamma, G. J. De Vries, N. R. Carlson* Amherst College, Box 210, P.O. Box 5000, Amherst, MA 01002-5000, *Program in Neuroscience and Behavior, Univ. of Massachusetts, Amherst, MA 01003.

Previous evidence suggested that the density of vasopressin-immunoreactive (AVP-ir) fiber innervation in the lateral septum (LS) is two to three times greater in males than in females, whereas in the lateral habenular nucleus (LHb) this difference is much smaller. Since the fibers in the LS and LHb come from the same group of AVP-ir cells, it was hypothesized that temporal differences in the development of these target areas may influence the development of sexual dimorphism in their AVP-ir fiber innervation. To study whether there are differences in the time of the last division (birth date) of cells in the LS and LHb, embryos were exposed to the cell birth marker 5-bromo-2-deoxyuridine (BrdU) by injecting pregnant Long-Evans rats on one of nine gestational days ranging from embryonic day 10 to day 18 (E10 to E18). At three months of age, the labeled brains were removed, sectioned, and immunocytochemically stained for AVP as well as BrdU. In general, it was found that AVP-ir fibers innervate bands of cells within the LS and LHb with birth dates distinct from surrounding cells. Furthermore, in the LS these cells were born much later (E17) than in the LHb (E14). These findings are consistent with the earlier formulated hypothesis, and suggest that the AVP innervation of target cells born earlier, such as the LHb, is less sexually dimorphic than the innervation of target cells born later, such as the LS.

542.10

ONTOGENY OF THE MALE RAT CREMASTER NUCLEUS: A CT-HRP AND CGRP IMMUNOHISTOCHEMICAL STUDY. B.W. Newton*. Department of Anatomy, University of Arkansas for Medical Sciences, Little Rock, AR 72205

The cremaster nucleus (CN) lies in the L1,2 spinal cord and is involved in testicular descent and thermoregulation via the cremaster muscle. The adult male rat CN has a pattern of calcitonin gene-related peptide-immunoreactivity (CGRP-IR) which is *opposite* to the remainder of the ventral gray horn; i.e., most CN motoneurons lack CGRP-IR but are surrounded by CGRP-IR afferents. To determine the position of neonatal male Sprague-Dawley (S-D) rat CN motoneurons and when they receive CGRP-IR afferents, the CN was retrogradely-filled with CT-HRP and/or immunostained for CGRP, using the PAP technique, on postnatal days (P)1,2,4,6,10,12,21,42, and 60. CT-HRP reveals that CN motoneurons shift slightly from a lateral to a central position in the L1,2 ventral gray horn and that prominent medial and lateral dendritic extensions appear on P2 and P4, respectively. Dorsal dendritic extensions are not prominent in neonatal life. CGRP-IR afferents are present within the CN at all timepoints examined, but show great increases in numbers and arborizations on P6 and P12. An adult pattern of CGRP-IR afferents within the CN is achieved by P21-42. The numbers of CN motoneurons which express CGRP-IR very slowly increases as the pups age and reaches ca. 3.5% of the P60 S-D CN motoneuron population. These data show that CGRP-IR afferents present in the neonatal CN surround the dendrites and soma of CN motoneurons and can, therefore, modulate the activity of the neonatal CN. This research is supported by NSF Grant IBN-9211368.

542.11

RECOVERY OF RAT HIPPOCAMPAL REGIONS FROM EARLY HYPOTHYROID RETARDATION: A STUDY OF VOLUME, SURFACE AREA, CELL NUMBER AND CYTOCHROME OXIDASE STAINING. A. Farahvar* and E. Meisami, Physiology Dept., Univ. of Illinois, Urbana, IL 61801.

Effects of early hypothyroid retardation and recovery from this condition was studied in CA1-CA4 regions of the hippocampus in 25- and 90-day rats. Newborn rat pups were rendered hypothyroid [PTU (propylthiouracil, 0.1%) in drinking water] and studied at days 25 and 90. Some pups were allowed to recover by removal of PTU at day 25 and observed at day 90. The surface area and volume but not cell number of hippocampal regions increased significantly between days 25 and 90 in controls. In the latter, CA1-CA4 regions occupied 52, 8, 32, and 9% respectively of total hippocampal surface area. Early hyothyroidism significantly reduced surface area and volume of all regions; remarkably these deficits were substantially normalized in the recovery animals. The growth of the hippocampal regions in the recovery rats was compensatory. Cell counts indicated a selective loss of cells in the CA3 region of the hypothyroid rats which was not restored in recovery animals. These quantitative changes and the pattern of staining of rat hippocampal regions by cyto-chrome oxidase will be presented for the normal and experimental animals. Results indicate selective vulner-ability as well as marked growth plasticity in hippocampal regions.

542.12

ANTERIOR ROOF DEAFFERENTATION OF THE PREOPTIC AREA INCREASES EAR WIGGLING BUT NOT LORDOSIS IN 19-DAY-OLD FEMALE RATS. G.S. Benedict, H. Grossman, & C.L. Williams. Departments of Psychology, Columbia University & *Barnard College, New York, NY 10027.

During the first week of life, both male and female rats display lordosis and ear wiggling without prior hormone priming (Williams, Behav. Neurosci., 1987). Work from our lab has shown that lordosis and ear wiggling become sexually dimorphic and more dependent on ovarian hormones for elicitation by postnatal days (PD) 10-18. Defeminization ultimately results from the presence of perinatal testicular androgen, although behavioral consequences are not manifested until one week later perhaps following the maturation of inhibitory neural systems. Our previous work suggests that the development of serotonergic afferents to the ventromedial hypothalamus contributes to the defeminization and the maturation of hormonal dependence of lordosis but not of ear wiggling (Benedict & Williams, Soc. Neurosci. Abst., 1992). Another possible source of inhibition may be forebrain afferents to the preoptic area, since dorsal deafferentation of this region unmasks both lordosis and ear wiggling in adult male rats (Yamanouchi and Arai, Neuroendocri., 1985). Using a Halász knife, we deafferented the roof of the preoptic area bilaterally rostral to the anterior commissure in male and female rats on PD 17, then tested them for lordosis and ear wiggling two days later. The knife cut had little effect on lordosis. However, it significantly increased the frequency of ear wiggling in hormone-primed (1 µg estradiol benzoate + 0.5 mg progesterone) female rats. There were little or no effects of the knife cut in males or in oil-treated females. Taken together, these results combined with previous findings suggest that the sexual differentiation of lordosis and of accompanying proceptive behaviors such as ear wiggling is subserved by the hormonally-driven development of at least two different processes.

REGENERATION IV

543.1

EXPRESSION AND REGULATION OF HEAT SHOCK PROTEINS FOLLOWING FACIAL NERVE AXOTOMY IN RATS. U.E. Olazábal, M. Reddington and G.W. Kreutzberg. Max-Planck-Institute for Psychiatry, Martinsried, Germany.

Cellular heat shock proteins (hsps) are induced in response to various stresses, including neurotoxicity and mechanical injury. We carried out this study to investigate the expression of constitutive hsp70kD (hsp70c) and hsp60kD in the facial nucleus following transection of its nerve at the stylomastoid foramen in male rats. The operated and control facial nuclei were removed by microdissection from intact controls and at 1h, 3h, 6h, 12h, 24h, 3d, 7d, and 14d after axotomy (N=6 per group) prepared in SDS sample buffer and normalized with respect to total facial nucleus protein content. Hsp70c and hsp60 were detected by Western blotting using antibodies to these hsps (StessGen) and enhanced chemiluminescence. Densitometric analysis showed that hsp70c levels were induced in the facial nucleus as early as 6h after axotomy (25.7% increase), reached maximum levels at 3 days (47.5% increase) and remained elevated thereafter. No changes in immunoreactive hsp70c were noted in contralateral unoperated nuclei. Preliminary analysis of hsp60 levels has indicated a similar time course of induction following axotomy. These results suggest that axotomy induces heat shock proteins in cells of the facial nucleus and that these events may be important in the early dynamic phases of regeneration. Immunocytochemical studies are under way to determine which cell types may be participating in this regulation.

543.2

FLUTAMIDE BLOCKS THE EFFECTS OF ANDROGENS ON HAMSTER FACIAL NERVE REGENERATION. K.J. Jones* Departments of Physical Therapy, and Anatomy and Cell Biology, University of Illinois at Chicago, Chicago, IL 60612.

In adult male hamsters, systemic administration of androgens at the time of facial nerve crush at the stylomastoid foramen accelerates the rate of facial nerve regeneration. In this study, the hypothesis that androgens affect facial nerve regeneration through a receptor-mediated mechanism was tested using the anti-androgen, flutamide, which blocks binding of the steroid to its receptors. Three-5 days after castration, hamsters were subjected to right facial nerve crush axotomies at the level of the stylomastoid foramen, and divided into 3 treatment groups: 1 testosterone propionate (TP) subcutaneous implant, 1 TP implant plus 1 daily injection of flutamide (10 mg/0.1 ml propylene glycol), or blank implants. Postoperative times ranged from 3-7 days. Stereotaxic injections of 3H-amino acids into the right facial nucleus were done, and the radioactivity in 1-mm segments of the dissected nerves determined and plotted as a function of time after injury. The furthest distance point at which the radioactivity was $\geq$ 2 S.D. above background defined the outgrowth distance of the fastest growing axons. The results indicate that flutamide blocks the TP-induced increase in outgrowth distances of regenerating facial axons, such that the values obtained for the flutamide and TP-treated animals are equivalent to those values obtained from the castrated only animals and significantly lower than TP-treated animals. Supported by NIH grant NS28238.

543.3

LOCAL CEREBRAL PROTEIN SYNTHESIS IN THE REGENERATING HYPOGLOSSAL NUCLEUS IN EUTHYROID AND HYPERTHYROID RATS. C. R. Dermon, G. E. Deibler, J. Jehle, L. Sokoloff, and C. Beebe Smith*, LCM, NIMH, Bethesda, MD 20892.

The rate of protein synthesis in the developing central nervous system is stimulated by thyroid hormones whereas in the mature brain sensitivity to thyroid hormones is lost. Following axotomy cranial nerve cells undergo chromatolytic changes and increases in rates of both glucose utilization and protein synthesis. We hypothesized that during regeneration neurons revert to an earlier developmental state that includes re-establishment of sensitivity to thyroid hormones. This question was examined by determining rates of protein synthesis in regenerating hypoglossal nucleus after axotomy in euthyroid and hyperthyroid rats. In 12 female, Sprague-Dawley rats (160-250g) the right hypoglossal nerve was cut at the level of the digastric muscle. Following axotomy six rats were treated daily for seven days with 100 µg of L-thyroxine (i.p.) and six were treated with vehicle. On post-axotomy Day 7 local rates of cerebral protein synthesis ($lCPS_{leu}$) were determined with the *in vivo* autoradiographic L-[1-^{14}C]leucine method (Smith et al., *PNAS* 85:9341, 1988). $lCPS_{leu}$ was measured in the nucleus as a whole at the level of appearance of the central canal. In both the vehicle- and thyroxine-treated rats $lCPS_{leu}$ was significantly increased in the axotomized hypoglossal nucleus ($P < 0.002$, paired t-tests). The percent increase, however, was not greater in the thyroxine-treated rats as we had hypothesized; in fact, it was somewhat smaller.

543.4

MATRIX REMODELING AND NERVE ACTIVITY AT FROG NEUROMUSCULAR JUNCTIONS. E. A. Connor*, K. Qin, H. Yankelev, and D. DeStefano. Univ. of Massachusetts, Amherst, MA.

Connective tissue in junctional regions of skeletal muscle undergoes striking remodeling after denervation. Interstitial cells accumulate near denervated neuromuscular junctions as do several matrix molecules including fibronectin and tenascin. This remodeling of matrix by interstitial cells may influence synapse regeneration. In previous experiments aimed at understanding the process that produces the connective tissue remodeling, we determined that postsynaptic blockade of muscle activity did not induce the interstitial cell accumulation. Here we examined the role of nerve activity in regulating connective tissue remodeling. Nerve activity was blocked by tetrodotoxin (TTX). Placebo or TTX pellets (9ug TTX) were placed adjacent to nerves to cutaneous pectoris muscles. Blockade of synaptic transmission was assessed by nerve stimulation proximal and distal to the pellet. Synaptic transmission was blocked for 14 days in TTX-treated muscles. We determined that presynaptic blockade of synaptic transmission by TTX did not induce an accumulation of interstitial cells. The distribution of interstitial cells in innervated muscles treated with either placebo or TTX pellets was not different from normal innervated muscles. In addition, there were no junctional accumulations of tenascin or fibronectin in TTX-treated innervated muscles. These data suggest that the loss of evoked nerve activity is not the signal that initiates the remodeling of connective tissue after denervation. Connective tissue remodeling in denervated muscle may occur by an activity-independent mechanism or by a mechanism activated by phagocytosis of degenerating axons.

543.5

HUMAN MUSCLE FIBERS REGENERATING IN VIVO AND IN VITRO EXPRESS STRONG ß-AMYLOID PRECURSOR PROTEIN mRNA WHILE NORMAL ADULT FIBERS DO NOT. E. Sarkozi[1], V. Askanas[1], J. McFerrin[1], S.A. Johnson[2], W.K. Engel[1]*. [1]USC Neuromuscular Center, U. So. Cal. School of Medicine, Los Angeles, CA 90017; [2]Andrus Gerontology Center, USC.

ß-amyloid protein (Aß) may be important in the pathogenesis of Alzheimer's disease (AD). Although accumulation of Aß and its precursor protein (ßAPP) has been considered confined to brain and its blood vessels, we have recently demonstrated Aß, ßAPP and ßAPP-mRNA accumulated in vacuolated muscle fibers of inclusion-body myositis (IBM) patients (Askanas, et al., 1992, Sarkozi, et al., 1993). We have now studied expression of ßAPP mRNA in a) muscle biopsies of patients with polymyositis 6, IBM 10, morphologically nonspecific myopathy 3, Duchenne dystrophy 1, b) normal muscle biopsies 5, and c) muscle fibers cultured from satellite cells of normal biopsies. In the various diseases, regenerating muscle fibers, identified by strong desmin immunoreactivity, expressed very strong ßAPP mRNA by in situ hybridization, utilizing a ^{35}S-labelled cRNA probe transcribed from a human ßAPP-751 DNA clone (Ponte, et al., 1988). Adult normal (non-regenerating) muscle fibers did not have identifiable ßAPP mRNA by in situ hybridization or northern blot. In contrast, cultured (regenerating) muscle fibers very strongly expressed ßAPP mRNA by in situ hybridization and northern blot, the former showing more ßAPP mRNA in myoblasts than in myotubes. Conclusion: generation of ßAPP is greatly increased in regenerating muscle, suggesting that ßAPP may play an important role in human muscle development.

543.6

IMMUNOCYTOCHEMICAL LOCALIZATION OF SKELETAL-MUSCLE-SPECIFIC TRANSCRIPTION FACTOR MEF2C IN HUMAN ADULT NORMAL AND PATHOLOGIC MUSCLE BIOPSIES. V. Askanas[1]*, M. Bilak[1], D. Leifer[2], W.K. Engel[1]. [1]USC Neuromuscular Center, Univ. of So. Cal. School of Medicine, Los Angeles, CA 90017; [2]Dept. Neurol., Harvard Medical School.

Human MEF2C (myocyte-specific enhancer binding factor 2C) belongs to a new class of MADs-box factors that interact with the MEF2 regulatory element and may mediate cell lineage, myogenesis and muscle formation. The expression and role of muscle transcription factors in normal and diseased human muscle have not been studied to our knowledge. Using a well-characterized polyclonal antiserum against MEF2C, we immunocytochemically studied muscle biopsies of 16 patients: polymyositis 4; inclusion body myositis (IBM) 6; morphologically non-specific myopathy 1; Duchenne muscular dystrophy 2; amyotrophic lateral sclerosis 2; normal muscle 5. In normal muscle, all nuclei and the cytoplasm of the postsynaptic domain of neuromuscular junctions had strong MEF2C immunoreactivity (IR). Regenerating muscle fibers (identified by double-labeling with desmin), in all pathologic biopsies that contained them, had strong cytoplasmic MEF2C-IR in addition to strong MEF2C-IR in the nuclei. IBM vacuolated muscle fibers had increased MEF2C immunoreactivity in and around nuclei. Conclusion: Our studies demonstrate novel localizations of MEF2C, suggesting that in human muscle MEF2C may be involved in a) transcription of postsynaptic proteins, and b) selective pathological processes.

543.7

MUSCLE REINNERVATION AFTER NERVE TRANSECTION AND ENTUBULATION REPAIR. F.C. Pereira and C.F. Da-Silva*. Neurobiology Lab., Dept. of Histology, University of Sao Paulo, Sao Paulo, Brazil.

The aim of the present study was to quantify the reestablishment of the neuromuscular junctions using the technique of entubulation repair as a paradigm to study peripheral nerve regeneration. Sixteen adult C57BL/6J mice received sciatic nerve transection at mid-thigh level and both proximal and distal nerves stumps were sutured into a 6 mm long polyethylene tube, ID 0.76 mm, leaving a nerve gap distance of 4 mm. Following 2 to 40 weeks after surgery, animals were perfused with fixative and the EDL muscle from the operated side was removed and processed histochemically for visualization of both cholinesterase sites and nerve fibers according to the protocol of Pestronk & Drachman (Muscle & Nerve, 1:70, 1978). Sixteen additional animals suffered sciatic nerve crush and were treated in the same fashion for comparison. A total of 2800 randomly selected synaptic areas were classified according to their innervation pattern. For morphometric analysis, camera lucida drawings of impregnated terminals were made and, with the aid of a computer system (Biographics), the number of branching points of terminal arborization and total length of terminal arborization from the first branching point were evaluated.Two weeks after nerve transection and repair, the EDL muscle was entirely denervated. After six weeks the muscle was reinnervated and axon sprouting among the muscle fibers led to polyinnervation of the EDL muscle. After 40 weeks, 80% of the muscle fibers were mono-innervated, and 80% of these endplates were monoinnervated by axons coming from the nerve trunk (a situation found in all muscle fibers of the EDL muscle from non-operated animals). Data from the morphometric analysis showed no difference in the nerve terminal branching pattern between non-operated and entubulizated animals. It is noteworthy that the same ratio of mono to polyinnervated fibers (4:1) was also found in animals with the same survival time (40 weeks) in which the sciatic nerve was simply severed by crushing. **These results show a similar pattern of long term muscle reinnervation after nerve transection with entubulation repair and crush lesion.** (Supported by FAPESP Grant).

543.8

IN VIVO GROWTH OF SCHWANN CELLS THROUGH A SILICONE TUBE IN THE ABSENCE OF AXONS REQUIRES BOTH ISOLATED "NERVE" SEGMENTS TO BE LIVING. Roger D. Madison[1] and Simon J. Archibald*[2]. [1,2]Departments of Neurosurgery, [1]Neurobiology, Duke University Medical Center and [1]Veterans Affairs Hospital, Durham, NC 27710.

It is known that axons co-migrate with Schwann cells during successful peripheral nerve regeneration through a nerve guide tube, and there is evidence that axons will not regrow in the absence Schwann cells. Conversely however, it has recently been shown that Schwann cells can form a tissue cable through a tubular prosthesis in the absence of any axonal ingrowth (A. A. Zalewski et. al. Neurosci. Abst. 1990, 529.6). In the present study we tested the ability of cells to form a bridge within a 12 mm silicone tube connecting two isolated denervated "nerve" segments in 10 adult rats. An 8 mm segment of sciatic nerve was removed, a 4 mm segment of the nerve acted as a "proximal" segment and the remaining 4 mm of the nerve acted as a distal segment. Both segments were sutured into the tube and the other end of each segment was tightly ligated to prevent axonal ingrowth. In 5 animals one segment was frozen to kill all cells. Although tissue cables were formed in all animals, Schwann cells (by + S100 staining) were only found in animals with both segments living. The continuous bands of Schwann cells were highly upregulated for NGF receptor. NS22404-08 (RDM) and VA Merit Review Program.

543.9

TRAJECTORY OF REGENERATING AXONS ACROSS ENCLOSED GAPS
GM Koschorke*, V. Mathur, R. Sood, TM Brushart, Curtis Hand Center, Union Memorial Hospital, Baltimore, Maryland 21218, USA

Synthetic tubes have been used clinically to bridge gaps in peripheral nerve. Axons regenerate in adequate numbers across short gaps (up to 3cm in primate). However, axonal dispersion within the tube contributes to non-specific target reinnervation and poor function (NSci.Abst. 16: 806). This study was designed to measure the dispersion of axons crossing a gap and the effects of gap distance and distal stump orientation on this dispersion. In 20 female SD rats both sciatic nerves were cut and the tibial and peroneal fascicles were precicely oriented within silicon tubes. This was accomplished by sewing the adjacent proximal or distal stumps to opposite sides of a thin silicon sheet, and inserting the sheet partway in the end of the tube along a slit in the tube's diameter. Four groups were prepared: correct alignment (CA), 2mm and 5mm gap, reverse alignment (RA) (proximal tibial aligned with distal peroneal), 2mm and 5mm gap. After 6 weeks, HRP-WGA was applied to the peroneal nerve distal to the tube. Cross sections of the proximal tube were drawn and digitized to determine the percentage of distal peroneal fibers originating from proximal peroneal and tibial fascicles respectively. In the 2mm CA group, 34% of axons entering the peroneal fascicle distally had crossed the midline from the proximal tibial stump, while 44% had crossed at 5mm. In RA groups (preliminary data) 78% originated from co-linear tibial nerve at 2mm and 77% at 5mm. Conclusions: 1. axonal growth is initially straight 2. significant dispersion occurs at 2mm 3. no significant increase in dispersion is demonstrated between 2 and 5mm 4. no tropic or trophic mechanism corrects fascicular malalignment.

543.10

NERVE EXPANSION:THE OPTIMAL ANSWER FOR THE SHORT NERVE GAP.
T.G. Skoulis , J.K. Terzis.* MRC, EVMS, P.O. Box 1980, Norfolk, VA 23501.

The management of a short nerve gap still presents a dilemma to the reconstructive surgeon. Specific aims: to establish an experimental model of nerve gap, to investigate the effects of expansion on a normal nerve, as well as on the proximal vs the distal segment of a transected nerve prior to repair, and to correlate these data to 3 control groups: tension free nerve repair, end-to-end coaptation under tension and regeneration through an interposition nerve graft. The rats were randomized in 7 groups. I: expander placed under the nerve but not expanded. II: slow expansion of an intact nerve. III & IV: slow expansion of the distal or the proximal end of a transected nerve. V: interposition graft. VI: coaptation under tension. VII: tensionless coaptation of a transected nerve. The functional recovery was assessed by these modalities: Behavioral, Electrophysiological (CAP) and Morphological. Our findings suggest that significant elongation does takes place. The CAP can be elicited from an expanded normal nerve as well as from a repaired nerve after proximal and/or distal expansion. The results were much better with a proximal expanded nerve segment. The functional return compared very favorably with the tension free repair.

543.11

INFLAMMATORY CELLS AND MEDIATORS IN NERVE REGENERATION CHAMBERS.
Lars B. Dahlin*, P. Thomsen and N. Danielsen, Dept. Hand Surg., Malmö Gen. Hosp., Univ. Lund, S-214 01 Malmö and Dept. Anatomy and Cell Biology, Medicinaregatan 3, Univ. Göteborg, S-413 90 Göteborg, Sweden.

The inflammatory response was quantitatively evaluated during peripheral nerve regeneration. The fluid accumulating in silicone nerve regeneration chambers (inserted in rats) was collected during the early period of regeneration of transected sciatic nerves. This fluid was analyzed with respect to inflammatory cells and mediators (leukotriene B4; LTB4, interleukin-1α, IL-1α). Leucocytes were detected during the entire observation period (up to 7 days after implantation). The highest concentration was detected after 24 h. The predominant cell type during the initial 5 days was the polymorphonuclear granulocyte. Analysis of the concentration of LTB4 demonstrated two peaks, at 24 h and 5 days. The IL-1α concentration displayed an early and relatively smaller peak after 24 h and a second and much larger peak after 7 days. The present study demonstrates that the early stage of nerve regeneration in the silicone chamber model is concomitant with an inflammatory reaction.

543.12

TRANSCRIPTIONAL REGULATION OF INTRACELLULAR SIGNAL MEDIATOR ENZYMES AFTER NERVE INJURY.
K. Ohno, T. Saika, and H. Kiyama* Dept.of Neuroanatomy, Biomedical Research Center, Osaka University Medical School, 2-2 Yamadaoka, Suita, Osaka 565. JAPAN.

In motoneurons, it is well known that peripheral nerve injuries enhance the expression of some neuropeptides. However, intracellular mechanism in the transcriptional regulation after the nerve injury remains unclear. In this respect, we focused on intracellular signal mediator enzymes, which are supposed to be involved in the regulation of gene transcription and intracellular signal transmission. The transcriptional regulation of these enzymes in the facial motor neurons was examined after the nerve injuries. Oligodeoxynucleotide probes specific to mRNAs of phospholipase C (PLC) isozymes, cAMP dependent protein kinase (PKA) subunits, calmodulin dependent kinase II (CaMKII) and protein kinase C (PKC) isozymes were labeled with ^{35}S-dATP at 3' terminal of the probes enzymatically and used for in situ hybridization histochemistry. After uni-lateral lesion the facial nerve, animals (male rats 150g) were sacrificed at various time points. The mRNA signal level was measured by image analyzer. Among all probes examined, PLCα and PKA regulatory subunits (RII α and β) mRNAs' level were significantly increased after the nerve injury, while PLCβ mRNA was repressed. These results suggested that when nerves got injury, transcription of PKA was more up-regulated than those of PKC and CaMKII. Among PLC isozymes, PLCβ is thought to play important role in intracellular signal transmission, while a function of PLCα has been unclear. The down-regulation of PLCβ may lead to down regulation of IP3/DG cascade, whereas up-regulation of PLCα may be indicating that an involvement of PLCα in the neuronal response against the nerve injury.

543.13

PREDEGENERATION ENHANCES NERVE REGENERATION IN A RAT SCIATIC Y-CHAMBER MODEL.
O. Zhao*, J. M. Kerns and N. Danielsen, Dept. Hand Surg., Malmö Gen. Hosp., Univ. Lund, S-21401, Malmö, Sweden and the Dept. Anat., Rush Med. Coll., Chicago, IL, USA

We studied rat peroneal and tibial nerve regeneration in a silicone Y-chamber model. The two distal nerves were either a 7 day predegenerated nerve segment (PNS) from the donor side or a fresh nerve segment (FNS). The proximal stump of the cut peroneal or tibial nerve was inserted into the proximal inlet of the chamber and the PNS and FNS of the corresponding nerve were inserted into the distal outlets. After 14 days survival, the regenerates in the distal tunnels were nearly equal in size (i.e. PNS≈FNS) for both peroneal and tibial groups. A great number of cells, whose nuclei appeared with Acridine orange were located throughout the regenerates. Schwann cells identified by S-100 protein were more abundant close to the nerve stumps. At 28 days postoperative, the size of the regenerate towards the PNS was 26% greater than that towards the FNS for the tibial nerve group and 14% greater for the peroneal nerve group. The density and number of regenerated myelinated axons in the distal nerve segment was greater on the PNS side for both the tibial (97% and 88%, respectively) and peroneal (221% and 221%, respectively) nerve groups. In contrast, the elevated density and number of non-vascular nuclei (mainly Schwann cells) was relatively constant for both PNS and FNS. It is suggested that the early activation of Schwann cells in the PNS segment is responsible for the enhanced regeneration and maturation observed in this Y-chamber model.

543.14

THE EFFECTS OF ACELLULAR GRAFTS ON NERVE REGENERATION.
N. Danielsen*, J. M. Kerns, O. Zhao, M. Kanje and G. Lundborg, Dept. Hand Surg. Malmö Gen. Hosp., Univ. Lund, S-214 01 Malmö; Dept. Animal Physiol., Univ. Lund, Sweden and Dept. Anatomy, Rush Med. Coll., Chicago, IL.

The use of nerve grafts to bridge a nerve defect is a well accepted clinical principle. The purpose of the grafting procedure is to offer the outgrowing axons the best possible microenvironment. The main candidates responsible for providing this microenvironment are the Schwann cells and the factors (e.g. basal lamina) these cells produce. We have examined the effects of various acellular grafts (created by repeated freeze/thawing) on early nerve regeneration. A 10 mm defect in the rat sciatic nerve was bridged by either acellular nerve grafts (ANG), or acellular muscle grafts (AMG), or acellular predegenerated grafts (APNG). The axonal regeneration distance was assessed by the sensory pinch test. The excised specimens were examined by immunohistochemistry for neurofilaments and conventional light microscopy. The results indicate that the APNG data best fitted a linear regression line with a delay period of 2.7 days. The data from the ANG and AMG groups were best expressed in exponential terms and both these groups had a prolonged delay period of at least 6 days. APNG grafts had a regeneration rate and delay period not significantly different from that of fresh (cellular) nerve grafts. The stimulatory effects of predegeneration may be due to an increase in the number of activated Schwann cells producing factors enhancing regeneration. Such factors apparently remain bound in the tissue stroma even if the cells are killed prior to grafting.

543.15

COLLATERAL SPROUTING FROM AN INTACT NERVE INTO AN ANASTOMOSED PREDEGENERATED STUMP. J.M. Kerns*, N. Danielsen, Q. Zhao, M. Kanje and G. Lundborg. Dept. Anatomy, Rush Med. Coll., Chicago, IL. and Depts. Hand Surg. Malmö Gen. Hosp., Animal Physiol., Univ. Lund, Sweden.

The ability of an attached nerve stump to attract collateral sprouts from an intact peripheral nerve was studied in the rat. Either a fresh (FNS) or predegenerated (PNS, 7d) segment was anastomosed end-to-side to an intact sciatic nerve midthigh with or without a perineural window. The presence of sprouts was evaluated by the sensory pinch test and histological examination. In another series the peroneal PNS was attached to the tibial fascicle and muscle contractility was measured at 90 dpo. Pinch test results showed a significantly greater response using PNS vs. FNS in the no-window group (5/8 vs. 0/5) at 14 dpo and in the window group (7/7 vs. 0/2) at 35 dpo. Microscopy showed new axons in the stump, presumably by collateral sprouting from intact axons rather than regeneration from damaged axons. Muscle tetanic force in the reinnervated tibialis anterior was greater using window anastomosis (69%) vs. no-window (49%). In conclusion, collateral sprouting can occur from an intact nerve, with or without a perineural window. Predegeneration may augment this via the neurotrophic action exerted by proliferating non-neuronal cells. This may have clinical application in nerve repairs. Supported by the Fogarty International Center and Swedish Medical Research Council.

543.16

SPECIFIC REINNERVATION OF VIBRISSAL FOLLICLES AFTER PARTIAL DENERVATION OF THE WHISKERPAD IN ADULT MICE. M-E. Corthésy, E. Welker, H. Van der Loos*. Institute of Anatomy, University of Lausanne, 1005 Lausanne, Switzerland.

We previously established that after transection, in the adult mouse, of the sensory nerve to vibrissal follicles of row C, all regenerating fibers reinnervate the follicles of that row only (Corthésy et al., Soc.Neurosci.Abstr.'92). Do reinnervating fibers result from sprouting of axons innervating follicles of rows B and D? Fast blue (○), and diamidino-yellow (◉) were injected in 7 mice 40 d. after transection (cartoon) and in 2 controls as follows: one tracer was injected in the 3 caudalmost follicles of row C and the other in those of each of rows B and D. Labelled cells were counted in the gasserian ganglion, and the results are summarized below.

CONTROLS	EXPERIMENTALS
● 0.33 - 2.33 %	0 - 2.26 %
◉ 61 - 66 %	65 - 77 %
○ 34 - 35 %	19 - 35 %
n= 301 - 307	n= 174 - 307

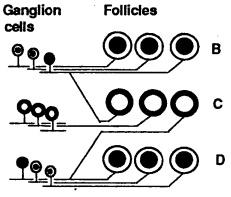


Table: ranges of the percentages of blue (○), yellow (◉) or double labeled (●) ganglion cells. 'n' is the range of total number of labeled cells per ganglion. The proportion of double-labeled cells is very similar in both groups of mice. Conclusion: reinnervation of row C is almost entirely due to specific regrowth of C row nerve fibers. Support: Swiss NSF 31-30932.

543.17

TRANSECTED CORTICOSPINAL AXONS REGROW FOLLOWING CO-IMPLANTATION OF CARBON FILAMENTS AND CULTURED FETAL CNS TISSUE. M.F. Dauzvardis*, T. Khan, S. Sayers, T. Trausch, and B. Dunlap. Rehabilitation R&D Center, VA Hines Hospital, Hines, IL 60141.

Carbon filament implants have been shown to promote limited axonal regrowth following partial and total transection of the rat spinal cord. In an attempt to amplify the axogenic properties of carbon filaments we co-implanted small diameter carbon filaments with cultured fetal spinal cord or cortex into the site of a mid thoracic spinal cord lesion which had completely transected the corticospinal tract.

Fetal cortex or fetal spinal cord harvested from E-16 rat embryos was cultured on bundles of approximately 10 thousand, (5 micron diameter) carbon filaments which were affixed to the bottom of petri dishes. After 3 days the carbon was cut into 4 millimeter sections and implanted into midthoracic spinal cord lesion sites. Two animals received, cultured cortex/carbon filament implants and two received cultured spinal cord/carbon filament implants. After cortical injection of WGA-HRP, labeled axons were observed as far as 4 millimeters below the implant site in all 4 animals, a finding not observed in surgical control animals. These results indicate that enhancing carbon filaments with various fetal cell populations may lead to increased recovery following partial spinal cord transection.

This research was supported by funds from the Rehabilitation R&D Center, VA Hines Hospital and the Amoco Foundation.

543.18

FK506, AN IMMUNOSUPPRESSANT, INCREASES FUNCTIONAL RECOVERY AND AXONAL REGENERATION IN THE RAT FOLLOWING AXOTOMY OF THE SCIATIC NERVE. B.G. Gold*†^, T. Storm-Dickerson†, D.R. Austin†, and K. Katoh†. †Center for Res. on Occup. & Environ. Toxicol. and ^Dept. Cell Biol. & Anat., Oregon Health Sci. Univ., Portland, OR 97201.

FK506 is a new immunosuppressive agent isolated from *Streptomeyces tsukubaensis*. The possibility that it may affect neurons is suggested by its ability to inhibit calcineurin since a prominent substrate for this phosphatase is the growth-associated protein, GAP-43. In fact, a preliminary report (Steiner et al., *Soc. Neurosci. Abst.* 12:603,1992) indicates that FK506 increases GAP-43 phosphorylation. Thus, we asked whether FK506 alters functional recovery in the rat sciatic nerve following nerve transection (axotomy). Rats given a sciatic nerve crush received daily subcutaneous injections of FK506 (10 mg/kg); axotomized control animals received saline. Clinical signs of recovery in the hind feet were manifested two days earlier in FK506-treated than in saline-treated animals; movement of the toes and walking (on the hind feet and toes) were first observed at 16 and 17 days, respectively, in saline-treated rats and at 14 and 15 days, respectively, in FK506-treated rats. Interdigit distances in the hind feet showed a return toward normal position of the toes (increased interdigit distances) at 18 days in FK506-treated rats. Light and electron microscopy (at 18 days) demonstrated that the sciatic nerve and its terminal branches from FK506-treated animals contained more myelinated fibers and larger regenerating axonal sprouts compared to saline-treated animals; myelination was further advanced, the endoneurium appeared more organized, and there were fewer remaining macrophages. Nerve outgrowth determinations (measured by radiolabeling the L5 dorsal root ganglion at 9 days and examining the distance traveled by fast axonal transport in sensory axons on day 10) indicated an increased rate of axonal elongation in FK506-treated rats. Taken together, the results suggest that FK506, by increasing the rate of axonal regeneration, may be beneficial for the treatment of peripheral nerve injuries. Supported by NS19611.

543.19

Alternative Splicing of Fibronectin in Injury and Repair of the Rat Peripheral Nervous System. G.A. Mathews* and C. ffrench-Constant
Wellcome/CRC Institute of Cancer and Developmental Biology, Tennis Court Road, Cambridge, CB2 1QR, U.K.

The primary gene transcript for fibronectin (FN) can be alternatively spliced in three regions to yield distinct forms of FN (Kornblihtt et al., 1985; Norton and Hynes, 1987). In rats two exons (EIIIA and EIIIB) can be either included or excluded, whereas a third (V) can be excluded (VO), or partially or completely included (V95 or V120). Inclusion of EIIIA & EIIIB in FN mRNA is associated with early embryogenesis (ffrench-Constant & Hynes, 1989) and wound healing (ffrench-Constant et al., 1989). We have assessed whether embryonic patterns of FN mRNA splicing are present following crush injury to the adult rat sciatic nerve, a model system for studying nervous system regeneration. RNase protection experiments were performed using probes designed to distinguish different splicing patterns of EIIIA, EIIIB and V. We have analysed RNA from 13.5 day rat embryos and from discrete segments of crushed and uncrushed nerves at four different time points after injury (1 day, 3 days, 1 week, and 2 weeks). Embryonic FN mRNA was largely EIIIA+, EIIIB+, and V120+. Compared to uncrushed nerve in which inclusion of EIIIA and EIIIB was insignificant, as early as 1 day post-crush the inclusion of both these regions in FN mRNA was upregulated in segments of nerve distal to and including the crush site. While control nerve RNA had comparable levels of V95+ and V120+ FN mRNA, crushing led to upregulation of the V120+ form. *In situ* hybridization experiments are underway to discover the cell types that synthesize these different FN variants and their localization within the nerve. These results show that embryonic forms of FN, which may be more appropriate for axon outgrowth and cell proliferation, are reexpressed as a result of peripheral nervous system injury.

This work is supported by the Wellcome Trust.

543.20

RECOVERY OF LOCOMOTOR FUNCTION AND RESTORATION OF DESCENDING BRAINSTEM PROJECTIONS IN SPINAL CORD-TRANSECTED LAMPREY. G.R. Davis* and A.D. McClellan, Division of Biological Sciences, Univ. of Missouri, Columbia, MO 65211

Spinal-transected larval lamprey (Petromyzon marinus) recover the ability to swim. We studied locomotor activity in whole animals and in vitro brain-spinal cord preparations of normal animals and animals 2-32 wks after spinal cord transection at 10% of body length (BL). Also, brainstem neurons were retrogradely labeled by HRP applied to the spinal cord to correlate the number and extent of restoration of descending projections with behavioral recovery.

In normal animals, the amplitude of lateral displacment of the body during swimming increased toward the tail. Prior to 8 wks post-transection (PT), these amplitudes were attenuated in the caudal body but by 8 wks PT were restored to the normal range. Coordinated locomotor muscle activity was present at 20% BL at 2 wks PT, and first appeared at 40% BL at 3 wks PT, and at 60% BL by 4 wks PT. In vitro locomotor activity was recorded from ventral roots at 20% BL by 4 wks PT, and first appeared at 40% BL at 8 wks PT, and at 60% BL at 16 wks PT. In normal animals, HRP applied to 20%, 40%, and 60% BL retrogradely labeled about 1250, 900, and 825 brainstem neurons, respectively, in 12 nuclei. In spinal-transected animals, the mean number of cells labeled from 20% BL increased from 14 at 3 wks PT to 1086 (not different from normal animals) at 32 wks PT. The mean number of cells labeled from 40% BL increased from 17 at 8 wks PT to 430 (different from normal animals) at 32 wks PT. Approximately 50 brainstem neurons were labeled from 60% BL at 32 wks PT. Thus, some brain-stem neurons, including some Muller cells, regenerate their axons more than 50mm caudal to the original transection (Davis & McClellan, Br. Res., 1993).

In conclusion, locomotor muscle activity appeared at progressively more caudal body levels with increasing recovery time, but at greater distances below the lesion than would be predicted by the extent of anatomical regeneration of brainstem projections. Thus, regeneration, as well as other mechanisms such as mechanosensory inputs, contribute to the time course of behavioral recovery in spinal-transected lamprey.

(Support: NIH NS29043, APA MB1-9108)

544.1

CYCLOSPORIN EXPOSURE IS CYTOTOXIC TO CULTURED CORTICAL NEURONS. S.-I. Chi*, B. Sklow, M.P. Goldberg, and D.W. Choi. Tzu-Chi Med. Res. Ctr., Hualien, Taiwan, and Center for the Study of Nervous System Injury, Dept. of Neurology, Washington Univ. Sch. of Medicine, St. Louis, MO 63110

Cyclosporin A is a potent immunosuppressant which is widely used in organ transplantation, but can be associated with a neurological syndrome characterized by encephalopathy, seizures, and white-matter lesions. We examined whether cyclosporin exposure would exhibit direct neurotoxicity in murine cortical cell culture.

Application of cyclosporin injured neurons (but not astrocytes) in a time- and concentration-dependent manner. 24-72 hour exposure to 10-30 μM cyclosporin caused progressive neuronal loss, assessed by phase-contrast microscopy and release of lactate dehydrogenase into the bathing medium. 1 μM cyclosporin did not produce significant neuronal injury. The neuronal loss produced by 72 hour exposure to 30 μM cyclosporin was partially attenuated by addition of the NMDA antagonist, MK-801 (0.5 μM) and by the 21-aminosteroids, U74500A and U74600F (1-5 μM).

These observations suggest that cyclosporin neurotoxicity may involve NMDA receptor activation and lipid peroxidation. The neurotoxicity of cyclosporin may be especially important in CNS transplantation studies, in which disruption of the blood-brain barrier might allow increased access of cyclosporin to potentially vulnerable neurons.

544.2

5' BROMODEOXYURIDINE AS A PRELABEL FOR THE ANALYSIS OF SURVIVAL AND MIGRATION OF FETAL HIPPOCAMPAL SUSPENSION GRAFTS. A.K. Shetty*, R.D. Madison, J. Bradley and D.A. Turner. Neurosurgery and Neurobiology, DUMC and VAMC, Durham, NC 27710.

The efficacy of bromodeoxyuridine (BrdU) as a nuclear marker of grafted hippocampal cells for morphological and quantitative studies of graft development was investigated. Timed pregnant rats were labeled with daily pulses of BrdU (50 mg/Kg.bw/day) between gestation days 15 and 19, at which time hippocampal cell suspensions were either cultured or grafted into normal adult hippocampus. The survival and migration of the labeled cells in grafts were quantitatively analyzed by three-dimensional serial reconstruction of host brain sections (Neurolucida).

Evaluation of cell cultures revealed labeling of 90% of all cells and 92% of neurons by the pulsed BrdU protocol; the development of labeled neurons appeared normal and comprimable to unlabeled neurons. In grafts, neuronal phenotypes predominated over astrocytes among labeled cells, as judged by morphology and double immunolabeling for BrdU and glial fibrillary acidic protein. Grafted neurons were generally confined to the transplant mass and showed minimal migration into the host. Absolute graft cell survival varied according to location: grafts projecting partly into the ventricle or white matter tracts (corpus callosum or fimbria) showed better absolute survival (20% and 25% respectively) than the grafts localized entirely within the hippocampus (8%). The results indicate that the pulsed BrdU labeling protocol consistently labeled a great proportion of neurons, which appeared to develop normally and were identifiable for quantitative studies of graft development.

Serial reconstruction of labeled cells proved useful to assess both migration and absolute survival of fetal grafts in host brain. The location of the grafts critically influenced the survival of grafted cells: white matter regions appeared to provide a more permissive local host environment than cell body layers within normal hippocampus. **Supported by NINDS RO1 NS29482-01 and VAMC Merit Review.**

544.3

HUMAN DOPAMINERGIC GENE EXPRESSION IN COS CELLS. *F.G. Kaddis[1,2], C.R. Freed[1,2]*, M.J. Weber[3], C.K. Surratt[2], D.J. Vandenbergh[2], J. Mallet[4], P. Horellou[4], G.R. Uhl[2]. 1 Depts. Med. & Pharm., U. Col. Sch. Med., Denver, Col., 80262; 2 Mol. Neurobiol. Br., ARC,NIDA,NIH & Depts. Neurol.& Neurosci., JHUSM, Balto., MD. 21224; 3 CNRS, Toulouse, France; 4 CNRS, Gif-sur-Yvette, France*

cDNAs encoding 4 human genes selectively expressed in dopaminergic neurons, co-transfected into COS cells, can confer interesting dopaminergic properties. Full-length cDNAs for tyrosine hydroxylase (hTH), dopamine transporter (hDAT), and vesicular transporter (hSVMT) express well in COS cells using the CMV promoter expression plasmid pcDNA1. A functional L-aromatic amino acid decarboxylase (hDDC) cDNA was also produced by ligating partial cDNAs into pcDNA1. Transient hDDC expression, tested in COS cells electroporated 3 days before assay with 20 μg of phDDC, revealed levels 600% of values in nontransfected cells. DDC in stably-expressing COS cells transfected with 6 μg of hDDC, 12 μg hTH and hDAT, 6 μg of hSVMT, and 1 μg of neomycin-resistance expression plasmids was tested after 2 weeks' neomycin selection. These cells express NSD-1015-reversible DDC activity at more than 400% background. These results point to the feasibility of CMV-promoter driven coexpression of these four dopaminergic genes in transfected cell populations. Multiply-transformed cells may prove useful for transplantation in Parkinson's disease.

544.4

REGULATION OF C-FOS GENE EXPRESSION OF THE TRNSPLANTED GENETICALLY MODIFIED CELLS IN THE RAT BRAIN. K. Asakuno[1,2], M. Isono[1]*, S. Sato[1], K. Kohno[2] and S. Hori[1]. [1]Dept. of Neurosurgery and [2]Dept. of Biochemistry, Oita Medical University, Oita 879-55, Japan.

In many genetic diseases, replacement of the absent or defective protein provides significant therapeutic benefits. However it is very difficult, in vivo, to regulate the expression of genetically modified gene. In this study, we established the stable transfectant from normal rat kidney fibroblast (NRK-49F) which had been transfected with a human metallothionein promoter (hMT-IIA)/human genomic c-fos fusion gene to produce c-fos protein (Fos). In these cells, the c-fos basal level of expression was observed and its expression could be modified by addition of metals in vivo. We transplanted these cells into striatum of the rat brains and investigated whether the human Fos expression could be modified by extrinsic addition of Zinc in the brain. We found that the transplanted cells could survive relatively well. In addition, the cells in rats fed by water contained Zinc strongly expressed Fos compared to that in rats without Zinc. Our result suggested that the gene expression could be regulate in the brain by the external agents, and this model has implication for future gene therapy.

544.5

THE EFFECT OF GLUTATHIONE PEROXIDASE TREATMENT ON CULTURED MESENCEPHALIC NEURONS. F. Pagan, C. A. Colton, and D. L. Gilbert*. Dept. of Physiology and Biophysics, Georgetown Univ. Med. School, Washington, DC 20007 and Clin. Neurosci. Branch, NINDS, NIH, MD 20892.

Neuronal survival was compared in the presence and absence of the antioxidant enzymes, superoxide dismutase (SOD) and glutathione peroxidase (GLP). Neuronal cultures were grown in the presence of SOD, GLP or under untreated, control conditions. Primary neuronal cultures were prepared from day 13 gestational (E13) rats using the papain digestion method. After 4 days, the cultures were fixed and immunocytochemical techniques using antibodies against tyrosine hydrolase (TH) and microtubule associated protein 2 (MAP2) were used to assess the survival of dopaminergic neurons and the degree of branching. The intensity of antibody reactivity was analyzed using peroxidase-coupled secondary antibodies and a modified ELISA assay. Photomicrographs were taken to confirm the ELISA data. Results show that GLP increased the level of TH and MAP2 immunoreactivity in primary mesencephalic cultures. The survival of TH-positive neurons was increased. Also, the degree of neuronal branching was dramatically increased in the presence of GLP. Thus, survivability of transplanted neurons might be enhanced if anti-oxidant protection mechanisms are used.

544.6

REINITIATED GROWTH FROM MATURE VENTRAL MESENCEPHALIC GRAFTS. I. Strömberg* and Maria Johansson. Department of Histology & Neurobiology, Karolinska Institutet, Stockholm, Sweden.

The ability of mature dopaminergic neurons in ventral mesencephalon to reinitiate growth after receiving an uninnervated target was studied in the intraocular grafting model. Fetal ventral mesencephalic tissue was grafted to the anterior chamber of the eye. Two weeks, six weeks or one year after the implantation, fetal striatal tissue was placed in contact with the nigral graft or grafted alone. Ingrowth of tyrosine hydroxylase (TH) immunoreactive nerve fibers into the caudate grafts were studied 6 weeks after grafting striatal tissue. The final size of the caudate grafts was significantly larger in eyes grafted with caudate alone than in eyes where striatal tissue was placed together with 1-year or 6-week-old dopaminergic grafts. Caudate grafted to 2- week-old nigra was larger, but not significantly, than that grafted to mature nigra. TH-immunohistochemistry revealed innervation of the caudate in all cases, except for the group where caudate was grafted alone. In caudate grafted to 2-week-old nigral transplants, TH-positive nerve fibers innervated a larger volume, had a patchy appearance and the density was higher than that seen in caudate transplants grafted to mature nigral grafts. In caudate grafts placed adjacent to 6 weeks old nigral grafts, the density and volume was larger than that seen in caudate grafted to 1-year-old nigra, but smaller than in caudate placed adjacent to immature nigral tissue. In striatal grafts placed together with 1-year-old nigra a tendency to patchy ingrowth was seen, but here the patches were large and the nerve terminal density was low. In conclusion, growth from nigral transplants can be reinitiated independent of age, but a lower density and smaller volume of the outgrowth is observed from mature grafts than from immature grafts.

544.7

HERPES SIMPLEX VIRUS VECTOR-MEDIATED GENE TRANSFER TO CNS NEURONS. M.A. Bender[1], N.A. DeLuca[1], B.B. Kaplan[2*], R. Ramakrishnan[3], M. Levine[3], R.Y. Moore[2], W.F. Goins[1], and J.C. Glorioso[1]. Dept of Molecular Genetics & Biochemistry[1], Psychiatry[2], University of Pittsburgh, Pittsburgh, PA 15261, Dept. of Human Genetics, University of Michigan, Ann Arbor, MI 48109[3].

We have developed herpes simplex virus (HSV)-based vectors for gene transfer to the CNS. HSV is attractive as a gene transfer vehicle for CNS neurons due to the natural propensity of the virus to establish lifelong latent infections within these neurons, and the virus can accommodate large amounts of foreign DNA. We have manipulated the virus vector to be non-neurotoxic by deletion of immediate early lytic gene functions, yet the vector readily establishes latency in substantial numbers of neurons in which the viral genome persists for the lifetime of the host. Using quantitative PCR techniques on DNA isolated from HSV-infected hippocampal tissue, we have demonstrated that the number of viral genomes remains relatively constant from 7 days to at least 8 weeks after infection.

We have employed a defective HSV vector, which contains an expression cassette with the strong human cytomegalovirus immediate early gene promoter (HCMV IEp) driving the β-galactosidase reporter gene (*lacZ*), to effect gene transfer to primary bovine adrenal chromaffin cells. Expression of the transgene can be detected in these cells in culture at 7 days. Stereotactic inoculations of the vector into rat CNS gives strong transgene activity only at early times (2-5 days), yet the recombinant viral genomes persist in the infected brain for at least 1 year after inoculation. In an attempt to achieve long-term expression of a foreign gene, we are testing a transcriptional activation system, GAL4-VP16, which is capable of inducing genes bound in chromatin structures. When binding sites for GAL4-VP16 are placed within a promoter, the GAL4-VP16 transactivator protein can stably boost transcriptional levels several hundred-fold, which we have observed in transient assays in culture. This system is currently being applied to the expression of the tyrosine hydroxylase gene in an attempt to acheive persistent TH expression from the defective HSV vector in the CNS.

544.8

TRANSPLANTATION OF AN O-2A GLIAL PROGENITOR CELL LINE. P.L. Kuhn[1], J.L. Norman [1*] and R.D. McKinnon[2], California State U., Chico, CA 95926[1] and R.W. Johnson Med.School, UMDNJ, Piscataway NJ 08854[2].

Transplantation of Central Glial-4 (CG-4) cells, an established line of bipotential oligodendrocyte type-2 astrocyte (O-2A) progenitors from rat cortex, was used to establish a paradigm for further analysis of the effect of trophic factors on CNS myelin formation. CG-4 cells were marked with the E. coli gene encoding β-galactosidase using a recombinant retroviral vector, then injected into the thalamus of post-natal day 2 (P2) rat pups. Brains from recipient animals were harvested at regular intervals, stained using X-gal reagent, then transplants (blue cells) were located by light microscope examination. Results provided evidence of migration of CG-4 cells *in vivo*. In a second procedure, unmarked CG-4 cells were injected into the thalamus of P2 Shiverer (Shi) mutant mice. Homozygous Shi mutants lack the gene encoding myelin basic protein (MBP), which forms the major dense line (MDL) in myelin membrane. Shi/Shi pups were identified by Polymerase Chain Reaction using oligonucleotide primers specific for the wild type and Shi MBP deletion. Recipient brains were harvested at various intervals, and immunohistochemical staining revealed the presence of transplanted CG-4 (MBP-immunoreactive) cells in the CNS of homozygous mutant tissue sections. The presence of MDL was examined by EM after ferrocyanide-reduced osmium tetroxide post-fixation and immunogold labeling of MBP. Results provide evidence of cell differentiation and expression of MBP, suggesting that CG-4 cells follow the normal *in vivo* development program of their O-2A counterparts.
Supported in part by a Pilot Research Award to RMcK from the US National Multiple Sclerosis Society.

544.9

COMPARISON OF THE RATE OF MATURATION OF NEURONAL CELLS DERIVED FROM THE HUMAN CELL LINE NT2 TRANSPLANTED INTO NEONATAL AND ADULT NUDE MICE S.R.Kleppner*, J.Q.Trojanowski, K.A.Robinson, V.M.-Y.Lee Inst. of Neurological Sciences and Dept. of Path. and Lab. Med., Univ. of PA, Philadelphia, PA 19104

Immunohistochemical studies of neuronal cells derived from the NT2 cell line (NT2N cells) implanted into adult nude mouse brain reveal that these cells mature beyond their *in vitro* phenotype. Levels of highly phosphorylated heavy neurofilament (NF-H), and of adult isoforms of tau, increased dramatically between 6 weeks and 4-6 months post-implant respectively. Heavy phosphorylation of NF-H is a late developmental event, and adult isoforms of tau are not found in the fetus. Neither heavily phosphorylated NF-H nor adult tau is found in NT2N cells *in vitro*. Other properties of the implanted NT2N cells include neurite outgrowth and expression of many neuron specific proteins including MAP2, all three neurofilament proteins, tau, vimentin, and N-CAM. In addition, the immediate environment appears to influence the extent of neurite outgrowth. In this study we compare these findings with the results of implanting NT2N cells into neonatal animals. The cells appear to become incorporated more extensively into the young brain, often extending processes for greater distances. In addition, the cell bodies seem to be more widely distributed throughout the brain. While the NT2N cells initially express the same array of proteins as in the adult, they may mature at a faster rate. Our data suggest that the developing brain may provide a more amenable environment for the NT2N cells than the adult brain. Ongoing studies address the issue of the effect of the immediate environment on the phenotype of these cells.

544.10

PURIFIED NEURONS CAN BE TRANSPLANTED IN THE ADULT CNS. J.CADUSSEAU[1], S. ROSTAING[1], V. DEVIGNOT[2], C.E. HENDERSON[3] and M. PESCHANSKI[1*] [1]INSERM CJF91-02, Créteil; [2]INSERM U261, Paris; [3]UPR-CNRS8402/INSERM U249, Montpellier; France.

Fetal neural transplants generally include both a set of neurons and their cellular environment. This procedure restricts the number of neurons of interest that can be grafted and leads to the implantation of cells that may be not only unnecessary but also detrimental. This study was undertaken to determine whether purified neurons would survive transplantation into the adult host brain. Spinal neurons have been purified by the panning procedure which is based on the transient expression of the low affinity receptor for the NGF specifically on immature motoneurons. Fetal purified neurons were transplanted into the brain parenchyma of an adult host previously depleted of neurons by the injection of an excitotoxin. During the first week post-grafting the transplants were characterized by a high density of almost exclusively neurons. These neurons displayed an immature morphology. During the second week post-grafting the cellular density of the graft decreased, the neurons grew and non-neuronal cells appeared. Small capillaries were observed throughout the transplant. Astroglial processes entered from the periphery of the graft and proceeded within the clusters of neurons whereas highly reactive astrocytes were observed along the capillaries. At the end of the second week post-grafting, microglial cells were evenly distributed throughout the transplant. These cells displayed a quiescent morphology and a density similar to that observed in the normal parenchyma. Older transplants (31/2 months) contained heterogeneous neuronal populations with a majority of large multipolar cells particularly rich in Nissl bodies. Many neurons could be immunostained for CGRP, a typical marker of motoneurons. Purified neurons, deprived of their fetal environment and ectopically transplanted, are therefore able to mature and to acquire normal morphological and biochemical characteristics. The host integrates the graft by complementing it with astroglial, microglial and vascular elements.
Supported by "Association Française contre les Myopathies".

544.11

NERVE GROWTH FACTOR-EXPRESSING PC12 BRAIN GRAFTS FOR USE IN ANIMAL MODELS OF PARKINSON'S AND ALZHEIMER'S DISEASES. D.C. Rohrer,[1] V.I. Nipper,[2] C.A. Machida,[2,3*] G. Nilaver.[4] [1]Div. Neurosurgery and Depts. [4]Neurology, [3]Biochem. and Mol. Biol., Oregon Health Sciences University, Portland, OR 97201; [2]Div. Neurosci., Oregon Regional Primate Res. Ctr., Beaverton, OR 97006.

An optimal cell line for use in transplantation models of Alzheimer's and Parkinson's diseases would: (i) be immortal and pluripotent; (ii) differentiate to functionally replace degenerating pathways; and (iii) induce localized neuronal sprouting of surviving host cells through the release of trophic factors. PC12 cells propagate in culture, neurodifferentiate in the presence of nerve growth factor (NGF), and produce dopamine and/or acetylcholine. However, while NGF-treated PC12 cells can ameliorate neurotransmitter deficits in rats, they degenerate post-implantation without sustained NGF availability. We have introduced an 820 bp cDNA recombinant encoding mouse NGF (mNGF), under control of the human metallothionein IIA zinc-inducible promoter, into PC12 cells via calcium phosphate transfection. Southern blot analysis of PCR products has verified incorporation of the transgene into the host genome in 36 stable transfectant cell lines. Northern blot analysis and mNGF immunocytochemistry (ICC) have identified several transfectants exhibiting substantial IIA promoter "off-on" response, and high inducibility of mNGF transcription/translation. Grafts into the striatum of dietary zinc-supplemented rats are being examined by *in situ* hybridization and ICC to verify inducible post-transplant neurodifferentiation. The potential application of these genetically-programmed cells for use in human neurodegenerative diseases is being explored in established rat models of Parkinson's and Alzheimer's diseases.

544.12

REGULATION OF DIFFERENTIATION IN A NEURONAL CELL LINE DERIVED FROM MEDULLARY RAPHE. L.A. White*, M.C. Castro, M.J. Eaton, & S.R. Whittemore, The Miami Project and Dept. Neurological Surgery, Univ. Miami, Miami, FL 33136

A neuronal cell line, RN46A, was derived by infecting embryonic day 13 rat medullary raphe with a retrovirus encoding the temperature-sensitive mutant of SV40 large T antigen (T-ag). At non-permissive temperature (33°C), RN46A cells express robust nuclear T-ag immunoreactivity. Low levels of neuron-specific enolase (NSE) and 160 kDa neurofilament protein (NF-M) were detected, while NF-L, NF-M, and glial-specific proteins were not expressed. After shifting to differentiating conditions, RN46A cells ceased dividing, differentiated with neuronal morphology and enhanced NF-L, NF-M, NF-H, and NSE expression. NF expression could be potentiated by activating protein kinase A. A subset of differentiated RN46A cells expressed tryptophan hydroxylase (TPH), which could be enhanced by treatment with $ACTH_{4-10}$. Differentiated RN46A cells did not survive beyond 8 days in vitro if grown on bare tissue culture plastic, but would survive through 10 days on collagen-fibronectin. This survival could be further potentiated with 0.1% rat serum. Initial experiments have shown that RN46A cells can survive at least 2 weeks in vivo following transplantation into the adult and neonatal CNS. Ongoing experiments are evaluating the factors which further potentiate RN46A survival and differentiation both in vitro and in vivo. Supported by The Miami Project to Cure Paralysis and NS26887.

544.13

THE ADULT CNS RETAINS THE POTENTIAL TO DIRECT SPECIFIC DIFFERENTIATION OF A TRANSPLANTED NEURONAL PRECURSOR CELL LINE. L.S. Shihabuddin*, S.R. Whittemore and V.R. Holets. The Miami Project, Univ. of Miami School of Medicine, Miami, FL 33136.

The survival and differentiation of the conditionally-immortalized neuronal cell line, RN33B, was examined following transplantation into the adult rat brain. RN33B cells were transplanted into the cerebral cortex, hippocampal formation, and cerebellum of allogeneic hosts. These three regions have well characterized and morphologically distinct laminar cell layers. Transplanted cells, identified using immunohistochemistry to detect ß-galactosidase expression, were seen in animals up to 24 weeks post-transplantation (the latest time point examined). Stably integrated cells with various morphologies consistent with their transplantation site were observed. In the cerebral cortex, many RN33B cells had differentiated with morphologies similar to small and medium pyramidal neurons and stellate cells. In the hippocampal formation, many RN33B cells had differentiated with phenotypes similar to endogenous neurons at the integration site. They assumed morphologies similar to pyramidal neurons characteristic of CA1 and CA3 regions of the hippocampus, granular neurons of the granular cell layer of the dendate gyrus, and polymorphic neurons of the hilar region. In the cerebellum, RN33B cells integrated only in the granular layer. These results suggest that the RN33B cells have the developmental capacity to behave as neuronal precursor cells. Most interesting, these studies indicate that the adult brain retains the capacity to direct the differentiation of neuronal precursor cells in a direction which is consistent with that of endogenous neurons. Supported by The Miami Project to Cure Paralysis, The Daniel Heumann Spinal Cord Research Fund, and NS26887.

544.14

GENERATION OF PRIMATE NEUROSPHERES AND GENETIC MODIFICATION OF RAT NEUROSPHERES IN CULTURE. C.N. Svendsen*, J.Fawcett and S.B. Dunnett. Departments of Experimental Psychology and Physiology, University of Cambridge, England.

Mouse embryonic cells continually divide when grown in serum free culture conditions in the presence of epidermal growth factor (EGF) forming free floating balls described previously as neurospheres (Reynolds *et al*, J. Neurosci. 12:4565, 1992). We have taken striatal tissue from E16 rat and E79 marmoset embryo's and grown them for 5-10 weeks in the presence of EGF (10-20ng/ml) on Teflon coated glass coverslips in serum free DMEM/F12 medium supplemented with B27 (GIBCO). Neurospheres formed rapidly in rat cultures (within 5 days) and needed to be passaged every week to avoid overgrowth but grew more slowly in marmoset cultures often taking 15-20 days for the first divisions to occur. After 5 weeks in culture rat neurospheres were passaged once before being genetically modified by infection with a recombinant retrovirus encoding the lac z gene and neomycin resistance. These infected neurospheres continued to divide in culture and staining for beta galactosidase at 2 days post infection revealed approximately 3% infection rate. 7 days following infection new spheres had developed and beta galactosidase staining showed that some of these contained over 70% infected cells while others were non-infected suggesting that infected cells continued to divide and form new spheres. Neomycin added to infected cultures for 4 days resulted in an increased number of infected cells relative to non infected cells. These results show that both rat and monkey neurospheres can be successfully generated from embryonic tissues and that rat spheres can be genetically modified using a retrovirus. We are currently assessing the stability of gene expression with time in culture and following transplantation into neonatal and adult rat brain.

544.15

TRANSPLANTATION OF POLYMER ENCAPSULATED CALF CHROMAFFIN CELLS IN THE SHEEP SUBARACHNOID SPACE: A PRECLINICAL STUDY FOR THE TREATMENT OF PAIN. P. Aebischer[1,2*], V. Padrun[1], M. Burki[1], J. Favre[1], J.M. Joseph[1], F. Mosimann[1], M. Augstburger[1], J.P. Gardaz[1], A.D. Zurn[1], B. Winistörfer[1], J. Sagen[2], J. Mills[3], B. Zielinski[3], M. Goddard[3]. [1]Surgical Research Div., Lausanne Univ., Switzerland; [2]Univ. of Illinois, [3]Div. of Biology & Med., Brown Univ.

The transplantation of polymer encapsulated bovine chromaffin cells into the lumbar subarachnoid space has been recently reported to exert antalgic effects in various rat models (Sagen et al., J. Neurosci., in press). In order to prepare for adapting this technique for humans, sheep were implanted with encapsulated calf chromaffin cells. Adrenal glands were harvested surgically in 10 young calves. Chromaffin cells were isolated the same day using a digestion-filtration technique. The cells were then loaded 48 hrs later into thermoplastic-based permselective capsules which were subsequently mounted on a silicone catheter. The capsules were maintained separately in hormonally-defined media for 7 more days, during which time catecholamine release measurements and sterility tests were conducted. The capsule system was then implanted in the lumbar subarachnoid space of 10 adult sheep using a closed, cannula introducer system. Four weeks postimplantation the capsule system was retrieved. Comparable catecholamine release was detected from both the retrieved and the in vitro cohort capsules. The evoked catecholamine release from the densely loaded capsules (around 2 x 10^6 cells) reached 2 nM/ capsule/30 min and was within 10% of the preimplant values. Good viability of densely packed cells was observed in the retrieved capsules. These data suggest that the technique of polymer encapsulation of bovine chromaffin cells can be scaled-up to a viable human size implant.

544.16

OUTGROWTH FROM DIFFERENTIATED CHROMAFFIN CELLS INDUCED BY NGF-PRODUCING FIBROBLAST GRAFTS G.R. Chalmers[1*], L.J. Fisher[1], K. Niijima[2], P.H. Patterson[2] and F.H. Gage[1]. [1]Dept. of Neurosciences, UCSD, La Jolla, CA, 92093 and [2]Biology Division, Caltech, Pasadena, CA, 91125.

Adrenal chromaffin cells (ACC) implanted within the striatum have been used as a point source of catecholamines in the dopamine depleted brain. One method to potentially enhance the effectiveness of such grafts may be to induce process outgrowth from the ACCs into the host brain. The present study examined whether ACC transdifferentiated to express a neuronal phenotype *in vivo* by NGF or bFGF could be induced to extend processes into host brain tissue. Adult 6-OHDA treated rats received injections into the denervated striatum of either: 1) ACC+NGF-producing fibroblasts, 2) ACC+control fibroblasts, 3) NGF-producing fibroblasts, or 4) control fibroblasts. Grafting was followed 8 weeks later by 3 adjacent grafts of NGF-producing or control fibroblasts. A further 8 weeks later grafts were processed for NGF receptor (NGFr), and tyrosine hydroxylase (TH) immunoreactivity (IR). Consistant with previous work, ACC cografted with NGF-producing fibroblasts developed extensive processes, whereas ACC cografted with control fibroblasts did not. Further, only the NGF grafts placed at a short distance from ACC+NGF cografts were TH and NGFr-IR. In contrast, NGF grafts placed distal to ACC+control fibroblast grafts were TH-IR negative. In a similar experiment, rats received grafts of ACC+bFGF-producing fibroblasts and adjacent grafts of either NGF-producing or control fibroblasts. ACC+bFGF grafts contained numerous TH-IR ACC only if adjacent to NGF-producing grafts. Further, the NGF grafts adjacent to ACC+bFGF cografts were TH and NGFr-IR, while control grafts adjacent to ACC+bFGF cografts were IR-negative. NGF thus appears to be capable of acting as a tropic factor for ACC, inducing process outgrowth from transdifferentiated ACC beyond the boundary of the grafted tissue and into the host brain *in vivo*. Further, once transdifferentiated, a source of NGF is required for the continued survival of implanted ACCs.

544.17

STABILITY OF XENOGENEIC CHROMAFFIN CELLS TRANSPLANTED TO THE CNS FOLLOWING SYSTEMIC CHALLENGE BY SKIN GRAFTING K. Czech, J. Sagen and G.D. Pappas. Dept. of Anat. & Cell Biol., University of Illinois at Chicago, Chicago, IL 60612.

The use of xenogeneic sources for donor tissue in transplantation procedures has the potential to solve the problems associated with donor availability. One obstacle preventing use is the risk of a serious rejection episode. However, previous studies in our laboratory have shown that short-term immunosuppression with Cyclosporine A (CsA) can enhance long-term survival of bovine chromaffin cells transplanted to the CNS of Sprague-Dawley rats. The objective of this study was to assess the stability of xenogeneic grafts in the CNS following systemic challenge. We transplanted isolated bovine chromaffin cells to the rat midbrain and analyzed survival 8 weeks later. All groups received CsA treatment beginning 2 days prior to transplantation. One group of animals received continuous CsA treatment, while another group received only 3 weeks CsA treatment. Half of the animals in each group were challenged systemically with bovine chromaffin cells injected intradermally six weeks following CNS implantation. We obtained robust and well-integrated transplants containing densely packed, healthy chromaffin cells in animals that were not systemically challenged. However, following challenge with systemic bovine chromaffin cell skin grafts in continually immunosuppressed animals, chromaffin cells in the CNS grafts were found in various stages of degeneration. Some apparently healthy chromaffin cells were also present. In addition, the graft and surrounding host tissue were edematous and the host-graft border was infiltrated by numerous astrocytic processes and cellular debris. Animals receiving only short-term immunosuppression at the time of systemic challenge showed no chromaffin cells surviving in the CNS grafts. The graft sites appeared cystic and vacuolated. In summary, systemic challenge with bovine chromaffin cells causes disruption of stable and well-integrated bovine chromaffin cell transplants in the CNS even with CsA treatment. Supported by NIH grants NS25054 and NS29831.

544.18

IN VIVO MICRODIALYSIS OF CHROMAFFIN CELL TRANSPLANTS POSSESSING ANTIDEPRESSANT ACTIVITY. C.E. Sortwell*[1], F. Petty[2], G. Kramer[2], M. Waddill[2] and J. Sagen[1]. [1]Dept. of Anat. and Cell Biol., Univ. of IL at Chicago, Chicago, IL 60612; [2]VA Medical Center, Dallas, TX 75216.

Studies in our laboratory have demonstrated the ability of monoaminergic grafts in the frontal neocortex to alleviate depression in rodent models. Specific monoaminergic antagonists reverse these antidepressant behavioral effects, and immunocytochemical analysis has revealed robust survival of monoaminergic cells for at least six months after grafting. These findings indirectly suggest that the antidepressant effect of the monoaminergic grafts is due to the release of monoamines into the host parenchyma. The purpose of the present study was to more directly determine whether bovine adrenal chromaffin cell grafts release catecholamines locally to the surrounding host neocortex. Female rats were baseline assessed for immobility scores in the Forced Swimming Test (FST), a popular measure of antidepressant activity. All rats were then stereotaxically implanted with either isolated bovine chromaffin cells, isolated bovine adrenal fibroblasts, Hank's solution or no transplant to the right frontal neocortex. All rats were immunosuppressed daily with Cyclosporine A for three weeks. Five to six weeks following transplantation rats were again assessed for immobility scores in the FST. Only chromaffin cell transplanted rats displayed significantly lower FST immobility scores; no effect of the three control graft conditions was observed. Each rat was implanted with a dialysis probe at a 500 µm distance away from the graft site and perfused through the probe with artificial cerebrospinal fluid for extracellular fluid sample collection. Perfusates from the chromaffin cell implanted rats contained significantly higher levels of catecholamines than the three control transplant conditions. Immunocytochemical results suggested that the grafts survived well. These results provide direct evidence that bovine chromaffin cells provide a releasible pool of catecholamines for antidepressant activity when grafted into the frontal cortex of rats. Supported by NIMH MH47491.

545.1

A LOCAL AREA NETWORK FOR THE TRANSPORTATION OF IMAGES FROM MICROSCOPY LABS TO GRAPHICS WORKSTATIONS. R. F. Gasser, Jr., G. B. Smith, K. J. Graham, R. F. Gasser, Sr., T. G. Weyand*, and R. R. Mize, Department of Anatomy and Computer Imaging Laboratory, Louisiana State University Medical Center, New Orleans, LA 70112.

The LSUMC Computer Imaging Laboratory (CIL) has developed an Ethernet based local area network (LAN) for the transportation of images from central imaging facilities to remote graphics workstations. The network includes a file server, five computer-based imaging stations (Magiscan MD image analyzer, Leica confocal laser microscope, 2 PC based image capturing and digitizing stations, JEOL JEM 1210 electron microscope), and three remote graphics workstations (Macintosh IIci, Quadra 950, and Silicon Graphics Personal Iris). The server and each of the workstations run PC, Macintosh, UNIX, or OS9 versions of NFS (Network File Server) and TCP/IP (Transmission Control Protocol/Internet Protocol). This software allows each station to send and receive files, remotely login to other machines, and to mount remote filesystems from NFS servers using the file transfer protocol (FTP). In practice, we transport images in a variety of formats (TIFF, TGA, PICT, raw) from each of our imaging devices to the Macintosh and Iris graphics workstations for further manipulation using NIH Image, Adobe Photoshop, Aldus Persuasion, Skandha, and Synu software packages. A variety of images are generated, including whole body and regional 3D reconstructions of human embryos, tissue sections labeled by antibody immunocytochemistry, tissue labeled by mRNA probes for in situ hybridization experiments, fluorescent labeled muscle fibers reconstructed from laser confocal optical sections, and electron micrographs. Once generated, images are archived on either Infinity optical disks or Exabyte DAT tapes. The system permits broad access to the instrumentation and promotes collaboration so that investigators can view and analyze images that have been generated at one site and transported via Internet to another. Supported by LEQSF 90-93RD-A-09 (RGSr), research equipment bond funds from the State of Louisiana, and RR-02800 and NIH EY-02973(RM).

545.2

IN VIVO MICROELECTRODE LOCALIZATION IN THE BRAIN OF THE ALERT MONKEY. F.K.D.Nahm*[1,2], A.Dale[2], T.D.Albright[1], D.G. Amaral[1]. The Salk Institute[1], La Jolla, CA, 92037, UCSD[2], San Diego, 92093.

We have developed a new technique for *in vivo* localization of microelectrodes during single-unit recording in the alert monkey. This technique utilizes a combination of x-ray and magnetic resonance imaging.

Four hollow glass beads were filled with a solution that rendered them visible in both x-ray and magnetic resonance (MR) images. The filled beads were surgically implanted on the skull of one monkey (*Macaca mulatta*). Three coronal MR scans offset by 1 mm along the AP axis were obtained. This yielded a total of 94 3-mm thick MR sections, which were subsequently displayed and stored on a graphics workstation. The subject was then implanted with recording chambers for single-unit studies. A microelectrode was advanced into the subject's brain and a series of x-ray images obtained. These images were used to reconstruct the 3-D position of the microelectrode relative to the four reference beads. The 3-D position of the microelectrode was then mapped onto the MRI data base using the reference beads for registration of x-ray and MR data. The microelectrode tip was visualized during the course of the experiment by an icon graphically superimposed on the appropriate MR section.

We attempted to verify the accuracy of this procedure for 10 microelectrode sites. Each site was marked with a microlesion. Post-mortem histological analysis revealed that the neuroanatomical loci of the microlesions were highly consistent with those predicted by our *in vivo* technique. This technique, which has a precision no less than 625 μm, should prove especially useful in long-term neurophysiological and neuropsychological studies in which knowledge of the location of brain probes is of crucial importance.

Supported by the McDonnell-Pew Center for Cognitive Neuroscience at San Diego, National Eye Institute, and NIMH.

545.3

UNBIASED STEREOLOGICAL ESTIMATION OF TOTAL SYNAPSE NUMBER AND TOTAL NEURONAL NUMBER IN THE NEOCORTEX OF THE CYNOMOLGUS MONKEY. P.R. Mouton*, L.C. Walker, C.A. Kitt and D.L. Price. The Johns Hopkins Univ. Sch. of Med., Balto., MD 21205

Using unbiased stereological methods, we have estimated the total number of synaptophysin-immunoreactive, presynaptic boutons (synapses) and the total number of neurons in the neocortex of a ten-year old male cynomolgus monkey (*Macaca fascicularis*). The monkey was perfused under deep Nembutal anesthesia with 4% paraformaldehyde followed by phosphate-buffered saline. The brain was blocked coronally *in situ* into 5-mm slabs (12 total slabs), using a specially designed stereotaxic blocking apparatus. From the rostral face of each slab, 40-μm sections were cut on a freezing-sliding microtome. Systematically sampled sections were processed for synaptophysin immunocytochemistry or stained with cresyl violet. Particles (synapses or neuronal nucleoli) were counted in unbiased, 3-D optical disectors spaced uniformly through the entire neocortex. We found ~1.5-2.0 trillion (10^{12}) total synapses and 1.5-2.5 billion (10^9) total neurons in the neocortex of the cynomolgus monkey. On systematically sampled and stained sections, these stereological analyses can be carried out in 2-3 hours with <10% within-sample variance. These and other unbiased stereological methods are being used currently to correlate neuropathological changes with indices of cognitive dysfunction in normal aging and Alzheimer's and Parkinson's diseases.

545.4

IMAGE ANALYSIS OF CYTOARCHITECTURAL VARIATION IN THE HUMAN PRIMARY VISUAL CORTEX. E. Armstrong*[1] and M. Roos[2] Dept. of Cellular Pathology, A.F.I.P., Washington, D.C. 20306 and Dept. of Anatomy, Mahidol University, Bangkok, Thailand (1), Topographic Engineering Center, Fort Belvoir, VA 22060 (2).

The development of signatures and boundaries for cortical regions requires a better understanding of the architectural variation within a region. To analyze the variation in cellular arrangements, we digitized Gallyas perikarya stained sections at a resolution of 15 um per pixel. Cortical layers II-VI were segmented from the background and vectors were drawn from upper layer II to lower layer VI. Vectors were placed perpendicular to the pial surface where all 6 layers could be seen. The feature vectors, gray levels, were smoothed horizontally and resampled to 35 dimensions.

We were particularly interested in variation as a function of distance between profiles and that which is a function of rotation. The Mahalanobis distance was used to study the variance of the profiles while controlling for the correlation of the densities within the vectors. The smallest variation was between topographically close feature vectors.

545.5

PROBABILITY RAT BRAIN ATLASES: A TOOL FOR INTERPRETING FUSED NEUROANATOMICAL MAPS. J. Nissanov*[1], S. Bhasin[1], G. Gregoriou[1], D. McEachron[1], R. Paz[2], L. Rioux[3], O.J. Tretiak[1]. [1]Imaging and Computer Vision Center, Drexel U., Philadelphia, PA. [2]Dept. de Biologia Cellular, U. Complutence, Madrid, Spain. [3]Dept. of Psychiatry, U. of Pennsylvania, Philadelphia, PA.

It is technologically feasible to align and average digital images of homologous brain section from different rats. If the images are of autoradiograms produced with the same ligand, the resulting image would offer an enhanced signal-to-noise ratio as well as other advantages. The impediment to this approach is interanimal variability: The same coordinate point in two homologous images of sections from different animals centered in a common coordinate system does not typically correspond to the same neuroanatomical location. We report on an approach to overcome this difficulty which utilizes probability templates to extract data from fused maps. The templates define areas on an average image likely to belong to a given region of interest.

This approach was tested by delineating 20 different structures 3 times by each of 4 neuroscientists on each of 4 animals. The multiple delineations defined a fuzzy map for each structure in each of the animals. The maps can be thought of as fuzzy sets with each pixel assigned a value, an association strength, equal to the inclusion frequency in the set of delineations.

For each structure, the fuzzy maps were averaged across homologous sections of different animals to produce a probability map. A template, defined as areas of greater than 50% probability, was generated and projected onto the fuzzy maps. It was found that the projected template defined a region of an average association strength of 73%. When compared to regions defined directly on the fuzzy maps by selecting pixels with a greater than 50% association strength, the regions defined by the probability template were about 70% smaller but sufficiently large to reliably analyze densitometrically. This demonstrates that probability templates could be used to extract data from fused maps. (Supported by NIH 2P41RR01638).

545.6

A METHOD FOR THE COMPLETE STAINING OF IDENTIFIED NEURONS AT WILL. M. DESCHENES* AND D.PINAULT Research Center for Neurobiology, Laval Univ. Sch. of Med. Québec City, Canada G1J 1Z4.

Since a long time neuroanatomists have used tracers *in vivo* to study the neuronal connections and the morphology of neurons. Currently available techniques have one major disadvantage; it is indeed difficult or impossible to trace all the fine processes that belong to a single cell labelled from an injection site. This is generally due to strong background staining or to a tangle of labelled neuronal profiles at the core of the injection site. Though complete labelling of a cell can sometimes be achieved by intracellular staining, distal axonal processes often remain unstained and this technique is difficult to perform *in vivo*. We present here a promising alternative which consists in the labelling of a small number of neurons (1-5 cells) by the extracellular iontophoresis of biocytin using fine micropipettes. Glass micropipettes (tip diameter≈ 2μm) were filled with a solution of K-acetate (0.5 M) plus 2% biocytin or neurobiotin. These electrodes had impedance of 15-25 MΩ and they were used to record extracellularly the activity of rat thalamic reticular neurons. Once a cell had been identified by electrophysiological criteria, the tracer was ejected extracellularly by iontophoresis (positive current pulses of 100-300 nA, 1 sec on / 1 sec off, for 10 to 30 min.). After a survival period of 1-4 hours rats were perfused and the tissue was processed histochemically according to the protocol described by Horikawa and Armstrong (J. Neurosci. Methods 25: 1-11, 1988).

This technique produced complete labelling of all the cellular processes of a small number of cells, including their axonal terminals. Very little deposit, if any, was observed at the injection sites. Because the extracellular iontophoresis of biocytin is easy to perform and because biocytin histochemistry is compatible with most immunohistochemical protocols, the above technique represents a powerful tool for the study of the fine structure of identified elements in the CNS. Supported by the MRC of Canada.

545.7

COMBINING INTRACELLULAR INJECTION OF NEUROBIOTIN WITH PRE-EMBEDDING IMMUNOCYTOCHEMISTRY USING SILVER-INTENSIFIED GOLD PROBES TO ANALYSE THE SYNAPTOLOGY OF FUNCTIONALLY-CHARACTERISED NEURONS. H.M. Young*, W.A.A. Kunze, S. Pompolo, J.B. Furness and J.C. Bornstein. Department of Anatomy & Cell Biology and Department of Physiology, University of Melbourne, Parkville, 3052, Victoria, Australia.

We have developed methods to examine the neurochemistry of synaptic inputs to functionally-characterized myenteric neurons in the guinea-pig ileum. Electrophysiologically-characterized myenteric neurons were filled with Neurobiotin using intracellular microelectrodes, and the tissue was fixed in 4% formaldehyde, 0.05% glutaraldehyde and 15% picric acid in phosphate buffer. The tissue was washed in 50% ethanol, exposed to 0.1% sodium cyanoborohydride, incubated in avidin-biotin-horseradish peroxidase and then exposed to antibodies against calbindin or calretinin. Thereafter, the Neurobiotin-filled neurons were revealed using the DAB reaction. The tissue was examined at the light microscope level to determine the morphology and projections of the Neurobiotin-filled neurons, and then incubated in gold-labelled secondary antibodies. Following osmication, the gold probes were silver intensified and the tissue was embedded flat in resin. The tissue was re-examined at the light microscope level. Neurons containing a DAB reaction product could be distinguished from neurons containing a silver-intensified gold reaction product using oblique lighting instead of transmitted light. Ultrathin sections were taken through the injected neurons and examined. At the ultrastructural level, Neurobiotin-filled cell bodies and their processes (labelled with DAB) were easily distinguished from the silver-intensified gold-labelled structures. Gold-labelled terminals of enteric interneurons were observed to make synapses and close contacts with Neurobiotin-filled nerve cell bodies and their processes. This technique is valuable for the neurochemical identification of synaptic inputs to morphologically- and/or functionally-characterized neurons.

545.8

GABA AND GLYCINE INNERVATION OF ABDUCENS MOTONEURONS. A SERIAL ELECTRON MICROSCOPIC ANALYSIS. F. Lahjouji, H. Bras, A. Barbe and G. Chazal*. CNRS, UPR 418, 280 Bd Ste Marguerite, 13009 Marseille, France.

We report in this study the results concerning the GABA innervation of 13 somata, 63 proximal dendrites and axonal processes of abducens motoneurons. We also demonstrate the coexistence of GABA and glycine in the same terminals.

The motoneurons were retrogradely or intracellularly identified by HRP. The presence of GABA and glycine was detected using a post-embedding immunocytochemistry method on consecutive ultrathin sections. The percentage of synaptic covering was calculated for each motoneuronal compartments and terminal population.

The motoneuronal membrane was contacted by axon terminals (ATs) containing round or pleomorphic vesicles. The GABA ATs, filled with pleomorphic vesicles, were the largest of all the ATs. The percentage of synaptic covering was 33% on somata, 43% on proximal dendrites and reached 61% on axon hillock. It decreased to 18% on the initial segment. On all the motoneuronal compartments, the axon hillock had the highest GABAergic synaptic covering.

Glycine was detected in ATs in synaptic contact with the somata. On the population of ATs analyzed, 11% were GABAergic, 8% contain glycine and 9% contain both neurotransmitters. The double labeled ATs had the largest length of apposition. The percentage of synaptic covering ranged from 4.7 to 14% for the GABA ATs and from 2.2 to 12.1% for the glycine one.

These data showed that GABA and glycine both participate in the inhibition of abducens motoneurons.

545.9

FRACTAL DIMENSION OF DENDRITIC ARBORS OF GENICULATE NEURONS IN THREE-DIMENSIONAL SPACE. J. Gemmill*, L.-A. Coleman and M.J. Friedlander. Neurobiology Research Center, University of Alabama at Birmingham, Birmingham, AL 35294 USA.

The fractal dimension (**D**) of neuronal dendritic structure is a useful measure of territorial coverage (Montague and Friedlander, PNAS, 86:7223-7227, 1989) and provides objective means for comparing neurons independent of scale. However, most such analysis has been limited to 2 dimensions (2D). Neuronal dendritic arbors cover 3 dimensional (3D) space, and thus calculation of **D** in 3D is a more appropriate descriptor. We estimated the 3D fractal dimension of Area 18 projecting dorsal lateral geniculate nucleus (LGNd) neurons in the developing cat. Area 18 projecting neurons in the LGNd A layers were identified by retrograde transport of fluorescent markers and injected with HRP to reveal their dendrites. Dendritic arbors of injected neurons at 5 ages were analyzed using a 3D reconstruction system. Various methods for measuring **D** in 3D were implemented and evaluated. The Barnsley "box-counting" method places 3D grids over the neuron and counts the total number of boxes intersected by the structure. Average **D** was found to be 1.65 over the range of scales 13-247μ, and **D** was less than 1.12 over the range of scales 2-11μ.. A method for analyzing growth paths in 2D (Katz and George, BMB, 47:273-286, 1985) was extended to 3D using a Monte Carlo simulation for 3D random walks. **D** measured this way represents total length achieved by a dendritic segment within a volume, i.e. growth. If the measure is normalized to have the same number of sample points per segment, **D** can be used as a measure of "local wiggle ". An additional method which finds the intersection of Scholl spheres with the branches of the neuronal structure to compute **D** (Schierwagen, BMA, 49:709-722, 1990) was determined to be too sensitive to noise. However, the frequency distribution of Scholl sphere intersections shows that although the number of branches remains constant with age, the density maximizes at distances farther away from the soma at older ages. Thus, measuring neuronal **D** in 3D results in an objective description of structural density.

Supported by: NIH Grant EY-05116

545.10

TOMOGRAPHIC RECONSTRUCTION OF STRIATAL SPINY DENDRITES USING HIGH AND INTERMEDIATE VOLTAGE ELECTRON MICROSCOPY C.A. Ingham[1], G.W. Arbuthnott[1], R.A.Baldock[1], S.J. Young[2], C.J. Wilson[3], M.E. Martone[2], G.E. Soto[2], S.P. Lamont[2], M.H. Ellisman[2] SPON: Brain Res. Assoc. [1]PVS & MRC HGU, Edinburgh, U.K. [2]SDMIR & Dept. Neurosci., San Diego, USA. [3]Anat. & Neurobiol., UT Mem. Memphis USA.

Dendritic spines on rat neostriatal spiny neurons are small and densely packed so that accurate measurements of their density, shape and size are impossible using the light microscope (LM). Tomographic methods have been applied to derive 3D reconstructions of spiny dendrites in semi-thin sections imaged with a 1000 kV electron microscope (EM). The 3D data is then evaluated using computer aided visualization and analysis methods (Wilson et al, *Neuroimage* 1 (1992) 11-22). In the present study, similar procedures were employed to study images acquired with a 400 kV EM. The method was assessed using material previously analysed with the LM in which we compared equivalent parts of rat spiny cell dendrites from intact and dopamine denervated striata (Ingham et al, *Exp. Brain Res.* 93 (1993) 17-27). 3D reconstructions of 2μm sections of Golgi-stained, gold-toned dendrites showed that, although spine density estimates differ in the EM and LM, the relative decrease in spine density in denervated compared to control dendrites was similar. The results suggest that documenting changes in spine density, and perhaps spine shape, under experimental conditions will be possible using EM tomography.

545.11

IMAGING OTOCONIAL MICROSTRUCTURE USING THE ATOMIC FORCE MICROSCOPE. R. Hallworth*, J.B. Campbell+, M.L. Wiederhold and P. Steyger. Dept. Otolaryngology.-HNS, UTHSCSA, San Antonio, TX 78284 and +Southwest Research Institute, San Antonio, TX 78228.

Otoconia are small (1-20 μm) calcium carbonate crystals found in the gravitation-sensing organs of the vertebrate vestibular system. A small percentage of the otoconial mass is protein, but is thought to be important in determining otoconial shape. We have employed an atomic force microscope (AFM) to examine the detailed structure of otoconia at very high resolution. The AFM (Digital Instruments Nanoscope II, at Southwest Research Institute, San Antonio, TX.) measures the forces of attraction or repulsion between specimen and probe without the need for tissue processing methods. Otoconia from the red-bellied newt (*Cynops pyrrhogaster*) were examined. Amphibia exhibit two otoconial polymorphs. *Prismatic* otoconia are slab- or wedge-shaped and are found mainly in the saccule. *Barrel-shaped* otoconia are round, with tapered three-faceted ends and are found in the utricle. Prismatic otoconia are primarily of the calcium carbonate crystal form aragonite; barrel-shaped otoconia are calcite.

In both crystal isoforms, a series of fine parallel lines aligned with the long axis were observed. The lines were irregular in both width and spacing, ranging from 1-10 nm wide and 10-100 nm in separation. The surface also displayed a series of axial ripples of somewhat larger size. Transverse to these were a second series of parallel lines, similar to the axial lines, that follow the ripple contours. On edges or facets, the surface consists of small (10-50 nm) protuberances, ovoid in prismatic and rectangular in barrel-shaped otoconia, that appear to be the terminations of an underlying fibril extending axially along the otoconium. The observations suggest an internal structure of otoconia and a mechanism for their synthesis.

{Work supported by the Department of Otolaryngology - HNS, UTHSCSA, and the VA Medical Research Fund.}

545.12

IMAGING OF CNS-pHi WITH CELLULAR RESOLUTION: pHe INFLUENCES pHi IN CULTURED ASTROCYTES, ACUTE BRAIN SLICES AND THE INTACT BRAIN. A.Them, A.Villringer, A.Bernatowicz, U.R.Büttner*, U.Dirnagl. Dept. of Neurology, University of Munich, 8000 Munich 70, F.R.G.

We used the fluorescent dye BCECF and a confocal laser scanning microscope (Bio-Rad MRC 600; KrAr-Laser) to image the intracellular-pH (pHi) in brain cells. At λex=488nm; the emission changes indicate relative changes in pHi. Wistar rats were used to obtain primary astrocytes in culture (age: 2-4 weeks; n=57 cells examined), brain slices (2-4 days; n=332) or an intact brain preparation of anesthetized rats with a closed cranial window implanted (2-3 month; n=9). The preparations were incubated with BCECF-AM (2 μM in CO_2/HCO_3^- buffered artificial cerebrospinal fluid (aCSF); DMSO < 0,01% Molecular Probes) for 30 min. In slices and the intact brain, cellular resolution was achieved up to a depth of >50 μm. pHi-changes were stimulated by switching the superfusing medium to aCSF equilibrated to different pCO_2's, or 15mM NH_4Cl, or hypercapnia in the intact animal. In the slice, a rise of pHe from 7,4 to 7,9 and 8,3 influenced pHi in the same direction and corresponding magnitude in 97% of 94 stimulations and in 88% of 188 stimulations respectively. Alcalotic pHe and NH_4Cl caused acidosis upon return to normal pHe. Hypercapnia in the intact brain was associated with intracellular acidosis. The effect of pHe on pHi may indicate a large conductance for acid base equivalents or a sensitivity of pH regulating enzymes to pHe in the preparations studied.

Supported by the DFG and the Wilhelm Sander Foundation.

545.13

OPTICAL IMAGING OF INTRINSIC SIGNALS AND EXTRACELLULAR RESISTANCE IN HIPPOCAMPAL SLICES: CORRELATIVE MEASURES OF CELL SWELLING. J.S. Henn and D.A. Turner* Neurosurgery and Neurobiology, DUMC and VAMC, Durham, NC, 27710.

Repetitive synaptic stimulation and osmotic manipulation have both been previously shown to result in transiently altered light transmission through select regions of a hippocampal slice. We are investigating mechanisms that underlie these stimulation and osmolarity induced intrinsic optical signals, particularly the relationship between intrinsic signals, cell swelling and the relative ratio of intracellular and extracellular spaces in CA1, CA3 and DG.

Using a 12-bit cooled CCD camera and image subtraction, the absolute changes in light transmission through submerged slices were measured ($\Delta T/T$ in %). Extracellular resistance was also directly measured in CA1 stratum radiatum as an index of cell swelling ($\Delta R/R$ in %). When switching the bathing media from normal aCSF (290 mosm) to dilute aCSF (260 mosm, n=3), $\Delta T/T$ in CA1 radiatum increased 9.4 $\pm$ 0.2% while the overall $\Delta R/R$ increased 20.1 $\pm$ 3.3% (14.3 $\pm$ 4.1% due to the dilute solution alone). These changes occurred throughout the hippocampus with CA1 > CA3 > DG. Increased osmolarity (320 mosm with either sucrose or dextrose; n=6) resulted in no net overall change in $\Delta T/T$ (0.3 $\pm$ 6.1%) but decreases in $\Delta R/R$ of 19.8 $\pm$ 12.3% (with increases of 4.8 $\pm$ 1.2% due to the solution alone). Control experiments (normal aCSF to normal aCSF, n=3) showed that $\Delta T/T$ in CA1 radiatum increased 3.2 $\pm$ 1.7% while $\Delta R/R$ increased 0.8 $\pm$ 3.7%. Studies of afferent stimulation of CA1 radiatum showed increased light transmission of 10-15%, though similar signals could not be induced in CA3 or DG.

The asymmetry of these responses suggest that the baseline ratio of intracellular to extracellular space (particularly high in CA1) is critical to the generation of apparent cell swelling-induced optical signals, and that this ratio may be biased by osmotic manipulation. **Supported by NINDS #29482 and VAMC Merit Review.**

545.14

NEAR-INFRARED AND INFRARED SPECTROSCOPIC MEASUREMENTS OF ANIMAL CRANIA AND BRAIN CORTICAL SLICES E. N. Lewis,[‡] A. M. Gorbach,[*§] A. W. Toga[§] and I. W. Levin[‡] [‡]Laboratory of Chemical Physics, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD 20892. [§]Laboratory of Neuroimaging, UCLA Neurology, Los Angeles, CA 90024

In order to evaluate the feasibility of thermoencephaloscopy (TES) based on the measurement of infrared (IR) radiation from an animal cranium and to provide a basis for the IR spectroscopic imaging of the brain, spectra were collected between 1-4 μm (10,000-2,500 cm^{-1}) of various preserved slices of monkey and rat brain tissue and of pieces of monkey, rat and human skulls and human dura matter. Transmission spectra were recorded using interferometers equipped with liquid nitrogen cooled indium antinomide (InSb) and mercury cadmium telluride (MCT) detectors for near-IR and mid-IR data collection. Samples were translated within the IR beams using a micro-manipulator. This technique provided a means to collect multiple spatially resolved spectra with a limiting spatial resolution of approximately 1 mm, although significantly higher spatial resolution is possible.

The spectra revealed the maximum transmittance for human crania to be approximately 0.5% between 1-1.2 μm and that the average transmission in the 3-5μm spectral region was significantly less than 0.1%. The values are notably higher for both monkey and rat skulls. These data show that the TES signal is based on thermal conduction between the skull and cortex, and not on the direct measurement of photons through the skull. Transmission spectra collected from spatially resolved regions and different thicknesses of cortical slices show absorption bands due to lipids, proteins and peptides and reveal measurable differences between white and grey matter. The spectra will be interpreted in terms of variations in the biochemical makeup of different regions of the cortex. The feasibility of obtaining high fidelity vibrational spectroscopic images will be discussed.

545.15

LOCALIZATION OF DOMOIC ACID INDUCED NEURAL DEGENERATION IN THE PRIMATE FOREBRAIN AS REVEALED BY CONVENTIONAL SILVER METHODOLOGIES AND BY A NOVEL FLUORESCENT TECHNIQUE. L. Schmued*, A. Scallet, S. Ali, W. Slikker Jr.. Division of Neurotoxicology, National Center for Toxicological Research, FDA, Jefferson AR 72079

Cynomologous monkeys were given a single intravenous dose of the sea food contaminant, domoic acid (1 to 4 mg/kg), and the survivors were perfused with fixative one week later. The forebrains were first examined for evidence of neuronal degeneration using a modified de Olmos' cupric-silver technique. This technique revealed degenerating neurons within several forebrain regions. Within the hippocampus, extensive cell and terminal damage was found within Ammon's horn and the dentate hilar region, while the dentate granule cells exhibited limited degeneration and then only after the highest doses. Major hippocampal afferents such as the enthorhinal cortex also exhibited noticeable degeneration. Some hippocampal efferent targets were also affected. Although no degeneration was seen in the mammillary bodies, extensive cell and terminal degeneration could be seen in the lateral septum. Conspicuous cell and terminal degeneration was also observed in the lateral dorsal nucleus of the thalamus. Degenerating cells and terminals were visable in the piriform cortex. Argyophilia was also seen in the median eminence and the subfornical organ.

The aforementioned degeneration was confirmed by a recently developed novel fluorescent technique for revealing neuronal degeneration. The method employs a photo oxidized fluorescein derivative which we call Fluoro-Jade-D. This technique correlates well with the degeneration observed with the suppressed silver techniques, and is considerably less time consuming or capricious. It also appears to detect degeneration at shorter intervals after insult than is detectable with conventional methodologies. It may also be useful in resolving the role of oxidative free radicals in neurotoxicity.

545.16

NEUROTOXICITY OF DOMOIC ACID TO HIPPOCAMPAL MOSSY FIBERS OF CYNOMOLGUS MONKEYS: COMPUTER IMAGING AND MEASUREMENT OF SILVER DEGENERATION STAIN **R.L. Rountree* and A.C. Scallet Division of Neurotoxicology, National Center for Toxicological Research/FDA, Jefferson, AR 72079-9502**

Domoic acid is a potent excitotoxin that is structurally related to glutamic acid and kainic acid. Domoic acid enters the food chain after production in certain algae or seaweed, which are then consumed by filter feeders such as mussels and clams that serve as human foods. Application of the Nadler and Evenson (1983) method, a silver stain specific for degenerating axon terminals and cell bodies, revealed a highly specific, dose-dependent and focal argyrophilic area restricted to the CA2 stratum lucidum (dose 0 - 1.0 mg/kg). Grains were defined by both size and optical density parameters and were counted by a semi-automated computer-based image analysis system. When the number of silver grains, from full-screen digital images from each of four to six sections per monkey (16 monkeys), was counted there was a significant relation ($p < 0.05$) between dose and grain count, based on Friedman's two-way analysis of variance. Compared to a control value of 444.6 ± 76.8 (mean ± SEM), grain counts increased by 22%, 61% or 74% of control for the 0.25, 0.5 or 1.0 mg/kg dose levels respectively. This method of silver stain measurement should prove useful in the quantitative assessment of neurotoxicity risk from domoic acid.

545.17

REGIONAL DISTRIBUTION OF GFAP IN RAT AND MOUSE BRAIN. P.M. Martin*[1] and J.P. O'Callaghan[2]. Curriculum in Toxicology, Univ. of North Carolina, Chapel Hill[1], NC 27599 and Neurotoxicology Division, U.S. Environmental Protection Agency[2], RTP, NC 27711.

Traditionally, astrocytes in intact and damaged brains have been examined using immunocytochemistry, with changes in morphology and staining intensity used as indications of gliosis. Immunocytochemistry is a qualitative method, however, and there is a need for a simple and inexpensive method of quantifying changes in GFAP. Moreover, it would be useful to obtain both qualitative and quantitative data from the same animals, although aldehyde-based fixatives used to prepare brain tissue for immunocytochemistry make that tissue unsuitable for use in biochemical assays. One solution to this problem is to use ethanol as a fixative, which would permit both immunocytochemical and biochemical assays to be done on brain tissue from a single animal. The purpose of this study was to examine, quantitatively and qualitatively, the regional distribution of GFAP in normal adult rats and mice. Six Long-Evans rats and six C57B16/J mice were perfused with a 70% ethanol/saline solution. Half of each brain was dissected into eleven regions, and GFAP concentration was measured in each region using a sandwich ELISA. The other half of each brain was paraffin-embedded and used for GFAP immunostaining. Large regional differences occurred in the brains of both rats and mice, with the highest concentrations of GFAP found in the brainstem, and the lowest concentrations found in the striatum and cortex of both species. Rats, however, had lower concentrations of GFAP in the hippocampus, relative to the cerebellum and olfactory bulbs, while mice had higher concentrations of GFAP in the hippocampus compared to these two areas. Specific patterns of $GFAP^+$ cells and fibers corresponded to the regional distribution of GFAP in both rats and mice, with the greatest number of $GFAP^+$ cells found in the cerebellum, hippocampus, hypothalamus, and olfactory bulbs. $GFAP^+$ fibers were found in the molecular layer of the cerebellum and in several regions of the brainstem. These data show that it is possible to assay GFAP concentrations in tissue prepared for immunocytochemical analysis. Assaying GFAP represents a simple approach to quantifying gliosis in the intact and injured central nervous system of rats and mice. (Supported by U.S. EPA Training Agreement T901915).

546.1

GABA$_B$ RECEPTORS ENABLE THETA PATTERN STIMULATION OF PERFORANT PATH TO INDUCE POLYSYNAPTIC LTP IN THE HIPPOCAMPAL FORMATION. D.V. Lewis* and D.D. Mott, Depts. of Pediatrics (Neurology), Neurobiology and Pharmacology, Duke Univ. Med. Center, Durham, N.C. 27710.

We have previously demonstrated that GABA$_B$ receptors enhance signal transmission through the dentate gyrus when stimuli are delivered at 5-7 hz, a frequency in the range of hippocampal theta rhythm. Here, we show that this enhancement of transmission enables LTP induction at multiple sites in the hippocampal circuitry (polysynaptic LTP).

Polysynaptic LTP was induced by delivering theta pattern stimulus trains (4 pulses x 100 hz delivered at 5 hz) to the perforant path in the hippocampal slice preparation. Throughout the train, population spikes in the granule cell layer of the dentate gyrus persisted, producing polysynaptic responses in area CA3. Following the train, both the EPSP slope and population spike in the dentate gyrus, evoked by a perforant path test stimulus, exhibited a lasting potentiation. In area CA3 the commissural/associational response to a Schaffer collateral test stimulus, but not the response to a mossy fiber test stimulus, showed a similar enhancement. Blockade of GABA$_B$ receptors with CGP 35348 (1 mM) caused population spikes in the dentate gyrus to fade rapidly during the train. CGP 35348 blocked LTP induction in the dentate gyrus and by preventing transmission of the signal, blocked the development of LTP in area CA3 as well.

High frequency perforant path stimulation (100 hz, 50 pulses) produced LTP in the dentate gyrus, but not in area CA3. We conclude that induction of polysynaptic LTP is dependent upon the stimulation paradigm, being preferentially induced by theta pattern stimulation, and that GABA$_B$ receptors enable theta pattern stimulation to produce polysynaptic LTP.

Supported by NIH grant NS 27488.

546.2

SYNAPTIC INHIBITION LIMITS ASSOCIATIVE INTERACTIONS BETWEEN AFFERENTS DURING THE INDUCTION OF LONG-TERM POTENTIATION AND DEPRESSION R.A. Tomasulo*, J.J. Ramirez, and O. Steward, Dept. of Neuroscience, Univ. of Virginia, Charlottesville, VA 22908

Long-term potentiation and associative long-term depression depend for their induction upon associative interactions between synapses that converge on individual dendrites. The presumed medium of intersynaptic communication is dendritic depolarization. We tested the hypothesis that GABA-A-mediated synaptic inhibition limits the spatial domain in which these interactions occur. Stimulating electrodes were placed in the medial and lateral entorhinal cortices of urethane-anaesthetized rats, to activate inputs to the middle and distal portions of dentate granule cells. Two recording pipettes were placed in the ipsilateral dentate hilus. A control electrode contained only 0.9% NaCl. A test electrode containing 8 mM bicuculline methiodide in 0.9% NaCl was placed 1.5 mm posterolateral in the same dentate gyrus. This established a local blockade of GABA-A neurotransmission, as described previously (Brain Res 561:27). We measured the initial pEPSP slope of the responses from each input at each site during 4 20-minute periods separated by 3 sets of conditioning trains. The trains (8 400 Hz, 17.5 msec trains at 15 sec intervals) were delivered first to the lateral site, then the medial, then both simultaneously. Each experiment, therefore, yielded two examples of homosynaptic, heterosynaptic, and paired tetanization at each recording site. Bicuculline significantly enhanced synaptic plasticity in all three categories (p = .012, .028, and .018 for homo-, hetero-, and paired respectively). Long-term depression averaged -16% at the bicuculline sites, while long-term depression did not appear at the control sites. We conclude that associative interactions are significantly constrained by synaptic inhibition. Support: BNS8818766 from NSF to OS and K08NSO1438 from NINDS to RAT.

546.3

MODIFICATION OF GABAERGIC SYNAPTIC RESPONSE FOLLOWING ASSOCIATIVE PAIRINGS IN RAT HIPPOCAMPAL SLICE. C.-J. Lee* and D. L. Alkon, NINDS, NIH, Bethesda, MD 20892.

Alkon *et. al.* reported that an inhibitory GABAergic synaptic response is transformed into an excitatory response following pairings of exogenous GABA with postsynaptic depolarization in *Hermissenda* (PNAS, 89:11862). They suggested that the GABAergic inhibitory synapse may also play a role in associative learning in the mammalian nervous system. To initially explore this possibility, we examine here modification of GABAergic IPSPs following associative pairings of postsynaptic depolarization with presynaptic volleys. Intracelluar recordings were made from CA1 pyramidal cells of an IPSP following EPSP in response to electrical stimulation of Schaeffer collaterals. Pairing was effective with high frequency stimulation (100 Hz for 100 msec) of Schaeffer collaterals while a postsynaptic cell was depolarized by the injection of a positive current step (200 msec, 1-2 nA). The pairing was repeated 10 times at 0.2 Hz. Our study showed that a new depolarizing component (NDC) emerged during the hyperpolarizing phase after the pairings. The appearance of the NDC after pairings is similar to the transfomation of IPSP into EPSP observed in *Hermissenda* in that an initially inhibitory synaptic response to a given input was transformed into an excitatory response. This component elicited after each presynaptic volley persisted up to 30 mins. It appeared between 20 - 40 msec following each stimulus onset, and it did not develop after unpaired stimulation. Pharmacological studies showed that 1) the NDC appeared after pairings even in the presence of the non-NMDA receptor antagonist (DNQX) 2) the NDC did not appear in the presence of both non-NMDA (DNQX) and NMDA receptor antagonists (APV), and finally, 3) the NDC did not appear after pairings when the IPSP was initially absent in the postsynaptic response to Schaeffer collateral stimulation. These findings suggest that the NDC is mediated through GABAergic receptors but also requires the activation of NMDA receptors during pairings.

546.4

LTP IN HIPPOCAMPAL CA1 PYRAMIDAL CELLS IS ACCOMPANIED BY OPPOSITE PRE- AND POSTSYNAPTIC CHANGES OF GABAERGIC INHIBITION. R.Rai*, G. Kovacs and A. Stelzer. Dep. of Pharmacology, SUNY Brooklyn, Brooklyn, NY 11203.

Possible pre- and postsynaptic changes of synaptic inhibition during LTP were examined in the CA1 hippocampal subfield. To evaluate postsynaptic changes, intracellular recordings were performed in apical dendrites of CA1 pyramidal cells in stratum radiatum in the transverse hippocampal slice from adult guinea-pigs. Schaffer collateral stimulation revealed a decrease of the early IPSP in 48 of 57 apical dendrites (no change in 5 of 57 and increase in 3 dendrites) during LTP (>30 min following tetanization). The iontophoretic GABA$_A$ response (both depolarizing and hyperpolarizing) was reduced in 20 of 23 apical dendrites (unchanged in 3 dendrites). Modification of the early IPSP and of the iontophoretic GABA$_A$ response was not observed when the tetanus was applied in the presence of APV (50 μM). Interneuron excitability was measured in somatic recordings of interneurons in strata lacunosum / moleculare (L/M) and alveus / oriens (A/O) (identified by physiological and staining (LY) criteria). Tetanization produced a lasting (>30 min) increase in EPSPs in L/M interneurons in 12 of 15 cells (no change in 2 and a decrease in one cell) and in A/O interneurons in 23 of 26 (no change in 3 cells). In sum, LTP in CA1 pyramidal cells is accompanied by a reduction of GABA$_A$-receptor sensitivity, but enhancement of interneuron excitability. Impairment of GABA$_A$-receptor function may, however, represent the prevailing mechanism in the modification of synaptic inhibition during LTP and disinhibition may represent a pivotal factor underlying tetanization-induced long-lasting potentiation of excitability of CA1 pyramidal cells.

546.5

LONG-TERM POTENTIATION OF INHIBITORY SYNAPTIC TRANSMISSION IN RAT VISUAL CORTEX. Y. Komatsu*. Dep. of Physiology, Kyoto Prefectural Univ. of Med., Kamigyoku, Kyoto 602, Japan.

Activity-dependent long-term modification of inhibitory synaptic transmission was studied in rat visual cortex slices. Inhibitory postsynaptic potentials (IPSPs) evoked by layer IV stimulation were intracellularly recorded from layer V cells while excitatory synaptic transmission was blocked by N-methyl-D-aspartate (NMDA) and non-NMDA receptor antagonists. High-frequency stimulation (50 Hz, 1 s) was applied to layer IV 10 times at 10 s intervals as a conditioning stimulation. The conditioning stimulation with intensity of 5 times the threshold intensity (5 T) to evoke an IPSP induced long-term potentiation (LTP) of IPSP in all tested cells (7/7) sampled from developing rats (P20-30 days). In mature rats (P60-70 days), the same conditioning stimulation (5 T) rarely induced LTP (1/7) and even stronger conditioning stimulation (10-15 T) induced LTP in only half of tested cells (3/6). This age dependence suggests that LTP of IPSPs contributes to the postnatal development of selective responsiveness of visual cortical cells through visual experience.

546.6

SYNAPTIC POTENTIATION IN CULTURED CORTICAL NEURONS: LONG-LASTING MODIFICATION OF SPONTANEOUS SYNCHRONIZED PERIODIC FIRING BY PATTERNED ELECTRICAL STIMULATION. E.Maeda[1], H.P.C.Robinson[2], Y.Kuroda[3] and A.Kawana*[1]. [1]NTT Basic Research Labratories, Midori-cho, Musashino-shi, Tokyo, 180 Japan, [2]Physiological Lab., University of Cambridge and [3]Tokyo Metropolitan Institute for Neuroscience.

In low $[Mg^{2+}]_o$, spontaneous synchronized bursts of action potentials and $[Ca^{2+}]_i$transients are observed in cultured cortical neurons, depends on NMDA-type synaptic transmission(Robinson et al. submitted). We examined the effects of electrical stimulation on the efficiency of synaptic transmission under these conditions. Electrical activity of cultured neuron networks was recorded extracellularly using planar electrode array(PEA) substrates at 2-4 weeks after plating, from up to 16 electrodes simultaneously. The PEA was composed of 64 indium tin oxide electrodes in an 8×8 grid at 1 mm intervals, fabricated as described by Jimbo et al.(IEEE trans. on BME, in press). The source of each burst, as estimated from the relative delays between electrodes, varied from burst to burst. After section of the network in half using a UV laser spot, synchronous burst firing had different frequencies and phases in the two halves. In initially silent cultures, synchronous bursting could be induced by local electrical stimulation through the PEA.

The average interburst interval and its variance increased with $[Mg^{2+}]_o$(0-200 μM). Synchronous bursts disappeared at 2mM $[Mg^{2+}]_o$ and only partial or weak synchronicity was observed at greater than 100μM $[Mg^{2+}]_o$. Electrical stimulation simultaneously at several sites in weakly synchronized networks (single 100 μsec pulses at 0.1-1 Hz for 2-10 min) produced strongly synchronized bursting which lasted for at least 15 min after cessation of the stimulus. The frequency and duration of bursts were also increased. In some preparations, spontaneous synchronized bursts could be initiated by electrical stimulation even at 2 mM $[Mg^{2+}]_o$. These results suggest that long-lasting potentiation of synaptic transmission in cultured cortical neuron networks can be induced by focal electrical stimulation.

546.7

ONTOGENY OF LONG-TERM POTENTIATION. J.D. Bronzino*, K.S. Abu-Hasaballah, R.J. Austin-LaFrance, and P.J. Morgane‡. Dept. of Engineering and Computer Science, Trinity College, Hartford, CT 06106; ‡The Worcester Foundation for Experimental Biology, Shrewsbury, MA 05145.

The LTP phenomenon has been studied in a host of animals and brain areas, however, studies examining the establishment and maintenance of LTP in the developing rat have been limited primarily to the *in vitro* slice preparation or intact, anesthetized animals. To date, no study has examined the development of LTP in the hippocampal formation in an intact, behaving preparation. This study was undertaken to examine the development of LTP at the perforant path/dentate granule cell synapse in freely-behaving rats during the preweaning (15 days of age), early post-weaning (30 days of age) periods of development, and adulthood (90 days of age). Population spike amplitude (PSA) measures showed significant enhancement 15 mins. after tetanization in all age groups. This measure was found to decay to baseline levels 5 hrs. after tetanization in 90-day old animals and 18-24 hrs. after tetanization in 30-day old animals. PSA measures obtained from 15-day old animals were still increasing at 96 hrs. after tetanziation. EPSP slope measures enhanced by approximately 35% in all age groups 15 mins. after tetanization. This measure decayed to baseline by 5 hrs. post-tetanization in 90-day old animals and 18-24 after tetanization in 30-day olds. 15-day animals continued to show enhancement of the EPSP slope measure (to over +300%) throughout the post-tetanization period (96 hrs). The results indicate that the freely-moving 15- and 30-day old animals are capable of establishing and maintaining LTP of the perforant path/ dentate granule cell synapse and that the levels of synaptic transmission enhancement are significantly greater in these younger animals than that reported in adults. This may indicate that GABAergic inhibition of dentate granule cell activity is not funtionally mature at either 15- or 30-days of age.

Supported by NSF Grant # BCS-9208128

546.8

LTD IN INTACT HIPPOCAMPUS: USE- AND NMDA RECEPTOR-DEPENDENCE. E. Thiels*[1], G. Barrionuevo[1] and T.W. Berger[2]. [1]*Depts. of Behavioral Neuroscience and Psychiatry, U. Pittsburgh, Pittsburgh PA 15260, and* [2]*Dept. of Biomedical Engineering, USC, Los Angeles CA 90089.*

Induction of long-term depression (LTD) at cortical synapses studied *in vitro* has been shown to require (1) presynaptic activity contiguous with suppressed postsynaptic activity, and (2) postsynaptic calcium influx, mediated at some synapses by NMDA receptor activation. Here we describe properties of use-dependent LTD involving the commissural-CA1 pyramidal cell synapse in hippocampus of anesthetized rats.

Repeated paired-pulse stimulation (PPS; 0.5Hz) of commissural input to CA1 pyramidal cells *in vivo* using 25-ms interstimulus intervals (ISIs), which produce suppression of CA1 pyramidal cell activation to the second impulse, resulted in robust LTD: only 200 pairs caused the amplitude of CA1 population spikes (evoked using low frequency, 0.1Hz, single impulses) to decrease from a baselne level of 4.6±0.3mV to 0.7±0.2mV at 60min (N=12). This effect was specific to short ISIs: 200 pairs using 1000-ms ISIs, which produce comparable pyramidal cell activation by the first and second impulse, failed to reliably modify evoked response amplitudes; the corresponding values were 4.9±0.3mV and 4.9±1.4mV (N=6).

The LTD observed using 25-ms ISIs was long-lasting: response levels continued to be depressed to 1.0±0.5mV 180min after PPS (N=5). In addition, the LTD was reversible by brief high-frequency commissural stimulation (100Hz for 1s; HFS): response amplitudes increased from 0.7±0.3mV 60min after PPS to 7.7±1.2mV 30min after HFS (N=5). A second train of PPS caused responses to decline to 3.4±1.4mV 60min after PPS. Finally, induction of LTD was dependent on NMDA receptor activation: microinjection of the NMDA receptor antagonist D-APV (100μM) in str. radiatum near the recording site blocked development of PPS-induced LTD, whereas microinjection of vehicle solution (150mM NaCl) did not. Response amplitudes in the presence of APV were 3.1±0.3mV before and 3.5±0.5mV 30min after PPS (N=4), compared to 4.0±1.0mV and 1.6±0.5mV, respectively, in the presence of NaCl (N=4). Taken together, these findings suggest that principles of LTD induction identified in *in vitro* preparations apply to LTD in intact brain, and moreover, that the network properties of intact brain readily provide for the implementation of those principles. (Supported by ONR, AASERT, NIMH, NIH BMSR, and NS24288).

546.9

LONG-TERM POTENTIATION OF SYNAPTIC DEPRESSION IN THE CNS OF THE SNAIL *HELIOSOMA TRIVOLVIS*. S. N. Chawla, T. Bloom, M. A. Peck, L. M. Aronica, D. D. Anderson and V. C. Kotak*. University of Pennsylvania, Biological Basis of Behavior, Philadelphia, Pa 19104-6196.

In the hippocampus LTP model, enhancement in synaptic efficacy involves an excitatory glutaminergic pathway that may be important to learning and memory (Bliss and Lynch, 1988; Zalutsky and Nicoll, 1990). Induction of such LTP can be interrupted by co-activation of GABAergic neurons (Douglas et al. 1983). However, characterization of inhibitory LTP *per se* is ill defined. In goldfish, an inhibitory circuit for LTP mediated by glycine is recently demonstrated (Korn et al. 1992). To examine synaptic potentiation, the CNS ganglionic cells in an isolated preparation of the pond snail, *Heliosoma trivolvis*, were impaled while an afferent nerve trunk was stimulated extracellularly. A single high frequency tetanic shock to the nerve trunk (100Hz, 5 sec) produced prolonged depression recorded intracellularly. This inhibition included an at least 50% reduction in the baseline neuronal firing, and an increase in IPSPs. In other neurons, tetanic shock to the afferents completely silenced the action potentials. Such synaptic depression facilitated progressively for at least 15 minutes. Since direct afferent inhibition is unlikely, this result indicates a strong potentiation in an inhibitory neural pathway. Bath application of 500 μM GABA produced a reversible inhibition while the GABAa receptor antagonist pircrotoxin depolarized the cells and increased their input resistance. In light of this, and the identification of GABA (Richmond et al. 1991) and FMRFamide (Haydon, 1991) in the CNS of *Heliosoma*, attempts are being made to characterize the tetanic inhibition in relation to these two transmitters/modulators.

Supported by the Biological Basis of Behavior Program for Undergraduate Research.

546.10

HOMOSYNAPTIC LONG-TERM POTENTIATION AND DEPRESSION OF CO-ACTIVE INPUTS ONTO SINGLE CA1 NEURONS *IN VITRO*. Michael Weliky*. Duke University Medical School, Box 3209, Durham, NC 27710.

Different stimulus protocols have been required to induce homosynaptic long-term potentiation (LTP) and depression (LTD) in the hippocampus. LTP has been typically induced by short bursts of high frequency stimulation while LTD induction has required extended periods of low frequency stimulation. Experiments were performed in hippocampal slices prepared from rats (19-32 days). Bursts of high frequency stimulation, applied for 30 to 60 seconds to CA1 afferents, induced either LTP or LTD of synaptic responses in single CA1 pyramidal cells. A multielectrode array, consisting of sixteen 12.5 um wires arranged into two parallel rows, was used to stimulate multiple afferent pathways converging onto the apical dendrites of single CA1 pyramidal cells. After synchronous tetanization, both LTP and LTD were induced in different pathways (n=11 slices), or only LTP was induced (n=12 slices). When picrotoxin was applied (50-100 uM) (n=9 slices) only robust LTP was induced in all slices except one, in which LTD was also induced. In both normal and PTX treated slices stimulus intensities were adjusted so that baseline synaptic responses spanned the same amplitude range of 15 to 130 pA. In normal slices, single responses could be alternately depressed or potentiated by pairing tetanization with intracellular current injection or voltage clamping to different membrane potentials (n=5 slices). Hyperpolarization increased the probability of inducing LTD while depolarization induced LTP. This suggests that 1) synchronous bursting of CA1 inputs, which occurs during sharp wave and theta EEG activity, can induce heterogeneous synaptic changes in target CA1 neurons, 2) these synaptic changes follow a Hebb rule of a covariance form, and 3) dendritic inhibition may play a role in regulating these changes by selectively hyperpolarizing local regions of the dendritic tree in which LTD is induced.

546.11

A POSSIBLE 'PRIMING' REQUIREMENT FOR LOW-FREQUENCY STIMULATION-INDUCED HOMOSYNAPTIC DEPRESSION (LTD) IN AREA CA1 IN VIVO. B.R. Christie*, W.C. Abraham and M.F. Bear. Department of Psychology, University of Otago, Dunedin, New Zealand.

Homosynaptic LTD of CA1 Schaffer collateral synapses has been shown to occur following 900 conditioning pulses at 1-3 Hz in vitro (Dudek & Bear, 1992, 1993). We investigated whether a similar effect could be demonstrated in the same pathway in vivo. 2-3 month old rats were anesthetized with sodium pentobarbital and prepared with stimulating electrodes in the stratum radiatum of CA1 of both hemispheres to activate separate CA3 afferents to a single population of CA1 pyramidal cells. Negative-going field EPSPs were recorded in stratum radiatum of one hemisphere, in response to alternating baseline stimulation of the two input pathways. Stimulation of the ipsilateral input at 1 Hz for 900 pulses, followed 45 min later by stimulation at 3 Hz for 900 pulses, induced a small (11% ± 4%, n=6) but significant LTD that was input specific and lasted as long as it was tested (30 min). Curiously, 900 pulses at either 1 Hz (n=8) or 3 Hz (n=4) alone produced no significant changes, nor did 1800 pulses (given as 2 sets of 900 pulses 45 min apart) at 3 Hz (n=3). This suggested that homosynaptic LTD in vivo might require 'priming' stimulation, as has been shown for associative LTD in the lateral perforant path (Christie & Abraham, 1992). Thus 'priming' stimulation (5 Hz, 80 pulses) was first delivered to the ipsilateral input (producing no change by itself), and then 15 min later 900 pulses at 3 Hz were given to the same input. This protocol resulted in LTD of 13% ± 4% (n=5), a magnitude similar to that observed in CA1 slices of adult rats (but without priming). Our experiments thus confirm the existence of low-frequency stimulation-induced homosynaptic LTD in field CA1 of intact rats, although in this preparation it appears to require 'priming' by prior synaptic activity.

Supported by grants from the NZ Health Research Council (B.R.C. & W.C.A.) and Human Frontier Science Programme (W.C.A. & M.F.B.).

546.12

AN UNUSUALLY PROLONGED RESPONSE BY THE IMMEDIATE EARLY GENE KROX 20 TO LTP-INDUCING STIMULATION. W.C. Abraham*, M. Dragunow, P. Lawlor, S.E. Mason, J. Demmer, W. Tate. Departments of Psychology and Biochemistry, University of Otago, Dunedin and Department of Pharmacology, University of Auckland Medical School, Auckland, New Zealand.

Tetanization leading to LTP at perforant path-dentate gyrus granule cell synapses was followed by a dramatic increase of mRNA and protein for the zinc-finger-containing transcription factor Krox 20, measured with Northern blots and immunohistochemistry. The induction was confined to the nuclei of cells in the ipsilateral dentate gyrus. Krox 20-like immunoreactivity increased within 20 min of tetanization of the perforant path, was maximal 1-8 h after stimulation, and was still elevated at 24 h before returning to baseline by 48 h after LTP induction. This prolonged time course of protein induction contrasts with the more transient induction after LTP of other immediate early genes, including the related molecule Krox 24. Krox 20 and Krox 24 showed a similar transient duration of mRNA induction (1-2 h), suggesting that the prolonged Krox 20 protein induction is due to greater stability of this protein. Low-frequency stimulation did not induce either LTP or Krox 20, and the induction of Krox 20 after tetanization was prevented by the NMDA antagonists CPP and MK801, which also blocked LTP induction. Stimulus paradigms known to induce LTP of differing durations produced corresponding differences in Krox 20 induction. Thus perforant path tetanization induces an NMDA receptor-mediated expression of Krox 20, which may play a role in the stabilization of LTP.

Supported by grants from the NZ Health Research Council (W.C.A., M.D. & W.T.), N.Z. Neurological Foundation (J.W.) and Human Frontier Science Programme (W.C.A. & W.T.).

546.13

PROTEIN KINASE C (PKC)-DEPENDENT PHOSPHORYLATION ACCOMPANIES INDUCTION OF LONG-TERM POTENTIATION (LTP) IN CA3 OF THE RAT HIPPOCAMPUS. H. Son[1], Paul Davis[2] and D.O. Carpenter[1]*
[1]Wadsworth Labs, NYS Dept. of Health and School of Public Health, [2]Dept. of Medicine, Albany Medical College, Albany, NY 12201.

LTP is a form of synaptic plasticity which may be an indicator of memory formation in the mammalian brain. We have applied physiological and biochemical measurements to determine the role of PKC in the process of LTP in the CA3-mossy fiber (MF) synapse of rat hippocampal brain slices. The MF synaptic terminals have PKC but not growth-associated protein 43 (GAP-43), while the pyramidal neurons have both. Brief high frequency stimulation (HFS) of mossy fibers induced LTP which lasted more than 90 min. The LTP was blocked by 10 μM D-sphingosine, an inhibitor of PKC. Phorbol-12,13-diacetate (PDAc) (1 μM), a PKC activator, increased the synaptic response by 395%. When HFS was given after full activation of PKC by PDAc, there was no post-tetanic potentiation or LTP. Double-pulse facilitation, which reflects available Ca^{2+} in the presynaptic terminal, was reduced by PDAc, suggesting involvement of presynaptic PKC in the induction of LTP. After LTP the phosphorylation of a 42 kd-protein was decreased for up to 10 min and increased after 30 min. This almost certainly is postsynaptic GAP-43. Phosphorylation of GAP-43 was also increased by phosphatidylserine and free Ca^{2+} at concentrations less than 10^{-6}M, and decreased by calmodulin (CaM) in a concentration dependent manner. Calmodulin binding inhibitor reversed the decreased phosphorylation of GAP-43 at the early stages of LTP. Therefore, it is likely that presynaptic PKC is required for the induction of LTP whereas postsynaptic PKC and GAP-43 are necessary for the persistence of LTP. After HFS phosphorylation of GAP-43 by PKC may be regulated by CaM via internal $[Ca^{2+}]$.
Supported by NS23807-08.

546.14

LONG-TERM POTENTIATION IS ASSOCIATED WITH AN INCREASED ACTIVITY OF Ca^{2+}/CALMODULIN-DEPENDENT PROTEIN KINASE II. K. Fukunaga[1]*, D. Muller[2], L. Stoppini[2] and E. Miyamoto[1]. [1]Dept. of Pharmacol., Kumamoto Univ. Sch. Med., Kumamoto 860, Japan, [2]Dept. of Pharmacol., Centre Med. Univ., 1211 Genève 4, Switzerland.

Among the molecular mechanisms that have been proposed to contribution to long-term potentiation (LTP) in hippocampus are the activation and autophosphorylation of Ca^{2+}/calmodulin-dependent protein kinase II (CaM kinase II). We investigated whether changes in CaM kinase II activity and autophosphorylation may occur as a result of LTP induction in hippocampus. High, but not low-frequency stimulation applied to two groups of CA1 afferents resulted in a long lasting increase in the Ca^{2+}-independent and total activities of the enzyme as well as an increase in the ratio of Ca^{2+}-independent to total activity. The effect was obtained using two different substrates and observed in both hippocampal slices and hippocampal organotypic cultures. It could be blocked by preincubation of slices with an NMDA receptor antagonist, D-2-amino-5-phosphonopentanoate. Treatment of slices with calyculin A, a phosphatase inhibitor, modified the activity of the enzyme, but LTP could still be induced and a further increase in Ca^{2+}-independent CaM kinase II activity still be observed. Finally, in the experiment with ^{32}P-labeled slices, we demonstrated a significant increase in the autophosphorylation of the enzyme with the concomitant induction of LTP. In agreement with several previous studies, these results support the idea that CaM kinase II may contribute to LTP in CA1 region of hippocampus.

546.15

B-50/GAP-43 AND NEUROGRANIN PHOSPHORYLATION AS MARKERS FOR PRE- AND POSTSYNAPTIC PKC-ACTIVITY DURING LTP. Ramakers, G.M.J., De Graan, P.N.E., Kraaij, D.A., Urban, I.J.A. and Gispen, W.H. (SPON: European Neuroscience Association) Rudolf Magnus Institute, Department of Medical Pharmacology, Utrecht University, Vondellaan 6, 3521 GD Utrecht, The Netherlands.

Several lines of evidence indicate that protein kinase C (PKC) plays an important role in the molecular mechanisms underlying long-term potentiation (LTP). However, the location of the PKC activity relevant for different phases of LTP is still largely unknown. To distinguish between pre- and postsynaptic PKC activity we monitored the in situ phosphorylation state of identified PKC substrates. As presynaptic marker we used protein B-50 (a.k.a. GAP-43, F1, neuromodulin), a protein implicated in different forms of synaptic plasticity and in the mechanism of neurotransmitter release. As postsynaptic marker we used neurogranin (a.k.a. p17, BICKS, RC3), a protein which shares an 18 amino acid sequence with B-50, containing the unique PKC-phosphorylation site.

Rat hippocampal slices (450 μm) were labelled for 90 min with ^{32}P-orthophosphate and were stimulated either by tetanic stimulation (100 Hz, 1 sec) or by treatment with the phorbol ester 4-β-phorbol-12,13-dibutyrate (4-β-PDB, 10^{-6}M). After field potential recordings, in situ B-50 and neurogranin phosphorylation were analysed in each individual slice by specific immunoprecipitation. An increase in B-50 phosphorylation could be detected 60 and 90 min, but not 120 min after the tetanus. 4-β-PDB stimulation induced a time- and concentration-dependent increase in B-50 and neurogranin phosphorylation, which could be blocked by PKC-inhibitors. 4-β-PDB induced stimulation of B-50 and neurogranin phosphorylation could be detected within 5 min and was maximal after 30 min. 4-α-Phorbol-12,13-didecanoate was ineffective in stimulating B-50 and neurogranin phosphorylation.

The time-curve of B-50 phosphorylation resembles that reported for increased glutamate release during LTP. Whether these two phenomena are causally related remains to be determined. Our data confirm that neurogranin is an in vivo substrate for PKC and show that it is now possible to monitor temporal and spatial differences in the phosphorylation state of specific PKC substrates within a single slice during LTP.

546.16

GENISTEIN REDUCES NOREPINEPHRINE-INDUCED LONG-LASTING POTENTIATION IN HIPPOCAMPAL DENTATE GYRUS

P.J. Voulalas* and J.M. Sarvey, Department of Pharmacology, USUHS, Bethesda, MD 20814.

Tyrosine kinase inhibitors have been demonstrated previously to block LTP in CA1. Both ß-adrenergic and NMDA receptors are required for induction of NELLP and LTP in the dentate gyrus. We examined (1) the effects of tyrosine kinase inhibitors on NELLP and (2) the effect of isoproterenol (IP) on tyrosine phosphorylation. Rat hippocampal slices were exposed in an interface chamber to 110μM genistein 30 min prior to bath application of 1μM IP. Pretreatment with genistein reduced potentiation of the medial perforant path granule cell population spike (PS) to 50% of that elicited in the absence of genistein. IP (1μM) transiently increased tyrosine phosphorylation of at least two proteins, detected by Western blot with an anti-phosphotyrosine antibody. The onset of increased phosphorylation directly parallels the increase in the amplitude of the PS, both peaking 5-10 min after exposure to IP. Phosphorylation decreased by 20 min, when the PS was still maximally potentiated. These results imply a similar underlying mechanism for the induction of LTP and NELLP.

LONG-TERM POTENTIATION VII

547.1

FURTHER CHARACTERISATION OF THE BLOCK OF LTP BY THE mGluR ANTAGONIST MCPG IN THE HIPPOCAMPAL CA1 REGION. N. Berretta, Z.A. Bortolotto, Z.I. Bashir, E.J. Coan* & G.L Collingridge. Dept. of Pharmacology - University of Birmingham - Birmingham B15 2TT (UK).

We have shown previously that the glutamate metabotropic receptor (mGluR) antagonist (±)-α-methyl-4-carboxyphenylglycine (MCPG) prevents the induction of LTP by a single tetanus (100 Hz, 1s) to the Schaffer collateral-commissural pathway (SCCP), but spares STP (Bashir *et al.*, Nature 1993 *in press*).

Further experiments have been performed in order to verify whether MCPG could block LTP under more extreme conditions of induction. Extracellular synaptic potentials were recorded from *stratum radiatum* of hippocampal slices. In the continuous presence of MCPG the induction of LTP was blocked even when tetanic stimulation consisted of 6 tetani (each comprising 100 Hz, 1s) delivered every 10 min. Following the tetani STP decayed to baseline in approximately 50 min (n=4). Moreover, a single tetanus (100 Hz, 1s) delivered in the presence of MCPG (500 μM) and picrotoxin (50 μM), to block $GABA_A$ receptor-mediated inhibition, induced STP but not LTP in 3 out of 4 slices.

It has been reported that in the spinal cord (+)-MCPG is the active isomer (Jane *et al.*, Neuropharmacology 1993 *in press*). In order to investigate the stereoselectivity of MCPG in blocking LTP induction, tetanic stimulation of the SCCP was delivered in the presence of (+)-MCPG (500 μM). In this experimental condition the tetanus induced only STP. However, after washout of (+)-MCPG a tetanus delivered in the presence of (-)-MCPG (500 μM) induced LTP, which was followed for up to 60 min (n=3). (+)-MCPG was ineffective in blocking LTP of a separate input in the same slices when it was applied immediately after the tetanus suggesting that for the induction of LTP it is necessary that the mGluRs are active during tetanic stimulation.

Supported by: CAPES-Brazil, MRC, Wellcome Trust.

547.2

ACTION OF A METABOTROPIC GLUTAMATE RECEPTOR ANTAGONIST ON LTP IN THE DENTATE GYRUS OF FREELY MOVING RATS. G.Riedel*, S.Vieweg and K.Reymann. Dept. Neurophysiol., Federal Institute for Neurobiology, POB 1860, 3010 Magdeburg, Germany

Long-term potentiation (LTP), a sustained increase in synaptic efficacy induced by high frequency electrical stimulation, requires postsynaptic activation of N-methy-D-aspartate (NMDA) receptors (Collingridge et al. 1983, *J.Physiol.*334:33-46) in CA1 and dentate gyrus of the hippocampal formation. Early reports supported an additional role for metabotropic glutamate receptors (mGluRs) for the late of LTP (Behnisch et al. 1991, *Neuroreport* 2:386-388). We investigated this hypothesis using the new selective and competitive mGluR antagonist (RS)- ∝ -methyl-4-carboxyphenylglycine (MCPG; gift from J. C. Watkins) (Eaton et al. 1993, *Eur.J.Pharmacol.* 244: 195-197).

The drug (0,02mg in 5μl) was injected intracerebroventricularly in awake rats 30 minutes before application of the tetanus (10 bursts, 200 Hz, 15 pulses, bursts intervall 10 s). The injection of MCPG has no effect on the baseline test responses. Tetanic stimulation of perforant path fibers resulted in a postsynaptic potentiation which lasted for 1 - 2,5 hours and showed a permanent decay in amplitude (n=6). After 2,5 hours, posttetanic test responses remained stable at baseline levels for up to 24 hours. Control experiments, in which saline (0,9%) was applied 30 minutes before the tetanus, showed stable LTP lasting for 24 hours or longer. These results suggest that in the dentate gyrus activation of mGluRs is essential for long-lasting synaptic changes in vivo. Blockade of the mGluR resulted only in a posttetanic and short-term potentiation (STP) of synaptic transmission. This confirms and extends the recent findings of Bashir et al. (1993, *Nature* in press) who proposed a role for mGluR in the induction of LTP in CA1 and CA3 in vitro.

547.3

SYNAPTIC ACTIVATION OF METABOTROPIC GLUTAMATE RECEPTORS IS INHIBITED BY LOW LEVELS OF NMDA. G. J. Pacelli* and S.R. Kelso, **Department of Biological Sciences, Committee For Neurosciences, University of Illinois at Chicago, 60680.**

Several subtypes of glutamate receptors have been shown to be involved in various phases of long term potentiation (LTP) in CA1 hippocampal cells. Recently Zorumski et al demonstrated that treatment of hippocampal slices with NMDA (≤ 1 uM) can inhibit LTP formation. One possibility for the mode of action of this low level NMDA is an effect on a metabotropic response. This hypothesis is suggested by the ability of NMDA to inhibit LTP even if it is applied after a high frequency stimulation normally capable of eliciting LTP . To test this hypothesis we attempted to physiologically activate metabotropic glutamate receptors. As a monitor we examined afterhyperpolarizations (AHPs) which normally are depressed with metabotropic receptor activation. We found that high frequency bursts applied to the Schaffer/commissural fiber system significantly depressed AHPs (10.9 ± 1.7 mV before; 10.2 ± 1.7 mV after; p=0.014, n=9). These bursts also induced LTP of the synaptic response to a test stimulus on that pathway (mean change 159% of control, n=9). We also found that subsequent application of 1 uM NMDA to the bathing solution not only inhibited the formation of LTP (mean change 83% of control, n=8), but it blocked the stimulus-induced depression of AHPs (7.9 ± 1.3 mV before; 8.3 ± 1.1 mV after; p=0.164, n=5). These results are consistent with a role of the metabotropic glutamate receptor in LTP formation. They also suggest that inhibition of LTP formation by low levels of NMDA may be mediated through an effect of NMDA on metabotropic responses. Supported by Klingenstein Foundation.

547.4

NOVEL NMDA ANTAGONIST NPC 17742 BLOCKS LONG-TERM POTENTIATION IN DENTATE GYRUS IN ANESTHETIZED RATS. D. Côté, P.A. Hetherington, F. Balcomb and M.L. Shapiro*. Dept. of Psychology, McGill University, Montréal, Québec, Canada, H3A 1B1.

NPC 17742 [2*R*,4*R*,5S-(2-amino-4,5-(1,2-cyclohexyl)-7-phosphonoheptanoic acid)] is a novel, competitive N-methyl-D-aspartate (NMDA) receptor antagonist and is the active isomer of NPC 12626 [2-amino-4,5-(1,2-cyclohexyl)-7-phosphonoheptanoic acid]. Because it crosses the blood-brain barrier, NPC 17742 is a convenient competitive NMDA antagonist for behavioral pharmacology studies of learning and memory. However, little is known about its physiological effects, specifically, its effect on long-term potentiation (LTP) in the hippocampus. The effect of peripherally administered NPC 17742 on the induction of LTP in the dentate gyrus was tested in male Long Evans rats, anesthetized with urethane and α-chloralose. NPC 17742 (i.p. in DMSO) prevented the induction of LTP of the perforant path - dentate gyrus synapses, 90 to 120 minutes after the injection. In some experiments, NPC 17742 decreased the population spike and increased slope of the EPSP, suggesting an increase in inhibition. NPC 17742 also blocked LTP in the hilar ipsilateral longitudinal associational pathway of the dentate gyrus. Furthermore, a block of LTP occurred with as little as 5 mg/kg i.p., given 90 minutes pre-tetanus. LTP was induced 90 to 120 minutes after control rats were injected with DMSO alone. The results of this study demonstrate the efficacy of NPC 17742 for preventing LTP by blocking NMDA receptors. The characteristics of NPC 17742 combined with the results of this study point to the usefulness of NPC 17742 as a tool for studying the involvement of NMDA receptors in learning and memory.

547.5

ON THE INDUCTION OF LTP BY mGluR ACTIVATION

Z.A. Bortolotto, W.W. Anderson* & G.L. Collingridge
Dept. of Pharmacology, Birmingham University, Birmingham, U.K.

In the CA1 region of the hippocampus, transient exposure to (1S,3R)-aminocyclopentane dicarboxylate (ACPD) can induce a slow-onset potentiation which occludes with tetanus-induced long-term potentiation (LTP). This potentiation is unaffected by the N-methyl-D-aspartate (NMDA) antagonist D-2-amino-5-phosphonopentanoate (AP5) but is prevented by kinase inhibitors (staurosporine and K-252b) and thapsigargin (Bortolotto & Collingridge, 1993 Neuropharmacology 32, 1-9).

We now report that ACPD-induced potentiation is not prevented by 50 μM nimodipine (n=3). ACPD induces potentiation of a pure α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor-mediated response (n=3) but not of a pure NMDA receptor-mediated response (n=6) (recorded in the presence of 50 μM picrotoxin and an appropriate excitatory amino acid receptor antagonist).

Low frequency stimulation (1 Hz, for 15 min) delivered immediately after the application of ACPD prevents the development of the slow-onset potentiation (n=5). This inhibition is prevented by the application of 50 μM AP5 (n=4).

(2S, 3S, 4S)-α-(Carboxycyclopropyl)-glycine) (L-CCG-1, 10 μM; n=8), which is potent at activating mGluR2, does not mimick the effects of ACPD.

We conclude that ACPD can induce a component of LTP which involves modification of AMPA receptor-mediated synaptic transmission. This component is slow to develop fully and probably involves the stimulation of phospholipase C-coupled mGluRs.

Supported by CAPES (Brazil) and the MRC

547.6

ACTIVATION OF METABOTROPIC GLUTAMATE RECEPTORS INDUCES LONG-TERM DEPRESSION OF GABAERGIC INHIBITION IN HIPPOCAMPAL CA1 NEURONS.

N.T. Slater*, Y.-B. Liu, J.R. Moyer and J.F. Disterhoft. Depts. of CMS Biology and Physiology, Northwestern Univ. Med. School, Chicago, IL.

The activation of metabotropic glutamate receptors (mGluRs) in hippocampal pyramidal neurons evokes a variety of effects on voltage- and transmitter-gated channels. We have examined the actions of the mGluR agonist 1S,3R-ACPD on CA1 pyramidal neurons in rabbit hippocampal slices *in vitro*. Slices were maintained at 31°C and cells were impaled with sharp microelectrodes for intracellular recording. The bath application of the mGluR agonist 1S,3R-ACPD (5-20 μM) for 20 min produced a reversible depolarization, blockade of spike accommodation and increase of input resistance. Synaptic stimulation of the str. radiatum evoked epileptiform discharges in the presence of 1S,3R-ACPD which only partially reversed 60 min after washout of 1S,3R-ACPD. A long-lasting enhancement of the EPSP was also observed in the presence of the NMDA antagonist D-AP5 (20 μM). This mGluR-induced long-term potentiation of the AMPA-mediated EPSP could result either from a direct enhancement of EPSPs, as recently proposed (Bortolotto & Collingridge, 1993), or to a reduction of the temporally-overlapping $GABA_A$ conductance. Both components of pharmacologically-isolated biphasic IPSPs were irreversibly reduced by 50% by 1S,3R-ACPD, whereas AMPA-mediated EPSPs were not potentiated by 1S,3R-ACPD. The long-term depression of IPSPs by 1S,3R-ACPD was not blocked by the external application of the PKC inhibitor calphostin C (1 μM), the mGluR antagonist L-AP3 (1 mM), or thapsigargin (1 μM). However, following intracellular injection of GTP-γ-S, 1S,3R-ACPD had no effect on the early $GABA_A$-mediated IPSP; the late, $GABA_B$-mediated component was directly blocked by GTP-γ-S injection (Andrade et al., 1986). These results demonstrate an important component of the long-term potentiation of synaptic excitability produced by activation of mGluRs may result from a G protein-mediated depression of GABAergic inhibition.

547.7

A DEVELOPMENTAL SWITCH OCCURS IN THE LONG-TERM EFFECTS OF METABOTROPIC GLUTAMATE RECEPTOR ACTIVATION IN RAT HIPPOCAMPUS.

B.L. Trommer*, P.A. Colley, J.F. Pasternak, J.J. Kennelly and N.T. Slater. Departments of Pediatrics and Neurology, Evanston Hospital, Evanston, Illinois, and Physiology, Northwestern University Medical School, Chicago, Illinois.

Activation of metabotropic glutamate receptors (mGluRs) has recently been proposed to play a role in long-term potentiation (LTP) in the hippocampus and long-term depression (LTD) in the cerebellum. While mGluR-activated intracellular messenger systems display profound developmental changes, little is known of the physiologic correlates of these changes. Population spikes (PS) were recorded extracellularly in the CA1 and dentate gyrus (DG) cell body layers in response to electrical stimulation of the Schaffer collateral or medial perforant path in rat hippocampal slices. The 1S,3R-isomer of the mGluR agonist ACPD (10 μM) was bath applied for 20 min, and the effects on PS amplitude studied for a further 60 min after the washout of the drug. In the CA1, the effects of ACPD were age-dependent: in young rats (P10-15) ACPD produced a rapid depression of PS amplitude which did not reverse upon washout of the drug (20% of control), whereas in older animals (P29-33), a lasting potentiation was produced (156% of control). This switch in the actions of ACPD occurred between P16 and P24. Subsequent induction of LTP by tetanic stimulation could be achieved at all ages, with the degree of potentiation being greatest in the intermediate age group. In the DG, LTD (68% of control) was induced by ACPD in young animals, but in adults only a reversible reduction of PS amplitude was observed. These results demonstrate that the effects of mGluR activation in hippocampus are both age-dependent and region-specific: mGluR activation evokes LTD in immature DG and CA1, and LTP in adult CA1.

547.8

LATERAL PERFORANT PATH-CA3 LTP IS MU OPIOID RECEPTOR-DEPENDENT, NMDA RECEPTOR-INDEPENDENT AND LONG-LASTING

B. E. Derrick, E. J. Barea-Rodriguez and J. L. Martinez, Jr.*
Department of Psychology, The University of California, Berkeley, CA 94720.

Previously we reported that LTP of lateral perforant path-CA3 responses is naloxone-sensitive and displays onset kinetics similar to LTP observed at the mossy fiber-CA3 synapse (SNA 18: 1497, 1992). We investigated the receptors involved in perforant path-CA3 LTP in anesthetized rats and the development of perforant path LTP in awake rats. Lateral and medial perforant path-CA3 responses evoked selectively by stimulation of the dorsomedial and ventrolateral aspects of the angular bundle preceded the onset of dentate field EPSPs, phase reversed in the CA3 pyramidal layer, and followed brief trains of 100 Hz, and thus were monosynaptic and locally generated in area CA3. The non-competitive NMDA receptor antagonist (+)-CPP (1 and 10 nmol) blocked LTP of the field EPSP slope in medial perforant path responses (9/10 animals), but was without effect on lateral perforant path LTP (8/8 animals). Lateral perforant path responses were, however, blocked by the mu opioid receptor antagonist CTOP (3 nmol). The time course of LTP in these pathways was investigated in rats with implanted electrodes. Following 5-7 days of stable baseline responses and a single session of high-frequency stimulation (five 100-500 msec trains at 400 Hz), LTP of medial perforant path slopes decayed to baseline amplitudes by 2-3 days. By contrast, LTP of lateral perforant path slopes decayed initially over the first 5 days but remained at potentiated levels (> 20% increase) for over 10 days. Measures of EPSP slope were uncorrelated with activity. Thus lateral perforant path-CA3 is opioid receptor-dependent and NMDA receptor-independent. Importantly, opioid receptor-dependent LTP at lateral perforant path-CA3 synapses is relatively long-lasting as compared to the NMDA antagonist-sensitive LTP observed at medial perforant path synapses.

Supported by DA 04195 and the Rennie Fund of the University of California.

547.9

NMDA RECEPTOR ACTIVATION IS NOT ESSENTIAL FOR THE INDUCTION OF LTP AT THE LATERAL PERFORANT PATH-DENTATE GYRUS SYNAPSE. *J.A. Reyes*, B.E. Derrick and J.L. Martinez, Jr.* Dept. of Psychology, University of California, Berkeley, CA 94720.

Although LTP at lateral perforant path-dentate gyrus synapse is naloxone-sensitive, the sensitivity of LTP in this pathway to NMDA receptor antagonists is unclear. We investigated the role of NMDA receptors in LTP of perforant path-dentate gyrus responses evoked by selective stimulation of the dorsomedial or ventrolateral aspects of the angular bundle. Following high-frequency stimulation (400 Hz 100 msec X 5) medial perforant path-dentate EPSP slopes reached maximal amplitude immediately and remained stable over a 1-hr period, while lateral perforant path-dentate LTP increased in amplitude over a 1-hr period. This latter pattern is characteristic of LTP development at synapses sensitive to opioid receptor antagonists, such as lateral perforant path- and mossy fiber-CA3 synapses. Local application of (+)-CPP (10 nmol) to the dentate molecular layer blocked both medial and lateral perforant path LTP measured 20 min post tetanus. However, the blockade of lateral, but not medial, perforant path LTP could be reversed by increasing the stimulation current intensity. Systemic administration of the competitive NMDA receptor antagonist (+)-CPP (10 mg/kg i.p.) blocked completely LTP of medial, but not lateral, perforant path response induced with high-intensity stimulation. These data suggest that activation of NMDA receptors is essential for the induction of medial, but not lateral, perforant path-dentate LTP. We suggest NMDA receptors may modulate induction of opioid receptor-dependent LTP at the lateral perforant path-dentate synapse. This is in contrast to LTP at lateral perforant path- and mossy fiber-CA3 synapses, which is unaffected by NMDA receptor antagonists.

Supported by DA04195 and the Rennie Fund of the University of California.

547.10

ROLE OF NMDA RECEPTORS AND VOLTAGE DEPENDENT Ca^{2+} CHANNELS (VDCC) IN TEA INDUCED SYNAPTIC ENHANCEMENT . K.M. Huber*, M.D. Mauk, and P.T. Kelly. Dept. of Neurobiology and Anatomy, Univ. of Texas-Houston Medical. School., TX 77225.

Our analyses of tetraethylammonium (TEA) induced synaptic enhancement (potentiation) in isolated hippocampal CA1 region has demonstrated two routes of Ca^{2+} entry into postsynaptic neurons which contribute to synaptic potentiation. In contrast to previous findings (Aniksztejn & Ben-Ari, Nature 349,67-69; Huang & Malenka, J. Neurosci. 13(2), 568-76), we observed that TEA potentiation was attenuated, but not blocked by the VDCC antagonist nifedipine (20 ± 8%; n = 8) compared to controls (41 ± 8%; n = 9). Moreover, TEA potentiation was strictly dependent on NMDA receptor activation. TEA application in D-APV resulted in a slight depression of synaptic transmission (-15 ± 8%). A second TEA application to the same slices in the absence of D-APV induced robust potentiation (62 ± 7%; n = 10). TEA potentiation was also blocked by the noncompetitive NMDA antagonist MK-801 (1 ± 6%; n = 9). One hallmark of NMDA-dependent LTP is its synapse specificity. The NMDA dependence of TEA potentiation predicts that its induction should require presynaptic stimulation with concurrent postsynaptic depolarization and display synapse specificity. When stimulation was stopped 5 minutes before and resumed 20-30 min after TEA applications, transmission did not potentiate (-8 ± 4%, n = 5). TEA potentiation was also synapse specific. In two pathway experiments, we observed long-lasting potentiation only in the pathway that was stimulated during TEA application (32 ± 5%, n = 8), while unstimulated pathways displayed short-lasting decremental potentiation (6 ± 2%; n = 8). These results are consistent with the dependence of TEA potentiation on NMDA receptor activation. We also tested if TEA potentiation, like tetanus induced LTP, used similar Ca^{2+} -dependent enzymes as such as protein kinases. Incubation of slices in the broad range and membrane permeable kinase inhibitor K-252a before and during TEA applications blocked potentiation (-15 ± 7%; n = 10). These results indicate that TEA induced potentiation and LTP have many similarities including their dependence on NMDA receptor activation, protein kinase activity, and synapse specificity.

In contrast to results in isolated CA1 regions, TEA potentiation in slices with intact CA3-CA1 connections was only partially reduced by D, L- APV (35 ± 9%, n = 9) compared to control potentiation (68 ± 14%, n = 11). Moreover, nifedipine reduced, but did not block TEA potentiation in intact slices (43.4 ± 9%, n = 7). Therefore, in intact slices, Ca^{2+} influx via VDCCs or NMDA receptors can contribute to TEA induced synaptic enhancement.

547.11

SEROTONIN SUPPRESSES LTP IN CA1 INDUCED BY THETA BURST STIMULATION. N. Otaky[1] and U. Staubli*[2]. [1]Dept. Psychology, McGill University, Montreal, PQ H3A 1B1; [2]Center for Neural Science, New York University, New York, N.Y. 10003.

Much evidence has accumulated concerning the effects of NMDA receptor antagonists on learning, supporting the hypothesis that LTP is the neurobiological variable involved in information encoding. Drugs acting on serotonin (5-HT) receptors may also provide a means for testing the link between LTP and memory. It has been reported that agonists of the 5-HT receptor cause hyperpolarization of hippocampal neurons, possibly via an action on the potassium channels utilized by the $GABA_B$ receptor. There is also evidence that 5-HT increases the excitability of GABA interneurons. This predicts that serotonin could increase the hyperpolarization present during the early stages of theta burst stimulation, which in turn would reduce the NMDA currents elicited by the early bursts. A recent report has confirmed that 5-HT is effective in blocking LTP induced by a single primed burst (Corradetti et al., 1992). Studies in our laboratory indicate that 5-HT substantially decreases the degree to which a theta burst response is increased by a prior burst given 200 msec earlier. It is well established that much of this between burst facilitation reflects the contribution of currents mediated by the NMDA receptor. Subsequent experiments conducted in the presence of APV confirm the interaction between 5-HT and the NMDA receptor mediated component of the burst response. The concentration range over which 5-HT is effective in this regard does not detectably affect dendritic field responses but reduces the magnitude of LTP produced by successive pairs of theta bursts. From these observations serotonin receptor agonists would be expected to impair learning. Considerable evidence in the literature suggests that this is the case.

547.12

NONHYDROLYZABLE ANALOGS OF ATP INHIBIT INDUCTION OF LONG-TERM POTENTIATION (LTP) IN MOUSE HIPPOCAMPAL SLICES.

A. Wieraszko*. CSI/IBR Center for Dev. Neurosci., Dept. of Biol., CUNY at Staten Island, N.Y. 10301

We have previously reported that ATP is released from electrically stimulated hippocampal slices (Wieraszko et al. Brain Res.,485: 244,1988), and that exogenous ATP was able to induce Long Term Potentiation (LTP) in hippocampal synapses (Wieraszko and Seyfried, Brain Res., 491: 356,1989). One of the possible mechanisms of ATP-induced LTP could be an interaction of ATP with purinergic (P2) receptors. To evaluate this assumption the influence of nonhydrolyzable antagonists of P2 receptors on LTP induced by high frequency stimulation (HFS) was investigated in mouse hippocampal slices. Each of the antagonist tested was added to the slice chamber 30 min prior of HFS. 5'adenylylimidodiphosphate (AMP-PNP), a slowly hydrolyzable analog of ATP completely blocked induction of LTP at 37.8 μM, but was ineffective at 4.7 μM. Cicarbon Blue, an antagonist of P2z receptor, while not effective at 10 μM, blocked induction of LTP at 40 μM. A potent antagonist of P2y receptors, 2-methylthio-ATP inhibited LTP at the concentration above 7.0 μM. ATP-γ-S, a slowly hydrolyzable analog of ATP blocked induction of LTP at concentrations above 35.0 μM.α,β-methyl ATP, an ATP receptor antagonist of P2x receptors was unable to block LTP even at 40.0 μM. The pattern of LTP inhibition, exerted by ATP receptor antagonists was similar. While all of them blocked LTP, they were unable to block short lasting (2-5 min) increase of the potential immediately after HFS. These results suggest that P2 receptors, while not involved in triggering of short-lasting facilitation of synaptic transmission, play animportant role in LTP. The research is in the progress to identify the subtypes of P2 receptors participating in LTP. Supported by NIH grant # NS27866 and PSC-CUNY grant #662171.

547.13

P TYPE Ca CHANNEL BLOCKER ω-AgaIVA BLOCKS INDUCTION OF LONG-TERM POTENTIATION (LTP) AND REDUCES DEPOLARIZATION INDUCED Ca RISE IN HIPPOCAMPAL CA1 PYRAMIDAL NEURONS. H. Kato, K.I. Ito, H. Miyakawa.* Dept. Physiol., Yamagata Univ. Sch. Med., Yamagata 990-23, JAPAN.

Although it has been shown that LTP in CA1 hippocampal pyramidal cells requires Ca elevation and can be block by APV, a NMDA receptor antagonists, source of Ca is still a matter of dispute. Ca imaging studies revealed that Ca entry through voltage-gated Ca channel is dominant over entry through NMDA receptor channel in synaptically induced Ca rise(Miyakawa et.al., 1992), and that even APV sensitive component of Ca rise could be due to entry through voltage-gated Ca channels(Regehr & Tank, 1992). Here we report effects of synthesized ω-AgaIVA, a P type Ca channel blocker, on LTP induction and Ca accumulation, using extracellular recordings and Ca imaging technique in guinea-pig hippocampal brain slice preparation.

ω-AgaIVA (60nM) did not block Schaffer input induced EPSPs. With the presence of ω-AgaIVA, tetanic activation of Schaffer inputs(100Hz, 1sec) did not result in increase in field EPSP amplitude recorded at str. radiatum in CA1 field, while it induced increse in population spike amplitude. Nifedipine (50μM), L type Ca channel blocker, failed to block LTP. To measure transient rise in Ca related to depolarization induced spikes, we injected Fura-2, Ca indicator dye, to a single Pyramidal neuron and measured fluorescence intensity simultaneously with membrane potential. Spike related Ca rise (Jaffe et.al.,1992) was smaller with the presence of ω-AgaVIA (20% reduction) at the soma, proximal apical dendrites and basal dendrites. Nifedipine also reduced spike related Ca rise by 20%, but the reduction was restricted to the region closer to the soma.

This study strongly suggest that Ca entry through P type Ca channels plays crucial roles in inducing LTP in hippocampal CA1 pyramidal neurons.

547.14

Angiotensin II and losartan modulation of posttetanic and long-term potentiation. D. L. Armstrong*, E. Garcia, B. Quinones, M. Villanueva, and M. J. Wayner. Division of Life Sciences, The University of Texas at San Antonio, San Antonio, Texas 78249-0662.

The results of these experiments demonstrate that angiotensin II (AII) blockade of long-term potentiation in hippocampal slices can be inhibited by losartan application to the slice perfusion fluid. Previous *in vivo* experiments have shown that losartan, an AT_1 receptor antagonist, inhibits AII actions in the hippocampus when administered peripherally, or when injected intrahippocampally in combination with the peptide. Transverse hippocampal slices were prepared using male, Sprague-Dawley rats and, after a 1-2 hr incubation period, slices were transferred as needed to a constant perfusion recording chamber. Population spikes were extracellularly recorded from the stratum granulosum following application of 0.1 ms duration stimulation pulses to the perforant path. The amplitude from the initial positive wave of the spike to peak negativity was measured once a minute for a 30 min baseline period. One second tetanic stimulation trains (100 Hz) were used to induce LTP. Under control conditions, a 30 to 40% increase in spike amplitude was observed in 7 out of 10 slices. In the presence of 487 nM AII, both LTP and posttetanic (PTP) responses were blocked in all slices tested (n=8). Complete blockade of LTP, but not PTP, was observed with 47.8 nM AII (n=5), while 4.78 nM AII had minimal effect on LTP (n=4). When 2.48 μM losartan preceded the AII application, the effects of the peptide were eliminated (n=6). In experiments where only losartan was applied a 60% increase in PTP amplitude was observed. This interesting effect indicates that losartan could be acting at presynaptic sites on perforant path terminals.

547.15

Antidromically-Conditioned E-S Potentiation is not Blocked by $GABA_A$ Inhibitors. Lee W. Campbell*, Jennifer M. Jester, Terrence J. Sejnowski. Computational Neurobiology Laboratory, Salk Institute, La Jolla, CA

Long-term potentiation (LTP) of the population spike in excess of that predicted by increase in the slope of the field excitatory post-synaptic potential (EPSP) has been described as a component of theta-burst LTP (Exp Brain Res 79:633-641). An increase in the ratio of excitation to inhibition, a reduction of tonic inhibition, and a decrease in spike threshold are thought to contribute to this EPSP-to-spike (E-S) potentiation. Associative LTP using antidromic stimulation as the conditioning stimulus produces a stable increase in the population spike amplitude, but no change in the slope of the EPSP (Soc Neuro Abstr 17:533.15). Do changes in tonic or synaptic inhibition underlie the associative form of E-S potentiation as they are thought to do in theta-burst E-S potentiation?

The EPSP and population spike were recorded from the CA1 layer of rat hippocampal slices. The antidromic conditioning stimulus was 50 bursts of 5 pulses at 100 Hz with an interburst interval of 200 ms delivered to the alveus. The Schaffer-collateral pathway was stimulated once per burst. In some slices the $GABA_A$ blockers picrotoxin (10 μM) and/or bicuculline (10 μM) were added to the bath prior to testing. When paired together, the antidromic and orthodromic stimuli produced a potentiation of the population spike, even in the presence of the $GABA_A$ blockers (138% ± 16.7; mean ± s.e.m). In contrast, the E-S potentiation component of theta-burst LTP is blocked by $GABA_A$ inhibitors.

Intracellular recordings from the CA1 layer reveal an increase in excitability following associative E-S potentiation. Paired t-tests suggest that a depolarization of the resting membrane potential (RMP) accounts for the increased excitability (ctrl -63.7 ± 0.326 mV, 15 min. post tetanus -61.2 mV ± 0.438; mean RMP ± s.e.m, $P = 0.04$, $F_1 = 9.08$). Spike threshold, input resistance, and time constant of the membrane were not effected by associative E-S potentiation.

547.16

DEHYDROEPIANDROSTERONE SULFATE AFFECTS LTP IN AREA CA1 OF THE RAT HIPPOCAMPUS. J.H. Meyer*, G. Wittenberg, R.D. Randall and D.L. Gruol. Dept. of Neuropharmacology and Alcohol Research Center, The Scripps Research Institute, La Jolla CA. 92037.

Neurosteroids such as dehydroepiandrosterone sulfate (DHEAS) affect both $GABA_A$-mediated IPSPs as well as excitatory neurotransmission. We have explored whether DHEAS can influence paired-pulse facilitation and long-term potentiation (LTP) in a rat hippocampal slice preparation.

Hippocampal slices from Sprague-Dawley rats were placed in an interface type slice chamber and perfused with artificial cerebrospinal fluid (ACSF). Extracellular synaptic field potentials in response to Schaffer collateral stimulation were recorded from the CA1 stratum radiatum. Paired pulse stimulation (inter-pulse interval 20-200 ms) was used during baseline recordings. Slices were then perfused with either ACSF (control slices) or 10 μM DHEAS (experimental slices) and paired pulse responses again obtained after a 1/2 hour period. Changes in synaptic responses of control slices were compared to changes noted in DHEAS-exposed slices. Subsequently, single pulse responses were recorded at 1 minute intervals in each slice both before and after induction of LTP. LTP responses noted in control slices were then compared to LTP responses seen in DHEAS-exposed slices. Analysis of the paired pulse responses indicated that DHEAS had no effect upon facilitation at any inter-pulse interval, arguing against any pre-synpatic effect of the steroid. DHEAS, however, did significantly augment the magnitude of LTP induced in area CA1. Such results may account for behavioral effects of DHEAS in which this neurosteroid has been noted to enhance memory (Soc. Neurosci Abstr 18; 1160). Supported by AA6420 and AA0756.

547.17

DENDRITIC LOCALIZATION OF LTP AND LTD

C.M. Coussens * & T.J. Teyler, Neurobiology, NE Ohio Col. of Med., Rootstown, OH 44272.

This experiment analyzed the distribution of LTP and LTD in dendrites of CA1 hippocampus. A "rake" stimulating electrode was placed orthogonal to the Schaffer collaterals and a recording electrode was placed in the cell body layer. Baseline recordings were obtained from each of the 6 stimulating electrodes spanning stratum radiatum/ stratum lacunosum-moleculare (every 100μm). Then, a 50 Hz tetanus was delivered to a mid-point electrode - ~300μm from the fissure. At 20 min post-tetanus, LTP was seen at the tetanus site and at distal synapses, whereas LTP toward the cell body was rarely noted. LTD was seen in 4 out of 10 slices at sites adjacent to the boundary of potentiated synapses.

When depotentiating stimulation (3-5 Hz for 900 pulses) was given to the previously tetanized site, depotentiation was observed there and, to a lesser degree, at adjacent previously potentiated synapses. These results show that LTP is distributed over a large extent of the dendrite, but depotentiation is a more focal phenomenon. These results may reflect the anatomy of the afferents activated, diffusion of second messengers, or activation of voltage dependent processes along the dendrite.

Supported by NINDS #28698

547.18

THE EFFECTS OF A NITRIC OXIDE SYNTHASE INHIBITOR ON LONG TERM POTENTIATION IN AREA CA1 OF RAT HIPPOCAMPUS S. M. Nicola, J. A. Cummings and R. C. Malenka* Neuroscience Graduate Program, University of California, San Francisco, CA 94143

The search for a retrograde messenger thought to be necessary for LTP has focused recently on nitric oxide (NO). To test the hypothesis that NO production is required for LTP, we attempted to obtain LTP in area CA1 of hippocampal slices that had been incubated in the NO synthase inhibitor N^{ω}-nitro-L-arginine (NOArg, 100–500 μM) for 1.5–11 hr at room temperature (21–23°C). The LTP induction protocol, given at 27–29°C, consisted of two 100 Hz, 1 s tetani at increased stimulus intensity. No significant reduction in LTP magnitude was observed in NOArg-treated (n=14) *vs* control (n=11) slices (LTP at 50–60 min post-tetanus: control=162±16%, NOArg=135±14%, P>.2). The study was then repeated in a blind manner, such that the experimenters did not know whether slices had been incubated in NOArg (100 μM) until after data analysis was complete. The induction protocol used in the blind study (four 100 Hz, 1 s tetani at control stimulus intensity, temperature 25–29°C, slices from 14–18 day old rats) resulted in LTP that was identical in magnitude in both control (n=11) and NOArg-treated (n=12) slices (control LTP=149±9%, NOArg LTP=148±12%, P>.5). To assess whether the NOArg was effective in permeating cells and inhibiting NO synthase, we incubated slices from 14 or 15 day old rats blindly in 100 μM NOArg or in control medium exactly as previously, applied 100 μM NMDA for 10 min at 27–29°C, and determined the level of cGMP per mg protein. Consistent with others' findings, NOArg completely blocked the NMDA-stimulated increase in cGMP observed in control slices (n=3). Our results suggest that NO synthase activity is not required for the induction of LTP under these experimental conditions.

547.19

THE SIGN OF LONG-LASTING EAP MODIFICATIONS IN THE DENTATE GYRUS IS PREDICTED BY THE SEQUENCE OF ISO-CCK APPLICATION TO THE RAT HIPPOCAMPAL SLICE. D.Dahl*, J. Li, and T. Anderson. The University of Texas at Dallas, Richardson, TX 75083.

Cholecystokinin (CCK) and baclofen (BAC, a γ-aminobutyric $acid_B$ [$GABA_B$] agonist) similarly potentiate paired-pulse facilitation in the dentate gyrus (DG), suggesting the involvement of a common substrate. Given the synergy of BAC-isoproterenol (ISO-a β-adrenergic agonist) in induction of long-lasting potentiation in the DG, CCK-ISO modulation of neuronal responses may also be expected.

In the hippocampal slice preparation, application of 75 nM ISO for 15-30 min induced a potentiation of EAPs that reversed to baseline upon a 30 min wash with ISO-free artificial cerebrospinal fluid (ACSF). However, when the wash was followed by a 15 min application of 1 μM CCK, potentiation of the EAP reoccurred that persisted through a second 30 min of wash with drug-free ACSF.

When 1 μM CCK was added after 15 min of ISO, such that there was a concurrent application of ISO-CCK, then the ISO-induced potentiation reversed to a long-lasting depression that persisted through 30 min of wash with drug-free ACSF.

Concurrent application of ISO and a β-adrenergic antagonist (1 μM propranolol or 1 μM timolol) or the *N*-methyl-D-aspartate antagonist APV (10 μM) blocked the reversible ISO-induced potentiation and also the postwash potentiation associated with CCK. The depression associated with concurrent ISO-CCK was also blocked by application of the antagonists.

These results suggest an interaction between β-adrenergic receptors and CCK in the modification of DG granule cell responses.

(Supported by a grant from the Whitehall Foundation to D.D.)

547.20

THE INVOLVEMENT OF α-TOCOPHEROL IN LONG-TERM POTENTIATION. Z. Xie* and B. R. Sastry, Neuroscience Research Laboratory, Dept. of Pharmacology and Therapeutics, The University of British Columbia, Vancouver, B. C., Canada, V6T 1Z3.

An increase in lipid peroxide content in hippocampus has been implicated in the impairment of learning ability in vitamin E-deficient rats. In the present studies, the ability of α-tocopherol phosphate (0.2 mM, applied for 5 min), a major antioxidant, to induce long-term potentiation (LTP) of the stratum radiatum stimulation-induced excitatory postsynaptic potentials (EPSPs) in CA1 neurons in guinea pig hippocampal slices was examined. α-Tocopherol induced a slowly developing LTP of the EPSP without changing the membrane potential and the input resistance (n=16), or the fast and the slow inhibitory postsynaptic potentials (IPSPs) (n=8). α-Tocopherol failed to induce a further potentiation of the EPSP during a pre-established tetanus-induced LTP (n=10). The chelation of postsynaptic Ca^{2+} with BAPTA (n=6) or the inhibition of protein kinase C (PKC) by sphingosine (n=6) or K-252b (n=6) prevented the α-tocopherol-induced LTP. AP3 (100 μM, n=8), but not APV (40 μM, n=6), blocked the α-tocopherol-induced LTP. α-Tocopherol also did not significantly alter the depolarization of CA1 neurons induced by glutamate (applied in normal medium containing APV, n=6) or by NMDA (applied in Mg^{2+}-free medium containing CNQX, n=6). Tetanic stimulation of the stratum radiatum failed to induce LTP of the EPSP in hippocampal slices of vitamin E-deficient rats (n=10); application of α-tocopherol also did not induce LTP in these slices (n=6).

These results indicate that vitamin E is involved in LTP in the hippocampal CA1 neurons. The activation of the metabotropic glutamate receptors, postsynaptic Ca^{2+} and the activation of PKC may be required for this LTP. It appears that in vitamin E-deficient rats LTP-induction is compromised not only because α-tocopherol is depleted but also since something else is adversely affected.

548.1

MOLECULAR CHARACTERIZATION OF CALCIUM CHANNEL SUBTYPES EXPRESSED IN *APLYSIA* NEURONS. B. H. White* and L. K. Kaczmarek, Yale University School of Medicine, Dept. of Pharmacology, New Haven, CT 06510.

To study the localization and regulation of neuronal calcium channels in the sea hare *Aplysia californica* we have begun to develop molecular probes that will recognize them. As a first step we have used reverse transcription coupled to the polymerase chain reaction (RT-PCR) to amplify partial sequence of the Ca^{++}-channel α_1-subunits expressed in *Aplysia* head ganglia, abdominal ganglion, and bag cell neurons. Primer sets targeting the regions IS6 to IIS5 and IVS1 to IVS6 amplify multiple Ca^{++}-channel fragments from *Aplysia* ganglia and bag cell neuron RNA. We have focussed our attention on two bag cell neuron Ca^{++}-channel fragments spanning the region from IS6 to IIS5 (BCCa-I and BCCa-II). These fragments are 50% identical at the amino acid level and appear to be related to distinct subtypes of mammalian neuronal Ca^{++}-channels. The BCCa-I sequence is 65% identical to an N-type α_1-subunit from rabbit brain (BIII, Fujita et al. (1993) *Neuron* 10, 585-598) over the IS6-IIS5 region, while the BCCa-II sequence is 66% identical to an L-type rat brain Ca^{++}-channel found predominantly in neuroendocrine tissues (RBα_1, Hui et al. (1991) *Neuron* 7, 35-44 and Chin et al. (1992) *FEBS Lett.* 299, 69-74). Fusion constructs containing the nonconserved I-II linker regions of BCCa-I and BCCa-II have been prepared for the generation of subtype-specific antibodies.

548.2

CALCIUM CHANNEL β_3 SUBUNIT SPLICE VARIANT ALTERS INACTIVATION KINETICS OF α_{1B} CALCIUM CHANNELS. E.C. Johnson, P.F. Brust, A.F. McCue, L.E. Chavez-Noriega*, S.B. Ellis, and M.M. Harpold, SIBIA, Inc. La Jolla, CA 92037.

HEK293 cells transiently expressing the $\alpha_{1B\text{-}1}\alpha_{2b}\beta_{1\text{-}2}$ recombinant calcium channel yield ω-CgTx GVIA sensitive, N-type calcium currents (Williams et al. 1992, Science 257:389-395). The deduced $\beta_{1\text{-}3}$ subunit amino acid sequence is identical to that of $\beta_{1\text{-}2}$ for 444 amino acids, they diverge for 34 amino acids and $\beta_{1\text{-}3}$ extends an additional 120 amino acids. We compared the properties of the whole cell currents of HEK cells expressing the $\alpha_{1B\text{-}1}\alpha_{2b}\beta_{1\text{-}2}$ and $\alpha_{1B\text{-}1}\alpha_{2b}\beta_{1\text{-}3}$ calcium channels. The current voltage curves from each were essentially identical, and peaked at 10-20 mV. Both have similar sensitivities to holding potential, with half maximal inactivation at approximately -57 mV. Both have similar voltage dependence of activation (measure from tail currents) with half maximal activation at approximately 5 mV. Both have similar kinetics of activation; the slowest kinetics, approx. 3 ms, occurs at approx. 0 mV. The tail currents in both could be well fit by a single exponential and had essentially identical voltage dependence (0.1 ms @-100 mV to ~0.57 ms @-30 mV). In contrast, the voltage dependence of the kinetics of inactivation is significantly different at all voltages examined. At a test pulse from -90 mV to 0, +10, +20 or +30 mV the single exponential fit of the inactivation was 85, 96, 135, and 180 ms respectively for $\alpha_{1B\text{-}1}\alpha_{2b}\beta_{1\text{-}2}$ and 202, 185, 268, 354 ms respectively for $\alpha_{1B\text{-}1}\alpha_{2b}\beta_{1\text{-}3}$. Not only is the inactivation substantially slower with the $\beta_{1\text{-}3}$ subunit ($p<0.008$ @ +10 mV) but the standard deviation is up to 4.6 times larger in the $\beta_{1\text{-}3}$ calcium channel. These differences in the voltage dependence and variability of inactivation might be due to phosphorylation differences between $\beta_{1\text{-}2}$ and $\beta_{1\text{-}3}$.

548.3

THE α_2 AND β SUBUNITS OF THE ω-CgTx GVIA RECEPTOR ARE REQUIRED FOR EFFICIENT CELL SURFACE EXPRESSION AND AFFECT THE ω-CgTx BINDING AFFINITY. P.F. Brust, S. Simerson, S. Schoonmaker, C. Deal, G. Velicelebi, E.C. Johnson* and S.B. Ellis, SIBIA, Inc., La Jolla, CA 92037.

The α_2 subunit of high-voltage-activated calcium channels has been shown to potentiate the current magnitude without a discernable change in the electrophysiological properties of the channel. In contrast, the β subunit potentiates the current magnitude with noticeable effects on both the current activation and inactivation kinetics. Previously, we showed that the $\alpha_{1B\text{-}1}\alpha_{2b}\beta_{1\text{-}2}$ subunit combination formed an ω-CgTx GVIA sensitive, N-type calcium channel when equimolar concentrations of each expression plasmid were transiently coexpressed in HEK293 cells. We have subsequently altered the molar ratios of $\alpha_{1B\text{-}1}$, α_{2b} and $\beta_{1\text{-}2}$ plasmids to achieve equivalent mRNA levels. The effects of the altered plasmid ratios were assessed by ω-CgTx GVIA binding and electrophysiological analyses. Coexpression of the three subunits with the altered ratios resulted in 45,000 receptors/cell, whereas coexpression of the $\alpha_{1B\text{-}1}$ and $\beta_{1\text{-}2}$ subunits, the $\alpha_{1B\text{-}1}$ and α_{2b}, or the $\alpha_{1B\text{-}1}$ subunit alone resulted in significantly fewer receptors per cell, 5750, 2250 and 800 sites/cell, respectively. The difference in the current density observed between the $\alpha_{1B\text{-}1}\alpha_{2b}\beta_{1\text{-}2}$ combination was 94 pA/pF compared to 5.6 pA/pF for the $\alpha_{1B\text{-}1}\beta_{1\text{-}2}$ combination. These results suggest that coexpression of the $\alpha_{1B\text{-}1}$, α_{2b} and $\beta_{1\text{-}2}$ subunits is required for efficient expression of the channel complex on the cell surface. The determined K_d value of each receptor combination for ω-CgTx GVIA also differed, 55 pM, 18 pM, 85 pM and 32 pM for the $\alpha_{1B\text{-}1}\alpha_{2b}\beta_{1\text{-}2}$, $\alpha_{1B\text{-}1}\beta_{1\text{-}2}$, $\alpha_{1B\text{-}1}\alpha_{2b}$ and $\alpha_{1B\text{-}1}$ combinations, respectively. These results demonstrate that both the α_{2b} and $\beta_{1\text{-}2}$ subunits affect the binding characteristics of ω-CgTx GVIA.

548.4

PHARMACOLOGICAL PROPERTIES OF THE N-TYPE VSCC IN TRANSFECTED HEK 293 CELLS. D. Bleakman*, P.F. Brust, D. Bowman, C.J. Grantham, S.B. Ellis, R.J. Miller, & M.M. Harpold, Lilly Research Centre Ltd., Windlesham, Surrey, U.K. and SIBIA Inc. La Jolla, CA 92037. U.S.A.

Recently, Williams et al., (Science 1992, 257,389-395), cloned and transfected an N-type Ca^{2+} channel from human brain into a human kidney cell line (HEK 293). We have investigated the properties of this channel using combined whole cell voltage clamp electrophysiology and fura-2 based microfluorimetry. Cells were depolarized (Vh=-80mV to Vt=+10mV) and an increase in the $[Ca^{2+}]i$ was observed concurrent with a rapidly activating, slowly inactivating current (range 150pA to 2 nA). Ca^{2+} currents were inhibited reversibly by Cd^{2+} (1μM) and irreversibly by w-CgTxGVIA (5μM). Ca^{2+} influx was not observed in untransfected HEK 293 cells. w-CgTx-GVIA produced concentration- and time- dependent inhibition of Ca^{2+} influx into transfected HEK 293 cells. The threshold for this effect was 1nM and complete inhibition of influx was observed at 1μM. In contrast, the P-type Ca^{2+} channel blocker w-Aga IVA (300nM) had no effect on evoked Ca^{2+} influx. Flunarizine (1μM), a mixed function Ca^{2+} channel antagonist, inhibited Ca^{2+} uptake in these cells. However, the L-type calcium channel antagonists, nifedipine (1μM), verapamil (1μM) and diltiazem (1μM) were inactive. In conclusion, the transfected Ca^{2+} channel has characteristics of an N-type Ca^{2+} channel.

548.5

THE INVOLVEMENT OF N, P AND L-TYPE CALCIUM CHANNELS IN NEUROTRANSMITTER RELEASE INDUCED BY DIFFERENT DEPOLARIZATION STRENGTHS. I.A. Pullar*, J. Harvey, S. Wedley and J.D. Findlay, Lilly Research Centre Ltd., Eli Lilly and Company, Windlesham, Surrey, U.K.

Keith et al. (Biochem. Pharmacol. 1993, 45, 165-171) showed that the block of noradrenaline release by the N-type Ca^{2+} channel antagonist, w-conotoxin GVIA (CgTx), depended on the degree of depolarization. We have investigated the Ca^{2+} channels involved in the release of acetylcholine (ACh) and 5-HT from rat cortical slices induced by 30 and 50mM K^+. Slices (350μm), prelabelled with ^{3}H-choline or ^{3}H-5-HT, were exposed to the antagonists for 15min prior to depolarization with K^+ for 4min. CgTx (0.3μM), which produced a maximal (c.50%) block of ACh and 5-HT release induced by 30mM K^+, was less effective (max. 20%) at blocking 50mM K^+ stimulated release. The block produced by the P-channel antagonist, w-Aga IVA, (0.3μM), however, was independent of stimulus strength (10% ACh, 40% 5-HT). The L-type Ca^{2+} channel antagonist, nifedipine (1.0μM), was ineffective at blocking ACh or 5-HT release. The block of 5-HT release obtained with the two toxins was additive whereas with ACh synergism was observed. The results indicate that, when depolarized with 30mM K^+, the Ca^{2+} required for 5-HT release enters through both the P and N channels whereas with ACh the P channel is not involved unless the N channels have been previously blocked. At 50mM K^+ the P and N channels only account for 50-60% of release. L channels are not involved in the release of either of the transmitters.

548.6

FATTY ACYLATION OF A PUTATIVE PRESYNAPTIC CALCIUM CHANNEL SUBUNIT FROM TORPEDO. C.B. Gundersen*, A. Mastrogiacomo and J.A. Umbach, Dept. Pharmacology and Jerry Lewis Neuromuscular Research Center, UCLA School of Medicine, Los Angeles, CA 90024.

Recent investigations revealed that the candidate presynaptic calcium channel subunit ($CCCS_1$) of Torpedo is post-translationally modified (Soc. Neurosci. Abst. 18 435, 1992). While pilot investigations failed to provide evidence that this protein is glycosylated (Asn residue 25 being a candidate for N-glycosylation), subsequent efforts indicated that $CCCS_1$ is fatty acylated: First, when $CCCS_1$ cRNA is expressed in Xenopus oocytes, one can detect the presence of 27kDa and 34kDa immunoreactive species of $CCCS_1$. The 27kDa species is soluble while the 34kDa species is membrane bound. When the 34kDa species is treated either with 1M hydroxylamine (pH 7.0) or 0.1M KOH in methanol, its mass is reduced to 27kDa. This result is compatible with $CCCS_1$ being fatty acylated. Complementary experiments reveal that if ^{35}S-labeled $CCCS_1$ is injected into oocytes, approximately half of the injected protein is acylated within 24h to generate the 34kDa species. Moreover, this 34kDa species (but not the 27kDa species) is membrane bound. From these results, we conclude that oocytes harbor an acyltransferase that can fatty acylate $CCCS_1$ and that this acylation is responsible for the membrane association of this protein. Finally, similar experiments have revealed that $CCCS_1$ from Torpedo is fatty acylated and the identity of these acyl groups is being determined.

548.7

NEURONAL CALCIUM CHANNELS SUBTYPES EXPRESSED IN RAT INSULINOMA AND HUMAN PANCREATIC BETA-CELLS. A. Pollo*, V. Magnelli, M. Lovallo, E. Sher# and E. Carbone. Dept. of Anatomy and Human Physiology, Univ. of Turin, Italy. (#)CNR Ctr. Cytopharmacology, Univ. of Milan, Italy.

We have investigated the coexpression of different Ca channel subtypes in the rat insulinoma cell line RINm5F and in human pancreatic ß-cells using whole-cell and single channel current recordings. Whole-cell currents in RINm5F cells could be attributed to at least one LVA and three HVA channel subtypes, similar for activation/inactivation kinetics and pharmacology to those already described in neurons. HVA Ba currents started to activate at -30mV, peaked at +10 and reversed at +50. The dihydropyridine (DHP) antagonist nifedipine (10μM) reversibly blocked only 55% of the total current at 0mV, while ω-conotoxin (ω-CgTx, 3.2μM) irreversibly blocked a smaller fraction (10-20%). The residual DHP- and ω-CgTx-insensitive current was shown to be sensitive to ω-Agatoxin IV A which depressed 10-30% of the total currents. ω-Aga action was partially relieved by high and frequent depolarizations. In human ß-cells, DHP action was stronger (85% block at -10mV), while ω-CgTx and ω-Aga were poorly effective. Single channel recordings in cell-attached and outside-out patches of RINm5F cells revealed a dominance of L-type channels with prolonged openings of large conductance (22-25pS in 100mM Ba) in the presence of Bay K 8644 (1μM). In 25% of the patches, however, L-type channels of smaller conductance (10-12pS) as well as DHP-insensitive channels were also observed. DHP-insensitive channels in ω-CgTx-treated cells showed prolonged activity for 300-500ms with short mean open times (1-2ms) and multiple conductance levels (9 to 20pS) that disappeared at low holding potential (-50mV).

548.8

CODISTRIBUTION OF NCAM AND DIHYDROPYRIDINE RECEPTOR ALPHA-2 SUBUNIT DURING EARLY MYOGENESIS *IN VITRO*.
F. Rieger* and S. Vandaele, Groupe de Biologie et de Pathologie Neuromusculaires, INSERM U.153/CNRS 0064, Paris (France) and Unité de neurocytologie moléculaire, Département de Pathologie, Université de Montréal (Canada).

We further characterized the monoclonal antibody (Mab) 3007 previously selected by its ability to immunoprecipitate dihydropyridine binding sites associated with voltage-dependent calcium channels of skeletal muscle (Vandeale et al., 1987). Mab 3007 immunoprecipated only the α2-subunit from iodinated solubilized T-tubule membranes, in conditions where the five subunits constituting the dihydropyridine receptor are dissociated. When used to immunocytochemically decorate rabbit muscle sections, Mab 3007 revealed a labelling pattern corresponding to the dihydropyridine receptor localization in the T-tubule network. Lastly, when incubated with solubilized membranes from ^{35}S-methionine-labelled rabbit muscle cell culture, Mab 3007 precipitated a unique polypeptide corresponding to the α2 subunit.

Using Mab 3007 and an anti-NCAM polyclonal antiserum, the surface distribution of the α2-subunit and its topographical relationship with the NCAM were then studied immunocytochemically during early myogenesis *in vitro*. On aligned myoblasts before fusion, the α2-subunit and the NCAM appaered uniformly distributed. On contracting myotubes, both molecules were organized in clusters grouped into "super-clusters". A high percentage of α2-subunits and of NCAM "super-clusters" were colocalized. Within the colocalized "super-clusters", NCAM and α2-subunit cluster staining patterns were exactly superposed. These results suggest that, at this stage of myotube differentiation, the α2-subunit is first directed towards the myotube plasma membrane before being restricted to the T-tubules, and that it may be cotransported with NCAM. It might also be possible that these two molecules interact during early myogenesis *in vitro*.

548.9

THE α-1 SUBUNIT OF AN N-TYPE VOLTAGE SENSITIVE CALCIUM CHANNEL IS PRESYNAPTIC TO PURKINJE CELL DENDRITES. R. Dado[1]*, R. Feddersen[2], D.R. Witcher[3], C. Honda[1], H. Orr[2], K.P. Campbell[3] and R. Elde[1], Dept. Cell Biol. and Neuroanat.[1], Lab. Med. and Path.[2], Univ. Minn., Minneapolis, MN 55455; Howard Hughes Med. Inst. & Dept. Physiol. & Biophys.[3], Univ. Iowa, Iowa City, IA 52242

Ca^{++} spiking in Purkinje cell (PC) dendrites is known to be mediated by P-type voltage sensitive calcium channels (VSCCs). However, Westenbroek et al., (1992) have recently suggested that dendritic Ca^{++} spiking may also be conducted via an N-type channel. In the present study, we used in situ hybridization and immunocytochemistry to determine the identity of cells whose membranes contain N-type channels. In situ hybridization studies of PCs using oligonucleotide probes selective for each class of α-1 subunits (A, B, C & D; see Overstreet et al., Soc. Neurosci. Abstr., 1993) revealed abundant expression of transcripts for A and D classes, but class B and C transcripts were undetectable over PCs. However, transcripts for all 4 classes were expressed by neurons which provide synaptic input to PCs, including cerebellar granule cells and cells in the inferior olivary nucleus. Confocal microscopy of preparations stained with class B specific antibodies (see Elde et al., Soc. Neurosci. Abstr., 1993) in combination with antibodies to calbindin revealed a striking arrangement of class B α-1 immunoreactive (CBα-1-ir) puncta which were presynaptic to dendrites and spines of PCs. The position of many of these CBα-1-ir puncta was consistent with the known distribution of terminals of climbing fibers. Lesions of inferior olivary neurons (the source of most climbing fibers to the cerebellum) with 3-acetylpyridine destroyed a major portion of the CBα-1-ir puncta. In addition, CBα-1-ir puncta were present, but restricted to the shaft of climbing fiber axons in the cerebellum of transgenic mice lacking PCs due to the expression of the SV-40 large T antigen under a PC specific promoter.

In conclusion, PCs express transcripts which encode α-1 subunits of P- and L-type VSCCs; they do not express a class B transcript which encodes an N-type VSCC. Thus, Ca^{++} spiking in PCs is unlikely to be mediated by this N-type VSCC. Instead, the present data suggest that N-type VSCCs particpate in neurotransmitter release from climbing fiber terminals. Supported by DA 06299 and DA 08131.

548.10

AXONAL TRANSPORT OF AN α-1 SUBUNIT OF AN N-TYPE VOLTAGE SENSITIVE CALCIUM CHANNEL IN SCIATIC NERVE AND DORSAL ROOTS OF RATS. R. Elde[1], R. Dado[1], D.R. Witcher[2], C. Wetmore[1]* and K.P. Campbell[2], Dept. of Cell Biol. and Neuroanat.[1], Univ. of Minn. , Mpls, MN 55455; Howard Hughes Med. Inst. & Dept. Physiol. & Biophys.[2], Univ. Iowa, Iowa City, IA 52242.

Voltage sensitive calcium channels are importantly involved in neurotransmitter release. However, little is known concerning the mechanisms by which these channel proteins are targeted to the presynaptic membrane. Antisera were obtained from rabbits immunized with a peptide (residues 851-867 of the class B α-1 subunit; Dubel et al. 1992) coupled to thyroglobulin or a fusion protein (residues 720-1139; Witcher et al. 1993). After affinity purification, immunostaining was observed in neurons which expressed transcripts for this subunit as determined by in situ hybridization (see Dado et al.; Overstreet et al., Soc. Neurosci. Abstr., 1993). Rats were deeply anesthetized and the sciatic nerve or lumbar dorsal roots were constricted with a tight ligature. After recovery ranging from 2-48 hrs, animals were sacrificed and longitudinal sections of sciatic nerves and dorsal roots were immunostained and observed using confocal microscopy. Class B α-1 immunoreactivity (CBα-1-ir) appeared as intensely stained, but sparsely distributed particles within the axoplasm of unperturbed nerves. After constriction of the sciatic nerve, CBα-1-ir appeared as large clusters of tightly packed particles within the axoplasm of individual axons proximal to the constriction. This suggested that the particles of CBα-1-ir travel by anterograde axonal transport, although not in the fastest phase, since the majority of CBα-1-ir clusters were located proximal to clusters of particles immunostained with antisera to neuropeptides (CGRP, SP), dopamine β-hydroxylase, and synaptotagmin. Distal to the constriction no CBα-1-ir clusters were observed, suggesting, that in contrast to neuropeptides, dopamine β-hydroxylase and synaptotagmin, retrograde transport of CBα-1-ir did not occur. Constriction of dorsal roots gave similar results. In addition, CBα-1-ir was observed in nerve fibers and terminals in the dorsal horn of the spinal cord and in the dorsal column nuclei, suggesting that CBα-1-ir is delivered to nerve terminals by anterograde axonal transport in primary afferent neurons. Supported by DA06299 and DA08131

548.11

STRUCTURAL DETERMINANTS OF ION SELECTIVITY IN BRAIN CALCIUM CHANNEL Yasuo Mori* Man-Suk Kim†, Takashi Morii§ and Keiji Imoto† *Dept. of Pharmacology and Cell Biophysics, Univ. of Cinti., Cinti., OH 45267-0575 †Dept. of Medical Chemistry, Kyoto Univ., §Dept. of Polym. Sci., KIT, Kyoto 606, Japan

Under physiological conditions, calcium channels must be highly selective in allowing large Ca^{2+} fluxes. Little direct information concerning the structural basis of this selective permeability has been available. To address this issue, Glu residues in the SS2 segment of the internal repeat III and IV of the brain calcium channel BI (Glu-1,469 and Glu-1,765) have been subjected to singel point mutations. The mutants expressed in *Xenopus* oocytes were tested for macroscopic current properties and sensitivities to inorganic blockers using 40 mM Ba^{2+} as the charge carrier. The mutation that replaces Glu-1,469 with Gln (E1469Q) caused a marked shift in reversal potential to the negative direction (from +58 mV to +38 mV). Although smaller in degree, the mutation that replaces Glu-1,765 with Gln (E1765Q) also caused a shift (to +50 mV). While the IC_{50} was considrably low for the wild-type (0.73 μM), the mutation E1469Q made the IC_{50} value for Cd^{2+} more than 200 times larger (201 μM), without affecting sensitivities to other inorganic blockers very much. The mutation E1765Q did not significantly change sensitivities to inorganic blockers including Cd^{2+}. These results indicate that the mutations altered the strict selectivity for divalent cations over monovalent cations, and imply that the Glu residues differentially take part in forming the selectivity filter of the calcium channel.

548.12

ELECTROPHYSIOLOGICAL AND PHARMACOLOGICAL COMPARISON OF A FAMILY OF NEURONAL CALCIUM CHANNELS. A.Stea*, T.W.Soong, C.D.Hodson, S.J.Dubel, W.J.Tomlinson, and T.P. Snutch. Biotechnology Laboratory, University of British Columbia, Vancouver, B.C., V6T 1Z3.

Several different Ca channel α1 subunits have been cloned from rat brain (designated rbA, rbB, rbC, rbD, and rbE). Four of these channels have been expressed in Xenopus oocytes by nuclear injection of cloned DNA. The rbA current in 40 mM Ba rapidly activated (τ ~ 5 ms) and showed slow inactivation (τ ~ 500 ms), whereas the rbB current activated very slowly (τ ~ 60 ms) and hardly inactivated over 800 ms. The rbC current showed variable activation rates (τ ~ 20 ms) with little inactivation over 800 ms. The rbE-II current activated completely within 20 ms (τ ~ 3 ms) and showed relatively fast inactivation (τ ~ 100 ms). The voltage dependent properties of the four Ca channel α1 subunits was significantly different. Half of the rbA and rbB currents were activated during a step to ~ -1 mV (holding potential -100 mV), while half the rbC current was activated at + 4 mV. In contrast half the rbE current was activated at a more hyperpolarized potential of -15 mV. In 4 mM Ba the rbE current activated in the range of some low voltage-activated channels. The currents also differed in their sensitivity to holding potential. Approximately one-half of the rbA, rbB and rbC currents were inactivated at a holding potential of -30 mV while this potential completely inactivated rbE-II (half-inactivation ~ -60 mV). Pharmacological characterization showed the rbC current was sensitive to dihydropyridines (L-type). The rbB current was insensitive to dihydropyridines and highly sensitive to ω-conotoxin GVIA (ω-CgTx) identifying it as a N-type Ca channel. The rbE current was not affected by either dihydropyridines or ω-CgTx but was sensitive to Ni. The electrophysiological properties of rbE-II are similar to a subset of T-type channels in hippocampal and thalamic nuclei. The rbA current was not completely blocked by AgaIVA at concentrations known to block P-type channels (200 nM).

Our results confirm that a family of Ca channel α1 subunits encodes Ca channels with diverse electrophysiological and pharmacological properties.

548.13

EXPRESSION AND LOCALIZATION OF A CALCIUM CHANNEL WHICH IS A MEMBER OF THE LOW VOLTAGE-ACTIVATED FAMILY. T.W.Soong, A.Stea, C.D.Hodson, S.J.Dubel, S.R.Vincent, and T.P. Snutch *. Biotechnology Laboratory, University of British Columbia, Vancouver, B.C., V6T 1Z3.

A rat brain Ca channel previously cloned in this laboratory (designated rbE-II) has a primary structure that is more related to the class A and B proteins (53-54% identity) than the class C or D proteins (~ 23% identity). Nuclear injections of the rbE-II clone (in PMT2 vector) into Xenopus oocytes gave robust currents (average > 500 nA) with 4 mM Ba as the charge carrier. These inward currents activated quickly ($\tau \sim 2$ ms) and inactivated fairly rapidly ($\tau \sim 100$ ms). The threshold for activation of the rbE-II current was -50 mV and more than half the channels were open at a step to -25 mV. In addition the rbE-II current was very sensitive to holding potential with half the channels inactivated at a holding potential of -65 mV. Application of nifedipine (10 uM), Bay K8644 (10 uM), or ω-conotoxin GVIA (1 uM) were without significant effect on the rbE-II current. AgaIVA (200nM) caused a small decrease in the current (~ 30%) which was not reversible with depolarizing pulses. Octanol (100 uM) and amiloride (1 mM) had little effect on the current. However both Ni and Cd at (10-30 uM) blocked more than 50% of the rbE-II current. Coexpression of a brain β subunit with rbE-II caused no major changes in the waveform or pharmacology of the rbE-II current but caused significant shifts in the voltage-dependent properties of this current. rbE-II + β currents were half activated at a step to -30 mV (from -100 mV) and were half-inactivated at a holding potential of -80 mV.

In situ hybridization showed that high levels of the rbE-II transcripts were found in the hippocampal pyramidal cells and the granule cells of the dentate gyrus. Moderate levels were found in the olfactory bulb, striatum, and amygdala. Expression was also noted in the intralaminar, parafasicular, and reticular nuclei of the thalamus and the substantia nigra and inferior olive. Cerebellar expression was in the granule and Purkinje cells. In many brain regions expressing rbE-II low voltage-activated Ca conductances sharing some of the characteristics of rbE-II have been described.

Taken together the electrophysiology,pharmacology, and localization of rbE-II identify it as a novel type of low voltage-activated Ca channel.

548.14

DETECTION OF THE CARDIAC CALCIUM CHANNEL mRNA IN DEVELOPING SKELETAL MUSCLE BY IN SITU HYBRIDIZATION. Y. Yang* and N. Chaudhari. Dept. of Physiology, Colorado State University, Fort Collins, CO 80253.

During the early development of skeletal muscle, mRNA for the cardiac dihydropyridine-sensitive calcium channel is detectable in fetal skeletal muscle tissue (Devel. Biol., 155:507, 1993). To define the cellular sources of this cardiac calcium channel expression, we analyzed fetal mouse skeletal muscle tissue by in situ hybridization. A 151 base antisense RNA located in the loop between repeat II and repeat III was used as a probe. A 586 base antisense RNA from Myo-D was used as a positive control. All probes were labeled with ^{35}S. Autoradiography reveals that the cardiac calcium channel mRNA is expressed in cells that are Myo-D positive, i.e. myoblasts and myotubes. In addition, the cardiac calcium channel mRNA is expressed in cells that are presumably fibroblasts between muscle bundles. The concentration of the cardiac calcium channel mRNA diminishes during skeletal muscle development. We have also detected the cardiac calcium channel mRNA in the G-8 fetal skeletal myoblast cell line by Northern blot hybridization. These results demonstrate that the cardiac calcium channel mRNA is expressed in myoblasts, myotubes as well as fibroblasts in developing skeletal muscle and raise the question of whether this channel may be functionally important during the early development of skeletal myofibers. Supported by NIH grant GM42652.

548.15

ISOLATION AND CHARACTERIZATION OF HUMAN GENOMIC CLONES FOR VOLTAGE-SENSITIVE CALCIUM CHANNELS. **H. H. Jung and H. Chin* Lab of Neurochemistry, NINDS, NIH, Bethesda, MD 20892**

Recent molecular cloning studies have identified the four distinct classes ($\alpha_{1\text{-}A}$, $\alpha_{1\text{-}B}$, $\alpha_{1\text{-}C}$, and $\alpha_{1\text{-}D}$) of cDNAs that encode the α_1 subunit of voltage-sensitive calcium channels. The $\alpha_{1\text{-}D}$ cDNA, corresponding to the DHP-sensitive, L-type calcium channels, has been isolated from a variety of excitable and nonexcitable tissues, including brain, neuroblastoma cells, panceatic β-cells, and kidney. In contrast, expression of the $\alpha_{1\text{-}B}$ mRNA encoding the ω-conotoxin-sensitive, N-type channels seems to be restricted to the nervous system and the cells of neuronal origin.

Since comparative analysis of the 5' upstream regulatory region of the L- and N-type calcium channel genes is likely to yield insights into the molecular mechanisms involved in cell type- or tissue-specific expression of voltage-sensitive calcium channels, we have begun to isolate the corresponding human genomic clones. In an earlier study, we reported isolation of a human genomic clone containing 5' upstream region of L-type $\alpha_{1\text{-}D}$ subunit gene, and mapped its location to the short arm of human chromosome 3 (Chin *et al.*, Genomics **11**, 914-919). We have now obtained two overlapping human genomic clones (insert size, 12 kb and 14 kb), and sequenced a 2-kb genomic fragment. The latter contained a 5' upstream region of the N-type calcium channel $\alpha_{1\text{-}B}$ subunit gene including the initiation methionine residue. Currently we are further characterizing the promoter regions of both the human $\alpha_{1\text{-}B}$ and $\alpha_{1\text{-}D}$ subunit genes to identify regulatory elements invoved in neurospecific expression of N-type calcium channel genes.

548.16

MOLECULAR CHARACTERIZATION OF VOLTAGE-SENSITIVE CALCIUM CHANNELS EXPRESSED IN HUMAN RETINA GLIAL CELLS. **J.-J. Hwang[1], D. G. Puro*[2], and H. Chin[1]. [1]Lab of Neurochemistry, NINDS, NIH, Bethesda, MD 20892 and [2] Dept. of Ophthalmology and Physiology, Univ. of Michigan School of Medicine, Ann Arbor, MI 48105.**

DHP-sensitive L-type calcium channel complexes, which are composed of α_1, α_2-δ, and β subunits, are present in a variety of excitable and nonexcitable tissues. The cDNA corresponding to the pore-forming α_1 subunit was initially isolated from skeletal muscle. Subsequently, two additional L-type α_1 subunit genes have been identified; the first gene (α_{1C}) encoding the cardiac and smooth muscle calcium channels, and the second (α_{1D}) corresponding to the L-type channels in neurons, endocrine cells and a number of other cell types. Our earlier electrophysiologic study showed that glial cells also express DHP-sensitive L-type calcium channels (Puro and Mano, J. Neurosci., **11**: 1873, 1991).

To further examine the molecular identity of the glial L-type calcium channels, we have carried out RT-PCR using primers specific for the calcium channel subunits. RNA samples were isolated from the glial cells that had been derived from the adult postmortem human retina and maintained in culture. Immunocytochemical markers (a specific MAb, anti-glutamine synthetase, and anti-GFAP) indicated that virtually all of the cells in culture are Müller glial cells. Sequencing analysis of the PCR subclones has indicated that the L-type calcium channel α_1 subunit in Müller glial cells is identical to the α_{1D} that has been cloned from human neuroblastoma cells (Williams *et al.*, Neuron **8**: 71, 1992). In addition, the brain-specific splice variant of the L-type α_2 subunit, $\alpha_{2\text{-}b}$, is also present in retinal glial cells. PCR products corresponding to either the N- type α_1 subunit or P-type α_1 subunit have not been detected. Further studies are currently underway to determine the subunit composition of glial L-type calcium channels.

CALCIUM CHANNEL PHYSIOLOGY

549.1

VOLTAGE-GATED CALCIUM CURRENTS IN POST-NATAL CAT RETINAL GANGLION CELLS. S-J. Huang, D.W. Robinson*, R.P. Scobey, and L.M. Chalupa. Dept. of Neurology and The Center for Neuroscience, University of California, Davis, CA95616, USA.

We have previously reported data describing the development of sodium and the potassium conductances in fetal and early post-natal cat retinal ganglion cells (RGCs). In order to provide a quantitative description of the observed spiking properties of these cells it is important to understand the type and quantity of calcium conductances present. RGCs were isolated from the retina of post-natal cats by enzymatic dissociation after retrograde labeling with rhodamine latex microsphere injections into the LGN and SC. To date we have concentrated on the activation and inactivation of L-type calcium current. Whole-cell patch clamp recordings were made from identified RGCs and L-type calcium currents were isolated by ion substitution and holding potential. All RGCs expressed L-type current varying in magnitude from cell to cell (8-400pA). The normalized conductance-voltage relationship was well fitted in all cases by the Boltzmann equation, giving a half point of activation of -0.1mV and a slope of 7.5. Based on Hodgkin-Huxley kinetics, L-type calcium currents were fitted with m^2h and the time constants of activation obtained, ranged from 0.41ms at 27mV to 2.1ms at -20mV. Steady-state inactivation was fitted by a Boltzman equation giving a half point of inactivation of -46mV and a slope of 13.

Supported by NIH grant EY-03991 and DWR is a HFSP Fellow.

549.2

USE OF THE RAT ADRENAL CHROMAFFIN CELL FOR THE STUDY OF ADRENERGIC EXCITATION-SECRETION COUPLING. B. Hollins & S.R. Ikeda.* Dept. of Pharmacology and Toxicology, Medical College of Georgia, Augusta, GA 30912.

Chromaffin cells were isolated from the adrenal medulla of male adult Wistar rats and used within 1 to 3 days. Studies were carried out to:1) identify Ca^{2+} channel types and 2) detect vesicle release. Voltage-activated Ca^{2+} currents were studied with whole-cell voltage-clamp. Currents were activated from a holding potential of -80 mV with 10 mM Ca^{2+} in the external solution. Under these conditions, inward currents first appeared near -40 mV and reached a maximum near 0 mV. A prominent shoulder in the I/V curve was evident near -20 mV. The L-type channel blocker, nifedipine, and the N-type channel blocker, ω-conotoxin, each blocked about one third of the current. The remaining current was resistant to ω-Aga-IVa, a P-type channel blocker.

Quantal catecholamine release in response to several stimuli could be detected amperometrically from single chromaffin cells using carbon fiber electrodes (Chow et al., *Nature* **356**: 60-63, 1992). Release was evidenced by a series of discrete current spikes which appeared following application of acetylcholine, 55 mM K^+, GABA or histamine. These results indicate that both Ca^{2+} currents and vesicular release of catecholamines are conveniently measured in rat adrenal chromaffin cells. The use of voltage-clamp combined with carbon fiber voltammertry should allow for the detailed study of adrenergic excitation-secretion coupling. Supported by NIH grant HL-43242.

549.3

BIOPHYSICAL PROPERTIES OF THE PURIFIED "N-TYPE" Ca^{2+} CHANNEL IN BILAYERS.

M. De Waard, D.R. Witcher, and K.P. Campbell*. Howard Hughes Medical Institute and Dept. of Physiology and Biophysics, Univ. of Iowa, College of Medicine, Iowa City, IA 52242.

The ω-conotoxin GVIA (ω-CgTx) receptor has been purified from rabbit brain. The receptor is composed of four subunits of molecular masses 230 K (α_1), 140 K (α_2), 95 K and 57 K (β_3) under reducing conditions. Immunoaffinity adsorption experiments using polyclonal antibodies directed to the unique cytoplasmic loop between domains II and III of an α_1 class B subunit indicates that the purified receptor solely contains ω-CgTx sensitive α_1 isoforms. The receptor has been reconstituted into lipid bilayer at the tip of patch electrodes and the biophysical properties of the channel have been analysed. Upon incorporation into lipids, the purified receptor displays properties similar and dissimilar to the native "N-type" Ca^{2+} channel. Conserved properties of the channel include selectivity ($P_{Ba/PCa}$, P_{Na}, P_K) and pharmacology (ω-CgTx sensitivity, DHP and phenylalkylamine insensitivity). Properties that are at variance include a sustainable activity without detectable signs of inactivation or "run-down", conductance variability and poor or absence of voltage-dependency. Our data suggest striking similarities between the properties of the purified ω-CgTx receptor from rabbit brain and the purified DHP receptor from rabbit skeletal muscle in bilayers. The results stress the importance of cellular regulatory components in the definition of some of the biophysical properties of the native "N-type" Ca^{2+} channel.

549.4

OUTWARD CURRENTS CONTAMINATING LOW CALCIUM WHOLE CELL PATCH RECORDINGS FROM BULLFROG DORSAL ROOT GANGLION CELLS. A.M. Holohean*, C.A. Rodriguez, J.C. Hackman and R.A. Davidoff. DVAMC and Department of Neurology, Univ. Miami Sch. Med., Miami FL 33101.

Whole cell Ca^{2+} current recordings were made from acutely dissociated dorsal root ganglion cells (*Rana catesbiana*) using the following solutions: *Internal* (mM): 110 CsCl, 10 EGTA, 5 $MgCl_2$, 40 HEPES, 2 ATP-Mg, 0.3 GTP, pH 7.4 with CsOH; *External*: 5 $CaCl_2$, 135 TEA-Cl, 10 Glucose, 0.0007 TTX, pH 7.4 with TEA-OH. Currents were elicited by depolarizing the cell in 10 mV increments from a holding potential of -50 mV. When external Ca^{2+} was reduced to 0 mM, inward Ca^{2+} currents were eliminated and outward currents were isolated and studied. Two types of outward currents were identified. In one group of cells only a slow activating, non-inactivating, nifedipine-sensitive (>95% block) current (I_{slow}: 678.6 ± 79.4 pA) was seen. A second group of cells exhibited a composite current consisting of a slow activating, non-inactivating, nifedipine-sensitive current (I_{slow}: 516 ± 116 pA) as well as a fast-activating, transient, nifedipine-insensitive current ($I_{transient}$: 590.8 ± 106.2 pA). Whole cell capacitance measurements (C_m) demonstrated that the former group of cells (C_m = 74.2 ± 7.7 pF) were larger than latter group of cells (C_m = 41.3 ± 2.7 pF). All outward currents were blocked by the addition of Cd^{2+}. (Supported by DVAMC Funds MRIS #1769 and #3369 and USPHS grant #NS17577).

549.5

CALCIUM REGULATION OF INTRACELLULAR pH IN PRIMARY CULTURE PITUITARY INTERMEDIATE LOBE MELANOTROPES. SJ Morris*, DM Beatty and BM Chronwall Cell Biol. and Biophys. Div. and Mol. Biol. and Biochem. Div., SBS, Univ. of Missouri-Kansas City, Kansas City, MO 64110-2499

The regulatory activities of both intracellular calcium ($[Ca^{2+}]_i$) and intracellular pH (pH_i) have greatly increased interest in the study of their interdependence. We have designed an epifluorescence video microscope which will image the fluorescence from two ratio dyes, indo-1 (for $[Ca^{2+}]_i$) and SNARF-1 (for pH_i) at video rates. Using this imaging system, we examined primary cultures of pituitary intermediate lobe melanotropes loaded with both dyes. Following experimentation, cells were positively identified by fluorescence immunohistochemistry.

K^+-induced depolarization of melanotropes produced increases in $[Ca^{2+}]_i$ due to activation of L-type Ca-channels. A secondary Ca^{2+} peak or oscillations were often seen. After treatment with CCCP, K^+ depolarization produced a rise in intracellular $[Ca^{2+}]_i$ as well as oscillations. After thapsigargin or cyclopiazonic acid treatment, K^+ depolarization produced a primary Ca^{2+} elevation but the secondary Ca^{2+} changes disappeared. This suggests that they were due to Ca^{2+} release from an endoplasmic reticulum-type of intracellular store.

All of these increases in $[Ca^{2+}]_i$ were also directly coupled to a rise in intracellular H^+. The close association between intracellular Ca^{2+} and H^+ suggests that the observed pH_i changes were due to release of H^+ upon binding of Ca^{2+} to intracellular buffers. This direct, obligate coupling of intracellular Ca^{2+} and H^+ opens the possibility that pH-dependent cellular processes are directly activated by sudden increases in intracellular Ca^{2+} levels. This second-messenger type of signaling system would be activated whether the Ca^{2+} was released from intracellular stores or entered the cell *via* plasma membrane Ca^{2+} channels.

Supported by grants from the Kansas Affiliate-AHA, Loeb Charitable Trust, NSF grant IBN-9211912, NIH grant GM44071 (SJM), and NIH grant NS28019 (BMC).

549.6

REGULATION OF VOLTAGE-GATED NEURONAL L-TYPE Ca^{2+} CHANNELS BY MEMBRANE DEPOLARIZATION *J. Liu, A. Rutledge and D. J. Triggle*, Department of Biochemical Pharmacology, School of Pharmacy, State University of New York at Buffalo, Buffalo, NY14260*

We defined the regulation of neuronal Ca^{2+} channels by membrane depolarization, a pathologic stimulus. Rat pituitary cells (GH4C1) were exposed to K^+ depolarizing medium for periods to 120 min. Radioligand binding, $^{45}Ca^{2+}$ flux and Western blot analysis revealed that L-type Ca^{2+} channels undergo a number of modifications in response to membrane depolarization. The most prominent modifications are the increased sensitivity of Ca^{2+} channels to 1,4-dihydropyridine antagonists and the rapid down-regulation of Ca^{2+} channels on the cell surface. Under depolarizing condition the affinity of Ca^{2+} channels for PN200-110 is increased approximately 20-fold, which arises from an increased association rate. Depolarization for 2 hr elicits an approximately 90% reduction of Ca^{2+} channels on the cell surface. Similar observations were made with chick retinal neurons and rat cerebellar granule cells. This down-regulation is rapid, recoverable, and the recovery does not require protein synthesis. This regulation involves a transient movement of channels to the cell interior since the total cellular content of channels was unchanged. Monoclonal antibody study showed that depolarization did not alter the level of Ca^{2+} channel α2 subunit in the cell membrane, which suggests that Ca^{2+} channel regulation does not involve the whole channel complex. Additionally, depolarization induces an up-regulation of muscarinic receptors and Go α subunits. This study shows that prolonged stimulation of Ca^{2+} channels leads to removal of Ca^{2+} channels from cell surface and stronger inhibition. *(Supported by NIH grant HL16003)*

549.7

EFFECT OF SURFACE CHARGE ON PERMEATION IN N-TYPE CALCIUM CHANNELS OF FROG SYMPATHETIC NEURONS. W. Zhou and S. W. Jones*. Dept. Physiology and Biophysics, Case Western Reserve University, Cleveland, OH 44106.

The peak whole-cell calcium current appeared to saturate with increasing $[Ba^{2+}]_o$. However, the voltage-dependence of activation shifted, as expected for 1 negative surface charge per ~110 Å^2. If the pore is assumed to see the same surface charge as the voltage sensor, the current increases cooperatively with local concentration, with calculated $[Ba^{2+}]_o$ > 1 M at the mouth of the channel. This seems unrealistic. Therefore, we estimated the surface charge seen by the channel pore, from the change in the instantaneous I-V for fully activated channels upon changes in ionic strength (replacing N-methyl-D-glucamine by sucrose). The surface potential change was estimated by adjusting the control I-V to match the I-V at a different ionic strength, allowing for (1) a change in local $[Ba^{2+}]_o$ and (2) a shift along the voltage axis. A surface charge of 1 per ~1500 Å^2 was calculated from the measured change in surface potential, using the Grahame equation. (Activation shifted with ionic strength as expected for 1 charge per ~90 Å^2). This suggests that the channel pore sees much less surface charge than the voltage sensor. Thus, as others have concluded, Gouy-Chapman theory is at best an approximation. The current-local $[Ba^{2+}]_o$ relation, corrected for the effective surface charge seen by the pore, shows a slight tendency to saturate at high $[Ba^{2+}]_o$.

549.8

INTRAMEMBRANE CHARGE MOVEMENT IN PURKINJE CELL FROM NEW BORN MICE. T. SHIMAHARA*, F. BOURNAUD and K. MELLITI. Lab. of Neurobiol. Cell. Moléc, CNRS, 91198 Gif-sur-Yvette, France

It has been proposed that a large portion of the intramembrane charge movement recorded in skeletal muscle upon membrane depolarization is involved in the excitation-calcium release coupling mechanism. The intramembrane charge movement is likely to arise from a slight rearrangement of intramolecular charges within dihydropyridine (DHP) receptors, located in the t-tubule membrane. Immunocytochemical studies have revealed the presence of DHP-sensitive calcium channels in neuronal somata of rat brain. We have analysed the biophysical and pharmacological properties of intramembrane charge movement in Purkinje cells. Experiments were performed on enzymatically dissociated cells from 15-17 day-old new born mice. Asymmetric current was recorded with whole cell clamp technique. We observed that 1) the relationship between pulse potential and amount of charge movement was described by a two-state Boltzmann equation. 2) the maximum amount of charge movement at the onset of the depolarizing pulse (Q_{on} max) was 29 ± 2.3 nC/μF (n=18). 3) Nifedipine (1-5μM) and -Aga-IVA (1μM) blocked respectively 29.9 % and 19.4 % of Q_{on} max.

Supported by a grant from A.F.M.

549.9

VOLTAGE-DEPENDENT CALCIUM CURRENTS IN YOUNG AND AGED RAT DORSAL ROOT GANGLION (DRG) NEURONS AND THE EFFECTS OF TETRAHYDROAMINOACRIDINE (THA). K.M. Kelly+*, N. Esmaeil+ and R.L. Macdonald+#, Depts. of Neurology+ and Physiology#, U. of Michigan, Ann Arbor, MI 48104.

There is relatively little information available on aging-related changes in the function of individual voltage-dependent Ca^{2+} channels (VDCCs). Aging-related alterations in the function of these channels and increased Ca^{2+} influx to neurons may be involved in the development of significant cognitive dysfunction such as impaired learning and memory. We have compared VDCC currents in young and aged rat DRG neurons and have tested the effects of THA, a centrally active anticholinesterase that has been used in the palliative treatment of patients with Alzheimer's disease. THA also has effects on K^+ and Na^+ currents, and inhibits VDCCs in young rat DRG neurons (Kelly et al., Neurosci Lett 1991;132:247-250).

Whole cell patch clamp recordings were obtained from acutely dissociated rat DRG neurons at 20 d, 18 mo and 30 mo of age. Sprague-Dawley rats were used at 20 d and (F344xBN)F1 rats at 18 and 30 mo of age. Neurons from 30 mo old animals had T-type currents of decreased amplitude and decreased activation and inactivation rates compared to younger animals. High threshold currents had increased amplitudes compared to younger animals. Current block by nifedipine (L-type) and ω-conotoxin (N-type) was effective in all age groups but did not abolish all currents. THA (250 μM) completely blocked all VDCC currents in all age groups. These results indicate aging-related changes of VDCCs in rat DRG neurons and the ability of THA to block these currents in both young and aged animals.

549.10

EXTRACELLULAR CALCIUM-INDUCED CHANGES IN THE MEMBRANE ELECTRICAL PROPERTIES OF THYROID PARAFOLICULAR CELLS. S.H. Hsuing, H. Tamir, & D.S. McGehee* NY State Psych Inst. Dept of Anat & Cell Biol in the Ctr for Neurobiol and Behav, Columbia Univ P&S, 630 W 168th St, NY, NY 10032.

Increases in serum calcium levels are known to stimulate calcitonin and serotonin (5-HT) release from the parafolicular C-cells of the thyroid gland. Calcitonin is known to lower serum Ca^{2+} levels; however, relatively little is known about the mechanism of release of this hormone. We are examining the processes underlying the sensitivity of these cells to extracellular Ca^{2+} in isolated primary cultures. Assays of calcitonin and 5-HT release indicate that the threshold for Ca^{2+}-induced release is approx. 5mM, with maximal stimulation at 10mM. Measurements of membrane potential, using whole cell perforated patch recording, indicate that 10mM Ca^{2+} induces a slow depolarization of the membrane with an associated increase in conductance. This leads to a rhythmic series of action potentials (APs) that continues until washout of the high Ca^{2+}. The interspike interval is 5-10 sec and the half width of each AP is nearly 2 sec. In voltage clamp (V_m=-60mV), application of 10mM Ca^{2+} to these cells stimulates an inward current that does not return to baseline until washout. Ongoing experiments are directed at determining the possible role of voltage-gated Ca^{2+} channels (VGCCs) in this response. Pretreatment of the cells with the nonspecific Ca^{2+} channel blocker lanthanum (La^{3+}, 20μM) completely inhibits 10mM Ca^{2+}-induced depolarization. The dihydropyridine antagonist of L-type VGCCs nimodipine (10μM), also inhibited the depolarization induced by 10mM Ca^{2+}, but to a lesser extent than the La^{3+}. It is suggested that the Ca^{2+} induced changes in membrane electrical properties underly the observed increases in hormone release from these cells. Support provided by grant MH375775 to HT and NS09395-01 to DM

549.11

CHARACTERIZATION OF WHOLE CELL CALCIUM CURRENTS IN "INTACT" SUBSTANTIA NIGRA NEURONS. T. DeFazio*, L. Byerly, and J.P. Walsh, Andrus Gerontology Center & Dept. of Biological Sciences, University of Southern California, Los Angeles, CA 90089-0191

Whole cell recordings were obtained from substantia nigra pars compacta (SNc) neurons using the thick slice patch clamp technique. In current clamp mode most cells exhibited broad action potentials (2-5 msec), tonic activity (~9 Hz), large afterhyperpolarizations, inward rectification, and low threshold calcium spikes (LTS). These properties are consistent with the electrophysiological criteria for identifying dopaminergic SNc neurons in brain slices. The input resistances were an order of magnitude greater than those observed using microelectrode techniques.

Voltage clamp recordings were contaminated by large fast biphasic current spikes, presumably due to action potentials escaping the poor voltage control of the membrane potential. Tetrodotoxin (TTX) and tetraethylammonium (TEA) attenuated the spikes in both voltage clamp and current clamp, but did not abolish them. Substituting cesium for potassium as the main cation in the internal solution eliminated the remnants of the uncontrolled spikes and permitted the isolation of labile voltage dependent inward currents. The currents were enhanced by substituting barium for calcium in the external solution, suggesting that this current is calcium mediated. The slow activation of the current may be due to the poor voltage control of the extensive dendritic arbor of these neurons and implies that at least some of the calcium channels are dendritically located.

Supported by NIA Grant 5PO1 A60979.

549.12

LOCAL POSITIVE CALCIUM FEEDBACK MEDIATES AGONIST-EVOKED CALCIUM WAVE PROPAGATION. Samuel S.-H. Wang*, Adawia A. Alousi, and Stuart H. Thompson. Neurosciences Program and Hopkins Marine Station, Department of Biological Sciences, Stanford University, Pacific Grove, CA 93950.

In mouse neuroblastoma cells (line N1E-115), muscarinic receptor stimulation leads to the production of IP_3 and consequent calcium (Ca) release from stores. We used fluorescence video microscopy of fura-2/AM to view Ca waves in single cells. Exposure to 1 mM carbachol leads to steep Ca rises after a latency of 1–30 seconds. This latency was longer for lower agonist doses and was often followed by a Ca wave that crossed cells at speeds of 10 to >100 μm/sec. Waves were slowed by heavy loads of the fast calcium buffers fura-2/AM and 5,5'-Br_2-BAPTA/AM ($k_{on} \approx 10^8\ M^{-1}s^{-1}$), but not by the slow buffer EGTA/AM ($k_{on} \approx 10^6\ M^{-1}s^{-1}$), showing that the rate at which a buffer binds Ca determines whether it is able to block wave propagation. Therefore, a limiting positive feedback step in wave propagation is the fast and local diffusion of Ca. Conversely, direct biochemical measurement and analysis of the latency to rise in Ca indicate that IP_3 is too long-lived ($t_{1/2} \approx 10$ seconds) to exist in the spatial gradients necessary for it to act as a rate-limiting messenger. We suggest that agonists cause global accumulation of IP_3 to threshold, followed by a regenerative wave in which Ca diffusion to neighboring release sites is one rate-determining step. Supported by NIH NS14519 and MH10088 and Sterling Drugs.

549.13

VOLTAGE-DEPENDENT CALCIUM ENTRY IN RAT SOMATOTROPHS. B.T. Lussier and P. Mollard*. CNRS - URA 1200, Université de Bordeaux II, 33076 Bordeaux Cedex, FRANCE.

The hypothalamic neuropeptide, growth hormone releasing hormone (GHRH), stimulates the release of growth hormone from somatotrophs of the anterior pituitary. This action of GHRH is associated with a small depolarization and an increase in the concentration of free intracellular Ca^{2+} ($[Ca^{2+}]_i$). Our aim was to investigate voltage-dependent Ca^{2+} entry in single purified rat somatotrophs. To this end $[Ca^{2+}]_i$ was measured using the Ca^{2+}-sensitive fluorescent dye indo-1 while monitoring or clamping membrane potential using "perforated" patch-clamp techniques. The following observations were made: i) a 5 s exposure to 10^{-7} M GHRH produced two electrical responses consisting of either a delayed, small and prolonged depolarization (5 to 10 mV), or the initiation of action potentials; ii) the sustained depolarization was associated with a prolonged increase in $[Ca^{2+}]_i$, while action potential gave rise to transient $[Ca^{2+}]_i$ spikes; iii) increases in $[Ca^{2+}]_i$ were dihydropyridine (DHP)-sensitive whereas sustained depolarizations were not; iv) under voltage clamp conditions, short (1 s) depolarizing (-60 to -10 mV) steps produced $[Ca^{2+}]_i$ spikes, while long (20-30 s) depolarizing (-50 to -40 mV) steps produced detectable steady inward Ca^{2+} currents and the associated sustained DHP-sensitive elevations in $[Ca^{2+}]_i$. We conclude that the two patterns of GHRH-dependent $[Ca^{2+}]_i$ increase in rat somatotrophs are due to voltage-dependent DHP-sensitive Ca^{2+} entry.

549.14

SPATIAL PATTERNS OF CALCIUM ENTRY INTO CULTURED RAT CEREBELLAR NEURONS ARE CORRELATED WITH INITIATION SITES FOR DEGENERATION. V.P. Bindokas*, D. Bleakman†, and R.J. Miller. Dept. Pharmacol. and Physiol. Sci., Univ. Chicago, 947 E 58th St. Chicago IL 60637; †Presently: Lilly Research Centre, U.K.

Calcium overload in neurons is linked to excitotoxic damage manifested by alterations in cell shape and subsequent degeneration. We have studied the patterns of $[Ca^{2+}]_i$ increases in cultured neurons evoked by activation of voltage-sensitive or ligand-gated Ca^{2+} channels. Cultures enriched in Purkinje cells are described in detail (Brorson et al 1992, J. Neurosci 11:4024). Cells were loaded with either fura-2 AM or fura salt via patch pipet. A digital microfluorimetric imaging system was used to collect ratio pairs at 1Hz or log average Ca^{2+} data from regions of interest at 2Hz. Voltage-sensitive Ca^{2+} channels were activated by fast application of 50mM K^+ while kainate-gated Ca^{2+} rises were isolated by KA (200μM) in Na^+-free saline with 1μM TTX + 50μM TA3090 or by quenching of the fura-2 signal by KA-activated Co^{2+} entry. Based on observations from over 60 cells, the pattern of Ca^{2+} rise elicited by 50K or KA displays a range of overlap including examples of apparent segregation. The Ca^{2+} rise in Purkinje cell dendrites elicited by 50K was largely eliminated by 100nM ω-AgaIVA. Sustained periods of high $[Ca^{2+}]_i$ (>700nM) often lead to swelling of neuronal somas and selective swelling (and pinching off) of neurites to produce beaded varicosities or "blebs". Often blebs formed at, or near, the sites of initial $[Ca^{2+}]_i$ rises produced by KA. Bleb loci were correlated with regions stained by the styryl dye RH414 which labels synaptic vesicles, with points of contact between neurites, and with regions rich in mitochondria as revealed by rhodamine 123 staining. These results support the notion that blebs tend to form in regions of Ca^{2+} overload and demonstrate spatial heterogeneity in Ca^{2+} signals.

549.15

CURRENT-DEPENDENT INACTIVATION OF A VERY LONG BARIUM TAIL CURRENT IN CULTURED RAT HIPPOCAMPAL NEURONS. M.L. Mazzanti*, O. Thibault, N.M. Porter and P.W. Landfield. Dept. Pharmacol., Univ. Kentucky, Coll. Med., Lexington, KY 40536.

In recent years we have described very long calcium (Ca^{2+}) tail currents in adult rat hippocampal slice neurons using sharp electrode voltage clamp. This tail current is dihydropyridine sensitive and is also sensitive to current-dependent inactivation, (Pitler, Landfield, 1987; Brain Res.; Campbell *et al*, 1990; Soc. Neurosci.). Elsewhere in this meeting (Thibault *et al*), we show that single L-type Ca^{2+} channel activity is closely correlated with these tail currents following repolarization.

The present studies utilized whole cell patch clamp methods to investigate similar very long tail current in cultured hippocampal neurons using 10 mM Ba^{2+} as the charge carrier. Whole cell recordings were evoked with 150 ms depolarizing steps to +10 mV from a holding potential of -70 mV. Tail currents lasting hundreds of milliseconds, with amplitudes between 50-200 pA, usually occurred following repolarization to -70 mV. In cells with a peak amplitude of at least 600-700 pA, the percent inactivation during step showed a highly significant negative correlation ($p<.005$) with long tail current amplitude. Parallel studies, using chelating agents, different concentrations of Ba^{2+}, and I-V analyses also show that the amplitude and duration of the tail currents are inversely proportional to the amount of Ba^{2+} current influx during the depolarizing step.

These studies indicate that the Ca^{2+}/Ba^{2+} tail currents are highly sensitive to current-dependent inactivation and that long tail currents appear to show inactivation proportional to Ba^{2+} currents during the depolarizing step (Supported by AG10836, AG04542 and Miles, Inc.).

549.16

Prolonged High Open Probability of Hippocampal L-Type Calcium Channels Following Repolarization is Unmasked by Low Ba^{2+} and Ca^{2+} Concentrations. O. Thibault*, N.M. Porter and P.W. Landfield. Dept. Pharmacol., Univ. Kentucky College of Medicine, Lexington, KY,40536.

Over the past several years, we have described long-lasting, dihydropyridine-sensitive, calcium (Ca^{2+}) tail currents in CA1 neurons of non-dissociated rat hippocampal slices. However the interpretation of these long tail currents is not fully clear. A few studies have seen single Ca^{2+} channel openings following a depolarization step, but these openings occurred with low frequency and often only in response to particularly strong depolarizations.

We report here that low concentrations of barium (Ba^{2+}) or Ca^{2+} in the pipette greatly enhance the probability of single L-type channel openings following a moderate depolarizing step. Single Ca^{2+}-channel currents were recorded in cultured neurons from rat hippocampus in the cell-attached patch configuration with pipettes filled with 110 mM Ba^{2+}, 20 mM Ba^{2+} or 5 mM Ca^{2+}. We found that repolarization openings (ROs) are infrequent in 110 mM Ba^{2+}, but substantially more frequent, and continue for hundreds of milliseconds following repolarization, in 20 mM Ba^{2+}. The ROs also are correlated with very long whole cell Ca^{2+} tail currents under a variety of conditions.

This increase in post-repolarization channel openings is seen also with 5 mM Ca^{2+} as the charge carrier, and occurs after brief depolarizations (5 ms) to physiological voltages (+10 mV). Thus, these data suggest that, under relatively physiological conditions, substantial Ca^{2+} channel activity normally persists long after a depolarization. (Supported by AG10836, AG04542 and Miles, Inc.).

549.17

RECOVERY OF N-TYPE CALCIUM CHANNELS FROM INACTIVATION. M. A. Werz* and S. W. Jones. Dept. Physiol. & Biophys., Case Western Reserve Univ., Cleveland, OH 44106.

Whole-cell N-currents of frog sympathetic neurons (with 2 mM Ba^{2+}) have components inactivating at markedly different rates (τ=0.15 s, τ=1.5 s, and noninactivating on a ~3 s time scale). In cells dialyzed with the phosphatase inhibitor okadaic acid, a more rapidly inactivating component develops (τ=15 ms) (*Soc. Neurosci. Abstr.* **18**:431, 1992). We are studying recovery from inactivation in control cells and cells dialyzed with okadaic acid, to determine the connectivity of the several inactivated states. Recovery was assessed using pairs of depolarizing steps to -10 mV, separated by a variable interval (10 ms - 60 s). Step durations of 35 ms, 0.35 s, and 3.5 s were used, to optimize selective inactivation of the different components. Following 3.5 s steps, three prominent components of recovery were observed (τ=0.09, 0.6, and 10 s). For shorter steps, a larger fraction of the recovery was rapid, without detectable changes in the τ's. Recovery was similar for components inactivating at different rates (comparing the amplitudes of each component, for each pair of voltage steps). Recovery from inactivation was also similar in control and okadaic acid-dialyzed cells. We conclude that the different components of inactivation do not correspond to distinct, independent calcium channel types. If the components reflect "modes" of gating, either mode switching is relatively rapid, or each mode contains multiple inactivated states.

549.18

REGULATION OF INTRACELLULAR CALCIUM CONCENTRATION AND ELECTRICAL BURSTING IN NEURONAL MODEL CELLS. Teresa Ree Chay.* Department of Biological Sciences, University of Pittsburgh, Pittsburgh, PA 15260.

A model that describes neuronal bursting activity is presented. This model is based on the hypothesis of use-dependent blocking of calcium-activated proteins (CAP) on the calcium channels. It is assumed that CAP may block the calcium channel only when it is in the open (conducting) configuration and that the rate limiting step is the conformational transformation between the blocked and unblocked states of the calcium channel. Since the charged CAP must bind/unbind to the charged channel pore, the conformational transformation depends not only on intracellular calcium ions but also on membrane potential. This slow conformational transformation gives rise to bursting of electrical activity. With this model we show how neurons response to hormones and neurotransmitters by altering the electrical activity. This alteration brings about the change in levels of intracellular calcium concentration, which in turn influences the activity of various protein kinases.

549.19

DISTRIBUTION OF IP3 AND RYANODINE RECEPTORS IN RAT NEOSTRIATUM. S. A. Alba, M. E. Martone*, M. H. Ellisman. Dept. of Neurosciences, Univ. California San Diego,CA 92093-0608.

In a previous study from this laboratory, two proteins that mediate the release of calcium from intracellular stores, the inositol 1,4,5-trisphosphate (IP3) and ryanodine receptors, were found to be differentially distributed in Purkinje cell dendrites: both proteins were found within the membranes of the endoplasmic reticulum in the dendritic shaft but only IP3 receptor was found in dendritic spines (Walton et al., J. Cell Biol., 1991,113, 1145). To extend these findings, we examined the distribution of the IP3 and ryanodine receptors in the rat neostriatum, which contains well-defined populations of spiny and aspiny neurons, to determine 1) which populations of striatal cells contain these two proteins and 2) whether they show a distribution within spiny dendrites similar to that found in cerebellum.

The distribution of IP3 and ryanodine receptors was studied at the light and electron microscopic levels in young adult rats by fluorescence and peroxidase immunocytochemistry using a polyclonal antiserum against IP3 (Mignery et al, Nature, 1989, 342, 192; provided by Dr. T. Südhof) and a polyclonal antiserum against a synthetic peptide of dog cardiac ryanodine receptor (Witcher et al., J. Biol. Chem. 1992, 267, 4963; provided by Dr. L. Jones). Within the neostriatum, labeling for both proteins was found within medium-sized cells with the ultrastructural characteristics of medium spiny neurons. In order to determine whether they were found in aspiny cells, some sections were also labeled for either choline acetyltransferase (ChAT) or parvalbumin, which mark two separate populations of striatal aspiny cells. Double-label immunofluorescence indicated that the ryanodine receptor colocalized with ChAT and parvalbumin while the IP3 receptor generally was not found in ChAT+ and parvalbumin+ cells. At the EM level, labeling for the IP3 receptor was found within cell bodies, dendrites and dendritic spines of spiny neurons. Ryanodine receptor labeling was seen in the cell body and proximal dendrites but did not appear to extend into spiny regions of the dendrite. Labeling for the ryanodine receptor also appeared to be present in astrocytic processes. The results of these experiments suggest that the IP3 receptor plays a specialized role in intracellular calcium regulation in spiny cells.

549.20

IMMUNOCYTOCHEMICAL LOCALIZATION OF CLASS A CALCIUM CHANNELS IN ADULT RAT BRAIN. R.E. Westenbroek*[1], J. W. Hell[1], T. Sakurai[1], T. P. Snutch[2], and W. A. Catterall[1]. [1]Dept. of Pharmacology, University of Washington, Seattle, WA 98195, and [2]Biotechnology Laboratory, University of British Columbia, Vancouver, B.C., V6T 1W5.

The class A neuronal calcium channel gene encodes an α1 subunit expressed at high levels in cerebellar Purkinje cells like P-type calcium channels. A polyclonal antibody (CNA1) that recognizes the α1 subunit of the rbA rat brain calcium channel specifically immunoprecipitates high affinity receptor sites for the spider toxin ω-AgaIVa and recognizes a 260 kDa glycoprotein in rat brain membranes that is phosphorylated by cAMP-dependent protein kinase. CNA1 was used in combination with the immunofluorescence and indirect peroxidase-anti-peroxidase techniques to investigate the distribution of class A calcium channels in rat brain. Calcium channels recognized by CNA1 are localized in cerebellar Purkinje cell somas and along the entire length of the dendrites. There is also intense punctate staining along the extent of the Purkinje cell dendrites, apparently at synaptic sites. In addition, the CNA1 immunoreactivity is localized along the full length of apical dendrites of hippocampal pyramidal neurons with relatively little labeling of somata. In the dorsal cortex there is labeling of the apical dendrites of pyramidal cells. This staining is relatively even along the entire length of the dendrites. These observations contrast with the uneven pattern of dendritic labeling seen in cortical pyramidal neurons using an antibody to N-type calcium channels and with studies of L-type calcium channels which have been shown to be localized predominantly in the cell body and proximal dendrites of most neurons. These results indicate that the ω-AgaIVA sensitive calcium channels recognized by CNA1 have a distinct distribution from L-and N-type calcium channels in central neurons, which may correlate with distinct functional roles for these channel subtypes.

550.1

OPEN CHANNEL CURRENT FLUCTUATIONS AND GATING OF Ca^{2+}-ACTIVATED K^+ CHANNELS. M.I. Glavinovic,*[1] and J.M. Trifaro.[2] Depts. of Anesthesia,[1] Anesthesia Research[1] and Physiology,[1] McGill University, Montreal Que. and Dept. of Pharmacology,[2] University of Ottawa, Ottawa, Ont., CANADA.

Surface charges on the channel protein or on the lipids can through electrostatic interactions influence both gating and permeation of ion channels. The extent of these influences depends on the spatial relationship between the charges and the gate or the channel pore. It has been suggested for Ca^{2+}-activated K^+ channels that the greater the number of Ca ions bound to the ligand binding sites the longer the duration of the channel openings (Barrett et al., J. Physiol. 331,211-230,1982). Therefore the gating itself can through electrostatic forces influence permeation. The current flow through these channels shows considerable fluctuations that are voltage dependent and appear to be caused by conformational fluctuations of the segment of the channel protein (Glavinovic, Soc. Neurosci. Abstr. 16, 360, 1990). In this study we examine the relationship between the duration of the channel openings and the open channel current fluctuations.

In excised inside-out patches from bovine chromaffin cell membranes the variances of the current fluctuations, the critical frequencies and the zero frequency asymptotic values of the Lorentzians fitted to the power spectra *were not* dependent on the duration of the channel openings. This suggests that the segment of the channel protein that is the source of the current fluctuations (and possibly the whole channel) is/are electrostatically well screened from the Ca^{2+} binding sites.

550.2

GATING AND SELECTIVITY OF APAMIN-SENSITIVE, CA-ACTIVATED K CHANNELS OF SMALL CONDUCTANCE IN RAT ADRENAL CHROMAFFIN CELLS. YoungBae Park and Bertil Hille*
Dept. of Physiology & Biophysics. U. of Washington, Seattle, WA 98195

The ionic selectivity and gating of apamin-sensitive Ca-activated K (SK) channels was studied in dissociated rat adrenal chromaffin cells using whole-cell and inside-out macropatch recording technique. The pipette solution contained (mM): 120 KAsp, 20 KCl, 5 Mg_2Cl, 20 HEPES, 0.1 EGTA, 3 K_2ATP, 0.1 Na_3GTP, and 0.08 leupeptin. Whole-cell tail currents following 1-2 s depolarization showed bell-shaped dependence on depolarization potential and were absent in Ca-free external solution or in 100 μM external Cd^{2+}. Reversal potentials of tail currents changed 58 mV per 10 fold change in $[K^+]_o$. Tail currents were largely blocked by extracellular application of apamin (200 nM), (+)-tubocurarine ($K_{½}$ 23.6 μM) and TEA ($K_{½}$ 5.4 mM). The relative permeability (P_x/P_K) of SK channels estimated from reversal potential changes was: Tl^+ 1.87, K^+ 1.0, Rb^+ 0.81, Cs^+ 0.16, NH_4^+ 0.11, and Na^+, Li^+ & methylamine < 0.01. With mixtures of Tl^+ and K^+, reversal potential and zero-current slope conductance showed anomalous mole-fraction dependence. Ca^{2+}-sensitivity and voltage dependence of SK channel gating were tested using inside-out macropatches. $K_{½}$ and Hill coefficient were 0.40 ± 0.09 μM (n=6) and 1.68 ± 0.28 (n=6) at -80 mV, respectively and independent of membrane potential in the range of -120 to -20 mV.
Supported by NS08174 and the McKnight and W.M.Keck Foundations.

550.3

CALCIUM BLOCK OF CALCIUM-ACTIVATED POTASSIUM CHANNELS. Kevin Chinn* and Cynthia Lee Martin, Searle, 4901 Searle Parkway, Skokie, IL 60077.

The mechanisms by which ions permeate and/or block membrane channels can influence both the physiology and pharmacology of the channel. It has previously been found in human T lymphocytes that calcium-dependent potassium current (IK_{Ca}) exhibits a linear current-voltage (I-V) relationship between -100 mV and 0 mV (Nguyen et al., Neurosci Abs 18:74, 1992). In whole-cell voltage clamp experiments with activated human lymphocytes we have found using voltage ramps (-200 mV to +40 mV) that while I-V relationships positive to -100 mV are linear, at voltages more negative than -130 mV outward rectification occurred (decreased slope conductance). In fact, a distinct hook shape often occurred in the I-V curve at such voltages which cannot be explained by Goldman rectification. Kca current depended upon external Ca concentration ($[Ca]_o$). Increasing $[Ca]_o$ from 2 to 9 mM decreased IK_{Ca} in the area of pronounced rectification by approximately 50%. Decreasing $[Ca]_o$ from 2 to 0.2 mM increased IK_{Ca} by approximately 20%. Both rectification and Ca dependence might be explained by Ca block of the channel as occurs with Na channels (Yamamoto et al., Biophys. J. 45:337, 1984).

550.4

CALCIUM INACTIVATION OF A SMALL CALCIUM-DEPENDENT POTASSIUM CHANNEL IN THE NEUROBLASTOMA-GLIOMA CELL LINE NG108-15. M. Harrington, S. Gutowski, P.C. Sternweis and F. Belardetti* Dept. Pharmacology, U.T. Southwestern, Dallas, TX 75235.

In NG108-15 cells, bradykinin (BK) transiently opens a Ca-activated K^+ conductance ($I_{K(Ca)}$) through activation of phospholipase C (PLC) mediated by the G protein $G_{q/11}$ (Wilk-Blaszczak *et al.*, 1993). PLC in turn generates inositoltrisphosphate (IP_3) which raises $[Ca^{++}]_i$, thus opening $I_{K(Ca)}$. We are investigating the detailed mechanism of regulation of this channel in inside-out patches bathed in K^+/EGTA/Ca^{++}. Whereas a BK-sensitive K^+ channel is readily observed in cell-attached patches (Higashida and Brown, 1988), inside-out patches exposed even briefly to millimolar $[Ca^{++}]_i$ during the patch isolation rarely contained Ca-sensitive outward channels. In contrast, if $[Ca^{++}]_i$ was maintained at or below micromolar levels, a voltage-independent 15-20 pS K^+ channel was reliably observed, whose P_o was steeply dependent on $[Ca^{++}]_i$ (half-activation around 1 μM). Potentially, G proteins could also be directly involved in its regulation. In conclusion this neuronal channel is an interesting example of multiple, convergent modulatory actions. These actions form the basis for the fine-tuning of the cell's excitability.

550.5

LEAD (Pb^{2+}) ENHANCES POTASSIUM CHANNEL ACTIVITY IN ACUTELY ISOLATED RAT CORTICAL ASTROCYTES. T.O.Jalonen*, M.Aschner[1], C.J.Charniga, A.J.Popp, and H.K.Kimelberg. Department of Surgery, and [1]Department of Pharmacology and Toxicology, Albany Medical College, Albany, N.Y. 12208.

It has previously been shown that Pb^{2+} (10 μM) causes swelling and enhances K^+ efflux in cultured, neonatal rat cortical astrocytes (Aschner et al., *Brain Res.Bull.*, 26:639,1991). To study the mechanisms of the direct effects of Pb^{2+} on the K^+ channel activity, we used the patch-clamp technique to record single-channel potassium currents in acutely isolated GFAP(+) rat cortical astrocytes. The cells were isolated from thin cortical slices of 3 to 30 days old rats with gentle papain treatment modifying the method described by Tse et al. (*J.Neurosci*, 12:1781,1992). In control recordings one of the voltage-sensitive K^+ channels, similar to that known to exist in cultured rat cortical astrocytes (Jalonen and Holopainen, *Brain Res.*, 484:177,1989), was seen to open with occasional, fast single steps, being open only about 4 % of the time. Within two minutes after direct administration of 0.2 to 40 μM Pb^{2+} on either the extra- or intracellular side of an excised cell membrane, the K^+ channel was seen to open with up to a 9-fold increase in the channel open probability, an increase in opening frequency and multiple (2-4) simultaneous channel openings. This increased single-channel activity could partly explain the enhanced K^+ efflux seen in astrocytes upon Pb^{2+} exposure. (Supported by NS 23750 (H.K.K.), H.Schaffer Foundation (A.J.P.), NIHES05233 (M.A.) and BRSG grant NIHSO7RR05394-31 (T.O.J.)).

550.6

IONIC CURRENT MODELING OF LIGHT RESPONSES FROM THE *HERMISSENDA* B PHOTORECEPTOR. K.T. Blackwell*[1], H.P. Dettmar[1], T.P. Vogl[1], D.L. Alkon[2]. [1]Environmental Research Institute of Michigan, Arlington, VA 22209; [2]Neural Systems Section, National Institute of Neurological Disorders and Stroke, NIH, Bethesda, MD

A lumped-parameter resistance-capacitance computer model of the four neuron circuit of Hermissenda crassicornis (Werness et al., Biological Cybernetics, 68:125, 1992) has been an excellent tool for examining the information flow and dynamics of biological associative learning. More detailed knowledge of mechanisms may be gained from examination of channel current interactions in a channel model of biological associative learning. Such a model requires characterization of the major voltage-dependent currents, which undergo persistent and progressive modification during associative learning, as well as the light-induced (CS) currents and GABA-induced (UCS) currents. We report here a channel level model of the light-induced currents of the Hermissenda B cell, which integrates the voltage-dependent currents modeled by Sakakibara, et al. (Biophysical J., 1993, In Press.) and the light-induced sodium and potassium currents measured under voltage-clamp conditions by Chen & Alkon (Soc. Neurosci. Abstr. 13:1397, 1987). The sodium currents, whose kinetics are independent of membrane potential, but are dependent on light intensity, are modeled by assuming only a single rate-limiting chemical reaction between rhodopsin isomerization and channel activation. We develop a model of light-induced release of calcium which activates the calcium-dependent potassium current characterized by Sakakibara et al. The resulting calcium induced potassium currents have an early outward component and a later apparently inward component, whose time course and amplitude are consistent with the early and late light-induced potassium currents measured by Chen and Alkon.
Supported in part by ONR N00014-92-C-0018 and NIH/NINDS N01NS02389.

550.7

FACILITATION OF CALCIUM DEPENDENT POTASSIUM CURRENT. Stuart H. Thompson*. Hopkins Marine Station, Department of Biological Sciences, Stanford University, Pacific Grove, CA 93950.

Voltage clamp and Ca imaging methods were used to study the relationship between $(Ca)_i$ and potassium current activation in neurons isolated from the central nervous system of the nudibranch mollusc *Doriopsilla*. In these neurons, the activation of Ca-dependent K current (I_C) provides a linear index of $(Ca)_i$ immediately adjacent to the membrane. Loose patch voltage clamp was used to record currents from patches enriched in I_C and it was found that the current facilitates by factors as great as 30X during depolarizing pulses repeated at 1 Hz. This process resembles the facilitation of neurotransmitter release. Outward current facilitation requires Ca channel gating and Ca influx, and it is blocked by injected Ca buffer. The Ca indicator fluo-3 and conventional fluorescence video microscopy were used to image Ca concentration changes near the membrane and the interpretation of data was aided by a computer simulation of Ca active transport, diffusion, and binding. Analysis indicates that the accumulation of residual Ca is responsible for outward current facilitation. Recovery from facilitation ($t_{1/2}$ app. 15 sec) is strongly temperature dependent and slowed by removing external Na. This result indicates that the near-membrane Ca signal is shaped by Na-Ca exchange. Supported by BNS-9021217.

550.8

IDENTIFICATION OF THE RAPIDLY-ACTIVATING K^+ CURRENTS, I_A AND I_D, IN NEURONS OF THE RAT BASOLATERAL AMYGDALA. M.D. Womble* & H.C. Moises. Dept. of Physiology, Univ. of Michigan, Ann Arbor, MI 48109.

The rapidly activating K^+ currents, I_A and I_D, differ in their decay rates and sensitivity to blockade by 4-aminopyridine (4-AP). These currents contribute to action potential repolarization and the regulation of firing patterns in several types of central mammalian neurons. We investigated the role of rapidly activating, 4-AP sensitive currents in neurons of the basolateral amygdala (BLA), recorded at room temperature in a slice preparation of the rat ventral forebrain. In current-clamp mode, low concentrations of 4-AP (50-100 μM) produced a small broadening of current-evoked action potentials, a change that was further enhanced with subsequent application of 4-AP at higher concentrations (2-4 mM). In single-electrode voltage-clamp, low levels of 4-AP (50-100 μM) inhibited a rapidly activating, slowly decaying outward current, with an average peak amplitude of 269 ± 77 pA (±S.E.M.) and decay tau of 415 ± 48 ms (n = 7). Increasing the 4-AP concentration to 2-4 mM resulted in inhibition of a second rapidly activating outward current, with a larger peak amplitude (612 ± 210 pA) and much more rapid rate of decay (tau of 51.6 ± 7.6 ms; n = 5). Thus, BLA neurons possessed 2 rapidly-activating outward currents, separatable by their decay rates and 4-AP sensitivities, that contributed to action potential repolarization. These were a slowly decaying, 4-AP sensitive current similar to the I_D of other central neurons, and a rapidly decaying, relatively 4-AP insensitive current similar to I_A. (Supported by DA03365 & AG10667 to H.C.M.)

550.9

GABA INHIBITION OF TRANSIENT POTASSIUM CURRENT (A-CURRENT) IN CULTURED MOUSE HIPPOCAMPAL NEURONS. R.-L. Wu* and M.E. Barish, Division of Neurosciences, Beckman Research Institute of the City of Hope, Duarte, CA 91010.

We have observed in whole-cell voltage clamp recordings that application of γ-aminobutyric acid (GABA; 50 μM) to cultured mouse hippocampal neurons results in reversible inhibition of A-current. Hippocampal cells were dissociated on embryonic days 15-17 and studied after 3-7 days in culture. Since $GABA_A$ responses in embryonic neurons are depolarizing, two procedures were adopted to minimize the possible effects of GABA-induced depolarization of imperfectly-clamped membrane on availability of A-current: a) recordings were made using a potassium sulfate-based internal solution with which the reversal potential for the $GABA_A$-activated chloride current was approx. -72 mV, and b) A-current was measured after $GABA_A$-activated chloride current had desensitized to a steady state. Under these conditions the pharmacological profile of A-current inhibition indicated a dependence on $GABA_A$ receptor activation – inhibition was mimicked by muscimol (50 μM) and blocked by bicuculline (100 μM), but not affected by phaclofen (100 μM).

The mechanism of A-current inhibition is under investigation.

550.10

CALCIUM-BINDING MOLECULES CREATE EXTREME SPATIAL AND TEMPORAL LOCALIZATION OF CA^{2+} SIGNALLING IN SACCULAR HAIR CELLS OF *RANA PIPIENS*. W.M. Roberts*. Inst. of Neuroscience, Univ. of Oregon, Eugene, OR 97403.

In this theoretical study, I investigate how the high concentration of a mobile cytoplasmic Ca^{2+}-binding molecule in frog saccular hair cells (W. Roberts, *Nature*, in press) creates an environment in which Ca^{2+} signalling is highly localized in space and time. By capturing Ca^{2+} in <10 μs, it causes the local $[Ca^{2+}]_i$ to reach a new steady level nearly instantaneously after the opening or closing of each Ca^{2+} channel, and allows each K_{Ca} channel to respond only to Ca^{2+} influx from a few of its nearest neighbors within the dense cluster of Ca^{2+}- and K_{Ca} channels at presynaptic active zones. The delay between the opening of Ca^{2+} channels and K_{Ca} channels, which is a crucial part of electrical resonance in these cells, must therefore depend entirely upon intrinsic properties of the K_{Ca} channels, and not partly on the time-course of Ca^{2+} accumulation as previously modelled by Hudspeth & Lewis (*J. Physiol.* **400**:275-97). Their model can be modified to accurately reproduce voltage clamp data and electrical resonance assuming instantaneous changes in $[Ca^{2+}]_i$, but cannot be reconciled with other measurements of the K_{Ca} channels' steady-state Ca^{2+} dependence because it fails to take into account the random and extreme fluctuations in $[Ca^{2+}]_i$ that each K_{Ca} channel experiences as nearby Ca^{2+} channels open and close. To understand fast Ca^{2+} signalling in these cells, it is necessary to recognize that K_{Ca} channels and other Ca^{2+} receptors are exposed to stochastic changes in $[Ca^{2+}]_i$ that are much larger and more rapid than suggested by measurements of the whole-cell Ca^{2+} current. Supported by NIH grant NS27142, a McKnight Endowment Fund for Neuroscience Scholars Award, and an Alfred P. Sloan Foundation Fellowship.

550.11

POTASSIUM CURRENT COMPOSITION AND KINETICS IN TOADFISH SEMICIRCULAR CANAL HAIR CELLS. A.Steinacker,[1]* and S.M. Highstein[2]. [1]Institute of Neurobiology, Univ Puerto Rico Med. Sci. Cam. San Juan, PR 00901 [2]Depts. Oto./Anat.Neurobiol. Wash. Univ. Sch. Med. St. Louis, MO 63110.

The response dynamics of the semicircular canals are commonly assumed to be slow in comparison to those of the auditory system. In the hair cell, response dynamics should be determined by the properties of the hair cell bundle and the ionic currents. We have therefore measured hair bundle length and ionic currents in the toadfish (Opsanus tau) semicircular canal hair cells and compare them to comparable measurements made in the same animal for auditory (saccular) hair cells (Steinacker and Romero, **Brain Res.** 574: 229-236, 1991). Whole cell patch clamp was done at 15 °C from isolated canal hair cells using several voltage protocols, calcium concentrations and pharmacological agents to distinguish between the potassium currents. The pipette contained (mM) KCl 165, $CaCl_2$ 0.1, $MgCl_2$ 1.5, EGTA 10, Hepes 5, K^+ ATP 2.5 at pH 7.2 and the bath teleost Ringer with 4 mM $CaCl_2$. Two major outward currents were found, an A current and a IKCa. Current activation and deactivation was rapid in comparison to saccular hair cells. The A current, with rapid activation and inactivation at resting potentials, was a prominent feature of canal cells and rare in saccular cells. Little evidence of the delayed rectifier so prominent in the saccular cells was seen in the canal cells. Resonant frequencies of canal hair cells were significantly higher than in saccular cells. Stereociliary length, is long (around 100 microns) in canal cells, as expected the slow response dynamics. Saccular stereociliary lengths do not exceed 15 microns, except for those few in a peripheral border. These data suggests that canal and saccular cells are coding different aspects of a sensory stimulus. The high resonant frequency and rapid current kinetics may play a role in determining the high acceleration sensitivity observed in canal afferents (Highstein and Boyle, J. Neurosci. 10: 1557-1569, 1990).

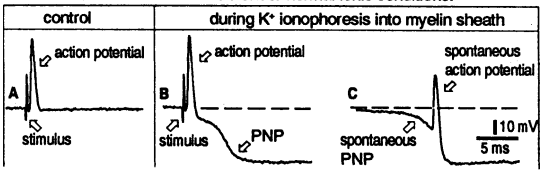
550.12

ACTION POTENTIALS ACTIVATE AN INTERNODAL POTASSIUM CONDUCTANCE IN RAT MYELINATED AXONS. G.David*, J.N. Barrett and E.F. Barrett. Dept. of Physiology & Biophysics, Univ. of Miami Med. Sch., P.O Box 016430, Miami, FL 33101.

Voltage changes associated with currents crossing the internodal axolemma of rat phrenic nerve fibers were monitored with a microelectrode inserted into the myelin sheath, to investigate activation of K channels in the internodal axolemma. Electrode location was verified by spread of ionophoresed dye throughout the myelin sheath of the impaled internode. The amplitude of the recorded action potential (AP) increased with increasing depth of microelectrode penetration into the myelin, but the resting potential remained near 0 mV.

Following ionophoresis of K^+ (or Rb^+, but not Na^+) from the microelectrode, APs were followed by a prolonged negative potential (PNP, Fig. B) lasting hundreds of ms. PNPs also arose spontaneously. Spontaneous APs sometimes occurred during the onset of the PNP (Fig. C), suggesting that the underlying axon was depolarized during the PNP. PNPs were associated with an increased conductance of the internodal axolemma, and were suppressed by tetraethylammonium (TEA) and 4-aminopyridine. These results suggest that PNPs are produced by a regenerative K current that enters the internodal axolemma via K channels opened by APs or subthreshold depolarizations. A similar mechanism may account for the ectopic APs observed after ischemia or prolonged repetitive firing, when extra-axonal $[K^+]$ may be greatly elevated.

APs recorded within the myelin sheath without artifically elevating $[K^+]$ were followed by a brief, positive, TEA-sensitive afterpotential, suggesting that APs can also activate internodal K channels under normal ionic conditions.



550.13

REMOVAL OF INTRACELLULAR POTASSIUM ALTERS K^+ CHANNEL SELECTIVITY IN CHICK DORSAL ROOT GANGLION NEURONS. M.J. Callahan and S.J. Korn*. Physiol. and Neurobiol. Dept., U. Connecticut, Storrs, CT 06269.

In whole cell patch clamp recordings from embryonic day 14 chick dorsal root ganglion neurons, replacement of intracellular K^+ with NMG^+ or Na^+ resulted in the development of a slow inward tail current (I_{cat}) carried by Na^+. I_{cat} was not observed when K^+_i was replaced by Cs^+. I_{cat} was activated by step depolarization for as little as 1 ms to potentials more positive than -40 mV, and inactivated in a time and voltage-dependent manner with prepulse durations on the order of seconds. I_{cat} was blocked by external Ba^{2+} (2 mM), but was unaffected by removal of $[Ca^{2+}]_o$, addition of Cd^{2+} (300 μM), or addition of intracellular EGTA (10 mM). Addition of as little as 3 mM K^+ to the intracellular solution blocked I_{cat} by ~90%; 20 mM K^+_i blocked I_{cat} by 100%. I_{cat} was not blocked by 10 mM Cs^+_i, TEA_i or TEA_o, but was significantly blocked by 3 mM Rb^+_i. Reversal potential measurements revealed that I_{cat} conducted Na^+, Cs^+ Rb^+ and K^+, but not Li^+, NMG^+, or TMA^+. Taken together, these data suggest that I_{cat} is a Na^+ conductance carried via a K^+ channel whose selectivity was altered upon removal of intracellular K^+. The activation and inactivation kinetics suggest that this channel may be a delayed rectifier K^+ channel.

Supported in part by The Whitaker Foundation, the PMA Foundation and the UCONN Research Foundation.

550.14

SPONTANEOUS FIRING IN THE NG108-15 NEUROBLASTOMA CELL LINE BY INTRACELLULAR pH ALTERATION. V. Kowtha*, K. Iwasa, H. Bryant, V. Krauthamer, D.Stenger. CSBE/NRL, Washington, DC,Dpt.Physiol.,USUHS,Bethesda, MD, LCB/NIDCD/NIH,Bethesda, MD,& EB/DPS/OST/CDRH/FDA, Rockville, MD.

Clay et al., (20th Ann Soc. Neuroscience Meeting 16:361, 1990) have shown stable spontaneous firing in the giant squid axon induced by changes in intracellular pH. We have recently observed similar automaticity (firing frequency ~ 2 Hz) when the intracellular pH was altered in the NG108-15 cell line plated in defined reduced and serum-free culturing conditions. When the cells were bathed in 20-50 mM NH_4Cl for a period of 1-2 minutes, a transient depolarization was observed (~ 5mV). When the cells were reperfused with normal extracellular recording medium (CONTROL), gradual hyperpolarization was observed (5-10 mV). The rate of hyperpolarization was a function of the NH_4Cl concentration. Stable spontaneous firing was observed in 3/8 attempts in both culturing conditions 15-20 minutes post CONTROL. Weak subthreshold oscillations were observed in cases where spontaneous firing was not observed. The changes in intracellular pH with the NH_4Cl pulse was characterized using SNARF-Calcein AM (pH sensitive dye, Molecular Probes, Eugene, OR). Intracellular pH was calibrated using a mixture of weak acid and weak base (butyric acid and trimethylamine) respectively (Thomas et al., Pfluegers Arch. 413:550,1987). The pH_i changes were biphasic, with an intial sharp increase followed by a slow rate of decline above baseline. With the reintroduction of CONTROL, a drop to near baseline and below was followed by a slow increase over baseline. In all cases, the threshold of firing was reduced and repetitive firing was observed at an increased current level.

550.15

CHARACTERIZATION OF POTASSIUM CURRENTS IN PYRAMIDAL NEURONS ACUTELY DISSOCIATED FROM ADULT RAT NEOCORTEX. M. H. O'Regan and R.R. Gala*. Dept. Physiology, Wayne State Univ. Sch. of Med., Detroit, MI 48201.

Potassium channels play a major role in regulating the excitability of mammalian central neurons. The ability of neurons to exhibit diverse firing patterns likely results from the presence of various potassium channels which differ in their voltage and calcium dependence and kinetics. The present study examined potassium currents using the whole cell configuration of the patch clamp technique. Cells were isolated from layers III to VI of rat sensorimotor cortex and were identified as regular spiking pyramidal neurons based upon their morphology and passive membrane characteristics. The most prominent outward current (recorded in the presence of TTX) activated rapidly, was sustained during the 150 ms test voltage pulse, and was sensitive to TEA. A transient current could be isolated which activated at more negative test potentials, was inactivated following a depolarizing prepulse, and decayed with a time constant of 9 ms. This current was reduced following the application of 4-AP. Blockade of Ca^{2+} channels by the addition of Co^{2+} to the bath solution resulted in an apparent enhancement of the transient current, presumably because of the loss of a counterbalancing inward Ca^{2+} current. Additionally, the sustained potassium current was reduced, indicating the presence of a sustained Ca^{2+} dependent component. The isolated currents displayed differences in their voltage dependence, activation and inactivation kinetics, and pharmacology which indicates varied roles in the regulation of neuronal activity. Since the activation of potassium channels is generally inhibitory, quiescent states in neocortex, such as that in the ischemic penumbra following the disruption of cerebral circulation, may result from the selective pathological influence of potassium currents.

550.16

OUABAIN-INDUCED HYPERPOLARIZATION OF MYENTERIC NEURONS INVOLVES ACTIVATION OF K^+-CHANNELS. J.-Q. Kong, J.A. Leedham, W.W. Fleming and D.A. Taylor*. Dept. Pharmacol. and Toxicol., WVU Hlth. Sci. Ctr., P.O. Box 9223, Morgantown, WV 26506-9223.

A reduction in resting membrane potential (RMP) of 'S' neurons in myenteric ganglia has been associated with the development of tolerance to opioids (Leedham et al., JPET 263: 15, 1992). To examine the contribution of Na-pumping on the RMP of myenteric 'S' neurons, studies were undertaken to determine the effect of ouabain-induced pump inhibition on these neurons. Standard intracellular recording techniques were employed with neurons exposed to drug via superfusion in the physiological salt solution. Of the 40 neurons exposed to ouabain (0.1 - 5.0 μM), 16 were hyperpolarized by 9.7 ± 2 mV and the remaining neurons either unaffected (13/40) or depolarized (9/40) by ouabain (10.4 ± 2.8 mV). Higashi et al. (J. Physiol. 389: 629, 1987) observed similar effects of ouabain in rabbit nodose ganglion cells due to activation of K^+-channels. To evaluate this possibility, the K^+-channel antagonist, TEA was used. TEA (0.5 mM) alone evoked modest (≤ 5mV) and inconsistent changes in RMP of 'S' neurons. However, in the presence of TEA, ouabain (5 μM) led to a substantial (23.4 ± 2.4 mV) depolarization in 11 of 12 neurons and no hyperpolarizations. In 5 additional cells, impalements with electrodes containing sodium acetate were obtained to induce intracellular sodium loading and elevation in sodium pump activity which led to a substantial resting hyperpolarization of ≥20 mV and a marked increase in ouabain-induced depolarization (34.2 ± 6.2 mV) in the presence of TEA. These data suggest that ouabain may activate 'S' neuron K^+-channels as well as inhibit the sodium pump. Under K^+-channel blockade or accelerated pump activity, the inhibitory effect of ouabain can be clearly observed. Supported by PHS grant DA 03773.

550.17

EFFECTS OF POTASSIUM CHANNEL OPENERS ON CATECHOLAMINE SECRETION AND Ca^{2+} MOBILIZATION IN CULTURED BOVINE ADRENAL CHROMAFFIN CELLS. Y.Masuda, H.Houchi* and M.Oka. Dept. of Pharmacology, Tokushima Univ. Sch. of Med., Tokushima, 770, Japan.

Potassium (K^+) channel openers have shown to produce relaxation of vascular smooth muscle. In contrast to the Ca^{2+}-channel blockers which have direct actions on the voltage-dependent Ca^{2+} channel, K^+ channel openers inhibit the voltage-dependent Ca^{2+} channels indirectly by activating the K^+ channel. K^+ channel openers may have additional actions on many excitable cells other than vascular smooth muscle. In cultured bovine adrenal chromaffin cells, K^+-channel openers, cromakalim and pinacidil stimulated the efflux of $^{86}Rb^+$ from the cells. 31 mM K^+ and carbamylcholine also increased the $^{86}Rb^+$ efflux which was dependent on the extracellular Ca^{2+}. These K^+-channel openers inhibited catecholamine secretion and $^{45}Ca^{2+}$ influx induced by 31 mM K^+ and carbamylcholine. Moreover, these K^+ channel openers were found to have inhibitory effect on Ca^{2+} release from the intracellular pools induced by carbamylcholine.

550.18

INWARD AND OUTWARD POTASSIUM CURRENTS IN CULTURED MOTONEURONS. J. G. McLarnon*, M. Michikawa and Seung U. Kim. Department of Pharmacology & Therapeutics, and Division of Neurology, Department of Medicine, Faculty of Medicine,The University of British Columbia, Vancouver, B. C., V6T 1Z3.

Electrophysiological studies have characterized unitary inward rectifier and calcium-dependent K^+ currents in identified (by immunostaining) mouse motoneurons. A 25 pS inward rectifier channel K(IR) was identified in cell-attached recordings with 140 mM K^+ in the patch pipette. The mean open times of K(IR) were decreased, with an exponential dependence, when patch hyperpolarization was increased. Channel open probability exhibited a sigmoidal dependence on potential with increased open probability at depolarizing potentials. Inactivation of K(IR) was evident with hyperpolarizing steps and ensemble averaged currents were lower with increased patch hyperpolarization. The rate of inactivation was faster with larger hyperpolarizing potential steps. External Cs^+ blocked K(IR) in a manner consistent with the predictions of a sequential open channel block model. Unitary properties of a large conductance calcium-dependent K^+ channel K(Ca) were also studied using inside-out patches. Channel conductance was 220 pS with symmetrical (140 mM) K^+ across patches and was 110 pS when external K^+ was 5 mM. The mean open times of K(Ca) were exponentially dependent on potential for both patch hyperpolarization and depolarization. Channel open probability exhibited a sigmoid dependence on potential with increased open probability at depolarized patch potentials. External, but not internal, TEA blocked K(Ca) currents at a concentration of 2 mM.

550.19

INWARD RECTIFIER CHANNELS IN EMBRYONIC SKELETAL MUSCLE CELLS IN CULTURE. F.L. Moody-Corbett*, S. Virgo, and S. Hancock. Division of Basic Medical Science, Memorial University of Newfoundland, St. John's, NF, A1B3V6.

Potassium inward rectifier (IR) current which is present in a number of cell types is important in establishing membrane excitability. Several lines of evidence suggest that there is more than one class of IR channels. We have previously described the macroscopic IR current in embryonic frog muscle cells in culture (Moody-Corbett and Gilbert,Dev. Br. Res., 55 (1990) 139). The purpose of the present study was to determine the characteristics of the IR channels underlying these macroscopic currents.

Skeletal muscle cells were dissected from stage 19-23 *Xenopus* embryos and grown in culture. Single channel events were recorded using a List patch clamp. Electrodes were filled with (in mM) 140 KCl, 5 $NaCl_2$, 1 $CaCl_2$, 10 HEPES, and .03 tetrodotoxin (TTX) and the extracellular recording solution contained (in mM) 140 NaCl, 5 KCl, 1 $CaCl_2$, 1.2 $MgCl_2$, 10 HEPES, and .03 TTX. The cells were held near resting membrane potential (RMP) and voltage steps applied for .1 or 5 sec.

Two classes of IR channels were analyzed. Both types had a high open probability at RMP and were seen together in a single patch. One class had a single channel γ of 26 pS, and was present in 60% of the patches examined. The other channel class had a γ of 10 pS and was much less frequently observed. Preliminary results suggest that the small γ channel was more prevalent in the youngest cells examined. The results suggest that at least two classes of IR channels exist in embryonic muscle cells.

550.20

THE EFFECT OF AXOTOMY ON K CHANNELS OF X-ORGAN NEURONS SOMATA. B. Mendiola, R. Godínez, R.F. Valdiosera and P. Huizar*. Depto. de Fisiología, CINVESTAV-IPN, México, D.F. 07000.

When X-organ neurons of the crayfish are axotomized at about 150 μm from the cell body, the neurons hiperpolarize and the input resistance decreases to about half the control values. Also, the action potential disappears or a slow depolarizing response remains. These effects could be explained by the opening of a background potassium channel and the inactivation of the channels responsable for the action potential (Ca, I_K and I_A channels). These effects are reverted by the extracellular application of 2 mM dibutyryl cyclic AMP to the neurons. In this study we have used cell-attached and inside-out patch clamp recordings to characterize the effects of axotomy and cyclic AMP on potassium channels of these neurons. The results suggest that in axotomized neurons, the channel responsible for the hiperpolarization is an inward rectifier channel since it is the most frequently observed in membrane patches of these neurons. In inside-out recordings, the most prominent effect of the application of the catalytic subunit of PKA and ATP, is an increase in the number of open channels of the delayed rectifier type, with a decrease in the number of the inward rectifier type. (Supported by CONACYT grant 1245-N9203).

ACETYLCHOLINE RECEPTORS: EXPRESSION OF MUSCARINIC RECEPTORS

551.1

DIFFERENTIAL EXPRESSION OF MUSCARINIC ACETYLCHOLINE RECEPTOR mRNAS AND THEIR SUBTYPES DURING DEVELOPMENT OF EMBRYONIC CHICK HEART AND RETINA. L.A. Parenteau* and N.M. Nathanson. Department of Pharmacology, University of Washington, Seattle, Washington, 98195.

Previous studies have shown there are two subtypes of muscarinic acetylcholine receptors (mAChR) expressed in embryonic chick heart, and suggested that at least two subtypes of mAChR are expressed in embryonic chick retina. We used solution hybridization to examine the level of mRNAs encoding cm2 and cm4 mAChR in chick heart and retina during embryonic development. There is five to ten times more cm2 mRNA than cm4 mRNA in heart at all stages examined. The levels of cm2 mRNA varied from 10 to 30 molecules per cell, while cm4 mRNA varied from 1 to 5 molecules per cell. In contrast, there is five to ten times more cm4 mRNA as cm2 mRNA expressed in retina. The levels of cm4 mRNA varied from 5 to 9 molecules per cell, while cm2 mRNA varied from 0.5 to 2 molecules per cell. The cm2 and cm4 receptor protein levels were measured by an immunoprecipitation assay using subtype specific antibodies generated against glutathione-S-transferase/mAChR fusion proteins. Data from these experiments confirmed that cm2 is the predominant subtype of mAChR expressed in embryonic chick heart, while cm4 is more highly expressed than cm2 in embryonic chick retina. In addition, our results suggest there is at least a third subtype of mAChR expressed in retina, since a significant fraction of the total population of mAChR in retina is not immunoprecipitated by anti-cm2 and anti-cm4 antibodies.

551.2

CHARACTERISATION OF THE MUSCARINIC RECEPTOR m4 GENE STRUCTURE AND ISOLATION OF ITS TRANSCRIPTIONAL CONTROL ELEMENTS. I.C.Wood, C.A.Harrington* and N.J.Buckley. Dept of Physical Biochemistry, National Institute for Medical Research, London, NW7 1AA.

Gene cloning studies have revealed the presence of five subtypes of muscarinic acetylcholine receptors. The coding exons for each of these genes have been obtained but the precise gene structure and transcriptional control elements for these genes have not yet been determined.

In order to obtain the transcriptional control elements for one of these genes, the m4 gene, a cosmid clone containing the m4 coding region and approximately 25 kb of upstream DNA sequence was isolated from a rat genomic library. A functional analysis using stably transfected cell lines demonstrated that this clone contained the transcriptional start site for the m4 gene and at least some of the tissue specific regulatory elements.

As only DNA sequence information from the coding region was available the positions and sizes of the upstream exons had to be determined using a selection of techniques including nuclear run-on analysis, a modified RACE analysis and PCR sequencing strategies. The data indicate that the transcription start site for the m4 gene is approximately 6 kb upstream from the initiating ATG and that the 5' untranslated sequences are contained within two exons which are separated from the coding exon by a 5.5 kb intron. DNA sequence analysis of the region immediately 5' of the transcription start site shows that the m4 gene lacks both TATA and CAAT boxes, however several consensus binding sites for other transcription factors have been identified.

551.3

REGULATION BY NERVE GROWTH FACTOR OF m4 MUSCARINIC ACETYLCHOLINE RECEPTOR mRNA EXPRESSION IN PC12 CELLS. N.H. Lee* and C.M. Fraser, Department of Molecular and Cellular Biology. The Institute for Genomic Research, Gaithersburg, MD 20878.

PC12 cells undergo differentiation to sympathetic neuron-like cells in response to nerve growth factor treatment. Concomitant with this phenomenon is an up-regulation of m4 muscarinic acetylcholine receptors (mAChRs) in plasma membrane homogenates (Jumblatt and Tischler, Nature 297:152-154). We now report on the effects of nerve growth factor on m4 mAChR mRNA expression in PC12 cells. Steady state levels of m4 mAChR mRNA were quantitated by Northern blot analysis and normalized to glyceraldeyhyde-3-phosphate dehydrogenase mRNA levels. Incubation of cultures of PC12 cells with nerve growth factor (50 ng/ml) induced an up-regulation of m4 mAChR mRNA that was significant after day 3 (1.8-fold) and maximal by days 12-15 (3 to 4-fold). Quantification of m4 mAChRs in intact cells demonstrated that induction of m4 mAChR mRNA levels was correlated with m4 mAChR up-regulation. m4 mAChR density was 43 fmol/mg protein under basal conditions and maximally increased by approximately 3-fold on days 12-15 of nerve growth factor treatment. The contribution of transcriptional and post-transcriptional events on nerve growth factor-induced up-regulation of m4 mAChR mRNA is currently under investigation by utilizing nuclear run-on and RNase protection assays, respectively. Preliminary results indicate that nerve growth factor may stabilize m4 mAChR mRNA in PC12 cells. The half-life of m4 mAChR mRNA in untreated cells was 2 hours and increased 3-fold following 9 days of nerve growth factor treatment. This study indicates that nerve growth factor up-regulates m4 mAChRs by controlling the expression of receptor mRNA levels.

551.4

EXPRESSION OF MUSCARINIC RECEPTOR SUBTYPES IN PC12 CELLS: EFFECTS OF DEXAMETHASONE. R. Basiboina, C. Hale, and R. Strong*. Department of Cellular and Structural Biology, University of Texas Health Science Center and GRECC, Audie L. Murphy Memorial Veterans Hospital, San Antonio, TX 78284 and Abbott Laboratories, Abbott Park, IL 60064.

Our laboratory is studying the role of transmembrane signal transduction in modulation of tyrosine hydroxylase gene expression in the adrenal chromaffin cell. Our initial attempts to use PC12 cells, a cell line derived from a tumor of the rat adrenal medulla, as a model system to study the function of muscarinic cholinergic receptors were impeded by the fact that they possess relatively low numbers of receptors. We considered the possibility that they would express higher receptor numbers if they were grown under conditions that more closely approximate the hormonal environment of the adrenal medulla. PC12 cells grown in the presence of nerve growth factor (NGF) express a phenotype similar to that of sympathetic neurons and show an increase in both muscarinic and nicotinic cholinergic receptors. On the other hand, corticosteroids direct the PC12 cell toward a chromaffin cell-like phenotype. In the present study, we examined the effects of dexamethasone on the expression of muscarinic receptor subtypes.

Preconfluent PC12 cells were treated for 15 days with dexamethasone. Muscarinic receptor RNA was determined from Northern blots of poly-A RNA extracts using cDNA probes for the various receptors. Dexamethasone treatment doubled the total number of muscarinic receptors as measured by atropine displaceable ^{3}H-QNB binding. Northern analysis of poly-A RNA extracts, using cDNA probes for the muscarinic receptor subtypes, detected the presence of only the m1 and m4 mRNA. However, dexamethasone treatment was only associated with an increase in the level of m4 mRNA. These findings parallel those of studies of the effects of NGF on muscarinic receptors and further show that the increase in muscarinic receptors is primarily due to an increase in the m4 subtype.

551.5

ENDOTHELIN-1 INCREASES MUSCARINIC ACETYLCHOLINE RECEPTOR mRNA AND c-FOS mRNA IN CEREBELLAR GRANULE CELLS. F. Fukamauchi*[1,2] and D.-M. Chuang[2] Medical Research Institute, Tokyo Medical and Dental Univ., 2-3-10, Kandasurugadai, Chiyoda-ku, Tokyo 101, Japan[1] and Section on Molecular Neurobiology, Biological Psychiatry Branch, NIMH, NIH, Bethesda, MD 20892, U.S.A.[2]

We have previously demonstrated that cerebellar granule cells express endothelin-1 (ET-1) receptors which mediate phosphoinositide hydrolysis and neurotransmitter release. To gain further insight into the functional role of ET receptors in these neurons, we have investigated the effects of ET on the mRNA levels for m2- and m3-muscarinic acetylcholine receptors (mAChRs). ET (10 nM) treatment of granule cells increased m2- and m3-mAChR mRNA levels with a maximal effect at 2 hr; the mRNA levels then declined and returned to the untreated level between 8 and 24 hr. The ET-induced up-regulation of m2- and m3-mAChR mRNAs was abolished by a 15-min pretreatment with phorbol dibutyrate (PDBu), a condition known to inhibit ET-induced phosphoinositide hydrolysis. Long-term (15 hr) treatment with PDBu, which was expected to deplete protein kinase C, also blocked the ET up-regulation, although PDBu alone elicited a significant increase of m3-mAChR mRNA. These results suggest a role of IP_3 production and /or protein kinase C activation in the effect of ET. The ET up-regulation was preceded by a transient increase of c-fos mRNA which peaked at 30 min after ET stimulation. Pretreatment with 2-aminopurine or cycloheximide abolished the ET-induced increase of c-fos mRNA as well as mAChR mRNAs and proteins, suggesting that induction of c-fos is involved in the ET-mediated mAChR up-regulation.

551.6

m2, m3 AND m5 MUSCARINIC ACETYLCHOLINE RECEPTORS mRNA EXPRESSION IN RAT MONONUCLEAR CELLS. P. Costa, D. J. Traver, A. F. Castoldi and L. G. Costa.* Dept. of Environmental Health SC-34, University of Washington, Seattle, WA 98195.

Several radioligand binding studies have demonstrated the presence of muscarinic acetylcholine receptors (mAChR) in lymphocytes from mice, rats and humans. The specific subtype(s) of mAChR present in rat lymphocytes are still unknown, although the m2 or m3 subtypes have been suggested. We analyzed the expression of m2, m3 and m5 mAChR subtypes in white mononuclear cells from rat blood and spleen. Northern blot hybridization and polymerase chain reaction on the reverse transcribed RNA (RT-PCR) were performed on the white mononuclear cells as well as other rat tissues used as positive controls (cerebral cortex, corpus striatum, hippocampus, brainstem, heart). No mRNAs for m2 or m3 subtypes could be detected by Northern blot in the white mononuclear cells. The more sensitive assay of RT-PCR gave a positive signal for the m3 subtype in both blood and spleen mononuclear cells. The level of expression of m3 was found to be about 1/1000 compared to the level of m3 mRNA in the hippocampus. No mRNA for m2 or m5 mAChR could be detected even using RT-PCR, suggesting the absence of the m2 and m5 subtypes in white mononuclear cells. (Supported in part by ES-04696).

551.7

GENERATION OF HERPES SIMPLEX VIRUS RECOMBINANTS FOR ALTERING EXPRESSION OF NEUROTRANSMITTER RECEPTORS IN INFECTED CELLS.
L. Gatzke*1, W. W. -G. Jia1, X. Wu1, M. Cynader2,and F. Tufaro1,2. Departments of 1Microbiology and Immunology and 2Ophthalmology, University of British Columbia, B.C. Canada.

The efficient introduction of genes into nerve cells is important both for the study of brain function as well as for therapy of neurological disorders. We have generated several HSV recombinants that express the human m1 muscarinic acetylcholine receptor gene (vTKhm1). Expression from this gene is driven by the CMV major I/E enhancer promoter inserted into the TK gene of HSV-1 vhsA, which contains a lac Z gene in UL41. The insertion was confirmed by Southern blots, and transcription from the CMV promoter was measured by RNase protection assays. Muscarinic receptor transcripts originating from the CMV promoter were detected from 2 to 20 h post-infection in vero cells. Ligand binding experiments with radiolabeled N-methyl scopolamine show a fifty fold increase in receptor expression by 12 h post-infection compared with control cells infected with wild type virus. Moreover, new receptors appeared as early as four h post-infection. Efforts are underway to use this recombinant herpes virus for functional studies of neurotransmitter receptors in a variety of neuronal populations.

ACETYLCHOLINE RECEPTORS: MUSCLE

552.1

Cloning of an Alpha-like Neuronal Acetylcholine Receptor (AChR) Subunit from *Aplysia californica*. R. E. McCaman* and J. K. Ono, Division of Neurosciences, Beckman Research Institute of the City of Hope, Duarte, CA 91010 and Dept. of Biological Science, California State University, Fullerton, CA 92634.

Studies of neurotransmitter receptors in *Aplysia* carried out on individual, identified neurons suggest a rich diversity of receptor subtypes differentiated by ionic selectivity and pharmacological sensitivity for each transmitter. Identification of the molecular bases of this diversity should provide unique insights into how structure relates to function in such arrays of neuronal receptors. Using a variety of techniques (polymerase chain reaction, screening a cDNA library from the circumesophageal ganglia, and 5' rapid amplification of cDNA ends) we have obtained a 2.5 Kb sequence (A15) that has 2 putative start codons. This sequence exhibits marked similarity to previously cloned sequences encoding AChR alpha-subunits from other species such as *Drosophila*, mouse and man. RNA extracted from specific *Aplysia* neurons was reverse transcribed using an A15 sequence-specific primer. The resultant cDNA served as a target for amplification with PCR using various pairs of A15 sequence-specific primers. The results of these experiments indicate that the message encoding our cloned sequence is specifically expressed by those neurons exhibiting the nicotinic AChR in *Aplysia* that selectively gates Na^+ channels and is inhibited specifically by hexamethonium. These studies demonstrate the feasibility of correlating the structure of cloned subunits with the known functional types of native neurotransmitter receptors present in individual, identified neurons. *Supported by NIH grants to REM and to JKO, Beckman Research Funds to REM and CSU mini-grants to JKO.*

552.2

BINDING OF THE ACHR TO SH2 DOMAINS OF FYN AND FYK PROTEIN TYROSINE KINASES S. L. Swope* and R. L. Huganir. Dept. of Neuroscience, Howard Hughes Medical Inst., The Johns Hopkins Univ. Sch. of Med., Baltimore, Md. 21205

The nicotinic acetylcholine receptor (AChR) is phosphorylated on tyrosine both in vitro and in vivo. To identify the protein tyrosine kinase (PTK) which phosphorylates the receptor, we have cloned and characterized two PTK that are highly expressed in Torpedo electric organ, a tissue enriched in the AChR. One of the kinases was the homolog of neuronal fyn while the other was a novel kinase we have named fyk due to its homology to fyn and yes PTK. Both kinases were shown by coimmunoprecipitation to be specifically associated with the AChR. In this study, we examined the molecular basis for the interaction of fyn and fyk with the AChR. SH2 domains, originally identified in the src class of PTK, interact with phospho-tyrosine containing proteins. Therefore, fusion proteins containing the SH2 domains of fyn and fyk were prepared and used as affinity reagents. The SH2 domains of fyn and fyk specifically bound the AChR. The association of the AChR with the SH2 domains was blocked by phospho-tyrosine and phenylphosphate, but not by phospho-serine or phospho-threonine indicating that the interaction was dependent on tyrosine phosphorylation of the AChR. The AChR β, γ, and δ subunits are each phosphorylated on a single tyrosine on the major intracellular loop. Upon dissociation of the AChR into subunits, the δ but not the β or γ subunits bound to the SH2 domain of fyn and fyk. These data suggest that the association of the AChR with fyn and fyk is mediated by an interaction of the tyrosine-phosphorylated intracellular loop of the of the receptor δ subunit with the SH2 domain of the PTKs.

552.3

CYTOSKELETAL ELEMENTS MAY BE INVOLVED IN THE RESENSITIZATION OF THE MOTOR ENDPLATE FOLLOWING PROLONGED CARBACHOL EXPOSURE. J.C. Hardwick and R.L. Parsons. Department of Anatomy and Neurobiology, University of Vermont, Burlington, VT 05405.

We have suggested that recovery of endplate sensitivity following a 5-10 min exposure to 540 μM carbachol may require the recruitment of AChRs from a readily available pool to replace receptors which were irreversibly inactivated by prolonged agonist exposure (Hardwick & Parsons, Br J Pharmacol, 108:741, 1993). To test whether elements of the subsynaptic cytoskeletal system are involved in the recruitment of new receptors, we studied the influence of colchicine pretreatment (10^{-4}M for 16-18 hr) on the extent of MEPC amplitude recovery following a 10 minute application of 540 μM carbachol. All experiments were done using potassium-depolarized twitch fiber endplates from snake costocutaneous muscles voltage clamped to -100 mV. In preparations treated with colchicine, but not exposed to carbachol, MEPC amplitudes and decay time constants were similar to control preparations. Following carbachol exposure, mean MEPC amplitude (but not the decay constant) was significantly less at colchicine-treated endplates than at control endplates. Prior to carbachol application, the single channel conductance was similar at control and colchicine-treated preparations. However, at colchicine-treated endplates, following recovery from carbachol exposure, large (41 pS) and small (28 pS) ACh-activated channels were recorded. These results suggest that colchicine, presumably by disrupting subcellular cytoskeletal organization, interferes with the conversion of the single channel conductance from a small to large conductance state in the replacement AChRs. Supported by NIH grants NS 08580 to J.C.H. and NS 23978 to R.L.P.

552.4

REMOVAL OF THE DISULFIDE LOOP STRUCTURE FROM *Torpedo* nACHR SUBUNITS ALTERS FUNCTIONAL PROPERTIES OF THE RECEPTOR. E.C. Walcott * T.Nishizaki, and K. Sumikawa. Dept. of Psychobiology, University of California, Irvine, CA 92717.

We have previously demonstrated that the formation of a conserved disulfide bond between Cys-128 and Cys-142 residues on β subunits of the *Torpedo* nicotinic acetylcholine receptor (AChR) is not necessary for receptor assembly; however, is required for efficient insertion of receptors into the plasma membrane. When site-directed mutant β mRNA was injected together with normal α,γ,δ mRNAs into *Xenopus* oocytes and assayed using two-electrode voltage clamp, expressed receptors show >94% reduction in ACh response as compared to normal AChRs, suggesting that many AChRs remain within intracellular compartments (*J Biol.Chem.* 267(9):6286,1992).

To further investigate the role of tertiary structure in AChR function, we analyzed mutant receptors lacking the conserved disulfide loop structure in the β subunit at the single channel level, using patch clamp in the outside-out patch configuration. The single channel current/voltage (I/V) relations indicated that the conductance of mutant AChRs (17.1±1.8 pS, n=3) was lower than that of wildtype AChRs (30.7±4.9pS, n=23). Mean open channel time (1.5±0.3mS, n=3) did not differ from normal AChRs. These results indicate that the extracellular tertiary structure of the β subunit of the AChR is important for receptor function. Current studies are aimed at defining the functional roles of tertiary structure in γ and δ mutant subunits. (Supported by NIMH- NRSA 14599-17 and the Muscular Dystrophy Association).

552.5

NICOTINIC ACETYLCHOLINE RECEPTOR ASSEMBLY MAY BE REGULATED THROUGH PHOSPHORYLATION OF A SECONDARY PROTEIN S. Jayawickreme and T. Claudio*. Dept. Cellular & Molecular Physiology, Yale Univ. Sch. of Med., New Haven, CT 06510.

The nicotinic acetylcholine receptor (AChR) is a pentameric complex composed of four different subunits with a stoichiometry of $\alpha_2\beta\gamma\delta$. The efficiency of AChR subunit assembly can be enhanced 2-fold by treating cells (either muscle cells or non-muscle cells expressing recombinant AChR) with the protein kinase A (PKA) activator, forskolin. We have shown that although γ and δ subunits undergo phosphorylation by PKA, only phosphorylation of the γ subunit correlates with an increase in assembly efficiency (Green et al. (1991) Neuron 7:659-666). The two consensus PKA sites (serine) of γ were singly or doubly mutated to alanines. Mouse L fibroblasts expressing wildtype $\alpha\beta\delta$ plus mutant γ were established. ^{32}P-orthophosphate labelling of cells showed phosphorylation of the two single mutant γ subunits. The expression of all three mutant receptors was, however, stimulated by forskolin demonstrating that phosphorylation of either or both sites does not lead to enhanced assembly of AChR subunits. To test whether or not phosphorylation of δ can assume the role of γ in stimulating assembly, a cell line expressing wildtype αβ plus doubly mutated γ was established. When treated with forskolin, assembly of subunits was still enhanced, indicating that stimulation is not mediated through phosphorylation of AChR subunits and suggesting that it may occur through phosphorylation of a protein which is involved in AChR subunit assembly.

552.6

TYROSINE KINASE INHIBITORS BLOCK AGRIN-INDUCED ACETYLCHOLINE RECEPTOR CLUSTERING ON CULTURED CHICK MYOTUBE. Z. Qu* and R. L. Huganir, Howard Hughes Medical Institute, The Johns Hopkins University School of Medicine, Baltimore, MD 21205.

The nicotinic acetylcholine receptor (AChR) is a ligand-gated ion channel that mediates signal transduction at the neuromuscular junction. The AChR from Torpedo electric organ and mammalian skeletal muscle is a pentameric complex of four subunits in the stoichiometry of $\alpha_2\beta\gamma\delta$. A number of experiments have shown that phosphorylation of neurotransmitter receptors plays an important role in the regulation of synaptic transmission. Recent studies have demonstrated that the AChR is regulated by phosphorylation by several serine and tyrosine kinases. Our previous data suggest that tyrosine phosphorylation of the AChR is regulated by innervation and the neuronal protein agrin. Agrin, a synaptic basal lamina protein released by motor neurons, causes the AChR, a 43kDa protein and other components on cultured chick myotube to aggregate, forming specializations that resemble the postsynaptic apparatus at the neuromuscular junction. We have attempted to further define the relationship between tyrosine phosphorylation and acetylcholine receptor clustering. A specific tyrosine kinase inhibitor, herbimycin, has been used to inhibit agrin-induced tyrosine phosphorylation of the AChR and possibly other proteins and results in inhibition of agrin-induced AChR clustering. In addition, herbimycin disperses previously formed agrin-induced receptor clusters on cultured chick myotubes. These results suggest that tyrosine phosphorylation may play an important role in acetylcholine receptor clustering and developmental regulation of synaptic formation at the neuromuscular junction.

552.7

ACETYLCHOLINE RECEPTOR CLUSTERING IN MYOTUBES IS REDUCED BY ELEVATED LEVELS OF THE 43K PROTEIN. C. M. Yoshihara and Z. W. Hall*. Dept. of Physiology, University of California, San Francisco, CA 94143-0444.

A key event in the development of the vertebrate neuromuscular junction is the formation of high density clusters of acetylcholine receptors (AChR) in the postsynaptic membrane. AChR clustering has been thought to occur by their association with a cytoplasmic, peripheral membrane protein (43K protein) that occurs in approximately 1:1 stoichiometry with the AChR. We have sought to examine the regulatory role of the 43K protein in AChR clustering by overexpressing it in muscle cells, in which it normally occurs. We isolated C2 muscle cell lines in which some cells overexpress the 43K protein and found that myotubes with increased levels of the 43K protein have small AChR clusters and that those with the highest levels of expression have a drastically reduced number of clusters. The reduction in cluster size and number was not due to a decrease in AChR expression. Examination of surface AChR expression in individual myotubes by autoradiography revealed no difference between high and low expressors. Moreover, the 43K protein expressed in the myotubes appears to be functional. It is able to form co-clusters with AChRs in myotubes and in transiently transfected COS cells. Thus, increased expression of the 43K protein appears to interfere with normal AChR cluster formation by muscle cells. Our results suggest that the 1:1 stoichiometry of the 43K protein and the AChR is important for AChR clustering. This work was supported by grants from the NIH, the Muscular Dystrophy Association, and by a President's Fellowship from the University of California.

552.8

INCREASES IN THE PROPORTION OF SLOWLY DEGRADING ACETYLCHOLINE RECEPTORS IN RAT MUSCLE CELL CULTURES. J.P. O'MALLEY* & M.M. SALPETER Section of Neurobiology & Behavior, Cornell University, Ithaca NY 14853

Cultured rat myotubes express two AChR populations decaying with half-lives of 1 day (rapidly decaying AChRs or R_f) and 3 days (slowly decaying AChRs or R_s) in a ratio of 9:1 respectively, which is similar to that seen at the denervated neuromuscular junction (nmj). At the nmj reinnervation leads to a downregulation of R_f and an upregulation of R_s.

To determine if neuronal factors regulate the relative expression of R_f and R_s, we investigated the role of ATP (released from the nerve during transmission) and dB-cAMP (which resembles innervation in its effect on AChR stability [Salpeter et al Annls NY Acad Sci, 1993]). Myotubes were treated with either 1 mM ATP or with 1 mM dB-cAMP three days after seeding on Matrigel substrates. Cultures plated on Matrigel can be maintained for prolonged periods of time, even after the initiation of spontaneous activity. The treatment was continued for 0 to 13 days before the AChRs were labeled with [^{125}I]-α-bungarotoxin. The loss of label was then measured for the next 13 days in the presence of the test drugs. We found that ATP progressively increased the expression of both R_f and R_s relative to controls, with the increase in R_s slightly exceeding that of R_f. Conversely dB-cAMP markedly suppressed R_f expression but had little effect on R_s.

Thus, like innervation, both cAMP and ATP increased the relative amount of R_s expressed in the myotubes. However cAMP suppressed R_f while ATP upregulated AChR favoring R_s. We are presently comparing these drug-induced changes in AChR expression with innervation to determine if alone, together or in combination with muscle activity they can induce the same developmental changes as innervation. Supported by NIH grant NS09315 (MMS) and MDA postdoctoral fellowship (JOM)

552.9

Heterogeneous Expression of Acetylcholine Receptor (AChR) Subunit mRNAs in Muscles is Independent of Fusion. X. Su, S. Berman, T. Sullivan and S. Bursztajn*. Mailman Research Center, McLean Hospital and Harvard Medical School, 115 Mill Street, Belmont, MA 02178.

Muscle progenitor cells differentiate into myoblasts and subsequently myotubes upon expression of muscle specific genes which are induced by myogenic factors. We have been interested in studying whether muscle specific genes are switched on uniformly in nuclei of myoblasts and myotubes. In situ hybridization study with myoD (myogenic factor) and U1 (intron splicing factor) gene probes showed uniform expression of these genes among nuclei of myoblasts, fusing myoblasts, and myotubes. However, study with AChR subunit (α, γ, δ) specific intron or exon-intron probes revealed that AChR subunit genes were expressed heterogeneously by the nuclei. The overall expression level of subunit genes was doubled after cell fusion and declined in mature muscle cells. The result indicates that the presence of myogenic factors does not induce full expression of AChR subunit genes in all muscle nuclei. The result can be interpreted to mean that the nuclei of muscle cells are programmed for heterogeneous expression of AChR genes before and after fusion.

552.10

INFLUENCE OF SPONTANEOUS MUSCLE ACTIVITY ON ACETYLCHOLINE RECEPTOR EXPRESSION IN CULTURED MAMMALIAN MYOTUBES. C.G. Carlson*, A.K.Q. Hasan, S.D. Adkins, M.J. Blake, A.M. Bode, S. Loyland. Depts. of Physiology and Pharmacology/Toxicology, Univ. N. Dakota School of Medicine, Grand Forks, N.D. 58202.

The influence of chronic exposure to tetrodotoxin (TTX) and Veratridine (VER) on Acetylcholine Receptor (AChR) expression was examined in long term cultures of mouse myotubes maintained on a laminin substrate. A significant effect ($p<0.05$) of drug exposure (between Culture Day (CD) 7 and 17) on total surface AChRs (I-125 alpha - bungarotoxin binding) was observed in 6 of 7 individual culture runs. TTX significantly ($p<0.05$) upregulated total surface AChRs by 54% on CD 13 in 3 of 5 culture runs and by 85% on CD 17 in 4 of 7 culture runs. VER failed to significantly influence total surface AChRs in 6 of 7 culture runs and produced a significant increase in only 1 of 12 comparisons performed at CD 13 and 17. Northern transfers indicated a substantial downregulation of embryonic AChR subunit mRNA's between CD 13 and 23 in VER treated myotubes. In contrast to effects on total surface AChRs, VER substantially downregulated and TTX produced only a slight increase in gamma subunit mRNA at CD 13 to 15 (2 of 2 culture runs). These results suggest that the disparate actions of TTX and VER on surface AChR expression are not due to opposing actions on a common activity - dependent mechanism (e.g., intracellular calcium accumulation) of AChR regulation. Supported by a grant from M.D.A.

552.11

TRANSCRIPTIONAL ACTIVATION OF THE MOUSE MYOGENIN GENE BY DENERVATION IS CONFERRED BY 5' *CIS*-ACTING ELEMENTS. G. Gibney, J. M. Sasner* and A. Buonanno. Laboratory of Developmental Neurobiology, NICHD, NIH, Bethesda, MD 20892.

Denervation of mouse hindlimb skeletal muscle results in a rapid accumulation of myogenin mRNA which precedes increases in nicotinic acetylcholine receptor (nAChR) subunit transcripts by 16-24 h. (Eftimie *et al.* (1991) Proc. Natl. Acad. Sci. *88*, 1349.) Since myogenin activates transcription of nAChR genes *in vitro* through E-box binding, it is probable that activation of the myogenin gene is more proximal to denervation in the pathway leading to induction of receptor subunit genes. We are employing *in vivo* and *in vitro* approaches to identify transcriptional regulatory elements of the mouse myogenin promoter-enhancer which confer activation by denervation. Transgenic mice have been created which carry a 3.7 kb upstream sequence of the myogenin gene driving a chloramphenicol acetyltransferase (CAT) reporter. These transgenic mice appropriately express reporter with respect to tissue- and developmental-specificity. Expression of the transgene (MYG3700.CAT) in adult hindlimb muscle increases 10-fold within 24 h after denervation; maximal levels of 40-fold are obtained by 4 d. We have generated stable C2 myoblast lines carrying successive 5'-deletion mutants of MYG3700.CAT as an efficient means of delineating *cis*-acting elements mediating the activity response. Effects of activity on myogenin gene transcription are analyzed by electrically stimulating C2 myotube cultures derived from the cell lines. To extend our analysis *in vivo*, we are testing the myogenin-CAT lines by myoblast implantation into mouse hindlimbs. Using this technique, we can demonstrate activation of nAChR α-subunit and myogenin reporter genes by denervation of the implanted hindlimb; in parallel experiments, activity of a myosin light chain reporter remains relatively unaffected by denervation. With this combined approach, we expect to determine which *cis*-acting control elements within the myogenin promoter confer differential gene expression with respect to electrical activity.

552.12

THE TRANSMEMBRANE TOPOLOGY OF THE NICOTINIC ACETYLCHOLINE RECEPTOR: A MOLECULAR MODELLING APPROACH. M.O. Ortells[1] and G.G. Lunt*. Biochemistry Department, University of Bath, Bath, BA2 7AY, UK.

The nAChR is formed from five subunits, each with an extracellular N-terminal domain, a transmembrane region (TM), and an extracellular C-terminal domain. The TM is believed to be composed of four α-helices, M1 to M4. The four-helix bundle structure is based mainly on hydrophibicity profiles. The best characterised of the nAChR TMs is M2 and there is a wealth of information that identifies this as the region that lines the ion-channel. Most of these data are only compatible with an α-helix conformation for M2. The molecular modelling started from the ion channel region using the α-1 Torpedo sequence of M2. The final model of the ion-channel comprises a five helical bundle with a left-handed twist. The angle between adjacent helices is around 12°. The diameter of the narrowest part of the pore, from E237 to S240 is 7.5Å and its length is 8Å. The channel is wider at the extracellular end. When chlorpromazine is docked into the channel it can interact with three labelled residues in the *Torpedo* receptor.

To model the remainder of the TM, we used the X-ray structure of myohaemeryhrin as a template for a four-helix bundle. Five copies were superimposed onto the ion-channel using the shared M2 helix. In this model, M4 is the most exposed to the membrane, and the twist imposed on M2 allows it to be perpendicular to the membrane.

Interestingly M1 of one bundle overlaps in one region with M3 of the neighbouring bundle. Different orientations of the bundle (with the position of M2 fixed) gave the same results, although they superimpose in different regions. Given this conformation, it is also evident that M3 is quite exposed to the membrane environment, thus it is surprising that this segment has not been labelled by lipophilic reagents as much as M4.

Our models suggest may suggest that neither of these two transmembrane regions are α-helices but may be (partially) β-sheets. In this case, these two domains must protrude into either the extracellular or cytoplasmic regions. No models of these alternatives have yet been made (for example, to form a ten β-sheet barrel), but clearly this must be a high priority for future studies.

[1] Fellow of the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET, INIBIBB), ARGENTINA, and partially supported by Fundación Antorchas (ARGENTINA) and The British Council

EXCITATORY AMINO ACIDS: EXCITOTOXICITY II

553.1

CYCLOTHIAZIDE POTENTIATION OF OXYGEN AND GLUCOSE DEPRIVATION-INDUCED ^{45}Ca UPTAKE AND LDH RELEASE IN CEREBROCORTICAL CELL CULTURE. A.W. Probert* and F.W. Marcoux. Neuroscience Pharmacology, Parke-Davis Pharmaceutical Research, Division of Warner-Lambert Co. 2800 Plymouth Road, Ann Arbor, MI 48105.

Cerebrocortical culture exposures to combined oxygen and glucose deprivation ($\downarrow O_2$/Gluc = 1% O_2 / 1mM D-glucose) increases ^{45}Ca uptake acutely and causes neuronal death (LDH release 24 hours later). During glucose deprivation, increasing hypoxic intervals (150 - 240 mins) increased ^{45}Ca uptake and LDH release. CPP (100μM), an NMDA antagonist, blocked both measures effectively (90-100% inhibition) following $\downarrow O_2$/Gluc intervals $\leq$210 minutes. NBQX (10μM), an AMPA antagonist, was less effective than CPP, inhibiting ^{45}Ca uptake (45-54% inhibition) and LDH release (72-79% inhibition) up to 170 mins, but not thereafter. Cyclothiazide (30μM), an inhibitor of AMPA receptor desensitization, potentiated (41-379% increase) both ^{45}Ca uptake and LDH release after $\downarrow O_2$/Gluc of $\leq$170 mins, but that potentiation did not exceed levels observed after longer deprivation intervals ($\geq$190 min) in controls. After $\downarrow O_2$/Gluc intervals of 150-170 mins NBQX and CPP blocked cyclothiazide's potentiation of ^{45}Ca uptake and LDH release (83-100% inhibition). Following $\downarrow O_2$/Gluc intervals of 190-210 mins, NBQX, which had no effect on ^{45}Ca uptake and LDH release in controls, partially blocked (54-60% inhibition) both measures in cyclothiazide treated cultures. CPP, which inhibited ^{45}Ca uptake and LDH release after $\downarrow O_2$/Gluc of 190-210 mins in controls, partially blocked (64-86% inhibition) both measures in cyclothiazide treated cultures. Whereas CPP (38-61% inhibition) but not NBQX blocked ^{45}Ca uptake and LDH release after 240 mins of $\downarrow O_2$/Gluc in controls, neither CPP nor NBQX inhibited these measures in cyclothiazide treated cultures. These findings suggest that AMPA receptors can potentiate ^{45}Ca uptake and LDH release after $\downarrow O_2$/Gluc in cerebrocortical cultures.

553.2

CYCLOTHIAZIDE ENHANCES AMPA- AND KAINATE-INDUCED CELL DEATH IN CULTURED RAT CORTICAL NEURONS. G.W. Campbell* and D.M. Rock. Neuroscience Pharmacology Dept., Parke-Davis Research, Division of Warner Lambert Co., Ann Arbor MI 48106.

Cyclothiazide and related compounds, which are clinically useful anti-hypertensive agents, have been shown in electrophysiological studies to reduce glutamate-induced desensitization and prolong excitatory postsynaptic currents. We studied the effects of cyclothiazide on α-amino-3-hydroxy-5-methylisoxazole-4-proprionic acid (AMPA)- and kainate (KA)-induced cell death in cultured rat cortical neurons. To quantify the degree of cell death, activity of the cytosolic enzyme lactate dehydrogenase (LDH) was measured from samples of medium taken from cultures following long applications (20-24 hrs) of either AMPA or KA in the presence or absence of cyclothiazide.

Cyclothiazide (10 and 30 μM) caused a pronounced shift in the EC_{50} of both AMPA and KA concentration response curves and an increased the maximal responses of both AMPA and KA. CPP (100 μM), a competitive NMDA receptor antagonist, reduced the increase in maximal response, suggesting that the increase in maximal response may be due to the action of released glutamate on the NMDA receptor.

These studies suggest that cyclothiazide, which affects glutamate-induced desensitization, can cause pronounced effects making long applications of both AMPA or KA more effective in producing neuronal cell death.

553.3

BRAINSTEM INFUSION OF GLUTAMATE RECEPTOR ANTAGONISTS SUGGESTS A ROLE OF NON-NMDA RECEPTORS IN SPONGIFORM DEGENERATION OF THE GERBIL COCHLEAR NUCLEUS. B.T. Faddis* and M.D. McGinn. Dept. Otolaryngology, Univ. of Calif. Sch. of Med., Davis, CA 95616.

A naturally occurring spongiform degeneration of the gerbil cochlear nucleus has been shown to be strongly dependent on auditory functional activity, suggesting an excitotoxic origin. We previously showed that unilateral brainstem infusions of kynurenic acid reduced degeneration when compared to the contralateral side (Soc. Neurosci. Abst., 17:1484). Here we report that brainstem infusions of 100 μM and 540 μM CNQX, a non-NMDA receptor antagonist, reduces lesion number density by 28% compared to the contralateral side ($F_{1,27}$=9.290, p=0.0052 and $F_{1,27}$=9.332, p=0.0052). A mixture of 540 μM CNQX + 10 mM MK-801 was also effective ($F_{1,27}$=10.727, p=0.0032), but no more so than CNQX alone. 10 mM 5,7-dichlorokynurenic acid (KYN) caused a 37% reduction in lesion number density ($F_{1,27}$=7.761, p=0.0094). Infusions of saline, 100 μM and 10 mM MK-801 and 100 μM KYN showed no significant effect. These results suggest that the induction of spongiform lesions is mediated by non-NMDA glutamate receptors and not by NMDA receptors.

553.4

ELECTROPHYSIOLOGICAL CORRELATES OF AMPA-INDUCED NEUROTOXICITY OF NEONATAL RAT PURKINJE CELLS (PCs). I. C. Strahlendorf* and H. K. Strahlendorf. Depts of Physiology and Neurology, Texas Tech University Health Sciences Center, Lubbock, TX, 79430.

It is becoming increasingly evident that AMPA is a neurotoxin. The rat cerebellar PC is an excellent model neuron of AMPA toxicity because it is rich in AMPA receptors and relatively devoid of NMDA receptors. We electrophysiologically characterized the events associated with AMPA-induced toxicity utilizing whole cell patch clamp recordings of early postnatal PCs (5-21 days) in the rat cerebellum in vitro slice preparation.

AMPA exposure (30μM for 30 min., [trigger phase]) caused a marked depolarization (inward current) with increased conductance that displayed varying degrees of desensitization. During the expression phase (90 min following AMPA exposure), membrane potentials of PCs often returned to control levels; but within 65-80 min, a sustained depolarization preceded by Ca spikes and an increased frequency of inward synaptic currents was evident. These events and the morphological correlates could be prevented with the continuous application of the AMPA antagonist CNQX, although treatment during the trigger phase alone was ineffective. Furthermore, depolarization alone induced by voltage clamping PCs at -10mV for 30 min was ineffective in mimicking AMPA toxicity. These studies provide electrophysiological correlates for the morphological changes that previously have been described by Garthwaite and colleagues. (Excitatory Amino Acids and Neuronal Plasticity, Plenum Press, Ed. Ben-Ari [1990], p. 505.)

553.5

ALTERATION OF THE GLUR-B AMPA RECEPTOR SUBUNIT FLIP/FLOP EXPRESSION AND STOICHIOMETRY IN KAINATE-INDUCED EPILEPSY AND ISCHEMIA. H.Pollard, A. Heron+, J. Moreau, C. Charriaut-Marlangue, M. Khrestchatisky, J. Epelbaum*, and Y. Ben-Ari. INSERM, U 29, 123 Brd de Port Royal, 75014, Paris, France.

In the hippocampus, glutamatergic pathways are altered following seizure activity or transient global ischemia, both leading to selective neuronal degeneration. Glutamatergic receptors, and notably AMPA receptors, may play an important role in the pathological outcome. AMPA receptors are assembled from GluR-A, GluR-B, GluR-C and GluR-D polypeptides which exist in flop and flip variants, the latter allowing larger glutamate currents. Using in situ hybridization we show that following kainate administration (ip or intraamygdala) there is a rapid (3 hours) and transient increase (50%) of GluR-B flip mRNA levels in CA1, CA3 and dentate gyrus. This early phase is followed by a second, persistent (1-3 days), GluR-B flip increase in seizure-resistant areas (CA1 and dentate gyrus) and a 40% decrease in the vulnerable CA3 area. Following global ischemia, the levels of GluR-B flip and flop variants are dramatically reduced (90-100%) in the subiculum and CA1, two areas particularly sensitive to ischemic insult. In keeping with the properties of GluR flip variants, it is suggested that altered subunit stoichiometry may lead to long-lasting enhanced efficiency of synaptic transmission in the epileptic hippocampus. Since GluR-B containing receptors are Ca^{2+} impermeable, our results also suggest altered Ca^{2+} permeability in the vulnerable pyramidal neurons of areas CA3 and CA1 in the epileptic and ischemic hippocampi respectively.*Sponsoring author, INSERM, U 159. + CNRS, U 641.

553.6

ENHANCED EXPRESSION OF THE GLUR6 KAINATE RECEPTOR SUBUNIT INDUCES EXCITOTOXIC LOSS OF CA3 AND HILAR NEURONS IN HIPPOCAMPAL SLICE CULTURES. P. Casaccia-Bonnefil*, E. Benedikz, H. Shen, H.J. Federoff^, and P.J. Bergold. SUNY-Health Science Center at Brooklyn, Brooklyn, NY, 11203; ^Albert Einstein School of Medicine, Bronx, NY, 10461

HSVGluR6 microapplication into stratum pyramidale of CA3 induced epileptiform discharge both in vivo and in vitro (see Bergold, et al., and During, et al., these abstracts). PCR amplification was performed using primers specific to the HSVGluR6 genome to assay the loss of HSVGluR6-transduced cells. HSVGluR6-transduced cells were lost between 3.5 and 4.5 hours after microapplication which is at a similar time as the end of the epileptiform discharges. 20-24 hours after microapplication loss of non-transduced CA3 and hilar neurons was observed. Non-transduced cell loss was blocked using NMDA antagonists or tetrodotoxin, suggesting that it required synaptic transmission and NMDA receptor activation. A threshold of HSVGluR6-transduced cells was necessary to induce the delayed loss of non-transduced CA3 neurons. Microapplication of additional HSVGluR6 virions resulted in a larger neuronal loss. Co-application of the GABA-A antagonist, picrotoxin, reduced the threshold of HSVGluR6-transduced cells. Enhanced GluR6 expression in a limited number of neurons likely results in induction of a epileptiform discharge and loss of both HSVGluR6-transduced and non-transduced neurons.

553.7

GLUTAMATE-MEDIATED EXCITOTOXIC DEATH OF CULTURED STRIATAL NEURONS IS MEDIATED BY NON-NMDA GLUTAMATE RECEPTORS. Q. Chen*, C. Harris, C.S. Brown, D.J. Surmeier and A. Reiner. Dept. Anat and Neurobiol., Univ. of Tennessee - Memphis, Memphis, TN 38163.

Recent interest has focused on the role of glutamate-mediated excitotoxicity in neurodegenerative disorders of the basal ganglia. The *in vitro* data on the receptor mechanisms involved in mediating this process, however, have been inconclusive. Some studies have indicated that excitotoxins acting at NMDA receptors kill striatal neurons but others have indicated that NMDA receptor mediated excitotoxic death of striatal neurons is minimal in the absence of cortex (Galarraga et al., Brain Res., '90). In the present study, we used a pharmacological approach to carefully examine this issue in two week old cultures of striatal neurons dissociated from E17 rat embryos. The sensitivity of these neurons to 1hr exposure to glutamate agonists and antagonists was determined by monitoring cell loss in identified regions of the growth dishes 24hrs after exposure.

We found that glutamate was a moderately potent excitotoxin in striatal cultures devoid of cortical tissue. The EC50 for neuronal loss following glutamate exposure was near 100μM. At saturating concentrations (500-1000μM), glutamate produced a 20-40% loss of identified neurons. This effect did not appear to be the result of NMDA receptor activation for two reasons. First, the NMDA-agonist quinolinic acid (QA) at concentrations up to 10mM did not produce neuronal loss. Depolarization with 35mM KCl to relieve the Mg2+ block of NMDA channels did not alter QA toxicity. Second, the specific NMDA receptor antagonist AP-5 did not antagonize the glutamate-induced loss at concentrations up to 10 times that of glutamate. In contrast, the ionotropic glutamate receptor antagonist CNQX did attenuate the loss produced by100μM glutamate. These results suggest that while glutamate at high micromolar concentrations is toxic to neostriatal neurons, non-NMDA receptors -- rather than NMDA receptors -- are crucial to its expression. NS-28889 (DJS); NS-19620, NS-28721 (AR).

553.8

NIMODIPINE ATTENUATES KAINATE-INDUCED CELL DEATH AND INCREASES IN $[Ca^{2+}]_i$ IN MURINE CORTICAL CULTURES. A.D. Lindsay* and J.H. Weiss. Dept. of Neurology, University of California, Irvine, 92717.

High concentrations of dihydropyridine (DHP) Ca^{2+} channel blockers attenuate neurodegeneration of cortical cultures produced by prolonged AMPA/kainate exposures, (Weiss et al., *Science* 247:1474). The effect of chronic kainate exposure on intracellular calcium ($[Ca^{2+}]_i$) and cell death was studied in murine cortical cultures in the presence and absence of the DHP, nimodipine (NP).

FURA-2 imaging (25° C, static 2 ml bath) revealed stable $[Ca^{2+}]_i$ levels of 10-50 nM for up to 8 hr. (maximum imaging duration for all experiments) in the absence of agonist. Addition of 30-100 μM kainate (KA) caused an initial rapid increase in $[Ca^{2+}]_i$ of 80 to 400 nM followed by a decay over several minutes to a stable plateau level (≈25-60% of the peak change in $[Ca^{2+}]_i$) which most cells were able to maintain for up to 8 hr. Both the peak and plateau levels varied widely between different cells. 24 hour exposure to 100 μM KA at 25° C killed 20-40% of the cells (as assessed by trypan blue staining of same cells used in imaging study). There was no correlation between the changes in $[Ca^{2+}]_i$ produced by KA and the likelihood of cell death.

20 μM NP caused a very slight (<20 nM) decrease in resting $[Ca^{2+}]_i$ levels and a pronounced attenuation of both the peak (up to 60%) and plateau (up to 50%) KA-produced increases in $[Ca^{2+}]_i$ (as compared to sister cultures with no NP). NP also reduced or eliminated KA-induced cell death. This suggests that Ca^{2+}influx through DHP-sensitive Ca^{2+}channels may be instrumental in chronic kainate neurotoxicity. In addition, there was a small population of cortical neurons which NP did not protect from KA-toxicity or KA-induced increases in $[Ca^{2+}]_i$. Perhaps the primary source of KA-induced Ca^{2+} influx in these unusual cells is through either DHP-insensitive Ca^{2+} channels or through Ca^{2+} permeable AMPA/kainate channels.

Supported by grants from the Alzheimers Assoc. and the Paralysis Project.

553.9

KAINATE INDUCES DIRECT Ca^{2+} ENTRY IN A SUBPOPULATION OF CULTURED CORTICAL NEURONS. L.M.T.Canzoniero*, S.Sensi, D.Turetsky and D.W.Choi. Dept. of Neurology and Center for the Study of Nervous System Injury, Washington Univ. School of Medicine, St. Louis, MO 63110.

Kainate-stimulated Co^{2+} uptake, a marker for divalent cation-permeable AMPA receptors, can be detected histologically on about 15% of neurons in murine cortical cell cultures, and these Co^{2+}-positive cells exhibit heightened vulnerability to kainate toxicity (Turetsky et al., Soc. Neurosci. Abstr. 18: 81,1992). Here we used fura-2 microfluorimetry to confirm that kainate can induce Ca^{2+} influx into Co^{2+}-positive neurons directly via agonist-gated channels.

Most cortical neurons exposed to 50 μM kainate in normal buffer solution (130 mM Na^+) responded with a marked increase in intracellular free Ca^{2+}, $[Ca^{2+}]_i$. However, if extracellular Na^+ was replaced by equimolar N-methyl-D-glucamine to prevent Ca^{2+} influx occurring subsequent to Na^+ influx (for example, via voltage-gated Ca^{2+} channels, or reverse operation of the Na^+-Ca^{2+} exchanger), only 20/160 neurons responded to kainate with an increase in $[Ca^{2+}]_i$. 18/20 of these responding cells took up Co^{2+} during a subsequent kainate exposure. These results support the hypothesis that excess Ca^{2+} entry through Ca^{2+}-permeable AMPA receptors (presumably lacking the edited form of the GluR2/GluR-B subunit) mediates the kainate-induced death of Co^{2+}-positive cortical neurons.

Supported by NIH grant NS 30337 to DWC.

553.10

(1S, 3R)-ACPD ATTENUATES NMDA-INDUCED NEURONAL CELL DEATH VIA A REDUCTION IN SUSTAINED CA^{2+} ACCUMULATION. G.J. Birrell, M.P. Gordon, R.D. Schwarz and F.W. Marcoux* Neuroscience Pharmacology, Parke-Davis Pharmaceutical Research, Division of Warner-Lambert Co. 2800 Plymouth Rd. Ann Arbor, MI 48105.

Glutamate excitotoxicity is linked to increases in neuronal Ca^{2+} and although blockade of ionotropic receptors results in the inhibition of glutamate-induced neuronal injury, the role played by metabotropic receptor activation is unclear. This study examined whether (1S,3R)-ACPD, a potent and selective metabotropic receptor agonist is neurotoxic, and whether (1S,3R)-ACPD has any effect on NMDA-induced neuronal cell death in primary cerebrocortical cultures derived from fetal rats.

Although causing small increases in Ca^{2+} accumulation, exposure of cultures to (1S,3R)-ACPD alone was not neurotoxic. The presence of (1S,3R)-ACPD during exposure to NMDA attenuated the resulting sustained accumulation of intracellular Ca^{2+} and delayed neuronal cell death. Reductions in sustained Ca^{2+} accumulation were associated both with Ca^{2+} efflux, in the absence of neuronal degeneration, and inhibition of delayed Ca^{2+} influx. The protective effects of (1S,3R)-ACPD were blocked by pre-treatment of cultures with pertussis toxin.

These results suggest that activation of metabotropic glutamate receptors may stimulate intracellular processes capable of limiting excitotoxic neuronal damage.

553.11

SUCCESSFUL TREATMENT OF EARLY EXCITOTOXIC/ISCHEMIC NEURONAL INJURY BY PERMEANT Ca^{2+} CHELATORS *IN VITRO* AND *IN VIVO*. M. Tymianski*, M.C. Wallace, I. Spigelman[1], M. Uno, P.L. Carlen, C.H. Tator, M.P. Charlton, University of Toronto, Toronto, Canada and [1]UCLA School of Dentistry, Los Angeles, CA.

We report the first successful treatment of early excitotoxic and ischemic neurodegeneration *in vitro* and *in vivo* by cell permeant analogs of BAPTA (a Ca^{2+} chelator) having a range of Ca^{2+} affinities. The chelators attenuated glutamate-induced intracellular Ca^{2+} increases and early neurotoxicity in neuronal explant cultures. When infused intravenously in rats, permeant fluorescent BAPTA analogs accumulated in neurons in multiple brain regions, including cortical and hippocampal neurons. BAPTA-AM, infused into rats *in vivo*, reduced Ca^{2+}-dependent spike-frequency adaptation and post spike-train hyperpolarizations in CA1 neurons taken from treated animals. This effect was reproduced by direct injections of BAPTA into untreated neurons, illustrating the dependence of this phenomenon on intracellular Ca^{2+} buffering. The effects of three different chelators (BAPTA, 5,5'-difluoro BAPTA and 4,4'-difluoro BAPTA) on Ca^{2+}-dependent membrane excitability varied with their Ca^{2+} affinity (K_d), with higher K_d compounds having lesser effects. When the different chelators were used to treat rats prior to the induction of focal cortical ischemia, they were highly neuroprotective as gauged by significant reductions in cortical infarction volumes and neuronal sparing. The extent of the chelators' protective effects *in vivo* also parallelled their affinity for Ca^{2+}. These data provide a novel therapeutic approach to neuronal ischemia, and the most direct evidence to-date that intracellular Ca^{2+} excess triggers early neurodegeneration *in vivo*.

553.12

ELECTROPHYSIOLOGICAL CONSEQUENCES OF INTRACELLULAR Ca^{2+} CHELATION IN HIPPOCAMPAL NEURONS *IN VITRO*.

I. Spigelman*, M. Tymianski[1] and M.P. Charlton[1], UCLA School of Dentistry & Brain Research Institute, Los Angeles, USA and [1]University of Toronto, Toronto, Canada.

Excessive increases in the intracellular concentration of free calcium ($[Ca^{2+}]_i$) are widely believed to be partial mediators of neuronal damage in cerebral ischemia. We have used intracellular Ca^{2+} chelators *in vivo* to prevent excessive neuronal $[Ca^{2+}]_i$ increases during ischemia. We showed that following intravenous injection, cell permeant (AM) analogs of BAPTA (1,2 bis-(2-aminophenoxy) ethane N,N,N',N'-tetraacetic acid) accumulate in hippocampal CA1 and neocortical neurons at concentrations sufficient to produce electrophysiologically measurable effects and act as neuroprotectants in experimental focal cerebral ischemia (Tymianski et.al., this session). To further examine the actions of BAPTA-AM analogs, intracellular recordings were made from rat CA1 neurons in slices. Bath application of 30 μM BAPTA-AM (high Ca^{2+} affinity, $K_d \approx 100$ nM) had no significant effect on cell input resistance (R_i) or spike amplitude. However, BAPTA-AM decreased spike frequency adaptation and post-spike train slow afterhyperpolarizations (AHPs) measured after 20-30 min of drug application. Furthermore, BAPTA-AM reduced the peak amplitude of evoked excitatory postsynaptic potentials (EPSPs) by 68%. In contrast, similar applications of 5,5'-Br_2BAPTA (low Ca^{2+} affinity, $K_d \approx 3.6$ μM) reduced EPSPs only by 21% and had little effect on R_i, spike adaptation or AHPs.

These data confirm that the actions of BAPTA analogs on membrane excitability depend on their Ca^{2+} affinity. We hypothesize that the neuroprotective effects of BAPTA analogs occur through buffering of excessive $[Ca^{2+}]_i$ increases. Additional actions of BAPTA *in vivo* may be due to attenuation of synaptic transmission. The data also suggest that the use of low Ca^{2+} affinity chelators like 5,5'-Br_2BAPTA as neuroprotectants may be advantageous since they would buffer excessive increases in $[Ca^{2+}]_i$ while exerting little effect on membrane excitability and synaptic transmission.

553.13

ELEVATION OF INTRACELLULAR CALCIUM LEVELS IN DISSOCIATED NEWBORN RAT BRAIN CELLS CAUSED BY β-N-METHYLAMINO-L-ALANINE (L-BMAA). D.M. Brownson[1], S.W. Leslie[2], T.J. Mabry[1], and J.S. Randall*[2] [1]Dept. of Botany, Col. of Nat. Sci. and [2]Div. of Pharmacol. and Toxic., Col. of Pharmacy; The Univ. of Texas, Austin, TX 78712.

This study investigated the ability of β-N-methylamino-L-alanine (L-BMAA), a non-protein amino acid present in edible seeds of the cycad plant *Cycas circinalis*, to increase intracellular calcium in dissociated newborn rat brain cells loaded with fura-2. In a dose-dependent manner, L-BMAA (in 10 mM $NaHCO_3$) produced an increase of intracellular calcium levels with an EC_{50} value of about 1 mM. The L-BMAA-mediated calcium increases were dependent on the extracellular calcium concentration. In the absence of extracellular calcium, L-BMAA did not increase intracellular calcium. These findings indicate that L-BMAA stimulated extracellular calcium entry but did not cause release of calcium from intracellular sources. L-BMAA-mediated calcium influx was not attenuated either by Mg^{2+} (1 mM) or MK-801 (400 nM), NMDA-receptor antagonists. L-BMAA-mediated calcium increases were highly dependent on the presence of bicarbonate (3-40 mM). The bicarbonate dependence was not due to the alkalization of the buffer medium or the increases in the sodium concentration. These results indicate that the neurotoxicity of L-BMAA is dependent upon the extracellular bicarbonate concentration and suggest the involvement of a carbamate derivative of L-BMAA. Since L-BMAA produces an elevation of intracellular calcium levels, the mechanism of neurotoxicity may be similar to that proposed for other excitatory amino acids although it does not appear to be NMDA receptor-mediated. [Supported by NIH grant R01 AG10637, NATO grant CRG 910429 and NIAAA grant R37 AA05809]

553.14

GLUTAMATE INCREASES INTRACELLULAR FREE MAGNESIUM IN RAT CORTICAL NEURONS *IN VITRO*. S.Rajdev*, J.Brocard and I.J.Reynolds, Dept. Pharmacology, Univ. Pittsburgh, Pittsburgh, PA 15261 and Dept. de Neurobiol., Ecole Normale Superieure, 75005 Paris, France.

Although Mg^{2+} plays an important role in many cellular functions, the factors regulating Mg^{2+} homeostasis are still not well understood. We investigated intracellular free Mg^{2+} ($[Mg^{2+}]_i$) changes in rat forebrain neurons using the Mg^{2+}-sensitive fluorescent dye, magfura-2. Resting $[Mg^{2+}]_i$ in neurons was 0.63 ± 0.03mM (n=94). Addition of glutamate (with 1μM glycine) increased $[Mg^{2+}]_i$ (by 0.4-11mM) in all the neurons studied. The threshold glutamate concentration required to produce $[Mg^{2+}]_i$ increase was 3μM and maximal response was approached with 100μM glutamate. This effect of glutamate on $[Mg^{2+}]_i$ is apparently mediated by NMDA receptor activation, since it was blocked by dizocilpine and 5,7-DCKU. The elevation of $[Mg^{2+}]_i$ by glutamate involved two separate processes. The principle component was independent of extracellular Mg^{2+} ($[Mg^{2+}]_o$), but was almost eliminated by removal of $[Ca^{2+}]_o$, and may be due to displacement of Mg^{2+} from intracellular sites by Ca^{2+}. A second smaller component was independent of $[Ca^{2+}]_o$, but required $[Mg^{2+}]_o$, and was potentiated by removal of $[Na^+]_o$, suggesting presence of a Na^+-Mg^{2+} exchange mechanism.

Kainate, KCl and veratridine were much less effective in increasing $[Mg^{2+}]_i$. As the agonists that most effectively increase $[Mg^{2+}]_i$ are excitotoxic to neurons following short exposure, this data may indicate a role for $[Mg^{2+}]_i$ in glutamate excitotoxicity.

Supported by American Heart Association in-grant-aid.

553.15

A PARADOXICAL REQUIREMENT FOR EXTRACELLULAR Mg^{2+} IN GLUTAMATE TOXICITY. K.A. Hartnett*, S. Rajdev, P.A. Rosenberg, E. Aizenman & I.J. Reynolds, Depts. of Neurobiol. & Pharmacol., Univ. of Pittsburgh Sch. Med., Pgh, PA 15261, & Dept. of Neurol., Children's Hosp. & Harvard Med. Sch., Boston, MA 02115

The presence of extracellular Ca^{2+} is an obligatory requirement for neuronal death following exposure to glutamate in rodent cortical cultures (Choi, *J. Neurosci.* 7: 369; 1987). We recently observed that glutamate elevates intracellular Mg^{2+} in addition to Ca^{2+} in neurons. In this study we used conditions that elevate $[Mg^{2+}]_i$ independently of $[Ca^{2+}]_i$ to investigate a possible role for Mg^{2+} in excitotoxicity. In Ca^{2+}-free solutions, and with N-methyl-D-glucamine (NMDG) substituting for Na^+, the presence of 9 mM Mg^{2+} was sufficient to produce neuronal death after a 20 min exposure to 100 μM glutamate. Under identical conditions, intracellular Mg^{2+} concentrations measured with Magfura-2, and neuronal cell volume, were both observed to increase following glutamate application. Toxicity was also induced by 200 μM NMDA and was prevented by: (i) 10 μM dizocilpine, (ii) omitting Mg^{2+} or Cl^- from the extracellular solution, and (iii) substituting choline for NMDG. Thus, in solutions devoid of Na^+ and Ca^{2+} glutamate-mediated neuronal death may be dependent on the influx of both Mg^{2+} and Cl^-.

553.16

DEPENDENCE OF NMDA NEUROTOXICITY IN CORTICAL CULTURES ON EXTRACELLULAR CHLORIDE AS WELL AS CALCIUM. M. Nguyen-Huynh, V. Zaleskas, and P.A. Rosenberg*. Dept. Neurol., Children's Hospital & Harvard Medical School, Boston MA 02115.

We are interested in the specific mechanisms underlying NMDA receptor mediated neurotoxicity (NNT). A previous study has shown that removal of chloride confers partial protection against NNT in addition to having shown the dependence of NNT on calcium [Choi (1987) J. Neurosci. 7: 369-379]. In this study we examine the chloride dependence of NNT further, as this might be important in understanding the mechanism of NNT.

Using thirty minute exposures of neuron-enriched astrocyte-poor cortical cultures to 0.5 - 1 mM NMDA in a tris buffered saline (pH 7.2), we found that removal of chloride (by substitution of sulfate salts for chloride salts, except for calcium chloride) provided complete protection against NMDA neurotoxicity, assayed by counting surviving neurons 20-24 hours after exposure (n=4). Similarly, removal of calcium also conferred protection in these cultures. In conventional astrocyte-rich cultures, removal of chloride provided substantial protection against NMDA toxicity, assayed by both counting of surviving neurons and by assay of ^{3}H-ouabain binding (71 $\pm$ 22% survival, n=15). Removal of calcium in the presence of chloride was also neuroprotective. In low chloride medium, increasing the external calcium concentration to 12 mM (by addition of the nitrate, acetate, or gluconate salts of calcium) did not consistently increase the toxicity of NMDA (70 $\pm$ 30% survival versus 91 $\pm$ 12% survival in normal calcium, n=3).

It appears likely that one or more chloride transport pathways are involved in NMDA receptor mediated neurotoxicity.

Supported by the American Heart Association.

553.17

EXPRESSION OF PARVALBUMIN IN CULTURED CORTICAL NEURONS USING A HERPES SIMPLEX VIRUS (HSV-1) VECTOR SYSTEM ENHANCES NMDA-INDUCED NEUROTOXICITY. Dean Hartley*, Rachael Neve, John Bryan, Donna Ullrey, Sun-Yung Bak, Matthew During, and Alfred Geller, Childrens Hosp, Boston, MA; McLean Hosp, Belmont, MA; Yale Univ. School Medicine. New Haven CT.

Glutamate neurotoxicity in cortical neurons is thought to primarily be mediated by the influx of calcium, consequently maintaining calcium homeostasis is of critical importance for cell survival. Parvalbumin is a small calcium-binding protein expressed in a subset of "rapid-firing" GABAergic neurons in the CNS. Using a HSV-1 vector system capable of introducing genes into post-mitotic neurons (TINS 14:428, 1991), we investigated whether expression of parvalbumin would alter NMDA-induced neurotoxicity. After two weeks, cultured cortical neurons were either mock infected or infected with pHSVlac (expresses b-galactosidase) or pHSVparv (expresses parvalbumin). After 24 hr the medium was exchanged with medium containing 0, 5, 10, 12.5, 15, or 300 mM NMDA + 10 mM glycine for an additional 24 hr. Cell death was measured by the efflux of lactate dehydrogenase into the medium. Interestingly, the introduction of parvalbumin into these cells significantly ($P<0.05$) enhanced neurotoxicity at NMDA concentrations of 5, 10, and 12.5 mM when compared to cultures infected with pHSVlac or mock infected. This enhancement was attenuated by the addition of tetrodotoxin. Furthermore, expression of parvalbumin in cortical neurons enhanced the release of both aspartate and glutamate. These date suggest that the expression of parvalbumin in cortical neurons enhances neurotransmitter release and thereby augments NMDA neurotoxicity.

553.18

EXCITOTOXIC NEURONAL DEATH IN CORTICAL CELL CULTURE IS NOT DUE TO APOPTOSIS R.F.Regan*, S.S.Panter, A.Witz, J.L.Tilly and R.G.Giffard Letterman Army Inst of Research, San Francisco, CA 94129 and Depts of Gyn and Ob, and Anesthesia, Stanford Univ Sch of Med, Stanford, CA 94305

Cell death by apoptosis, common during brain development, is also a postulated mechanism of pathologic neuronal loss. Neuronal injury after activation of glutamate receptors was evaluated for ultrastructural, biochemical, and pharmacologic evidence of apoptosis. Exposure to 500 μM glutamate for 10 min produced rapid mitochondrial swelling and disruption, increased nuclear and cytoplasmic lucency and dilatation of endoplasmic reticulum. Chromatin clumping without localization to the nuclear membrane and membrane lysis were marked by 2 hr. Exposure to low concentrations of AMPA or kainate for 3-6 hr resulted in distinctly different morphology. Both led to cytoplasmic condensation, vacuolization and moderate chromatin clumping throughout the nucleus. After 12 hr mitochondrial swelling and increased nuclear and cytoplasmic lucency were noted. No increase in internucleosomal DNA cleavage over baseline levels was produced by any of these injury paradigms, assessed by autoradiography after 3' P^{32} labelling and gel electrophoresis. Neuronal death was not attenuated by cycloheximide or actinomycin-D. These results suggest that exposure to exogenous excitatory amino acids does not induce apoptosis in primary cultures of murine neurons.

553.19

GLUTAMATE INDUCES APOPTOTIC CELL DEATH IN PRIMARY CULTURES OF THE NERVOUS SYSTEM. A.M. Marini*, F. Finiels-Marlier, B. Dipasquale‡, S. Paul and R.Youle‡. CNB, NIMH, and ‡Biochemistry Section, Surgical Neurology Branch, NINDS, Bethesda, Md 20895.

Primary cultures of cortical neurons and cerebellar granule cells are susceptible to the toxic effect of glutamate. We studied these neurons following exposure to toxic concentrations of glutamate using Nomarski optics which identifies apoptotic cells as round and highly refractive bodies. The control level of apoptotic refractive cells in cortical neurons was 3-5% whereas in cerebellar granule cells it was 20-30%. The percentage of apoptotic refractive cells increased to 15-20% in cortical neurons and up to about 50% in cerebellar granule cells following glutamate (150 and 30μM respectively) exposure at 2 and 4 hours. Apoptotic refractive changes were attenuated by cycloheximide (1μg/ml) or the NMDA receptor channel blocker MK-801 (1μM); both reduced the percentage of apoptotic refractive cells to control levels. In thymocytes, the apoptotic refractive changes identified by Nomarski optics correlate with internucleosomal DNA degradation. Therefore, we extracted the DNA from glutamate-treated cultures, but found no evidence of the internucleosomal DNA degradation pattern. Our findings show that the early nuclear changes detected using Nomarski optics, characteristic of apoptotic cell death, result from glutamate exposure of the cultured neurons. However, we were unable to detect the DNA degradation pattern that other neurotoxins induce in cultured neurons (ref. Dipasquale et al., 1991, Biochem. Biophys. Res. Commun. 181:1442-1448).

554.1

A FLUORIMETRIC ASSAY FOR THE DETERMINATION OF ANTHRANILIC ACID IN BIOLOGICAL MATERIALS. P. Guidetti* and R. Schwarcz. Md. Psych. Res. Center, Baltimore, MD 21228.

Anthranilic acid (ANA), a tryptophan metabolite present in urine and peripheral tissues, has been shown to provide a major source of 3-hydroxyanthranilic acid, an effective bioprecursor of the endogenous excitotoxin quinolinic acid, in the rat brain (J. Neurochem. 55: 738, 1990). Using a novel isolation procedure, we have now examined the presence of ANA in various rat tissues and body fluids. Tissue sames were homogenized (1:80, w/v), and serum and urine samples were diluted ((1:80 and 1:4000, v/v, respectively) in water. After acidification and centrifugation, the supernatant was purified through a strong cation exchange resin. The fraction containing ANA was subjected to reversed phase HPLC. ANA was detected fluorimetrically (sensitivity limit: 50 fmoles) and identified by its retention time using three different mobile phases. ANA was thus found in serum (131 ± 7 nM) and urine (9.9 ± 1.8 nmol/mg creatinine). In peripheral organs, ANA levels were highest in the liver and in the kidney (1.8 pmol/mg prot.) and lowest in the adrenal (0.52 pmol/mg prot.). In the brain, only small regional differences in ANA were found among 10 brain areas, ranging from 0.51 to 0.98 pmol/mg prot. Residual blood was not responsible for the presence of ANA in tissue. The role of endogenous ANA in the biosynthesis and function of quinolinic acid remains to be examined.

Supported by USPHS grant NS 28236.

554.2

EXCITOTOXIC EFFECTS OF ANTHRANILIC ACID IN MATURE ORGANOTYPIC CULTURES OF RAT HIPPOCAMPUS. N.A. Shapira, R. Schwarcz[1], B.C. Christie-Pope* and W.O. Whetsell, Jr., Dept. of Pathology, Vanderbilt Univ. School Med., Nashville, TN 37232 and [1]Maryland Psychiatric Research Center, Baltimore, MD 21228.

In the rat brain, 3-hydroxyanthranilic acid (3-HANA), the immediate bioprecursor of the excitotoxin, quinolinic acid (QUIN), is preferentially produced from anthranilic acid (ANA) (J. Neurochem. 55:738, 1990). Like 3-HANA (Soc. Neuroscience Abstr. 16:461.3, 1990), ANA may ultimately exert excitotoxic effects in the CNS after conversion in QUIN. To investigate this possibility, mature organotypic cultures of rat hippocampus were exposed to ANA. While relatively short exposure times (up to 2 days with up to 10 mM ANA) produced little or no morphological alteration as assessed by light- and electron-microscopy, longer exposure times (3-6 days with up to 10 mM ANA) resulted in dose-dependent neuronal damage. Changes characteristic of excitotoxic damage were observed with as little as 100 μM ANA. At the highest dose used (10 mM), quantitative ultrastructural image analysis demonstrated an increase in post-synaptic dendritic area of more than 20-fold compared to controls. Concomitant incubation of sibling cultures with the selective NMDA-receptor antagonist, 2-amino-7-phosphonoheptanoic acid (APH) at 1 mM, prevented morphological changes induced by ANA (100 μM, 1 mM or 10 mM). These results indicate that ANA, possibly through its conversion to QUIN, can function as an excitotoxin in brain. ANA, a regular biochemical constituent in brain (see Guidetti and Schwarcz, this meeting), may therefore have a role in the evolution of excitotoxic brain disorders (Lab. Invest. 68:372, 1993). Supported by USPHS grants NS16102 and NS28236.

554.3

ENHANCEMENT OF GLUTAMATE (GLU)- OR QUINOLINIC ACID (QUIN)-INDUCED EXCITOTOXICITY IN HIPPOCAMPAL CULTURES BY AMINOOXYACETIC ACID (AOAA). W.O. Whetsell, Jr.* and N.A. Shapira

The capacity of GLU and some of its chemically structural analogs to induce excitotoxic neurodegeneration in mammalian CNS has received increasing scientific attention over the past three decades. The low excitotoxic potency of GLU compared to other excitotoxic agents has also been of interest (JNEN 51:351, 1992) since there is evidence that excitotoxicity of GLU or some GLU-analogs may play a role in certain chronic human neurodegenerative disorders. The mechanism(s) by which excitotoxicity may act in neurodegenerative disorders is not understood. It has been suggested, that among other possibilities, interference with intraneuronal energy metabolism (J. Pharmacol. 257:870, 1991) sufficient to induce metabolic stress might predispose neurons to the effects of low concentrations of excitotoxic substances. In whole-animal experiments, administration of AOAA, a substance which may interfere with kynurenic acid synthesis (Synapse 9:129, 1991) or with intraneuronal energy metabolism (Annals Neurol. 31:119, 1992), has been shown to induce excitotoxic CNS lesions which can be prevented by specific excitotoxic-receptor antagonists. The present study examines the effects of AOAA on mature organotypic cultures of rat hippocampus either alone or in the presence of GLU or an endogenous GLU analog, QUIN. Results indicate that while AOAA (100 μM) alone, GLU (100 μM) alone or QUIN (10 μM) alone produce little or no morphological change for at least three days, a combination of AOAA-plus-GLU or AOAA-plus-QUIN at the same concentrations (100 or 10 μM) produce a 5- to 10-fold increase in post-synaptic dendritic area (compared to controls) as shown by ultrastructural image analysis and a 2- to 5-fold increase in percent of open space due to cell swelling as shown by light microscopic image analysis. This response is blocked by the NMDA-receptor antagonist, 2-amino-7-phosphonoheptanoic acid (APH). In this model, AOAA increases the susceptibility of hippocampal neurons to neurodegeneration induced by GLU or QUIN. (Supported by USPHS Grant NS-28236)

554.4

ANTAGONISM OF QUINOLINIC ACID INDUCED TOXICITY ON STRIATAL DIAPHORASE NEURONS FOLLOWING ADMINISTRATION OF KYNURENINE, PROBENECID AND NICOTINYLALANINE. C.A. Harris[1], R.J. Boegman[1]*, K. Jhamandas[1] and R.J. Beninger[2]. Departments of [1]Pharmacology and [2]Psychology, Queen's University, Kingston, Ont. K7L 3N6.

Quinolinic acid (QUIN), an endogenous excitotoxin, and kynurenic acid (KYNA), a potent endogenous excitatory amino acid antagonist, are both synthesized via the kynurenine pathway which is the primary metabolic route of tryptophan to niacin.

In the present study we examined the sensitivity of striatal diaphorase (nitric oxide synthase) containing neurons to an infusion of QUIN (15 nmol) administered into the striatum of rats pretreated with various drug regiments aimed at elevating brain KYNA levels. The effects of increasing the accumulation of KYNA while inhibiting the synthesis of QUIN with ip or icv nicotinylalanine (NAL), precursor loading with ip kynurenine (KYN) and the blockade of organic acid transport with ip probenecid (PRO) were examined alone and in combination.

Rat brain KYNA levels increased from 61.8 ± 52.1 pmol/g wet wt to 5638.6 ± 1203.9 pmol/g wet wt following the administration of NAL (500 mg/kg), KYN (450 mg/kg) and PRO (200 mg/kg).

The survival of NADPH-diaphorase positive cells two days following an infusion of (15 nmol) QUIN was 11.9 ± 7.2 %. A combination of NAL + KYN + PRO increased the survival of diaphorase neurons to 50.4 ± 5.3 % following infusion of QUIN. These result show that it is possible to increase neuronal survival in vivo by altering endogenous levels of KYNA.

Supported by Medical Research Council.

554.5

EXCITOTOXIC EFFECT OF QUINOLINIC ACID ON NIGROSTRIATAL DOPAMINERGIC NEURONS : MODULATION BY INHIBITION OF NITRIC OXIDE SYNTHASE? B.P. Connop[1], R.J. Boegman[1], K. Jhamandas[1], R.J. Beninger[2] and J.V. Milligan[1]. Departments of [1]Pharmacology and Toxicology and [2]Psychology, Queen's University, Kingston, Ont. K7L 3N6 (Canada)

Quinolinic acid (QUIN), a metabolite of the Kynurenine pathway has been implicated in a number of neurological disorders. QUIN has been found to be an potent excitatory amino acid which acts on the N-methyl-D-aspartate subtype of glutamate receptor.

Previous results show that the cholinergic neurons of the nucleus basalis magnocellularis are sensitive to QUIN with an EC(50) of 104 nmol infused. In order to determine the sensitivity of the dopaminergic neurons, QUIN was infused into the substantia nigra compacta of male Sprague Dawley rats in the absence and presence of a nitric oxide synthase inhibitor. Four days after the QUIN infusion, striatal tyrosine hydroxylase activity was found to decrease and had an EC(50) of 16 nmol. This suggests that the dopaminergic neurons are more sensitive than the cholinergic neurons to the excitotoxic effects of QUIN.

Initial studies were performed with the nitric oxide synthase (NOS) inhibitor N-nitro-L-arginine methyl ester (L-NAME). It was found that two interperitoneal injections of L-NAME eight hours apart caused a maximal inhibition of the NOS enzyme twenty four hours after the last injection. A dose-response curve of L-NAME showed that a dose of 250 mg/kg resulted in an 89% inhibition of the brain isozyme.

Preliminary studies indicate that 24 hour pretreatment of rats with 250 mg/kg L-NAME prior to infusion of 40 nmol QUIN did not offer any protection to the dopaminergic neurons projecting to the striatum.

(Supported by the Parkinson's Foundation and PMAC)

554.6

PROTECTION AGAINST QUINOLINIC ACID INDUCED NEURONAL DAMAGE OF STRIATAL DIAPHORASE NEURONS BY CO-INFUSION WITH PICOLINIC ACID. B.E. Kalisch, R.J. Boegman, K. Jhamandas, J. Goh* and R.J. Beninger†. Department of Pharmacology and Toxicology and Department of Psychology†, Queen's University, Kingston, Ontario, Canada, K7L 3N6.

Lesions produced by acute administration of the endogenous neurotoxin quinolinic acid (QUIN) can be antagonized by co-injection with picolinic acid (PIC), a monocarboxylic acid originating from the same pathway that yields QUIN (Cockhill, J. *et al.*, 1992, Brain Research, 599: 57-63). The present study examined the effects of long-term exposure to low doses of QUIN on diaphorase (nitric oxide synthase) containing neurons of the rat striatum. Exposure to QUIN (6 nmol/h) for 7 days resulted in a severe depletion of NADPH diaphorase-positive neurons. Infusion of PIC (18 nmol/h) for 7 days was not toxic to these neurons. Comparable co-infusion of PIC with QUIN prevented the depletion of NADPH diaphorase-positive neurons induced by QUIN. These results demonstrate that PIC can prevent neuronal damage induced by sub-chronic infusions of QUIN. The effects of long-term exposure to QUIN alone or in combination with PIC on nitric oxide synthase activity in rat striatum is currently under investigation.

(Supported by the Medical Research Council of Canada)

554.7

ANTI-EXCITOTOXIC EFFECT OF 4-CL-KYNURENINE IN THE RAT HIPPOCAMPUS IN VIVO. H.-Q. Wu*, F.G. Salituro[1] and R. Schwarcz. Md. Psych. Res. Ctr., Baltimore, MD 21228 and [1]Marion Merrell Dow Res. Inst., Cincinnati, OH 45215.

In the rat brain, 4-Cl-kynurenine (4-Cl-KYN) can be enzymatically converted to 7-Cl-kynurenic acid (7-Cl-KYNA), a potent antagonist of the glycine site associated with the NMDA receptor (Soc. Neurosci. Abstr. 18: 482.12, 1992). The neuroprotective properties of 4-Cl-KYN vis-á-vis the excitotoxin quinolinic acid (QUIN) were now studied in the rat hippocampus. Local co-infusion of 4-Cl-KYN (≥ 40 nmoles) or 7-Cl-KYNA (≥ 4 nmoles) with QUIN (8 nmoles) completely prevented QUIN-induced neuronal death, as assessed histologically and biochemically. Pretreatment for 2 h significantly enhanced the neuroprotective potency of 4-Cl-KYN (but not of 7-Cl-KYNA). The importance of the conversion of 4-Cl-KYN to 7-Cl-KYNA was further substantiated by local injection of the selective gliotoxin fluorocitrate (1 nmol/1 μl). Fluorocitrate interfered with the production of 7-Cl-KYNA from 4-Cl-KYN and greatly attenuated the neuroprotective effect of 4-Cl-KYN without influencing the effect of 7-Cl-KYNA. These data demonstrate that astrocytes are responsible for synthesizing and releasing neuroprotective quantities of 7-Cl-KYNA from 4-Cl-KYN. More generally, astrocytes may therefore be targeted for the delivery of neuroprotective kynurenic acid analogs in vivo.

Supported by USPHS grant NS 16102.

554.8

LIMBIC SEIZURES CAUSE INCREASE IN KYNURENIC ACID NEOSYNTHESIS AND ATTENUATION OF VULNERABILITY TO EXCITOTOXINS IN THE RAT STRIATUM. H. Baran* and R. Schwarcz. Maryland Psychiatric Research Center, Baltimore, MD 21228.

In rats, seizures induced by kainic acid (KA; 10 mg/kg, s.c.) cause increased striatal kynurenine aminotransferase (KAT) activity (+ 66%) one week later (Soc. Neurosci. Abstr. 17: 356.10, 1991). Since kynurenic acid, the product of KAT, is anticonvulsant and neuroprotective, we studied the vulnerability of striatal neurons to intrastriatal quinolinic acid (QUIN) or KA at 7 days after a systemic KA injection. Rats pretreated with KA proved to be substantially less vulnerable than controls to local injections of QUIN (60 and 120 nmoles) and KA (2.5 nmoles), as assessed both histologically and biochemically (decrease in glutamate decarboxylase activity). A subconvulsive dose of KA (6 mg/kg) failed to increase KAT activity in the striatum and to protect against QUIN toxicity (60 nmoles). The correlation between increased striatal KAT and neuroprotection was further examined using another convulsant, pilocarpine (325 mg/kg, i.p.). Pilocarpine, too, elevated striatal KAT activity by 7 days (+ 90%) and attenuated the toxicity of intrastriatal QUIN (60 nmoles). Taken together, the resistance of the striatum to an excitotoxic insult following limbic seizures may be related to increased endogenous neuroprotection by kynurenic acid. These data may also pertain to the integrity of striatal tissue in epileptic disorders.

Supported by USPHS grant 16102.

554.9

KYNURENINE PATHWAY ENZYMES FOLLOWING CNS IMMUNE STIMULATION (POLIOVIRUS INFECTION OF THE SPINAL CORD). K. Saito*, J.S. Crowley, M.Saito, S.P. Markey, J.H. Vickers[1], and M.P. Heyes Lab. of Clinical Science, NIMH; and Food and Drug Administration[1] Bethesda, MD 20892.

Quinolinic acid (QUIN), kynurenic acid (KYNA) and L-kynurenine (L-KYN) accumulate in brain following immune stimulation (*Brain* 115,1249), and are attributed to induction of indoleamine-2,3-dioxygenase (IDO). Direct conversion of L-tryptophan to QUIN by CNS tissue occurs in conditions of brain inflammation, but not by normal brain (*J. Neurochem* 60, 180). Therefore, to investigate whether induction of enzymes distal to IDO may determine L-KYN conversion to QUIN, poliovirus was inoculated directly into the spinal cord as a model of inflammatory CNS disease (*FASEB J.* 6: 2977). Induction of spinal cord IDO (35.9-fold) accompanied increases in kynurenine-3-hydroxylase (2.4-fold) and kynureninase (2.3-fold), which were highly correlated to CNS QUIN levels and measures of inflammatory lesions. 3-Hydroxyanthranilate-3,4-dioxygenase activity was unaffected. CSF KYNA accumulated in proportion to both IDO activity and L-KYN levels, whereas kynurenine aminotransferase activity was unaffected. Inhibitors of the kynurenine pathway, 6-chlorotryptophan and 4-chloro-3-hydroxyanthranilic acid (*Biochem. J.* 291 11), attenuated [$^{13}C_6$]-QUIN formation when co-infused intracisternally with [$^{13}C_6$]-L-tryptophan in the infected animals. These results support roles for induction of IDO, kynurenine-3-hydroxylase and kynureninase in accelerating the synthesis of QUIN, L-KYN and KYNA during brain inflammation. Because macrophages can convert L-tryptophan to QUIN, and because kynurenine pathway enzymes may be localized in different cellular compartments, substrate flux may be regulated not only by local enzyme activites and the presence of inflammatory cell infiltrates, but also the cellular and sub-cellular distribution of substrate and enzymes.

554.10

CONTROL OF QUINOLINIC ACID BIOSYNTHESIS IN THP-1 CELLS J.F. Reinhard Jr*., M. Jansen, J.B. Erickson, S.Y. Chang and E.M. Flanagan Division of Pharmacology, The Wellcome Research Laboratories, 3030 Cornwallis Rd., Res. Triangle Park, NC 27709

Quinolinic acid (quin) is a neurotoxin formed *in vivo* from tryptophan (TRP). Quinolinate is synthesized in liver and in macrophages through a 5 enzyme pathway. The first step in quin formation is indoleamine dioxygenase (IDO). The present experiments were conducted to determine whether IDO was important for controlling extrahepatic quin synthesis. A human, monocytic leukemia-derived cell line (THP-1 cells) was pretreated for 48 hours with phorbol myristate acetate (PMA) before receiving human γ-interferon (IFN). Quin synthesis was determined by incorporation of [$^{13}C_6$]-TRP into [$^{13}C_6$]-quin using gas chromatography-mass spectrometry during the final, 48 hour, incubation. Kynurenine hydroxylase (KH) and IDO activities were determined on the cell pellets.

Treatment	IDO activity	KH activity	[$^{13}C_6$]-QUIN
None (DMSO)	0.2 ± 0.04	8.9 ± 0.3	99 ± 0.3
PMA	5.6 ± 0.5	8.9 ± 1.5	149 ± 0.3*
IFN	31.9 ± 3.5*	12.4 ± 3.0	96 ± 0.6
PMA + IFN	124.5 ± 19.3*	14.7 ± 2.6*	172 ± 0.1*

(* = $p<0.05$, compared with control). The data represent enzyme activity (pmol/min*mg protein) or media quin (nM) following a 48 hour incubation with 25 μM [$^{13}C_6$]-TRP. The media quin values are corrected for differences in cellular protein content. These data indicate that extrahepatic cellular quin synthesis is limited by factors other than IDO since increases in IDO did not result in equivalent increases in quin. The data suggest that other enzymes in the biosynthetic pathway, e.g. KH and kynureninase, are important for controlling quin synthesis.

554.11

Pretreatment with mega-dose methylprednisolone increases quinolinate toxicity in the rat striatum. T.A. Uhler, D.M. Frim, P. Pakzaban, J. Schumacher, W. S. Rosenberg* and O. Isacson. Neuroregeneration Laboratory, McLean Hospital, Belmont, MA, 02178; Neurosurgery and Neurology Services, Massachusetts General Hospital and Harvard Medical School, Boston, MA

The mechanism of neuronal death after ischemic or traumatic insult is unknown but may be due to energy depletion and glutamate toxicity. One possible pathway leading to cell death after such insults may be lipid peroxidation caused by inability to metabolize superoxides in an energy depleted state. High doses of glucocorticoids or lazaroids have been shown to be potent antioxidants, capable of mitigating the effects of superoxides on lipid membranes *in vitro*. We investigated these antioxidants for neuroprotective effects in a rat striatal excitotoxic lesion model. Animals were lesioned by striatal infusion of 60 nmol of quinolinic acid, an NMDA receptor agonist. Animals received twice daily i.p. injections of either normal saline, lazaroid U-78517F (Upjohn) (20mg/kg), or methylprednisolone succinate (MPSS) (Upjohn) (30mg/kg) beginning the evening before surgery and continuing through postoperative day 1. Animals were sacrificed 7 days after lesioning and brains were histologically evaluated for lesion size by Nissl staining of coronal sections. Mean maximal cross-sectional lesion area for saline treated animals was 3.62 ± 1.03 mm^2 (mean ± S.D.; n=7). Treatment with lazaroid U-78517F did not affect lesion size (4.23 ± 0.92 mm^2; n=8); however, MPSS caused a significant ($P<0.05$) 56% increase in mean maximal cross-sectional lesion area (5.76 ± 1.17 mm^2; n=8). The detrimental effect of mega-dose MPSS is likely due to deleterious glucocorticoid effects outweighing any positive antioxidant effect. The failure of lazaroid U-78517F to decrease lesion size may be due to poor intraperitoneal uptake or poor brain penetration. Alternatively, these findings are consistent with the possibility that oxidation damage may play only a minor role in NMDA receptor-mediated toxicity.

554.12

NEURONAL DYSFUNCTION SECONDARY TO CHRONIC EXPOSURE OF QUINOLINIC ACID. T.J. Bazzett*, R.L. Albin, J.B. Becker & R.C. Falik. Depts of Neurology and Biopsychology, University of Michigan, Ann Arbor, MI 48014.

Rats received implants of bilateral intrastriatal chronic dialytic delivery devices. The right striatum received 185 nmol quinolinic acid (QUIN) per day for three weeks. The left striatum received buffered saline solution (VEH).

Nissl staining revealed loss of cells in a region approximately 200 μm radial from the dialysis probe in the QUIN treated striatum. Cytochrome oxidase staining was reduced in this same area. Beyond this region, the number of nissl stained cells and cytochrome oxidase staining appeared normal. There was a marked decrease in parikarya that stained positive for calbindin or parvalbumin immunoreactivity throughout most of the QUIN treated striatum. Calbindin staining was reduced also in surrounding cortex and parvalbumin staining was reduced in the globus pallidus. The VEH treated striatum was normal. Previous work has shown that the decreased nissl and cytochrome oxidase staining persisted at least one month after QUIN treatment, indicating these changes are permanent. Experiments in progress will determine the relative permanence of decreased calbindin and parvalbumin staining after cessation of QUIN treatment. Supported by NS19613, NS01300, NS22157 and The Reproductive Science Program 5 T32 HD07048.

554.13

β-AMYLOID 25-35 (βA) AND QUINOLINIC ACID (QA) INJECTIONS INTO THE VENTRAL PALLIDUM/SUBSTANTIA INNOMINATA (VP/SI) OF YOUNG MALE F344 RATS: BEHAVIORAL, NEUROCHEMICAL AND HISTOLOGICAL EFFECTS. E.M. Sigurdsson,[1]* J.M. Lee[1,2] and S.A. Lorens.[1] Depts. Pharmacology[1] and Pathology,[2] Loyola Univ. Chicago Med. Center (Bldg. 135), Maywood IL 60153

Alzheimer's disease is associated with the loss of cholinergic neurons in the basal forebrain and is characterized histopathologically by amyloid deposition and neurofibrillary tangles in several, but not all, cortical and subcortical regions. In order to determine whether basal forebrain injections of βA are neurotoxic, we compared the effects of bilateral injections into the VP/SI of βA (1.0 nmole/3.0 μl) and of QA (75.0 nmole/3.0 μl). Control rats received vehicle infusions (VEH; 3.0 μl saline). Exploratory behavior in an open field (OF) and the acquisition of a one way (spatial) conditioned avoidance response (CAR) were analyzed 13-16 d postoperatively. QA rats were more active in the OF than either the βA or VEH rats (75%; $p<0.12$), and, at the start of the test session, exited the central square more rapidly (mean = 7 sec; $p<0.05$) than the VEH rats (20 sec). In contrast, the βA rats remained in the central square for a longer time (47 sec; $p<0.05$) before moving to a wall square. The βA rats emitted their first CAR after fewer trials than the VEH rats (4.7 v 7.3; $p<0.05$), while the QA rats committed fewer errors to reach criterion than the VEH animals (3.6 v 5.6; $p=0.05$). The rats were sacrificed 22-23 d postoperatively. Choline acetyltransferase activity (ChAT-Act) in the QA rats was reduced in both the right and left amygdala (26-29%; $p<0.05$), but not in the frontal cortex. βA did not affect ChAT-Act. Cavitation and glial proliferation (1.5 mm^3) were observed surrounding the QA injection site. In contrast, no histological differences were discerned between the βA and VEH rats. These results suggest: 1) that intra-VP/SI injections of QA can produce functional changes which are associated with histological evidence of neurotoxicity and a reduction in amygdaloid but not frontal cortical ChAT-Act; and, 2) that βA VP/SI injections can induce behavioral alterations in the absence of histological or neurochemical signs of neurotoxicity.

554.14

DOMOIC ACID NEUROTOXICITY IN CYNOMOLGUS MONKEYS: EFFECT OF DOSE ON HIPPOCAMPAL NEURONAL AND AXON TERMINAL DEGENERATION A.C. Scallet*, Z. Binienda, F.A. Caputo, S. Hall[1], M.G. Paule, R.L. Rountree, L. Schmued, T. Sobotka[1] and W. Slikker, Jr. Div. of Neurotoxicology, National Center for Toxicol. Research/FDA, Jefferson, AR 72079 and [1]Center for Food Safety & Applied Nutrition/FDA, Washington, DC 20204

Domoic acid (Dom, a seafood contaminant derived from certain algae and seaweed) is a tricarboxylic amino acid structurally related to kainic and glutamic acids. To identify neurohistological biomarkers of Dom exposure in relation to dose, adult and juvenile monkeys were given Dom (0.25-4 mg/kg, IV) or saline after an overnight fast and evaluated by a modified Fink-Heimer procedure, as well as GFAP and c-fos immunohistology. Juveniles tolerated up to 4 mg/kg Dom. Some adults (≥1 mg/kg) developed bradypnea, became moribund, and were perfused, revealing elevated c-fos expression within 3-7 hours of i.v. Dom. The remainder were perfused one week later; two adults (1 and 1.25 mg/kg) and one juvenile (4 mg/kg) had extensive neuronal and terminal degeneration and gliosis throughout the hippocampus and subiculum. Other adults (≤1 mg/kg) as well as juveniles (≤2 mg/kg) had small focal areas of terminal degeneration restricted to CA2 stratum lucidum. The results suggest that major hippocampal damage occurs only at near-lethal doses of Dom, while lower doses selectively target an area previously reported to contain the highest concentration of pre-synaptic Kainic-acid receptors in brain.

554.15

EFFECTS OF DOMOIC ACID ON BRAIN METABOLIC MARKERS AND ANATOMY IN RATS. N.M. Appel*, J.P. O'Callaghan, L.M. Freed, S. Wakabayashi, S.I. Rapoport, J.J. De George and J.F. Contrera. FDA CDER DRT, Laurel, MD; NIH NIA LNS, Bethesda, MD and USEPA, Research Triangle Park, NC.

Domoic acid (Dom) is a neurotoxic excitatory amino acid (EAA) which appears to act as an agonist at the kainic acid subtype of EAA receptors. Dom has been identified as the contaminant in mussels causing adverse neurological and gastrointestinal effects, and in some cases death, to people who consumed them. Incorporation of [^{3}H]palmitic acid ([^{3}H]PA) and [^{14}C]arachidonic acid ([^{14}C]AA) has been used to study regional structural and functional integrity in brain by autoradiography. Glial fibrillary acidic protein (GFAP) and argyrophilia have been used as markers of neurotoxic effects of drugs and xenobiotics in brain. Seven days following i.p. Dom (2.25 mg/kg) regional [^{3}H]PA incorporation was unchanged. In contrast [^{14}C]AA incorporation was significantly increased throughout the brain. Moreover in Dom-treated rats that manifested seizures, [^{14}C]AA incorporation was further increased in many brain regions (e.g. parietal cortex, thalamus, caudate putamen hippocampus, and septum) when compared to Dom-treated rats which did not display seizures. Widespread glial hypertrophy and increased regional concentrations of GFAP-like immunoreactivity were noted in Dom-treated rats but only in those rats manifesting seizures. Similarly only Dom-treated rats that manifested seizures displayed argyrophilia. Unlike the other markers, however, argyrophilia was discretely localized. These data suggest Dom causes widespread alterations in brain metabolism, which could be predictive of more focal neurotoxic effects.

554.16

NEUROBEHAVIORAL ASSESSMENT FOLLOWING LOW DOSE EXPOSURE TO DOMOIC ACID (DOMA) IN THE RODENT. **T.Sobotka*, R.Brodie, Y.Quander, S.Hall, M.Bryant and C.Barton. Food and Drug Administration, Washington, D.C.**

DOMA, a kainic acid analog, has been identified as the neurotoxin in amnesic shellfish poisoning characterized by gastrointestinal and neurologic distress, the latter including seizure, loss of memory and neuropathology. The full spectrum of neurotoxicological changes elicited by DOMA is still poorly understood. Studies are being conducted to delineate the neurobehavioral profile of animals exposed to DOMA, particularly at the lower range of the dose-response curve. In the initial series of experiments adult male rats, dosed i.p. with 0, 0.25, 0.75 or 2.25 mg DOMA/kg, were tested for changes in motor behavior, sensory reactivity and behavioral inhibition at representative intervals over a period of 2 weeks after dosing. Animals were then necropsied for a standard pathological evaluation. Overt signs of neurotoxicity, ranging from mild salivation and scratching to profuse salivation, pronounced head/forebody jerking, tremor and clonus, were noted 1 to 4 hrs after dosing with 2.25 mg/kg. Approximately 43% of the animals at this dose became moribund or died within 2 days. Behaviorally, general motility was significantly affected by treatment in an apparent biphasic fashion. Animals were hypoactive within 1 hour of dosing (0.75 mg/kg and 2.25 mg/kg) but then hyperactive at 6 hrs (2.25 mg/kg), 24 hrs (0.75 mg/kg) and 1 week (2.25 mg/kg) after dosing. Activity was at control level 2 weeks after dosing. Within the activity test sessions, habituation was significantly decreased immediately after dosing (0.75 mg/kg and 2.25 mg/kg) and at 24 hrs after dosing (2.25 mg/kg). Open field exploratory behavior was inconsistently affected by treatment. Auditory startle responding was unaffected but prepulse inhibition of startle was transiently enhanced 2 hours after exposure to DOMA but only at the 0.75 mg/kg dose. At term, neuropathological changes were in the hippocampus and the anterior cerebral hemisphere predominantly in the 2.25 mg/kg dose group. Significant treatment-related pathology was not found in other tissues examined.

554.17

DOMOIC ACID (DOM) PHARMACOKINETICS IN THE MONKEY: CORRELATION WITH NEUROPATHOLOGICAL EFFECTS. W. Slikker, Jr.*, J.A. Sandberg, L. Holder, Z. Binienda, F.A. Caputo, S. Hall, M.G. Paule, R.L. Rountree, L. Schmued, T. Sobotka and A.C. Scallet. Divisions of Neurotoxicology and Chemistry, NCTR/FDA, Jefferson, AR 72079 and CFSAN/FDA, Washington, D.C. 20204.

In order to develop a data-base for risk assessment of DOM, a seafood neurotoxicant, young (2.1 kg avg. wt.) and adult (4.9 kg) cynomolgus monkeys were administered a single bolus dose of DOM (0.25, 0.5, 1.0 and 4.0 mg/kg) or saline vehicle intravenously. Blood samples were collected before and up to 4 hrs after dosing. Model-independent pharmacokinetic analysis of HPLC-derived DOM plasma concentrations yielded the following values (mean ± SE): Vd_B (l/kg), 0.19 ± 0.03 and T 1/2 (hr), 0.99 ± 0.13. AUCs (μg/ml x hr) increased with dose (n=9; r=0.74). For severe neuropathological subgroup (N=3), AUCs ranged from 16-91. For less severe neuropathological subgroup (N=4), AUCs ranged from 2.8-11.5, and were correlated with histological damage 7 days after dosing (r=0.94). Clearance (ml/min/kg) ranged from 0.7-4.5 and inversely correlated with weight (age) of the monkeys (r=0.92). These data indicate that DOM exposure, as measured by plasma pharmacokinetic analysis, is predictive of subsequent central nervous system damage and that young monkeys exhibit less exposure and less neurotoxicity than comparably dosed adults.

554.18

Sigma sites 1 and 2 are not involved in neuroprotection governed by sigma ligands in primary hippocampal cultures. A.S. Lesage*, K. De Loore, and J.E. Leysen. Dept. of Biochemical Pharmacology, Janssen Research Foundation, Belgium

Various sigma ligands were tested for their induction of protection against glutamate induced excitotoxicity when chronically added to neuronal cultures derived from rat embryonic hippocampal tissue. IC50 for protection were calculated as the dose resulting in 50% inhibition of specific release of lactate dehydrogenase. The IC50 values for binding to the sigma sites 1 and 2 were determined on hippocampal membranes, via displacement of [^{3}H]-ditolyl-ortho-guanidine (DTG). We found that sabeluzole, opipramole, tiospirone, fenpropimorf and haloperidol were equipotent in binding to the sigma sites and protection against glutamate in hippocampal cultures. DTG and PD128298 showed high affinity for the sigma sites but were not protective up to a concentration of 10 μM. To investigate whether protective compounds acted as agonists and non-protective compounds as antagonists, we incubated the hippocampal cultures with the protective compound in the presence of 900 nM non-protective DTG or PD128298. The neuroprotection of ifenprodil, opipramole, tiospirone, fenpropimorf, sabeluzole, and haloperidol was not affected by the presence of DTG or PD128298. This suggests that neuroprotective properties of sigma ligands are probably not governed through their binding at the sigma sites on hippocampal neurons in culture. Therefore the correlation between potency in neuroprotection and potency for sigma site binding of compounds, is not the result of agonism and antagonism.

554.19

ROLE OF SIGMA RECEPTORS IN DEXTROMETHORPHAN MEDIATED NEUROPROTECTION. M. DeCoster*, E. Knight and F. Tortella. Walter Reed Army Inst. Res., Washington, DC 20307.

The neuroprotective effects of dextromethorphan (DM) have been demonstrated in *in vitro* models of neurotoxicity and *in vivo* models of brain and spinal cord injury (Tortella, TIPS 10: 1989). The neuroprotective mechanism(s) of action for DM remain undefined although several possibilities, including non-competitive antagonism of NMDA receptors, blockade of calcium channels, attenuation of post-injury hypoperfusion, and selective activation of specific high-affinity DM binding sites and/or sigma receptors, have been suggested. Using an *in vitro* model of neuronal toxicity, i.e. glutamate-induced cell death of cultured rat cortical neurons, we have initiated studies exploring the possible role of sigma receptors in the mechanism of action of DM neuroprotection. LDH measurements obtained from 12-15 day old primary neurons exposed to 80 μM glutamate for 45 minutes reveal significant neuronal death measured 24 h post glutamate. The neuroprotective EC_{50} for DM is 5 μM. The mixed sigma/PCP ligand (+)-SKF10047 and the selective benzomorphan sigma-1 ligand (+)-pentazocine are also neuroprotective (EC_{50}s = 2 and 3 μM, respectively). In contrast, the selective, non-benzomorphan sigma-1 ligand (+)-3-PPP is without effect at concentrations as high as 100 μM. Preliminary results indicate that DTG, which expresses high affinity for sigma-1 and sigma-2 sites, is also neuroprotective (100 % at 100 μM) while carbetapentane, a ligand possessing high affinity to specific DM sites as well as sigma-1 sites, was weakly neuroprotective (11% at 100 μM). Collectively, while certain sigma ligands are neuroprotective and others are not, their role in the neuroprotective mechanism of action of DM remains equivocal.

EXCITATORY AMINO ACIDS: EXCITOTOXICITY IV

555.1

EXCITOTOXICITY IN A NOVEL CONTINUOUS NEURAL CELL LINE. A. Cologer[1], S. Lu[1], N. G. Simon*[1] and G. I. Chovanes[2]. [1]CMBB, Lehigh Univ., Bethlehem, PA 18015 and [2]Lehigh Valley Hospital Center, Allentown, PA 18105.

Continuous neural cell lines that express glutamate receptors can potentially be used as an in vitro model for studying the mechanisms mediating excitotoxicity. Four major classes of glutamate receptors have been identified, along with subtypes of each. Pharmacological studies of a novel cell line, termed NH2 (a fusion between N18TG2 neuroblastoma and mouse hypothalamic neurons) indicate that it may express AMPA and/or metabotropic receptor subtype(s). Exposure to glutamate (2.5 mM to 20.0 mM) produced dose-dependent cell death (8.0% to 26.6%, respectively). Quisqualate (0.25 mM to 2.0 mM), a potent agonist targeting AMPA receptors and metabotropic receptor subtype linked to phosphoinositol, also produced dose-dependent death (17.7% to 52.1%). Additionally, exposure to ACPD, an agonist activating another metabotropic receptor subtype linked to cAMP, produced dose-dependent death (11.7% to 29.8% death in response to 0.03 to 1.0 mM ACPD). NMDA, however, did not have cytotoxic effects on NH2 cells. These findings suggest that NH2 cells may express AMPA and/or potentially two subtypes of the metabotropic receptor.

Supported by the D.R. Pool Trust.

555.2

POSSIBLE ROLE OF POLYAMINES IN GLUTAMATE-INDUCED NEUROTOXICITY IN THE NT2 HUMAN CELL LINE. M. Munir*, Y.M.-Y. Lee, L. Lu and P. McGonigle **Depts. of Pharmacology and Pathology, University of Pennsylvania, Philadelphia, PA 19104.**

NT2 cells are a clonal line of human teratocarcinoma cells that are terminally differentiated into neuron-like cells following exposure to retinoic acid. The fully differentiated cells express both NMDA and non-NMDA glutamate receptors and exhibit an excitotoxic response to glutamate. In this study, we have further characterized the excitotoxic response to glutamate using LDH release and MTT reduction. In 4 week old differentiated cells, glutamate-induced toxicity was dose-dependent with an EC_{50} of 30 μM and maximum toxicity produced at 3 mM. Increasing the duration of exposure to 5 mM glutamate from 0.5 hr to 6 hr did not significantly increase toxicity. The LDH release produced by a maximal dose of glutamate increased with the age of the differentiated cells. The density of NMDA receptors measured with [^{125}I]-MK-801 also increased with age suggesting that changes in receptor density may be primarily responsible for enhanced glutamate-induced toxicity in older cells. To examine the role of polyamines in excitotoxicity, cells were pretreated with the enzyme inhibitor DFMO to block the synthesis of polyamines. DFMO treatment significantly inhibited the toxicity produced by 2 mM glutamate and produced no toxicity when administered alone. In contrast, the parent polyamine putrescine enhanced the toxicity produced by glutamate and produced significant toxicity when administered alone. DFMO treatment did not attenuate the toxicity produced by putrescine. These results provide support for the hypothesis that induction of polyamine synthesis following NMDA receptor stimulation may play an important role in excitotoxicity. The NT2 cell line appears to be an ideal model system in which to study this phenomenon.

(Supported by USPHS GM 34781)

555.3

Transfection of HEK 293 cells with cloned NMDA receptors leads to cell death. N.J. Anegawa, D. Lynch, M. Batshaw*, D.Pritchett Depts. Pharmacology, Neurology and Pediatrics, Univ. of Pennsylvania, and Children's Seashore House, Philadelphia, PA 19104.

NMDA receptors have been implicated in cellular toxicity and recently, multiple putative NMDA receptor subunits have been cloned. We have investigated the effects of transfecting combinations of cloned NMDA receptors on cell viability. Human Embryonic Kidney 293 cells were transfected by the $CaPO_4$ method and 48 hrs after transfection, the number of viable cells were counted by trypan blue staining. Transfection with NR 1A and NR 2A cDNAs together resulted in dramatic cell death (37% of viable cells compared to plasmid transfected control). Transfection with NR 1a plus NR 2B or 2C resulted in lower levels of cell death (51 and 76% viable cells). Transfection of single subunits (NR 1a, 2A , 2B or 2C alone) did not result in cell death. Treatment with 1-10uM of MK 801 in the 1a+2A transfection partially protected the cells from degeneration (75% viable cells) However, 100uM APV or 100uM 7-Chlorokynurenate failed to protect the cells. Furthermore, transfecting NR 2A with a NR 1a cDNA in which asparagine (residue 598) was mutated to glutamine (a mutant known to decrease calcium permeability, Burnashev *et al.* Science 257: 1415, 1992)lead to a decrease in cell death (71% viable cells) These results demonstrate that transfecting combinations of cloned NMDA receptor subunits can lead to Ca^{++} dependent cell death. This work was supported by DA05432, Pfizer Fellowship.

555.4

GLUTAMATE RECEPTORS MEDIATE INWARD CURRENT, CYTOSOLIC CALCIUM ELEVATION, AND EXCITOTOXIC INJURY IN P19 CELLS. D.M. Turetsky*, J.E. Heuttner, L.M.T. Canzoniero, S.L. Sensi, M.P. Goldberg, D.I. Gottlieb and D.W. Choi. Depts. of Neurology, Cell Biology & Physiology, Anatomy & Neurobiology; and Center for the Study of Nervous System Injury, Washington Univ. Sch. of Med., St. Louis, MO 63110.

We examined the expression of glutamate receptor currents and receptor-mediated toxicity in P19 cells, a mouse teratocarcinoma cell line which can be induced to differentiate into glutamate receptor-bearing neuron-like cells by treatment with retinoic acid (MacPherson et al., Soc. Neurosci. Abst. 18, 612, 1992; Turetsky et al., J. Neurobiol., in press).

Undifferentiated P19 cells exposed to 10 μM retinoic acid for 4 d in suspension culture, then plated onto primary cultures of cortical glia, developed neuronal morphology and immunoreactivity for neurofilaments. Patch clamp recordings from cells 7-10 days after plating revealed inward currents evoked by NMDA or kainate. The former were potentiated by glycine, and blocked by MK-801 or Mg^{2+}; channels had a conductance of about 45 pS and permeability to both Na^{2+} and Ca^{2+}. The latter were blocked by CNQX. Fura-2 microfluorimetry showed that NMDA, kainate, and high K^+ all induced an increase in intracellular free Ca^{2+}. Exposure to 500 μM NMDA for 24 hours destroyed 80-95% of these P19 cells with EC_{50} about 70 μM; death was blocked by MK-801 or D-APV. 24 hr exposure to 500 μM kainate destroyed 60-75% of the cells; death was blocked by CNQX.

555.5

ACTIVATION OF CALPAIN BY NMDA RECEPTOR STIMULATION IN ORGANOTYPIC HIPPOCAMPAL SLICE CULTURE. S. Del Cerro*, Amy Arai, Markus Kessler, Ben Bahr, Peter Vanderklish, Santiago Rivera and G. Lynch. Center for the Neurobiology of Learning and Memory, University of California, Irvine, CA 92717, U.S.A. We have shown before that activation of the calcium-activated protease calpain is involved in the development of neuronal damage after ischemia or hypoxia. The present experiments sought to determine whether hippocampal slice cultures provide a suitable preparation for further studies of this type examining whether calcium influx through NMDA receptors can elicit activation of calpain. Slices were prepared from 10 and 13 day old neonates and maintained in culture for 1-6 weeks. Calpain activation was monitored by the appearance of a prominent 150 kD breakdown product of spectrin (SBDP) on Western blots. The SBDP concentration rose rapidly during the first day after cutting but then decreased to a low level by day 7 (4-6% of total spectrin immunoreactivity) and remained stable thereafter. Application of 100-500 μM NMDA for 5-30 min produced an increase in the SBDP proportional to the exposure time. The effect of NMDA was comparable at all culture durations (7-42 days) and was prevented with 20 μM MK-801. NMDA-induced calpain activation was strongly dependent on the extracellular calcium concentration; spectrin breakdown was negligibly small at 2 mM but readily detectable and similar in magnitude at 4 and 6 mM calcium. These results indicate that activation of calpain in response to NMDA receptor stimulation is similar in organotypic hippocampal slice cultures and in acute slice preparations from adult animals, and that slice cultures are a potential model for studying processes involved in neuropathology.
(Supported by grant AG-00583 from the National Institute of Aging).

555.6

N-METHYL-D-ASPARTATE-INDUCED NEUROPROTECTION IN CEREBELLAR GRANULE CELLS: EVIDENCE FOR A DEVELOPMENTALLY-EXPRESSED NEUROPROTECTIVE PROTEIN(S). P. Damschroder-Williams and S. M. Paul*. Section on Molecular Pharmacology, NSB, NIMH, NIH, Bethesda MD 20892

Exposure of cerebellar granule cells to subtoxic concentrations of NMDA results in a robust neuroprotective state as measured by exposure to toxic concentrations of glutamate (Chuang et al., Mol. Pharm. 1992) or MPP^+ (Marini and Paul, PNAS 1992). We have now characterized this neuroprotective state as to the kinetics of induction and reversal, sensitivity to protein synthesis inhibitors and time in culture. Preincubation of cerebellar granule cells with NMDA (10-100μM) results in a concentration-dependent (EC_{50} ~ 25μM) neuroprotective state against glutamate toxicity. With continuous NMDA (50μM) exposure maximal induction occurs between 12-16 hours; where the EC_{50} for glutamate toxicity is "right-shifted" almost 3 orders of magnitude (50μM in untreated vs. >50mM in NMDA-treated cells). The $t_{\frac{1}{2}}$ for reversal of the NMDA-induced neuroprotective state is ~ 24 hrs. As previously reported, preincubation of cells with cycloheximide blocks the neuroprotective state induced by NMDA. Finally, the neuroprotective state induced by NMDA decreases with time in culture; being maximal at 8-14 DIV and absent at >21 DIV. The ability of NMDA to induce the expression of a neuroprotective protein(s) in cerebellar granule cells may be developmentally regulated.

555.7

POSSIBLE ROLE OF EXCITOTOXICITY IN DEGENERATIVE EFFECTS OF 3-AP. M.G. Pierson*, D. Li, D. Glaze and J. Kwon. Cain Foundation Laboratories, Dept. of Pediatric Neurology, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030.

Selective neuronal degeneration due to 3-acetyl pyridine (3-AP) primarily affects inferior olive, other extrapyramidal sites and hippocampus. We hypothesized such effects are mediated by excitotoxic rather than metabolic processes. Electroencephalography was performed of 30 d Wistar rats 3 h prior to, and for 6 h after injection at three doses (62, 125, and 250 mg/kg, i.p.). Both cortical surface electrodes and hippocampal depth electrodes were employed. Brain was examined for signs of localized excitatory/degenerative reactions during the first 24 h. Staining included immunostaining for Fos protein and heat shock protein (HSP_{70}), acid fuchsin staining for damaged neurons and cupric silver staining for degenerating neurons. Brain was also examined 1 week after injection to identify lesions.

Indications of excitatory and/or degenerative effects were dose-dependent. Following highest dosage, EEG's indicated seizure activity during first 6 h. Induced Fos immunoreactivity, which locates excitatory events, was visible in hippocampus, striatum, and raphe nuclei, but not in inferior olive 4 h after injection. These sites and inferior olive could be selectively stained by acid fuchsin at 4 h. Only in inferior olive did induction of HSP_{70} occur so quickly. At 1 week there were lesions in all of the above sites, but especially in inferior olive.

Data suggest excitatory events precede lesions in all sites. The odd fact that HSP_{70}, but not Fos was induced at 4 h may indicate the speed with which inferior olivary cells are killed by 3-AP. Theoretically 3-AP may cause a buildup of quinolinic acid. Thus, the effects on toxicity of antagonism of NMDA type glutamate receptors were examined.

555.8

OXIDATIVE INJURY: A ROLE IN DICARBOXYLIC AMINO ACID-MEDIATED GLIOTOXICITY. R.J. Bridges*, J. Choi, L. Ralston, and M. Reyes. Department of Neurology, Irvine Research Unit in Brain Aging, Univ. California, Irvine, CA 92717.

In addition to being neurotoxic, several dicarboxylic amino acid analogs of glutamate have been identified as gliotoxic. Although the pathological mechanism through which these compounds damage glial cells is as yet not well characterized, it is clearly distinct from excitotoxic neuronal injury and appears to be dependent upon the intracellular accumulation of the toxic amino acids. In the present study we have begun to examine the potential role of oxidative injury as a contributing factor in gliotoxic pathology. Cultures of type I neonatal rat cortical astrocytes exposed to L-α-aminoadipate or L-homocysteate, two of the most potent gliotoxic amino acid, exhibited *i*) a decrease in their intracellular levels of glutathione and *ii*) a an increase in oxidative damage to proteins as measured by a decrease in tryptophan fluorescence. Furthermore, modification of the levels of antioxidants in the cultures strongly influenced the vulnerability of the astrocytes to gliotoxic-mediated cell lysis (quantified by a release of lactate dehydrogenase). Thus, astrocytes with low levels of glutathione, an endogenous antioxidant, were more vulnerable to injury, while supplementing the cultures with the antioxidant α-tocopherol attenuated the lysis of the astrocytes. These findings support the hypothesis that oxidative injury contributes to the underlying pathological events that are triggered by the accumulation of excessive levels of the gliotoxic dicarboxylic amino acids in astrocytes.
This work was supported in part by NIA LEAD award F32 NS07876.

555.9

EXPOSURE TO OXYGEN FREE RADICALS POTENTIATES EXCITOTOXIC INJURY IN CORTICAL CULTURES. L.L. Dugan* and D.W. Choi. Dept. of Neurology and Center for the Study of Nervous System Injury, Washington Univ. School of Medicine, St. Louis, MO 63110.

Excitotoxicity and free radical damage have been separately implicated in the pathogenesis of neuronal cell loss in several neurological diseases. Nevertheless, these processes may not be separate: excitotoxicity may be partly mediated by free radical formation (Monyer et al., Neuron 5: 121, 1990), and free radical formation may enhance glutamate release (Pellegrini-Giampietro et al., J. Neurochem. 51: 1960, 1988). Here we examined the effect of oxygen free radicals on neuronal vulnerability to excitotoxicity.

Murine cortical cultures exposed to 40-60 μM H_2O_2 exhibited a 10-40% loss of reduced glutathione after 30 min, and increased levels of hydroxyl radicals after 1 hr, but no evidence of cell damage by phase-contrast microscopy or lactate dehydrogenase efflux to the bathing medium. However, cell death after subsequent submaximal exposure to NMDA or oxygen-glucose deprivation was enhanced. The NMDA concentration-toxicity relationship was shifted to the left enough that some neurons were destroyed by low concentrations of NMDA normally lacking toxicity. Some potentiation of kainate toxicity was also seen.

These results suggest that levels of free radicals too low to produce injury in isolation, can enhance neuronal vulnerability to excitotoxic injury.
• Supported by a Dana Research Fellowship to LLD and NIH grant NS 30337 to DWC.

555.10

A FREE RADICAL MECHANISM OF KAINATE-INDUCED NEURO - EXCITOTOXICITY IN CELL CULTURE: POSSIBLE INVOLVEMENT OF XANTHINE OXIDASE ACTIVATION. Yu Cheng and Albert Y. Sun*. Dept. of Pharmacology, Univ. of Missouri, Columbia MO 65212.

Kainic Acid (KA) has been used to study the neuroexcitotoxicity produced by glutamate. Although the mechanism of KA toxicity is not completely understood, KA-induced membrane depolarization and intracellular Ca^{++} elevation are thought to be essential in the toxicity. In our previous experiments we detected free radical formation following the administration of KA. In the present study the mechanisms involved in KA-induced free radical formation and cell degeneration were investigated using high density cortical neuron cultures. A free radical trapping agent, PBN, as well as the combined action of exogenously added superoxide dismutase and catalase attenuated KA neurotoxic effect. Both a voltage-gated Ca^{++} channel blocker, verapamil, and a calpain inhibitor significantly protected cortical neurons from cell death. Increase in xanthine oxidase (XO) activity following calpain activation may be one of the free radical generating systems in response to KA stimulation since allopurinol, a XO inhibitor, also protected cortical neurons from KA-induced cell death. It is possible that KA stimulation induces a cascade by activating first a calcium-dependent protease which then activates xanthine oxidase, subsequently leading to the generation of hydroxyl free radicals. This free radical production may cause cell death and tissue damage associated with neurodegenerative diseases.
(Supported in part by NIAAA Grant #2054.)

555.11

GLUTAMATE INDUCES THE PRODUCTION OF REACTIVE OXYGEN SPECIES IN SINGLE CULTURED NEURONS.

Ian J. Reynolds*, Michael J. Zigmond and Teresa G. Hastings. Departments of Pharmacology and Neuroscience, Univ. Pittsburgh, Pittsburgh PA 15261.

The excitotoxic effects of glutamate on central neurons may be mediated in part by the production of reactive oxygen species. The goal of this preliminary study was to investigate the time course and ionic dependence of glutamate-induced production of reactive oxygen species in single neurons cultured from fetal rat brain. Neurons were cultured from the cortex of e18 rat embryos and used after 21-28 days *in vitro*. Cells were loaded with 10μM dichlorodihydrofluorescein diacetate (DCF) for 15min at 37°C. Fluorescence was measured using a Meridian ACAS 570 confocal microscope using standard fluoroscein optics.

Addition of 100μM glutamate with 10μM glycine altered DCF fluorescence in approximately half of the neurons examined within 5min of glutamate application. Increases in fluorescence were typically localized to the margins of the cell soma, while decreases in DCF signal were often seen in the middle of the cell. Both effects of glutamate on DCF fluorescence were Ca^{2+}-dependent. H_2O_2 (30mM) increased DCF fluorescence evenly across the cell soma in contrast to the glutamate-induced changes.

These results demonstrate the feasibility of detecting the production of reactive oxygen species at the level of a single neuron. The mechanisms underlying these changes are currently under investigation.

Supported by a Grant-in-Aid from the American Heart Association.

555.12

ADDITIVE INHIBITION OF GLUTAMATE UPTAKE BY ARACHIDONIC ACID AND OXYGEN RADICALS VIA TWO DISTINCT MECHANISMS

A. Volterra*, D. Trotti, C. Tromba & G. Racagni Ctr. Neuropharmacol., Inst. Pharmacol. Sci., University of Milan, 20133 Milan, Italy.

Massive arachidonic acid (AA) release, uncontrolled formation of reactive oxygen species (ROS) and enhanced extracellular levels of excitatory amino acids are important neurotoxic events triggered by brain insults such as ischemia and trauma. Several evidences suggest a link between these events. We have previously shown that both AA (Volterra et al., J.Neurochem, 59, 600-606,1992) and ROS (Volterra et al., Soc.Neurosci.Abs.,18,647, 1992) rapidly inhibit high-affinity glutamate (GLU) uptake. Here we study the interaction between AA and ROS effects in primary astrocytic cultures from rat cerebral cortex. Within 10 min GLU uptake is inhibited by 20% with 15 μM AA, 35-40% with 50 μM AA and 25-30% with ROS generated by xanthine(500 μM)+xanthine oxydase(50 mU\ml) (X\XO); when added together, 15 μM AA+X\XO reduce uptake by 45% and 50μM AA+X\XO by 60%. Potent uptake inhibition by AA+X\XO mixtures is not due to cell damage, since they do not increase extracellular LDH. Chemical reaction between AA and ROS to form new inhibitory species is unlike, because sequential addition of AA and X\XO leads to identical inhibition. On the other hand, AA and ROS effects are blocked by distinct agents: scavenger enzymes superoxide dismutase (SOD, 90 U\ml)+catalase (CAT, 3000 U\ml) or disulfide-reducing agent dithiothreitol (DTT, 2 mM) abolish ROS inhibition with no effect on AA, while albumin (BSA, 0.1%) blocks AA but not ROS. Moreover, progressive uptake decline during prolonged exposure to 15 μM AA+X\XO is only partially reduced by agents acting on either AA or ROS alone, while is blocked by a mixture of them (SOD\CAT+DTT+BSA). Our data indicate that: (1) AA and ROS inhibit GLU uptake via two distinct mechanisms; (2) their effects are completely additive. Overlapping formation of AA and ROS during brain insults could lead to severe derangement of GLU uptake function.

555.13

FREE RADICALS ENHANCE BASAL RELEASE OF [^{3}H]D-ASPARTATE BY CEREBROCORTICAL SYNAPTOSOMES.

S.C. Gilman*, M.J. Bonner and T.C. Pellmar, Physiology Dept., AFRRI, Bethesda, MD 20889-5603.

Increased free radical generation is associated with a number of pathological conditions. To investigate the effects of free radicals on basal and high K^+-stimulated release of excitatory amino acids (EAAs) from presynaptic nerve terminals, synaptosomes were isolated from the guinea pig cerebral cortex. Peroxide generates hydroxyl free radicals by reacting with tissue iron (Fenton reaction). Iron alone also has been suggested to generate free radicals in neural tissue. We previously demonstrated that peroxide-generated radicals decrease Ca^{2+}-dependent [^{3}H]L-glutamate release from synaptosomes while increasing basal release. The present study uses [^{3}H]D-aspartate, which is not taken up into the synaptic vesicles, to evaluate the release of EAAs from the cytoplasmic pool.

Pre-treatment with peroxide or iron alone or peroxide in the presence of iron significantly increased the calcium-independent basal release of [^{3}H]D-aspartate. Pre-treatment with the iron chelator desferrioxamine had little effect on its own but significantly limited the enhancement by peroxide. High K^+ evoked release in the presence of Ca^{2+} was enhanced by peroxide but not by iron. These data suggest that peroxide increases non-vesicular basal release of EAAs through Fenton-generated hydroxyl radicals. This release could cause accumulation of extracellular EAAs and contribute to the excitotoxicity associated with some pathologies.

555.14

KAINIC ACID (KA) INDUCED TOXICITY IN CULTURED NEURONS OVEREXPRESSING CU/ZN-SUPEROXIDE DISMUTASE.

O. Bar-Peled*, L. Sklair-Tavron and Y. Groner, Molecular Genetics and Virology Dept., The Weizmann Institute of Science, Rehovot, Israel 76100.

The gene encoding Cu/Zn-superoxide dismutase (CuZnSOD), a key enzyme in the metabolism of oxygen free radicals, resides in the Down's syndrome (DS) region of chromosome 21. The possible involvement of CuZnSOD overexpression in the etiology of DS and in neuronal death was studied by analyzing neurons of transgenic (tg)-SOD mice harboring and overexpressing the human gene. Neurons derived from cortex and spinal cord of Tg-SOD and non-transgenic mouse embryos were cultured. Mature cultures were exposed to 50 μM KA for 16 h and their sensitivity to excitotoxicity determined. It was found that Tg-SOD neurons, both cortical and spinal cord cultures, exhibited higher sensitivity to the treatment with KA as compared to non-transgenic cultures. To determine the contribution of glial cells to this differential vulnerability we examined glial-neuronal cultures from Tg-SOD and control embryos. On the average 50 percent more neurons died when astroglia from Tg-SOD mice were used as feeder layer, indicating that these cells play an important role in the apparent sensitivity of Tg-SOD neurons to KA toxicity. In spinal cord cultures, large motoneurons (LMN) were by far more sensitive to KA toxicity as compared to the cortical and spinal cord neurons, but no marked differences were observed between Tg-SOD and control LMN. The significance of these observations to Familial Amytrophic Lateral Sclerosis will be discussed.

555.15

THE EFFECT OF NITRIC OXIDE SYNTHASE INHIBITOR ON THE BEHAVIOR AND BRAIN MORPHOLOGY, A DOSE RESPONSE STUDY.

S. Shapira*, B.A. Weissman and T. Kadar, Dept Pharmacology, IIBR, Ness Ziona IL-70450, Israel.

It has been shown that in spite of an obvious protective effect of nitric oxide synthase (NOS) inhibitor on ischemic brain tissue in vitro, no such effect can be confirmed in vivo. The present study was taken in order to explore the dose dependency of nitric oxide (NO) inhibition on brain ischemia in vivo. Groups of gerbils were given doses of the NOS inhibitor nitroarginine (5-50 mg/kg, ip). Four hours later, the animals were exposed to 5 min forebrain ischemia, and behavioral (up to 6 days post ischemia) and histological (at 6 days) parameters were evaluated. All nitroarginine treated groups showed significant decrease (10-12%) in body weight, starting with the 5 mg/kg group, and spontaneous hyperactivity, with the highest activity in the 25 and 50 mg/kg groups, in a dose dependent fashion. Marked ischemic injury was observed in all animals exposed to ischemia, but not in the nitroarginine (50 mg/kg, ip) treated gerbils that were not subjected to ischemia. A dose dependent pattern was observed also in a morphometric analysis of the dorsal hippocampus (cell count of CA1). It is concluded that gradual inhibition of NO production aggravates the ischemic brain injury already at a low (5 mg/kg) dose, and this effect reaches its peak between 25 and 50 mg/kg in a dose dependent manner There is a distinct correlation between behavioral and histologic parameters.

555.16

DELAYED NITRIC OXIDE PRODUCTION MEDIATES L-GLUTAMATE AND KAINATE NEURONAL DEATH IN CULTURED RAT CORTICAL NEURONS

X. Vigé, A. Carreau, J.P. Nowicki and B. Scatton
SYNTHELABO RESEARCH, Biology Department, 31 avenue Paul Vaillant-Couturier, 92220 Bagneux, France.

We have evaluated the neuroprotective effect of N^G-nitro-L-arginine (L-NNA) against L-glutamate (L-glu)- or kainate-induced neurotoxicity in cultured rat cortical neurons under different treatment schedules. A substantial nitric oxide (NO) synthase activity was detected in these cortical cultures. The NO-mediated increase in cellular cyclic GMP content induced by a 5 min treatment with either L-glu or kainate was fully antagonized by L-NNA (100 μM). Treatment of neurons with either L-glu (500 μM for 5 min) or kainate (100 μM for 24 h) produced a delayed neuronal death at 24 h, as measured by LDH release. Addition of L-NNA (100 μM) to the medium 5 min prior to and during L-glu exposure (5 min) decreased by only 23 % (not significant) the amino-acid-induced neurotoxicity in spite of a complete inhibition of NO synthesis under these conditions. When added 5 min before L-glu and again just after L-glu removal and kept in contact with the neurons for the following 24 h, L-NNA (100 μM) antagonized by 74 % the L-glu-induced neurotoxicity (IC_{50} = 4 μM). Similarly, permanent application of L-NNA (100 μM) protected neurons from kainate (100 μM for 24 h) toxicity by 85 %. MK-801 (1 μM) completely antagonized both L-glu- and kainate-induced neurotoxicity, indicating that delayed neuronal death is mainly mediated by NMDA receptor activation.

These results demonstrate that in our experimental conditions, NO synthesized in response to NMDA receptor stimulation plays a major role in L-glu- or kainate-induced neurotoxicity. The increased efficacy of L-NNA with prolonged application indicates that neuronal death occuring during the maturation phase that follows L-glu exposure is due to a delayed production of NO.

555.17

NITRIC OXIDE TOXICITY IN XENOPUS OOCYTES: ROLE OF CALCIUM. J.D. Connor*, J.M. Nave, J.E. Smith, J.M. Lewis. Dept. of Pharmacology, Hershey Med. Cntr., Penn State Univ. Col. of Medicine, Hershey, PA 17033.

Nitric oxide may be a mediator of "excitotoxicity", a process in which unregulated binding of glutamate to its receptors leads to neuronal death. Glutamate increases the concentration of free calcium in cytoplasm which stimulates nitric oxide synthetase. We tested the hypothesis that Xenopus oocytes could serve as a model system for evaluating this series of events in vitro. Our specific goals were to characterize toxicity produced in oocytes by nitroprusside, a nitric oxide precursor, and to determine if calcium influences toxicity. Stages V and VI oocytes were kept in Modified Barth's Solution (MBS, a salt mixture containing calcium) or in calcium-free MBS. Dose-response and time-action data for nitroprusside toxicity were obtained. Toxicity was measured quantally by counting cells that failed to exclude trypan blue, 1%. Nitroprusside was toxic to oocytes in MBS in the range of 10 to 100 mM. Interbatch variability was considerable. In calcium-free MBS, the TD50 for nitroprusside was 65% of that estimated for normal MBS, and response variability was much reduced. In summary, nitroprusside in mM concentrations is toxic to oocytes, and the response is decreased by withholding extracellular calcium. Co-operativity between nitric oxide and free intracellular calcium may underlie the process of excitotoxicity.

555.18

GLUTAMIC ACID (GLU) UPTAKE INHIBITION BY NITRIC OXIDE (NO). S. POGUN* and M.J. Kuhar. Neuroscience Branch, NIH-NIDA, Balto., MD 21224.

Glutamic acid is thought to be the major excitatory neurotransmitter in the brain, and GLU uptake is thought to be the mechanism of inactivation of released GLU. One effect of the stimulation of NMDA receptors is the production of NO which can act as a gaseous, diffusible messenger, with possible presynaptic action. In this study, we examine the effect of NO on ^{3}H-GLU uptake in hippocampal synaptosomes from male, Sprague-Dawley rats. ^{3}H-GLU uptake was carried out by standard procedures. Tissues were preincubated first at 37°C for 15 min and then at 30°C for 5 min. Uptake was begun by adding ^{3}H-GLU (3 nM) and terminated 3 min later by adding cold sucrose and collecting the tissues by rapid filtration. NO inhibited ^{3}H-GLU uptake in a time, temperature and dose-dependent fashion. Maximal inhibition was about 50% which occurred with 300 µM NO. Inhibitions did not occur at 0°C. The inhibition was prevented by coincubation with reduced hemoglobin. Potassium ferro- and ferricyanide did not inhibit uptake but rather enhanced it. The inhibition by NO could be reversed by washing the synaptosomes by centrifugation and resuspension, although there was not a total reversal under our conditions. Thus, it seems clear that NO could, at least in principle, inhibit GLU uptake at synapses. This could have implications for and help explain glutamate neurotoxicity since NO production would have the effect of prolonging the presence of GLU in sensitive areas. Also, if NO is the retrograde messenger involved in long-term potentiation, inhibition of GLU uptake could contribute to the strengthening of the synapse which is characteristic of LTP. NO inhibition of GLU uptake is an interesting potential new mechanism in regulating synaptic activity.

555.19

CORRELATING CHANGES IN INTRACELLULAR FREE CALCIUM CONCENTRATION WITH EXCITOTOXICITY AT A SINGLE CELL LEVEL. A. F. Strautman*, J. S. Althaus, P. F. Vonvoigtlander CNS Diseases Research, The Upjohn Co. Kalamazoo, MI 49001.

An increase in the intracellular calcium concentration is a commonly accepted result of many types of CNS trauma. Its involvement in neurotoxicity has been described in a number of *in vitro* excitotoxicity models. We have developed a procedure for measuring the changes in intracellular free calcium concentration ($[Ca^{+2}]_i$) using fura-2 with an image analysis system and correlating these responses with viability in the same cells 24 hours later. We are testing the hypothesis that a particular pattern of changes in $[Ca^{+2}]_i$ may lead to cell death. We are using two related neurotoxic treatments with a primary culture of cerebellar granule cells: glutamate receptor analogs (NMDA, kainic acid, AMPA) and peroxynitrite, a recently described toxin that forms when nitric oxide and superoxide combine. We find that after a 30 minute exposure and washout of kainic acid a sustained elevation of $[Ca^{+2}]_i$ is indicative of cell death at 24 hours. Since peroxynitrite reacts very quickly at physiologic pH , we have developed a procedure to locally apply peroxynitrite to the cells using a micropipette and syringe pumps. We find that peroxynitrite increases $[Ca^{+2}]_i$ and, despite evidence that cerebellar granule cells are resistant to other nitric oxide generating agents, is toxic in this model. We are exploring the mechanisms of the peroxynitrite induced $[Ca^{+2}]_i$ increase and further characterizing the relationship to the sustained increase following excitotoxic exposure.

555.20

NITRIC OXIDE (NO) INDUCES THE RELEASE OF INTRACELLULAR CALCIUM ($[Ca^{2+}]_i$) IN STRIATAL NEURONS. B.A. MacVicar*, S.A. Samanani, S. Duffy and S. Weiss. Neuroscience Research Group, University of Calgary, Calgary, Alberta, Canada T2N 4N1.

NMDA receptor activation in neurons releases NO which is implicated in excitotoxicity and synaptic plasticity. We have examined the role of NO in NMDA-induced changes of ($[Ca^{2+}]_i$). Imaging and measurement of fura-2 fluorescence was used to estimate $[Ca^{2+}]_i$ in striatal neurons cultured from the embryonic mouse. Repetitive NMDA activation induced sustained increases in $[Ca^{2+}]_i$ in approximately 50% of neurons (13-15 DIV). Inhibiting NO synthesis with Nω-Nitro-L-arginine (NARG, 1 µM) prevented the NMDA-evoked sustained increases in $[Ca^{2+}]_i$. L- but not D-arginine (100 µM) prevented the actions of NARG suggesting that NO produced by NMDA activation induces sustained increases in $[Ca^{2+}]_i$. We therefore examined the actions of NO itself on $[Ca^{2+}]_i$. Striatal neurons were superfused with Hepes buffered solution that was degassed by bubbling with argon. NO containing stock solutions were obtained by bubbling degassed solutions with NO which were then diluted in degassed solution to the appropriate concentrations. Superfusion of striatal neurons for >30 min with either degassed solution alone or with nitrite (5-200 µM) did not effect resting $[Ca^{2+}]_i$ in most cells. NO (5-50 µM) induced increases in $[Ca^{2+}]_i$ which gradually developed over 4-5 min and reached a stable plateau in the continued presence of NO. The NO-induced $[Ca^{2+}]_i$ plateau persisted in 0 Ca^{2+}-EGTA solution but quickly decreased to control levels if NO was removed. These results indicate that NO induces Ca^{2+} release from internal stores and may transduce the sustained increases of $[Ca^{2+}]_i$ from NMDA receptor activation. Supported by MRC Canada and Ciba-Geigy.

EXCITATORY AMINO ACIDS: PHARMACOLOGY V

556.1

EFFECTS OF THE NMDA ANTAGONIST KETAMINE IN SCHIZOPHRENIC PATIENTS A.C. Lahti, D.J. LaPorte, B. Mokriski, C.A. Tamminga*, Maryland Psychiatric Research Center, University of Maryland at Baltimore, Baltimore, MD 21228

The non-competitive NMDA antagonist PCP produces an approximation of the signs and symptoms of schizophrenia in normal humans. Its psychotomimetic effect often extends beyond the half life of the drug. To evaluate the nature, dose characteristics, and time course of an NMDA antagonist on mental status in schizophrenic patients, we have administered the PCP analogue ketamine in a randomized double blind design using 3 dosages of ketamine (0.1, 0.3 and 0.5 mg/kg) and a placebo, each administered over 60 seconds. Patients received two sets of challenges: 1) after haloperidol stabilization treatment and 2) after 4 weeks of neuroleptic withdrawal. Patients were evaluated using the BPRS and the SANS global at baseline and 20, 90, and 180 minutes post injection. In haloperidol treated patients, ketamine induced a dose-related, short lasting worsening in mental status, often reminiscent of the patients' acute symptoms. The BPRS total score and psychosis subscale were significantly increased compared to the placebo at 20 minutes after the two higher doses. Preliminary data (N=3) fail to show that haloperidol blocks the ketamine-induced worsening in psychosis. Several subjects evidenced longer lasting psychotomimetic effects such as flashbacks, dreams, and prolonged psychosis worsening. Immediate recall of verbal and non-verbal information (Logical Memory and Figural Reproduction, Wechsler Memory Scale-Revised) and other cognitive measures were collected before and 30 minutes following drug. Ketamine had no effect on memory consolidation or on any of the other cognitive tasks.

556.2

DEXTROMETHORPHAN ATTENUATES AND REVERSES MORPHINE TOLERANCE. K.J. Elliott*, A. Hynansky and C.E. Inturrisi. Dept. of Pharmacology, Cornell U. Med. College, New York, NY 10021.

Dextromethorphan (DM) is an antitussive agent and a functional NMDA receptor antagonist. Infusion of morphine (MOR) (sc osmotic pump at 30 mg/kg/24 hrs) for 7 days resulted in a greater than seven fold decrease in MOR's potency as measured with sc cumulative dose response curves using the tailflick (TF) test in male CD-1 mice. The MOR ED50 value increased from 3.7 mg/kg (2.3-5.5, 95% CI) prior to the initiation of the MOR infusion to 27.1 mg/kg (17.8-41.8) on day 7 of the infusion. In contrast, co-administration of DM by sc infusion (12 mg/kg/24 hrs) attenuated the development of tolerance such that on day 7 the MOR ED50 of the DM + MOR treated group at 6.9 mg/kg (4.6-9.9) was not significantly different from the MOR ED50 on day 1. DM given alone as above for 1 or 7 days did not affect the TF response nor did it alter the MOR ED50. Tolerance (a 10 fold increase in the ED50) produced by the implantation of two 25mg MOR pellets for 4 days was completely reversed by DM (30 mg/kg tid for 3 days) while saline injected animals remained tolerant when tested on day 8. These results demonstrate that a clinically available drug which is an antagonist at the NMDA receptor may have utility in the modulation of MOR tolerance. Supported in part by DA01457 and CA09512.

556.3

NMDA RECEPTOR ANTAGONISTS ATTENUATE ANALGESIC TOLERANCE TO MORPHINE, BUT NOT TO KAPPA OPIOIDS. C.E. Inturrisi, K. Elliott, N. Minami, Y.A. Kolesnikov, G.W. Pasternak and K.M. Foley*. Depts. of Pharmacology, Neurology and Neuroscience, Cornell U. Med. Coll. and The Cotzias Lab. of Neuro-Oncology, MSKCC, New York, NY 10021.

Daily administration of 5 mg/kg sc of morphine (MOR), a mu-opioid agonist, or U50488H (U50), a $kappa_1$-opioid agonist, for 5 days in CD-1 mice results in a 2 to 3 fold shift to the right of the respective analgesic (tailflick, TF) dose-response curves, indicating the development of tolerance. Concurrent administration of the competitive NMDA receptor antagonist, LY274614 (LY) at 24 mg/kg/24 hr, sc infusion or daily 6 mg/kg ip, attenuates the development of MOR tolerance, when the response to saline plus MOR is compared on day 5 with LY plus MOR. Daily administration of the noncompetitive NMDA antagonist, MK801 (MK) at 0.3 mg/kg ip, also attenuated MOR tolerance. Neither of these drugs modify the TF response or alter the ED50 for MOR. In contrast, coadministration of LY or MK, as above, failed to attenuate the development of tolerance to U50 or to the $kappa_3$-opioid agonist, naloxone benzoylhydrazone (NalBzoH). These results suggest that mu-opioid tolerance but not $kappa_1$ or $kappa_3$ opioid tolerance involves the mediation of NMDA receptors. Supported by DA01457, DA07242, CA09512 and the Winston Foundation.

556.4

NMDA RECEPTOR AND PROENKEPHALIN GENE EXPRESSION ARE MODULATED BY CHRONIC MORPHINE ADMINISTERED ALONE OR IN COMBINATION WITH NMDA ANTAGONIST. M. Brodsky*, K. Elliott, A. Hynansky, N. Minami, and C.E. Inturrisi. Pharmacology, Cornell U. Med. Coll., NY, NY 10021.

Changes in N-methyl-D-aspartate (NMDA) receptor and proenkephalin (PPenk) gene expression were studied in morphine (MOR)-tolerant and MOR-sensitive rats treated with the NMDA antagonist, LY274614 (LY) (Tiseo and Inturrisi, JPET, 1993). NMDAR1 (functional NMDA receptor subunit) and PPenk mRNA were measured in selected CNS regions by solution hybridization. At one week NMDAR1 mRNA levels were significantly lower in spinal dorsal horn (SC) and higher in lateral thalamus (LT) of MOR-treated as compared to placebo. Coadministration of MOR with LY attenuated both the behavioral manifestations of tolerance and MOR-induced changes in NMDAR1 mRNA in SC and Nucleus Raphe Magnus (NRM), but not in LT. PPenk mRNA levels were significantly higher in NRM and Nucleus Paragigantocellularis (PGi) of MOR-treated rats, and coadministration of MOR with LY prevented this induction in NRM but not in PGi. Infusion of LY for 7 days to placebo rats had no effect on behavior or on the NMDAR1 and PPenk mRNA levels in these CNS regions. Altered NMDAR1 and PPenk expression in CNS regions comprising nociceptive pathways may contribute to the development of tolerance to morphine and its attenuation by LY. Supported by DA05130, DA01457 and CA09152.

556.5

(+)MK-801 PREVENTS THE DEVELOPMENT OF CHRONIC TOLERANCE TO ETHANOL ON A CIRCULAR MAZE TEST. S. Rafi-Tari, J.F. Liu, P.H. Wu, H. Kalant, J.M. Khanna and G.A. Cottrell*. Department of Pharmacology, University of Toronto, and Addiction Res. Foundation, Toronto, Ontario, Canada M5S 1A8

Much evidence indicates that the development of tolerance to ethanol involves learning. (+)MK-801 (MK) and ketamine, that disrupt learning and memory, also prevent the development of tolerance to the motor impairment effect of ethanol (E). However, in addition to its effect on learning, (+)MK-801 impairs motor function. Therefore the circular maze test, that depends on learning but is largely independent of motor function, was used to assess the effect of MK on chronic E tolerance. Forty male Sprague Dawley rats, trained and met criterion performance (21 ± 3 % of error responses) on a circular maze test, were ranked and divided into five balanced groups [saline (S)-saline; S-E; MK (0.15 mg/kg)-S; MK (0.15 mg/kg)-E (1.0 g/kg) or MK (0.075 mg/kg)-E (1.0 g/kg). The treatment consisted of an i.p. injection of S or MK followed 30 min later by another i.p. injection of S or E. Thirty min after S or E, rats were given a practice session on the maze. MK (0.15 mg/kg) given before the daily administration of E and practice on the maze, prevented the development of tolerance but a lower dose (0.075 mg/kg) of the drug did not. These results suggest that blockade of NMDA receptor activity prevents the development, rather than the expression, of tolerance to ethanol. Supported by NIAAA grant #1 R01-AA08212-03.

556.6

2-HEXYL-3-INDOLEACETAMIDE (FGIN-1-27) ACTING AT THE MITOCHONDRIAL DBI RECEPTOR COMPLEX (MDRC) ANTAGONIZES THE AMNESIC EFFECT OF MK-801. E. Romeo*, D. Konkel, I. Zivkovich, A. Korneyev, and A. Guidotti. Fidia-Georgetown Institute for the Neurosciences, Georgetown Univ Med Sch, Washington, DC 20007.

FGIN-1-27, a specific ligand for the glial MDRC increases the rate of brain pregnenolone synthesis in a manner that is blocked by PK11195 (JPET 262:971, 1992). Here we examined whether FGIN-1-27 pretreatment, by modulating brain steroidogenesis, has an effect on MK801 (0.6μmol/kg,i.p.) and triazolam (3.2μmol/kg,i.p.) induced memory impairment in the passive avoidance test in rats. FGIN-1-27 is devoid of any anterograde or posterograde action but, in a dose dependent manner (57-228μmol/kg,p.o.), prevents the learning impairment induced by MK801 but not by triazolam. Pretreatment with 47 μmol/kg,i.p., of pregnenolone sulfate (PS) - a steroid that positively modulates glutamate receptor function and has memory enhancing effects (PNAS 89:1567, 1992) - antagonizes the amnesic effect of MK801 but not that of triazolam. PK11195 (28μmol/kg,i.p.), which per se is devoid of action, antagonizes the effect of FGIN-1-27, while flumazenil (3.1μmol/kg,i.p.) antagonizes only the effect of triazolam. The antagonism of the MK801 amnesic action by FGIN-1-27 was reproducible in two other memory and learning rat models: the radial maze and the Morris water maze test. After injection of PS, this steroid reaches a brain concentration of approximately 1 μM which correlates well with the concentration of PS effective as a positive allosteric modulator at glutamate receptors (Mol. Pharmacol. 40:333, 1991). In addition we could demonstrate that 10^{-6}M PS decreases the binding of [^{3}H]MK801 to rat cortical membranes in the presence or absence of glutamate and glycine. In contrast FGIN-1-27 was unable to modify the binding of [^{3}H]MK801 in the same membrane preparation.

Supported in part by NIH grant RO 1 MH49486-01.

556.7

PARTIAL AGONISTS AT THE GLYCINE SITE COUPLED TO NMDA RECEPTORS AND INHIBITORS OF NITRIC OXIDE SYNTHETASE BLOCK LATE-PHASE FORMALIN-INDUCED LICKING IN MICE. L. Seguin, S. Le Marouille-Girardon and M.J. Millan*. I.D.R.S., 7 rue Ampère, 92800 Puteaux - France.

NMDA receptors in the dorsal horn of the spinal cord mediate amplification processes ('wind-up') underlying persistant pain and both channel blockers and NMDA recognition site antagonists display antinociceptive properties. The ion channel coupled to the NMDA recognition site also possesses a glycinergic site and recent data suggest that Nitric Oxide (NO) plays a role in the cellular expression of actions mediated by NMDA receptors. Thus, herein, we compared the antinociceptive actions of the following drugs: (+)-MK 801, a channel blocker; CPP, a NMDA recognition site antagonist; MDL 29,951, a glycine site antagonist; (+)-HA 966 and D-cycloserine, partial agonists at the glycine site and L-NAME and 7-nitroindazole, inhibitors of NO synthetase. Mice were injected in the dorsal hind paw with 20 μl of 5% formalin and licking quantified 0-5 min (early phase, EP) and 35-50 min (late phase, LP) thereafter. The rotarod test (in separate mice) was used to evaluate motor behaviour. Drugs were given s.c., 30 min pre-testing. Doses are Inhibitory $Dose_{50}$s (mg/kg, s.c.). (+)-MK 801 and CPP were equipotent against EP/LP pain at doses (0.03/0.03 and 2.1/2.4, respectively) close to those required for inducing ataxia; 0.05 and 2.9, respectively. In contrast, MDL 29,951 inhibited LP (1.0) but not EP licking (>10) and did not induce ataxia (>10). (+)-HA 966/D-cycloserine also selectively blocked LP (1.6/15.3) as compared to EP (33.3/>40.0) licking. Further, (+)-HA 966 likewise blocked LP licking (2.2) when administered after the EP. (+)-HA 966/D-cycloserine were inactive in the rotarod test (>40.0/>40.0). L-NAME/7-nitroindazole selectively blocked LP (2.8/9.8) versus EP (>40/>40) licking without inducing ataxia (>40.0/>40.0). In conclusion, as compared to channel blockers and NMDA antagonists, antagonists and partial agonists at the glycine site and inhibitors of NO synthetase may elicit antinociception against prolonged pain in the absence of motor discoordination.

556.8

PREGNENOLONE SULFATE BLOCKS NMDA ANTAGONIST-INDUCED DEFICITS IN A PASSIVE AVOIDANCE MEMORY TASK IN RATS. C. Mathis,* S.M. Paul and J.N. Crawley. Section on Behavioral Neuropharmacology and Section on Molecular Pharmacology, National Institute of Mental Health, NIH, Bethesda, MD 20892.

Neurosteroids, including pregnenolone sulfate (PS), potentiate NMDA receptor activation and display memory-enhancing properties (Irwin et al., 1992; Flood et al., 1988). The present study investigates the ability of PS to enhance retention performance of a passive avoidance task and to prevent the behavioral deficits induced by an NMDA receptor antagonist, 3-((+/-)-2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid (CPP).

PS (0.084 - 1680 pmol) or CPP (0.8 - 1.6 nmol) was administered intraventricularly to previously cannulated rats, 15 minutes before the training trial of a step-through passive avoidance task. PS had no significant effect alone on the retention performance 24 hours later, at the doses tested, although there was a trend towards improved retention. CPP significantly impaired retention performance at the doses of 1.2 and 1.6 nmol.

Following the same behavioral procedures, combinations of CPP (1.2 nmol) with PS (0.084 - 840 pmol) were administered, to investigate the interactions of PS with an NMDA receptor antagonist. PS significantly reduced CPP-induced deficits on the retention performance at doses of 420 and 840 pmol. In addition, PS (840 pmol) prevented the ataxic effects of CPP (1.2 nmol) in a rotorod test.

These results demonstrate that the neurosteroid PS potently antagonizes deficits induced by an NMDA antagonist on a memory task and on motor function, and support a positive modulatory role for PS on NMDA receptors.

556.9

EFFECTS OF AN NMDA GLUTAMATE RECEPTOR BLOCKER ON THERMOREGULATION IN THE RAT. C. V. Gisolfi* and F. Mora. Dept. of Physiology & Biophysics, Univ. of Iowa, Iowa City, IA 52242, USA, and Dept. of Physiology, Faculty of Medicine, Univ. Complutense of Madrid, 28040 Madrid, SPAIN.

Because (a) N-methyl-D-aspartate (NMDA) glutamate receptors are located in the anterior hypothalamus (AH), (b) glutamate is a putative neurotransmitter in the AH involved in neurochemical circuits of hormone regulation, (c) dopamine is involved in temperature control and interactions between dopamine and glutamate exist in the brain, the possibility exists for glutamate to participate in thermoregulation. To test this hypothesis, we measured heart rate, blood pressure, activity, and core body temperature via telemetry in male Sprague-Dawley rats (n=8) exposed sequentially to 1 h periods at ambient temperatures of 22°C, 40°C, and 22°C after i.p. injections of saline and 25, 50, and 100 mg/kg D,L-2-amino-5-phosphonopentanoic acid (AP5) administered randomly on different days. There were no effects of the NMDA blocker on heart rate, blood pressure, or activity. All doses of the blocker tended to elevate core body temperature above values observed following injection of the saline control, but there were no significant differences. We conclude that glutamate does not act in thermoregulatory pathways through NMDA receptors. Other glutamate receptors, such as AMPA and metabotropic receptors, are under investigation.

Supported by SDGICYT PB90-0252.

556.10

THE NMDA RECEPTOR ANTAGONISTS (±)-CPP AND CGS19755 ATTENUATE THE BEHAVIOURAL AND NEUROCHEMICAL EFFECTS OF AMPHETAMINE IN MICE. L.J. Bristow, L. Thorn, P.H. Hutson and M.D. Tricklebank*. Merck Sharp and Dohme Research Laboratories, Neuroscience Research Centre, Terlings Park, Harlow, Essex CM20 2QR, U.K.

We have previously shown that the glycine/NMDA receptor antagonist, R(+)-HA-966, attenuates drug-induced mesolimbic dopaminergic hyperactivity in rodent brain. We now show that the competitive NMDA receptor antagonists, (±)-CPP AND CGS19755, have similar properties.

Mice were injected i.p. with (±)-CPP or CGS19755 (1 - 10 mg/kg) and 30 min later given (+)-amphetamine sulphate (5 mg/kg, s.c.). Motor activity was then recorded over the following 2 h using individual photocell cages. In separate groups of mice, dopamine synthesis was determined by measurement of DOPA accumulation following inhibition of aromatic acid decarboxylase with NSD 1015. Animals were injected with (±)-CPP (10 mg/kg, i.p.) or CGS19755 (5 mg/kg) 30 min prior to (+)-amphetamine (2.5 mg/kg, s.c.). Thirty min later, all mice received NSD 1015 (100 mg/kg, i.p.) and then killed after a further 30 min and the nucleus accumbens dissected.

The hyperlocomotion induced by (+)-amphetamine was dose-dependently antagonised by both (±)-CPP and CGS19755 at doses having no effect on locomotor activity *per se* (ED50s = 5.8 mg/kg and 2.4 mg/kg respectively).(±)-CPP and CGS19755 also reduced the amphetamine-induced increase in DOPA accumulation in the nucleus accumbens by 59% and 66% respectively and neither compound had any effect on dopamine synthesis in the absence of amphetamine.

The results provide further evidence that NMDA receptor antagonists attenuate the activation of mesolimbic dopamine systems by amphetamine.

556.11

INTRACEREBRAL SPERMINE PRODUCES IFENPRODIL SENSITIVE HYPERACTIVITY IN RATS. Gh. Perrault, E. Audi, B. Zivkovic* and D.J. Sanger. Synthelabo Recherche (LERS), 31 ave P. V. Couturier, 92220 - Bagneux, France

The potential physiological importance of polyamines (such as spermine), in the central nervous system has been emphasised by the finding that these substances act at a site associated with NMDA receptors. However, few interactions between polyamine-induced effects and NMDA antagonists have been investigated in in vivo studies. In the present experiments, bilateral infusion of spermine (5,10,20 µg/side) into the nucleus accumbens produced in rats dose-dependent increases in exploratory activity (recorded in photobeam activity cages) which lasted for 60 min. This spermine-induced effect was antagonised by pretreatment, with an injection of ifenprodil (3,10,30 mg/kg, ip) an anti-ischaemic drug which shows NMDA antagonist properties probably mediated by activity at the polyamine site. These are the first behavioral findings showing the polyamine antagonist properties of ifenprodil, which, like other NMDA antagonists, shows anticonvulsant and central depressant effects but does not induce any behavioral stimulation or PCP-like discriminative stimulus properties. However, further experiments are needed to investigate whether this present effect is associated with the polyamine modulatory site of NMDA receptors or with other polyamine receptors.

556.12

KETAMINE-LORAZEPAM INTERACTIONS IN HEALTHY HUMANS. J.H. Krystal*, L.P. Karper, A. Abi-Dargham, D.C. D'Souza, A. Bennett, M.B. Bowers, D.S. Charney. Yale U. Dept. Psychiatry, VA Medical Center, West Haven, CT 06516

Lorazepam is a putative treatment for ketamine-induced psychosis, perceptual alterations, and cognitive impairments. To formally assess this capacity, the interactive effects of ketamine and lorazepam were studied in humans. METHODS: In an ongoing study, healthy subjects (n=18) completed four test days in which they received pretreatment with lorazepam 2 mg or placebo two hours prior to ketamine (0.26 mg/kg i.v. bolus followed by 0.65 mg/kg/hr i.v.) or placebo. RESULTS: Lorazepam did not significantly reduce increases in BPRS "positive" or BPRS "negative" symptoms of psychosis. Lorazepam reduced ketamine-induced perceptual alterations similar to dissociative states. Neither medication reduced gross orientation or produced motor impairment on the finger-tapping test. Ketamine increased perseverative error on the Wisconsin Card Sorting Test, an effect reduced by lorazepam. Lorazepam exacerbated ketamine impairments in delayed recall. Lorazepam did not alter ketamine-induced increases in plasma levels of cortisol or prolactin. IMPLICATIONS: Lorazepam is not an antidote for all ketamine effects. Lorazepam reduces ketamine effects on sensory integration and performance impairments on tests sensitive to frontal cortical impairment. However, lorazepam failed to improve ketamine-induced psychosis, negative symptoms, and memory impairments.

556.13

STRYCHNINE-INSENSITIVE GLYCINE ANTAGONISTS ATTENUATE A CARDIAC ARREST INDUCED MOVEMENT DISORDER. D.D. Truong, M.J. Hussong, A. Starr* and R.R. Matsumoto. Department of Neurology, University of California Irvine, Irvine, CA 92717.

Until the recent development of several novel and selective glycine antagonists, our ability to rigorously test the relationship between glycine and myoclonus was hampered by the lack of pharmacological tools. We now report the efficacy of four strychnine-insensitive glycine antagonists (HA966, felbamate, ACEA1011, ACEA1021) in attenuating the auditory-induced myoclonus exhibited in our cardiac arrest-induced animal model of post-hypoxic myoclonus. Male Sprague Dawley rats underwent cardiac arrest and resuscitation, subsequently exhibiting post-hypoxic myoclonus with salient features similar to the human form of the disorder. After the arrest and resuscitation, vehicle injections (saline i.p., DMSO i.p., water p.o.) were all without effect on the auditory-induced myoclonus exhibited by these animals. However, there was a significant effect of dose ($P<0.05$) for felbamate (500, 1000 mg/kg p.o.; Carter Wallace, Cranbury, NJ), ACEA1011 (10, 25, 50 mg/kg i.p.; E. Weber, UCI, Irvine, CA) and ACEA1021 (25, 50 mg/kg i.p.; E. Weber, UCI, Irvine, CA). Preliminary studies with a single dose of HA966 (10 mg/kg; RBI, Natick, MA) also showed marked attenuation of myoclonus. These data suggest the potential therapeutic usefulness of these strychnine-insensitive glycine antagonists in myoclonus, and possibly other ischemia-related, seizure and movement disorders.

556.14

ANTI-PARKINSONIAN EFFECTS OF CENTRALLY-ADMINISTERED GLYCINE ANTAGONISTS. BARBARA S. SLUSHER*, KEVIN C. RISSOLO, PAUL F. JACKSON AND LINDA M. PULLAN. DEPARTMENT OF PHARMACOLOGY, CNS SECTION, ZENECA PHARMACEUTICALS, WILMINGTON, DE 19897.

There has been a recent surge of interest in the potential utility of glutamate antagonists in Parkinson's Disease (PD). Degeneration of nigrostriatal dopamine neurons in PD results in overactivity of the glutamate neurons of the subthalamo-pallidal pathway. NMDA- and AMPA-type glutamate antagonists have been shown to reverse parkinsonian symptoms in several primate and rodent PD models. In theory, antagonists of the glycine site of the NMDA receptor should display similar antiparkinsonian activity, with less propensity for adverse side-effects. In this report, we examined the effects of glycine antagonists on reversal of akinesia associated with monoamine depletion in mice. Male Swiss-Webster mice were injected with 5 mg/kg reserpine (i.p.; t = -19 hr), 250 mg/kg a-methyl-p-tyrosine (i.p.; t = -30 min) and 20 mg of a glycine antagonist (i.C., at the level of the globus pallidus; t = 0). Motor activity was assessed for one hour using an Automex capacitance activity monitoring system. Known glycine antagonists including (R)-HA-966, Merck's L689560, 5,7-dichlorokynurenic acid and 7-chlorokynurenic acid caused a significant (720%*, 599%*, 390%* and 324%*, respectively; *$p<0.01$) stimulation of locomotor activity, compared to vehicle controls. ZENECA has recently reported a series of novel pyridazinoindole glycine antagonists. Two compounds from this series, 7,9-dichloro-8-methoxypyridazino[4,5-b]indole-1,4-dione and 7,9-dichloropyridazino-[4,5-b]indole-1,4-dione, also caused significant (349%** and 341%**, respectively; **$p<0.05$) stimulation of locomotor activity. Co-injection of glycine antagonists with the glycine agonist D-serine (100 mgs) completely blocked locomotor stimulation. D-Serine alone showed no effect. In addition, all glycine antagonists tested did not cause significant hyper-stimulation when centrally-administered to normal non-repserpinized mice. These data suggest that glycine antagonists may have therapeutic utility in the treatment of idiopathic PD.

556.15

AMPA PREVENTS NMA-INDUCED ELECTROPHYSIOLOGICAL CHANGES IN THE RAT CEREBRAL CORTEX - AN IN-VIVO STUDY. J.Addae*, L.Noray, S.Joseph, R.Saunders and G.N.Melville, Department of Physiology, The University of the West Indies, St.Augustine, Trinidad & Tobago.

It has been reported that the effect of topical application of N-methyl-D,L-aspartic acid (NMA) on the Somatosensory Evoked Potentials (SEPs) can be prevented by quisqualic acid (quis). This study set out to determine whether the effect of quis was via the ionotropic or metabotropic site. Male Sprague Dawley rats were anaesthetised with urethane. Following caniotomy, a cup of paraffin wax was constructed over the exposed cortex and the SEPs from forepaw stimulation recorded. Application of NMA at 0.5mM for 2 mins completely abolished the SEPs (n[number of rats]=20). Applying AMPA at 0.05mM for 5 mins increased the SEP amplitude by 18.9 ± 4.3% (mean ± sem), and totally prevented the effect of 0.5mM NMA (n=11). AMPA at 0.01mM for 5 mins had no appreciable effect on the SEP amplitude or the subsequent application of 0.5mM NMA (n=5). In comparison, the lowest concentration of quis to prevent subsequent NMA (0.5mM) effect was 0.25mM (n=13). This concentration of quis increased the SEP amplitude by 18.3 ± 4.3%. These results suggest that activation of the AMPA receptor-complex can inhibit NMA effects in the rat somatosensory cortex; this may be important in neuroprotection against NMDA-mediated neurotoxicity in the cerebral cortex.

556.16

SOME CORRELATES OF MK-801 TOXICITY WHEN ADMINISTERED TO PREWEANLING RATS. A. Weeks, T. Benevides, S. Rioux, G. McClenaghan, P. Frankham B. Fairey, B. Milburn, L.Ridley, D. Bannerman, R. Telford, S. Kish and M.J. Saari*. Neuroscience Research Unit Nipissing University, 100 College Drive, North Bay, Ontario, CANADA.

This study examined the effects of chronic MK-801 injections during the early developmental period. Male Wistar rat pups received daily injections of MK-801 (1.0 mg/kg; 0.1 mg/kg or saline vehicle;sc.) on postnatal days 7 to 12. Significant mortality was observed following the 1.0 mg/kg but not the 0.1 mg/kg dose. On sacrifice, significant reductions in organ and body weights were seen but only with the 1.0mg/kg group. The mortality following the 1.0 mg/kg injections occurred four to six days following the termination ofthe injections. The toxic effects of the MK-801 injections were blocked by pre-administration of NBQX (10mg/kg; sc.). Activity levels were affected by the MK-801 in a dose related manner. ACKNOWLEDGEMENT: The authors would like to thank NOVO NORDISK for their contribution of NBQX.

556.17

EVALUATION OF THE REINFORCING AND DISCRIMINATIVE STIMULUS EFFECTS OF THE PUTATIVE POLYAMINE-SITE NMDA ANTAGONIST ELIPRODIL. K.S. Nicholson, R.L. Balster* and D.J. Sanger. Department of Pharmacology & Toxicology, Medical College of Virginia, Richmond, VA 23298 USA and Synthelabo Recherche, 92220-Bagneux, France.

Eliprodil (SL 82.0715) has been shown to have noncompetitive NMDA antagonist effects in a number of *in vitro* models and it has *in vivo* anticonvulsant and neuroprotectant effects. Evidence implicates the polyamine modulatory site on the NMDA receptor as the site for eliprodil's NMDA antagonist effects (Carter et al. J Pharmacol Exp Ther 253:475, 1990). Because noncompetitive NMDA antagonists of the PCP-type produce psychotomimetic effects and are abused, eliprodil was evaluated for PCP-like abuse potential in two animal models. Eliprodil was tested for i.v self-administration in four rhesus monkeys who regularly lever-pressed for 10 µg/kg injections of phencyclidine (PCP). When doses of 3-100 µg/kg/injection of eliprodil were substituted for PCP for 4 consecutive days, rates of responding did not exceed those when saline or the emulphor vehicle were substituted, providing evidence that eliprodil did not have reinforcing effects under these conditions. Eliprodil (10 and 20 mg/kg i.p.) was also tested for substitution in eight rats trained to discriminate 3 mg/kg PCP from saline under a standard 2-lever drug discrimination procedure. None of the rats selected the PCP lever after either dose of eliprodil, yet 20 mg/kg decreased rates of responding showing that a behaviorally active dosage range was tested. These results suggest that eliprodil may not produce disturbing PCP-like subjective effects or abuse liability that would limit its clinical usefulness. Supported in part by NIDA grant DA-01442.

556.18

THE DISCRIMINATIVE STIMULUS EFFECTS OF EXCITATORY AMINO ACID AGONISTS IN NMDA-TRAINED RATS. D.M. Grech* H. Li, W.H.W. Lunn and R.L. Balster, Department of Pharmacology & Toxicology, Medical College of Virginia, Virginia Commonwealth University, Richmond, VA 23298 and Lilly Research Laboratories, Eli Lilly & Company, Indianapolis, IN 46285.

Sixteen male Sprague-Dawley rats were trained to discriminate 30 mg/kg i.p. NMDA from saline using a 2-lever operant procedure. Responding was maintained under a FR 32 schedule of food reinforcement. Substitution tests were completed with NMDA (3-56 mg/kg), the transmitter candidates: L-glutamate (30-560 mg/kg), L-aspartate (30-300 mg/kg), L-homocysteic acid (L-HCA) (100-1500 mg/kg) and L-cysteine (30-1000 mg/kg), monosodium glutamate (MSG) (100-3000 mg/kg), as well as kainic acid (0.1-3 mg/kg) and the selective NMDA receptor agonist, (D,L,-tetrazol-5-ylglycine) (LY 285265) (0.01-1.0 mg/kg). Only NMDA and LY 285265 fully substituted for the NMDA training dose (30 mg/kg). LY 285265 was approximately 100 fold more potent than NMDA for producing NMDA-like discriminative stimulus effects. Partial substitution occurred with MSG, L-glutamate, and L-HCA, resulting in mean maximum levels of 49-59% NMDA-lever responding, however, response rate decreases were only obtained with 3000 mg/kg MSG suggesting that behaviorally active doses of the other compounds may not have been studied. L-cysteine, kainic acid and L-aspartate also failed to substitute for NMDA or produce decreases in response rates. Unlike the majority of the excitatory agonists tested, full substitution occurred only with NMDA and LY 285265, suggesting that selective NMDA receptor activation is the basis for the NMDA discriminative stimulus. These results also suggest that LY 285265 may be a potent systemically active selective agonist for NMDA receptor stimulation. This research was supported by NIDA grants DA 01442 and DA 07027.

556.19

EFFECTS OF PHENCYCLIDINE TREATMENT IN VIVO ON THE PHENCYCLIDINE BINDING AND GLUTAMATE UPTAKE IN THE MOUSE CEREBRAL CORTEX. P. Saransaari*, S.M. Lillrank and S.S. Oja. Tampere Brain Res. Ctr., Dept of Biomed. Sci., Univ. of Tampere, SF-33101 Tampere, Finland.

The effects of a psychotomimetic drug, phencyclidine (PCP), on glutamatergic neurotransmission were studied in mice. The binding of tritiated 1-[1-(2-thienyl)cyclohexyl]piperidine (TCP) to cerebral cortical membranes and the uptake of [^{3}H]glutamate by cortical synaptosomal preparations were assessed after PCP treatment (1 mg/d/mouse for 3 days) with implanted minipumps. The binding capacity B_{max} of TCP significantly increased but the binding constant K_D remained the same after PCP exposure, indicating that more binding sites became available. The basic properties of the binding remained unaltered but the actions of glutamate, glutamate receptor agonists and glycine were potentiated in PCP-treated mice. The uptake of glutamate was saturable, consisting of both high- and low-affinity transport components. After PCP exposure the transport constant K_m of the high-affinity component increased but that of the low-affinity component was not changed. The maximal velocity V of the high-affinity transport increased while that of the low-affinity transport decreased. Moreover, inhibition by structural analogues was potentiated, suggesting modification of the glutamate transporter by PCP treatment. The results show that chronic PCP treatment, used as a model of psychosis, markedly affects the studied glutamatergic parameters.

Supported by the Emil Aaltonen Foundation, Finland.

556.20

DIFFERENTIAL EFFECTS FOLLOWING REPEATED ADMINISTRATION OF COMPETITIVE AND NON-COMPETITIVE NMDA RECEPTOR ANTAGONISTS ON STARTLE RESPONDING, NMDA-INDUCED CONVULSIONS AND LETHALITY IN MICE. J. P. Tizzano*, J. A. Johnson, K. I. Griffey and J. L. Grider. Lilly Research Laboratories, Eli Lilly and Company, Greenfield, IN 46140.

Competitive and non-competitive NMDA receptor antagonists protect against convulsions and neuronal injury in animal models. In this study, drug-related changes in auditory and tactile startle responding, NMDA-induced convulsions and lethality (12.5 nmol, intracerebral) were evaluated in mice following single or daily (7 days) injections with the competitive NMDA receptor antagonist, CPP (0, 10, 20, or 40 mg/kg, i.p.) or the non-competitive NMDA receptor antagonist, MK-801 (0, 0.1, 0.5, or 1 mg/kg, i.p.). CPP induced moderate tolerance to the depression in auditory startle responding at 40 mg/kg, whereas tactile startle responding was unaffected by repeated administration. In contrast, MK-801 induced a dose-related sensitization to the enhanced auditory and tactile startle response. Following an intracerebral injection of NMDA, mice treated repeatedly with CPP or MK-801, did not exhibit tolerance or sensitization to the anticonvulsant or lethal effects over this time period. The present results dissociate the effects of repeated CPP or MK-801 administration on auditory and tactile startle responding from those on NMDA-induced convulsions and lethality.

557.1

GLUTAMATE RECEPTOR EXPRESSION IN RAT STRIATUM: EFFECT OF DEAFFERENTATION

J.B. Penney, U. Wüllner, M.V. Catania, D.G. Standaert, C.M. Testa, G.B. Landwehrmeyer, L.S. Dure* and A.B. Young, Neurology Service, Massachusetts General Hospital, Boston, MA 02114.

Although the recent progress in molecular cloning has tremendously increased our knowledge about the diversity of glutamate receptors (GluR), little is known about the regulatory mechanisms that control GluR expression. Using *in situ* hybridization with oligonucleotide probes selective for the respective mRNAs, we studied GluR1-4 (flip/flop), NMDA R1, 2a, 2b, and mGluR1-5 subunit mRNA expression in the rat striatum after unilateral frontal cortical ablation. In addition, [3H]AMPA, [3H]Kainate and [3H]Glutamate (NMDA- and Metabotropic) ligand binding studies were performed. Striatal NMDA and AMPA but not kainate receptor binding sites were increased 3 days after cortical ablation and remained increased for at least 60 days. Metabotropic receptor binding to a low affinity quisqualate site was slightly, but significantly reduced. On the other hand, after a decrease at 3 days, only small increases of almost all GluR subunit mRNAs were found at 15 and 60 days in the dorsolateral striatum. No switch in subunit composition occurred. It is conceivable, that additional posttranscriptional mechanisms contribute to the increase in ligand binding sites. Contrary to the receptor subunits, GFAP mRNA expression was markedly enhanced at 3 days and returned to near normal at 60 days, indicating a distinct temporal reaction to deafferentation in astrocytes and neurons.

Supported by USPHS grant NS19613 and DFG grant WU184/1-1 and LA 702/2-1.

557.2

LOCALIZATION OF NON-N-METHYL-D-ASPARTATE GLUTAMATE RECEPTORS IN NORMAL AND ALZHEIMER HIPPOCAMPAL FORMATION.

Anne B. Young,* John B. Penney, Craig D. Blackstone and Bradley T. Hyman. Neurology Service, Massachusetts General Hospital and Harvard Medical School, Boston, MA 02114 and Department of Neuroscience, Howard Hughes Medical Institute Research Laboratories, Johns Hopkins University School of Medicine, Baltimore, MD 21205 USA.

The hippocampi and adjacent temporal cortices of 24 human brains were examined with antibodies to the GluR1, GluR2/3 and GluR4 subunits of the AMPA-preferring glutamate receptor. GluR1 immunoreactivity was most dense in dentate gyrus with lower density in other hippocampal and cortical regions. GluR 2/3 immunoreactivity was the most intense of the three antibodies, with high levels throughout most hippocampal subfields, where it was localized to cell bodies, proximal axons and dendrites. GluR4 immunoreactivity was very sparse in all regions. In Alzheimer's disease cases, the general pattern of staining was similar to that seen in controls. GluR1 and GluR4 immunoreactivity was seen in some but not all neuritic plaques. All 3 antibodies recognized some neurons undergoing neurofibrillary degeneration.

Supported by USPHS grants AG08487 (BTH), AG08671 (ABY, JBP), GM07309 (CDB), and the Howard Hughes Medical Institute (CDB).

557.3

IN SITU HYBRIDIZATION ANALYSIS OF AMPA RECEPTOR SUBUNIT GENES IN THE DEVELOPING RAT SPINAL CORD.

M. W. Jakowec* and R. G. Kalb. Dept. of Neurology, Yale University, New Haven, CT. 06510.

We have used *in situ* hybridization to study the areal and developmental expression pattern of the AMPA subtype of glutamate receptors genes in the rat spinal cord. Lumbar spinal cord from various postnatal ages and adults, were analyzed using riboprobes against the GluR1-4 subunits of the AMPA receptor. In the adult both GluR1 and GluR2 messages are highly expressed within the substantia gelatinosa but are essentially absent from all other regions of the spinal grey matter. The GluR3 and GluR4 messages are present in most adult spinal neurons (with the exception of the substantia gelatinosa which has only light staining with GluR4). In marked contrast, during early postnatal life the GluR1 and 2 messages are highly expressed throughout the entire grey matter and subsequently decline of the first months of life. The GluR3 and 4 messages are not developmentally regulated as they are present at stable levels throughout the grey matter in both neonates and adults. These findings suggest that AMPA receptors transiently expressed by neurons throughout the spinal grey matter during development. These AMPA receptors have a unique subunit composition with major contributions by GluR 1 and 2. The distinctive spatio-temporal expression of GluR 1-4 may confer unique electrophysiological properties upon developing spinal neurons that may have important implications for activity-dependent neuronal development. Supported by the NIH and the Muscular Dystrophy Association.

557.4

GLUTAMATE RECEPTOR SUBTYPES ARE DIFFERENTIALLY EXPRESSED IN ASTROCYTES FROM DIVERSE BRAIN REGIONS.

D. Fan*, A.B. Johnson, J.A. Kessler and R.S. Zukin. Dept of Neuroscience, Albert Einstein College of Medicine, Bronx, NY 10461.

Electrophysiological and pharmacological studies indicate that AMPA/kainate (non-NMDA) type glutamate receptors occur in astrocytes, whereas NMDA receptors do not. We used northern blot and *in situ* hybridization to examine the pattern of kainate/AMPA and NMDA receptor expression in astrocytes from different brain regions. Astrocytes from 1-2 day old neonatal rat hippocampus, striatum, cerebellum and cerebral cortex were maintained in culture in serum containing medium for 14-18 days. RNA harvested from these cultures was analyzed on northern blots and fixed monolayer cultures were hybridized *in situ* under conditions of high stringency with ^{32}P-labelled cDNA probes (northern analysis) or ^{35}S-labelled RNA probes (*in situ* hybridization) directed against GluR1, GluR2 and GluR3 (AMPA/kainate) and NMDAR1 receptor mRNAs. Under these conditions, there is no detectable cross-hybridization among these probes. In blots and cells hybridized to GluR1 and GluR3, labelling was observed in astrocytes from hippocampus, cerebellum and the cortex. In contrast, GluR2 and NMDAR1 expression were not detectable. In preliminary experiments (n=3), GluR1 expression was of highest density in the cerebellum and lowest in the hippocampus. These results indicate that GluR1 and GluR3 are the glutamate receptor subunits mRNA actively expressed in rat hippocampal, cerebellar and cortical astrocytes.

557.5

DISTRIBUTION OF NMDA RECEPTOR SUBUNITS ON RAT HIPPOCAMPAL, CEREBELLAR AND CORTICAL NEURONS IN CULTURE AND IN BRAIN SLICES USING SUBUNIT SPECIFIC ANTIBODIES.

B. Mulac-Jericevic, T.A. Benke, N.L. Peterson*and K.J. Angelides. Department of Cell Biology, and Division of Neuroscience, Baylor College of Medicine, Houston Texas.

The distribution of N-methyl-D-aspartate (NMDA) receptors plays an important role in neuronal signaling. The complex can be assembled from several subunits. Previously, the distribution of these individual NMDA receptor subunits mRNA in mouse and rat brain has been examined by in situ hybridization. NR1 subunit mRNA was found to be widely distributed throughout most brain regions, while the expression of the NR2C mRNA was observed in cerebellum and olfactory regions exclusively. We have now studied the distribution of NMDA receptor subunits using NR1 and NR2C subunit specific-antibodies. Antibodies were generated against N-terminal synthetic peptides, and their specificity characterized by several methods. Cultured neurons from hippocampus and cerebellum were labelled with anti-NR1 peptide antibodies. The labelling pattern in cultured hippocampal neurons showed a homogeneous distribution of NR1 subunits with some punctuate regions on both cell bodies and dendrites. Antibodies specific for NR2C subunit strongly labelled cerebellar granule neurons but not hippocampal neurons consistent with the exclusive localization of NR2C mRNA. Cortical slices were examined using scanning laser confocal fluorescence microscopy after labelling with anti-NR1 and NR2C subunit specific antibodies. CA1 pyramidal cell bodies were strongly labelled while dendrites, identified by anti-MAP-2 antibodies, were labelled less by NR1 specific antibodies. Anti-NR1 subunit antibodies showed very weak labelling of CA3 region dendrites compared to cell bodies. In cerebellum anti-NR1 peptide antibodies labelled granule neurons in both the outer and inner molecular layers. Purkinje neurons were lightly labelled. Similarly, antibodies specific for NR2C peptide labelled granule neurons in both the outer and inner molecular layer of the cerebellum. The pattern of labelling confirms results of in situ hybridization but offers additional insight into the receptor organization on the neuron surface. Supported by NIH grant NS28072 and a Human Frontiers of Science Award to K.J.A.

557.6

NMDA RECEPTORS AND SUBUNIT mRNAS EXPRESSED IN CULTURED CORTICAL NEURONS.

J. Zhong, S. L. Russell, D. B. Pritchett, P. B. Molinoff, and K. Williams*. Department of Pharmacology, University of Pennsylvania School of Medicine, Philadelphia, PA 19104-6084.

Ifenprodil, a novel NMDA receptor antagonist, discriminates, with a >100-fold selectivity, two subtypes of NMDA receptor that are differentially expressed in rat forebrain during postnatal development (Williams *et al.*, Neuron 10: 267-278, 1993). The *in vitro* expression of NMDA receptor subtypes has been investigated using binding assays with [^{125}I]I-MK-801 on membranes prepared from cultured cortical neurons. In cultures maintained for 7 days *in vitro*, ifenprodil inhibited the binding of [^{125}I]I-MK-801 in a monophasic fashion with high affinity (IC_{50} = 0.4 μM). In 21-day cultures, the inhibitory effects of ifenprodil were biphasic, having high- (IC_{50} = 0.5 μM; 75%) and low-affinity (IC_{50} = 100 μM; 25%) components. Expression of NMDA receptors having a low affinity for ifenprodil was reduced in cultures maintained for days 7–21 in medium with low concentrations of serum (1% or 0%). Serum deprivation did not alter the level of expression of receptors having a high affinity for ifenprodil. A solution hybridization/RNase protection assay was used to measure NMDA receptor subunit mRNAs in cultured neurons. The level of mRNA encoding the NR1 subunit increased during days 1–21 *in vitro*. NR2A mRNA was low or undetectable in 7-day cultures but increased progressively between days 7 and 21. NR2B mRNA levels remained relatively constant over time. NR2C mRNA was not detected in cultured cortical neurons. Delayed expression of the NR2A subunit in cultured neurons may be responsible for the generation of receptors having a low affinity for ifenprodil, as has been proposed for receptor expression in rat forebrain.

(Supported by USPHS grants NS 30000 and GM 34781).

557.7

IMMUNOAFFINITY PURIFICATION OF GLUTAMATE RECEPTORS OF THE NON-NMDA AND NMDA TYPE. N. Brose*[1], G.P. Gasic[1], S.W. Rogers[2], T. Moran[3], J.H. Morrison[3], R. Jahn[4] and S.F. Heinemann[1]. [1]Salk Institute, La Jolla, CA 92037; [2]Univ. Color. Health Sci. Ctr., Denver, CO 80262; [3]Mount Sinai Sch. Med., New York, NY 10029; [4]Yale Univ., New Haven, CT 06510.

We employed monoclonal antibodies directed against the non-NMDA glutamate receptor subunit Glu R2 and against the NMDA receptor subunit NMDA R1 to purify native non-NMDA- and NMDA-type glutamate receptors from rat brain membranes. Receptors were purified from detergent extracts using affinity columns that had been coupled with 6 mg immobilized IgG (either CI 3A11 anti-Glu R2 or CI 54.2 anti-NMDA R1). Purified fractions were analyzed by SDS-PAGE and Western blotting. Purifed non-NMDA glutamate receptors contained several protein species, as judged by silver stain, with the predominant species exhibiting molecular weights of ~100-105 kDa. Purified NMDA receptors contained a major protein component of ~116 kDa and several less abundant proteins. Western blotting demonstrated that the major protein components of purified non-NMDA receptors corresponded to Glu R1-4, while the most abundant band in purified NMDA-receptors was NMDA R1. Western blots using antibodies to several known glutamate receptor subunits showed that NMDA receptors form distinct protein complexes and do not contain non-NMDA receptor subunits. Similarly, receptors containing Glu R2 do not contain NMDA receptor subunits or glutamate receptor subunits of the kainate-preferring type (Glu R 6 and 7, KA2). These data suggest the existence of at least three structurally distinct groups of glutamate receptor protein complexes.

557.8

IMMUNOCYTOCHEMICAL LOCALIZATION OF A METABOTROPIC GLUTAMATE RECEPTOR IN NEURONAL CULTURES. P.CORSI*, Z.VIDNYANSZKY, T.J. GORCS, J.TAKACS and J.HAMORI. Ist.Fisiologia Umana, Medical School*, Bari 70100, Italy; Lab. Neurobiology, Semmelweis University, Budapest, Hungary.

In order to analyze the cellular localization of the metabotropic glutamate receptor (mGluR1a), the immunoreactivity to the mGluR1a at the light and EM levels has been investigated in primary neuronal cultures of cerebral cortex, hippocampus and basal forebrain from embryonic mouse. The polyclonal antibody utilized for the visualization of the mGluR were raised against the C-terminal amino acid sequence region 1194-1199 of the mGluR1a. At the EM level, a prevalent neuronal localization can be observed, the neuronal cell bodies with their processes appeared immunoreactive; the immunoreaction was mainly restricted to the cytoplasma, the nuclei devoid of staining. Similarly, the nerve terminals and the synaptic membranes appeared heavily stained accordingly with its predicted membrane localization. However, few differences can be pointed out in the different cultures, i.e. in the basal forebrain cultures the mGluR1a localization is both pre and post-synaptic, while in the hippocampal cultures it appears always post-synaptically with an enrichment at the post-synaptic density.

557.9

DISTRIBUTIONS OF NMDA RECEPTOR SUBTYPES CORRESPOND TO SPECIFIC RECEPTOR SUBUNITS. D. T. Monaghan*, H.C. Clark, and B.E. Schneider. Department of Pharmacology, Univ. Nebraska Medical Center, Omaha, NE 68198.

There are four pharmacologically-distinct populations of NMDA receptors. These are typified by receptors in the lateral thalamus ("antagonist-preferring"), medial striatum ("agonist-preferring"), midline thalamic nuclei and cerebellum. Using radioligand-receptor binding autoradiography and *in situ* hybridization of subunit-specific oligos, we find that the anatomical distribution of each of the four native receptor populations corresponds to a specific NR2 subunit. The NR2A transcript has a distribution very similar to the "antagonist-preferring" NMDA receptor subtype labelled by [^{3}H]antagonists (CPP and CGP39653), both displaying high levels in the VP nucleus of lateral thalamus and the deep cerebral cortical layers. NR2B subunit messenger were the only NR2 species found in regions enriched in "agonist-preferring" sites (medial striatum and lateral septum). NR2C subunits were largely restricted to the cerebellum which contains a pharmacologically-distinct receptor subtype. NR2D subunits were restricted to those diencephalic nuclei which contain another pharmacologically-distinct NMDA receptor subtype (the midline thalamic nuclei: intermediodorsal, paratenial, & paraventricular, and the anteroventral and medial geniculate nuclei). In addition, NR1 subunit transcripts containing "insert 1" were found in many regions containing high affinity for antagonists (thalamus, deep parietal cortex). Together with pharmacological studies of NMDA receptor subunits expressed in *Xenopus* oocytes (see Buller et al. abstract), the NR2 subunits appear to account for much of the pharmacological diversity of native NMDA receptors. Supported by NIH grant NS 28966.

557.10

THE NR2 SUBUNIT CONTRIBUTES TO THE PHARMACOLOGICAL DIVERSITY OF NATIVE NMDA RECEPTORS

A.L. Buller*, R.A. Morrisett and D.T. Monaghan Dept. Pharmacology, Univ. Nebraska Medical Center, Omaha, NE 68198.

Four distinct NMDA receptors, identified in radioligand binding studies, are typified by receptors in the lateral thalamus (LTH; "antagonist-preferring"), medial striatum (MS; "agonist-preferring"), midline thalamic nuclei (MT) and cerebellum (CBG). The relationship between these receptor subtypes and recently identified NMDA receptor subunits (NR1a-g; NR2A-D) has not been determined. We now report that the pharmacological differences between native NMDA receptor subtypes depends, in large part, on the particular NR2 subunit present in the NR1-NR2 heteromer.

The pharmacology of heteromeric NMDA receptors expressed in *Xenopus* oocytes (NR1-NR2A, NR1-NR2B, NR1-NR2C) was compared to that of native receptor subtypes in the LTH, MS and CBG. The NR1-NR2A receptor displayed higher affinity for all antagonists tested, while the NR1-NR2B receptors showed higher affinities for agonists, patterns analogous to that observed for NMDA receptors in the LTH and MS, respectively. Relative to NR1-NR2A and NR1-NR2B, oocyte-expressed NR1-NR2C receptors had a lower affinity specifically for both D-3-(2-carboxypiperazin-4-yl)-1-propenyl-1-phosphonic acid (D-CPPene) and homoquinolinate (HQ). These differences were identical to that observed for CBG versus forebrain NMDA receptors. Together with the observation that NR2 subunit mRNAs co-localize with distinct NMDA receptor subtypes (see Monaghan et al., this meeting), these data suggest that native NMDA receptor subtypes differ in their molecular composition and that NR2 subunits significantly contribute to the pharmacological diversity of NMDA receptor subtypes.

557.11

EXPRESSION OF METABOTROPIC GLUTAMATE RECEPTORS COUPLED TO ADENYLATE CYCLASE IN CEREBELLAR NEURONS. J.T. Wroblewski*[1], S. Ikonomovic[1], M.R. Santi[1], B. Wroblewska[2] and D.R. Grayson[1]. [1]Fidia-Georgetown Institute for the Neurosciences and [2]Department of Biology, Georgetown University, Washington D.C. 20007.

Primary cultures of cerebellar granule cells express metabotropic glutamate receptors (mGRs) coupled to the stimulation of phosphoinositide hydrolysis (mGR1 and mGr5) and those coupled to the inhibition of adenylate cyclase (mGR2, mGR3 and mGR4). Treatment of granule cells with forskolin in presence of the phosphodiesterase inhibitor 3-isobutyl-1-methylxantine caused a dose-dependent increase of cAMP accumulation. This effect was inhibited in a dose-dependent manner by the mGR agonists glutamate, ibotenate, and *trans*-1-amino-1,3-cyclopentanedicarboxylic acid (ACPD), with the maximal inhibition reaching about 50% of the forskolin-induced response. The inhibitory action of these agonists was not prevented by Mg^{2+}, MK-801 or CNQX, antagonists of ionotropic glutamate receptors, but it was blocked by pre-treatment of granule cells with pertussis toxin. The observed pharmacological profile suggested the presence of a G protein-coupled metabotropic receptor which closely resembled that reported for the expressed mGR2 and mGR3 receptors. The forskolin-induced cAMP accumulation was further decreased by 2-amino-3-phosphonopropionate (AP3) and 2-amino-4-phosphonobutyrate (AP4), suggesting the presence of mGR4 receptors. This pharmacological profile was compared with the appearance of mRNA for the particular mGRs. Using quantitative PCR assay with internal standards we have determined that cerebellar granule cells cultured for 9 days in 25 mM K^+ exhibit the highest level of mRNA for mGR4, followed by a two-fold smaller level of mRNA for mGR3. In contrast, the level of mRNA for mGR2 was 50-fold smaller than for mGR3. It may be concluded that in cerebellar granule cells two metabotropic glutamate receptors, mGR3 and mGR4 contribute to the control of cAMP concentrations.

557.12

THE EXPRESSION OF AN NMDA RECEPTOR GENE IN GUINEA PIG MYENTERIC PLEXUS. D.L. Broussard*, X. Li, D.B. Pritchett, P.G.C. Bannerman, D. Pleasure. Children's Hospital of Phila, Univ. of PA Sch. of Med., Phila, PA 19104.

Glutamate may mediate the release of ACh within the enteric nervous system (ENS) via NMDA-type receptors (Am J. Physiol. 261:G693,1991). We investigated the expression of the gene for the NMDA receptor in guinea pig myenteric plexus using RT-PCR. Total RNA was isolated from guinea pig myenteric plexus (enteric neurons and glia), cultured guinea pig enteric glia and rat brain. Following reverse transcription, PCR was performed with primers that, in rat and human neural tissues, amplify a 332 nucleotide sequence that overlaps the L-glutamate binding site of the NMDA type 1 receptor (PNAS 90:2174,1993). UV illumination of an ethidium bromide gel demonstrated a PCR product from the myenteric plexus and brain, but not enteric glia. This band was positive by Southern blotting with a ^{32}P-labelled oligonucleotide homologous to a sequence that, in the rat, is flanked by the primers. This is the first report of the expression of an NMDA receptor gene within the ENS, and supports a glutamatergic neuronal pathway within the ENS. (NIH #HD28815 & R.W. Johnson Foundation)

557.13

ABSOLUTE AMOUNTS OF THE mRNAs ENCODING THE METABOTROPIC GLUTAMATE RECEPTORS IN CEREBELLAR GRANULE NEURONS *IN VITRO*. M.R. Santi*[1,2], S. Ikonomovic[1], J.T. Wroblewski[1] and D.R. Grayson[1]. [1]Fidia-Georgetown Institute for the Neurosciences and [2]Department of Biology, Georgetown University, Washington D.C. 20007.

We have designed receptor specific primer pairs and internal standards corresponding to unique regions of the mGR1, mGR2, mGR3, mGR4 and mGR5 metabotropic glutamate receptor cDNAs. Using a competitive PCR derived assay with internal standards, we have been able to quantitate the absolute amounts of each mRNA in cerebellar granule cells maintained in primary culture in different concentrations of KCl (10 mM vs. 25 mM KCl) as a function of time in each paradigm (i.e. 3, 6, 9 and 13 Days *In Vitro* (DIV)). Different concentrations of KCl depolarize cerebellar granule neurons to different extents which has been shown to affect their survival properties and their responsiveness to various glutamate receptor agonists. We determined that the amounts of the mGR1 mRNA were constant in 10 mM KCl (1.2 amol/μg RNA) and higher than the values obtained in cultures maintained in 25 mM KCl (maximum of 0.86 amol/μg RNA at 6 DIV). mGR2 mRNA levels were lower but increased to a maximum (0.8 amol/μg RNA) after 9 DIV. In 25 mM KCl, mGR2 mRNA is even lower attaining a maximum after 6 DIV (0.33 amol/μg RNA). mGR4 mRNA levels, which were consistently the most abundant, were approximately the same during the initial time periods in both paradigms but differed significantly by the 9th DIV (20 amol/μg RNA in 10 mM KCl vs. 9.3 amol/μg RNA in 25 mM KCl). Preliminary results obtained using mGR5 specific primers indicated that the content of this mRNA is also higher in cultures maintained in 10 mM KCl than in 25 mM KCl. In contrast, mGR3 mRNA levels were consistently higher in cultures maintained in 25 mM KCl (0.92 amol/μg RNA in 10 mM KCl vs. 4.9 amol/μg RNA in 25 mM KCl). Collectively, the data indicate a complex temporal pattern of expression of this gene family which correlates with known metabotropic second messenger responses (either the stimulation of $InsP_3/Ca^{2+}$ cascade or inhibition of adenylate cyclase) to glutamate in the different KCl paradigms tested.

557.14

STABLE MAINTENANCE OF GLUTAMATE RECEPTORS AND OTHER SYNAPTIC COMPONENTS IN ORGANOTYPIC HIPPOCAMPAL SLICE CULTURES. K.B. Hoffman[1], B.A. Bahr[1], S. Rivera[2], P.W. Vanderklish[1], R.A. Hall[1], M. Kessler*[1], A. Arai[1], C. Gall[2] & G. Lynch[1], [1]Center for Neurobiology of Learning/Memory and [2]Dept. of Anatomy and Neurobiol., Univ. of Calif., Irvine, CA 92717

Cultured hippocampal slices retain many *in vivo* features with regard to neuronal circuitry, synaptic plasticity (e.g. LTP), and pathology, while remaining accessible to a variety of manipulations. The present study characterized glutamate receptors and other synaptic markers in organotypic hippocampal slice cultures using *1*) [^{3}H]AMPA and [^{3}H]MK-801 binding to membranes by filtration and autoradiography, *2*) immunostaining with anti-GluR1, -R2/3, -R4, and -NMDAR1, and *3*) *in situ* hybridization using [^{35}S]cRNA probes specific for receptor subunit mRNAs. Hippocampal slices (400 μm) obtained from 11 days postnatal rats were cultured on Biopore membranes in serum-containing media at 37°C and harvested after 0.4 - 30 culture days (CDs). GluR1, GluR2/3, and NMDAR1 antigenicity levels as well as [^{3}H]AMPA binding transiently decreased between 0.4 and 2 CDs, recovered by CD 5, and remained stable through at least CD 30. Similar profiles were exhibited by other synaptic markers (synaptophysin, $NCAM_{180}$, and $NCAM_{140}$) as well as by spectrin and MAP2. The GluR4 subunit, in contrast, was not detectable during the first 10 days in culture but then developed gradually. The slices expressed low (K_D = 500 nM) and high (12 nM) affinity [^{3}H]AMPA binding sites at a ratio of 10:1, as found in the adult hippocampus. The expected astrocyte/GFAP response to the slice cutting occurred at CD 2-5; GFAP then remained stable through CD 30. In summary, following an initial and brief depression, synaptic components were expressed at steady-state expression levels in long-term hippocampal slice cultures, thus, allowing the investigation of their functional and regulatory properties (AFOSR 92-J0307 supported).

557.15

ALTERATIONS IN HIPPOCAMPAL GLUTAMATE RECEPTOR EXPRESSION FOLLOWING CHRONIC EPILEPTIFORM ACTIVITY *IN VITRO* A. Gerfin-Moser, F. Grogg, B. H. Gähwiler, P. Streit* and S. M. Thompson. Brain Research Institute, University of Zürich, CH-8029 Zürich, Switzerland

We have used organotypic hippocampal slice cultures as a model system to investigate the long-term consequences of epileptic activity. Cultures were prepared with standard techniques from 5 day old rat pups, and allowed to mature for 14-18 days. Sustained epileptic activity was elicited by treatment with 500 µM picrotoxin in serum-free medium for 2-3 days. The expression of glutamate receptor (GluR) mRNAs was examined after 48 hrs by *in situ* hybridization using ^{35}S-labelled oligonucleotides as probes. The most striking change in mRNA expression was observed for two AMPA-sensitive GluR subunits (GluR-A & GluR-B). mRNA levels for these subunits decreased in convulsant treated cultures to less than half the levels observed in control sister cultures. However, this downregulation was reversible if treated cultures were subsequently kept in normal medium for five days. mRNA expression reached almost control levels again, which shows clear evidence of recovery. Nissl staining of hybridized picrotoxin treated cultures showed virtually no nuclear or perikaryal damage to the neurons, indicating that cell death did not account for the decrease in hybridization signal intensity. The mRNA expression of various $GABA_A$ receptor subunits was not found to be altered following long-term convulsant treatment, consistent with unaffected inhibitory synaptic transmission in CA3 pyramidal cells (Müller et al., 1993, Proc. Natl. Acad. Sci. USA, 90; 257-261). In further experiments, the mechanisms underlying modulation of GluR expression and their relation to excitotoxic injury will have to be established.

Supported by the Swiss National Foundation (grants nos 31-27641.89 and 31-28652.90) and Sandoz Pharma AG, Basel.

557.16

N-METHYL-D-ASPARTATE RECEPTOR (NMDAR) mRNA EXPRESSION IN NGIO8-15 CELLS UNDERGOING SYNAPSE FORMATION. H. Yamamoto, K. Akama, D. Kersey and J. Moskal*. The Chicago Institute for Neurosurgery and Neuroresearch, Chicago, IL 60614.

NG108-15 cells provide an excellent model system to study molecular mechanisms underlying synapse formation. NG108-15s are a clonal cell line derived from murine neuroblastoma, N18TG2, and rat glioma, C6, cell lines. Upon dibutyryl-cyclic AMP (dBcAMP)-induced differentiation, NG108-15 cells become "synaptically competent" and make functional synapses when co-cultured with rat myotubes. We used this system to examine the expression of NMDAR mRNA by Northern analysis. A rat NMDAR cDNA was generously provided by Dr. S. Nakanishi (Moriyoshi et al., *Nature* (1991) 354, 31-37). NMDAR mRNA could be detected in dBcAMP-treated NG108-15 and N18TG2 cells, but was absent in rat myotube cultures. When non-synapse forming, dBcAMP-treated, N18TG2 cells were co-cultured with rat myotubes NMDAR mRNA levels were negligible. However, a marked (approx. 10-fold) increase in NMDAR mRNA levels was detected when the NG108-15 cells were co-cultured with rat myotubes, compared to NG108-15 cells cultured alone. These results suggest that, while NMDAR mRNA induction may be due to the influence of muscle-associated factors on NG108-15 cells, the increase could be a necessary component of the molecular events leading to the formation of functional synapses.

557.17

AGE, SEX, ESTROUS CYCLE AND STRESS DEPENDENT CHANGES IN HIPPOCAMPAL GLUTAMATE RECEPTORS. G.Ö.Peker*, L.Kanıt, O.Algan, N.Özdemir, B.E.Okur. Dept. of Physiol., Ege Univ. Sch. of Med., Bornova, 35100 İzmir, Turkey.

Glutamate (glu) receptor system is uniquely involved in development, learning-memory, neurodegeneration and neuroendocrine regulation. Hippocampus, in particular, is an important site for plasticity where different neurotransmitter systems interact with others and with various hormones under changing circumstances. This study was undertaken to investigate whether L-3H-glu binding is sexually dimorphic, prone to activational effects of gonadal and cortico-steroids and vulnerable to ageing induced changes. Beginning from their fourth week of life, 20 male and 25 female virgin Sprague Dawley rats were exposed to 45 min duration of immobilization and bright light, three times a day, five days a week, for 12-14 weeks. L-3H-glu binding was assayed in hippocampal homogenates of the experimental, age-matched control and 25 aged (>20 months) male rats. Plasma corticosterone levels at decapitation were determined and vaginal smears were monitored for 30 days to assess estrous cycles. ANOVA revealed that hippocampal glu receptor binding (HGRB) is sexually dimorphic with higher values for males ($p<0.012$), estrus phase elevates binding ($p<0.022$) and chronic stress enhances binding dramatically ($p<0.006$). T-test showed a prominent ($p<0.0005$) decrease in aged rats. Our results imply that the HGRB is prone to manipulation by sex- and stress-related endocrine effects which may occur as organizational and/or activational genomic action and glu receptors are highly involved in biological ageing.

558.1

REGULATION OF NMDA RECEPTOR PHOSPHORYLATION BY ALTERNATIVE SPLICING OF THE C-TERMINAL DOMAIN. W.G. Tingley, L.A. Raymond, K.W. Roche, A.K. Thompson and R.L. Huganir*. Department of Neuroscience, Howard Hughes Medical Institute, The Johns Hopkins University School of Medicine, Baltimore, MD 21205.

NMDA receptors in the brain trigger synaptic plasticity, modulate synaptogenesis, and induce excitotoxic neuronal damage. Precise regulation of NMDA receptors is therefore essential for normal brain function and for neuronal survival. A variety of studies have suggested that protein phosphorylation of NMDA receptors may regulate their function. Multiple NMDA receptor subunits have recently been cloned (NR1, NR2A-D). We have examined the phosphorylation of the NR1 subunit by Protein Kinase C (PKC) in cells transiently expressing recombinant NR1 and in primary cultures of cortical neurons. PKC phosphorylates several distinct sites on the NR1 subunit. The majority of these sites are contained within the C-terminal domain, and are deleted in several splice variants of NR1. We are currently examining the functional effects of phosphorylation of recombinant wild-type and mutant NMDA receptors expressed in human embryonic kidney (293) cells. Our results demonstrate that the major sites for PKC phosphorylation of NR1 are regulated by alternative splicing of NR1 mRNA, suggesting that alternative splicing of phosphorylation sites may represent a general mechanism for regulating the phosphorylation of glutamate receptors. In addition, these results imply that the C-terminal domain of NR1 is located intracellularly, which contradicts current topology models for glutamate receptors.

558.2

PROTEIN TYROSINE KINASES (TKs) IN THE POSTSYNAPTIC DENSITY (PSD) AND PHOSPHORYLATION OF THE NMDAR1. S.Y. Lin*[1,2], K. Wu[1,2], T.W. Kim[1,2], Y. Huang[3], J.L. Xu[1], P.C.Suen [1,2] and J.B. Black[1,2]. [1]Dept. of Neurosci. & Cell Biol., UMDNJ/RWJ Med. Sch., Piscataway, N.J. 08854; [2]Grad. Program in Physiol. & Neurobiol., Rutgers Univ., Piscataway, N.J. 08854 and [3]Div. of Neurosci., NYSPI, New York, N. Y. 10032.

Protein tyrosine kinases play critical roles in cell growth and differentiation. Recent studies showing high TK activity in rat brain suggest that the enzyme(s) may be involved in neural signal transduction as well. Remarkably, however, potential synaptic functions of specific TKs and substrates are unexplored. We examined TKs and substrates in the PSD, which is critical for synaptic function. Our initial results revealed that the specific TK activator, Mn^{2+}/Vanadate, enhanced phosphorylation of several proteins in the PSD. The phosphate groups incorporated were resistant to treatment with 1M KOH, suggesting binding to tyrosyl residues. The presumptive TKs and substrates were greatly enriched in the cortical PSD, compared with those in the homogenate or synaptic membrane fractions. Western blot analysis, using highly specific rabbit anti-phosphotyrosine antiserum, indicated that the PSD isolated from cerebral cortex, cerebellum and olfactory bulb contained several region-specific phosphotyrosyl proteins. Since several lines of evidence suggest that tyrosine kinases and NMDA receptor(s) may be involved in long-term potentiation, we examined TK-mediated phosphorylation of NMDA receptor 1(NMDAR1), which we recently localized to the PSD. The receptor was phosphorylated in a Mn^{2+}/Van-dependent manner, and the phosphorylation was specifically inhibited by an antibody against the NMDAR1. Consequently, tyrosine phosphorylation may regulate NMDA receptor function. Taken together, our findings suggest that TKs and substrates are components of the PSD and that these synaptic molecules may play important role(s) in synaptic function through postsynaptic mechanisms. (NS10259 and HD23315)

558.3

PHOSPHORYLATION OF GLUTAMATE RECEPTORS IN CULTURED HIPPOCAMPAL NEURONS. S.E. Tan and T.R. Soderling*. Vollum Institute, Oregon Health Sci. Univ., Portland, OR. 97201.

Recent gene ablation studies strongly support a role for CaM-kinase II in hippocampal learning and memory (Silva et al, Science 257:201-206, 206-209). ^{32}P-labeling of non-NMDA glutamate receptors (GluRs) was studied in cultured hippocampal neurons stimulated 2-15 min. with agonists which selectively stimulate either CaM-kinase II, protein kinase C (PKC) or cAMP-dependent protein kinase A (PKA). Treatment of neurons with glutamate/glycine (Glu/Gly), ionomycin (Iono), or 12-O-tetradecanoyl- phorbol-13-acetate (TPA) increased ^{32}P-labeling of immunoprecipitated GluRs (GluR1-4) by 145%, 180% and 227%, respt. Glu/Gly and Iono, but not TPA, also increased ^{32}P-labeling of CaM-kinase II by 175% and 195%, respectively. Of these three agonists, only TPA stimulated phosphorylation of MARCKS (225%), a specific substrate of PKC. Forskolin treatment gave a 3-4 fold increase in the active catalytic subunit of PKA but did not result in the ^{32}P-labeling of GluR1-4, CaM-kinase II or MARCKS. Phosphorylation of GluRs in response to Glu/Gly was blocked by a specific NMDA antagonist (APV) or by a cell- permeable inhibitor of CaM-kinase II (KN-62). These results are consistent with our hypothesis that Ca^{2+}-influx through the NMDA-type ion channel can activate CaM-kinase II (Fukunaga et al. J. Biol. Chem. 267:22527-22533) which in turn can phosphorylate and enhance non-NMDA receptor ion channels (McGlade-McCulloh et al. Nature 362:640-642). Such a mechanism could contribute to the postsynaptic component of synaptic plasticity such as long-term potentiation.

Supported by NIH grant NS 27037.

558.4

PERSISTENT INTRACELLULAR PHOSPHATASE ACTIVITY REGULATES NMDA RECEPTOR/CHANNEL FUNCTION. D.N. Lieberman*[1] and I. Mody[2]. [1]Dept. Neurology & Neurological Sci., Stanford Univ., Stanford, CA, and [2]Depts. of Anesthesiology & Neurology, UT Southwestern Med. Ctr., Dallas, TX.

Currents through single NMDA channels were recorded in the cell-attached configuration from acutely dissociated adult rat dentate gyrus granule cells. L-aspartic acid (100-200 nM) and glycine (3 μM) were used to elicit openings primarily to the 50 pS level in an extracellular solution containing 1.8 mM Ca^{2+} but no Mg^{2+}. When the phosphatase inhibitor okadaic acid (10 μM) was perfused onto the neurons, mean open time, burst, cluster and super-cluster durations increased significantly (61%, 150%, 1250%, and 162% respectively), as did the number of openings per these complex groupings (30-72%). No changes in mean channel conductance were detected. Dwell time distributions were not altered by lower concentrations of okadaic acid (0.5-1 μM) which are known to completely inhibit phosphatases type 1 and 2A. Thus, the low potency of okadaic acid on NMDA channel kineties is consistent with the involvement of phosphatase 2B (the Ca^{2+}-dependent phosphatase calcineurin) in the intracellular regulation of the NMDA receptor/channel complex. This hypothesis was tested by eliminating Ca^{2+} entry through the channel. When patch electrodes containing no Ca^{2+} and 10 mM EDTA were used to record channel activity, single channel conductance increased by 35%, but okadaic acid (10 μM) was ineffective in producing changes in channel kinetics. Furthermore, when extracellular Ca^{2+} was substituted by equimolar Ba^{2+}, okadaic acid was equally ineffective.

These findings are consistent with the hypothesis that NMDA channels are regulated by a persistent phosphorylation/dephosphorylation process, and that Ca^{2+} entering via the channel reduces channel activity through the activation of an intracellular phosphatase.

Supported by NINDS grant NS-27528 (I.M.), and a Howard Hughes Predoctoral Fellowship (D.N.L.).

558.5

MODULATION OF NMDA RECEPTORS BY PROTEIN PHOSPHATASES IN CULTURED HIPPOCAMPAL NEURONS. L.-Y. Wang, B. A. Orser and J. F. MacDonald*. Depts. of Physiol., Pharmacol. and Anaesth. Univ. of Toronto, Toronto, Canada M5S 1A8

In the mammalian central nervous system, *N*-methyl-D-aspartate (NMDA) receptors play crucial roles in synaptic plasticity, neuronal development and neurotoxicity. Biochemical modulation of these receptors by protein kinases and phosphatases may have profound effects on the synaptic efficacy at excitatory synapses. We have investigated the actions of calyculin A, a potent inhibitor of phosphatase 1 (PP1) and 2A (PP2A) on NMDA receptors in nystatin perforated patches. Macroscopic currents activated by NMDA were potentiated by this phosphatase inhibitor. In the absence of added coagonist glycine, calyculin A also increased the rate of NMDA receptor desensitization. Single channel recording in the cell-attached configuration revealed that this inhibitor significantly increased channel open probability. In contrast, direct application of PP2A to cytoplasmic side of excised inside-out patches decreased the open probability of these channels. These data suggest that the inhibition of PP 2A by calyculin A may cause a net increase in the levels of phosphorylation of NMDA receptors. This effect is functionally similar to activating some protein kinases. Thus, synaptic efficacy can be potentially regulated by the endogenous activities of both protein kinases and phosphatases. "Supported by a grant to MRC of Canada Group in Nerve cells and Synapses".

558.6

ATP PREVENTS 'RUNDOWN' OF WHOLE-CELL NMDA CURRENTS BY SUPPORTING PHOSPHORYLATION RATHER THAN BY LOWERING $[Ca^{2+}]_i$ IN CULTURED RAT SPINAL DORSAL HORN NEURONS. Y.T. Wang*, Y. Pak and M.W. Salter. Div. Neurosci., Hosp. Sick Children and Dept. Physiol. Univ. Toronto, Toronto, Canada M5G 1X8.

Intracellular regulation of N-methyl-D-aspartate (NMDA) receptors was investigated by means of simultaneous patch-clamp recording and fluorescent measurement of $[Ca^{2+}]_i$ with fura2 in cultured spinal dorsal horn neurons. NMDA currents were evoked by pressure-ejection of L-aspartate (250 μM, 100 ms) at regular intervals every 1-2 min. During recordings using nystatin-perforated patch configuration NMDA currents were constant after stabilization of the perforation. However, during recordings using whole-cell configuration, when the intracellular solution contained BAPTA 10 mM, NMDA currents showed 'rundown': the currents declined progressively and stabilized at approximately 50% of the control level within 15 min of the start of recording. Also, each application of L-aspartate caused a transient increase in $[Ca^{2+}]_i$. When Mg-ATP (4 mM) was included in the intracellular dialysate, the rundown of NMDA currents was prevented and there was an increase in current amplitude. In addition, ATP reduced baseline levels of $[Ca^{2+}]_i$ and prevented L-aspartate-evoked increases in $[Ca^{2+}]_i$. These same effects on $[Ca^{2+}]_i$ were produced by raising the concentration of BAPTA to 30 mM without ATP. However, this concentration of BAPTA failed to affect the rundown of the NMDA currents. Inclusion of ATPγs, an ATP analog which is a substrate for phosphorylation, mimicked the effects of ATP on NMDA currents. But rundown was not affected by ATP-PNP, an ATP analog that is not a phosphorylation substrate. These results suggest that intracellular ATP prevents rundown of the NMDA currents not by lowering $[Ca^{2+}]_i$ but by supporting phosphorylation. (Supported by the Canadian MRC and the Nicole Fealdman Memorial Fund.)

558.7

A NOVEL MECHANISM OF AMPA RECEPTOR REGULATION: IONICALLY TRIGGERED KINASES & PHOSPHATASES

B.A. Pasqualotto*, R.A. Lanlus, and C.A. Shaw. Departments of Physiology and Ophthalmology and Neuroscience Program. University of British Columbia, Vancouver, Canada.

Agonist or depolarizing stimulation induce decreases in AMPA receptor number in slices of adult rat neocortex. The dependency of these agents on protein phosphorylation (Pasqualotto, Lanius, & Shaw, NeuroReport 4(4), 1993) raises the question of how such stimuli can stimulate protein kinase activity. One common feature of these regulatory agents is that they both cause transient, but profound, changes in local ionic concentrations within the cell. The molecular cascade of events linking cellular stimulation to subsequent receptor regulation was examined by investigating the effect of changes in the concentrations of several ions on the specific binding of ^{3}H-CNQX in slices of adult rat neocortex. Ca^{2+} led to concentration-dependent decreases in ^{3}H-CNQX binding which could be blocked by the protein kinase inhibitors H7 and protein kinase inhibitor peptide. In contrast, Cl^- led to concentration-dependent increases in ^{3}H-CNQX binding which could be blocked by the phosphatase inhibitors sodium-ortho-vanadate and sodium-ß-D-glycerol phosphate. K^+ and Na^+ had no effect on ^{3}H-CNQX binding. These results suggest that Ca^{2+} and Cl^- may be acting as signals which trigger kinase(s) and phosphatase(s) involved in the regulation of AMPA receptors. We suggest that such ionically triggered enzyme activty may play a key role in the regulation of many receptor populations.

558.8

PROTON SENSITIVITY OF RECOMBINANT NMDA RECEPTOR SUBUNITS.

Stephen F. Traynelis* and Stephen F. Heinemann. Molecular Neurobiology Laboratory, Salk Institute, La Jolla, CA, 92037.

NMDA receptor function in mammalian neurons is strongly inhibited by extracellular protons. Reported IC50 values in neurons suggest that both physiological regulation of interstitial H^+ and pathological changes in extracellular pH will influence NMDA receptor (NR) function. In order to better understand the structural basis for H^+ modulation of NR function, we have studied the effects of extracellular [H^+] on agonist-evoked currents recorded under voltage-clamp from the recombinant NR subunits expressed in *Xenopus* oocytes. Responses of homomeric NR1 subunits to glutamate (plus glycine) were inhibited by extracellular H^+ with an IC50 corresponding to pH 7.4 (40 nM; n=31). Three additional variants of NR1 that arise from differential splicing of the 3'- end of the RNA (NR1-2a,3a,4a) showed similar sensitivity to extracellular [H^+] (n=22). However, the presence of an alternatively-spliced 63 bp exon near the 5'- end of the RNA markedly reduced proton inhibition, regardless of the exon configuration of the 3'- terminal. IC50 values for all NR1 splice variants with this 5'- insert (NR1-1b,2b,3b,4b) were similar (pH 6.6; 250 nM; n=41). The differential H^+ sensitivity imparted to NRs by the 63 bp 5'- exon, which codes for a highly charged amino acid sequence, suggests that receptors containing subunits with this insert will be insensitive to changes in interstitial pH. In contrast to the 50% inhibition at physiological pH of NR1 subunits lacking the 5'- exon, NR1 subunits that contain this insert are fully functional under normal conditions.

Coexpression of NR1 subunits with NR2A or NR2B did not alter the proton sensitivity of NR1 subunits either with or without the 5'- exon (n=38). Furthermore, proton inhibition was independent of voltage (n=44), extra/intracellular [Ca^{++}] (n=54), agonist (glutamate/aspartate/NMDA; n=113), or agonist (glutamate or glycine) concentration (n=69).

558.9

SENSITIVITY OF CLONED NMDA RECEPTORS TO REDOX COMPOUNDS.

A.Omerovic*, J.P.Leonard and S.R.Kelso. Dept. of Biol. Sci.,Univ. of Illinois at Chicago, Chicago IL 60680.

We have studied the effects of redox compounds dithiothreitol (DTT), a reducing agent, and 5,5-dithio-bis-2-nitrobenzoic acid (DTNB) and nitric oxide (NO), oxidizing agents, on homomeric and heteromeric NMDA receptors expressed in Xenopus oocytes. NMDA currents were recorded under voltage clamp at -80 mV.

Redox modulation was observed in both homomeric (in oocytes injected with mouse zeta1 cRNA) and heteromeric (in cells coinjected with zeta1 and mouse epsilon1 or epsilon3 cRNA) NMDA receptors. In cells expressing homomeric receptors, DTT (4 mM; 5 minutes) induced an enhancement of the NMDA current to 138 ± 6 % of control. The effect was completely reversible in 5 minutes after washout of DTT. DTNB and the NO donor S-nitrosocysteine (SNOC) did not significantly inhibit NMDA currents in these cells.

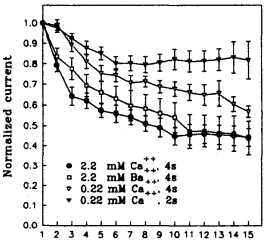
Heteromeric receptors (zeta1+epsilon1 and zeta1+epsilon3) showed a greater sensitivity to redox compounds: the average enhancement of the NMDA current by DTT was 154 ± 11 % and 355 ± 32 % of control, respectively. The effect was long lasting for both heteromers (in zeta1/epsilon3 it recovered only partially 60 minutes after washout of DTT). The increased NMDA current returned to control level only after DTNB application. SNOC (100 uM, 5 min) induced inhibition of the NMDA currents, but only after previous application of DTT. However, DTNB (1mM, 5 min) effectively reduced NMDA currents without previous reduction of receptor. Interestingly, both DTNB and NO effects were partialy reversible, which would not be expected if they act solely on vicinal cysteine residues.

558.10

NMDA-RECEPTOR CURRENTS UNDERGO USE-DEPENDENT INACTIVATION MEDIATED BY INTRACELLULAR CALCIUM.

A.F. Schinder and M. Montal*. Dept. of Biology, UCSD, La Jolla, CA 92093.

Repetitive stimulation of glutamate (glu) receptors elicits increasingly smaller ionic currents in hippocampal neurons. To investigate mechanisms underlying this phenomenon, voltage clamp (V_m= -80 mV) whole-cell currents evoked by locally perfused glutamate (100 µM) were recorded on hippocampal neuronal cultures from rat embryos. Pharmacological studies show that these currents are mainly carried by NMDA-receptor channels. In presence of 2.2 mM extracellular Ca^{++} ($[Ca^{++}]_e$), repetitive glu applications of 4 s/min. elicited progressively smaller currents whose size and shape stabilized after 10 episodes (figure). When $[Ca^{++}]_e$ was replaced by Ba^{++} (2.2 mM) similar effects were obtained. Reducing $[Ca^{++}]_e$ to 0.22 mM attenuated both the interepisode and intraepisode inactivation -as shown by the ratio between peak and steady state currents. Inactivation was prevented by shortening the length of the glu pulse to 2 s. When the membrane potential was held at +50 mV during the interepisode intervals, inactivation was partially and transiently abrogated. We conclude that NMDA-receptor channels inactivate in presence of intracellular divalent cations. This may arise from Ca^{++} binding either to the receptor or to closely associated regulatory proteins. This signaling pathway may involve a voltage-dependent phase.



Supported by NIMH.

558.11

DEVELOPMENTALLY RELEVANT PROPERTIES AND INHIBITION BY LEAD (Pb^{2+}) OF NMDA-INDUCED CURRENTS IN POSTNATAL, ACUTELY DISSOCIATED RAT HIPPOCAMPAL NEURONS.

K. Ishihara[1]* and E.X. Albuquerque[1,2]. [1]Dept. Pharmacol. Exp. Ther., Univ. Maryland Sch. Med, Baltimore, MD 21201; [2]Lab. Mol. Pharmacol., IBCCF, Fed. Univ. Rio de Janeiro, Brazil, 21944.

In previous studies using cultured fetal rat hippocampal neurons it was found that the amplitude of NMDA-induced currents increased with cell maturation, and that Pb^{2+} preferentially inhibited NMDA-induced currents in younger, cultured neurons (*JPET* 263:859 & 868, 1992). In this report, the ontogeny of the NMDA subtype of the glutamatergic receptor/ion channel, and developmental changes in its sensitivity to Pb^{2+} were investigated in hippocampal neurons acutely dissociated (with or without trypsin) from 3-30-day-old neonatal rats. NMDA-induced currents increased in amplitude until 10-12 days postnatal regardless of whether dissociation was carried out with or without trypsin. However, the NMDA-activated currents in the trypsin-treated group were smaller than those in the untreated group. The whole-cell currents induced by NMDA in the presence of glycine were composed of a fast and a slowly decaying current. The fast-decay component of the currents increased up to the age of 10-12 days after birth and then decreased, while the slow-decay component, also growing up to that point, plateaued instead. The current-voltage relationship for the NMDA-induced currents was not different between trypsin-treated and untreated groups. The EC_{50}s for NMDA and glycine in the enzyme-treated group were larger than in the other group. Pb^{2+} (10 or 30 µM) inhibited, dose dependently, NMDA-induced currents recorded from neurons dissociated at various stages of development. The Pb^{2+}-induced inhibition of NMDA-activated currents was not affected by trypsin treatment. The fast component, which was predominant at younger ages, was more susceptible to Pb^{2+} than the slow component, so that inhibition by Pb^{2+} was most pronounced in neurons dissociated at 3-4 days. These results indicate that sensitivity to NMDA of hippocampal neurons develops until the age of 10-12 days postnatal, and that Pb^{2+} is selectively toxic to immature neurons.

Support: USPHS Grants ES05730 and NS25296.

558.12

VARIATIONS IN PHYSIOLOGICAL RESPONSES OF THE HETEROMERIC NMDA RECEPTOR SUBUNITS EXPRESSED IN 293 HUMAN EMBRYONIC KIDNEY CELLS.

N. AGOPYAN*
Max-Planck Inst. f. med. Forsch., Abt. Zellphysiol., D-6900 Heidelberg, Germany.

When visualized by *in situ* hybridization, recently cloned heteromeric NMDA receptor subunits (NR1: Moriyoshi et al. 1991, Nature, 354:31; NR2: Monyer et al. 1992, Science, 256:1217) show differential expression patterns in the rat brain. To see whether or not the differences in regional expression patterns are also reflected in their physiological responses we recorded, in Mg^{2+}-free Ringer, 100 µM glutamate-induced whole-cell currents from 293 cells expressing heteromeric NR1-NR2 receptor channels.

In the absence of glycine, glutamate did not induce any detectable currents in NR1-NR2A, NR1-NR2B, and NR1-NR2C transfected cells. As the glycine concentration was increased NR1-NR2A, NR1-NR2B, and NR1-NR2C transfected cells produced an inward current which reached a maximum at 10 µM glycine. Dose-response curves revealed a higher glycine sensitivity in NR1-NR2C subunit (K_d : 0.67 µM). K_d's for NR1-NR2A and NR1-NR2B subunits were 4.15 µM and 1.12 µM respectively. Desensitization rate during a 1 sec pulse of glutamate and glycine was much faster in cells expressing the NR1-NR2A subunit than those expressing NR1-NR2B subunit. On the other hand cells expressing the NR1-NR2C subunit did not desensitize during the time course of application. The desensitization rate in NR1-NR2A and NR1-NR2B subunits was glycine dependent since in the absence of glycine preincubation NR1-NR2B did not desensitize while NR1-NR2A desensitized slower. The decay rate of the offset current after the glutamate pulse was also dependent on the subunit composition: In cells expressing NR1-NR2C and NR1-NR2B subunits offset rate was comparably slow (1.8 Hz) while in those expressing NR1-NR2A it was much faster (5 Hz). In conclusion the distinct physiological properties of heteromeric NMDA receptor subtypes may explain the existence of pharmacologically different NMDA receptors in the brain. *Supported by NSERC of Canada.*

558.13

PHARMACOLOGICAL PROPERTIES OF DOUBLE AND TRIPLE HETEROMERIC NMDA RECEPTOR COMBINATIONS.

K.A. Wafford*, C.J. Bain, B. Le Bourdelles, P.J. Whiting and J.A. Kemp Merck, Sharp & Dohme Research Laboratories, Terlings Park, Harlow, Essex CM20 2QR. England

The NMDA receptor plays a critical role in the control of neuronal transmission and cDNAs encoding the subunits which make up this receptor have recently been identified. We have isolated a cDNA clone from a human hippocampal cDNA library encoding the NMDA-R1 (NR1) subunit and this has been coexpressed in Xenopus oocytes with the rat NMDA-R2A (NR2A) and NMDA-R2C (NR2C) cDNA clones. Receptors formed from NR1+NR2A had EC_{50}'s for glycine and glutamate of 3.4μM and 5.2μM respectively. Receptors formed from NR1+NR2C had EC_{50}'s for glycine and glutamate of 0.14μM and 0.79μM respectively. By coexpressing NR1+NR2A+NR2C and examining the concentration response elicited by glycine a unique receptor was found to be preferentially expressed with an EC_{50} of 0.77μM together with a small proportion of NR1+NR2C receptors (<20%). Glutamate also had an intermediate affinity of 2.08μM on this receptor. As all three subunits coexist in the cerebellum, this trimeric combination may constitute a cerebellar form of the NMDA receptor.

558.14

FUNCTIONAL EVIDENCE FOR DIFFERENT NMDA RECEPTOR SUBTYPES IN NEURONES CULTURED FROM TWO REGIONS OF RAT BRAIN.

J. A. Kemp, R. G. Hill* and T. Priestley. Merck Sharp & Dohme Research Laboratories, Neuroscience Research Centre, Terlings Park, Eastwick Road, Harlow, Essex, CM20 2QR, U.K.

The discovery of multiple NMDA receptor subunits, some of which only occur in discrete brain regions, e.g. NR2C in the cerebellum, has highlighted the possibility of pharmacological subtypes of N-methyl-D-aspartate (NMDA) receptors. We have investigated this possibility by comparing the kinetics of NMDA receptor-mediated L-glutamate and glycine responses on whole-cell voltage-clamped rat cortical and cerebellar neurones in cell culture. There were marked differences between the two cell types in the time-constant for the agonist-evoked current relaxations (τ_{off}) (Table 1).

Table 1	Glutamate kinetics τ_{off} (ms)	Glycine kinetics τ_{off} (ms)
Cortex	467 ± 38 (11)	1107 ± 61 (19)
Cerebellum	170 ± 24 (12)♣	285 ± 27 (14)♣

♣ = significantly different from cortex, $P < 0.0001$ (t test)

These data indicate that glutamate and glycine have different affinities for NMDA receptors in these two cell types and, thus, provide functional evidence to support NMDA receptor heterogeneity in the rat central nervous system.

558.15

IL-1β POTENTIATES NMDA MEDIATED CURRENTS IN XENOPUS OOCYTES.

L.A. Leonard, G.E. Ringheim, M.C. Cornfeldt*, F. Wirtz-Brugger. Hoechst-Roussel Pharmaceuticals Inc., Somerville, NJ 08876-1258.

It has been proposed that a cytokine mediated acute phase inflammatory response may be involved in the neurodegenerative processes of Alzheimer's disease (AD). Many immune system proteins, including IL-1β, are elevated in AD (McGeer et al. Neurology. 42, 447-449, 1992). Excitatory amino acids (EAA) also play a pivotal role in chronic neuronal degeneration by inducing cytotoxicity. The potential synergistic relationship between EAA mediated currents and IL-1β was investigated in the present study. Xenopus oocytes microinjected with rat whole brain mRNA were incubated for 24 hours in culture solution (ND96+Ca^{++}) with or without the addition of 1nM m-IL-1β. These two populations of oocytes were then voltage clamped and perfused with various EAA's. The currents evoked by NMDA (100 and 500μM with 3μM glycine) were significantly potentiated ($p>0.05$) by 41 and 38 percent respectively in the IL-1β incubated group (N=48) as compared with controls (N=45). Currents elicited by GABA (50 and 100μM) and kainate (30μM) were unchanged by IL-1β. These findings may suggest a mechanism by which cytokines contribute to neurodegenerative processes in AD.

558.16

MODULATION OF N-METHYL-D-ASPARTATE RECEPTOR FUNCTIONS BY THE PENTADECAPEPTIDE.

V.K. Shukla, K.C. Marshall, H. Xiong, M. Dumont and S. Lemaire*. Departments of Pharmacology and Physiology, Fac. of Med., Univ. of Ottawa, Ottawa, Ontario, Canada K1H 8M5.

Histogranin, was originally identified as an adrenalomedullary pentadecapeptide possessing N-methyl-D-aspartate (NMDA) receptor antagonist activity. A chemically more stable analog, [Ser^1]histogranin has also been synthesized and shown to be as potent as histogranin in a rat brain membrane binding assay. Binding of [^{3}H]CGP-39653 to membrane preparations of rat brain was displaced by [Ser^1]histogranin in a biphasic manner with IC_{50} of 10.4 nM and 16.4 μM, representing 42% and 58% of the binding sites, respectively. Saturation binding experiments with [^{3}H]CGP-39653 in the abscence and presence of [Ser^1]histogranin (2 μM) indicated that the inhibitory effect of the peptide was noncompetititive, producing a decrease in the B_{max} (from 93.2 ± 8.5 to 62.1 ± 7.2 fmol/mg protein). Pressure injection of NMDA (5 mmol) and DL-α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) (5 mmol) on intracellularly recorded locus coeruleus neurons in an "in vitro" brain slice preparation induced pronounced increases in membrane depolarization amplitude and spike frequency. Coinjection of [Ser^1]histogranin (12.5 μmol) strongly blocked (85-100%) the effects of NMDA in 100% of tested cells (n=11) but had no or lower effects (20-50% reduction) in 30% of cells stimulated with AMPA. In the mouse "in vivo" model, [Ser^1]histogranin (10-100 nmol/mouse, i.c.v.) produced a dose-dependent blockade of NMDA (0.5-2 nmol)-induced convulsions. These results suggest that [Ser^1]histogranin is an allosteric modulator of NMDA receptor binding activity and a specific blocker of NMDA receptor functions. Supported by MRC.

PEPTIDES: BIOSYNTHESIS, METABOLISM, AND BIOCHEMICAL CHARACTERIZATION I

559.1

ISOLATION AND MOLECULAR CLONING OF *APLYSIA* FURIN AND PC2, TWO CANDIDATE PROHORMONE PROCESSING ENZYMES.

G.T. Nagle*, S.L. Knock, W.R.A. van Heumen, A.T. Garcia, E. Gorham, and A. Kurosky. Marine Biomedical Institute and Department of Human Biological Chemistry and Genetics, Univ. of Texas Med. Branch, Galveston, TX 77555.

The neuroendocrine bag cells and exocrine atrial gland of *Aplysia californica* express genes belonging to the egg-laying hormone (ELH) family and process the resulting precursor at mono-, di-, tri- and tetrabasic sequences. Some proportion of these cleavages may result from proteolysis by a furin-like or PC2-like enzyme; these enzymes have been identified in organisms as diverse as yeast and humans. The atrial gland is particularly interesting because the organ produces large amounts of ELH-related peptides and may be a relatively rich source of prohormone processing enzymes. An atrial gland λZAP cDNA library was constructed and screened using a furin-related PCR probe, and a clone encoding a furin-like protein was isolated. The insert has been partially sequenced, and is identical to a furin-related PCR product from the bag cells. The library was also screened using a *Lymnaea* PC2 probe (provided by A.B. Smit, Holland) and a clone encoding a PC2-like protein was isolated. These clones are relatively large as a result of extended 3′ untranslated regions. Supported by NIH NS 29261 and the Robert A. Welch Foundation (H-1190).

559.2

SECRETORY CELL CHARACTERIZATION AND LOCALIZATION OF PROHORMONE PROCESSING ENZYMES IN THE ATRIAL GLAND OF *APLYSIA CALIFORNICA*.

W.R.A. van Heumen, S.L. Knock*, G.T. Nagle, and A. Kurosky. Marine Biomedical Institute and Department of Human Biological Chemistry and Genetics, Univ. of Texas Med. Branch, Galveston, TX 77555.

Relatively little is known about the post-translational processing of egg-laying hormone (ELH)-related gene products in the atrial gland. A combination of morphologic techniques that included light microscopic histology and immunocytochemistry, transmission electron microscopy, and immunoelectron microscopy were used to localize ELH-related peptides in the atrial gland and to evaluate the characteristic morphology of their secretory cells. Results of these studies showed that there were at least three major types of secretory cells in the atrial gland one of which was immunoreactive to antisera against ELH-related precursor peptides. All three ELH-related precursor genes were expressed in type 1 cells and the processing of these precursors also occurred within the secretory granules of type 1 cells. In contrast to the bag cells, the N-terminal and C-terminal products of the ELH-related precursors of the atrial gland were not sorted into different types of vesicles. *In situ* hybridization studies confirmed the occurrence of furin and PC2 in the atrial gland. Supported by NIH NS 29261 and the Robert A. Welch Foundation (H-1190).

559.3

IDENTIFICATION AND CHARACTERIZATION OF THE LUQ PEPTIDES IN APLYSIA CALIFORNICA. R. S. Aloyz and L. DesGroseillers*. Department of Biochemistry, University of Montreal, Montreal, Canada, H3C 3J7.

We are investigating the role of neuropeptides in renal physiology. Left Upper Quadrant (LUQ) neurons and neuron L10 in the central nervous system of *Aplysia* have been shown to extensively innervate the kidney and regulate renal functions. Three neuropeptide precursor genes (LUQ-1, L5-67 and L10-M), which are differentially expressed in these cells, have previously been cloned. However, the nature of the physiologically relevant peptides is still unknown. We now report the characterization of the peptides isolated from the dorsal LUQ cells. Labelling of LUQ cells with radioactive amino acids and resolution of the peptide content of these cells on SDS-PAGE shows two prominent peptide bands of around 10 and 8 kd. The size of the bands roughly corresponds to the deduced molecular weights of the L5-67 propeptides and the longest of the mature products derived from it. To characterize the smaller -RFamide product, labeled peptides were separated on different HPLC systems. The major labeled peak comigrated with a synthetic peptide deduced from the cDNA sequence, on two different HPLC systems. Sequencing of the peak will confirm the nature of the peptide. We also looked for the presence of these two peptides in the kidney. Kidneys were first homogenized and the peptides were separated on Sephadex G-50. To identify the LUQ peptides, each fraction was screened by RIA, using antisera recognizing either the small -RFamide peptide or the longer peptide. These antisera will also be useful for determining the location of the peptide-containing cells by immuno-histochemistry.

559.4

PROTEOLYTIC PROCESSING OF THE *APLYSIA* A PEPTIDE PRECURSOR IN AtT-20 CELLS. P. A. Paganetti and R. H. Scheller*. Howard Hughes Med. Inst.; Dept. of Mol. and Cell. Physiol. Stanford Univ. Med. Center. Stanford, California 94305.

When the *Aplysia* ELH precursor is expressed in the neuroendocrine AtT-20 cells, the carboxyterminal derived peptides are packaged and stored in secretory vesicles, while the aminoterminal region of the precursor is constitutively secreted. When the highly homologous A peptide precursor is expressed in AtT-20 cells, both the aminoterminal and the carboxyterminal derived peptides are packaged in storage granules. This is likely to be due to the fact that the initial cleavage of the A peptide precursor occurs more slowly than the ELH precursor. We propose that in the A peptide precursor, the first cleavage occurs after the sorting site resulting in co-packaging of the multiple products derived from a single precursor protein. To determine the structural features of the prohormone responsible for this differential sorting, we made chimeric precursors and determined the rates of initial cleavage as well as the efficiency of storing the peptides products.

From these studies, we conclude that the differential sorting is not due simply to the amino acid sequence of the first processing site, but is also determined by more global aspects of the precursor structure.

559.5

STRUCTURE AND DISTRIBUTION OF RAT SUBTILISIN-RELATED ENDOPROTEASE PACE4 PREVALENT IN PITUITARY, BRAIN AND HEART. R. C. Johnson, D. N. Darlington* and R. E. Mains. Neuroscience Dept., The Johns Hopkins University School of Medicine, Baltimore MD 21205.

Low stringency screening of a rat hypothalamic cDNA library was performed using highly conserved 450 nt regions of rat prohormone convertases PC1, PC2 and furin. A clone containing a ~4 kb insert was identified which is about 90% identical to human PACE4 in amino acid sequence, with much lower similarity to rat PC1, PC2, furin, PC4, PC5 or PC6. The rat PACE4 sequence has the Asp-His-Ser catalytic site triad, the Arg-Gly-Asp routing signal and several sites for N-linked glycosylation. Rat PACE4 has a long COOH-terminal region which is very rich in Cys residues (15%). Rat PACE4 has a tissue and cell line distribution unlike any reported PC, with high expression in several brain regions, atrium, ventricle and anterior pituitary; little or no mRNA is detected in skeletal muscle, lung, liver, kidney, spleen, stomach, colon, duodenum, ovary, testes, pancreas and submaxillary gland. PACE4 mRNA is prevalent in AtT-20, GH3 and BRL cells, while undetectable in hEK-293, CHO, 3T3, COS, C127 and L cells using rat PACE4 probes. *In situ* hybridization revealed PACE4 mRNA in anterior and intermediate pituitary but not neural pituitary.

559.6

DISTINCT ROLES OF PC1 AND PC2 IN PROHORMONE and PROENZYME BIOSYNTHETIC PROCESSING. A. Zhou, S.L. Milgram & R.E. Mains* Dept. of Neuroscience,The Johns Hopkins University School of Medicine, Baltimore MD 21205.

Our previous work showed that overexpression of prohormone convertase 2 (PC2) in AtT-20 cells, a corticotrope cell line expressing high endogeneous levels of PC1 and low levels of PC2, could alter endoproteolytic processing of proopiomelanocortin (POMC) into a melanotrope-specific pattern, characterized by enhancing 2 cleavage steps [producing ACTH(1-13)NH2/CLIP and γLPH/β-endorphin] and adding two new cleavages to produce Lys0-γ3-MSH and β-endorphin(1-27). In a pulse-chase radiolabeling paradigm, the PC2 effects on processing were only seen after significant chase times. In the present study, PC1 was overexpressed in AtT-20 cells by stable transfection of PC1 cDNA. Results show that, unlike overexpression of PC2, PC1 increased the rate of conversion of POMC to ACTH through an intermediate in the first 1-2 hours of chase, further confirming that PC1 alters POMC processing primarily at the early steps and that PC1 and PC2 function in a strict temporal order. AtT-20 cells previously were established which stably expressed various forms of peptidylglycine α-amidating monooxygenase (PAM); PAM α-amidates peptides during biosynthesis. PAM itself is known to undergo endoproteolytic processing at several sites when expressed in AtT-20 cells. When such PAM expressing AtT-20 cells were stably transfected with PC2 cDNA (resulting in a double-transfected cell line), no additional cleavages were detected in PAM processing, while changes in POMC processing were seen as before, indicating a restricted substrate specificity for PC2.

559.7

CPE, PAM, PC1 AND PC2 mRNAs ARE EXPRESSED IN SUBSTANCE P and CGRP CONTAINING NEURONS OF THE RAT TRIGEMINAL GANGLION. C.M. Flores*, M.E. Goldstein and H. Gainer. Laboratory of Neurochemistry, NINDS, NIH, Bethesda, MD 20892.

The enzymatic, processing cascade leading to the formation of mature, bioactive peptides is thought to involve endoproteolytic cleavage of the propeptide precursor, removal of C-terminal basic residues and, in some cases, C-terminal amidation. Using *in situ* hybridization histochemistry in conjunction with immunocytochemistry, we determined whether the transcripts encoding any of the recently identified processing enzymes (i.e. CPE, PAM, PC1 and PC2) are coexpressed with the amidated neuropeptides SP or CGRP in neurons of the rat trigeminal ganglion (TGG). Consistent with investigations on other sensory ganglia, e.g. DRG (Goldstein et al., J. Neurosci. Res., 1991), the present studies demonstrate that the TGG contains two major cell types, distigulshable by their size and intermediate filament subtype expression: a population of relatively large cells that expresses NF-L (61%) and a population of relatively smaller cells that expresses peripherin (35%) with approximately 5% of cells coexpressing both proteins. The neuropeptides SP and CGRP were localized predominantly in the smaller, peripherin-positive population. Transcripts encoding each of the processing enzymes examined were detected in all TGG neurons, but only PAM mRNA appeared to be more highly expressed in SP and CGRP synthesizing cells. Moreover, each of these transcripts was detected as early as embryonic day 20 and increased throughout development, reaching maximal levels of expression in the adult animal, an ontogentic profile similar to that observed for SP and CGRP. These data indicate that the mRNA transcripts encoding CPE, PAM, PC1 and PC2 are all coexpressed with their putative neuropeptide substrates SP and CGRP. The finding that each of these enzymes is also expressed in cells not containing SP and CGRP suggests the existence of other neuropeptide substrates in these cells or, possibly, as yet unidentified functions for these enzymes.

559.8

RECOMBINANT ß-PROTACHYKININ (ß-PT) PRODUCED IN *E. COLI*: COMPARATIVE EXPRESSION AS ß-PT AND AS A ß-PT/MALTOSE-BINDING PROTEIN FUSION. A. Kohn*, R. Kannan, and V.Y.H. Hook. Dept. of Biochemistry, Uniformed Services University of the Health Sciences, Bethesda, MD. 20814.

Production of milligram amounts of recombinant neuropeptide precursors in *E. Coli* is desirable for studies of in vitro precursor processing at estimated in vivo precursor levels in the micromolar range. Expression of human ß-protachykinin (ß-PT) was accomplished with pET3c and pMAL-c expression vectors. The pET3c vector contains the T7 promoter and expresses the foreign protein, whereas the pMAL-c expresses the foreign protein as a fusion with the maltose binding protein (MBP). ß-protachykinin DNA was amplified by PCR from the ß-preprotachykinin cDNA with deletion of the signal sequence and with incorporation of appropriate restriction sites at 5' and 3' ends for subcloning into expression vectors. ß-PT in the pET3c vector was expressed in BL21(DE3) cells containing the bacteriophage DE3 and the gene for T7. ^{35}S-Methionine labeling studies showed that intact ß-PT was expressed upon induction with IPTG, but was rapidly degraded. Furthermore, ß-PT could not be detected by immunoblots or by Coomassie protein staining of SDS-PAGE gels. In contrast, the ß-PT/MBP fusion was expressed at high levels (milligrams), and was recognized by anti-PT (gift from Dr. J. Krause, Washington University) and anti-MBP sera. The ß-PT/MBP fusion contains a thrombin cleavage site at the junction of the ß-PT and MBP that may allow production of ß-PT for in vitro processing studies.

559.9

IDENTIFICATION OF CARBOXYPEPTIDASE H TRANSCRIPTS BY ANTISENSE *mRNA*. S.-R. Hwang* and V.Y.H. Hook. Dept. of Biochemistry, Uniformed Services University, Bethesda, MD.

Carboxypeptidase H (CPH; EC 3.4.17.10) is a metallopeptidase involved in the processing of prohormones. Previously, northern analysis using cDNA as probe showed that a single transcript of 2.2 or 2.4 kb is present in rats and human, respectively. However, multiple CPH mRNAs of 2.2, 2.4, and 3.3 kb have been demonstrated in bovine. In our studies, a full length rat antisense CPH mRNA was used to re-examine CPH transcripts among different tissues by northern analysis. We find that (i) mouse brain and AtT-20 cells contain mRNAs of 5.9, 2.6, and 2.2 kb, with the 2.6 kb form being predominant; (ii) rat brain and pituitary possess the 2.2 kb form as the main form among three transcripts; (iii) rat adrenal medulla contains primarily a 2.2 kb species among two transcripts (5.9 and 2.2 kb); (iv) bovine adrenal medulla contains primarily the 5.9 kb form, while in bovine pituitary the 2.2 kb mRNA appears as the major form. These results show a new CPH transcript of 5.9 kb, and indicate tissue-specific expression of multiple CPH mRNAs. To analyze transcripts, cDNA libraries from bovine adrenal medulla and pituitary were constructed using lambda Uni-ZAP^{TM}XR as vector. Northern analysis of transcripts generated from total library cDNAs by T3 RNA polymerase showed a distinct 4.2 kb transcript that was detected only from the adrenal medulla cDNA library. These results are consistent with the 5.9 kb mRNA in bovine adrenal medulla, but not in pituitary, and suggest that the 4.2 kb transcript may be generated from a cDNA representing the 5.9 kb CPH mRNA. Further studies will address differences among these transcripts.

559.10

ORDER OF DIBASIC PROCESSING IN PROENKEPHALIN. F. Liu, P.R. Housley and S.P. Wilson*. Department of Pharmacology, University of South Carolina School of Medicine, Columbia, S.C., 29208.

The opioid peptide precursor preproenkephalin (PPE) contains seven enkephalin sequences and is synthesized by epinephrine-producing adrenal chromaffin cells and a variety of peripheral and central neurons. After removal of a signal peptide, PPE is further processed at dibasic sites to yield its final products. Processing of human PPE in bovine chromaffin cells was examined using recombinant plasmids containing the human PPE cDNA under the control of the cytomegalovirus immediate early gene promoter. Preliminary studies showed that efficient transient expression of *E. coli* β-galactosidase was obtained in chromaffin cells with this promoter. Following transfection of hPPE into chromaffin cells, several proenkephalin-immunoreactive bands were observed on Western blots with the monoclonal antibody PE25 that recognizes human, but not bovine, proenkephalin sequences (Spruce *et al.*, *EMBO J.* 9: 1787-1795, 1990). The pattern of proenkephalin-derived peptides observed was similar to that of bovine PPE processing products. Site-directed mutagenesis was then employed to make a series of recombinant plasmids containing mutations in the hPPE sequence at dibasic processing sites. Conversion of Arg to Gln at hPPE(229) or hPPE(236) did not alter the pattern of proenkephalin-immunoreactive peptides, suggesting that these particular sites may not important for proenkephalin processing. Single and multiple mutations of other processing sites were also performed. These mutants will be used to determine the sequence of processing at the various dibasic sites of proenkephalin. Supported by the South Carolina Heart Association.

559.11

CARBOXY-TERMINAL CLEAVAGE OF PROHORMONE CONVERTASE 1: MECHANISM AND POTENTIAL SIGNIFICANCE Y. Zhou and I. Lindberg* Dept. of Biochem. and Mol. Biol. Louisiana State University Medical Center, New Orleans, LA 70112

Peptide hormones are initially synthesized in precursor form and must undergo a series of proteolytic processing events prior to release in biologically active form. Recent studies have revealed a new eukaryotic subtilisin-like proteinase family, of which prohormone convertase 1 (PC1) is a member. In both AtT20 and CHO cells, PC1 is synthesized as a glycosylated proprotein which undergoes a rapid amino-terminal cleavage to generate an 87 kDa form which is enzymatically active; at later times 87 kDa PC1 is converted to 74 and 66 kDa forms by carboxy-terminal cleavage (though this event is largely incomplete in CHO cells). In this work, possible mechanisms of this PC1 C-terminal cleavage event have been explored using homogeneous recombinant 87 kDa PC1. We found that 87 kDa PC1 could spontaneously convert to 74 and 66 kDa forms *in vitro*. The 74 kDa form apparently represented an intermediate product. These cleavages were calcium-activated, with a broad pH range. EDTA and PCMS could substantially block these conversions; so did D-Tyr-Ala-Lys-Arg-CH_2Cl and Cbz-Arg-Ser-Lys-Arg-AMC, implying that the cleavages might occur at dibasic residues. Dilution experiments suggested that an intermolecular interaction was involved in the cleavage; however, an intramolecular mechanism cannot be excluded at this point. Partially purified 74/66 kDa PC1 was characterized using Cbz-Arg-Ser-Lys-Arg-AMC. We found that 74/66 kDa PC1 was enzymatically active and activated by millimolar concentration of calcium. Compared to the 87 kDa form, 74/66 PC1 exhibited a narrow pH optimum (between 5.0 and 5.5) and appeared to be more sensitive to PMSF. Unlike the 87 kDa form, 74/66 kDa PC1 had an activity lag phase which varied depending on protein and calcium concentrations. We are currently exploring the possibility that C-terminal processing of 87 kDa PC1 represents further activation.

559.12

DISTRIBUTION AND CHARACTERIZATION OF A NEUROPEPTIDE PROCESSING ENZYME IN ADULT RAT BRAIN. E. Berman, K. Carr, L.D. Fricker*, and L. Devi. Departments of Psychiatry and Pharmacology, New York University Medical Center, New York, 10016 and *Department of Molecular Pharmacology, Albert Einstein College of Medicine, Bronx, New York, 10461.

Many peptide hormone and neuropeptide precursors undergo post-translational processing at mono and/or dibasic residues. An enzymatic activity capable of processing prodynorphin at a monobasic processing site designated 'dynorphin converting enzyme' has been previously reported in rat brain and bovine pituitary. A carboxypeptidase designated 'carboxypeptidase E' subsequently removes C-terminal basic residues. In this study the distribution of dynorphin converting enzyme activity and carboxypeptidase E activity has been compared with the distribution of immunoreactive dynorphin B-13 in 10 regions of rat brain. The distribution of these enzyme activities generally matches the distribution of immunoreactive dynorphin B-13 in most but not all brain regions. The dynorphin converting enzyme activity in the rat brain was subjected to ion exchange chromatography. The chromatography behavior, peptide inhibitor profile and pH optima of the purified dynorphin converting enzyme activity are consistent with the previously reported enzyme from the bovine pituitary and pituitary-derived cell lines. Taken together, these data support the possibility that, like carboxypeptidase E, the dynorphin converting enzyme is involved in the maturation of dynorphins, as well as other neuropeptides, and peptide hormones.

This work is supported by grants NS 26880 to L.D. and DA 03956 to K.C.

559.13

INABILITY OF PC2 TO PROCESS PROSOMATOSTATIN (PSS) IN GRANULATED SECRETORY CELL LINES. Y.C.Patel, A.S.Galanopoulou, N.G.Seidah, S.C. Patel*. Fraser Labs, McGill University and Clinical Research Institute, Montreal.

We have previously reported that PC1, but not PC2, can effect dibasic cleavage of rat PSS to SS-14 in constitutively secreting COS-7 cells (*JBC*, 268:6041, 1993). To test whether inactivity of PC2 in these cells reflects a requirement for secretory granules, we have now studied PSS processing in 3 secretory cell lines which express PC2 but not PC1 endogenously (GH3,GH4C1 rat pituitary; Y-1 mouse adrenocortical cells). PSS processing was correlated with PC2 mRNA under basal conditions or conditions that induced granule formation and/or which modulated PC2 expression (treatment of GH3/GH4C1 cells with EGF, insulin and ß-estradiol; treatment of Y-1 cells with ACTH 10^{-7} M, 24 h). Cells were transfected with rat PSS cDNA in pKS5. Cell extracts and media were analysed by HPLC and RIAs. PC2 mRNA by Northern analysis was expressed as fold change compared to PC2 mRNA in control GH4C1 cells taken as 1.

	GH3	GH3 treated	GH4C1	GH4C1 treated	Y-1	Y-1 treated
PC2 mRNA :	19	35	1	3	7	0.8
SS-14 (%) :	100	100	25	13	80	100
SS-28 (%) :	0	0	14	26	20	0
unprocessed(%):	0	0	61	61	0	0

CONCLUSIONS:(i) Induction of secretory granules has little effect on efficient PSS processing.(ii) Although basal expression of PC2 mRNA correlates with efficient PSS processing, the lack of coregulation of SS-14 conversion and PC2 levels argues against a functional role for this endoprotease in PSS processing and suggests the existence of other SS-14 convertases, possibly the recently discovered PC5.

559.14

RECOMBINANT PROENKEPHALIN: EXPRESSION, PURIFICATION, AND PROCESSING BY 'PROHORMONE THIOL PROTEASE'. M.R. Schiller*, K. Miller, and V.Y.H. Hook. Dept. of Biochemistry, Uniformed Services University, Bethesda, MD.

The major enkephalin precursor cleaving endoprotease in chromaffin granules has been identified as a novel 'prohormone thiol protease' (PTP). PTP cleaves at dibasic and monobasic processing sites to generate appropriate intermediates and the final (Met)enkephalin opiate peptide. Further studies of proenkephalin (PE) processing by PTP requires development of a radioassay with PE concentrations near estimated in vivo levels of 10^{-5}-10^{-4} M. To obtain milligrams of PE for these studies, the pET3c vector containing the T7 promoter was used to express recombinant PE. The signal sequence of rat preproenkephalin cDNA (gift from Dr.S.Sabol, NIH) was deleted and the PE DNA was subcloned into pET3c. PE expression in BL21(DE3) *E. Coli* cells was demonstrated by IPTG induction of a 33 kDa band that was recognized by a monoclonal anti-PE antibody (gift from Dr.B. Spruce, UK). Purification of PE under denaturing conditions utilized DEAE-Sepharose and preparative SDS-PAGE, followed by renaturation. ^{35}S-(Met)-PE was generated by the PE DNA in the riboprobe vector pSP65, followed by in vitro transcription and translation. Combination of purified bacterial PE and ^{35}S-(Met)-PE provides a radioassay with PE at µM concentrations (1-20 µM). PTP processing of PE estimates an apparent affinity for PE in the µM range and an apparent turnover number. To our knowledge, these are the first studies of processing enzyme affinity and activity for authentic prohormone.

559.15

CHARACTERIZATION OF A NEUROENDOCRINE α_1-ANTICHYMOTRYPSIN (ACT)-LIKE PROTEIN FROM PITUITARY AS AN INHIBITOR OF A NEUROPEPTIDE PRECURSOR PROCESSING ENZYME.

N. Tezapsidis*, T. Purviance, and Y.Y.H. Hook. Dept. of Biochemistry, Uniformed Services University, Bethesda, MD. 20814.

A cascade of proteolytic steps is required for converting neuropeptide precursors into active peptide neurotransmitters and hormones. Control of cellular proteases is often realized through endogenous protease inhibitors. We find that the 'prohormone thiol protease' (PTP) is the primary activity responsible for enkephalin precursor processing in bovine chromaffin granules; PTP is also detected in secretory vesicles of bovine pituitary. Our investigations of the regulation of PTP demonstrate that the enzyme is inhibited by α_1-antichymotrypsin (ACT) (human), and that ACT immunoreactivity is colocalized with PTP. The purified neuroendocrine ACT-like protein from pituitary potently inhibited, at nM concentrations, both PTP and chymotrypsin. Interestingly, comparison of kinetic $K_{i,app}$ values showed the bovine neuroendocrine ACT-like protein to be 3-4 times more potent than human liver ACT in inhibiting PTP. However, determination of kinetic constants also showed that the human liver ACT was approximately 6 times more effective than bovine neuroendocrine (pituitary) ACT-like protein for inhibition of chymotrypsin. It is possible that the differential potencies of the bovine neuroendocrine ACT-like protein compared to human liver ACT in inhibiting PTP and chymotrypsin may reflect species differences in ACT or possible tissue specific isoforms of ACT.

559.16

CORTICOTROPIN RELEASING FACTOR DEGRADATION BY PURIFIED PEPTIDASES.

J.C. Ritchie* and C.B. Nemeroff. Depts. of Pharmacol. and Psychiatry, Duke Univ. Durham, NC 27710 and Emory Univ., Atlanta, GA 30322.

The metabolism of neuropeptides by peptidases has been postulated to be the primary mechanism for the deactivation of these neuromodulators. Much is known concerning the action of endopeptidases on the smaller neuropeptides (15 amino acids or less). Corticotropin-releasing factor (CRF_{1-41}) is a large neuropeptide that plays multiple roles in integrating the body's response to stress. In addition to it's primary role as a hypophsiotropic hormone regulating pituitary ACTH synthesis and secretion, CRF has been shown to be widely distributed throughout the brain and to act as a putative neuromodulater.

In an attempt to elucidate the metabolism of CRF_{1-41} we have performed controlled digestions of this neuropeptide with purified preparations of Angiotensin Converting Enzyme (ACE), Aminopeptidase M, Endopeptidase 24.11 (Neprilysin), Cathepsin D, and Chymotrypsin. Digestions were carried out at 37°C for varying time periods in the presence of excess CRF_{1-41}, appropriate cofactors, positive controls, and specific inhibitors. The reaction products were then characterized on an HPLC system optimized for the detection of r/h CRF_{1-41} and its available synthetic fragments. All of the enzymes tested were capable of degrading CRF_{1-41} to various degrees and produced very different fragment patterns. Incubations for various time intervals and with specific inhibitors altered the patterns of peak generation indicating secondary cleavage sites for some of the proteases within the CRF molecule. No evidence was found for the generation of the putative convulsant peptides from the N-terminal end of the CRF molecule. These results support the hypothesis that the actions of CRF are terminated by enzymatic hydrolysis (either inter or intracellularly) and that the CRF molecule is susceptible to degradation in at least 4 areas (the amino-terminus, positions 15-16, 20-21, and 35-36) by endogenous peptidases.

559.17

DIFFERENTIAL PROCESSING OF SUBSTANCE P AND NEUROKININ A BY HUMAN VASCULAR SMOOTH MUSCLE.

L.Wang, P.E.Ward and H.J.Cooke*. Department of Physiology, The Ohio State University, Columbus, OH 43210.

Substance P (SP) and neurokinin A (NKA) can be released from nerve terminals into the smooth muscle layer of the blood vessel wall, and produce significant cardiovascular effects. However, little is known about the subsequent metabolism of these transmitters. We have found that incubation of SP and NKA with cultured human aortic and pulmonary artery smooth muscle cells results in rapid hydrolysis of these peptides. SP hydrolysis was entirely due to neutral endopeptidase-24.11 (NEP-24.11; EC 3.4.24.11) (0.23 ± 0.03 nmol/min/10^5 cells), which converted SP to SP[1-7] and other fragments. In addition to NEP-24.11-mediated NKA metabolism (0.16 ± 0.04 nmol/min/10^5 cells), NKA was also subject to N-terminal hydrolysis by aminopeptidase M (AmM; EC 3.4.11.2) (0.26 ± 0.06 nmol/min/10^5 cells). NEP-24.11 and AmM-mediated metabolism could be completely inhibited by phosphoramidon and amastatin, respectively. The identity of the cell surface membrane bound AmM was confirmed via immunoelectrophoresis. These data demonstrate that two specific cell surface peptidases may play a significant role in metabolizing neurokinins released within the vessel wall. Supported by HL45791.

559.18

AGE-RELATED ALTERATIONS IN THE POSTTRANSLATIONAL PROCESSING OF β-ENDORPHIN (β-ENDO) IN THE HYPOTHALAMUS OF FEMALE C57BL/6J MICE.

D.Joshi*, S.James, H.P.J.Bennett, M.M.Miller. Departments of Experimental Medicine, Anatomy, Obstetrics and Gynecology and Center for Studies on Aging, McGill University, Montreal, Quebec H3A 1A1, Canada.

β-endo (1-31), is the active opioid peptide product of proopiomelanocortin processing. Further processing of β-endo (1-31) yields β-endo (1-27), (1-26) and their acetylated forms which are inactive. β-endo modulates GnRH and LH levels during normal estrus cycles in rodents. The age-related decline in reproductive function has been linked to altered opiatergic influences on GnRH and LH. The purpose of this study was to determine whether differential processing of β-endo with age could contribute to the altered opiatergic activity in female C57BL/6J mice. Pooled (n=3) extracts of the arcuate nucleus (ARC) and the preoptic area (POA) of 3mo old normally cycling (diestrus), 12mo old irregularly cycling (diestrus), 24mo old persistent diestrus and 24mo old long-term ovariectomized (L-OVX) mice, and pituitary intermediate lobe standards were subjected in quadruplicate to reversed-phase high pressure liquid chromatography. Fractions corresponding to appropriate peaks were assayed for β-endo-like-immunoreactivity by sequence specific RIAs. The active as well as inactive forms of β-endo were present in both in the ARC and the POA at all ages although their ratio varied. β-endo (1-31) was the predominant form in young animals. However, by middle age, there was a significant ($p < 0.05$ ANOVA) increase in the proportion of inactive forms of β-endo associated with a decrease in the proportion of the active form. There was an increase in the ratio of inactive to active forms with age, which was partially reversed by L-OVX. These results suggest that there are marked alterations in the processing of β-endo by middle age in the C57BL/6J mouse. We conclude that changes in the proportional distribution of active and inactive forms of β-endo may in part account for the altered opiatergic influence on the LH surge with age. Supported by NIH RO1 AG07795 (MMM).

PEPTIDES: BIOSYNTHESIS, METABOLISM, AND BIOCHEMICAL CHARACTERIZATION II

560.1

ELECTROSPRAY MASS SPECTROMETRY ANALYSIS OF OPIOID PEPTIDE PRECURSORS

Dominic M. Desiderio*, Lin Yan, Genevieve Fridland, and Jih-Lie Tseng Department of Neurology and the Charles B. Stout Neuroscience Mass Spectrometry Laboratory, University of Tennessee, Memphis, 800 Madison Avenue, Memphis, TN, 38163.

The objective of this research program is to analyze selected human neuropeptidergic systems, and to use mass spectrometry methods to provide the highest level of molecular specificity for qualitative and quantitative neuropeptide measurements. Recently, we have developed electrospray (ES) mass spectrometry methods to analyze intermediate-sized precursor molecules from proopiomelanocortin, $POMC_{bovine,\ 1\text{-}265}$, which contains BE. Gel permeation and RP-HPLC produced partially-purified fractions from bovine pituitary that showed ME-like immunoreactivity (ME-li) after treatment with trypsin and CNBr. ES-MS analysis of an immunoreactive fraction showed three major components, with M.W. of *ca.* 6550, 8440, and 9855. Trypsin treatment of this mixture, when analyzed by FAB-MS, showed the presence of ions at *m/z* 558 and 1134 (among others) which correspond to the tryptic peptides $BE_{20\text{-}24}$ (=$POMC_{254\text{-}258}$) and $BE_{10\text{-}19}$ (=$POMC_{244\text{-}253}$). After HPLC, all tryptic peptides will be sequenced by MS-MS so that the intermediate-sized precursors can be located within the preproenkephalin $A_{bovine,\ 1\text{-}263}$ and the $POMC_{bovine,\ 1\text{-}265}$ precursor molecules. NIH GM 26666.

560.2

A MODIFIED IMMUNO-POLYMERASE CHAIN REACTION METHOD TO MEASURE MINUTE CENTRAL NERVOUS SYSTEM ANTIGENS.

F.E. Bloom*, P.P. Sanna, E. Weiss, and E. Merlo Pich, Department of Neuropharmacology, The Scripps Research Institute, La Jolla, CA.

The immuno-polymerase chain reaction (I-PCR) system described recently (Sano, T. *et al.*, 1993, Science, 258:120-122) combines immuno-specificity with PCR sensitivity to detect and quantify scant analytes. Originally, I-PCR was used to measure bovine serum albumin (BSA) immobilized on microtiter plates: a streptavidin-protein A fusion protein linked a reporter DNA to a specific anti-BSA antibody. The reporter DNA was then enzymatically amplified by PCR, greatly enhancing the sensitivity of a conventional ELISA assay. We have modified I-PCR and applied it to the measurement of TNF-α and other inflammatory peptides in the CNS. In our method, the target antigen is bound to a solid phase by an immobilized specific primary antibody. The captured antigen is then immunoreacted with a specific secondary antiserum (sandwich). These immune complexes are then incubated with a biotinylated tertiary antiserum which links the secondary antiserum to a biotinylated reporter DNA through an avidin bridge. The reporter DNA is then enzymatically amplified by PCR in the presence of a radiolabelled deoxynucleotide. The specific PCR products are then quantified by direct radioactivity measurement and microdensitometric analysis of the autoradiogram. Our I-PCR detects peptide in the pM concentration range, and may be useful for assessing any antigenic factor (i.e., cytokines, tumor antigens, etc.), especially in procedures such as intracranial microdialysis where sample volume is limiting. Partially supported by MH-47680.

560.3

NEUROPEPTIDE IMMUNOLESIONS EFFECTS ON PEPTIDE LEVELS: IMPLICATIONS FOR NEURODEGENERATIVE DISORDERS. M.L.de Ceballos* and M.Pernas.Neurodegeneration Group,Cajal Institute, CSIC,Madrid,Spain.

Administration of peptide antisera to neonatal rats results in long-lasting changes in peptide content. Neuropeptides of interest were substance P (SP), met-enkephalin (met-enk) and neurotensin (NT), highly enriched in basal ganglia and known to be altered in Parkinson's and Huntington's diseases. Purified preiummune sera or antisera against SP (SP Ab), met-enk (met-enk Ab) or NT (NT Ab)(IgGs, 500 ug/rat sc) were given to P2 rats and peptide levels were measured by a combined HPLC/RIA method in striatum and substantia nigra of 3 month-old rats. Met-enk Ab administration decreased striatal met-enk and SP levels and nigral SP content. SP Ab reduced SP levels in striatum and s.nigra, met-enk in striatum but increased NT concentrations in s.nigra. NT Ab decreased nigral NT and striatal met-enk levels, while SP content was reduced in striatum but enhanced in s.nigra.

These results suggest colocalization of several peptides in striatum. Met-enk Ab and SP Ab administration reproduced some peptide alterations observed in degenerative diseases.

560.4

ACCUMULATION OF ADRENAL NEUROPEPTIDES AFTER ANTIBODY - MEDIATED DENERVATION. S. Brimijoin[1], Å. Dagerlind[2], T. Hökfelt[2], P. Hammond[1] and G. M. Tyce[1]*. [1] Depts. of Pharmacology and Physiology, Mayo Clinic, Rochester MN 55905; [2] Department of Histology, Karolinska Institute, Stockholm, Sweden.

Surgical denervation of the rat adrenal gland is known to cause accumulation of certain neuropeptides in chromaffin cells. We used radioimmunoassay to measure NPY-like immunoreactivity (NPY-IR) and methionine-enkephalin-like immunoreactivity (Met-Enk-IR) in adrenals from rats treated with anti-acetylcholinesterase IgG. This treatment destroys the cholinergic input from preganglionic sympathetic nerves. Within 2 days of antibody injection, Met-Enk-IR in the adrenal gland increased 9-fold, while NPY-IR increased 50%. The peptide response could reflect: 1) the chronic synaptic inactivation that follows the degeneration of presynaptic terminals, or 2) the acute activation that accompanies terminal degeneration. To test these possibilities, the experiment was repeated in the presence of two drugs that, together, block muscarinic and nicotinic receptors (atropine, 4 mg/kg/day + chlorisondamine, 20 mg/kg/day). When the drugs were given alone by osmotic infusion for 2 or 4 days, adrenal Met-Enk-IR was unchanged, while NPY-IR fell by 30%. Measurements of urinary catecholamine excretion showed that drug treatment did prevent the paroxysmal release of norepinephrine and epinephrine that normally follows injection of acetylcholinesterase antibodies. The treatment also prevented the antibody-induced rise in adrenal NPY-IR, but it did not reduce the rise of Met-Enk-IR. We conclude that different transsynaptic mechanisms are responsible for regulating Met-Enk and NPY in adrenal chromaffin cells. (Supported by Grant NS29646).

560.5

SELECTIVE PEPTIDASE INHIBITORS ENHANCE RECOVERY OF SUBSTANCE P AND CALCITONIN GENE-RELATED PEPTIDE RELEASED FROM RAT SPINAL CORD SLICES J.J. Chen*, L.Barber, J. Dymshitz, M.R. Vasko. Dept.of Pharmacology & Toxicology, Indiana U. School of Medicine, Indianapolis, IN 46202.

The release of the neuropeptides, substance P (SP) and calcitonin gene-related peptide (CGRP) from sensory neurons is important in modulating nociception and inflammation. One potential problem in measuring release of these peptides is their rapid degradation by peptidases. This could significantly reduce the recovery of peptides released from neuronal tissues and contribute to the variability in results observed with different preparations. To determine whether peptidase activity affects the recovery of SP and CGRP, we studied the effects of various peptidase inhibitors on the release of these peptides from spinal cord slices.

Spinal cords from adult Wistar rats were rapidly removed, chopped into 300 micron pieces, and superfused with Kreb's buffer in the presence or absence of various peptidase inhibitors. Peptide release was stimulated by exposing the tissue to 500 nM capsaicin for 9 min. Superfusates were collected and assayed for SP and CGRP using radioimmunoassay. Release is reported as % of peptide content in the tissue/min.

The maximal recovery of peptides released from spinal cord slices was obtained by exposing the tissues to a combination of 20 µM bacitracin, 100 µM phenylalanylalanine (PHE-ALA) and 50 µM p-chloromercuriphenylsulfonic acid (PCMS). Resting and capsaicin-stimulated release of SP were 0.11±0.03 % and 0.44±0.06 %, respectively. Resting CGRP release was 0.20± 0.02 %, whereas capsaicin-stimulated release was 0.90± 0.11 %. When slices were exposed to 20 µM bacitracin alone or in combination with 1 µM phosphoramidon and 10 µM thiorphan, resting release of both peptides was approximately 5-fold less, while capsaisin-evoked release was approximately 2-fold less than maximal.

The results demonstrate that recovery of peptides released from spinal cord slices is dependent in part on peptidase activity. Moreover, the amount of peptides recovered during release experiments may vary depending on the specific peptidase inhibitors utilized. (Supported by PHS DA07176)

560.6

A NOVEL TRANSCRIPT ENCODING A SECRETED PROTEIN IS REGULATED BY SECRETAGOGUES IN THE PITUITARY. B.T. Bloomquist, D.N. Darlington, and B.A. Eipper*. Dept. Neuroscience, The Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205.

Gene regulation was studied in rat intermediate pituitary melanotropes consequent to 21 day chronic exposure to bromocriptine or haloperidol. These secretagogues are known to affect the expression of transcripts involved in the regulated secretory pathway. A novel transcript (#45) which has many characteristics of a secreted protein has been isolated. Expression of #45 mRNA is limited to neuroendocrine cell lines and tissues; tissue *in situ* hybridization analysis has shown #45 mRNA to be highly abundant in both the intermediate and anterior pituitary as well as in the paraventricular nucleus. Northern blot analysis and DNA sequencing have shown the #45 transcript to be about 720 basepairs in length not including the poly(A) tail. The cognate translation product of #45 would be 175 amino acids in length containing a putative signal sequence of about 30 amino acids. The mature sequence would contain two dibasic amino acid sites, two cysteine residues, one potential sulfation site, but no glycosylation sites. Neither the nucleic acid nor the amino acid sequences show any homology to reported sequences. *In vitro* translation has confirmed the presence of a signal sequence which, when cleaved, reduces the size of the *in vitro* translation product from 20 kDa to 18 kDa. Although #45 is coregulated with POMC, PAM, PC1, PC2, and CPE by dopaminergic input in the intermediate pituitary, #45 is regulated inversely to them in response to dexamethasone or CRH treatment in AtT-20 cells. Support: DA-00266, -00098, -05404, and Scottish Rite.

560.7

TIME-COURSE ADMINISTRATION OF NEUROLEPTICS DECREASES REGIONAL NEUROTENSIN METABOLISM IN INTACT RAT BRAIN SLICES. C.S. Konkoy* and T.P. Davis. Dept. Pharmacology, Univ. Arizona, Tucson, AZ 85724.

Treatment with antipsychotic agents has been shown to affect both neurotensin levels and the activities of enzymes implicated in the metabolism of neurotensin. In the present study, the effects of intraperitoneally administered antipsychotics and dopaminergic agents on neurotensin (NT) metabolism were examined in intact slices isolated from micropunched regions of rat brain. Significant degradation of NT 1-13 with the concomitant production of NT fragments, including NT 1-8, NT 1-11, and NT 9-13, occurred after incubating for 2h regionally dissected microslices with 100 µM NT 1-13. Degradation was high (nearly 50%) in nucleus accumbens (NA) and caudate-putamen (CP) and much lower in hippocampus and frontal cortex. Administration (i.p.) of haloperidol (3 mg/kg) 1h prior to slice preparation had no effect on levels of degradation in slices isolated from any of the four regions. Administration (i.p.) of haloperidol (3 mg/kg) or chlorpromazine (20 mg/kg) 24h prior to slice preparation significantly reduced the degradation of neurotensin in slices isolated from NA. Levels of degradation remained significantly reduced at 48h after haloperidol administration but returned to that of control levels at 96h. Neurotensin degradation in slices isolated from CP decreased following administration of haloperidol (3 mg/kg, 48h) or chlorpromazine (20 mg/kg, 24h), and increased following administration of methamphetamine (2 i.p. injections of 15 mg/kg, 12h). These data suggest that drugs which affect dopaminergic transmission influence the metabolism of neurotensin in specific brain regions. (Supported by NIH grant MH42600).

560.8

CORRELATION BETWEEN TOLERANCE IN NEUROTENSIN GENE EXPRESSION AND CATALEPTIC RESPONSE IN RATS FOLLOWING CONTINUOUS HALOPERIDOL TREATMENT K.M. Merchant* and D.J. Dobie. Dept. of Psychiatry, Univ. of Washington, Seattle, WA 98195.

Our previous studies have demonstrated that only cataleptogenic antipsychotic drugs increase the expression of neurotensin/ neuromedin N (NT/N) mRNA in the dorsolateral striatum (DLSt) of the rat. Interestingly, this response of the NT neurons was significantly reduced following chronic (28 d) continuous administration of the neuroleptic, haloperidol. The present study examined the time course of tolerance in the NT/N mRNA expression after continuous haloperidol treatment and its correlation with cataleptic behavior in the same animals. Male Sprague Dawley rats were treated with haloperidol (1 mg/kg/d) or saline via subcutaneously implanted osmotic minipumps for 3, 7, 14 or 28 days. For comparison, separate groups of rats were given a single IP dose of haloperidol or saline and sacrificed at 1 or 7 hours after the treatment. Cataleptic response was studied by the horizontal bar test at 24 and 48 hr after commencing the treatment and again on the day of the sacrifice. Neostriatal gene expression was examined by *in situ* hybridization histochemistry.

Induction of NT/N mRNA levels in the DLSt and catalepsy occurred after the acute treatment with haloperidol and appeared to persist following 3 days of continuous drug administration. However, by 7 days of drug treatment significant tolerance in both NT/N mRNA and cataleptic responses was observed. Statistically significant correlation was observed between tolerance in NT/N mRNA levels and that in cataleptic scores of each animal suggesting that haloperidol's induction of NT/N gene expression in the DLSt may contribute to the drug-induced catalepsy. To investigate further the specificity of the apparent correlation between NT/N gene expression and cataleptic behavior, alterations in the expression of enkephalin and tachykinin genes in adjacent brain sections were examined and compared with those in NT/N mRNA. (Support: Scottish Rite Schizophrenia Research Program)

560.9

FACTORS REGULATING PRODUCTION OF NEUROPHYSIN BY NEUROENDOCRINE TUMORS. Andrew S. Friedmann, Michael J. Fay, Xiaoming Yu, Vincent Memoli and William G. North*. Departments of Physiology and Pathology, Dartmouth Medical School, Lebanon, N.H. 03756.

Tumor production of neurophysin (HNP) is abnormal and leads to the formation of Neurophysin-Related cell-Surface Antigen (NRSA) that becomes incorporated into the plasma membrane of the tumor cells. Recently, the levels of vasopressin mRNA in one long-term culture derived from small-cell carcinoma of the lung (SCCL) were found to be influenced by glucocorticoids and cAMP. In this study we used another long-term culture of SCCL (H 69) and examined the influence of : isobutyl-methylxanthine (IBMX, $5x10^{-4}$M) plus forskolin (FSK, $25x10^{-6}$M); IBMX and 8-bromo-cAMP ($5x10^{-4}$M); dexamethasone (DEX, $1x10^{-7}$M); and a combination of IBMX, 8-bromo-cAMP, and DEX on the expression by these cells of immunoreactive HNP using quantitative immunocytochemistry. Cells were incubated with reagents or vehicle for 2 to 8 days. They were then fixed in acetone, reacted with affinity-purified rabbit polyclonal antibodies to HNP, and processed using the avidin-biotin method that included staining through peroxidase oxidation of 3,3'-diaminobenzidine (DAB). Staining for HNP was quantitated with a microcomputor imaging devise by relating the total area of scanned cells (A_T) to the area stained for HNP (A_S). Results demonstrate that glucocorticoids alone decrease the expression of neurophysin by SCCL, while cAMP increases this expression. Of particular interest was the finding that glucocorticoids, when used in combination with reagents that increase cellular cAMP, actually enhance the positive influence these reagents have on neurophysin expression by SCCL.

560.10

REGULATED SECRETION OF CGRP FROM THE CATECHOLAMINERGIC VESICLES OF TRANSFECTED PC12 CELLS. E. S. Schweitzer* and C.-J. Jeng. Dept. of Anatomy & Cell Biology, UCLA Medical School, Los Angeles, CA 90024.

We have introduced a construct (pTNDcal) containing the rat calcitonin gene under the control of the RSV LTR into PC12 cells. We examined the sorting and secretion of CGRP in stably transfected clones of this neuronal cell line by subcellular fractionation, immunofluorescence microscopy, and stimulation-release experiments. CGRP was quantitated using an RIA that is selective for CGRP and which does not detect calcitonin.

When transfected cell homogenates were subjected to fractionation on sucrose/ficoll density equilibrium gradients, the CGRP co-purified with markers for the large, dense-core secretory vesicles that contain catecholamines. The CGRP did not appear to be associated with other vesicles, including the low-density cholinergic vesicles or the small, clear, synaptophysin-rich vesicles.

This localization was confirmed by double-label immunofluorescence microscopy, which indicated that the CGRP co-localized with secretogranin I, a marker for the catecholaminergic vesicles, but not with synaptophysin or SV2.

Carbachol-induced depolarization of transfected cells caused a dramatic increase in the rate of secretion of CGRP, similar to that observed previously for the endogenous transmitter norepinephrine or transfected human growth hormone. We therefore conclude that the CGRP is selectively packaged into the catecholaminergic vesicles of PC12 cells, from which it is released in a highly regulated manner.

560.11

MICRODIALYSIS OF NEUROPEPTIDE Y USING THE DIFFERENCE METHOD. A.C. Thompson*[1], J.B. Justice, Jr.[2], & J.K. McDonald[1]. Dept. of Anatomy & Cell Biology[1], Emory Univ. Sch. of Med., & Dept. of Chemistry[2], Emory Univ., Atlanta, GA 30322.

Measurements of neuropeptide Y (NPY) by conventional microdialysis methods have been complicated by low levels of NPY recovery (< 1.5%, *in vitro*). An alternative microdialysis technique is the difference method (Lönnroth et al., Am. J. Phys., 256:E250-E255, 1989) in which the estimate of extracellular concentration is determined independently from the level of recovery. We have tested the feasibility of using the difference method to measure NPY *in vitro*.

Several conc. of NPY (4.2- 58.9 pg/µl) were perfused serially through 3 microdialysis probes (1, 2, & 5 mm of active dialysis membrane) placed in an NPY solution (mean conc. 14.6 +/- 2.1 pg/µl) at room temperature (unstirred). Five 15-min samples were collected at each perfusate conc. (flow rate = 1µl/min) and assayed for NPY by RIA. The difference between NPY levels (C) in the perfusate (C_{in}) and NPY levels in the dialysate (C_{out}) were determined and the relationship between C_{in}-C_{out} and C_{in} was assessed by linear regression. The resulting regression lines were found to predict reliably the conc. of NPY in the solution surrounding the dialysis probe (predicted conc. 14.5 +/- .2 pg/µl vs. actual conc. 14.6 +/- 2.1 pg/µl). In contrast, conventional microdialysis (no NPY in the perfusate) yielded undetectable levels of NPY. Thus, using the difference method shifted the conc. of the samples into the detectable range of the assay, and also provided an estimate of the solution conc. The former represents a new application of the difference method.

These results show that the difference method improves the measurement of NPY by microdialysis *in vitro*, and may be a useful method for NPY microdialysis *in vivo*.

Research supported by NIH HD26833 awarded to JKM.

560.12

Enzymatic Conversion of Pro-drug Forms of [D-Pen^2, D-Pen^5]Enkephalin and [D-Pen^2,L-Cys^5]Enkephalin. D.L. Greene*, V.S. Hau, V.J. Hruby, and T.P. Davis. Depts. of Pharmacology and Chemistry., The Univ. of Ariz., Coll. of Medicine, Tucson, AZ 85724.

To improve the blood-brain barrier (BBB) penetration of δ opioid receptor peptides [D-Pen^2, D-Pen^5]Enkephalin (DPDPE) and [D-Pen^2, L-Cys^5]Enkephalin (DPLCE), various pro-drug forms were synthesized to increase lipophilicity and drug delivery to the brain. This study examined the *in vitro* conversion of these pro-drug forms in serum and brain homogenate. Blood was collected from the rat/mouse abdominal aorta for serum harvesting and the brain resuspended at a 15% protein concentration. The peptide was added at a 100 µM concentration to the serum or brain. The samples (in triplicate) were incubated at 37 ± 0.1°C for 0, 30, 60, 120, and 240 min,and acetonitrile was added to stop all enzymatic metabolism. The samples were centrifuged, and supernatant was analyzed by HPLC. Resultant metabolic T1/2 (min) of the compounds examined are shown in the following table:

Peptide Structure	100% Serum	15%Brain
Phe-DPDPE	6.82	3.94
DPDPE-Phe-Ala-NH-C_6H_{13}	446.07	348.63
N,O diacetyl-Phe-DPDPE	12.10	7.75
Asp^6-DPDPE	294.00	33.54
DPLCE-Phe-OH	176.35	102.30
DPLCE-Arg-Pro-Ala	316.57	9.21
DPLCE-Arg-Gly-OH	53.08	40.45

These results show that pro-drug forms of DPDPE and DPLCE have longer metabolic/ conversion half-lives in the serum than in the brain. Since the blood would carry these compounds to the brain, a longer half-life of conversion in the serum than in the brain would allow more time for the pro-drugs to cross the BBB and be cleaved to the parent compound. Therefore, Asp^6-DPDPE show the most promising results as a pro-drug of DPDPE and DPLCE-Arg-Pro-Ala is the most promising pro-drug of DPLCE. (N.I.D.A. #DA06284)

560.13

DESIGN AND ANALYSIS OF A CONFORMATIONALLY CONSTRAINED CYCLO(16, 20)-[Lys^{16}, Asp^{20}]-BETA-ENDORPHIN ANALOGUE.

M.A. Scheideler* and J.W. Taylor Pharmaceuticals Division, Novo Nordisk A/S, 2760 Måløv, DENMARK* and Dept. of Chemistry, Rutgers State University, Piscataway, NJ 08855.

The 31-residue Beta-endorphin peptide is thought to consist of three basic structural units: The N-terminal (Met)enkephalin pentapeptide, a linker region (residues 6-12) and a potential C-terminal amphipathic sequence (residues 13-27) that contributes both to the receptor selectivity and potency of Beta-endorphin (properties reviewed by J.W. Taylor and E.T. Kaiser (1986) Pharm. Rev. 38, 291-315).

A Beta-endorphin analogue that is side-chain bridged in the C-terminal region was synthesized with the aim of confirming the requirement for an amphipathic helix, and establishing its role in receptor binding and signal transduction efficiency. CD spectra showed that this analogue possesses increased helical character in water. Receptor binding was comparable to that of Beta-endorphin (human) in a rat brain assay selective for the mu-opioid receptor, and in a neuroblastoma cell line (F11) which expresses this receptor. The peptides efficacy in inhibiting cAMP synthesis in F11 cells closely matched its affinity at the mu-opioid receptor expressed by these cells.

560.14

A TEN-FOLD MORE POTENT VIP AGONIST THAT DIFFERENTIATES TWO CNS VIP RECEPTORS. I.Gozes, G.Lilling, A.Davidson*, M.Fridkin and D.E. Brenneman. Chem. Path., Sackler Med. School, Tel Aviv Univ.; Israel; Weizmann Inst., Israel; Dev. Mol. Pharmacol. LDN, NICHD, NIH, Bethesda, MD, USA.

Vasoactive intestinal peptide (VIP) recognizes two functionally distinct receptors in the CNS, a low affinity receptor mediating VIP increases in cAMP formation and a high affinity receptor mediating VIP-associated maintenance of neuronal survival (J. Pharmacol. Exp. Therap. 257:959,1991; J. Mol. Neurosci. 4:1,1993). We have synthesized a conjugate of VIP and stearic acid (st-VIP; J. Clin. Invest. 90:810,1992), as a potential drug for noninvasive impotence treatment. We now show that st-VIP was 10-fold more potent than VIP in protecting spinal cord neurons against neuronal cell death induced by electrical blockade. This effect was sustained over a wide concentration range, in contrast to the VIP effect, which exhibited a narrow range of efficacy. Also, unlike VIP, st-VIP did not stimulate cAMP formation in astroglia. Binding experiments indicated increased affinity of st-VIP at the high affinity receptor and a decreased affinity to the cAMP-associated binding site. This strategy opens new avenues for design of analogues of therapeutic value.
Supported by Fujimoto Corp.

560.15

NON RADIOACTIVE ASSAY FOR PROTEIN PHOSPHATASE 2B (CALCINEURIN) ACTIVITY USING A PARTIAL SEQUENCE OF THE SUBUNIT OF cAMP DEPENDENT PROTEIN KINASE AS SUBSTRATE. A. Enz*, G. Shapiro, M. Zurini and M. Luyten, Preclinical Research SANDOZ PHARMA Ltd., CH-4002 Basle/Switzerland.

Calcineurin is a Ca^{2+}/calmodulin dependent protein phosphatase (protein phosphatase 2B) regulating the dephosphorylation step of phospho-proteins, which play important roles in neurotransmission and immunosuppression. A suitable i.e. quite specific substrate for calcineurin is a partial sequence of the R-II subunit of cAMP dependent protein kinase. The current assay for the determination of calcineurin activity is based on the measurement of the release of ^{32}P-phosphate from this peptide following its phosphorylation with ^{32}P-ATP, catalyzed by the catalytic subunit of cAMP dependent protein kinase. This very sensitive method consumes a high amount of radioactivity and is therefore problematic as screening method for calcineurin inhibitors. We developed an alternative non radioactive enzyme assay where both reactions, phosphorylation by protein kinase and dephosphorylation by calcineurin, are controlled and monitored by the simultaneous quantitative determination of phosphorylated and nonphosphorylated peptide using HPLC on RP18 colums and UV detection. The method allows the measurement of enzyme kinetics as well as the characterization of potential inhibitors. Examples of calcineurin inhibition by several derivatives of the immunosuppressant cyclosporine complexed with the corresponding binding proteins and compounds known from the literature will be presented. The method, although less sensitive than the radioactive, is an attractive alternative, since calcineurin is commercially available and the substrate is easily accessible in sufficient amounts. An important advantage of this new method, in addition to the obviation of radioactivity, is the higher specificity (better identification of substrate and product).

560.16

RAT BRAIN TRANSGLUTAMINASE. Y. Takeuchi*+,#, P. J. Birckbichler+, M. K. Patterson, Jr.+, B. Howell+ and Y. Ito#. +Biomed. Div. The Samuel Roberts Noble Fnd., Ardmore, Ok 73402 and #Tsukuba Res. Inst. in collaboration with Merck Res. Lab., Banyu Pharm. Co., Ltd., Tsukuba 300-33, JAPAN.

Transglutaminase(TGase) in neuronal system has been suggested to be involved in neurotransmitter release, long term potentiation, regeneration after nerve injury, and so forth. In order to have molecular basis on the enzyme in central nervous system, the enzyme was partially purified from the centrifugal supernatant of rat brain homogenate by ion-exchange, gel filtration and GTP-agarose affinity column chromatography. We found that two proteins with apparent molecular weight of 75-79kDa and 48kDa, respectively, which were immunopositive against tissue-type TGase. However, these proteins were not enzymatically active. GTP affinity chromatography showed that there are two different types of brain TGase. Partially purified enzyme was not immunologically detected by antibodies against various types of TGase including tissue type TGase, epidermal TGase and factor XIIIa. The results may suggest a novel type of TGase in mammalian brain.

PEPTIDES: BIOSYNTHESIS, METABOLISM, AND BIOCHEMICAL CHARACTERIZATION III

561.1

C-*fos* MEDIATES THE INCREASE IN STRIATAL NEUROTENSIN mRNA PRODUCED BY HALOPERIDOL. G.S. Robertson*. Department of Pharmacology, University of Ottawa, Ottawa, Ontario, Canada, K1H 8M5.

A single injection of the antipsychotic haloperidol dramatically elevates mRNA encoding the neuropeptide neurotensin (NT) in the dorsolateral striatum. This increase is preceded by enhanced expression of Fos, the product of the immediate-early gene *c-fos*. In order to determine whether these events are related, we examined a) the overlap between haloperidol-induced Fos-like immunoreactivity (FLI) and NT mRNA and b) the effect of inhibiting Fos expression on the haloperidol-induced increase in NT mRNA in the dorsolateral striatum. Nuclei displaying FLI were frequently found in striatal neurons expressing elevated levels of NT mRNA 2 hr after haloperidol (4 mg/kg). Injection of an antisense phosphorothioate oligonucleotide to *c-fos* into the striatum, 14 hrs prior to haloperidol administration (4 mg/kg), reduced both FLI and the *in situ* hybridization signal for NT mRNA observed in the dorsolateral striatum 2 hrs after haloperidol. However, the antisense oligonucleotide did not reduce either Jun-like immunoreactivity or enkephalin mRNA in adjacent sections. Furthermore, infusion of a sense phosphorothioate oligonucleotide to *c-fos* into the striatum did not reduce the increase in FLI and neurotensin mRNA produced by haloperidol (4 mg/kg). These results are consistent with the proposal of Merchant et al. (J. Neurosci., 12: 652, 1992) that *c-fos* mediates the increase in NT mRNA produced by haloperidol.

561.2

EXPRESSION OF THE PROHORMONE-CONVERTING ENZYME GENE, PC2, IN *XENOPUS* BRAIN: POSSIBLE CO-LOCALIZATION WITH PRO-TRH IMMUNOREACTIVE NEURONS IN BRAINSTEM. L. P. Pu, S. Ghose, E. A. Neale*, J. F. Mill, W. P. Hayes and Y. P. Loh. Labs. of Dev. Neurobiology (NICHD) and Molec. Biology (NINDS), NIH, Bethesda, MD 20892.

Prohormone processing at paired-basic residues has been shown to be tissue-specific. However, it is as yet unclear which processing enzymes are involved for each prohormone. In mammal, neural and endocrine tissues express the prohormone convertase, PC2, which has been shown to appropriately cleave several prohormones (eg., POMC, insulin) at paired-basic residues. To better understand neuropeptide biosynthesis at the level of processing, we have studied the expression of PC2 with respect to its anatomical relationship with the pro-TRH system in frog brain.

Xenopus pro-TRH can generate up to 7 TRH tripeptides via cleavage at paired-basic residues (Bulant et al. 1992, FEBS **296**, 292-296). Using a 600 base probe made by PCR and the published *Xenopus* PC2 sequence (Braks et al. 1992, FEBS **305**, 45-50), RNA blots of frog brain showed two bands of ~3 kb and ~5 kb, which is similar to mammal. Moreover, our *in situ* hybridization histochemical studies using a cRNA probe, detected high levels of PC2 gene expression widely in *Xenopus* brain. Some of the highest concentrations of PC2 mRNA were found in known neuropeptide-rich brain regions, including some but apparently not all areas that also express TRH.

Colocalization studies of pro-TRH by immunocytochemistry using specific antiserum (pCC10) (Jackson et al., 1985, Science **229**, 1097-1099) in combination with the PC2 cRNA probe produced the preliminary finding that PC2 may be colocalized with pro-TRH immunoreactive neurons in at least some brainstem regions. The evidence for a wide distribution of PC2 mRNA in frog brain and its possible colocalization with pro-TRH immunoreactivity in some neurons suggests that PC2 may be involved in the biosynthesis of TRH, as well as other neuropeptides in the CNS.

561.3

REGULATION OF THE TRANSCRIPTIONAL ACTIVITY OF DYNORPHIN AND TACHYKININ GENES IN RAT STRIATUM: A STUDY WITH DOPAMINE UPTAKE INHIBITOR GBR-12909. S. P. Sivam.* Department of Pharmacology & Toxicology, Indiana University School of Medicine, 3400 Broadway, Gary IN 46408.

This study examined the influence of the neurotransmitter, dopamine on the regulation of prodynorphin (PD) and preprotachykinin (PPT) peptidergic gene expression in the rat striatum. The dopamine uptake inhibitor, GBR-12909 (GBR) was administered daily (20 mg/kg) for one, two or four days to different groups of female Sprague-Dawley rats. The animals were killed one hour after injection. Striatal tissues were used for the biochemical determinations. The levels of peptides, dynorphin A (1-8) (DYN) and substance P (SP) were determined by radioimmunoassays. The abundance of PD-mRNAs and PPT-mRNAs was quantified by Northern blot analysis. The rate of transcription of PD and PPT genes was assessed by a nuclear transcription run-on assay using a nuclei preparation derived from fresh or frozen striatal tissue. The ^{32}P-labeled mRNA transcripts generated by the transcription run-on reaction were purified and hybridized to a slot-blotted membrane containing plasmids with rat cDNA for PD or PPT genes. GBR administration produced time-related increases in the levels of DYN and SP as well as PD-mRNA and PPT-mRNA. The increases in peptide and mRNA levels were temporally preceded by an induction of the rate transcription of PD and PPT genes as evidenced from the transcription run-on assays. The results further support the hypothesis that an enhanced dopaminergic activity positively regulate peptidergic activity in striatonigral DYN and SP neurons. Supported by USPHS grant NS26063.

561.4

SUB-REGIONAL EFFECTS ON NEOSTRIATAL NEUROPEPTIDE EXPRESSION BY ADRENAL GLUCOCORTICOIDS AND CHOLINERGIC TONE. J.Angulo*, M.Ledoux and B.McEwen. *Dept. Biological Sciences, Hunter College of CUNY, New York NY 10021 and Lab. of Neuroendocrinology, The Rockefeller University, New York NY 10021.

We have evaluated the effect of cholinergic tone and glucocorticoids on the expression of protachykinin (PT) and proneurotensin (PNT) mRNAs in the caudate-putamen (CPU) and accumbens (NAc) of the rat brain by in situ hybridization histochemistry. Removal of circulating adrenal steroids by bilateral adrenalectomy (ADX) decreased PT mRNA abundance in the dorsal aspect of the CPU (22% below sham controls) and this decrease was blocked by corticosterone (CORT) replacement. PT mRNA in the ventral CPU was unaffected by either ADX or CORT. In addition, administration of the cholinergic antagonist scopolamine to ADX rats increased PT mRNA in dorsal CPU 33% above sham. Concurrent administration of CORT and scopolamine increased this message 80% above sham. No effects were observed in ventral or anterior CPU and NAc. In contrast, PNT mRNA abundance was decreased by ADX in ventral and dorsal aspects of the CPU (CORT replacement blocked this effect in both areas). Scopolamine alone or with CORT elicited no effect. These results demonstrate that circulating adrenal steroids and cholinergic tone affect neuropeptide expression in regionally-specific compartments of the striatum.

561.5

ACUTE RESERPINE TREATMENT DECREASES EXPRESSION OF PPT mRNA IN RAT STRIATUM. S.Kumar, M.B.Harrison*, C.A.Hubbard and J.M.Trugman. Dept. of Neurology, Univ. of Virginia, Charlottesville VA 22908.

Acute dopamine (DA) depletion with reserpine and AMPT enhances D1 agonist stimulation of regional cerebral glucose utilization in the substantia nigra pars reticulata within 24 hrs. This is one indicator of denervation supersensitivity and reflects heightened metabolic activity in striatonigral neurons. To determine if alterations in neuropeptide expression could be involved in acute development of supersensitivity, we examined expression of preprotachykinin (PPT) and preproenkephalin (PPE) mRNA in the striatum 6 and 24 hrs following a single dose of reserpine. 11 rats received reserpine (5 mg/kg i.p. at time 0) and AMPT (100 mg/kg i.p. at 5 or 23 hrs) and were killed at 6 (n=6) or at 24 hrs (n=5), with 6 controls receiving vehicle. This protocol has been shown to produce 98-99% DA depletion. Alternate sections were processed for in situ hybridization with ^{35}S-labelled oligonucleotide probes for PPT and PPE mRNA, exposed to film and optical density values (OD) determined. Neither PPT nor PPE mRNA expression differed from control at 6 hrs (OD mean±se: PPT control 0.328±.027, 6 hr 0.342±.027; PPE control 0.423±.018, 6 hr 0.444±.028). At 24 hrs PPT mRNA expression had decreased to 62% of control (OD 0.202±.013;p<.002, ANOVA) while PPE was unchanged (OD 0.443±.023). In chronic DA depletion, PPT mRNA decreases and PPE increases. In the present study, the acute effects of DA depletion are selective for PPT. PPT mRNA is localized to striatonigral neurons which preferentially express D1 receptors and its product, Substance P, stimulates ACh release from striatal interneurons and increases DA release from nigrostriatal terminals. The selective decrease in PPT mRNA within 24 hrs of acute DA depletion suggests that decreased Substance P synthesis may mediate or correlate with enhanced sensitivity to D1 receptor stimulation.

561.6

OPPOSITE CHANGES IN TRH AND ITS DEGRADING ENZYME PYROGLUTAMATE AMINOPEPTIDASEII, DURING DEVELOPMENT OF KINDLING. P.de Gortari+°, A. Fernández-Guardiola#, A.Martínez#, M. Cisneros*, J.L.Charli° and P.Joseph-Bravo°*. *Inst. Biotecnología, Universidad Nacional Autónoma de México; #Inst. Mexicano de Psiquiatría and +U. Iberoamericana.

Pyroglutamate aminopeptidase II (PPII) is a neuronal ectoenzyme responsible for TRH degradation at the synaptic cleft. PPII is regulated under different endocrine conditions where TRH is involved (hyperthyroidism, estrous cycle) but only at the adenohypophyseal level; in vitro, TRH can downregulate PPII activity. TRH present in extrahypothalamic brain areas has been postulated to serve as a neuromodulator and levels of this peptide increase in amygdala, hyppocampus and cortex after electrical stimulation. We were interested in studying whether brain PPII could be regulated in conditions that stimulate TRHergic neurons such as kindling.Male Wistar rats with indwelling bipolar electrodes placed in left temporal lobe amygdala (AM) and frontal cortex;amygdaline after discharge established (average 300 uAmp) and kindling (1sec, 60Hz,1msec pulses) induced by daily electrical stimulation. EEG activity,duration and frequency of AM afterdischarge measured. Animals were sacrificed in behavioural kindling stages II,IV and V, brains kept at -80C. Regions (amygdala, hyppocampus, n. accumbens, frontal cortex, medula oblongata and hypothalamus) were dissected and assayed for TRH and PPII activity. TRH levels increased from stage II to V in amygdala, hyppocampus and frontal cortex being significantly higher than sham animals. In n. accumbens a significant decrease, compared to sham was observed at stage II, but increased throughout to stage V. In contrast, PPII activity increased considerably at stage II in all regions studied, compared to sham, decreasing thereafter (amygdala data-table). These results suggest that PPII activity in the central nervous system can be regulated in conditions known to affect TRHergic neurons. Supported by DGAPA-UNAM.

	SHAM	II	IV	V
(TRH mg/prot)	585 ± 140	984 ± 118	980 ± 108	1105 ± 37
(PPII sp.act.)	0.8 ± 0.3	7.3 ± 0.3	6.8 ± 0.9	2.1 ± 0.4

561.7

BRAIN PROTEOLYSIS OF OXYTOCIN "IN VITRO" AND "IN VIVO" CHANGES DURING AGING IN MALE RATS. R. Stancampiano, M.R. Melis & A. Argiolas*. Bernard. B. Brodie Dept. Neurosci., Cagliari Univ., 09124 Cagliari, Italy.

Oxytocin proteolysis was studied "in vitro" with purified synaptic membranes and "in vivo" after injection into the hippocampus of male Wistar Kyoto rats of different ages. When oxytocin was incubated "in vitro" with brain synaptic membranes obtained from 2, 6, and 12 month-old rats, no difference in the content of C-terminal and N-terminal fragments formed by aminopeptidase-like and endopeptidase-like enzymes, respectively, was found after HPLC separation and quantification by amino acid analysis. In contrast, the content of all fragments decreased by about 20-25% when membranes obtained from 18 and 24 month-old rats were used. When [^{3}H-Tyr^{2}]oxytocin was injected "in vivo" in the hippocampus of 2, 6, 12 and 18 month-old rats, no difference in the content of free [^{3}H]-tyrosine and other [^{3}H]-labelled fragments was found in the hippocampal peptidic extract after HPLC fractionation. However, the content of all radioactive fragments was about 50% lower in the extract from 24 month-old rats. The results suggest that oxytocin proteolysis in brain decreases during aging.

561.8

IN VIVO INDUCTION OF RAT C-FOS AND PREPROENKEPHALIN (PPENK) mRNAs BY PROTEIN SYNTHESIS INHIBITORS (PSI). Y.-S. Zhu* and C.E. Inturrisi. Dept. of Pharmacology, Cornell U. Med. College, New York, NY 10021.

In vivo, labile repressor(s) may regulate the immediate-early gene, c-fos. By use of specific, rapid and sensitive solution hybridization assays and Northern blot analysis, we demonstrate that treatment with PSI, either cycloheximide (CHX) or anisomycin (ANI), increased the levels of c-fos mRNA in the adrenal, liver, spinal cord, hippocampus and striatum, while the increase in PPenk mRNA was limited to adrenal and liver. Within 1 h after CHX (4 mg/kg, sc), c-fos mRNA was increased 20 fold and at 3 h it reached a peak 30 fold compared to saline and by 6 h it had declined to 10 fold. In contrast, PPenk mRNA was unchanged at 1 h after CHX, had increased 6 fold at 3 h and reached a peak (13 fold) at 6 h. A similar time course was observed after ANI treatment. Northern blot analysis indicates that the size of the c-fos & PPenk mRNAs was not altered by PSI. In adrenal, the CHX-induction of c-fos and PPenk mRNAs was dose-dependent in the range of 1 to 50 mg/kg, sc. Unilateral adrenal denervation did not alter the CHX-induction of c-fos or PPenk mRNAs in either adrenal medullae or cortex. These effects of PSI, which are tissue and gene specific, may be mediated by labile repressor(s) which regulate c-fos and PPenk gene expression in vivo. Supported by DA01457.

561.9

PITUITARY ADENYLATE CYCLASE ACTIVATING POLYPEPTIDES STIMULATE SUPERIOR CERVICAL GANGION NEUROPEPTIDE Y AND CATECHOLAMINE EXPRESSION AND SECRETION. V. May*, C. A. Brandenburg and K. M. Braas. Dept. of Anatomy & Neurobiology, University of Vermont College of Medicine, Burlington, Vermont 05405.

The expression of specific superior cervical ganglion (SCG) neurotransmitters and neuropeptides in primary neuronal cultures is stimulus-dependent. Pituitary adenylate cyclase activating polypeptides (PACAP38 and PACAP27) stimulate neuronal differentiation and second messenger levels in sympathoadrenal cells. The regulation of SCG neuropeptide Y (NPY) and catecholamine expression and secretion by PACAP38, PACAP27 and VIP was examined in SCG neuronal cultures. Neurons were treated with 10^{-11} to 10^{-6} M peptide for 2 to 25 days. NPY and catecholamine/tyrosine hydroxylase content, secretion and mRNA levels were compared. Nanomolar concentrations of PACAP27 and PACAP38 maximally stimulated sustained SCG neuronal NPY secretion throughout the 25 day treatment, while VIP required 1000-fold higher concentrations. The NPY secretory rate in PACAP treated neurons was 615.8 pg/ml medium/h compared to 67.25 pg/ml/h for control cells; secretion was linear with time. Neither PACAP38 nor PACAP27 changed SCG cell NPY content, although 10-fold stimulation of secretion was elicited. In contrast, NPY mRNA levels were increased by PACAP treatment. PACAP elicited concentration-dependent sustained stimulation of catecholamine secretion, similar to the stimulation of NPY secretion. Half-maximal release was attained with 10 nM PACAP38; maximal release at 100 nM PACAP38 was increased 6-fold. PACAP thus appears to be an important regulator of SCG NPY and catecholamine expression and secretion. (Supported by HD-27468)

561.10

EXAMINATION OF THE ROLE OF THE GABAERGIC SYSTEMS IN REGULATION OF STRIATAL NEUROTENSIN GENE EXPRESSION IN THE RAT by K. P. Decker*, P. Roy-Byrne, and K. M. Merchant. Department of Psychiatry, ZA-99, HMC, University of Washington, Seattle, WA 98104.

Acute neuroleptic administration has been shown to increase expression of neurotensin/neuromedin (NT/N) gene in the dorsolateral striatum and nucleus accumbens. The purpose of the present study was to examine modulation of these neuroleptics' effects on neurotensin expression by GABAergic agents. Muscimol, a specific $GABA_A$ agonist, was chosen for the current study. Adult male Sprague-Dawley rats were treated with; saline, haloperidol (1 mg/kg), muscimol (3.2 mg/kg), or haloperidol (1 mg/kg) plus muscimol (3.2 mg/kg). Animals were sacrificed one hour after medication administration. Expression of NT/N mRNA was examined by in situ hybridization using a [^{35}S]-labelled riboprobe complementary to NT/N mRNA.

In this acute treatment paradigm, densitometric analysis of film autoradiograms revealed that administration of muscimol alone had no effect on NT/N levels in the dorsolateral striatum. Additionally, muscimol did not appear to modulate haloperidol-induced increases in NT/N mRNA levels in the dorsolateral striatum. These data suggest that $GABA_A$ receptors may not be involved in acute regulation of striatal NT/T gene expression in dorsolateral striatal neurons. Further studies are underway to examine the effect of augmenting GABAergic transmission in NT/N gene expression. (Supported by Scottish Rite Schizophrenia Research Program).

561.11

INHIBITION OF PRO-CHOLECYSTOKININ (CCK) SULFATION BY TREATMENT WITH SODIUM CHLORATE ALTERS ITS PROCESSING AND DECREASES CELLULAR CONTENT AND SECRETION OF CCK 8. M. C. Beinfeld*. Dept. Pharmacol. & Physiol. Sci., St. Louis Univ. School of Medicine., St. Louis MO.

Pro-cholecystokinin (CCK) has three sulfated tyrosine residues. Sulfation of the tyrosine residue in CCK 8 is known to be important for its activity at CCK A receptors. The role of these sulfated tyrosines in the sorting and processing of pro-CCK was examined by treatment of CCK-secreting rat thyroid medullary carcinoma cells with 10 mM sodium chlorate (a non-toxic inhibitor of tyrosine sulfation). This treatment caused a 50% decrease in the cellular content of immunoreactive CCK and an 80% decrease in its secretion. Sephadex G-50 chromatography of cellular extracts and culture media showed a selective depletion of CCK 8, and a comparative sparing of CCK 33 and larger molecular forms. The sulfation of the tyrosines of pro-CCK is clearly important for the correct sorting and/or processing of pro-CCK. The pattern of immunoreactive CCK peptides seen with chlorate treatment is consistent with the substrate specificity of a recently identified putative CCK cleaving enzyme and suggests that unsulfated pro-CCK is not efficiently processed to CCK 8 *in vivo*. The large decrease in CCK content and secretion observed with sodium chlorate may also be due to inefficient sorting of unsulfated pro-CCK into secretory vesicles. Supported by NIH NS18667.

561.12

GENE EXPRESSION OF THE PROHORMONE CONVERTASES IN THE RAT BRAIN AND PITUITARY: COMPARATIVE DISTRIBUTION OF PC5 AND PACE4. R. Day*, M. Marcinkiewicz, M. Chrétien, N. G. Seidah. Clinical Research Institute of Montreal, Montreal, Quebec, Canada, H2W 1R7.

Recent advances in the molecular characterization of posttranslational processing enzymes have demonstrated a subtilisin-like enzyme family in mammalian tissues to be responsible for the endoproteolytic processing of inactive protein precursors to biologically active peptides, via selective cleavage of basic residues. Two of these processing enzymes or convertases, PC1 and PC2, are primarily expressed in neuronal and endocrine cells while another, furin, is ubiquitously expressed. PC5 and PACE4, two novel convertases, with very similar structures, have recently been described. Very little is known about their cellular distribution. In this study, we examined the mRNA expression of PC5 and PACE4 in the rat brain and pituitary by *in situ* hybridization using ^{35}S-labeled cRNA probes. The cellular distribution of PC5 was mostly neuronal. Brain regions expressing a relatively higher abundance of PC5 include the CA3 region of the hippocampus, discrete deeper cortical layers and the amygdaloid nuclei. As previously observed for PC1, the CNS gene expression of PC5 was less widespread and less abundant than that of PC2 mRNA. In the pituitary, PC5 mRNA was principally expressed in the anterior lobe gonadotrophs, though we could not exclude other endocrine cell types. Very little PC5 expression was observed in the intermediate and neural lobes. Thus, the localization of PC5 contrasts with that of PACE4, whose distribution appears to be ubiquitous. The observation of different combinations of convertase expression in distinct brain regions, suggests that specific convertase mixtures are involved in protein processing. Accordingly, particular combinations of convertases should yield region-specific differences in posttranslational processing.

561.13

SYMPATHETIC ACTIVATION IS INVOLVED IN NICOTINE- BUT NOT CAPSAICIN- AND BRADYKININ-INDUCED CGRP RELEASE FROM THE RAT TRACHEA X.-Y. HUA*, E.A. CHIANG, S. JINNO & T.L. YAKSH Anesthesiology, Univ. of California, San Diego, CA 92093-0818

We have previously demonstrated that application of capsaicin (Caps), bradykinin (BK) and nicotine (NIC) to the rat trachea induces a dose-dependent increase in CGRP release. Activation of postganglionic sympathetic terminals and subsequent release of prostaglandins (PG) have been reported to mediate BK-induced inflammatory responses. The present study investigates the possible contribution of sympathetic nerve and mast cells to the effects of Caps, BK and NIC on CGRP release by using the isolated rat trachea model[1]. The effects of BK ($5x10^{-6}$M) and NIC (10^{-5}M) but not Caps (10^{-7}M) are significantly reduced by indomethacin treatment. 6-OHDA treatment, which depletes catecholamine in the rat trachea, does not alter Caps- and BK-induced CGRP release but significantly attenuates NIC's effect. 48/80 pretreatment, which causes degranulation of a majority of mast cells in the trachea, does not significantly change the effects of the three agents. Neither norepinephrine nor neuropeptide Y (10^{-5}M) alter the basal or Caps-evoked CGRP release. ATP (10^{-5}M) induces an increase in CGRP outflow from the rat trachea. In conclusion, (1) Caps-evoked CGRP release is not mediated by PG production or by activation of sympathetic terminals and mast cells; (2) The fact that indomethacin, but not 6-OHDA or 48/80 treatment inhibits BK's effect suggests that BK-induced CGRP release is mediated by PG synthesis, which is independent of the presence of sympathetic nerve or mast cells; (3) Sympathetic activation may be involved in NIC-induced PG-mediated CGRP release.

1. *Soc Neurosci Abstr 18:986 (414.17), 1992.*

(This work is supported by TRDRP KT-19, Univ. of California. X.-Y. H.)

561.14

SEROTONINERGIC AND ADRENERGIC REGULATION OF SOMATOSTATIN GENE EXPRESSION IN THE RAT HYPOTHALAMUS. G. Pelletier* and S. Li. MRC Group in Molecular Endocrinology, CHUL Research Center and Laval University, Québec, G1V 4G2, Canada.

The role played by the serotoninergic and adrenergic systems in the regulation of somatostatin (SS) secretion is controversial. The aim of the present study was to evaluate the role of these two neurotransmitter systems on SS gene expression in the male rat periventricular nucleus. Quantitative in situ hybridization involving of a [^{35}S]-labeled cDNA encoding rat pre-proSS mRNA was perform at the cellular level to measure the amounts of SS mRNA per cell. The influence of the serotoninergic system was studied following the chronic administration (2.5 days) of serotonin (5HT), the 5-HT$_{1+2}$ receptor antagonist methysergide, the 5-HT$_2$ receptor antagonist ketanserin and ondansetron, a 5-HT$_3$ receptor antagonist. 5HT induced a 17% increase in the number of silver grains/cell while ondansetron and methysergide decreased the hybridization signal by 27% and 11%, respectively. Ketanserin had no effect on mRNA levels. The role of the adrenergic system was evaluated by the 2.5 day administration of the α-1 and α-2-adrenergic receptor blockers, prazocin and yohimbine, and the β-adrenergic antagonist propranolol. While propranolol had no effect on SS mRNA levels, both prazocin and yohimbine induced a decrease of 46 and 18%, respectively, in the number of grains per cell. The present data indicate that SS gene expression is positively regulated by the serotoninergic system probably via 5HT$_2$ and 5HT$_3$ receptors and the adrenergic system through α–1 and α–2 adrenergic receptors.

CATECHOLAMINE RECEPTORS: DOPAMINE RECEPTOR LOCALIZATION AND REGULATION

562.1

CELLULAR DISTRIBUTION OF D_{1A} AND D_2 DOPAMINE RECEPTORS USING FLUORESCENT DETECTION OF IN SITU cDNA SYNTHESIS. ***M.A. Ariano*, [1]S. Nair, and [1]J.H. Eberwine.***
Neuroscience, Chicago Medical School, N. Chicago, IL 60064; [1]Pharmacology, University of Pennsylvania, Philadelphia, PA 19104.

We have developed a fluorescence method to determine the in situ synthesis of cDNAs for different dopamine receptor clones by modifying the radionuclide-based in situ transcription (IST) technique (Science 240: 1661, 1988). The high sensitivity offered by fluorescent detection of labeled IST cDNA allows rapid cellular detection of low abundance mRNAs, such as those encoding the dopamine receptors.

Incorporation of rhodamine-coupled dUTP (FluoroRed™, Amersham) into dopamine receptor IST cDNA was performed after hybridization with 3' anti-sense DNA oligonucleotide primer generated against sequences in the third cytoplasmic loop of the dopamine receptors. Tissues were subsequently washed and examined using a fluorescent microscope, some 8 hours after initiation of the experiment. We could visualize fluorescent neurons in the neocortex, neostriatum, nucleus accumbens, and olfactory tubercle expressing nascent cDNA for the D_{1A} and D_2 receptor subtypes. The specificity of the fluorescent IST technique was further substantiated using stably transfected Chinese Hamster Ovary (CHO) cells, expressing only one of thep dopamine receptor subtypes (PNAS USA 87: 6723, 1990). Controls included hybridization using neuron specific enolase to detect neuronal profiles, or oligo dT to ascertain cells with poly-A tailed mRNAs. Both experiments showed robust numbers of fluorescent cells. An additional negative control, omission of primer, demonstrated the low background signal in the tissue slice. The fluorescent method has distinct advantages: 1) rapidity of analysis, 2) use in double labeling, and 3) potentially improved sensitivity.

562.2

DOPAMINE RECEPTOR EXPRESSION IN NEOSTRIATAL OUTPUT NEURONS. E.A. Nansen, M.A. Ariano[1], D.R.Sibley[2], D. Birt*, M.S. Levine. MRRC UCLA, Los Angeles, CA 90024, Dept. Neurosci. Chicago Med. Sch. N. Chicago IL 60064[1], Mol. Pharmacol. Unit ETB NINDS Bethesda, MD 20892[2].

The present study was designed to assess expression of dopamine (DA) receptors in neostriatal output neurons using fluorescent retrograde markers and polyclonal antibodies. Fluorescent labeled microspheres were stereotaxically injected into either the substantia nigra or the globus pallidus of rats. Following surgery (3-10 days), animals were sacrificed and brains were rapidly removed and frozen. Coronal sections (10-20 μm) were cut from the forebrain and processed with polyclonal antibodies raised against specific peptide sequences for the D_1, D_2 or D_3 subtypes of DA receptors. The secondary antibodies were conjugated to fluorescent markers in order to localize the DA receptor immunoreactivity in the fluorescent labeled neostriatal output neurons. The slides were analyzed on a Biorad MRC 600 confocal microscope capable of producing <1 μm thick optical sections.

The majority of identified striatopallidal neurons contained either D_1 or D_2 DA receptor immunoreactivity. It is possible, because striatonigral fibers also course through the pallidal injection sites, that some of the identified striatopallidal projection cells might be striatonigral output neurons if beads are transported in damaged axons. Most striatonigral neurons also contained immunoreactivity for either D_1 or D_2 DA receptors. D_3 immunoreactivity was localized to a smaller subpopulation of striatonigral cells than either the D_1 or D_2 immunoreactivity. These results provide evidence that different DA receptor subtypes are localized to both striatonigral and striatopallidal neurons. Supported by USPHS Grants NS 23079 (M.A.A.), HD 05958 (M.S.L.) and AG 10252 (M.S.L.)

562.3

Autoradiographic Localization of Dopamine Receptor Sites in Rat Brain Using [^{3}H]-Quinelorane (LY163502). S. L. Gackenheimer*, J. M. Schaus and D. R. Gehlert. CNS Pharmacology, Lilly Research Laboratories, Indianapolis, IN 46285.

Quinelorane (LY163502) is a selective D_2 agonist which has been extensively studied for its effects on the sexual behavior of male rats. When infused into the medial preoptic area of the rat brain, or injected SC, quinelorane improved levels of male sexual behavior (mounting and copulation)(J. Neural Trans. 68:153-70, 1987). Recent studies suggest that quinelorane may also interact with cloned D_3 receptors expressed in cell lines (Eur. J. Pharm 225: 331-37, 1992).

In the present study, quinelorane was radiolabeled and evaluated as a ligand for autoradiographic localization of dopamine receptors in the rat brain. Optimal labeling conditions were determined by a series of biochemical studies using slide mounted sections of rat forebrain. Nonspecific binding was determined by including 1 µM quinelorane in the incubation buffer. Once the binding conditions were determined, sections were labeled and exposed against a sheet of Hyperfilm-^{3}H for eight weeks. Quantitation of the binding was accomplished using ^{3}H-Micro-scales (Amersham) which were placed against the film along with the labeled sections. Developed films were analyzed by densitometry using the MCID (Imaging Research, Ontario, Canada) image analysis system.

Saturation analysis using sections of rat forebrain indicated that [^{3}H]quinelorane bound to a single site with an apparent K_d of 2 nM and a B_{max} of 40 fmol/mg tissue dry weight. On the concentration dependent portion of the curve, specific binding accounted for greater than 99 percent of the total binding. Gpp(NH)p reduced binding in a concentration dependent manner with an almost complete inhibition of binding at 10 µM. Localization experiments revealed a binding distribution simular to the distribution reported for other radiolabeled D_2 receptor agonists and antagonists. A high level of specific binding was seen in the glomerular layer of the olfactory bulb, caudate-putamen and islands of Calleja. Moderate levels of binding were seen in the olfactory tubercle, nucleus accumbens, dorsal lateral septum, zonal layer of superficial gray, ventral tegmental area and lobe 10 of the cerebellum. Low levels of binding were observed in the medial preoptic area, substantia nigra compacta, dorsal raphe and nucleus of the solitary tract.

In conclusion, [^{3}H]quinelorane binds to sections of rat brain with high affinity and exhibits very low nonspecific binding. The distribution of binding was consistent with the distribution of D_2 and D_3 receptors reported in rat brain. As such, [^{3}H]quinelorane represents a useful tool for further pharmacological and anatomical characterization of D_2 and D_3 receptors in rat brain.

563.4

DETERMINATION OF DOPAMINE D3 RECEPTOR DISTRIBUTION IN RAT BRAIN USING [^{125}I]-7-TRANS-OH-PIPAT P. McGonigle*, R.P. Artymyshyn, S. McElligott, M.P. Kung and H. Kung **Depts. of Pharmacology and Radiology, University of Pennsylvania, Phila., PA 19104.**

D3 receptors in sections of rat brain were measured with the new, D3-selective radioligand [^{125}I]-7-Trans-OH-PIPAT (PIPAT). Preliminary characterization of the binding of the radioligand was performed in sections of tissue mash derived from nucleus accumbens and olfactory tubercle. At 22°C, binding of 0.3 nM [^{125}I]-PIPAT reached equilibrium in 90 min and remained stable for at least one hour. Scatchard plots of saturation data were linear and yielded a K_d of .52 ± .02 nM, consistent with densitometric measurements made in the nucleus accumbens and islands of Calleja. Displacement curves were monophasic and the pharmacological profile of 7-OH-DPAT > quinpirole > domperidone > dopamine > clozapine is consistent with the labeling of a D3 receptor. The distribution of D3 receptors was measured in 20 µm coronal and sagittal sections labeled with 0.2 nM [^{125}I]-PIPAT. Nonspecific binding was measured in the presence of 5 µM 7-OH-DPAT. Sections were apposed to film for 18 or 72 hours. The highest densities of labeling were observed in the islands of Calleja, the rostral part of the nucleus accumbens and the molecular layer of lobules 9 and 10 of the cerebellum. Lower densities of labeling were found in: the caudal nucleus accumbens, substantia nigra, inferior olive, bed nucleus of the stria terminalis, interpeduncular nulceus, and selected thalamic and hypothalamic nuclei. Labeling in the caudate putamen was low and restricted to its rostral and medial aspects. These results are in general agreement with the localization of mRNA coding for D3 receptors as determined using *in situ* hybridization. (Supported by USPHS GM 34781 and NARSAD)

563.5

DISTRIBUTION OF DOPAMINE RECEPTOR MESSENGER RNA IN THE PRIMATE BRAIN. J. H. Meador-Woodruff*, J. Wang, S. P. Damask, and S. J. Watson. Mental Health Research Institute, University of Michigan, Ann Arbor, MI 48109-0720.

At least five dopamine receptor subtypes have been cloned, and each appears to have distinct pharmacological properties and a unique neuroanatomical distribution. In the rat, the D1 and D2 receptors appear to be enriched in motor, limbic, and neuroendocrine circuits, whereas the D3 and D4 receptors have been reported to be primarily localized to limbic regions. The D5 receptor has a particularly unusual and restricted distribution. Most neuroanatomical characterization of the mRNAs encoding these receptors has been performed in the rodent; relatively little has been done to describe the anatomical distribution of these messages in the primate and human brain. As part of our ongoing efforts to characterize dopamine receptor systems in brain, we have mapped the distributions of the mRNAs encoding the five dopamine receptors in the brain of the old world monkey by *in situ* hybridization, using radioactively-labeled riboprobes synthesized from corresponding human cDNAs. While many similarities in the distributions of dopamine receptor mRNAs exist between the rat and the monkey, significant differences are also obvious. These differences suggest that the dopamine receptors may function differently in discrete neural circuits in the primate than in the rat, indicating that generalization of dopamine receptor message data from the rat to the monkey and human should be made with caution. Supported by MH00818 and MH42251.

563.6

DOPAMINE RECEPTORS SUPERSENSITIVITY: EFFECTS OF CHRONIC L-DOPA AND CARBIDOPA TREATMENT IN RATS WITH 6-HYDROXYDOPAMINE LESION. Mir Ahamed Hossain* and Norman Weiner. Dept. of Pharmacology, University of Colorado Health Science Center, Denver, CO 80262.

Intracellular cAMP accumulation in slices from striatum and substantia nigra was measured following 30 days of L-DOPA/carbidopa treatment in rats with unilateral 6-OHDA lesion (90%) of the nigrostriatal tract. Following 6-OHDA lesion the basal cAMP accumulation was increased significantly (92%) in the striatal slices ipsilateral to the lesion, while remaining unaffected in the ipsilateral substantia nigra as compared to the contralateral intact hemisphere. Dopamine (DA) and tetrahydrobiopterin (BH_4) levels were depleted by 99% and 70% respectively in the ipsilateral striatum as we reported previously (Brain Res., 598: 121, 1992). After 30 days of L-DOPA/carbidopa treatment, the elevated levels of cAMP accumulation in ipsilateral striatum reduced significantly and returned to the control levels of the contralateral intact side of vehicle-treated group. The D-1 receptor agonist SKF 38393 (10uM) increased cAMP accumulation in striatal slices from lesioned and intact hemispheres of both vehicle and treatment group which was completely inhibited by the D-1 antagonist SCH 23390 (10uM). In contrast, the ability of SKF 38393 to enhance the cAMP accumulation was blocked by the D-2 agonist quinpirole (10uM) in striatal slices from the intact hemisphere but not in tissues from the lesioned side. In substantia nigra, no significant differences in cAMP accumulation were observed in presence of the agonist or antagonist. Our data indicates that chronic L-DOPA/carbidopa treatment reverses the D-1 receptor supersensitivity seen following 6-OHDA lesions, suggesting a D-1 receptor mediated action of L-DOPA. Following 6-OHDA lesion there appears to be an uncoupling of the inhibitory D-2 receptor from D-1 receptor associated cAMP accumulation. Chronic L-DOPA/carbidopa treatment does not reverse this uncoupling of D-1 and D-2 receptor.

563.7

FUNCTION AND REGULATION OF THE D_3 DOPAMINE RECEPTOR. D. Lévesque*, P. Sokoloff, M.-P. Martres, J. Diaz, V. Dimitriadou, C. Pilon and J.-C. Schwartz. Centre Paul Broca, INSERM U. 109, Paris 75014 and Lab. de Physiologie, Université René Descartes, Paris 75006, France.

The physiological role of the dopamine (DA) D_3 receptor has remained elusive because of the absence of functional correlates. We have transfected NG108-15 neuroblastoma X glioma hybrid cell line with a D_3 receptor cDNA. This cell line stably expresses D_3 receptors, which display high affinity for DA and low, but consistent regulation by guanine nucleotide. DNA synthesis, measured by incorporation of [^{3}H]thymidine, is increased by 70-80% upon stimulation by DA (EC_{50}= 2 nM), quinpirole (EC_{50}= 1 nM) or (+)7OH-DPAT (EC_{50}= 0.3 nM), and is antagonized by (+)UH 232. The effect of 100 nM quinpirole is nearly abolished by pretreatment with pertussis toxin, and is potentiated by co-stimulation of protein kinase C with the phorbol ester PMA (1 nM). There is no apparent coupling with classical intracellular second messengers. On the other hand, D_3 receptor activation transiently induces Fos immunoreactivity. Thus, transfected D_3 receptors in NG108-15 cells are coupled, through a pertussis toxin-sensitive G protein, to a so far unidentified primary effector that induces *c-fos* expression and mitogenesis. We also investigated the *in vivo* regulation of the D_3 receptor. Lesion with 6-OHDA produces a marked decrease of both D_3 receptor binding (-71%, with [^{3}H]7OH-DPAT) and D_3 receptor mRNA level (-52%, with quantitative PCR and *in situ* hybridization) in the nucleus accumbens ipsilateral to the lesion, whereas chronic haloperidol treatment (20 mg/kg, b.i.d., for 2 weeks) is without effect. Thus, unlike D_2 receptors, D_3 receptors do not upregulated after interruption of DA neurotransmission, supporting the involvement of these receptors in the antipsychotic effects, for which there is no tolerance. DL is holder of a fellowship from the Natural Sciences and Engineering Research Council of Canada.

563.8

D_1 AND D_2 DOPAMINE RECEPTOR DENSITIES IN GRAFTED RAT STRIATUM AFTER CHRONIC INTERMITTENT L-DOPA AND/OR THE MAO-INHIBITOR DEPRENYL (SELEGILINE). C.E. Adams*, A.F. Hoffman, J.L. Hudson, B.J. Hoffer and S.J.Boyson. Depts. of Neurology and Pharmacology, Univ of Colo Health Sciences Center, Denver, CO 80262.

It has been proposed that deprenyl may prevent the long term oxidative stress on native or grafted nigral tissue which is hypothesized to be caused by the use of L-dopa in Parkinson's patients. We examined this question in unilaterally 6-OHDA-lesioned rats which were injected twice daily in the following groups: S/V (sham/vehicle); G/V (grafted/V); G/L (G/L-dopa & benserazide); G/L/D (G/L/deprenyl); and G/D. All animals received drugs or vehicle for two weeks prior to grafting or sham graft; drug treatment followed for an additional 2.5 months. Immediately following the treatment period, the brains were processed for quantitative autoradiography. D_1 receptors were labeled with ^{3}H-SCH-23390 while D_2 receptors were labeled with ^{3}H-spiperone. Preliminary analysis of the data revealed significant increases in the density of D_2 but no change in the density of D_1 receptors within the lesioned compared to the intact striata of the S/V rats. Grafting of fetal nigral tissue normalized the density of D_2 receptors throughout the grafted striata of the G/V rats but led to a decrease in the density of D_1 receptors within the dorsomedial quadrant of the grafted striata compared to the intact side. Chronic treatment with L-dopa alone decreased the density of both D_1 and D_2 receptors within the ventromedial quadrants of the grafted striata in the G/L rats. Deprenyl treatment led to a lower density of D_1 receptors within the grafted compared to the intact striata of the G/D and G/L/D groups. Graft viability, as measured by reductions in apomorphine-induced rotations, was unaffected by drug treatments. Supported by NIH NS 09199 and NS 29203

562.9

LONG-TERM L-DOPA ADMINISTRATION TO RATS WITH 6-OHDA LESIONS: BEHAVIOR AND STRIATAL NEUROCHEMISTRY.

S. R. Wachtel* and E. D. Abercrombie. Center for Molecular & Behavioral Neuroscience, Rutgers University, Newark, NJ 07102.

Despite the standard use of L-3,4-dihydroxyphenylalanine (L-DOPA) therapy for Parkinson's disease, the reasons for the emergence of dyskinesias and declining efficacy with long-term treatment are not understood. We hypothesize that these adverse effects derive from repeated exposure of the denervated striatum to unregulated, supranormal levels of extracellular dopamine (DA) formed from L-DOPA. We therefore examined the effects of a 28 day regimen of L-DOPA+benserazide (50 mg/kg each, bid) on behavior and striatal neurochemistry in rats with unilateral 6-hydroxydopamine (6-OHDA) lesions of the nigrostriatal DA pathway. Ipsilateral rotation, monitored at 1 week intervals, was increased 3-4 fold at day 8 and then continued to increase at a slower rate for the remainder of the treatment duration. Twenty-four hrs after the last L-DOPA injection, microdialysis probes were used to monitor extracellular levels of DA and acetylcholine (ACh) bilaterally in the striatum. The increase in extracellular DA elicited by L-DOPA (50 mg/kg) in the DA-depleted striatum was not altered by long-term L-DOPA treatment as compared to untreated controls, despite the enhanced rotational response in the former group. L-DOPA administration did not alter striatal ACh output on the intact or the lesioned side in untreated controls. In contrast, ACh output was stimulated by L-DOPA selectively in the lesioned striatum of rats that had received the chronic L-DOPA. Thus, long-term L-DOPA administration, in this model, produces a potentiation of behavioral responding that is associated with a corresponding increase in ACh output in the lesioned striatum but not an enhanced L-DOPA-induced increase in extracellular DA. This indicates that post-synaptic changes may be critically involved in the emergence of unwanted side effects associated with long-term L-DOPA therapy in Parkinson's disease. [Supported in part by USPHS grants NS09206 (SRW) and NS19608 (EDA)]

562.10

DOES 6-HYDROXYDOPAMINE (6-OHDA) LESION OF THE NIGROSTRIATAL PATHWAY AFFECT STRIATAL D2 DOPAMINE (DA) RECEPTOR mRNA LEVELS?

K.-X. Huang*, T. Minowa, Y. H. Sohn, M.J.Twery, M.M. Mouradian and J.R. Walters. Exptl. Therap. Branch, NINDS, Bethesda, MD 20892.

The rat model of Parkinson's disease produced by 6-OHDA-induced lesion of the nigrostriatal DA pathway is associated with enhanced response to DA agonists and increased density of postsynaptic D2 receptors. Whether lesion alters steady-state levels of striatal D2 mRNA remains controversial. Solution hybridization/ribonuclease protection assay was utilized to address this issue. RNA was isolated from ipsilateral and contralateral striata. From a rat D2 cDNA clone, a ^{32}P-labelled antisense riboprobe was transcribed *in vitro* covering bases 404-829 which include the 87 bp alternatively spliced exon. Total RNA was hybridized with D2 and β-actin probes and digested by RNase A/T1. Protected hybrids were excised from the gel and radioactivity quantitated. In lesioned rats not screened by DA agonist injection, no significant differences were found in absolute counts for D2 long or D2 short mRNAs or in ratios of D2/β-actin mRNAs between lesioned and unlesioned striata in rats studied 2, 4, 8 or 19 weeks after lesion (n = 4-8). As expected, the D2 messages in the substantia nigra on the lesioned side were undetectable. These results indicate that postsynaptic changes induced by DA denervation are not associated with alterations in steady-state levels of D2 mRNA and are inconsistent with reports of increases in striatal D2 receptor and mRNA levels as measured by other methods. However, rats utilized in these latter studies have typically been pretreated with apomorphine to test the effectiveness of the lesion. In view of the agonist- and l-DOPA-induced priming phenomenon observed in experimental animals and in patients with Parkinson's disease, future studies should also investigate D2 mRNA levels following DA agonist pretreatment of 6-OHDA-lesioned rats.

562.11

CHANGES OF D1 AND D2 RECEPTORS IN ADULT RAT NEOSTRIATUM AFTER NEONATAL DOPAMINE DENERVATION: QUANTITATIVE RADIOLIGAND BINDING, IN SITU HYBRIDIZATION AND IONTOPHORETIC DATA.

F. Radja*, M. El Mansari, J.-J. Soghomonian, K.M. Dewar, A. Ferron, T.A. Reader and L. Descarries. Centre de recherche en sciences neurologiques (Départements de physiologie, psychiatrie et pathologie), Université de Montréal, Montréal, and Centre de recherche en neurobiologie, Université Laval, Québec, Québec, Canada.

The specific binding of [^{3}H]SCH23390 to D1 and of [^{3}H]raclopride to D2 dopamine (DA) receptors was measured by autoradiography in the rostral and caudal neostriatum (NS) of adult rats subjected to near total destruction of nigrostriatal DA neurons by intraventricular 6-hydroxydopamine soon after birth (3 days). Three months after this lesion, [^{3}H]SCH23390 binding was slightly but significantly decreased in the rostral NS (22%), but unchanged in the caudal NS. In contrast, [^{3}H]raclopride binding was considerably increased throughout the NS (10-40%). In the rostral NS, there were no parallel changes in D2 receptor mRNA levels, as measured by *in situ* hybridization on adjacent sections. Caudally, however, slight but significant increases in D2 mRNA were detected (10-20%). As assessed by quantitative iontophoresis, there was a marked enhancement (63%) of the inhibitory responsiveness of spontaneously firing units in the rostral NS to DA and the D1 agonist, SKF38393, in neonatally-lesioned versus control rats. On the other hand, responsiveness to PPHT, a potent D2 agonist, appeared to be unchanged. Such opposite changes in the number of D1 and D2 binding sites, expression of D2 receptor mRNA and electrophysiological responsiveness to DA and D1 and D2 agonists, suggested independent adaptations following the neonatal DA denervation of NS. They also emphasized that the effects of DA and D1 and D2 agonists in NS do not depend strictly on the number of D1 and D2 receptor sites. (Supported by the FRSQ, the Parkinson Foundation of Canada and MRC grants MT-6967 and MT-3544).

562.12

ONTOGENETIC SUPERSENSITIZATION OF DOPAMINE D_1 RECEPTORS OCCURS WITHOUT A CHANGE IN D_1 HIGH AFFINITY SITES.

[1]L. Gong, [1]R.M. Kostrzewa, [2]R. Brus, [1]E.A. Daigneault*, [3]R.W. Fuller and [3]K.W. Perry. [1]Dept. of Pharmacology, East Tennessee State Univ., Johnson City, TN; [2]Dept. of Pharmacology, Silesian Academy of Medicine, Zabrze, Poland; and [3]Lilly Research Labs, Eli Lilly Co., Indianapolis, IN.

To test whether SKF 38393 could ontogenetically prime dopamine D_1 receptors, intact and neonatal 6-hydroxydopamine (6-OHDA) lesioned rats (134 µg i.c.v.; desipramine pretreatment) were treated daily for the first 28 days from birth with the D_1 agonist, SKF 38393 HCl (3.0 mg/kg i.p.) or its vehicle. In intact rats SKF 38393 treatments had no effect on SKF 38393 HCl (3.0 mg/kg IP)-induced locomotor activity at 2 months. In D_1 neonatally primed 6-OHDA rats, enhanced locomotor responses were seen with the first SKF 38393 challenge dose at 2 months. This response increased further with weekly SKF 38393 treatments. There was no change from control in the percentage of high affinity D_1 sites in lesioned and primed rats. Striatal DA content was reduced >98% and 5-HT content was elevated >50% in neonatal 6-OHDA lesioned rats, regardless of whether SKF 38393 was co-administered ontogenetically. The findings indicate that ontogenetic SKF 38393 treatments partially prime D_1 receptors, but that this effect is not associated with a shift in the high affinity binding status of D_1 receptors. (Supported by NS 29505)

562.13

CHANGES IN DOPAMINERGIC FUNCTION FOLLOWING KINDLING.

R.A. Fox, C.D. Applegate and D.A. Cory-Slechta*. Departments of Neurology and Environmental Medicine, University of Rochester School of Medicine and Dentistry, Rochester, NY 14642.

The kindling phenomenon, an animal model of epileptogenesis, has been shown to increase the number of both D2 dopamine (DA) receptors and mRNA in the striatum and nucleus accumbens of rats. To determine whether the increases in DA receptors are of functional significance, male rats were trained, using standard operant food-reinforced drug discrimination (DD) procedures, to discriminate the stimulus properties of an autoreceptor dose (0.05mg/kg i.p.) of the selective D2 agonist quinpirole (QUI) from saline, and QUI dose-effect curves were determined. Animals were then matched according to ED50 values, and one rat in each pair was kindled while the other was a sham-operated control. Subsequent redetermination of the dose-effect curves revealed a decreased QUI sensitivity in control but not kindled rats. Likewise, dose-effect curves for apomorphine (APO) substituted for QUI were left-shifted in kindled rats relative to controls. These data suggest that kindling-induced increases in D2 receptor number appear to compensate for the loss of D2 sensitivity produced in controls by continuous administration of QUI in the DD procedure, and are consistent with a kindling-induced functional D2 autoreceptor supersensitivity. In addition, head down, sniffing behavior in response to a high dose of APO was increased in kindled rats, consistent with a possible kindling-induced D1 supersensitivity. Kindling-induced changes in DA systems, therefore appear to have functional consequences. Supported by ES05903 and ES05017.

562.14

EXPRESSION OF D2 AND D3 RECEPTORS IN LIMBIC SYSTEM OF NORMALS, SCHIZOPHRENICS AND ALZHEIMER'S CASES

J.N. Joyce*, A. Murray, H. Ryoo, S. Goldsmith. Dept. Psychiatry, Lab. Chemical Neuroanatomy, Univ. Penn. Sch. Medicine, Philadelphia, PA

We have previously described the autoradiographic mapping of D2-like receptors with [^{125}I]epidepride in human brain (Joyce et al., JPET, 253:1253,1991), showing high expression in limbic regions. It is unclear to what extent we were labeling D3 receptors. We took advantage of the binding of [^{125}I]epidepride to D2 and D3 receptors in human brain in the presence and absence of compounds that have differing affinities for D2 or D3 sites to visualize these receptors (Murray et al, 1992; EJP, 227:443). High ratios of D3/D2 receptors were found in the striosomes of the ventral striatum, the medial aspect of the internal globus pallidus, the basal forebrain, the A10 region of the midbrain. Within the limbic system there was high ratios in the central and amygdalo striato transition divisions of the amygdala and in the dentate gyrus (DG). The remainder of the hippocampus and medial temporal lobe show undetectable amounts of D3 receptors. Reductions in the number of D2-like receptors in the DG, subiculum and perirhinal regions are observed in Alzheimer's disease (Joyce et al, 1993; Neurosci. Lett). Changes in expression of D2-like receptors are not observed in the DG or subiculum in elderly schizophrenic cases. Altered laminar expression of receptors in the medial temporal lobe and within the "columns" of the temporal cortex were observed (Goldsmith and Joyce, this meeting). Even though elderly schizophrenics are demented, they do show similar patterns of D2 receptor expression. This is the first direct evidence that the expression of DA receptors is modified in the limbic lobe of the schizophrenics and could contribute to the psychotic symptoms. Examination of regions with high expression of D3 receptors are being examined in these same cases. **Funded by MH 43880, AG 09215.**

562.15

THE D_{2S} AND D_{2L} DOPAMINE RECEPTORS ARE DIFFERENTIALLY REGULATED IN CHO CELLS. Li-Juan Zhang*, Jean E. Lachowicz and David R. Sibley. Molecular Neuropharmacology Section, ETB, NINDS, NIH, Bethesda, MD 20892.

To investigate and compare the regulatory properties of the two isoforms of the D_2 dopamine receptor, we have stably expressed their cDNAs in Chinese Hamster Ovary (CHO) cells. Cell lines were selected which express similar levels of [^{3}H]methylspiperone binding activity. Both isoforms mediate a dose-dependent and pharmacologically-specific inhibition of adenylyl cyclase activity in both intact cell and membrane preparations. Pretreatment of both D_{2L} and D_{2S} receptor expressing cells with 100 μM dopamine produces a ~5-fold shift (lower affinity) in the EC_{50} for dopamine inhibition of cAMP accumulation with a ~25% decrease in the maximum response. Dopamine treatment also results in a ~20% decrease in the maximum receptor binding activity of the D_{2S} receptor expressing cells. In contrast, the D_{2L} receptors are up-regulated by about 3-fold in response to dopamine treatment. This difference in response between the D_{2S} and D_{2L} receptors is not cell line specific as other CHO clones expressing these isoforms show identical responses. The dopamine-induced up-regulation of D_{2L} binding is time-dependent, reaching maximal levels after 10 hr with a $t_{1/2}$ = 4 hr. This response is blocked by prior treatment of the cells with 1 μg/ml pertussis toxin for 24 hr and is not mimicked by cAMP analogs. To test whether protein synthesis is required for the D_{2L} receptor up-regulation, we pretreated the cells with 5 μg/ml cycloheximide to block mRNA translation. This was found to completely inhibit the up-regulation of D_{2L} binding activity, however, there was no effect on the desensitization of the adenylyl cyclase response. Our data indicate that dopamine can positively regulate the synthesis of the D_{2L} receptor isoform in CHO cells. The mechanism underlying this regulation and its apparent absence for the D_{2S} isoform is currently under investigation.

562.16

ANALYSIS OF THE ENHANCER REGION IN THE HUMAN D_{1A} DOPAMINE RECEPTOR GENE. M. T. Minowa, T. Minowa, J. R. Walters* and M. M. Mouradian. Experimental Therapeutics Branch, NINDS, Bethesda, MD 20892

We have previously reported that the human gene encoding the D_{1A} dopamine receptor has a housekeeping gene like promoter with multiple consensus sequences for Sp1 binding sites. The main enhancer activity is located between nucleotides -1197 and -1120 relative to translation initiation site where consensus sequences for AP2 are also present. Yet purified Sp1 does not bind to the enhancer of this gene as determined by gel shift and DNase I footprinting. Furthermore, nuclear factor(s) in two cell lines expressing this gene, NS20Y and SK-N-MC, which bind to the AP2-like sequence have a molecular size different than AP2 protein by UV cross linking. Our experiments suggest the existence of specific regulatory factors in D_{1A} expressing cells which bind to the regions between -1197 to -1154 and between -1154 to -1120 to enhance promoter activity. Mutations introduced at each of the putative AP2 sites supported this observation. Competitive co-transfection experiments indicated that plasmid vectors carrying these regions could effectively compete with the transcriptional activity of D_{1A} enhancer-CAT gene constructs.

562.17

ANALYSIS OF THE NEGATIVE MODULATOR OF THE RAT D_2 DOPAMINE RECEPTOR GENE PROMOTER. T. Minowa, M. T. Minowa and M. M. Mouradian*. Experimental Therapeutics Br., NINDS, Bethesda, MD 20892.

The D_2 dopamine receptor is classically recognized to be the primary mediator of the motor, behavioral and endocrine effects of central dopaminergic transmission. To investigate the molecular events leading to transcriptional regulation of the rat D-2 gene, we have cloned its 5′ flanking region and identified multiple transcription initiation sites (Minowa et al, Biochemistry 31:8389, 1992). We found an Sp1-like positive modulator between bases -75 and -29 and a negative modulator between -218 and -76. In the present study, we analyzed this silencer region further. DNase I footprinting and gel mobility shift assays using nuclear extract from NB41A3 cells, which express D_2 binding sites, indicated that the region between -116 and -80 is the only sequence in the silencer recognized by nuclear factor(s). This sequence includes three GTGGG repeats and one Sp1 consensus sequence. Addition of this region to the D_2 promoter-CAT gene construct pCATD2-75 decreased promoter activity up to 50%. UV cross linking using GTGGG repeat oligonucleotide with NB41A3 nuclear extract and purified human Sp1 suggested that a non-Sp1 factor in NB41A3 cells binds to this sequence and negatively regulates the D_2 gene.

562.18

CLONING OF EXON I OF THE RAT D_3 DOPAMINE RECEPTOR GENE. D-Q. Gao, T. Minowa, P. A. Jose*, M. M. Mouradian. Experimental Therapeutics Branch, NINDS, Bethesda, MD 20892 and Dept. of Pediatrics, Georgetown Univ. Med. Center, Washington, D.C. 20007.

The brain distribution and pharmacology of the D_3 type of dopamine receptors suggest important function in emotion, cognition and motor behavior. Understanding the molecular regulation of the gene coding for this receptor will assist in elucidating potential changes in its expression in pathological states. As an initial step in characterizing the promoter of the rat D_3 gene, we have cloned and sequenced the true exon I of this gene using an anchored RT-PCR technique with olfactory tubercle poly (A)$^+$ RNA as template. The first gene-specific primer was based on the sequence of the most upstream exon published in which the first ATG codon is located (Giros et al, Biochem. Biophys. Res. Comm. 176: 1584, 1991). A clone having a novel sequence of 88 bp upstream of the first coding exon was isolated. RT-PCR using sense primer based on the new sequence indicated the presence of this exon I in the D_3 message in nucleus accumbens and striatum as well. The G+C content of the D_3 exon I is only about 50% unlike the very high G+C content of the D_2 exon I although the two genes are highly homologous in their coding region.

562.19

THE ROLE OF cAMP-DEPENDENT PROTEIN KINASE IN THE DESENSITIZATION OF THE HUMAN D_1 DOPAMINE RECEPTOR. E.M. Landau*, C.L. Ma, E.C. Healy, R.D. Blitzer and C. Schmauss. Department of Psychiatry Bronx VA Medical Center and Depts of Psychiatry and Pharmacology, Mt. Sinai Medical Center, New York, NY 10029.

The human D_1 dopamine receptor (D_1R) was cloned from genomic DNA using PCR, and its sequence ascertained. The full length D_1R insert was cloned into the expression vector pRC/CMV. This plasmid construct was used for transient transfection of HEK 293 cells by means of the DEAE-dextran procedure (Federman et al. Nature 356:159, 1992). Activation of adenylyl cyclase in the transfected cells was measured by the conversion of [α-^{32}P]ATP to [^{32}P]cAMP (Jacobowitz et al. J. Biol. Chem. 268:3829, 1993). When stimulated by dopamine (DA, 10^{-8} to 10^{-4} M, for 30'), the transfected cells responded by increased adenylyl cyclase activity in a dose-dependent manner. Pre-incubation of the cells with DA (10 uM) for 30 or 60 minutes resulted in a reduction of the response to DA (10^{-4}, 30') by 34±7 % (n=7) and 67±5% (n=4) respectively. When pre-incubated in the presence of the cAMP-dependent kinase (A-Kinase) inhibitor Rp-cAMPS (1 mM, 5 hours), the desensitizing effect of DA (10uM for 1 hour) was decreased by 71+/-10 %. Smaller concentrations of Rp-cAMPS caused a smaller block of desensitization, whereas increasing the Rp-cAMPS concentration to 3 mM did not increase the blocking effect. It is concluded that the D_1R desensitization is largely but not completely caused by activation of A-kinase. (Supported by a VA Merit grant and NIH MH 45212)

562.20

SUPERSENSITIVITY OF DOPAMINE D_{2443} RECEPTOR MEDIATED INHIBITION OF cAMP ACCUMULATION IN FORSKOLIN TREATED LTK- CELLS. A. Westlind-Danielsson* and M.H. Johansson. Department of Neuropharmacology, CNS Preclinical R & D, ASTRA ARCUS AB, S-151 85 Södertälje, Sweden.

Cultured cells expressing the different forms of recently cloned dopamine (DA) receptors have introduced the possibility of studying various aspects of DA receptor-effector coupling and regulation not readily examined *in situ* in brain. The DA D2 receptors exists as two splice variants of the same gene, in rat denoted $D2_{415}$ and $D2_{444}$, in man denoted $D2_{414}$ and $D2_{443}$. Acute agonist activation of either form, examined in transfected cells, leads to inhibition of both adenylyl cyclase (AC) activity and cAMP accumulation. This response is markedly attenuated when mouse fibroblast Ltk- cells expressing $D2_{415}$ are grown in the presence of quinpirole for 1-24 hrs, whereas D2 antagonist treatment has no effect on the response (Bates et al., 1991, Mol. Pharmacol. 39:55-63). We used Ltk- cells expressing the $D2_{443}$ receptor in order to examine whether direct and persistent activation of the AC itself (raising cAMP levels) with forskolin (FSK) would regulate $D2_{443}$ receptor mediated inhibition in the opposite direction, that is producing a supersensitive receptor-effector response. FSK treatment for 4 hrs resulted in an increase of the maximal inhibition of cAMP accumulation (from 68 to 77%) produced by acute exposure to DA in the presence of FSK. Basal cAMP levels decreased by about 33% along with the maximal response to acute FSK challenge (≈23%). The results imply that not only can receptor-effector supersensitivity be induced in cell culture by positively manipulating this signal transduction system at the level of AC but the treatment also leads to desensitization of some of the cAMP enhancing characteristics of the system in parallel.

563.1

SUBCHRONIC TREATMENT WITH A 5-HT 1A AGONIST ENHANCES THE RESPONSE OF MESOCORTICAL DOPAMINE NEURONS TO STRESS. B. A. Morrow* and R. H. Roth, Departments of Pharmacology and Psychiatry, Yale University School of Medicine, New Haven, CT 06510

We investigated the effects of a 7 day treatment of a 5-HT 1A agonist, 8-OH DPAT (2-dipropylamino-8-hydroxy-1,2,3,4-tetrahydronaphthalene), on basal and stress-activated dopamine (DA) and serotonin (5-HT) turnover in the medial prefrontal cortex (mPFC) and the nucleus accumbens (NAS). Rats were treated with saline or 8-OH DPAT, 0.5 mg/kg i.p., twice a day for 7 days. On the 8th day, rats were either restrained or left in the home cage for 30 min, then sacrificed, and the mPFC, NAS, and striatum were harvested for analysis by HPLC-EC. 8-OH DPAT treatment lowered basal 5-HT turnover (5-HIAA/5-HT) in the mPFC, but not the striatum or NAS. No change in basal DA turnover (DOPAC/DA) was noted in any region tested. Stress increased DA metabolism in the mPFC and NAS of saline treated rats, as expected. An important observation was that 8-OH DPAT treatment and stress lead to a significantly greater increase in DA turnover compared to saline treatment and stress in the mPFC only. In the NAS, 8-OH DPAT did not cause a change in basal 5-HT metabolism or in the stress-induced increase in DA turnover. Additional data obtained utilizing chemical lesions of 5-HT neurons will be presented. We conclude that subchronic treatment with 8-OH DPAT, a 5-HT 1A agonist, selectively affects the mesocortical DA and 5-HT systems and their response to stress without altering the mesoaccumbal systems. Support in part by MH-14092 & MH-14276.

563.2

ELECTROPHYSIOLOGICAL RESPONSES OF MESENCEPHALIC DOPAMINE NEURONS TO DORSAL RAPHE STIMULATION IN 5-HT-DEPLETED RATS. J. Gervais and C. Rouillard* Lab. of Neurobiology and Dept. of Pharmacology, Laval University, Québec, Canada G1J 1Z4.

Several lines of evidence indicate that 5-HT afferences from the dorsal raphe (DR) nucleus and 5-HT drugs can modulate the spontaneous activity of mesencephalic DA neurons. We have previously demonstrated that DR stimulation induced two different types of response in both SNpc and VTA. Some DA cells exhibited an inhibition-excitation sequence while in others DA neurons, the initial response was an excitation followed by an inhibition. In SNpc, 56% of the DA cells recorded were initially inhibited, 31% were initially excited and only 13% were not affected by DR stimulation. In contrast, 63% of the VTA DA cells recorded were initially excited, 34% were initially inhibited and 2% were not affected. However, the DR also contain a fair proportion of non-5-HT elements. Therefore, the main objective of the present study was to determine the specific contribution of 5-HT fibres to the effects recorded in the midbrain after electrical stimulation of the DR. To lesion the 5-HT system selectively, rats were injected i.c. with 5,7-DHT alone or in combination with a daily i.p. treatment with PCPA. At least 10 days were allowed for neuronal degeneration to occur before performing *in vivo* extracellular single unit recordings. in chloral hydrate -anesthetized rats. DA neurons in the SNpc and VTA were recorded using single barrel micropipettes and were identified by their location, waveform, firing rate and pattern. DR stimuli consisted of monophasic square wave pulses of 800 µA intensity and 350 µsec duration delivered at a rate of 0.5 Hz. Destruction of DR 5-HT neurons almost completely abolished the inhibition in both SNpc and VTA (0 and 6% respectively), without important change in the number of DA cells excited. Consequently, the proportion of DA neurons which were not affected by DR stimulation have increased after 5-HT depletion (60% in SNpc and 31% in VTA). These data strongly suggest that the 5-HT input from the DR nucleus is mainly inhibitory and modulate in a different manner the DA neurons of nigrostriatal and mesolimbic systems.

563.3

SEROTONIN-DOPAMINE INTERACTION IN THE RAT VENTRAL TEGMENTAL AREA: AN ELECTROPHYSIOLOGICAL STUDY IN VIVO. E. Esposito*S. Pagannone and S. Prisco. Istituto di Ricerche Farmacologiche "Mario Negri", Consorzio "Mario Negri" Sud, S. Maria Imbaro (Chieti), Italy.

In the present study the influence exerted by the serotoninergic system on the activity of dopamine (DA) neurons in the ventral tegmental area (VTA) was investigated. Extracellular single unit recordings were performed in vivo on male Sprague Dawley rats, anesthetized with chloral hydrate. Dopamine neurons in the VTA were recorded using single barrel micropipettes and were identified by their location, waveform, firing rate and pattern. Cumulative intravenous injections of the mixed 5-HT_{1B}/5-HT_{1C} receptor agonists *m*-trifluoromethylphenylpiperazine (TFMPP) (1.25-160 µg/kg) and *m*-chlorophenylpiperazine (mCPP) (1.25-320 µg/kg) caused a dose-dependent inhibition of the firing rate of VTA DA neurons. The effect of mCPP (maximal inhibition 40%) was more pronounced compared to that of TFMPP (maximal inhibition 25%). Administration of the selective 5-HT_{1B} receptor agonist 7-trifluoromethyl-4(4-methyl-1-piperazinyl)-pyrrolo[1,2-a]quinoxaline (CGS 12066B) (1.25-160 µg/kg, i.v.) did not cause any change in the basal firing rate of VTA DA neurons. Pretreatment with the specific 5-HT_{1B} receptor antagonist cyanopindolol (100 µg/kg, i.v.) did not modify the inhibitory effects of TFMPP and mCPP on DA neurons in the VTA. Cumulative intravenous injections of mesulergine (2.5-80 µg/kg) and mianserin (2.5-160 µg/kg), two selective 5-HT_{1C} receptor antagonists, produced a significant increase in the basal firing rate of VTA DA neurons. Administration of mesulergine (40 and 80 µg/kg, i.v.) dose-dependently antagonized the rate decreasing effect produced by mCPP on VTA DA neurons. Taken together, these data indicate that serotonin exerts an inhibitory action on DA neurons in the VTA through the 5-HT_{1C} receptor subtype.

563.4

ELECTROPHYSIOLOGICAL EFFECTS OF (-)HA-966: EVIDENCE FOR AN INTERACTION WITH $GABA_B$ RECEPTORS. P.D. Shepard', S.T. Connelly and K.C. Grobaski. MD Psychiatric Res. Cntr., Baltimore, MD 21228.

HA-966 (± 1-hydroxy-3-amino-pyrrolidinone-2) has been shown to produce marked changes in both the firing rate and discharge pattern of mesencephalic dopamine (DA)-containing neurons in vivo (Shepard and Lehmann, JPET 261:387, 1992). These effects do not appear to be mediated by the NMDA antagonist properties of (+)HA-966 but rather through a γHB-like effect of the (-)enantiomer (McMillen et al., J. Neural Trans., 89:11, 1992). In the present series of experiments, electrophysiological techniques were used to explore the pharmacological mechanisms underlying the effects of (-)HA-966 on the activity of mesencephalic DA-containing neurons in the rat.

As previously reported, systemic administration of (-)HA-966 produced a dose-dependent inhibition in neuronal firing rate in chloral hydrate (CH) anesthetized rats (ED_{50} ≃ 5.5 mg/kg). Although similar results were obtained in ketaminized animals, the potency of the drug was significantly reduced (ED_{50} ≃ 13 mg/kg). Parallel experiments conducted using an in vitro brain slice preparation confirmed the ability of (-)HA-966 to suppress neuronal activity (EC_{50} ≃ 500 µM), however, addition of low concentrations of CH (10 µM) to the bathing media failed to potentiate the inhibitory effects of the drug. Bath application of the selective $GABA_B$ antagonist CGP-35348 (100 µM), completely reversed the inhibitory effects of (-)HA-966 in vitro. CGP-35348 (200 - 500 mg/kg., i.v.) was also effective in antagonizing the inhibitory effects of (-)HA-966 in vivo, although neuronal firing rate failed to return to pre-drug levels. Taken together, these data suggest that the inhibitory effects of (-)HA-966 are mediated locally, possibly through an interaction with $GABA_B$ receptors on DA-containing neurons. (Supported by NARSAD and MH-48543).

563.5

PACEMAKER ACTIVITY OF NIGRAL DOPAMINE NEURONS; INVOLVEMENT OF $GABA_B$-RECEPTORS AND EFFECTS ON DOPAMINE RELEASE H. Nissbrandt*, T. Kling-Petersen, A. Elverfors and G. Engberg. Department of Pharmacology, University of Göteborg, Medicinaregatan 7, S-413 90 Göteborg, Sweden.

Previous electrophysiological experiments have emphasized the importance of the firing pattern for the functioning of midbrain dopamine (DA) neurons. In the present study, extracellular recording techniques were used to investigate the significance of $GABA_B$-receptor activation for the firing properties of DA neurons in the substantia nigra (SN) in the rat. Intravenous administration of the $GABA_B$-receptor agonist baclofen in low doses (1-16 mg/kg), insufficient to decrease firing rate, was associated with a dose-dependent regularisation of the firing pattern, concomitant with a reduction in burst firing. Also, systemic administration of relatively low doses of γ-hydroxybutyric acid (GHBA; 50-300 mg/kg, i.v.) induced a regularisation of the firing pattern. Both the regularisation of the firing pattern and inhibition of firing rate produced by these drugs were antagonized by the $GABA_B$-receptor antagonist CGP 35348 (200 mg/kg, i.v.).

By combining electrophysiological recording of dopaminergic neurons in the SN and microdialysis in the striatum in the same rat and simultaneously it was possible to directly compare the effect of pacemaker-like activity on the extracellular DA concentration in the striatum. Systemic administration of a low dose of GHBA (200 mg/kg, i.v.), which did not change the firing rate of the dopaminergic neurons, but induced a pacemaker-like activity, decreased the DA efflux in the striatum with 30%.

The present results indicate that $GABA_B$-receptors are involved in the modulatory control of firing pattern, that relatively low doses of GHBA induces pacemaker-like activity and that pharmacologically induced pacemaker-like activity reduces the DA efflux in the striatum.

563.6

SUBTHALAMIC NUCLEUS LESIONS: EFFECTS ON MK-801 STIMULATION OF NIGROSTRIATAL DA NEURONS. A.R. West, J. Zhang, M.D. Kelland*, and A.S. Freeman, Cellular and Clinical Neurobiology Program, Dept. of Psychiatry, Wayne State Univ. Sch. of Med., Detroit, MI 48201.

Noncompetitive NMDA antagonists (e.g., MK-801, PCP, TCP) stimulate the firing rate of rat nigrostriatal (NSDA) and mesoaccumbal dopamine (DA) neurons after i.v. administration. The site(s) of the glutamatergic synapses involved in this effect is not known. It *is* known that the excitation is indirect, perhaps secondary to inhibition of midbrain interneurons (EJP 230:371, 1993). Because the subthalamic nucleus (STN) provides a glutamatergic input to the midbrain, and affects nigral DA and non-DA cell activity (Smith & Grace, Synapse 12:287, 1993), we have begun to evaluate its role in the effects of MK-801 on DA neuronal activity. Rats received unilateral injections of ibotenic acid or saline into the STN nucleus. After 7-9 days, rats were anesthetized and electrophysiological recordings were made from identified NSDA neurons. The response of NSDA cells to MK-801 (0.05-3.2 mg/kg, i.v.) was not significantly altered in lesioned rats (N = 9) compared to controls (N = 8). It was oberved that nigral DA neurons were relatively scarce in the lesioned rats. A preliminary cells/track analysis revealed that the number of spontaneously active nigral DA neurons in lesioned rats (N = 4) was about half that in controls; average firing rates were not altered. These results suggest that glutamatergic afferents from the STN nucleus are not essential for MK-801-induced stimulation of NSDA neuronal firing. It is possible, however, that remaining STN cells contribute to the effects of MK-801 in lesioned rats. Because the effects on MADA neurons are greater than on NSDA neurons, we are testing the effects of STN lesions on MADA cell responses to MK-801. (Support: DA07844, MH42136).

563.7

ELECTROPHYSIOLOGICAL EFFECTS OF THE SELECTIVE 5-HT-1A AGONIST, 8-OH-DPAT, ON MIDBRAIN DA NEURONS: A9/A10 DIFFERENCES. J. Zhang* and A.S. Freeman, Cellular and Clinical Neurobiology Program, Dept. of Psychiatry, Wayne State Univ. Sch. of Med., Detroit, MI 48201.

The 5-HT-1A agonist 8-OH-DPAT is known to excite a subpopulation of nigrostriatal (A9) DA neurons (Kelland et al., JPET 253:803, 1990). In the present study, *in vivo* single-unit recording methods were used to compare the effects of 8-OH-DPAT on A9 and A10 DA neurons in anesthetized rats. 8-OH-DPAT (4-128 μg/kg, i.v.) stimulated the firing rates of 9 of 16 (56%) A9 DA neurons and 21 of 22 (95%) A10 DA neurons. The stimulatory effects were of greater magnitude on A10 DA neurons. 8-OH-DPAT (128 μg/kg) also significantly altered A10 DA neuronal firing pattern, as evidenced by increased burst-firing and reduced regularity of firing. Sensitivity of A9 and A10 DA cells to quinpirole-induced inhibition was not altered by 8-OH-DPAT. Acute midbrain/forebrain hemitransection did not alter 8-OH-DPAT-induced changes in DA neuronal firing rate or pattern. In contrast to the effects of i.v. 8-OH-DPAT, microiontophoretic administration did not increase DA neuronal firing rate. Microiontophoretic 8-OH-DPAT, however, selectively increased burst-firing of A10 DA cells, and reduced the regularity of A9 and A10 DA neuronal firing.

In summary, the excitatory effects of i.v. 8-OH-DPAT are more pronounced on A10 DA neurons compared to A9 DA neurons. As concluded previously for A9 DA cells (Kelland et al., 1990), the 8-OH-DPAT-induced increases in A10 DA cell firing rate appear to be indirect, perhaps via disinhibition secondary to inhibition of raphe 5-HT cells. The excitatory effects of 8-OH-DPAT on DA neurons do not appear dependent on actions at forebrain sites or DA autoreceptors. (Support: DA07844.)

563.8

ELECTROPHYSIOLOGICAL EFFECTS OF CCK PEPTIDES ON SUBSTANTIA NIGRA PARS RETICULATA NEURONS. A.S. Freeman* and J. Zhang, Cellular and Clinical Neurobiology Program, Dept. of Psychiatry, Wayne State Univ. Sch. of Med., Detroit, MI 48201.

The ventral tier of nigral (A9) DA neurons sends dendrites ventrally into the pars reticulata (SNr). The SNr contains interneurons and output neurons, and a regulatory role for dendritically released DA on SNr neurons is likely (Waszczak and Walters, Science 220:218, 1983). Because CCK-8S is contained in DA neurons and can be released dendritically, it too may influence SNr cell activity. In this study, the effects of i.v. CCK-8S, CCK-US and CCK-4 on the firing rate of antidromically identified nigrothalamic (NTh) SNr cells and footpinch-sensitive SNr cells (putative interneurons) were tested in anesthetized rats.

The CCK-A/CCK-B agonist CCK-8S (8 and 16 μg/kg, N = 25) produced short-lived (≈4 min) inhibitions of the activity of the majority of SNr neurons sampled. These effects were similar in time-course but opposite in amplitude to those it exerts on midbrain DA neurons. In contrast, the CCK-B receptor agonists, CCK-8US (8, 16 and 64 μg/kg, N = 19) and CCK-4 (8, 16, 64 μg/kg, N = 12), each produced slowly developing increases in the firing rate of SNr neurons. These results suggest that both CCK-A and CCK-B receptors influence SNr neuronal activity. The location of the receptors for these responses cannot be determined from the present experiments. Additional studies are required to assess possible effects of *endogenous* CCK-8S on SNr neuronal function. Almost all footpinch-excited neurons were identified as NTh cells. This clouds interpretation of the results on the strict basis of a sharp division between output cells and putative interneuons. This may be related to the fact that NTh cells have collateral branches in the substantia nigra. Support: MH42136.

563.9

VENTRAL TEGMENTAL AREA AND PREFRONTAL CORTEX UNIT FIRING AFTER ACUTE TREATMENT WITH GAMMA-HYDROXYBUTYRATE IN ANESTHETIZED RATS. P. Jelenic[1], C. Labrie[2] and R. Godbout[2]*. Départements de psychologie[1] et de psychiatrie[2], Université de Montréal, Montréal (Québec) Canada H4J 1C5

Gamma-hydroxybutyrate (GHB) is a naturally occurring fatty acid with peculiar hypnotic properties as it can readily induce REM sleep. It has been shown that GHB modifies dopamine (DA) synthesis while sparing 5-HT and noradrenaline. The medial prefrontal cortex (PFC) is densely innervated by DA afferents from the ventral tegmental area (VTA) and both structures contain GHB uptake and binding sites. To better understand the possible link between the effects of GHB on (REM) sleep and on DA neurotransmission, this study analyzed the consequences of acute administration of GHB on firing rates of VTA and PFC neurons in urethane-anesthetized rats.

Confirming previous studies, GHB (250 mg/kg, i.p.) inhibited reversibly VTA cell firing (latency to 50% inhibition: 19-28 min; maximal firing decrease: 100% of baseline; latency to 90% recovery of firing: 56-82 min). PFC firing was also reversibly inhibited by GHB (50-500 mg/kg) in 75% of the 28 neurons tested, in a dose-dependent manner (latency to 50% inhibition: 11-25 min; maximal firing decrease: 72-85% of baseline; latency to 90% recovery: 24-193 min).

Since DA normally inhibits PFC firing *in vivo*, PFC activity should have been increased after GHB. Various possibilities are presently considered to explain these results, including the involvement of non-DA mechanisms. We are also presently investigating possible targets of GHB-sensitive VTA cells, including structures involved in the triggering of REM sleep.

Supported by the "Fonds de la recherche en santé du Québec"

563.10

NEURONAL INHIBITION BY IONTOPHORETICALLY APPLIED DOPAMINE OR GABA IN THE MEDIAL SEPTAL/MEDIAN PREOPTIC AREA IN RATS. F.P. Martial, S.N. Thorntor* and S. Nicolaïdis . CNRS URA 637, Neurobiologie des Régulations, Collège de France, 11 pl. M. Berthelot, 75231 Paris Cedex 05, France.

The median septum/preoptic area has been implicated in hydromineral balance regulation. Our previous electrophysiologically results have shown that neurons in this area are influenced by the hormones of this regulation ie angiotensin II and aldosterone (Society for Neurosciences abstract 561.1, 1991). GABA and dopamine are also known to modulate neuronal activity in this area. Therefore, we have investigated the effects of iontophoretic application of GABA, dopamine, D1 and D2 agonists on neuronal activity in this region.

Twenty-one male Wistar rats were anaesthetised with urethane and a 7 barrelled microiontophoretic electrode attached to an extracellular recording electrode was then advanced through the cortex into the medial septum/median preoptic are where unit activity was recorded.

In this area 115 spontaneously active neurons were tested for their responses to dopamine. Of these neurons 72 (62.6 %) were inhibited, 2 (1.7 %) were activated and the rest were not affected. Of the dopamine responsive neurons 12 were tested with the D1 agonist SKF 38393 which inhibited them all. The D2 agonist LY 171555 was tested in 19 of which only 11 (57.9 %) were inhibited with no effect on the others. In the same area 53 spontaneously active neurons were tested with GABA of which 30 (56.9 %) were inhibited. On 11 GABA responsive cells aldosterone was also applied iontophoretically and 8 had their sensitivity to GABA modified (either decreased or increased). The inhibition by dopamine was immediate whereas that of GABA slower.

These results suggest that the well known inhibitory effect of GABA can be modulated by aldosterone in the septo-preoptic area. Dopamine may also play an important role in this area as a potent inhibitor of neuronal activity (mostly through D1 receptors). (Supported by MH 43787)

563.11

DOPAMINERGIC MODULATION OF THE INPUT/OUTPUT FUNCTION OF RAT PIRIFORM CORTEX PYRAMIDAL CELLS.
R.E. Bergman*, E. Barkai and M.E. Hasselmo, *Department of Psychology, Harvard University, Cambridge, MA 02138.*

Computational models of schizophrenia which focus on dopaminergic modulation in cortical structures (Cohen and Servan-Schreiber, *Psych. Rev.* 99: 45-77) use an inaccurate representation of the input/output function of cortical pyramidal cells, implementing dopaminergic modulation as a shift in slope of a sigmoid function which is stable over time. In contrast, the input/output function of real cortical pyramidal cells depends strongly on previous activity. To obtain an accurate representation of dopaminergic modulation in biophysical simulations, we tested the effect of dopamine on the full f/I curve of piriform cortex pyramidal cells (n = 10, mean Vm = -74.5 mV, mean spike height = 98.7 mV, mean threshold = -50.2 mV).

All pyramidal cells showed adaptation of the spiking respone to 1 sec. intracellular current injection at a range of amplitues, with increased interspike interval at later stages of current injection. Perfusion with dopamine (10μM, n=5) decreased this adaptation, increasing the frequency of action potential generation. This effect appears to be due to suppression of an afterhyperpolarization (AHP) current with a reversal potential between 80 to 85 mV. In the presence of 200μM cadmium, the influence of dopamine on firing frequency and total spikes generated was blocked (n=3). Dopamine did not change the shape of action potentials generated. This data suggests that the influence of dopamine on neuronal adaptation is mediated by modulation of a calcium-dependent potassium current. In slices perfused with 20μM carbachol, causing partial suppression of adaptation, dopamine was still capable of increasing the spiking response, suggesting these modulators act on different systems. Dopamine appeared to increase the amplitude of EPSPs generated by stimulation of both afferent (layer 1a) and intrinsic (layer 1b) fibers.

563.12

EFFECTS OF DOPAMINE AND cAMP AGONISTS ON $I_{Ca}2+$ AND $[Ca^{2+}]_i$ IN CATFISH ISOLATED CONE HORIZONTAL CELLS. F.Zinebi and B.N.Christensen*. Dept. of Physiology & Biophysics, University of Texas Medical Branch, Galveston, TX 77555-0641.

$[Ca^{2+}]_i$ and $I_{Ca}2+$ were measured simultaneously in fura-2 loaded cells using the whole cell patch-clamp technique. Dopamine (DA), the DA1 agonist (SKF-38393), forskolin (For) and 8-bromoadenosine 3',5'-cyclic monophosphate (8bcAMP)), were applied to the cell by pressure ejection or under concentration-clamp conditions in normal Ca^{2+} or in 15 mM barium. These cells are believed to express only the DA1 receptor. Our results indicate that: a) these drugs showed no significant effect on $[Ca^{2+}]_i$ when applied to the cell clamped at the resting membrane potential (-65 mV); b) when the $I_{Ca}2+$ is maximally activated, DA can produce a simultaneous decrease or increase in $I_{Ca}2+$ and $[Ca^{2+}]_i$ whereas those drugs that stimulate cAMP only decrease $I_{Ca}2+$ and $[Ca^{2+}]_i$. We conclude that the decrease in both $I_{Ca}2+$ and $[Ca^{2+}]_i$ is due to DA activation of cAMP. The DA-induced increase in $I_{Ca}2+$ and $[Ca^{2+}]_i$ suggests a second role for this neurotransmitter that may involve a different regulatory pathway. Supported by grant NEI-01897.

563.13

ELECTROPHYSIOLOGIC EFFECTS OF 7-OH-DPAT ON NIGROSTRIATAL *VS* MESOLIMBIC DOPAMINE SYSTEMS. J-C. Liu, R.F. Cox[1], G.J. Greif, J.E. Freedman and B.L. Waszczak*. Dept. Pharm. Sci., Northeastern Univ., Boston, MA 02115 and [1]CNS Sect., Burroughs Wellcome Co., Research Triangle Park, NC 27709.

Since D_3 receptor mRNA has been localized in predominantly limbic regions, it was of interest to determine if 7-hydroxy-*N,N*-di-*n*-propyl-2-aminotetralin (7-OH-DPAT), a putative D_3-selective agonist, exhibits different effects in nigrostriatal and mesolimbic dopamine systems. Extracellular single unit activities of substantia nigra pars compacta (SNc) and ventral tegmental area (VTA) dopamine (DA) neurons, and caudate-putamen (CPu) and nucleus accumbens (NAc) postsynaptic neurons, were recorded in male rats anesthetized with chloral hydrate. I.v. (+)7-OH-DPAT (RBI) potently and completely inhibited the firing of both SNc and VTA DA neurons (ED_{50}'s: 3.5 ± 0.7 μg/kg, SNc; 3.9 ± 0.9 μg/kg, VTA). The active enantiomer, (+)7-OH-DPAT (BW Co.), was 2-3-fold more potent than the racemic drug (ED_{50}'s: 1.2 ± 0.3 μg/kg, SNc; 1.7 ± 0.4 μg/kg, VTA). There were no significant differences in potency for either drug in inhibiting SNc and VTA neurons. Effects were completely reversed by either i.v. haloperidol (2 mg/kg) or AJ-76 (1-8 mg/kg; Upjohn). In other studies, iontophoretically applied (+)7-OH-DPAT (0.01 M, pH 4, 1-64 nA) caused current-dependent inhibitions of spontaneously active or glutamate-driven CPu and NAc neurons (I_{50}'s: 5.6 and 4.3 nA, respectively). Again, no difference in potency between CPu and NAc cells was noted. Finally, in cell-attached patch-clamp recordings from freshly dissociated rat CPu neurons, an 85 pS K^+ channel known to be activated by DA and the D_2-like agonist quinpirole was observed with (+)7-OH-DPAT (0.2-1 μM) applied in the patch pipette. Although D_3 receptors have been assumed to play an important role in limbic areas, these results suggest that 7-OH-DPAT behaves similarly to D_2 agonists and does not show significantly different effects on nigrostriatal and mesolimbic systems. Supported by NIH grants NS 23541 (BLW) and MH 48545 (JEF).

563.14

NEUROLEPTIC-INDUCED INCREASE IN BURST FIRING OF DOPAMINE CELLS IS ATTENUATED BY HEMISECTION OF THE STRIATONIGRAL PROJECTION. M. L. Pucak* and A. A. Grace. Departments of Behavioral Neuroscience & Psychiatry, University of Pittsburgh, Pittsburgh, PA 15260.

Dopamine (DA) neurons can exhibit a burst-firing pattern which is associated with enhanced release of DA. In order to investigate whether DA antagonists increase burst firing via blockade of DA receptors within the substantia nigra, we have examined whether the increased percentage of spikes fired in bursts elicited by the DA antagonists haloperidol (HAL; 4 mg/kg, i.v.) or sulpiride (SULP; 32 mg/kg, i.v) in anesthetized rats is attenuated by hemisection of the striatonigral projection. Hemisection did not significantly alter the effects of haloperidol on the percent of spikes fired in bursts (average difference: intact=-1.9±4.7; hemisected=1.2±2.1); however, sulpiride was significantly less effective at increasing burst firing in hemisected rats (intact=4.2±1.6; hemisected=-0.5±1.9; $p<.05$). In addition, a smaller number of neurons exhibited significant increases in burst firing in response to either drug (haloperidol: intact=2/12, hemisected=0/12; sulpiride: intact=4/19, hemisected=1/17). Haloperidol also caused decreases in the percent of spikes fired in bursts in some DA cells (2/12), and this effect was not abolished by hemisection (2/12). Sulpiride decreased burst firing only after hemisection (1/17). These results suggest that the ability of DA antagonists to increase burst firing may be partially mediated indirectly via blockade of DA receptors located in forebrain regions, whereas their ability to decrease burst firing may be mediated locally within the substantia nigra, perhaps by blockade of DA receptors located on descending terminals. Therefore, although we have shown previously that hemisection does not alter the effects of haloperidol and sulpiride on DA neuron firing rate, removing descending feedback afferents does alter the effects of DA antagonists on DA neuron firing pattern.
Supported by MH09873 (MLP), NS19608, MH42217, and MH45156 (AAG).

563.15

ALPHA-ADRENERGIC DEPOLARIZATION OF RAT MIDBRAIN DOPAMINE NEURONS J. Grenhoff*, S.W. Johnson and R.A. North. Vollum Institute, Oregon Health Sciences University, Portland, OR 97201

Interactions between brain noradrenaline and dopamine (DA) systems have been described with a variety of methods, but the cellular mechanisms involved have not been characterized. We used intracellular recording with microelectrodes from rat midbrain slices to study the adrenergic pharmacology of DA neurons in the substantia nigra and ventral tegmental area. The α_1-adrenoceptor agonist phenylephrine (PE; 1-100 μM) produced depolarization and excitation in 67 % of principal (DA) neurons. The depolarization was accompanied by an increase in input resistance and persisted in tetrodotoxin (0.5 μM). In voltage clamp experiments, PE induced an inward current. Neither the α_2 agonist UK 14304 (1 μM) nor the ß agonist isoproterenol (10 μM) mimicked the effect of PE. The action of PE was antagonized by the α_1 antagonist prazosin (30 nM-1 μM). These results indicate the presence of excitatory α_1-adrenoceptors on midbrain DA neurons.

563.16

α_1-ADRENOCEPTOR ANTAGONISM MODULATES THE CHANGES IN FIRING PATTERN AND TRANSMITTER RELEASE INDUCED BY A SELECTIVE DOPAMINE (DA)-D_2-ANTAGONIST IN THE MESOLIMBIC, BUT NOT IN THE NIGROSTRIATAL DA SYSTEM. J.L. Andersson*, M. Marcus and T.H. Svensson. Karolinska Institute, Dept. Pharmacology, Box 60400, S-10401 Stockholm, Sweden.

Most antipsychotic drugs are, in addition to being DA-D_2-antagonists, also relatively potent α_1-adrenoceptor antagonists. Here, we have studied the effects on midbrain DA neurons of the selective DA-D_2-antagonist raclopride, alone and in combination with the selective α_1-adrenoceptor antagonist, prazosin, utilizing extracellular single cell recording techniques. In addition, in vivo voltammetry was used to measure extracellular DA concentrations in the nucleus accumbens (NAC) and the dorsolateral striatum (STRI) in anesthetized rats during these drug treatments. Raclopride induced a dose dependent increase in firing rate in ventral tegmental area (VTA) DA neurons, significant already at 10 μg/kg, and in zona compacta, substantia nigra (ZC-SN), DA neurons, not significant until 2560μg/kg. Burst firing was also increased in VTA DA neurons, at 40μg/kg, as well as in ZC-SN DA neurons, at 640μg/kg. A high dose of raclopride (2560μg/kg, cumulated dose) induced a 900% increase in extracellular DA concentrations in NAC, but only a 300% increase in STRI, probably secondary to increased neuronal activity. Thus, our electrophysiological results and voltammetry data demonstrate some limbic selectivity for the selective DA-D_2-antagonist. Prazosin pretreatment (0.3mg/kg, i.v. 15 min before) abolished the raclopride induced increase in burst firing in VTA DA neurons, while effects on ZC-SN DA neurons were unaffected. The prazosin pretreatment also caused a reduction in raclopride induced elevation of extracellular DA-concentrations in NAC, but not in STRI.
Thus, α_1-adrenoceptors seem to modulate the effect of DA-D_2-antagonism preferentially in the mesolimbic, but not in the nigrostriatal DA-system.

563.17

FACILITATION OF DENTATE GYRUS FIELD RESPONSES BY VTA STIMULATION IS ATTENUATED BY DOPAMINE ANTAGONISTS Henriksen, S.J.*, Steffensen, S.C. and Criado, J.R., Scripps Research Institute, La Jolla, CA 92037

The dentate gyrus is the first processing stage in the hippocampal trisynaptic circuit and occupies a strategic location for subcortical biasing of neocortex-hippocampus-neocortex information throughput. It is heavily innervated by cholinergic and GABAergic fibers from the medial septum (MS) and to a lesser extent by afferents from the supramammillary nucleus, noradrenergic fibers from the locus coeruleus, serotonergic fibers from the dorsal/median raphe and dopaminergic fibers from the ventral tegmental area (VTA). The MS, however, receives a dense dopaminergic innervation. We sought to determine the extent of the physiological dopaminergic input to the dentate gyrus. Stimulation of the VTA in anesthetized rats elicited a small field potential (latency=15ms) recorded in the hilar region of the dentate that produced interval- and activity-dependent facilitation (140%) of perforant path to dentate population spike (PS) amplitudes, but had no effect on PS latency or field EPSPs. Microelectrophoretic application of either the α2-antagonist, yohimbine, the β-antagonist, propranolol, the 5-HT1A antagonist, NAN 190, the 5-HT2 antagonist, ketanserine, the GABAa antagonist, bicuculline, the GABAb antagonist, saclofen, or muscarinic antagonists, atropine or scopolamine, into the dentate hilus had no effect on VTA facilitation. The ICV administration of the serotonin and noradrenaline neurotoxin, 5,7 dihydroxytryptophan, or the in situ MS injection of the neurotoxin, ibotenic acid, ten days prior to recording had no effect on VTA facilitation. Microelectrophoretic application of the D2 antagonist, eticlopride, but not the D1 antagonist, SCH23390, markedly decreased VTA facilitation (80%). Independent of VTA stimulation, eticlopride decreased PS amplitudes and increased recurrent inhibition, while SCH23390 increased PS amplitudes and decreased recurrent inhibition. These results suggest that there is a direct and important dopaminergic input from the midbrain that modulates dentate excitability. This work was supported by DHHS # P50-AA06420 to SJH.

563.18

INTRACEREBROVENTRICULAR (ICV) NOREPINEPHRINE, MICRODIALYSIS AND PERFORANT PATH-EVOKED POPULATION SPIKE POTENTIATION IN THE RAT DENTATE GYRUS. C.W.Harley*, M.D.Lalies and D.J.Nutt. Psychopharmacology Unit, School of Medical Sciences, Bristol University, Bristol, UK BS8 1TD

Norepinephrine (NE) exogenously applied to the hippocampus in vivo and in vitro potentiates the perforant path-evoked population spike in dentate gyrus. In vitro 10 μM NE is a threshold concentration for this effect (Lacaille, JC & Harley, CW (1985) Brain Res. 358: 210-220).

In the present study microdialysis probes and recording electrodes were implanted in dorsal dentate gyrus of urethane-anesthetized rats to monitor NE levels and perforant path-evoked potentials concurrently. NE, 5 μg in 2 μl artificial cerebrospinal fluid, which we previously found to be near threshold for icv NE-induced potentiation, was injected following a minimum of 60 minutes of baseline monitoring.

Exogenous levels of NE after the icv injection reached 30-700X basal NE levels in the first 15 minute sample. The increase in NE was accompanied by a step-wise potentiation of the population spike which was typically sustained during the postinjection period. NE levels declined steadily from the initial peak, falling by ca 50% in each 15 min sample after the first 30 minutes and returning to basal levels within two hours. Injections entering the medial, but not lateral, ventricles were ineffective in changing the population spike.

These data suggest effective exogenous concentrations for NE-induced potentiation in vivo are in the range of .3-1 μM. We hypothesize that similar concentrations might occur synaptically to produce the potentiation effect.

563.19

Characterization of a noradrenergic excitatory effect on cholinergic neurones of the nucleus basalis in guinea-pig brain slices. P. Fort, A. Khateb, A. Pegna, M. Mühlethaler and B.E. Jones*, Dept. of Physiology, CMU, 1211 Geneva 4, Switzerland and *Montreal Neurological Inst., McGill University, Montreal, Canada H3A 2B4.

Cholinergic neurones of the nucleus basalis represent a major relay of the ascending reticular activating system, receiving inputs from the brainstem and projecting in turn upon the cerebral cortex. One of the important brainstem afferent inputs to the cholinergic cells derives from the noradrenergic locus coeruleus neurons. The potential influence of the noradrenergic input was examined by bath application of drugs onto identified cholinergic basalis neurons recorded by intracellular technique in guinea-pig brain slices. Noradrenaline (NA) consistently produced a strong membrane depolarization that was accompanied by an increase in membrane resistance and a marked increase in firing rate. The effects were mimicked by L-phenylephrine, an alpha-1 receptor agonist, and blocked by prasozin, a selective alpha-1 antagonist, whereas no effects were produced by clonidine, the alpha-2 agonist. Isoproterenol, a beta agonist, also produced an effect upon the membrane, although weaker than that of NA or L-phenylephrine. Thus by potential action upon both alpha-1 and beta receptors, noradrenaline produces a depolarization of the cholinergic basalis neurons, which would tend to promote and accelerate tonic firing in these cells. (Swiss NSF, FFLB and Canadian MRC).

563.20

CONTROL OF CALCIUM CURRENT IN RAT TRIGEMINAL NEURON BY ADRENERGIC AND MUSCARINIC RECEPTOR AGONISTS. Seung-Yeol Nah* and Edwin W. McCleskey Dept. of Cell Biology and Physiology, Washington University School of Medicine, St. Louis, Missouri 63110, U. S. A.

The effect of adrenergic and muscarinic receptors agonists on Ca current of adult rat trigeminal ganglion neurons was investigated using whole-cell patch-clamp methods. The application of acethylcholine, carbachol, or oxotremorine (50 μM each) produced rapid, reversible reduction of the Ca current by 17 ± 6 %, 19 ± 3 %, and 18 ± 4 %, respectively. Atropine, a muscarinic antagonist, blocked carbachol-induced Ca current inhibition to 3 ± 1 %. Norepinephrine (50 μM) reduced Ca current by 18 ± 2 %, while clonidine (50 μM), an α_2-adrenergic agonist, inhibited Ca current by only 4 ± 1 %. Yohimbine, an α_2-adrenergic antagonist, did not block the inhibitory effect of norepinephrine on Ca current, whereas prazosin, an α_1-adrenergic antagonist, attenuated the inhibitory effect of norepinephrine on Ca current to 6 ± 1 %. This pharmacology contrasts with α-adrenergic modulation of Ca channels in sympathetic neurons, which is sensitive to clonidine but not blocked by yohimbine. Our data are consistent with modulation via an α_1-adrenergic receptor, but we cannot exclude the possibility of a novel α-adrenergic receptor. Treatment with pertussis toxin (250 ng/ml) for 16 hours greatly reduced norepinephrine- and carbachol-induced Ca current inhibition to 2 ± 1 % and 2 ± 1 %, respectively. These results demonstrate that norepinephrine, through an α-adrenergic receptor, and carbachol, through a muscarinic receptor, inhibit a Ca current in adult rat trigeminal ganglion neurons via pertussis toxin sensitive GTP-binding proteins.

SEROTONIN RECEPTORS: ONTOGENY AND REGULATION

564.1

TIME COURSE OF THE DOWNREGULATION OF SOMATODENDRITIC 5-HT1A AUTORECEPTORS PRODUCED BY IPSAPIRONE TREATMENT. K. McMonagle-Strucko* and R.J. Fanelli. Inst. for Dementia Research, Miles Inc., West Haven, CT 06516.

Previous work found that rats treated twice daily with ipsapirone (10 mg/kg IP) for 3 weeks resulted in a large decrease in 5-HT_{1A} binding in the dorsal raphe nucleus. Additional experiments were performed to determine the time course for the development and duration of the effects of ipsapirone treatment on 5-HT_{1A} receptors. In an initial study, rats were treated twice daily with ipsapirone (10 mg/kg IP) for 1 day, 1 wk, or 2 wks and sacrificed 24 hrs after the final injection. In a second study, rats were treated for 3 wks and sacrificed 1, 48, 72 hrs or 7 days following the final treatment. Quantitative analyses were done of autoradiograms of in vitro [^{3}H]8-OH-DPAT binding. The first study showed that the decrease in binding in the dorsal raphe nucleus was not apparent at times measured prior to 3 wks of treatment. In the second study, where rats were treated with ipsapirone for 3 wks and sacrificed at varying times following the final injection, large declines in 5-HT_{1A} were found in the dorsal raphe nucleus 1 and 48 hrs following treatment. When brains were obtained 72 hrs following ipsapirone treatment, 5-HT_{1A} binding was back to control levels.

564.2

CHANGES IN SEROTONIN RECEPTOR SUBTYPES INDUCED BY SHORT- AND LONG-TERM ANTIDEPRESSANT TREATMENT. M. Frankfurt*, C.R. McKittrick and B.S. McEwen. Laboratory of Neuroendocrinology, The Rockefeller University, New York, NY 10021.

Fluoxetine, a 5HT uptake inhibitor, and tianeptine, a 5HT uptake enhancer, are both clinically active antidepressants. We compared the effects of these drugs on 5HT receptor binding, following both short- and long-term administration. Fluoxetine or tianeptine (10mg/kg) or saline was administered i.p. to male rats daily for 4 or 21 days. Animals were killed 48 h after the last injection. Brains were frozen and sliced into 16 μm sections; adjacent sections were processed for quantitative autoradiography of various 5HT receptor subtypes. Receptor binding in 11 regions was analyzed using computer-assisted densitometry. Tianeptine treatment for 21 d, but not 4 d, significantly increases 5HT1B receptor binding in the dorsomedial nucleus of the hypothalamus (↑12.2%) and the sensory region of the parietal cortex (↑14.3%). Fluoxetine has no effect at either time point. Fluoxetine, but not tianeptine, for 21 d significantly decreases binding of paroxetine, which labels 5HT uptake sites, in CA3 of hippocampus (↓18.7%) and dentate gyrus (↓19.1%). Neither drug affects 5HT1A receptor binding after 4 d treatment. 5HT1A binding following 21 d and paroxetine binding following 4 d treatment are currently being analyzed. The changes described above suggest that alterations in 5HT receptors are involved in the mechanism of action of these antidepressants. Further investigation is required to determine how the apparently opposing effects of these drugs converge to produce an antidepressant effect.

[Supported by GMO754 (CRM) and MH41286 (BMc)]

564.3

INCREASED HIPPOCAMPAL SPROUTING OF 5,7-DHT LESIONED SEROTONERGIC NEURONS AFTER DELAYED TREATMENT WITH THE 5-HT_{1A} AGONIST TANDOSPIRONE. A. Shemer*, J. Bell, E. Azmitia and P.M. Whitaker-Azmitia. Pfizer, Inc., New York, New York 10017, Dept. of Biology, New York University, New York, NY, 10003 and Dept. of Psychiatry, SUNY, Stony Brook, New York, 11794.

Selective 5-HT_{1a} receptor agonists can release the growth factor S-100ß from immature astroglial cells. This growth factor promotes terminal growth of serotonergic neurons during critical stages of development. Adult serotonergic neurons are also capable of terminal re-growth after 5, 7 DHT lesions - a process which takes approximately six weeks. The current study was undertaken to determine if a 5-HT_{1a} agonist could accelerate the re-growth of a lesioned adult brain, presumably through the release of S-100ß.

Adult male Sprague-Dawley rats were pretreated with desipramine and lesioned by 5,7 DHT (4μg/4μl) injection unilaterally into the cingulum bundle and into the fornix-fimbria. Animals were treated for 12 days postlesion with saline or 10 mg/kg tandospirone or with saline for 9 days followed by 3 days tandospirone. On day 12 postlesion, animals were perfused and immunochemically stained for S-100ß, 5-HT_{1a} receptor and serotonin. All of the lesioned animals showed increased S-100ß and 5-HT_{1a} staining above unlesioned. However, only the lesioned animals treated with saline for nine days and three days with tandospirone showed a recovery of serotonin immunoreactivity.

564.4

EXPRESSION CHANGE OF 5-HT_{1A} mRNA IN THE HIPPOCAMPUS OF THE LONG-TERM ADRENALECTOMIZED RATS. B. Liao*, B. Miesak and E.C. Azmitia. Dept. of Biology, New York Uni., New York, N.Y. 10003

Age-matched female Sprague-Dawley rats weighing about 150 g were adrenalectomized (ADX) and maintained with free access to saline for two months. After two months, part of the ADX rats were given dexamethasone (dex) (10 ug/ml saline) 24 or 72 hr before perfusion. In situ hybridization histochemistry showed a dramatic decrease of 5-HT_{1A} mRNA in the dentate gyrus (DG) of the hippocampus in the ADX rats but no apparent change in the CA1 to CA4 regions compared to the sham ADX rats. The ADX rats given dex showed a progressive recovery of 5-HT_{1A} mRNA labelling in DG after exposure to dex for 24 and 72 hr. There was a corresponding decrease of Nissl stained cells in the granular cell layer of the DG in the ADX rats. Both the superior and inferior blades of the DG became shorter and thinner. Dex treatment reduced a recovery of Nissl staining, with a denser staining in the inferior blade than in the superior blade.

It has been suggested by Sloviter et al. that circulating adrenal steroids are essential for maintaining the structural integrity of DG granule cells in the hippocampus. We have found that the 5-HT_{1A} receptors in the hippocampus are linked to the production of S-100b, which is a neurotrophic factor and a glial mitogen that serves important roles in CNS development and maintenance. The present study shows that the expression of 5-HT_{1A} mRNA as well as Nissl stained cells in the DG were reduced after long-term ADX rats and progressively recovered after exposed to dex. Therefore, we speculate that the manipulation of 5-HT_{1A} receptors by adrenal steroids may underlie the plasticity of the granular neurons in the long-term ADX rats. Supported by NSF 88-12892.

564.5

DEXAMETHASONE INCREASES 5-HT$_{1A}$ RECEPTOR, VIMENTIN, p53, c-FOS AND CALBINDIN IMMUNOREACTIVITY IN HIPPOCAMPUS AND CORTEX OF THE LONG-TERM ADRENALECTOMIZED ADULT RAT.

E.C. Azmitia[1*], J. Bell III[1], B. Liao[1] and P.M. Whitaker-Azmitia[2], [1]Lab. of Mol. Neuroplasticity, Dept. Biol., New York Univ., NY, NY 10003; [2]Dept. Psychiat., SUNY, Stony Brook, NY 11794

Long-term adrenalectomy (LT ADX) leads to a loss in the number of neurons in the dentate gyrus and we have shown corresponding decreases in 5-HT$_{1A}$ receptor mRNA in situ hybridization in the dentate gyrus (DG). Treatment with dexamethasone (dex) for 24-72 hours in the long-term ADX rats produces a recovery of 5-HT$_{1A}$ receptor mRNA labeling and the reappearance of Nissl-positive cells in the granular layer. In this study, a variety of molecules associated with cell differentiation were tested in LT ADX with or without dex. for 72 hr.

Adult SD female rats (175-200 g) were bilaterally adx and kept on saline (Sal) for 2 months before being further divided into a saline group or a group given dex (10 µg/ml) in their saline drinking water for 72 hr. before death. A sham adx group was also studied. The animals were perfused transcardially with 4% paraformaldehyde, 0.1% $MgCl_2$ in 0.1M phosphate-buffered saline. The brains were removed and processed with antibodies raised against 5-HT$_{1A}$ receptor, vimentin, c-fos, p53, S-100ß, and calbindin. Sections were counter-stained with methyl green.

Astrocytic labeling in the hippocampus (dentate gyrus, cornu Ammonis (CA), subiculum, indusium griseum and fasciola cinerea) and cortex (temporal and parietal) were increased for 5-HT$_{1A}$ receptors, vimentin, p53 and c-fos in LT ADX given dex compared to both sham ADX and LT ADX. S-100ß labeling showed no apparent change in these cells. Calbindin stained neurons were decreased in LT ADX especially in the pyramidal dendrites of CA$_{1-2}$ and the molecular layer of the superior blade of DG. These changes were reversed by dex. A number of pyramidal neurons in subiculum, fasciola cinerea and CA$_{1-4}$ were both c-fos and p-53 positive in LT ADX and their number was increased after dex. These results are consistent with glucocorticoid regulation of glial and neuronal differentiation in the adult brain.

(Work supported by NIA Program Project AG10206)

564.6

MINERALO- AND GLUCOCORTICOID RECEPTORS SYNERGISTICALLY MEDIATE THE ACTION OF CORTICOSTERONE ON 5-HT1A mRNA EXPRESSION IN DENTATE GYRUS.

O.C. Meijer and E.R. de Kloet* Div. of Medical Pharmacology, Leiden/Amsterdam Center for Drug Research, University of Leiden, P.O. Box 9503, 2300 RA Leiden, The Netherlands.

Corticosterone (B) is well established as a regulator of several aspects of the serotonergic raphe-hippocampal system. The effect of different plasma concentrations of B on 5-HT$_{1A}$ receptor mRNA expression in the hippocampus and the dorsal raphe nucleus (DRN) was studied, in order to differentiate between the actions mediated via the high affinity mineralocorticoid receptor (MR) and via the lower affinity glucocorticoid receptor (GR).

Male Wistar rats were adrenalectomized (ADX) and in two groups of animals either 20% (Low B) or 100% B (High B) pellets were implanted subcutaneously. This led to plasma B levels at which either predominantly the MRs or both MRs and GRs were occupied, respectively. One week post surgery, significant effects were measured only in the granule cell layer of the dentate gyrus, where a negative correlation between plasma B levels and 5-HT$_{1A}$ receptor mRNA amounts was seen. ADX led to an 38% increase in expression, Low B replacement to normalization towards levels of sham-operated animals and High B to a pronounced decrease in expression of the message. A trend towards increased expression after ADX was observed for the CA3 pyramidal cells, whereas the neurons in the CA1 cell field and the DRN remained unresponsive.

These results suggest that endogenous B, via MR activation, suppresses 5-HT$_{1A}$ receptor mRNA expresssion in the dentate gyrus, and that elevations in plasma B can further down-regulate the 5-HT$_{1A}$ receptor mRNA via synergistic action of activated MRs and GRs.

564.7

EFFECTS OF DEXAMETHASONE ADMINISTRATION ON HIPPOCAMPAL 5-HT1A RECEPTOR GENE EXPRESSION

D.T.Chalmers*, J. Lopez, D. Vazquez and S.J.Watson Mental Health Research Institute, University of Michigan, Ann Arbor, Michigan, 48109-0720.

We have previously reported that hippocampal 5-HT$_{1A}$ receptor gene expression is increased in response to adrenalectomy (ADX) and is responsive to low dose corticosterone administration, implicating MR sensitivity. In the present study, we have employed dexamethasone (DEX), a selective GR agonist, to investigate the effects of GR occupation on 5-HT$_{1A}$ mRNA expression.

Rats were ADX (12) or SHAM treated (11). Five ADX animals received daily injections of DEX (50ug, i.p.) for 1 week, remaining ADX animals received saline. SHAM animals received injections of saline (6) or DEX (5). After 1 week ADX, 5-HT$_{1A}$ receptor mRNA expression was significantly increased in all hippocampal subfields. ANOVA analysis indicated significant effects of treatment in all subfields ($p<0.01$). In the same animals, 5-HT$_{1A}$ binding exhibited parallel, though proportionally smaller, increases in all hippocampal subfields post-ADX. Administration of DEX at the time of ADX significantly attenuated increases in 5-HT$_{1A}$ mRNA expression in all subfields ($p<0.05$, Fisher Test), although 5-HT$_{1A}$ mRNA levels remained significantly higher than SHAM levels in all subfields with the exception of CA1. However, unlike 5-HT$_{1A}$ mRNA expression, 5-HT$_{1A}$ binding levels were responsive to DEX administration only within selective hippocampal subfields, CA1 and dentate gyrus. We conclude that GR occupation negatively regulates 5-HT$_{1A}$ receptor mRNA expression within the hippocampus and that 5-HT$_{1A}$ receptor sites are more sensitive to modulation in those hippocampal subfields expressing the highest levels of GR receptors, CA1 and dentate gyrus.

564.8

EFFECT OF ADRENALECTOMY ON 5HT RECEPTOR SUBTYPE mRNA EXPRESSION IN THE RAT HIPPOCAMPUS AND RAPHE.

M.C. Holmes*, K.L. French, J.L.W. Yau and J.R. Seckl. Dept Med, Western Gen Hosp, Edinburgh Univ, Scotland.

Glucocorticoids alter serotonergic (5HT) activity in the raphe-hippocampal (HC) system, thereby affecting mood. Following adrenalectomy (ADX) there is a rise in corticosteroid receptors and increased binding of ^{3}H-5HT in HC subregions. We have investigated the effect of ADX on mRNA expression encoding 5HT1A, 5HT1C and 5HT2 receptors. In the anterior HC, 5HT1A receptor mRNA is highly and evenly distributed throughout the dentate gyrus and CA1-4 pyramidal cells, whereas 5HT2 receptor mRNA is expressed only in the hilus, stratum oriens of CA1 and stratum pyramidale of CA3-4. 5HT1C receptor mRNA expression is restricted to a few cells of the strata radiatum and oriens of CA3. None of the 5HT receptor subtype mRNA expression is altered by ADX at 6h, 2 or 14 days. In the posterior and ventral HC, 5HT1C and 5HT2 (but not 5HT1A) receptor gene expression was more highly expressed and widely distributed than in anterior HC. 2 d after ADX, 5HT1C receptor mRNA expression increased in the stratum pyramidale of CA3 (50±15% rise, $p<0.01$) and stratum oriens of CA1 (22±7%, $p=0.05$). Both dexamethasone and aldosterone replacement abolished the effect of ADX in CA3, only dexamethasone was effective in CA1. ADX did not alter 5HT1A or 5HT2 receptor gene expression in the posterior HC, nor 5HT1A (autoreceptor) mRNA expression in the dorsal raphe nucleus. Increased sensitivity to 5HT1C-mediated effects is seen in some models of depression; the glucocorticoid control of 5HT1C mRNA expression in the posterior HC may provide the basis for corticosteroid-5HT interactions in affective disorders.

564.9

REGULATION OF THE 5-HT$_{1A}$ RECEPTOR IN P11 CELLS EXPRESSING BOTH 5-HT$_{1A}$ AND 5-HT$_2$ RECEPTORS.

J.G. Hensler*, H.A. Miller and P.R. Molinoff. Depts. Psychiatry and Pharmacology, U. of Pennsylvania School of Medicine, Dept. of Veterans Affairs Medical Center, Phila., PA. 19104-6084.

P11 cells, which express 5-HT$_2$ receptors coupled to phosphoinositide (PI) hydrolysis (Ivins *et al.*, Mol. Pharm. **37**:622, 1990), were transfected with the gene for the human 5-HT$_{1A}$ receptor. Expression of the 5-HT$_{1A}$ receptor in P11 cells was verified by measuring the specific binding of ^{3}H-DPAT (K_d = 0.7-1.2 nM, B_{max} = 100-500 fmol/mg protein). In P11 cells expressing the 5-HT$_{1A}$ receptor, DPAT did not stimulate PI hydrolysis and 5-HT$_2$ receptor-mediated stimulation of PI hydrolysis was not modified. DPAT inhibited forskolin-stimulated cAMP accumulation in a concentration-dependent manner (EC_{50} = 1-3 nM). Maximal inhibition by DPAT (1 µM) was approximately 70-80%. Incubation of P11 cells with the 5-HT$_1$ receptor agonist 5-carboxamidotryptamine (5-CT; 100 nM) for 18 hr resulted in a marked enhancement of forskolin-stimulated cAMP formation and a significant increase in the EC_{50} value of DPAT to inhibit cAMP formation, with no change in the degree of maximum inhibition. The change in the potency of DPAT to inhibit forskolin-stimulated cAMP formation was not accompanied by changes in 5-HT$_{1A}$ receptor affinity or number. Exposure of cells to 5-HT (10 µM) for periods of time (15 min, 30 min, or 1 hr) sufficient to maximally stimulate PI hydrolysis through 5-HT$_2$ receptor activation, did not alter the potency or efficacy of DPAT to inhibit forskolin-stimulated cAMP formation. Treatment of P11 cells with the phorbol ester PMA (1 µM, 15 min) resulted in a significant decrease in the maximum inhibition of cAMP formation by DPAT (Control: Emax = 70 ± 7%; PMA pretreated: Emax = 34 ± 3 %; n=4). However, this desensitization was not accompanied by changes in 5-HT$_{1A}$ receptor affinity or number. These data suggest that although activation of protein kinase C by phorbol ester results in the desensitization of the 5-HT$_{1A}$ receptor expressed in P11 cells, stimulation of 5-HT$_2$ receptor-mediated PI hydrolysis does not. (Supported by USPHS grant MH 48125 and Dept. of Veterans Affairs).

564.10

REGULATION OF SEROTONIN RECEPTORS AND S-100ß IN PRIMARY CULTURES OF RAT CORTICAL ASTROCYTES.

R. P. Hellendall*, J. P. Liu, M. B. Wilkie, and J. M. Lauder. Dept. of Cell. Biol. and Anat., Univ. of N. Carolina, Chapel Hill, NC 27599.

Previous studies have indicated the presence of functional serotonin (5-HT) receptors in rat astrocytes. Activation by serotonergic ligands can induce the release of S-100ß, a calcium-binding protein, which may, in turn, serve as a trophic agent for serotonergic neurons (Whitaker-Azmitia *et al.*,'90; Liu and Lauder, '92). The current studies examined the potential role of serum factors, which include 5-HT, on the expression of astrocytic 5-HT receptors and how exposure to 5-HT receptor ligands may be correlated with intracellular levels of S-100ß . Purified cortical astrocytes were plated on glass coverslips. To control for high levels of 5-HT present in serum, cultures were switched to either defined medium (N2) or fresh BME/10% fetal calf serum (BME/FCS) at 1 DIV and grown for an additional 1, 2, or 4 DIV. Serotonergic ligands were applied at 2 DIV and maintained for 6, 24, or 72 hrs. In cultures grown in serum, 5-HT$_{1A}$ receptor protein/mg total protein was highest from 1-3 DIV and then significantly decreased by 5 DIV. This pattern was also found for S-100ß protein. When maintained in N2, elevated levels of receptor protein were found at 5 DIV whereas S-100ß protein remained unchanged. We were unable to detect 5-HT$_{1A}$ receptor transcripts under any culture paradigm. Treatment of cultures with 10nM 8-OH-DPAT (a 5-HT$_{1A}$ agonist) or DOI (a 5-HT$_{1C/2}$ agonist) down-regulated S-100ß protein following a minimum of 24 hours exposure while S-100ß mRNA was reduced following 72hr DPAT treatment. DPAT also significantly affected cAMP levels at each exposure period. The 5-HT antagonists NAN190 or mianserin produced elevated levels of S-100ß protein after 24 hrs. These data indicate that factors present in serum can downregulate astrocyte 5-HT$_{1A}$ receptor expression. Receptor activation may be associated with the modulation of expression of S-100ß, suggesting an astrocyte mediated mechanism regulating the viability of serotonergic neurons.

564.11

MODULATION OF CENTRAL 5-HT_{1A} AND 5-HT_2 RECEPTOR-MEDIATED FUNCTIONAL RESPONSES FOLLOWING 21 DAY TREATMENT WITH 8-OH-DPAT, AMITRIPTYLINE, DESIPRAMINE AND FLUOXETINE. Lucy Rényi* and Patricia Jimenez.
CNS Preclinical R&D, Department of Behavioural Pharmacology, Astra Arcus AB, S-151 85 Södertälje, Sweden.

We compared the modulation of some behavioural responses following 21 day treatment with the 5-HT_{1A} receptor agonist 8-OH-DPAT 2x0.1 mg/kg s.c., amitriptyline (AM) 2x30 mg/kg p.o., desipramine (DMI) 2x10 mg/kg p.o. and fluoxetine (FL) 2x2.5 mg/kg p.o. The following behavioural responses were evaluated: 1. the 5-HT_{1A} receptor-mediated inhibition of the cage-leaving response, elongated body posture, forepaw-treading. 2. the 5-HT_2 receptor-mediated wet-dog shakes (WDS) induced by DOI (1-(2,5-dimethoxy-4-iodo-phenyl)2-amino-propane). 3. salivation (SAL) and male to male mounting (M-to-MM) with penile erection (PE) after a challenge dose of 8-OH-DPAT. All the four compounds produced tolerance to the 5-HT_{1A} receptor-mediated responses. To examine the degree of tolerance, various challenge doses of 8-OH-DPAT were used (0.1-3.0 mg/kg s.c.). The rank order was as follows: AM >FL >8-OH-DPAT >DMI. The 21 day treatment with 8-OH-DPAT and AM, but not with DMI and FL, resulted in a supersensitivity of the WDS. A challenge dose of 8-OH-DPAT following 21 day treatment with 8-OH-DPAT, AM and DMI, but not with FL, produced SAL and M-to-MM with PE.

Thus, the 21 day treatment with 8-OH-DPAT produced a similar profile to that of AM. The pattern for DMI and FL was somewhat different.

564.12

EFFECT OF TREATMENT WITH ADINAZOLAM ON SEROTONIN RECEPTORS AND PHOSPHOINOSITIDE HYDROLYSIS IN RAT BRAIN. Y. Dwivedi, S.C. Pandey, M.P. Dubey, X. Ren, J.M. Davis* and G.N. Pandey. Illinois State Psychiatric Institute, Chicago, Illinois 60651

Adinazolam is an anxiolytic drug that also has antidepressant properties, but its mechanism of action is unclear. We determined the effect chronic treatment with adinazolam on serotonin and ß-adrenergic receptors subtypes in rat brain and the phosphoinositide signalling system in choroid plexus. Rats were treated with two doses of adinazolam, 5 mg/kg and 10 mg/kg, for 21 days and were decapitated 24 hours after the last injection. We determined $5HT_2$ and ß-adrenergic receptors in cortex, $5HT_{1C}$ receptors and receptor mediated PI hydrolysis in choroid plexus, $5HT_{1C}$ receptors in hippocampus, and $5HT_{1A}$ receptors in cortex and hippocampus. Treatment with adinazolam significantly increased 5HT stimulated PI hydrolysis (68%) in choroid plexus as compared with control rats. However, it did not have significant effects either on ß-adrenergic receptors as measured by ^{125}I-Cyanopindolol in rat cortex or $5HT_{1A}$ receptors in cortex or hippocampus. It also had no significant effect on the number and affinity of $5HT_2$ receptor binding sites in rat cortex. These results thus suggest that adinazolam is dissimilar to other antidepressants in its effects on $5HT_2$ and ß-adrenergic receptors. However, its effect on $5HT_{1C}$ receptors in choroid plexus and possibly hippocampus may be relevant to its therapeutic effects. We are currently in the process of determining $5HT_{1C}$ receptors in cortex, hippocampus and choroid plexus after chronic treatment with adinozolam.

564.13

DIABETES-INDUCED ALTERATIONS IN 5-HT RECEPTOR SUBTYPE ACTIVATION. L.L. Bellush* & E. Huh, Dept. of Psychology, Ohio University, Athens, OH 45701

Two experiments were conducted which compared the responses of streptozotocin-diabetic (STZ) rats and nondiabetic controls (CTRL) to selective 5-HT agonists. In Exp. 1, 8-OHDPAT-induced hypothermia and DOI-induced hyperthermia were measured in STZ and CTRL, half of which had been adrenalectomized (ADRX) 4 days prior to measurements. The response to 8-OHDPAT was attenuated, and the response to DOI exaggerated in STZ rats, while ADRX had opposite effects. The STZ-induced alterations resemble those previously associated with elevated circulating glucocorticoids (CORT) in clinical depression. Elevated CORT is also seen in STZ rats and could account for 5-HT receptor alterations, given the ADRX effects. In Exp. 2, STZ rats showed greater anxiety in a light/dark emergence test but responded to the anxiolytic effects of buspirone in a parallel fashion to CTRL. The differential sensitivity of STZ to 8-OHDPAT and buspirone, both 5-HT1A agonists, may be due to differential pharmacological profiles of the two drugs.

564.14

5-HYDROXYTRYPTAMINE$_{1B}$ RECEPTOR REGULATION AND ONTOGENY: BEHAVIORAL TOLERANCE AND DOWN-REGULATION OF [^{125}I]IODOCYANOPINDOLOL-LABELLED 5-HT_{1B} BINDING SITES IN THE CENTRAL NERVOUS SYSTEM. M. R. Pranzatelli* and P. Razi, Departments of Neurology, Pediatrics, and Pharmacology, Laboratory of Movement Disorder Pharmacology, The George Washington University, Washington, DC 20010.

Little is known about the regulation and ontogeny of 5-hydroxytryptamine$_{1B}$ (5-HT_{1B}) receptors, a putative terminal autoreceptor in the central nervous system. We studied the response of [^{125}I]iodocyanopindolol ([^{125}I]ICYP)-labelled central 5-HT_{1B} sites to chronic treatment with 5-HT agonists and antagonists at a dose of 10 mg/kg/d i.p. for 30 consecutive days in the rat. RU 24969 significantly reduced B_{max} 23 - 63% in cortex, hippocampus, striatum, brainstem, and spinal cord without a change in K_d except for a 1.7-fold increase in cortex and spinal cord. The putative 5-HT_{1B} agonist TFMPP, but not *m*-CPP or the 5-HT_{1B} antagonists pindolol or quipazine, also reduced the B_{max} of cortical 5-HT_{1B} sites. 100 μM GTPγS induced a significant 5-fold right shift in 5-HT affinity for striatal 5-HT_{1B} sites, but the magnitude of the shift was not altered by chronic treatment with RU 24969, *m*-CPP, or pindolol. The number of striatal 5-HT_{1B} sites increased with postnatal age through the first month. Neonatal 5,7-dihydroxytryptamine (5,7-DHT) lesions made by i.p. injection and shown to alter 5-HT uptake site density did not significantly alter 5-HT_{1B} binding site density in cortex, striatum, diencephalon, or brainstem. In behavioral studies, chronic treatment with RU 24969 but not *m*-CPP induced tolerance to RU 24969-evoked cage crossings. The data demonstrate functionally significant decreases in maximum number of 5-HT_{1B} receptors in response to chronic agonist treatments. They also suggest that *m*-CPP does not act as a 5-HT_{1B} agonist at the dose studied and the hypolocomotion it induces is not mediated by 5-HT_{1B} sites.

564.15

Transfected Human 5-HT_{1C}, but not 5-HT_2, Receptors Modulate Naturally Expressed 5-$HT_{1B\text{-like}}$ Receptor Function in CHO Cells. Saul Maayani[1], Kelly A. Berg[1*], Cynthia A. Sailstad[1] and Alan Saltzman[2]. Depts. of Anesthesiology[1], Mount Sinai Medical Center, CUNY, NY,NY 10029 and Molecular Biology[2], Rhone Poulenc-Rorer, Collegeville, PA 19426.

We have found that wild-type CHO cells naturally express a 5-HT receptor which inhibits forskolin-stimulated (FS) cAMP formation in a pertussis toxin sensitive manner. 5-HT agonists inhibited FS cAMP accumulation with the following rank order of potency and % maximal inhibition (EC_{50}, nM; % inhibit): 5-Carboxamidotryptamine (5-CT, 4; 76%) $\geq$ 5-HT (6; 81%) > CP 93129 (18; 82%) > pindolol (39; 61%) > sumatriptan (160; 63%) >>> 8-OH-DPAT (not active at 100 nM). The response to 100 nM 5-CT was blocked by 1 μM methiothepin but was insensitive to 1 μM spiperone or mesulergine. This pharmacology is consistent with a 5-$HT_{1B\text{-like}}$ receptor subtype. The cDNA for human 5-HT_{1C} and 5-HT_2 receptors were stably transfected separately into wild-type CHO cells and the effect of activation of these receptors on PI hydrolysis and on the 5-$HT_{1B\text{-like}}$ response was measured. Accumulation of inositol phosphates in response to 5-HT were (E_{max}, EC_{50}) 600%, 30 nM and 400%, 430 nM for 5-HT_{1C} and 5-HT_2 lines, respectively. Co-activation of 5-HT_{1C} and 5-$HT_{1B\text{-like}}$ receptors with 5-HT (0.1-100 nM) abolished the 5-$HT_{1B\text{-like}}$ response which returned when the 5-HT_{1C} receptor was blocked with mesulergine (1 μM). Furthermore, the 5-$HT_{1B\text{-like}}$ response to 5-CT was completely blocked by 1 μM DOI. In contrast, co-activation of 5-HT_2 receptors did not alter the 5-$HT_{1B\text{-like}}$ response. Although 5-HT_{1C} and 5-HT_2 receptors are thought to activate similar second messenger pathways, we postulate that inhibition of the 5-$HT_{1B\text{-like}}$ response may involve a second effector system that is coupled to the 5-HT_{1C} receptor but not to the 5-HT_2 receptor. (USPHS GM 34852; DA 06620; MH 48125)

564.16

Rapid Desensitization of Human 5-HT_{1C} Receptors Stably Expressed in CHO Cells. Kelly A. Berg[1], Barbara J. Ebersole[1*], Alan Saltzman[2], and Saul Maayani[1]. Depts. of Anesthesiology[1], Mount Sinai Medical Center, CUNY, NY , NY 10029 and Molecular Biology[2], Rhone-Poulenc Rorer, Collegeville, PA 19426.

Acute desensitization of receptor-mediated phosphatidylinositol turnover was characterized in a CHO cell line stably expressing 5-HT_{1C} receptors. CHO cells, transfected with a cDNA clone for the human 5-HT_{1C} receptor expressed a density of 170 fmol/ mg protein as determined by mianserin-sensitive [^{3}H]-mesulergine binding (Kd=1nM). Cells labeled for 24h with 1 μCi/ml [^{3}H] myo-inositol and incubated with varying concentrations of agonist and 20 mM LiCl for 10 min at 37° C accumulated total inositol phosphates (IP's) with the following rank order of potency and intrinsic activities (EC50,nM; intrinsic activity): LSD (20; 0.13) >5-HT (30; 1.0) >DOI (80; 0.56) > quipazine (2500; 0.56). For desensitization studies, cells were incubated in the presence of maximal concentrations of 5-HT, DOI or LSD at 37° C for 15, 30, 45, 60 or 120 minutes. IP accumulation was measured after addition of 20 mM LiCl and a further incubation of 10 minutes. Treatment with 5-HT produced a rapid, homologous desensitization which was maximal by 15 min reducing total IP accumulation to 20% of control levels. Long term incubation (24h) with 1 μM PMA did not alter the desensitization in response to 5-HT indicating that PKC activatidn does not mediate this phenomenon. In contrast to the effect of the full agonist 5-HT, treatment with the partial agonists DOI and LSD produced less desensitization at a slower rate. The maximal IP accumulation in response to DOI occurred at 30 min and was 40% of control levels, while that for LSD occurred at 60 min and was 60 % of control. Therefore we propose that the rate and magnitude of 5-HT_{1C} receptor desensitization is related to the relative drug efficacy of these 5-HT_{1C} agonists (5HT>DOI>LSD).
(USPHS GM 34852; DA 06620; MH 48125)

564.17

DIFFERENTIAL REGULATION OF $5HT_{1C}$ RECEPTORS BY SEROTONIN ANTAGONISTS WITH AND WITHOUT NEGATIVE INTRINSIC ACTIVITY. E.L. Barker and E. Sanders-Bush* Dept. of Pharmacology, Vanderbilt Univ., Nashville, TN 37232.

Receptor antagonists are classically thought to share a common binding site with agonists, but have no intrinsic activity when added alone. However, emerging evidence from many receptor systems indicates that some antagonists actually possess negative intrinsic activity capable of producing effects opposite of those produced by agonists. Agonist-induced stimulation of $5HT_{1C}$ receptors activates the phosphoinositide (PI) hydrolysis signalling cascade. In NIH 3T3 fibroblasts transfected with $5HT_{1C}$ receptor cDNA, we have identified two classes of receptor antagonists based on their effects on basal PI hydrolysis, i.e., effects in the absence of agonist. The receptor antagonists mianserin, mesulergine and ketanserin are active antagonists, inducing a dose-dependent decrease in basal PI hydrolysis. (+)-2-Bromolysergic acid diethylamide (BOL) is a neutral antagonist with no effect on basal values. Because $5HT_{1C}$ receptors display paradoxical down-regulation when exposed to receptor antagonists *in vivo* and *in vitro*, it was of interest to determine if the active and neutral antagonists differ in their ability to induce down-regulation of the receptor. These studies utilized primary cultures of choroid plexus epithelial cells as a model for the study of $5HT_{1C}$ receptor regulation. The active antagonist mianserin, but not the neutral antagonist BOL, was capable of inducing atypical down-regulation of $5HT_{1C}$ receptors. These studies are the first to show direct evidence that the $5HT_{1C}$ antagonists that induce down-regulation are truly active at the receptor. This activity may result in conformational changes in the receptor, leading to an alteration in turnover via an unidentified pathway. (Supported by USPHS grant MH34007.)

564.18

DOWN-REGULATION OF $5\text{-}HT_{1C}$ RECEPTORS AFTER CHRONIC CLOZAPINE TREATMENT - ROLE OF SPARE RECEPTORS. M. Kuoppamäki, E. Syvälahti, J. Hietala*. Dept. of Pharmacology, University of Turku, SF-20520 Turku, Finland.

Repeated administration of clozapine causes downregulation of $5\text{-}HT_{1C}$ receptors. This study further characterizes the down-regulation of $5\text{-}HT_{1C}$ receptors after chronic clozapine treatment (14 days,68h withdrawal). $5\text{-}HT_{1C}$ receptor agonist and antagonist binding sites in rat choroid plexus were measured by using [^{125}I]DOI and [^{3}H]mesulergine autoradiography, respectively. In addition, the $5\text{-}HT_{1C}$ receptor function was studied by measuring 5-HT-stimulated phosphoinositide hydrolysis. $5\text{-}HT_{1C}$ receptor agonist binding sites were significantly decreased by 29 % and 49 % after 10 and 25 mg/kg/day of clozapine, respectively, and $5\text{-}HT_{1C}$ receptor antagonist binding sites by 48 % and 59 % after these treatments. However, the 5-HT-stimulated phosphoinositide hydrolysis was, significantly decreased (- 27 %) only after the 25 mg/kg/day dose of clozapine. The results suggest a 20-30 % receptor reserve for $5\text{-}HT_{1C}$ receptors in rat choroid plexus.

564.19

CHRONIC LITHIUM GREATLY ENHANCES THE EXPRESSION OF C-FOS IN RAT BRAIN FOLLOWING ACTIVATION OF $5HT_2$, BUT NOT $5HT_{1A}$ SEROTONIN RECEPTORS. R.A. Leslie*, J.M. Moorman, and D.G. Grahame-Smith Oxford University-Smithkline Beecham Centre for Applied Neuropsychobiology, University Department of Clinical Pharmacology,Oxford, OX2 6HE, UK.

Lithium suppresses inositol-3 phosphate production by phosphatase inhibition. It also affects serotonergic function in the CNS; eg., downregulating $5\text{-}HT_{1A}$ and $5\text{-}HT_2$ receptors. We studied these actions using c-Fos immunocytochemistry following selective activation of 5-HT receptor subtypes. The $5\text{-}HT_2$ receptor agonist DOI (2,5-dimethoxy-4-iodophenylisopropyl-amine) produces a localised pattern of Fos-like immunoreactivity (FLI) in rat brain (Leslie et al., Neuroscience 53:457,1993). We now show that the $5\text{-}HT_{1A}$ agonist 8-OH-DPAT (8-OH-2-[di-*n*-propylamino] tetralin) induces FLI in a very different pattern; furthermore, chronic lithium treatment greatly enhances FLI in the cerebral cortex following $5\text{-}HT_2$, but not $5\text{-}HT_{1A}$ receptor activation. FLI was localised in 100µM brain sections of rats fed on control or lithium carbonate (0.1%) diet for one week prior to drug challenge (DOI = 8mg/kg, 8-OH-DPAT = 2mg/kg, or saline control i.p.). FLI was low to undetectable in controls. DOI alone produced a dense band of Fos-positive cells in layer Va of somatosensory cortex, while 8-OH-DPAT produced a much more homogeneous pattern in the cortex. Chronic lithium significantly enhanced FLI following DOI, but not 8-OH-DPAT, administration. The most marked effect was in the caudal piriform cortex, an area previously devoid of staining, where FLI was intense. Ritanserin (0.4mg/kg) completely abolished all FLI after DOI with or without lithium. Further studies are in progress to determine why lithium enhances serotonin receptor activity so selectively.

564.20

REGULATION OF $5\text{-}HT_2$ RECEPTORS AND $5\text{-}HT_2$ RECEPTOR mRNA AFTER IRREVERSIBLE INACTIVATION WITH EEDQ. R.K. Raghupathi*, R.P. Artymyshyn, R.A. Habboushe and P. McGonigle Dept. of Pharmacology, Univ. of Pennsylvania, Philadelphia, PA 19104.

$5\text{-}HT_2$ receptors respond in an anomalous fashion to treatments with various agents such as 5-HT reuptake blockers, depleting agents, neurotoxins and $5\text{-}HT_2$ antagonists. To better understand the regulation of the $5\text{-}HT_2$ receptor system, we administered (i.p.) the irreversible antagonist EEDQ (10 mg/ml) to rats (n = 42) which were subsequently sacrificed 0.5, 1, 2, 4, 7 and 14 days after injection. $5\text{-}HT_2$ receptor densities were measured using [^{3}H]-ketanserin under conditions that selectively labeled $5\text{-}HT_2$ receptors. $5\text{-}HT_2$ receptor mRNA levels were measured using *in-situ* hybridization with a 200-base [^{33}P]-labeled riboprobe directed against $5\text{-}HT_2$ receptor mRNA. In cortical regions with a high density of $5\text{-}HT_2$ receptors, receptor levels were decreased to 30% of control values 12-24 hrs after injection. Receptor levels gradually recovered to about 70% of control levels by day 7 but did not recover fully to control values by day 14. Cortical $5\text{-}HT_2$ receptor mRNA levels declined to about 40% of control by 24 hours and showed a gradual increase to control values by day 4. The observation that mRNA levels decrease concomitantly with receptor inactivation suggests that $5\text{-}HT_2$ receptor recovery after inactivation by EEDQ is mediated by post-translational regulatory processes and not by altered gene expression. This is in contrast to the $5\text{-}HT_{1A}$ system where we have observed an increase in mRNA levels preceding receptor recovery after EEDQ inactivation. (Supported by USPHS MH 43821)

SEROTONIN RECEPTORS: PHYSIOLOGY AND BEHAVIOR

565.1

$5HT_4$ AGONISTS: BIMU-1 AND BIMU-8 INDUCE ANALGESIA BY FACILITATING CENTRAL CHOLINERGIC TRANSMISSION. C. Ghelardini, L. Fanfani, N. Galeotti, A. Giotti, [1]C.A. Rizzi and A. Bartolini. Depts. of Pharmacol., Univ. of Florence I-50143, Firenze and [1]Boehringer Ingelheim, Milan, Italy. SPON: European Brain and Behaviour Society

There is accumulating evidence indicating that certain benzimidazolone derivatives including BIMU-1 and BIMU-8 potentiate electrically-stimulated twitch responses in guinea-pig ileum longitudinal muscle strip through enhancement of acetylcholine (ACh) release from cholinergic nerve endings (Rizzi et al., 1990; Craig and Clarke, 1990). Moreover the increased electroencephalogram energy (theta rhythm) in the rats induced by the activation of $5\text{-}HT_4$ receptors appears to be due to an amplification of ACh release (Boddeke and Kalkman, 1990). Since the amplification of cholinergic neurotransmission induces a central antinociceptive effect (Ghelardini et al., 1990, 1992), in the present work the ability of BIMU-1 and BIMU-8 for modifying pain theshold was investigated. The study was performed in both mice (hot-plate and acetic acid writhing test) and rats (paw-pressure test). Our data indicate that in all three tests BIMU-1 (10-30 mg/kg i.p.) and BIMU-8 (30 mg/kg i.p.) are able to induce antinociception without affecting either performance on rota-rod test or spontaneous locomotory activity measured with an Animex apparatus. The analgesic action of BIMU-1 (3 µg/mouse) and BIMU-8 (10 µg/mouse) occurs in the CNS, since also their intracerebroventricular administration yielded a statistically-significant increase in pain threshold. The analgesic action of both BIMU-1 and BIMU-8 was prevented by atropine (5 mg/kg i.p.), hemicholinium-3 (1 µg/mouse i.c.v.) and by the $5HT_4$ antagonist SDZ 205-557 (10 mg/kg i.p.). These results suggest not only the possibility of inducing antinociception by $5\text{-}HT_4$ agonists, but also that analgesia is mediated by the cholinergic system.

565.2

EFFECTS OF THE $5\text{-}HT_3$ ANTAGONIST MDL 72222 ON CISPLATIN-INDUCED PERIPHERAL NEUROPATHY. P.M. Whitaker-Azmitia,* A. Borella and X. Zhang, Dept. of Psychiatry, SUNY, Stony Brook, NY, 11794.

$5\text{-}HT_3$ antagonists are reported to be efficacious in inhibition of cisplatin-induced emesis. However, their efficacy in treating another major side effect of cisplatin therapy - peripheral neuropathy - has not been tested. In this study, we report that the antagonist MDL 72222 increases toxic effects in a developing model of cisplatin neuropathy.

5-day old rat pups were treated twice weekly for four weeks with 1 mg/kg cisplatin or 3 mg/kg MDL 72222 or both. Cisplatin significantly increased tailflick latency at PD17 (4.1 sec vs. 13.0 $p < .002$) and PD21 (5.1 sec vs. 22.3 $p < .005$). This effect was not reversed by MDL 72222 at either timepoint. In addition to the tail flick changes, the animals also showed significant inward turning of the hindfeet which was quantified by measuring the average distance between the toes minus the average distance between the heels. For control animals, this was .632 ± .066 mm and for cisplatin, .456 ± .065 mm ($p < .05$). However, MDL72222 worsened this condition with the toe-heel distance reduced to .091 ± .1 ($p < .001$). Immunochemical analysis of spinal cord showed that cisplatin caused significant loss of CGRP-positive cells which was not reversed by MDL 72222. Our work suggests that the peripheral neuropathy induced by cisplatin is not prevented by $5\text{-}HT_3$ antagonists.

565.3

SPIPERONE-INDUCED ACTIVATION OF SEROTONERGIC NEURONAL ACTIVITY CORRELATES WITH 5-HT_{1A} AUTORECEPTOR BLOCKADE IN AWAKE CATS. C.A. Fornal*, C.W. Metzler, W.J. Litto, F. Marrosu, and B.L. Jacobs. Dept. of Psychol., Prog. Neurosci., Princeton Univ., Princeton, NJ

Somatodendritic 5-HT_{1A} autoreceptors are believed to be involved in a negative feedback mechanism whereby the synaptic concentration of released serotonin can modulate impulse activity of central serotonergic neurons. These receptors apparently exert a tonic inhibitory influence on neuronal activity under physiological conditions, since systemic administration of the 5-HT_{1A} antagonist spiperone increases the firing rate of serotonergic neurons in awake animals (Jacobs & Fornal, Pharmacol. Rev. 43:563-578, 1991). The present study examined the relationship between spiperone-induced activation of serotonergic dorsal raphe (DRN) neurons and the 5-HT_{1A} autoreceptor-blocking actions of the drug. Neurons were recorded prior to, and for 6 hr following, spiperone injection (0.25 and 1 mg/kg, iv) using methods previously described (Fornal et al., Am. J. Physiol. 259:R963-R972, 1990). In addition, the inhibitory response of serotonergic neurons to a challenge dose (10 µg/kg, iv) of the selective 5-HT_{1A} agonist 8-OH DPAT was determined at 1-hr intervals for 6 hr after spiperone administration. Spiperone increased unit activity and blocked the action of 8-OH DPAT. Both effects were dose- and time-dependent. Furthermore, there was a strong positive correlation between these two actions of spiperone. These results suggest that the increase in DRN serotonergic neuronal activity produced by systemic administration of spiperone is due to blockade of the 5-HT_{1A} autoreceptor. Activation of this receptor represents the initial step in the engagement of a physiological negative feedback system. Supported by grants from the AFOSR (90-0294) and the NIMH (MH 23433).

565.4

EFFECTS OF (+)WAY 100,135 ON BRAIN SEROTONERGIC UNIT ACTIVITY IN BEHAVING CATS. C.W. Metzler*, C.A. Fornal, S.C. Veasey and B.L. Jacobs. Prog Neurosci., Princeton Univ., Princeton, NJ 08544.

Brain serotonergic neuronal activity is modulated by 5-HT_{1A} autoreceptors. Recently, we have shown that spiperone, a 5-HT_{1A} antagonist, increases serotonergic neuronal activity in awake animals, suggesting that these neurons are under tonic feedback inhibition under physiological conditions (see Fornal et al., this meeting). The present study examined the effects of another putative 5-HT_{1A} autoreceptor antagonist, (+)WAY 100,135 on the spontaneous activity of serotonergic neurons in the dorsal raphe nucleus (DRN) in behaving cats. Serotonergic neurons were identified on-line by their slow and regular discharge activity, complete cessation of firing during REM sleep, and response to systemic administration of the 5-HT_{1A} agonist 8-OH DPAT. (+)WAY 100,135 was administered intravenously in doses of 100, 500, and 1000 µg/kg. Unit activity was monitored prior to, and for 2 hr following drug injection. In contrast to the effects of spiperone, administration of (+)WAY 100,135 produced a suppression of serotonergic unit activity. The effect was apparent within one min post-injection and lasted for approximately 15-30 min. All three doses of (+)WAY 100,135 maximally decreased neuronal activity by approximately 30-40%. Thus, the effect of (+)WAY 100,135 on serotonergic activity does not appear to be dose-related. Control saline injections had no significant effect on neuronal activity. These results indicate that (+)WAY 100,135, unlike spiperone, does not increase serotonergic neuronal activity in awake animals, suggesting that the drug is ineffective in blocking the inhibitory action of released serotonin at the autoreceptor. Supported by grants from the AFOSR (90-0294) and the NIMH (MH 23433).

565.5

EFFECTS OF 5HT_{1A} LIGANDS ON CORE TEMPERATURE IN THE CONSCIOUS DOG. I. Hallett, V.C. Middlefell and G. Stack[1]*, Wyeth Research UK Ltd, Taplow, Berks, SL6 0PH, [1] Wyeth-Ayerst Research, Princeton, NJ.

5HT_{1A} agonists and partial agonists induce hypothermia in the mouse and rat (Bill et al., 1991, Br. J. Pharmacol., 103:1857-1864), and in man (Anderson et al., 1990, Psychopharmacology, 100: 498-503). The aim of this study was to determine whether a similar response was induced by 5HT_{1A} ligands in the conscious beagle dog.

Dogs of either sex were trained to stand in restraining slings while their rectal temperature (T) was continually recorded with a digital probe. In some experiments heart rate (HR) was derived from a lead II ECG. 8-OH-DPAT (10µg/kg s.c.) or saline (100µl/kg s.c.) was administered at 30 min intervals for 2h. The 5HT_{1A} partial agonist, gepirone (0.3 and 3.0 mg/kg p.o.), or lactose placebo was administered in gelatine capsules and the effects on T observed for 4h. The effects of the α_2 adrenoceptor agonist, UK14304 (30 and 100µg/kg s.c.) were also investigated as a positive control.

8-OH-DPAT did not decrease HR or T but rather evoked a 0.2° C increase in T associated with body tremor, following the 3rd and 4th drug doses. Previous studies have shown marked behavioural effects are induced by 8-OH-DPAT (30µg/kg i.v.). Gepirone (0.3 mg/kg) had no effect on T, whereas gepirone (3.0 mg/kg) evoked a 0.5° C decrease in T compared to controls 3 h after administration though this was accompanied by overt behavioural effects (panting, salivation, tremor and tucking in of the hind limbs) in one of the dogs. By comparison, UK14304 (30 and 100µg/kg) evoked falls in T of 1° C (at 2h) and 1.4° C (at 4h) respectively with an accompanying sedation.

Thus, our studies failed to show a hypothermic effect of 5HT_{1A} ligands in the dog except at doses producing pronounced motor effects.

565.6

EVIDENCE USING CONFORMATIONALLY RESTRICTED SUMATRIPTAN ANALOGUES, CP-122,288 AND CP-122,638, THAT 5-HT_{1D} RECEPTORS DO NOT MEDIATE BLOCKADE OF NEUROGENIC INFLAMMATION. W. S. Lee, F. M. Cutrer and M. A. Moskowitz*. Stroke Research Laboratory, Neurosurgery and Neurology Services, Massachusetts General Hospital, Harvard Medical School, 32 Fruit Street, Boston, MA 02114.

We compared the effects of two conformationally restricted sumatriptan analogues with sumatriptan (S) on the plasma protein extravasation within dura mater and the expression of c-fos protein in lamina I and II_o of trigeminal nucleus caudalis of pentobarbital-anesthetized guinea-pigs. The extravasation response (^{125}I-BSA) was induced by either unilateral trigeminal ganglion stimulation (0.6 mA, 5 ms, 5 Hz, 5 min) or substance P (SP; 1 nmol/kg, i.v.). The c-fos expression was elicited by the instillation of capsaicin (0.1 µmol) through a catheter placed into the cisterna magna.

In plasma protein extravasation response the threshold for CP-122,288 and CP-122,638 (1 and 0.1 pmol/kg, i.v., respectively) was remarkably lower than for S (7 nmol/kg), as was the dose at maximum response (1 and 0.1 nmol/kg for CP-122,288 and CP-122,638, respectively, and 240 nmol/kg for S). SP-induced plasma leakage was unaffected by either CP-122,288 or CP-122,638 or S, and metergoline (0.1 µg/kg) only partially (27%) reversed the effect of CP-122,288 (1 pmol/kg). Furthermore, CP-122,288 (1 nmol/kg) significantly reduced the number of c-fos labelled cells by 43.8%.

The marked potency differences between S and its analogues (despite similar 5-HT_{1D} receptor affinities) strongly suggest that the mechanism for all three drugs may well be distinct from their ability to activate the 5-HT_{1D} receptor subtype and that the two analogues are more selective for the prejunctional receptor.

565.7

Chronic 5HT_{1A} agonist treatment produces a trans-receptor up-regulation in rat brain 5$HT_{1D\beta}$ receptor mRNA and expression: relationship to anxiolytic activity. M. Teitler,* K.J. Miller, C.T. Casey, and B.J.Hoffman@ Dept. of Pharmacology, Albany Medical College, Albany, NY 12208; @ Laboratory of Cell Biology, NIMH, Bethesda, MD 20892

Ipsapirone, buspirone, and gepirone, three 5HT_{1A} receptor agonists, display anxiolytic effects in preclinical animal models of anxiety as well as in humans. The effects in humans are noted after chronic treatment, indicating that the anxiolytic effects of the drugs are presumably due to some compensatory mechanisms. Benzodiazepines are anxiolytic, presumably due to their actions in potentiating GABAergic transmission. In order to ascertain whether chronic 5HT_{1A} agonist treatment could alter GABAergic activity through a serotonergic mechanism we examined the effects of chronic ipsapirone treatment on the expression of 5$HT_{1D\beta}$ (5HT_{1B}) receptors in rat brain, using in situ hybridization and autoradiographical radioligand binding studies. 5$HT_{1D\beta}$ receptors are highly expressed in the rat substantia nigra: the nigra receives a major GABAergic projection from the striatum. Two week treatment with 10 mg/kg ipsapirone, twice daily, s.c. produced a six-fold increase in striatal 5$HT_{1D\beta}$ receptor mRNA. Accompanying this dramatic increase in mRNA expression was a 30% increase in ^{125}I-cyanopindolol-labelled 5$HT_{1D\beta}$ receptors in the substantia nigra. Acute treatments with ipsapirone did not produce any effects on mRNA or expressed receptor levels. The effects of other psychotropic drugs on 5$HT_{1D\beta}$ receptor mRNA and expression levels will be investigated. Supported by MH40716 (M.T.)

565.8

5-HT-1C AGONISTS SUPPRESS APOMORPHINE-INDUCED LOCOMOTOR ACTIVITY P. B. HICKS,* R. J. ZAVODNY AND K. A. YOUNG Department of Psychiatry & Behavioral Sciences, Texas A&M College of Medicine, Temple, Texas 76508.

We have previously reported that a low dose (0.1 mg/kg) of the 5-HT-1C antagonist mesulergine potentiates apomorphine-induced locomotor activity (AILA). This effect can be blocked by the 5-HT-1C/2 agonist DOI (Psychopharmacology 110: 97-102). However, DOI (agonist at both the 5-HT-1C and 5-HT-2 receptors) itself suppresses AILA at doses above 0.5 mg/kg. Recently, an antagonist (MDL 100,907) has been developed that is highly selective for the 5-HT-2 receptor. In the following experiments we tried to determine the role of 5-HT-2 and 5-HT-1C receptors in the suppression of AILA seen with DOI.

For the determination of AILA, serotonergic test drug(s) or vehicle were injected SC in Sprague-Dawley rats five minutes before administration of 0.25 mg/kg APO or vehicle and the number of times the animal crossed the lines of a test grid were counted in 5-min intervals. Ketanserin (5-HT-2 antagonist; 0.5 mg/kg) had no effect on AILA. Ketanserin (0.5 mg/kg) + DOI (1 mg/kg) resulted in marked suppression of AILA ($p<.01$). The potentiation of AILA by low dose mesulergine (0.1 mg/kg) was blocked by DOI at either 0.1 or 1.0 mg/kg ($p<.05$). MDL 100,907 had no effect on AILA. The suppression of AILA by DOI (1.0 mg/kg) was not reversed by MDL 100,907 (0.15 mg/kg). These data suggest that 5-HT-1C agonists suppress AILA, but are in direct conflict with a previous report that the 5-HT-1C agonist LSD markedly potentiated AILA (Fink and Oelssner, Eur. J. Pharmacol. 75: 289-296, 1981).

565.9

5-HT SYNTHESIS-SUPPRESSING AND DA SYNTHESIS-PROMOTING ACTIONS OF THE 5-HT$_{1B}$ RECEPTOR AGONIST TFMPP *IN VIVO*: PRE- *VS.* POSTSYNAPTIC 5-HT$_{1B}$ RECEPTOR-MEDIATED EVENTS.

S. Hjorth, Department of Pharmacology, University of Göteborg, Medicinareg. 7, S-413 90 Göteborg, SWEDEN*

5-HT$_{1B}$ receptors are found pre- as well as postsynaptically to the 5-HT neurones in the rat brain, particularly in the DA-rich limbic and basal ganglia areas. In preliminary studies, the 5-HT$_{1B}$ receptor agonist TFMPP was found to decrease 5-HT synthesis but simultaneously enhance DA synthesis in rat brain *in vivo*. The present experiments were carried out in an attempt to clarify the mechanism(s) underlying these actions. Adult male Sprague-Dawley rats (250-350 g) were used in the studies. TFMPP (0.1-10 mg/kg SC, 60 min. before death) dose-dependently reduced the 5-HT, and increased the DA, synthesis (5-HTP and DOPA accumul., respectively, after 30 min. decarboxylase inhibition) in the limbic forebrain and the corpus striatum. The 5-HT synthesis-suppressing action of TFMPP proved resistant to axotomy (unilateral hemitransection, 90 min. before death) and reserpine (5 mg/kg IP; 90 min. before death) pretreatment, indicating that the effect was directly mediated and independent of nerve impulses and intact monoamine stores. In contrast, the DA synthesis-promoting action of TFMPP was abolished by either of these procedures. Taken together, the results indicate *i)* that the reduction of rat brain 5-HT synthesis after TFMPP is mediated by 5-HT, tentatively 5-HT$_{1B}$, autoreceptors located on the serotonergic axon terminals, and *ii)* that the DA synthesis-promoting action of TFMPP is independent of its effect on 5-HT synthesis, and apparently requires intact nerve impulse traffic. The effects of TFMPP on 5-HT and DA synthesis may thus possibly reflect pre- and postsynaptic, respectively, 5-HT$_{1B}$ receptor (heteroreceptor?)-mediated events.

565.10

SEROTONIN (5-HT) HYPERSENSITIVITY ASSOCIATED WITH 5-HT HYPERINNERVATION IN ADULT RAT NEOSTRIATUM AFTER NEONATAL DOPAMINE (DA) DENERVATION. M. El Mansari, A. Ferron* and L. Descarries. Centre de recherche en sciences neurologiques (Départements de physiologie et de pathologie), Univ. Montréal, Montréal, Qc, Canada.

Destruction of nigrostriatal DA neurons by intraventricular 6-OHDA in newborn rats results in a 5-HT hyperinnervation of the adult neostriatum (NS) predominating in its rostral half. These rats also show a considerable increase in 5-HT2 binding sites in the rostral but not the caudal NS, and enhanced neuronal responsiveness to iontophoresed 5-HT and its selective 5-HT2 agonist DOI in the rostral NS. In view of recent results demonstrating that the 5-HT hyperinnervation is also present, although less prominent, in the caudal NS (Soucy et al., *this volume*), neuronal responsiveness to 5-HT and DOI was further assessed, by means of the IT50 index, in the caudal NS of neonatally lesioned rats. The NS of rats similarly lesioned as adults and hence not 5-HT-hyperinnervated was also examined. As previously found rostrally, marked increases in inhibitory responsiveness to 5-HT and to DOI were measured in the caudal NS of neonatally lesioned rats. In contrast, 15-30 days after adult lesions, the sensitivity to 5-HT and DOI was not different from control. Enhanced responsiveness to 5-HT and DOI after neonatal lesion was therefore related to the 5-HT hyperinnervation rather than the DA denervation *per se*. Enhanced responsiveness to DOI in the caudal NS suggested an up-regulation of 5-HT2 receptors, despite the earlier measurements of binding values equivalent to control. Indeed, some of these receptors are probably normally located on DA terminals, so that "control" values after DA denervation should be indicative of an increase in remaining intrastriatal locations. Since 5-HT2 antagonists suppress the spontaneous hyperactivity seen in these rats (Luthman et al., *J. Psychopharmacol.* 5:418, 1991), it is likely that the enhanced neuronal responsiveness to 5-HT asssociated with the increase in neostriatal 5-HT2 receptors is implicated in this abnormal behavior. (Supported by FCAR and MRC grant MT-3544).

565.11

RELEASE OF DOPAMINE FROM THE RAT OLFACTORY TUBERCLE IS MEDIATED BY ACTIVATION OF 5-HT$_3$ RECEPTORS. J. Del Río* and A. Zazpe. Dept. of Pharmacology, University of Navarra Medical School, 31080-Pamplona, Spain.

Serotonin (5-HT) and specific 5-HT$_3$ receptor agonists release dopamine (DA) from the striatum and limbic regions. It has been suggested that DA is released through a carrier-related mechanism and is not a consequence of 5-HT$_3$ receptor activation. We have studied the effect of 5-HT$_3$ receptor stimulation and blockade on K^+-evoked [^{3}H]DA release from slices of rat olfactory tubercle, a terminal field of mesolimbic DA projections. After superfusion of the slices with high K^+ (45 mM) and a washout period, tissue was incubated with [^{3}H]DA (0.1 μM) for 30 min. When DA efflux was stabilized, slices were stimulated twice (S_1 and S_2, 30 min apart) with KCl (20 mM, 6 min). Drugs were added 15 min before S_2 and the effect calculated as change in the S_2/S_1 ratio. The 5-HT$_3$ agonist 2-Me-5-HT (1-10 μM) induced a significant and concentration-dependent increase in S_2/S_1. Phenylbiguanide (PBG) and 5-HT (2 μM each) also increased significantly this ratio. Low concentrations of the 5-HT$_3$ antagonist ondansetron (20-200 nM) blocked, also in a concentration-dependent manner, the effect of 2-Me-5-HT and of PBG. Other 5-HT$_3$ antagonists such as granisetron and tropisetron (200 nM) also blocked the [^{3}H]DA efflux induced by 2-Me-5-HT (2 μM). Interestingly, the DA uptake blocker nomifensine (10 μM) did not modify at all the effect of 2-Me-5-HT (2 μM). The results suggest that, under conditions of K^+-induced neuronal depolarization, enhanced release of DA by serotonergic agonists is a 5-HT$_3$ receptor-mediated effect.

565.12

Food intake, locomotor activity, temperature and neuroendocrine effects of DOM in rats.

J.L. Hill, C.S. Aulakh , P. Mazzola-Pomietto, A. Reid* and D.L. Murphy. Laboratory of Clinical Science, National Institute of Mental Health, Bethesda, MD 20892.

Intraperitoneal administration of various doses of the phenylisopropylamine hallucinogen 1-(2,5-dimethoxy-4-methyl-phenyl)-2-aminopropane (DOM) to male Wistar rats produced dose-related decreases in one-hour food intake in the food-deprived-paradigm and locomotor activity. DOM also produced increases in rectal temperature and in plasma concentrations of prolactin, adrenocorticotropic hormone (ACTH) and corticosterone. DOM administration did not have any significant effect on growth hormone levels.

Since DOM has high affinity at both 5-HT2 and 5-HT1C receptor sites in radioligand studies, we futher studied the effects of various 5-HT receptor subtype selective antagonists on DOM-induced changes in rectal temperature, food intake and plasma hormones. Pretreatment with propranolol (ß-adrenoceptor antagonist that also has binding affinity for 5-HT1A, 5-HT1B and 5-HT1C sites) or MDL-72222 (5-HT3 antagonist) did not attenuate DOM- induced hyperthermia. In contrast, pretreatment with metergoline (5-HT1/5-HT2 antagonist), mesulergine and ritanserin (5-HT1C/5-HT2 antagonists) as well as spiperone (5-HT1A/5-HT2/D2 antagonist) significantly attenuated DOM-induced hyperthermia. These findings suggest that DOM-induced hyperthermia in rats is mediated by stimulation of 5-HT2 receptors. We will also present data on the effects of 5-HT antagonists on DOM-induced decreases in food intake and increases in plasma hormones.

565.13

Evidence that m-CPP-induced hyperthermia in rats is mediated by 5-HT1C receptors.

P. Mazzola-Pomietto, C.S. Aulakh, K.M. Wozniak, D. Johnson* and D.L. Murphy. Laboratory of Clinical Science, National Institute of Mental Health, Bethesda, MD 20892.

m-chlorophenylpiperazine (m-CPP), an active metabolite of the antidepresssant trazodone (Caccia et al, 1982) possesses an approximately 10-fold higher affinity for 5-HT1C versus 5-HT1A, 5-HT1B and 5-HT2 sites (Hoyer et al, 1988). Intraperitoneal administration of m-CPP (2.5 mg/kg) to male Wistar rats produced hyperthermia with a peak effect at 30 min. Pretreatment with propranolol and CGP361A (ß-adrenoceptor antagonists that also have binding affinity for 5-HT1A and 5-HT1B sites) as well as MDL-72222 and ondansetron (5-HT3 antagonists) did not attenuate m-CPP induced hyperthermia. In contrast, pretreatment with metergoline (5-HT1/5-HT2 antagonist), mesulergine, mianserin and ritanserin (5-HT1C/5-HT2 antagonists) as well as spiperone (5-HT1A/5-HT2/D2 antagonist) significantly attenuated m-CPP-induced hyperthermia. Futhermore, daily administration of m-CPP (2.5 mg/kg/day) produced complete tolerance to its hyperthermic effect by day 3. However, there was no cross-tolerance to 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI, a 5-HT 2 agonist that also has high affinity for 5-HT1C receptors)-induced hyperthermia when m-CPP-treated (2.5 mg/kg/day x 5) animals were challenged with DOI on day 6. While the antagonist data alone suggest that either 5-HT1C or 5-HT2 might mediate m-CPP-induced hyperthermia, the lack of cross -tolerance to DOI strongly suggests that m-CPP-induced hyperthermia in rats is mediated by stimulation of 5-HT1C receptors.

565.14

Evidence that DOI-induced hyperthermia in rats is mediated by 5-HT2 receptors.

D.L. Murphy, P. Mazzola-Pomietto, K.M. Wozniak, N.A Garrick*, J.L. Hill and C.S. Aulakh. Laboratory of Clinical Science, National Institute of Mental Health, Bethesda, MD 20892.

1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI) is a phenylisopropylamine hallucinogen which has been shown to compete strongly for [3H]-DOB-labelled 5-HT2 receptors with a similar rank order of potency at [3H]-mesulergine-labelled 5-HT1C receptors in homogenate binding assays (Titler et al, 1988). Intraperitoneal administration of DOI (1.0 mg/kg) to male Wistar rats produced hyperthermia with a peak effect at 60 min Pretreatment with propranolol and CGP361A (ß-adrenoceptor antagonists that also have binding affinity for 5-HT1A and 5-HT1B sites) did not attenuate DOI-induced hyperthermia. In contrast, pretreatment with metergoline (5-HT1/5-HT2 antagonist), mesulergine, mianserin and ritanserin (5-HT1C/5-HT2 antagonists) as well as spiperone (5-HT1A/5-HT2/D2 antagonist) significantly attenuated DOI-induced hyperthermia. On the other hand, pretreatment with MDL-72222 and ondansetron (5-HT3 antagonists) potentiated DOI-induced hyperthermia. Futhermore, daily administration of DOI (2.5 mg/kg/day) for 17 days did not produce either tolerance to its hyperthemic effect or modify m-CPP-induced hyperthermia in rats. These findings suggest that DOI-induced hyperthermia in rats is mediated by stimulation of 5-HT2 receptors.

565.15

Lack of cross-tolerance for hypophagia induced by DOI versus m-CPP suggests separate mediation by 5-HT2 and 5-HT1C receptors, respectively. C.S. Aulakh, P. Mazzola-Pomietto, B Hullihan-Giblin* and D.L. Murphy. Laboratory of Clinical Science, National Institute of Mental Health, Bethesda, MD 20892.

In radioligand studies, 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI) has been shown to have high affinity for 5-HT2 and 5-HT1C receptor sites (Titler et al, 1988) whereas m-chlorophenylpiperazine (m-CPP) possesses an approximately 10-fold higher affinity for 5-HT1C versus 5-HT1A, 5-HT1B and 5-HT2 sites. In the present study, we investigated the time course of development of tolerance to the hypophagic effects of DOI and m-CPP and futhermore, also checked for cross-tolerance between m-CPP and DOI. We studied the effects of daily injections of m-CPP (2.5 mg/kg), DOI (2.5 mg/kg) or saline on first hour food intake in separate groups of male Wistar rats using the food-deprived paradigm.

Intraperitoneal administration of DOI produced significant decreases in the first hour food intake on day 1 and on day 2 relative to saline-treated animals. Complete tolerance developed to DOI-induced hypophagia by day 3. However, there was no cross-tolerance to m-CPP-induced hypophagia when DOI-treated (2.5 mg/kg/day x 4) animals were challenged with m-CPP on day 5. Similarly, complete tolerance developed to m-CPP-induced hypophagia by day 3 but again, there was no cross-tolerance to DOI-induced hypophagia when m-CPP-treated (2.5 mg/kg/day x 4) animals were challenged with DOI on day 5. These findings suggest that m-CPP- and DOI-induced hypophagia is separately mediated by 5-HT1C and 5-HT2 receptors, respectively. We will also present data on 5-HT1C and 5-HT2 receptor binding in different brain areas in 4-day m-CPP (2.5 mg/kg/day) and DOI (2.5 mg/kg/day)-treated animals.

565.16

INVOLVEMENT OF CENTRAL 5-HT2 RECEPTORS IN THE MAINTENANCE OF TOLERANCE TO ETHANOL BY ARGININE-8-VASOPRESSIN P.H. Wu, J.-F. Liu, A.J. Lanca, L.A. Grupp* and H. Kalant. Dept. of Pharmacology, Univ. of Toronto, and Addiction Res. Foundation of Ontario, Toronto, Canada.

AVP and some of its analogs can maintain tolerance to ethanol (EtOH) after EtOH administration ceases, but only if mesolimbic 5-HT pathways are intact. The type(s) of 5-HT receptor required for this AVP effect on EtOH tolerance is not known. Male Sprague Dawley rats (N=120) were trained to criterion performance on a moving belt, and then implanted with an intracerebroventricular (i.c.v.) cannula. Saline (S) or 5,7-dihydroxytryptamine (5,7-DHT) was injected i.c.v. After 2 weeks of recovery, the rats were treated daily with saline or EtOH (1.8 g/kg i.p.) followed by practice sessions on the belt, until the EtOH-treated rats showed tolerance. At this point, EtOH treatment was discontinued and for the next 2 wks S or AVP (10 μg) was given s.c. daily, together with S or various 5-HT receptor agonists i.c.v., via Alzet osmotic minipumps. All animals were then tested under EtOH. Immunocytochemistry revealed that 5-HT neuronal terminals were destroyed by the 5,7-DHT while the cell bodies in the raphe nuclei were spared. In the EtOH tests, tolerance was found to be maintained by AVP combined with i.c.v. infusion of 5-HT, α-methyl-5-HT (5-HT_2 agonist), or 2-methyl-5-HT (5-HT_3 agonist), but not with 8-OH-DPAT (5-HT_{1A} agonist). These results indicate relatively selective involvement of 5-HT_2 or 5-HT_3 receptors in the maintenance of EtOH tolerance by AVP. Supported by NIAAA grant #1 R01-AA08212-03.

565.17

IN VIVO ASSESSMENT OF SUB-TYPE SPECIFIC $5HT_2$ ANTAGONISTS. P.A. Wisler* and P.C. Doherty. Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, IN 46285.

Homology among the subtypes of the $5HT_2$ receptor (2A, 2B, and 2C) has made it difficult to assess the selectivity of agonists and antagonists for the members of this sub-family. Here we report on the effects of various $5HT_{2A/2C}$ antagonists on 1-(m-chlorophenyl)-piperazine (mCPP) induced penile erection, a response mediated by the $5HT_{2C}$ receptor, and 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI) induced headshakes, a $5HT_{2A}$ mediated event. mCPP increased erections in a dose dependent fashion (0.1 to 1.0 mg/kg). DOI increased both the erectile response (0.1 to 0.3 mg/kg) and the number of head shakes (0.3 to 10.0 mg/kg). Induction of the latter response was associated with a significant decline in erections. A series of $5HT_{2A/2C}$ antagonists inhibited DOI induced (1.0 mg/kg) headshakes with the following order of potency: Mianserin > Ketanserin ≥ LY53857 > Ritanserin. Most of these antagonists were effective at blocking mCPP induced (0.3 mg/kg) erections: Mianserin ≥ LY53857 > Ritanserin. Ketanserin, at doses up to 30 mg/kg, had no effect on mCPP induced erections. However, it did unmask the ability of DOI to induce erections at doses greater than 0.3 mg/kg. These results suggest that this combination of behavioral tests can establish the in vivo selectivity of antagonists for the $5HT_{2A}$ and $5HT_{2C}$ receptors.

565.18

5-HT_2- AND NOT 5-HT_{1c}-RECEPTOR ACTIVATION MEDIATES THE HEAD-TWITCH RESPONSE IN CRYPTOTIS PARVA. N.A. Darmani*, O.R. Mock, L.C. Towns, and C.F. Gerdes. Departments of Pharmacology and Anatomy, Kirksville College of Osteopathic Medicine, Mo 63501.

Our initial studies suggested that the 5-$HT_{2/1C}$ agonist, DOI, produces both the head-twitch response (HTR) and the ear-scratch response (ESR) in mice via stimulation of 5-HT_2 receptors. However, challenge studies revealed that these behaviors are produced via two different receptors (possibly 5-HT_2 and 5-HT_{1C}) and due to a lack of selective agents one cannot designate a specific receptor for a particular response. The purpose of the present study was to investigate such behaviors in the least shrew (Cryptotis parva) which is more sensitive to DOI than rodents. Varying doses of DOI (0.31-2.5 mg/kg) was administered intraperitoneally to different groups of shrews and the induced behaviors were recorded cumulatively at 5-min intervals for the next 30 min. DOI produced a dose-dependent (bell-shaped) and time-dependent increase in the HTR frequency. The induced HTR was completely attenuated by the 5-$HT_{2/1C}$ antagonists ketanserin and spiperone. The 5-HT_{1C} antagonist with 5-HT_2 agonist action, lisuride, also produced the HTR in a bell-shaped dose- (0.63-2.5 mg/kg) and time-dependent fashion. Central injections of both DOI (0.2 μg) and lisuride (0.5 μg) also induced the HTR. Both peripheral and central administration of lisuride failed to produce the ESR. DOI significantly induced the ESR only at the highest dose tested (2.5 mg/kg, i.p.). Centrally administered DOI (0.2 μg) produced more ESRs relative to vehicle controls, however, the difference did not attain significance. At low doses (0.31 and 0.63 mg/kg), DOI had no effect on locomotor activity but significantly attenuated the behavior at larger doses. Both low and high doses of lisuride increased the motor activity. Spiperone dose-dependently suppressed locomotion whereas ketanserin had no effect. The present results suggest that the HTR is a 5-HT_2 receptor-mediated event and changes in locomotor activity do not affect the induced-HTR.

565.19

EXCITATION BY SEROTONIN (5-HT) OF INTERNEURONS IN PIRIFORM CORTEX: BLOCKADE BY A NEW, HIGHLY SELECTIVE 5-HT_2 ANTAGONIST.

G.J. Marek* and G.K. Aghajanian. Depts. of Psychiatry and Pharmacology, Yale University School of Medicine, New Haven, CT 06508.

Recent electrophysiological studies have revealed a subpopulation of interneurons located near the border of layers II and III in rat piriform cortex that are excited by serotonin (5-HT; Sheldon & Aghajanian, 1990). It has been suggested that this excitation is mediated by 5-HT_2 rather than 5-HT_{1C} receptors (Sheldon & Aghajanian,1991). However, the pharmacological agents available for analysis were limited in specificity. In the present study, we tested a new, highly selective 5-HT_2 antagonist MDL 100,907 which has a 300 fold greater affinity for 5-HT_2 than 5-HT_{1C} or α_1 receptors (Schmidt et al.,1992).

Bath application of incremental concentrations of MDL 100,907 (1-10 nM) for 30 min periods potently blocked the activation by 5-HT of interneurons recorded from rat piriform cortex. Schild analyses resulted in a pA_2 ranging from 0.43-0.59 nM, which is close to the published K_i of 0.36 nM (Schmidt et al.,1992). In addition, parallel shifts in the 5-HT dose response curve were observed with MDL 100,907. Moreover, the effects of a 1 hr bath application of MDL 100,907 at 1 nM were largely reversed after a 20 min washout period. In contrast, a kinetic analysis of ritanserin, a 5-$HT_{2/1C}$ antagonist, could not be performed as equilibrium was difficult to achieve, blockade continued to progress after the perfusion of the drug was completed, and no washout was observed. These data confirm that 5-HT_2 rather than 5-HT_{1C} receptors mediate the excitation by 5-HT of interneurons in the piriform cortex. In addition, these results suggest that MDL 100,907 may be an excellent tool in electrophysiological investigations to differentiate 5-HT_2 from 5-HT_{1C} responses.

566.1

FURTHER PHARMACOLOGICAL CHARACTERIZATION OF SIGMA RECEPTOR REGULATION OF NMDA-STIMULATED DOPAMINE RELEASE IN RAT STRIATUM. G. M. Gonzalez-Alvear* and L. L. Werling. Dept. Pharmacology, The George Washington University Medical Ctr., Washington, D.C. 20037.

We have previously reported that (+)pentazocine inhibited NMDA-stimulated [^{3}H]DA release from rat striatal slices in a concentration-dependent, biphasic manner. We have now investigated the effects of several additional sigma ligands, as well as atropine, in this system.

Striata were dissected, chopped, and washed in Mg^+-free modified Krebs-HEPES buffer, then incubated with 15 nM [^{3}H]DA for 30 min. Slices were loaded into a superfusion apparatus and superfused with buffer to establish a low, stable baseline release. Tissue was stimulated to release [^{3}H]DA by a 2 min exposure to 25 μM NMDA. After return to the non-stimulating buffer, tissue was stimulated a second time (S2) for 2 min in the presence or absence of test compound (drug).

BD737, a sigma agonist, was found to inhibit NMDA-stimulated DA release from rat striatal slices. In contrast, the novel sigma antagonist, BD1008, reversed inhibition of DA release by 100 nM (+)pentazocine. Further examination revealed that BD1008 reversed inhibition of DA release by all concentrations of (+)pentazocine tested. Since (+)pentazocine, at concentrations above 1 μM, has been reported to have activity at cholinergic receptors, we examined the effects of atropine on (+)pentazocine inhibition of DA release. Atropine did not antagonize the inhibition of DA release by (+)pentazocine, obviating any cholinergic receptor-mediated effects. In addition, since (+)pentazocine, at very high concentrations, has been reported to interact with the PCP receptor, we investigated the ability of BD1008 to antagonize the inhibition of DA release produced by the PCP receptor selective drug, MK-801. BD1008 did not reverse the inhibition of DA release by MK-801. These data suggest that both sigma and PCP receptors can regulate the release of DA from striatum. (Supported by a grant from NIDA to LLW and by NIGMS predoctoral fellowship to GMG.)

566.2

DIZOCILPINE (MK-801) EXCITES NEOSTRIATAL NEURONS IN THE FREELY MOVING RAT. K.C. Hooper*, D.A. Banks, L.L. Johnson, and G.V. Rebec. Prog. Neural Science, Dept. Psychology, Indiana University, Bloomington, IN 47405.

In addition to dopaminergic innervation, the neostriatum receives glutamatergic input from neocortex and thalamus. The interactions among these inputs, however, are complex. For example, NMDA receptors on dopaminergic terminals inhibit dopamine release, and glutamate release is inhibited by D2 dopamine receptors on corticostriatal terminals.

We have previously shown that stimulation of D2 dopamine receptors with quinpirole inhibits the activity of striatal neurons in awake, behaving rats, whereas SKF-38393, a D1 receptor agonist, produces inconsistent effects (Hooper et al., Soc. Neurosci. Abstr. 18:995, 1992). As an extension of this research, we have begun a series of experiments to assess the electrophysiological effects of dizocilpine (MK-801), a non-competitive NMDA glutamate antagonist.

In contrast to quinpirole, MK-801 (0.10 or 0.25 mg/kg) increased the firing rate of neostriatal neurons, and this effect was accompanied by a dose-dependent increase in behavioral activation. These results suggest an important role for both D2 dopamine receptors and NMDA glutamate receptors in the regulation of neostriatal neuronal activity.

Supported by the National Institute on Drug Abuse, DA 02451.

566.3

KAPPA OPIOID RECEPTOR ACTIVATION MODULATES EXCITATORY AMINO ACID RESPONSES IN ACUTELY ISOLATED NEURONS FROM THE DORSAL HORN. M. Kolaj*, R. Cerne and M. Randić. Dept. of Vet. Physiol. and Pharmacol., Iowa State University, Ames,IA 50011.

To understand how κ-opioid receptor agonists affect the excitability of spinal dorsal horn (DH) neurons, we examined the actions of Dynorphin $A_{1\text{-}17}$($Dyn_{1\text{-}17}$) and *trans*-3,4-dichloro-N-methyl-N-[2-(1-pyrrolidinyl)cyclohexo]benzene acetamide(U-50,488H) on excitatory amino acids (EAA)-activated current responses of freshly isolated neurons from the spinal dorsal horn (laminae I-IV) of rats (8-13d) under whole-cell voltage-clamp conditions. Here we report that the inward current responses of DH neurons induced by α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA,10-50μM), kainate (KA,10-100μM) and N-methyl-D-aspartate (NMDA,100μM) were modulated by $Dyn_{1\text{-}17}$ and U-50,488H in a complex manner. When applied simultaneously with or prior to an EAA, $Dyn_{1\text{-}17}$(5pM-1μM) reversibly reduced the responses of DH neurons to AMPA (85.7 ± 1.3% of control, 17/26 cells), KA (79.5 ± 6.5%, 8/12 cells) and NMDA (77.0 ± 0.7%, 5/9 cells). In addition, $Dyn_{1\text{-}17}$ reversibly enhanced latency and the rise time of the AMPA-induced current in 77% of the examined cells. The effects were antagonized by naloxone (0.1μM, 5/7 cells) and κ_1-opioid receptor antagonist, nor-binaltorphimine (0.1-100nM, 5/6 cells). Following removal of $Dyn_{1\text{-}17}$ the EAA-induced responses were potentiated (AMPA:by 120.1 ± 6.1%; KA:by 115.5 ± 4.4%; NMDA:by 121.8 ± 4.2%) in about half of the cells. U-50,488H (1μM) had also two distinct effects on the peak amplitude of the initial transient component of the AMPA- and the NMDA-induced currents consisting of an initial depression (AMPA:to 89.5 ± 2.5%, 6/9 cells; NMDA:to 80.7 ± 5.3, 6/7 cells) followed by a potentiation (AMPA:by 119.7 ± 5%; 7/7 cells, NMDA:by 115 ± 5, 3/7 cells). These results indicate that κ-opioid receptor agonists modulate the AMPA, KA and NMDA receptors signaling function in a proportion of the rat spinal DH neurons (Supported by NS-26352 and IBN-9209462).

566.4

IN VIVO DOPAMINE RELEASE IN THE RAT PREFRONTAL CORTEX IS INCREASED BY STIMULATION OF THE MEDIODORSAL THALAMIC NUCLEUS. M.G.P.Feenstra, W.van der Weij, J.J.Hamstra, M.H.A.Botterblom and R.M.Buijs*, Graduate School Neurosciences Amsterdam, Neth. Inst. Brain Research, 1105AZ Amsterdam ZO, The Netherlands.

In the prefrontal cortex (PFC) dopaminergic (DA) projections from the ventral tegmental area (VTA) largely overlap with excitatory projections from the mediodorsal nucleus of the thalamus (MDT). We studied the possible involvement of the MDT in the regulation of DA release by using microdialysis in the medial PFC. Basal DA overflow in awake animals was 1.2 pg/50 μl (bilateral canulae) or 0.6 pg/50 μl (unilateral). Both NMDA (1 mM, 20 min) and AMPA (0.1 mM) strongly increased DA overflow, but this was correlated with the induction of epileptic effects, suggesting massive, synchronized neuronal activity.

Stimulation of the MDT by local perfusion of bicuculline (0.1 mM for 20 min) through a dialysis canula increased DA overflow in the PFC 2-5 fold without inducing epileptic activity. Thus, this experimental approach provides a more physiological means to increase excitatory input and demonstrates the facilitatory influence of the MDT on PFC DA release. Ongoing experiments are being carried out to test the possible involvement of presynaptic glutamate receptors on DA terminals vs polysynaptic activation of DA neurons.

566.5

EFFECTS OF DIPYRIDAMOLE ON DOPAMINE UPTAKE AND RELEASE IN VITRO: POSSIBLE RESERPINE-LIKE ACTION. K.T. Delle Donne*, S. Devraj and P.K. Sonsalla. Department of Neurology, Univ. of Med. & Dent. of New Jersey-Robert Wood Johnson Medical School, Piscataway, NJ 08854.

Dipyridamole (DPR) is a drug commonly used as an adenosine transport inhibitor. To examine the effects of endogenous adenosine on dopamine (DA) release, mouse neostriatal slices were superfused with DPR *in vitro*. DPR (20μM) caused a 65% inhibition of K^+-evoked endogenous DA release, but only 8-10% of the inhibition could be blocked by 10μM cyclopentyltheophylline, a potent adenosine receptor antagonist. This suggests that inhibition of DA overflow by DPR is not mediated primarily by adenosine actions. Furthermore, DOPAC overflow was enhanced 6-8 fold over basal during the 30-min pretreatment of the slices with DPR, which indicates substantially increased turnover of DA. Reserpine (10μM) exhibited a similar profile. DOPAC overflow was increased 20-fold during the 30-min pretreatment and K^+-stimulated DA release was decreased by 75%. [^{3}H]DA uptake into mouse neostriatal synaptosomes was inhibited by DPR with an IC_{50} of 23.4 ± 1.1μM. Inhibition of [^{3}H]DA uptake by nitrobenzylthioinosine (NBTI), a more selective adenosine uptake inhibitor, was much less potent with maximal inhibition of 30 ± 8% at 100μM. In contrast to DPR, amfonelic acid (10μM), a classic DA uptake inhibitor, produced no change in DOPAC overflow and a 2-3 fold increase in basal DA outflow during the 30-min pretreatment; following K^+ stimulation, DA overflow was enhanced 2.5-fold. These results suggest that, in addition to its effects on adenosine transport, DPR may also have DA-depleting effects similar to those of reserpine which decrease the ability of dopaminergic terminals to accumulate DA and to sustain the depolarization-sensitive pool of DA.

566.6

THE EFFECTS OF DOPAMINE ANTAGONISTS ON THE CHANGES OF CCK-LIKE IMMUNOREACTIVITY INDUCED BY ACUTE METHAMPHETAMINE ADMINISTRATIONS IN THE RAT BRAIN. N.Kawai[1], Y.Takamatsu[1], H.Yamamoto[1], A.Baba[1], T.Moroji[1] and K.Yoshikawa*[2]. 1; Dept. of Psychopharmacology, Tokyo Institute of Psychiatry, Tokyo 156, and 2; Dept. of Molecular Neurobiology, Tokyo Metropolitan Institute for Neurosci., Tokyo 183, JAPAN.

The effects of the dopamine D1- and D2-antagonists on the changes of CCK-like immunoreactivity (CCK-LI) induced by acute MAP-administration were investigated in the rat medial frontal cortex (MFC). Single administration of selective D2-antagonists, sulpiride (SUL) and YM-09151-2 (YM), and D1/D2-antagonist haloperidol (HP) reduced the CCK-LI in the rat MFC, while selective D1-antagonist SCH23390 (SCH) did not. Acute administration of MAP also reduced the CCK-LI in the rat MFC. This effect of MAP was reversed by SCH and HP, but not by SUL. Interestingly, another selective D2-antagonist YM completely reversed the effect of MAP. In the behavioral study, SCH, HP and YM blocked the stereotyped behavior induced by MAP-administration. SUL partially blocked the MAP-induced Stereotyped behavior only with large doses (250mg/kg). These results indicates the differential roles of dopamine D1- and D2-receptors in controlling the cortical CCK-containing neurons.

566.7

THE CCK ANTAGONIST, LORGLUMIDE (CR-1409), DOES NOT ALTER DOPAMINE LEVELS IN RAT CAUDATE AS MEASURED BY MICRODIALYSIS. K.A. Kedzie and K.J. Renner*. Dept. Biomedical Sciences, Southwest Missouri State Univ., Springfield, MO 65804.

Dopamine (DA) signals associated with 100 mM KCl-induced slow wave depolarization (SWD) in the rat caudate (CPu) are blocked by CCK-A receptor antagonists (Brain Res. 594:47, 1992). Failure to elicit KCl-induced DA signals following CCK-A antagonist treatments may be due to a CCK-DA interaction. We investigated this possibility by determining the effects of lorglumide (LORG) on basal and locally stimulated levels of DA in the CPu. Dialysis probes were implanted into the CPu of male Sprague-Dawley rats anesthetized with chloral hydrate (400 mg/kg). Samples were collected at 20 min intervals and analyzed for DA using HPLC with electrochemical detection. LORG (640 μg/kg, Rotta Labs.) or saline were administered i.v. LORG did not significantly affect basal DA levels (n=9). Perfusion of 30 mM KCl 20 min following saline (n=8) or LORG (n=9) increased DA to 352 ± 51 and 386 ± 80 % of basal levels, respectively. The results suggest that CCK-A receptors do not mediate putative CCK-DA interactions in the CPu. The inhibition of SWD in the CPu by LORG does not appear to directly involve the dopaminergic system.

566.8

SYSTEMIC MORPHINE INCREASES DOPAMINE RELEASE IN THE MPOA OF MALE RATS. L. Matuszewich,* B. Finnerty, D. Lorrain and E. M. Hull. Department of Psychology, SUNY at Buffalo, Buffalo, NY 14260.

Morphine has been shown to affect dopamine activity in the mesolimbic dopamine (DA) tract. Infusions of morphine into the ventral tegmental area increased DA metabolism in the nucleus accumbens and also facilitated sexual behavior in male rat castrates (Mitchell & Stewart, 1990). Another brain site involved in the DA regulation of male copulatory behavior is the medial preoptic area MPOA (Hull, et al., 1986). This study examined the effects of morphine (15 mg/kg) on DA and its metabolites in MPOA.

The effects of a single systemic injection of morphine (15 mg/kg) were assessed through microdialysis. Samples collected every 20 minutes were analyzed using high performance liquid chromotography (HPLC) with electrochemical detection. Dopamine and its metabolites increased significantly in the MPOA following a morphine injection (DA, $p<.05$; DOPAC, $p<.01$; HVA, $p<.05$). This parallels the effects of systemic morphine on the mesolimbic DA tract.

Sponsored by NSF Grant # IBN9211660

566.9

THE NITRIC OXIDE PRECURSOR, L-ARGININE, INCREASES DA AND 5-HT RELEASE IN MEDIAL PREOPTIC AREA OF MALE RATS. D. S. Lorrain and E. M. Hull*. Psychology Department , SUNY at Buffalo, NY 14260.

The gas nitric oxide (NO) is an intercellular messenger that has been implicated in glutamate-induced dopamine (DA) release in striatal slices (Hanbauer et al., 1992), and norepinephrine and acetylcholine release in hippocampal slices (Lonart et al., 1992).

In the present experiment, sixteen animals received the NO precursor L-arginine (100 μM) into the MPOA through a microdialysis probe; half of these also received the inactive isomer D-arginine (100 μM) for 3 samples before L-arginine administration. Seven additional animals received the NO synthesis inhibitor N-monomethyl L-arginine (NMMA, 400 μM) through the dialysis probe; four of these also received L-arginine (100 μM) plus NMMA.

L-arginine significantly increased extracellular levels of DA, serotonin (5-HT), and the DA metabolites DOPAC and HVA. Furthermore, these increases were completely blocked by co-administration of the NO synthesis inhibitor NMMA. The inactive isomer D-arginine was ineffective. NMMA by itself decreased DA below baseline.

These data suggest that NO may modulate the release of DA and 5-HT in the MPOA. Both DA and 5-HT in the MPOA have been implicated in the control of male sexual behavior, with DA facilitating and 5-HT generally inhibiting copulation and genital reflexes (Hull et al., 1986 & 1992; Pehek et al., 1989; Warner et al., 1991). The present data, together with those cited above, suggest that NO may enhance the release of several neurotransmitters. Its function in the MPOA may be to modulate the prolonged DA release that accompanies (and facilitates) copulation. The mechanisms of this enhancement and its generality are at present unknown.

566.10

NEUROKININS ENHANCE EXCITATORY AMINO ACID-INDUCED ACTIVATION OF THE NEONATAL RAT SPINAL CORD *IN VITRO*.
Urban, L.* Naeem, S. Patel, I.A. and Dray, A. Sandoz Institute for Medical Research, London WC1E 6BN, UK.

Neurokinins (NK) facilitate excitatory amino acid (EAA) induced currents in single dorsal horn cells [Rusin et al., J. Neurophysiol., 68. (1992) 256). Here we have further studied the interactions between NKs and EAAs in the neonatal spinal cord.

Spinal cord with attached tail was prepared form 1-2 day old rats and maintained *in vitro*. Synaptic activity was recorded as a ventral root (VR) depolarization from the L_4 ventral root following drug superfusion.

Submaximal VR-responses were evoked by 10s application of EAA agonists. In the presence of subthreshold concentrations of NK-A and SPOMe, but not substance P NMDA and quisqualate responses were enhanced significantly, while kainate responses were unaltered. The enhancement was selectively blocked by MEN10 376 and CP 96345 respectively. In the presence of a cocktail of endopeptidase inhibitors (captopril, thiorphan and bestatin, each 1.0μM) subthreshold doses of substance P significantly enhanced NMDA and quisqualate responses which could be blocked selectively by CP96 345. The possibility that the enhancement of EAA effects was mediated via protein kinase C (PKC) activation was tested using the inhibitor staurosporine (1.0μM). Staurosporine blocked the facilitation of the EAA responses after a 30 minutes perfusion. At this time all NK-evoked responses were blocked while EAA-evoked responses were not affected. These data suggest that NK-2 receptor activation selectively enhances NMDA and quisqualate evoked depolarization of the ventral root. In circumstances where an excess of SP occurs in the spinal cord NK1 receptors may also produce a similar effect. NK-induced enhancement of EAA responses is mediated via PKC activation.

566.11

ONTOGENY OF AN INTERACTION BETWEEN DOPAMINE AND κ OPIOID SYSTEMS: EFFECTS ON MOTOR ACTIVITY AND SENSORY RESPONSIVENESS IN THE FETAL RAT. D. K. Simonik*, S. R. Robinson, and W. P. Smotherman. Laboratory of Perinatal Neuroethology, Center for Developmental Psychobiology, Binghamton University, Binghamton, NY 13902-6000.

Milk has behavioral effects in the fetal rat that are caused by changes in the dopamine and κ opioid systems. Administration of dopaminergic and κ opioid drugs to the fetal rat suggests that these systems interact on E21 of gestation. In the present study, fetuses were observed on E19, E20 and E21 to characterize the effects of DA D1 and κ opioid agonists and antagonists on fetal motor activity and sensory responsiveness. Fetal subjects were observed to assess spontaneous motor activity and sensory responsiveness in two behavioral bioassays. Changes in motor activity and the incidence of elicited facial wiping were used to measure responses to tactile and chemosensory stimuli. To characterize possible interactions between the DA and κ opioid systems, fetal subjects were injected ip with a D1 agonist (SKF-38393) and κ antagonist (nor-binaltorphimine), or a κ agonist (U50,488) and D1 antagonist (SCH-23390). Drug manipulations resulted in changes in fetal motor activity as well as responsiveness to tactile and chemosensory stimuli. The data support the interpretation that an interaction between the DA and κ opioid systems appears on E20 and is clearly present on E21; activity in the DA system promoted κ opioid activity to bring about changes in motor behavior and sensory responsiveness. A qualitatively different interaction between these two systems was evident on E19. These data suggest that pharmacological activation of the DA and/or κ opioid systems can produce behavioral effects in the rat fetus, and that the nature of the interaction between these two systems changes markedly during the last few days of gestation.

This research is supported by Grant HD 16102 to WPS and HD 28231 to WPS and SRR.

566.12

CLOZAPINE PRODUCES DIFFERENT EFFECTS FROM THOSE OF FLUPHENAZINE-N-MUSTARD (FNM) ON RECEPTORS AND mRNAs ASSOCIATED WITH DOPAMINERGIC AND OPIOID PEPTIDERGIC SYSTEMS OF MOUSE BRAIN. J.-F. Chen*, S.-P. Zhang, T.A. Connell, L.-W. Zhou, and B. Weiss. Division of Neuropsychopharmacology, Department of Pharmacology, Medical College of PA, Philadelphia, PA 19129.

Clozapine (CLZ) is a novel antipsychotic agent, having relatively few extrapyramidal side effects. To examine and compare the biochemical effects of CLZ with those produced by classical antipsychotics, we determined the effects of CLZ on the levels of D_1 and D_2 dopamine (DA) receptors and D_1 and D_2 receptor mRNAs, and on the levels of mu and delta opioid receptors and proenkephalin mRNA. Receptor densities were assayed by quantitative receptor autoradiography using radiolabeled ligands specific for each receptor. mRNA levels were measured by in situ hybridization histochemistry using [^{35}S]-labeled specific oligonucleotide probes. Continuous infusion of CLZ (19 μmol/kg/hr, s.c.) with Alzet minipumps increased the level of D_1 receptors in mouse striatum but failed to significantly change the levels of D_2 receptors or D_1 or D_2 mRNAs. Continuous treatment with CLZ also increased the densities of the mu and delta opioid receptors and increased the levels of proenkephalin mRNA. In contrast to the effects of CLZ, repeated administration of the relatively selective D_2 antagonist FNM slightly decreased D_1 receptors and markedly reduced D_2 receptors. It also reduced D_1 mRNA and increased D_2 mRNA. In addition, FNM decreased mu and delta opioid receptors and increased proenkephalin mRNA in rat striatum. This study shows that CLZ has effects on DA and opioid peptidergic systems that are different in some respects from those produced by selective D_2 DA antagonists. Further, the data support the hypothesis that altered opioid peptidergic activity may be involved in the antipsychotic activity of CLZ. Supported by MH42148.

566.13

TIME-COURSE OF CHANGES IN STRIATAL LEVELS OF DA UPTAKE SITES, DA D2 RECEPTOR AND PREPROENKEPHALIN mRNAs AFTER NIGROSTRIATAL DOPAMINERGIC DENERVATION IN THE RAT. M. Chritin, C. Feuerstein and M. Savasta*. INSERM-LAPSEN U.318, Pavillon de Neurologie, CHU de Grenoble, BP 217, 38043 Grenoble cedex 9, France.

Changes in striatal dopamine uptake sites, D_2 receptor and preproenkephalin (PPE) mRNA levels provoked by unilateral 6-hydroxydopamine-induced lesion of the nigrostriatal dopaminergic (DA) pathway were studied by quantitative autoradiography and in situ hybridization (ISH) in rats sacrificed at different post-lesional delays.

The disappearance of DA terminals as visualized with the labelling of dopamine uptake sites with [^{3}H]GBR 12935 became significant 36 hours after the lesion and was almost complete at a delay of 7 days. PPE mRNA amounts significantly increase (+24%) already at the shortest delay studied (9 hours after the lesion) while the labelling of the uptake sites on DA terminals was not affected. The time course increase of PPE mRNA levels was progressive until 21 days post-lesion where it reached its maximum (+132%) and remained stable up to the latest delay studied (60 days). Conversely D_2 mRNA contents remained unchanged up to 5 days post-surgery and then increased relatively quickly since at 7 days post-lesion their levels were near (+21%) the maximum observed which was reached at 21 days post-lesion (+32%).

In contrast with PPE mRNA variations, the amplitude of increase of D_2 mRNA contents were always weaker.

Moreover, whatever the post-lesional delay examined, quantitative modifications of DA uptake sites, D_2 and PPE mRNAs were globally more pronounced in the dorsolateral region of the striatum than in the medial one.

This study shows that 6-hydroxydopamine induced-lesion of the nigrostriatal pathway leads to the increase of the contents of the mRNAs coding for Met-enkephalin and DA D_2 receptor, but with a differential time course, the first one increasing quite immediately after the lesion while the second one is significantly modified only after 1 week. These results suggest a time-dependent differential sensitivity to the degree of DA denervation of both major components implicated in the striatopallidal output.

566.14

ATTENUATION OF HALOPERIDOL-INDUCED DOPAMINE RELEASE BY 8-OH DPAT IN THE RAT: A CORRELATE FOR THE ANTICATALEPTIC EFFECTS OF 8-OH-DPAT? H. Sommermeyer, J. De Vry, J. Greuel* and T. Glaser. Institute for Neurobiology, Troponwerke GmbH & Co. KG, Berliner Strasse 156, 51063 Köln, FRG

The classical neuroleptic haloperidol (HAL) has antipsychotic properties, but induces also extrapyramidal side effects. While the antipsychotic properties of neuroleptics may be due to their action on the mesolimbic and/or mesocortical dopamine system, their extrapyramidal side effects have been attributed to their action on the nigrostriatal dopamine system. It has been reported, that 8-hydroxy-2-(di-N-propylamino) tetralin (8-OH-DPAT), a selective serotonin$_{1A}$ receptor agonist, blocks the HAL-induced catalepsy (Invernizzi et al. (1988) Neuropharmacology 27: 515-518). In Wistar rats, catalepsy induced by 1 mg/kg (s.c.) HAL was reversed by combined administration of 0.3 mg/kg (s.c.) 8-OH-DPAT. For getting insight into the underlying mechanism of the anticataleptic effects of 8-OH-DPAT, the effects of HAL, 8-OH-DPAT, and a combination of HAL and 8-OH-DPAT were characterized by microdialysis measurements of dopamine (DA) release in the striatum of freely moving Wistar rats. HAL (1 mg/kg, s.c.) increased DA release maximal 1.8-fold, whereas 8-OH-DPAT (0.3 mg/kg, s.c.) had no effect on the DA level in the striatum. Interestingly, after simultaneous administration of HAL (1 mg/kg, s.c.) and 8-OH-DPAT (0.3 mg/kg, s.c.) no changes in the extracellular DA level were detectable. The attenuation of HAL-induced DA release in the striatum by 8-OH-DPAT is possibly a correlate for the reversal of HAL-induced catalepsy by this serotonin$_{1A}$ receptor agonist.

566.15

FUNCTIONAL EXPRESSION OF CLONED α_2C4- AND β_2-ADRENERGIC AND δ-OPIOID RECEPTORS IN *XENOPUS* OOCYTES. Angela K. Birnbaum*, Diane R. Wotta, Ping Y. Law, and George L. Wilcox. Dept. of Pharmacology, University of Minnesota, Minneapolis, MN 55455

The *Xenopus* oocyte is a useful functional expression system for many seven-transmembrane receptors coupled through G proteins. The majority of the receptors expressed and electrophysiologically detected in the oocyte couple through the IP_3 system via endogenous calcium-activated chloride channels. Although the oocyte has also been shown to be capable of expressing receptors that exert their actions through the cAMP second messenger system, electrophysiological detection was not possible in the absence of co-expressed cAMP-regulated ion channels. By injecting mRNA encoding the cystic fibrosis transmembrane conductance regulator (CFTR, a cAMP-regulated chloride channel), we have characterized the function of one opioid (δ) and two adrenergic (α_2C4- and β_2) receptors: activation of these cloned receptors increases the IBMX-induced, cAMP-mediated generated chloride currents that can be recorded by two-electrode voltage clamp.

G protein-coupled receptors couple to different G proteins depending on concentration of expressed receptors. Using relatively large amounts of injected mRNA (10ng), we have observed enhancement of the IBMX-induced current responses by adrenergic and opioid agonists. Concentration-dependent responses were observed for the agonists UK14304 (α_2), DADLE (δ), and isoproterenol (β_2) and antagonized by idaxozan, naloxone, and propranolol, respectively. In most cell and neural tissue systems, activation of α_2 and δ receptors is generally observed to decrease levels of cellular cAMP via G_i proteins. By contrast, we observed enhancement of the IBMX response. However, the interaction we observe using isobolographic analysis of co-activation of co-expressed α_2 and β_2 receptors is inhibitory suggesting that G_i protein coupling can also be detected with this system. Functional expression of G_i- and G_s-coupled receptors in the oocyte system may prove to be a useful adjunct to parallel studies in neural systems. [Supported by NIDA:T32-DA07234(AB);DA-04274& DA-00145(GLW);DA-07339(PYL)]

STORAGE, SECRETION, AND METABOLISM II

567.1

SOLUBLE GLUTAMIC ACID DECARBOXYLASE (GAD) IS PRESENT MAINLY AS A DIMER IN BRAIN. S. N. Sheikh and D. L. Martin*. Wadsworth Center, New York State Dept. of Health and Dept. Environmental Health & Toxicology, SUNY, Albany, NY 12201-0509

GAD is present in brain as at least two forms, GAD_{65} and GAD_{67}, which are the products of two genes. Current data indicates that GAD is a multimer and studies with denaturing polyacrylamide gels suggest that some GAD is dimeric, but the relative amount of multimeric and monomeric GAD in brain is unknown. We have investigated this by using non-denaturing gradient polyacrylamide (PAA) gels along with immunoblotting. Rabbit antisera were produced against synthetic peptides selected from the amino acid sequences of the two forms of GAD. Each antiserum produced a high level of anti-peptide antibodies when tested by ELISA and each recognized the appropriate form of GAD on immunoblots of SDS-PAGE gels. To investigate the subunit composition of soluble native GAD, rat brain supernatants were analyzed on 5-25% and 5-30% PAA linear gradient gels. Almost all soluble GAD was present in multimeric form; brain contains very little monomeric GAD. The molecular weight of multimeric native GAD was estimated as 134 ± 3.5 kDa, indicating that soluble GAD exists in a dimeric form in brain. The average molecular weight of native GAD on these gels did not change when brain supernatants were diluted, indicating that the GAD dimer does not result from an easily reversible aggregation of the subunits. Whether native GAD is present as both hetero- and homodimers is under investigation. Supported by grant MH35664 from USPHS/DHHS.

567.2

THE EFFECTS OF INTRACELLULAR CALCIUM AND EXTRACELLULAR AMMONIA ON ASTROCYTE INTERMEDIARY METABOLISM. WC Gamberino, WA Brennan, jr.* and KF LaNoue. Dept. of Cell. and Mol. Physiology, Penn State Coll. of Medicine, Hershey, PA 17033

Astrocytes play an important role in glutamine/glutamate metabolism and NH3 fixation in the brain. However, the regulation of these pathways in astrocytes is not clear. In many cells, the level of intracellular calcium, [Ca^{2+}i], is important in regulating glutamate metabolism through α-ketoglutarate (αKG) levels. CO_2 fixation furnishes citric acid cycle intermediates used in these pathways in astrocytes. In liver cells, increases in [Ca^{2+}i] indirectly stimulate CO_2 fixation. To elucidate the role of [Ca^{2+}i] in regulateing astrocyte CO_2 fixation, secondary cultures of neonatal rat astrocytes were incubated with a modified Krebs Ringer bicarbonate buffer containing 15 mM $H^{14}CO_3$ (10000 dpm/nmol) in one of three conditions: 1) low [Ca^{2+}i] of 80 nM induced by incubation with 5mM EGTA, 2) control resting [Ca^{2+}i] of 125 nM, and 3) high [Ca^{2+}i] of 350 nM induced by incubation with 100 μM ATP. To determine how NH3 fixation is affected by calcium levels, the same three conditions were applied to astrocytes in the same buffer containing 2mM NH_4Cl to simulate an ammonia load. The cellular extracts as well as the extracellular buffers were analyzed by anion exchange chromatography. The preliminary data indicate: 1) In contrast to hepatocytes, increased [Ca^{2+}i] does not increase CO_2 fixation in astrocytes. The most striking effect of increasing [Ca^{2+}i] was an increase in the rate of αKG efflux from astrocytes. This occurred at the expense of intracellular αKG and was specific for αKG. 2) The production of labelled pyruvate indicates that some of the fixed CO_2 cycles from oxalacetate back to pyruvate via phosphoenolpyruvate carboxykinase and pyruvate kinase. Furthermore, this cycling appears to be stimulated by low [Ca^{2+}i]. 3) The presence of 2mM ammonia in the buffer doubled levels of labelled glutamine both inside and outside the cells, but caused no increase in CO_2 fixation nor any significant decrease in either αKG levels or glutamate levels. The results suggest [Ca^{2+}i] may be an important modulator of astrocytes' capacity to return to neurons carbon lost as glutamate.

567.3

GAD_{67} PROTEIN IN RAT CEREBRAL CORTEX IS HIGHLY SENSITIVE TO CHANGES IN GABA CONCENTRATION. K. Rimvall* and D.L. Martin. Wadsworth Center for Labs and Research, New York State Dept. of Health, Albany, NY 12201.

Increased intracellular GABA levels (> 2.5-fold) in rat brain lead to large decreases in the amount of GAD_{67} protein (75-80%) through a mechanism involving either a change in GAD_{67} protein stability or GAD_{67} mRNA translation (Rimvall et al., J. Neurochem. 60:714-720, 1993). To determine whether more biologically relevant changes in intracellular GABA would still induce significant changes in GAD_{67} protein levels, rats were injected with varying doses of γ-vinyl-GABA (GVG; Vigabatrin®) and GABA and GAD_{67} protein levels in the cerebral cortices were determined by HPLC and quantitative immunoblotting. To distinguish between the neuronal pool of GABA and the whole-tissue GABA (i.e. neuronal and astrocytic), the analysis was done using both synaptosomal and whole-tissue preparations. After three daily injections of GVG (30 mg/kg and 150 mg/kg) both GABA- and GAD_{67}-levels reached steady-state. Whole-tissue GABA increased 1.5-fold (30 mg/kg) and 4-fold (150 mg/kg) and GAD_{67} protein levels decreased by 25% and 75%. Rats were then injected with seven different doses of GVG (30 to 150 mg/kg) for 5 days. With increasing GVG, we observed a gradual increase in whole-tissue and synaptosomal GABA and gradual decreases in total GAD activity and GAD_{67} protein levels. The levels of GAD_{65} protein remained constant att all GVG concentrations. GAD_{67} was very sensitive to GABA - for every 1% increase in nerve-terminal GABA we saw a 2% reduction in GAD_{67} levels. Our results demonstrate that the GAD_{67} protein is strictly controlled by intraneuronal GABA, and we suggest that this regulatory mechanism has important implications for the physiological regulation of inhibitory neurotransmission in the mammalian CNS. Supported by grant MH35664 (USPHS/DHHS).

567.4

NEURONAL GLUTAMATE METABOLISM AND ATP DEPLETION IN HIPPOCAMPAL SLICES. J.E. Madl*. Dept. Anatomy & Neurobiology, CSU, Ft. Collins, CO 80523.

ATP depletion may release Glu and other transmitters by reversing uptake systems, resulting in excitotoxicity and neuronal death. However, Glu is also a key metabolite in many metabolic pathways, including several that are important in ATP production. Lack of Glu due to release might inhibit these pathways and decrease further ATP synthesis. Effects of metabolic inhibitors on Glu and ATP in rat hippocampal slices were quantified by HPLC and immunocytochemical localization of Glu was used to examine sites of metabolism and release. ATP depletion induced by inhibition of glycolysis resulted in Glu loss consistent with: 1) Glu release and 2) conversion of Glu to Asp by transamination and part of the Krebs cycle. Immunocytochemical localization of Glu suggested conversion occurred in neuronal cell bodies, dendrites and terminals. During hypoglycemia, conversion of Glu to Asp provided some ATP, but the depletion of ATP remained severe enough to produce a large release of Glu. Inhibition of a different Glu-utilizing pathway, the malate-aspartate shuttle (MAS), produced severe depletion of ATP and Glu. Depletion of Glu by MAS inhibition was consistent with Glu release without the conversion of Glu to Asp that was prominent during hypoglycemia. In contrast to neurons, some glia were able to retain or accumulate Glu during insults, suggesting differences between glial and neuronal metabolism may result in glial uptake of Glu during metabolic insults. Glu metabolism by MAS and the Krebs cycle can contribute to ATP synthesis and reduce Glu release.

567.5

REGULATION OF BRAIN L-GLUTAMATE DECARBOXYLASE BY PROTEIN PHOSPHORYLATION AND DEPHOSPHORYLATION. Jun Bao*, Britto Nathan and J.-Y. Wu. Physiology and Cell Biology, University of Kansas, Lawrence, KS 66045.

γ-aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the vertebrate brain. In the brain, the rate limiting step in GABA biosynthesis is the decarboxylation of L-glutamic acid by L-glutamate decarboxylase (E.C.1.1.15, GAD). The level of GABA in the brain is highly regulated, primarily by GAD. Despite its importance, the precise mechanism underlying regulation of GAD activity is still not clear. Here we report that GAD activity was inhibited by the conditions favoring protein phosphorylation and this inhibition could be reversed by phosphatase treatment. Furthermore, this inhibition was resulted from the suppression of a Ca^{2+}-dependent phosphatase in the system. Direct evidence of phosphorylation of GAD was demonstrated by incorporation of ^{32}P into GAD protein. These results indicate that GAD activity is regulated by phosphorylation and dephosphorylation in the brain. Finally, a Ca^{2+}-dependent phosphatase, calcineurin, is implicated to be responsible for activation of GAD. [Supported in part by NIH grant NS-20978 and NSF grant BNS-88 20581]

567.6

D-AMPHETAMINE-INDUCED DISPLACEMENT OF [^{123}I]IBF EQUILIBRIUM BINDING IN NON HUMAN PRIMATES M. Laruelle , A Abi-Dargham*,M. Al-Tikriti, S. S. Zoghbi, Y. Zea-Ponce, R. M. Baldwin, D. S. Charney, P. B.Hoffer, R. B. Innis. Yale Univ. and West Haven VA Medical Center, West Haven, CT 06516

We previously showed that an *in vivo* binding equilibrium state can be reached and sustained during constant infusion of a SPECT radiotracer (Laruelle et al., Eur J Pharmacol, 230:119-123, 1993). This paradigm can be used to evaluate the effect of pharmacological challenge tests. Four SPECT experiments were performed on two baboons to evaluate the ability of d-amphetamine to induce displacement of [^{123}I]IBF (iodobenzofuran), a high affinity dopamine D2 antagonist. [^{123}I]IBF was administered as a bolus (3.7 ± 0.3 mCi, ± SEM, n=8) followed by a constant infusion (1.8 ± 0.2 mCi/h) for 480 min. Data were acquired on the CERASPECT camera. Equilibrium was reached at 150 min. D-amphetamine was injected i.v. at 240 min. These injections induced a slow and prolonged decrease of the striatal / occipital ratio which stabilized at a lower level 120 to 180 min after d-amphetamine injection. In both animals, a 0.1 mg d-amphetamine dose induced a modest decrease of the striatal/ occipital ratio (5-7%). At higher doses (0.3 to 1 mg/kg), d-amphetamine decreased this ratio by 12 ± 2% in one baboon and 26 ± 2% in the other baboon (baboon 1 versus baboon 2, n=3, p=0.021). As d-amphetamine has little affinity for D2 receptors, this effect is presumably mediated by the release of endogenous dopamine. These studies suggest that dynamic, non invasive, in vivo receptor imaging techniques can be used to obtain information about endogenous transmitter release .

567.7

IMMUNOLOGICAL TARGETING OF TOXINS TO CENTRAL NEUROPEPTIDERGIC NEURONS : EXPRESSION OF VASOPRESSIN, OXYTOXIN AND CORTICOTROPIN RELEASING FACTOR mRNA STUDIED BY IN SITU HYBRIDIZATION IN THE TARGET NEURONS. A. Burlet*, A. Rafai, B. Haumont-Pellegri, F. Tilders, J.P. Nicolas and C. Burlet. INSERM U308, 38, rue Lionnois - F-54000-Nancy (France).

We previously demonstrated that cellular toxins (ricin A chain and monensin) injected with a cytotoxic IgG2a monoclonal antibody (MAb) to a neuropeptide, namely vasopressin (AVP) or corticotropin releasing factor (CRF), may specifically penetrate the peptidergic neurons and induce disturbances of cellular functions. We studied here the effects of one microinjection of CRF-MAb, AVP-MAb or non specific IGg with toxins near the paraventricular nuclei (PVN) on the mRNA expression of AVP, oxytocin (OT) and CRF by in situ hybridization, using synthetic oligonucleotide probes labelled with dATP-^{35}S.

The immunological targeting of toxins to AVP neurons increased the mRNA expression of AVP and decreased the mRNA expression of OT in the target site when observed 24h latter. One week after the injection, the mRNA expression of AVP in the PVN neurons was markedly decreased whereas the mRNA expression of OT was increased. In the same experiments, the impairment of cellular functions of AVP neurons in PVN differently modified the mRNA expression of AVP and OT outside the injection site, at the SON level for example. The immunological targeting of toxins to CRF neurons decreased the mRNA expression of CRF in the target site but differently altered the mRNA expression of AVP and OT, according to the post-injection time which was observed.

The evolution of the mRNA expression of the neuropeptides may be explained by the impact of the acute neutralization of the neuropeptide which preceded the long-term impairment of the neuron biosynthetic functions.

567.8

LONG-LASTING INHIBITION OF BRAIN NITRIC OXIDE SYNTHESIS BY SYSTEMIC ADMINISTRATION OF NITRO-L-ARGININE METHYL ESTER X. Xu[1]*, F. Zhang[1], J. Hu[2], E. E. El-Fakahany[2] and C. Iadecola[1], Dept. of Neurology[1] and Psychiatry[2], Univ. of Minnesota, Minneapolis, MN 55455.

N^{ω}-substituted L-arginine analogues are widely used *in vivo* as inhibitors of brain nitric oxide synthase (NOS). However, the dose-response relationships and the time-course of the NOS inhibition after systemic administration have not been fully elucidated. We, therefore, studied the dose-response characteristics and the temporal profile of NOS inhibition induced by i.v. administration of nitro-L-arginine methyl ester (L-NAME). Rats were anesthetized with halothane and a cannula was placed in the femoral vein. After recovery from anesthesia, L-NAME was administered i.v. at 5, 10 or 20 mg/kg increments every 30 min until the desired cumulative dose was obtained (5, 10, 20 and 40 mg/kg; n=7 per dose). Rats were sacrificed 30 min after the last dose. NOS catalytic activity was assayed in forebrain homogenates using the method of Bredt and Snyder (PNAS, 86:9030, 1989). L-NAME attenuated brain NOS activity by 26±8% at 5 mg/kg ($p>0.05$), 40±5% at 10 mg/kg ($p<0.05$), 53±8% at 20 mg/kg ($p<0.05$) and 55±4% at 40 mg/kg ($p<0.05$). After L-NAME (20 mg/kg; single dose i.v.; n=5 per group) NOS activity was attenuated ($p<0.05$) by 33±6% at 30 min, 52±5% at 2 hrs, 55±2% at 24 hrs, 29±3% at 48 hrs and 24±3% at 96 hrs. We conclude that, intravenous administration of L-NAME leads to a dose-dependent and protracted inhibition of brain NOS catalytic activity. However, NOS inhibition is not complete even at the highest dose tested, a finding that may reflect a limited transfer of L-NAME across the blood-brain barrier. The finding that the inhibition persists for several days after a single administration is consistent with the hypothesis that nitro-L-arginine, the active principle of L-NAME, binds to NOS irreversibly (BBRC, 176:1136, 1991). Thus, L-NAME, administered systemically at concentrations commonly used in physiology experiments, leads to a partial but long-lasting inhibition of brain NOS activity *in vivo*. (Supported by the L. Sklarow fund and the American Heart Association)

567.9

PHOSPHOGLUCOMUTASE BECOMES GLYCOSYLATED AND MEMBRANE ASSOCIATED FOLLOWING DEPOLARIZATION IN PC12 CELLS AND CORTICAL SYNAPTOSOMES. N. A. Veyna*, P. Bounelis, and R. B. Marchase. Department of Cell Biology, The University of Alabama at Birmingham, Birmingham, AL 35294.

We have previously reported that the glycogenolytic enzyme phosphoglucomutase (PGM) is post-translationally modified by the addition of a glucose-1-phosphate moiety. In rat cortical synaptosomes and PC12 cells the levels of this cytoplasmic modification were greatly enhanced in response to secretory stimuli. We now present evidence that the stimulus-induced post-translational glycosylation of PGM is accompanied by the association of the protein with a membrane. Cortical synaptosomes that had been either maintained in control conditions (2.4 mM KCl) or exposed to depolarization (50 mM KCl) were fractionated into membranes and cytoplasm. Using Western blot analysis with a PGM-specific monoclonal antibody, increased immunoreactivity was found in the membranes of synaptosomes that had been depolarized for 5 seconds, with a concomitant loss of immunoreactivity in the cytoplasmic fraction. Under nondepolarizing conditions PGM immunoreactivity was present primarily in the cytoplasm. Freeze-thaw permeabilization was used to introduce [β^{35}S]UDP-glucose into synaptosomes prior to depolarization and fractionation. Under depolarizing conditions virtually all incorporated radioactivity was located in the membrane fraction, while under nondepolarizing conditions very little radioactive label was incorporated into either fraction. In addition, nondepolarized PC12 cells that were permeabilized with Triton X-100 for 3 minutes prior to fixation displayed minimal PGM immunofluorescence. Following depolarization, however, PGM immunoreactivity was retained within the cell in a punctate pattern. These data suggest that the population of PGM that becomes glycosylated in response to depolarization also becomes associated with a membranous structure in both synaptosomes and PC12 cells.

567.10

CONCENTRATION AND STORAGE OF BIOTIN IN THE OLFACTORY CORTEX F. Scalia*, S. Eisner and J.Y. Lettvin. Dept. of Anat. & Cell Biol., SUNY-Health Sci. Cntr., Brooklyn, NY 11203 and Dept. of Bioengineering, Rutgers Univ., Piscataway, NJ 08855.

Further characterization of the subfield of olfactory cortex in which regenerating optic nerve axons can synapse involves study of its cellular organization, neural connections, and chemoarchitecture. Biotin (BT) (vit. H) is an essential dietary cofactor in carboxylation reactions. Although brain tissues are enriched in BT, little further is known about its specific localization and role in CNS function. BT was detected in brain sections of adult Rana pipiens, where it formed compact, spherical, intracellular aggregates of 0.5-2.0 um diam., by means of incubation in avidin-HRP and subsequent reaction with Ni-Co-DAB, after glutaraldehyde fixation. In transverse sections, the aggregates formed a prominent, narrow layer positioned at the innermost level of layer I of the lateral prominence of the olfactory cortex. The plate extended as a ribbon along this boundary zone, separating the pars dorsalis and ventralis of the olfactory cortex for their full A-P extent. The prominence of this field was increased by intragastric or parenteral delivery of BT or biocytin (up to 100 ug/gm). The BT concentrating structures delimit the dorsal border of the optic recipient subfield of the olfactory cortex. (Supported by NIH grant EY05284.)

567.11

HUMAN KYNURENINE AMINOTRANSFERASE II: PURIFICATION FROM LIVER AND COMPARISON WITH THE BRAIN ENZYME. E. Okuno*, M. Nakamura, H. Baran[1], R. Schwarcz[1], and R. Kido. Wakayama Med. Coll., Wakayama 640, Japan and [1]Maryland Psychiatric Research Center, Baltimore, MD 21228.

Two kynurenine aminotransferases (KAT I and KAT II) are capable of producing the neuroinhibitory and neuroprotective compound kynurenic acid in the human brain. We have now purified KAT II to homogeneity from human liver using, in succession, heat treatment, $(NH_4)_2SO_4$ fractionation, DEAE-Sepharose, Butyl-cellulofine, Sephadex G-150, Hydroxyapatite and Mono-Q column chromatography. The purified enzyme showed a single protein band (MW 47kDa by SDS-PAGE). Purified liver KAT II was then compared to partially purified brain KAT II (cf. Brain Res. 542:307, 1991). Both enyzmes showed virtually identical substrate specificity and inhibition by amino acids. Moreover, there was agreement between liver and brain KAT II with regard to activation by acetate, pH optimum, K_m values for amino acids and keto acids, and molecular weight (assessed by sucrose gradient centrifugation). Anti-KAT II antibodies were produced and partially purified. Subsequent immunoblotting analyses confirmed that KAT II from human liver and brain are immunologically identical. In immunotitration experiments, the anti-KAT II antibody did not recognize human brain KAT I. Pure KAT II and its antibody can be expected to serve as valuable tools in further studies of kynurenic acid production in the human brain. Supported by USPHS grants NS 28236 and MH 44211.

567.12

BIOSYNTHESIS OF CONJUGATES OF PHENYLACETIC ACIDS IN THE RAT STRIATUM. L. E. Dyck*, D. A. Durden and A. A. Boulton. Neuropsychiatric Research Unit, Dept. of Psychiatry, Univ. of Saskatchewan, Saskatoon, Canada S7N 0W0

The trace acids, phenylacetic acid (PAA), m-hydroxy-PAA (mHPAA) and p-hydroxy-PAA (pHPAA) are present and heterogenously distributed in rodent brain. PAA added to cultures or injected into rats is neurotoxic. Endogenous PAA levels are normally low; two factors that might maintain low levels of PAA in vivo were investigated: first, formation of conjugates of PAA and second, transport of PAA out of the brain by a probenecid-sensitive system. The presence of conjugates of these acids was investigated by subjecting homogenates of rat striatum to hydrolysis. The transport inhibitor, probenecid, increased the concentrations of free mHPAA, free pHPAA and the total concentrations of all three acids indicating that all three trace acids can be removed from the rat brain by a transport system. PAA concentrations were increased ten-fold by hydrolysis (to 160 ± 27 ng/g), pHPAA increased only two-fold (to 54 ± 4 ng/g), and mHPAA was unaffected. These findings coupled with the failure of pargyline to decrease free or total PAA levels suggest that conjugation of PAA is an important factor regulating free PAA levels. The identity of the acid conjugates in the brain is unknown. Incubation of rat tissue homogenates with ^{3}H-glycine or ^{14}C-PAA showed that the kidney but not the brain could synthesize radiolabelled phenylacetylglycine. Supported by Saskatchewan Health.

567.13

EXISTENCE OF NEUROSTEROIDS IN HYPOTHALAMUS. H. Yamada[1], K. Kurokawa[1], J. Ochi[1] & K. Kataoka[2]*. [1]Dept. of Anatomy, Shiga Univ. of Med. Sci., Otsu 520-21, & [2]Dept. of Physiol., Ehime Univ. Sch. of Med., Ehime 791-02, JAPAN

The synthesis of bioactive steroids in neural cells has not been researched in detail yet. We report here our serial histochemical studies on (1) endogenous digitalis-like substance (EDLS) which is newly identified as *cis-trans-cis* type steroid natively found in plants, and (2) cytochrome P450 enzymes for steroid synthesis.

Male Wistar rats (180-250g b.wt.) were transcardially perfused with buffered fixative containing formaldehyde, after they were anesthetized with intraperitoneal injection of sodium pentobarbital (20mg/kg b.wt.). Then hypothalamic sections were processed for histochemical staining.

(1) EDLS causes vasoconstriction and natriuresis as a result of inhibition of Na^+,K^+-ATPase. The distribution of EDLS was analyzed by an immunohistochemical technique using digoxin-antibody. EDLS was found in the hypothalamic magnocellular neurons in the supraoptic and paraventricular nuclei and nerve terminals in the median eminence.

(2) P450scc(cholesterol side chain cleavage) and P450$11\beta$ (11β-hydroxylase) were immunostained. The antibodies were provided from Dr. S. Kominami & Dr. S. Takemori (Hiroshima Univ.). P450scc-immunoreactivities were seen in the somata of periventricular area; and P450$11\beta$ were observed in the supraoptic and paraventricular neurons. The positive reactivities of both P450 enzymes were also found in the nerve terminals in the median eminence.

567.14

NEUROSTEROIDS IN RAT RETINAE. P. Guarneri (a)*, R. Guarneri (a), C. Cascio (b), P. Pavasant (c), F. Piccoli (b) and V. Papadopoulos (c). (a) Inst. Experimental Medicine, CNR, Palermo; (b) Inst. Neuropsychiatry, 90129 Palermo, Italy; (c) Dept. Anatomy & Cell Biology Georgetown Univ., Washington D.C., 20007.

Brain glial cells are capable to synthesize steroids, named neurosteroids (Hu et al., PNAS 84:8215, 1987; Guarneri et al., PNAS 89:5118, 1992).

We used isolated rat retinae as a neural model to investigate neurosteroid synthesis and regulation independently from blood-borne steroids interference. Pregnenolone, progesterone and their metabolites (17α-OH progesterone, 17α-OH- pregnenolone, DOC, THDOC and 3α-OH-DHP) were identified. Using mevalonolactone (MVA) as sterol precursor, we observed a time-dependent pregnenolone synthesis in intact retinae incubated in Krebs-Hepes solution at 37°C in the presence of 20μM lovastatin to reduce endogenous MVA synthesis, and inhibitors of pregnenolone metabolism (20μM trilostane and 10μM SU 10603). The addition of 0.76mM aminoglutethimide, an inhibitor of P450scc enzyme involved in the cholesterol conversion into pregnenolone, blocked its formation; the addition of cAMP stimulants increase pregnenolone synthesis. Immunocytochemistry revealed a primary localization of P450scc enzyme at retinal ganglion cells and bipolar cells. The results demonstrate rat retinae is a favourable model for studying neurosteroidogenesis and its regulation independently from blood-borne steroids interference, and reveal for the first time the steroidogenic ability of neuronal cells.

568.1

COMPARISON OF SIGNAL TRANSDUCTION OF HUMAN DOPAMINE-D2- AND NEUROPEPTIDE Y-Y1-RECEPTORS EXPRESSED IN CHO CELLS.

G. Weng*, R.G. MacKenzie[1], D.H. VanLeeuwen[1], D. Larhammar[2], H. Ericson and C. Wahlestedt. Div. Neurobiology, Dept. Neurology and Neuroscience, Cornell Univ. Med. Coll., New York, NY 10021, [1]Dept. Pharmacology, Parke-Davis, Ann Arbor, MI 48106 and [2]Dept. Med. Genetics, Univ. Uppsala, Sweden.

In many cells, dopamine-D2-receptors and neuropeptide Y-Y1-receptors show distinct similarities with respect to signal transduction and second messenger formation. We therefore sought to determine whether stably transfected chinese hamster ovary (CHO) cells would respond to dopamine and neuropeptide Y in an identical fashion. For this purpose, CHO cells were transfected with human D2- (Grandy *et al., PNAS* **86**:9762, 1989) or human Y1-receptor (Larhammar *et al., J. Biol. Chem.* **267**:10935, 1992) cDNA. Successful transfection was assessed by *de novo* expression of specific binding sites labeled by [^{125}I]-iodosulpiride and [^{125}I]-peptide YY.

Cells with similar B_{max} values for the respective radioligand were then used for second messenger studies: (1) Inhibition of cAMP accumulation, (2) potentiation of ATP-induced [^{3}H]-arachidonic acid release, and (3) mobilization of Ca^{2+} measured by fura-2 fluorometry. While dopamine was capable of affecting all three second messenger phenomena in D2-receptor transfected cells, stimulation of Y1-receptor transfected cells by neuropeptide Y only resulted in $[Ca^{2+}]_i$ elevation. Since specific binding of the respective radioligands, as well as agonist-stimulated Ca^{2+} responses, were of a similar magnitude in the two types of transfected clonal CHO cells, it is possible that the two G-protein-coupled receptors under study might differ with respect to affinity to specific G-proteins rather than the stoichiometry of receptor:G-protein.

568.2

THE HUMAN NK1 RECEPTOR MEDIATES ARACHIDONIC ACID RELEASE IN TRANSFECTED CHO CELLS.

E. R. Jackson, K. Pratt, S. McLean & S.H. Zorn*. Central Research Division, Pfizer Inc., Department of Neuroscience, Groton, CT 06340.

The human neurokinin-1 (NK1) receptor exhibits high affinity for the undecapeptide Substance P (SP), the non-peptide NK1 antagonist CP-96,345 and lower affinity for the NK2 receptor agonist, neurokinin-A (NKA) and the NK3 receptor agonists eledoisin and senktide. In CHO cells stably expressing the rat NK1 receptor subtype, SP stimulates both phosphatidylinositol turnover (PI) and activation of adenylate cyclase activity (AC) (Takeda et al., J. Neurochem. 59, 1992). The present study was undertaken to study whether the human NK1 receptor also couples to multiple signal transduction pathways in CHO cells and also to arachidonic acid (AA) release. Substance P produced a dose-dependent release of arachidonic acid from cells prelabelled with [3H]-AA with an EC50 of 0.9 nM and a maximal response that was 8- fold higher than basal levels. The magnitude of this response was similar to that observed with 10 μM A23187 and 2 μM thapsigargin. Agonist concentration-response curves demonstrated a rank order of potency of SP> eledoisin>NKA,>> senktide. The response to 3.3 nM SP was completely blocked by the (+)- but not the (-)-enantiomer of CP-96,345 (pKi = 8.84 ± 0.06). Pertussis toxin had no effect on SP-induced AA release, PI turnover or AC activity. The AA response appeared to require the influx of calcium since it was sensitive to changes in the concentration of the divalent ion in the media. Although forskolin stimulated cAMP accumulation in these cells, it did not stimulate PI turnover or AA release. The results indicate that SP stimulation of the human NK1 receptor expressed in CHO cells leads to the activation of three second messenger systems (AC, PI, and AA release). The three responses are not mediated by pertussis toxin sensitive G-proteins. While the AA response is not due to stimulation of adenylate cyclase, cross-talk between the PI and PLA2 systems could not be excluded.

568.3

CHARACTERIZATION OF PHOSPHOLIPASE A_2 (PLA_2) IN RAT BRAIN. EVIDENCE FOR MULTIPLE MOLECULAR FORMS.

R. Diaz-Arrastia*, M.A. Clark#, and J.H. Schwartz, Center for Neurobiol. & Behav., Columbia Univ., New York, NY 10032, and #Schering-Plough Research Institute, Kenilworth, NJ 07003.

Release of arachidonic acid (AA) from membrane phospholipids in neurons is important in gating ion channels and also participates in long-term synaptic plasticity. The major enzymatic activity responsible for AA release is PLA_2. Studies in non-neural tissues demonstrate two classes of PLA_2s: high molecular-weight (MW) cytosolic enzymes and low MW secreted enzymes. Both classes are regulated in specific ways by a variety of physiologic conditions. To learn how neuronal PLA_2(s) are regulated, we undertook a biochemical analysis of the lipase(s) from rat brain. Brain tissue contains two major classes of PLA_2: (1) a high MW (M_r 100,000) enzyme which is both cytosolic and particulate, and (2) a low MW (M_r 14,000) enzyme which is soluble. The high MW enzyme is inactivated by deoxycholate, but is active in non-ionic detergents (200 μM Triton X-100). The enzyme is stable in thiol reducing reagents, is activated by Ca^{2+} ion in the micromolar range, and is optimally active at pH 8. The high MW enzyme preferentially hydrolyzes phosphatidylcholine (PC) over phosphatidylethanolamine (PE). Using conditions specific for the high MW enzyme, we have enriched activity 200-fold by hydrophobic column chromatography, ion-exchange chromatography, and gel filtration. In these respects the high MW enzyme resembles the cytosolic PLA_2 that has been cloned from monocytic cells. The low MW enzyme is active in the presence of ionic detergents (1 mM deoxycholate) and inactivated by thiol reducing reagents. It requires Ca^{2+} ion in the millimolar range and has a pH optimum of 9.5, and prefers PE > PC as substrate. It is thus similar to extracellular PLA_2s described in a variety of tissues. Since in non-neural tissues cytosolic PLA_2 are activated by phosphorylation while secreted PLA_2s are transcriptionally regulated, elucidation of how these enzymes are regulated in brain should yield important clues to mechanisms of signal transduction and synaptic plasticity.

568.4

CEINGE CLONE 3, A NEW IMMORTALIZED BRAIN CELL LINE, POSSESS BRADYKININ, ENDOTHELIN-1 AND PDGF RECEPTORS COUPLED TO INTRACELLULAR Ca^{2+} ELEVATION :

A. Fatatis*, § T. Russo, A. Bassi, E. Iannotti, R. Caporaso, G.F. Di Renzo and L. Annunziato. Dept. of Pharmacology and §Dept. of Biochemistry, II School of Medicine, "Federico" II University of Naples, Naples ITALY.

CEINGE clone 3 (Cl 3) is a cell line obtained by immortalization of cells derived from 14 days old fetal rat brains. Cell infection was performed using a murine leukemia virus (PyMuLV) carrying the gene encoding the polyoma middle T antigen. CEINGE Cl 3 cells are able to grow in agar and to induce the formation of tumors when inoculated into nude mice. CEINGE Cl 3 displays immunocytochemical staining with antibodies directed against vimentin and S-100 whereas no staining was observed with antibodies against neurofilaments and glial fibrillary acid protein. About 20% of the cells were positive to anti-galactocerebroside C. Two different degrees of morphological differentiation were obtained using 10μM retinoic acid (RA) alone or followed by a treatment with 10-100μM forskolin (FK). In the first case, after a period of 3-5 days, cells became spindle-shaped forming parallel rows. Treatment of RA-differentiated cells with FK induced a drammatic morphological change, showing a stellate cellular shape and long processes forming an evident network. Using single-cell fura-2 microfluorimetry, bradykinin (BK) and endothelin-1 (ET-1) were found to be able to elicit an $[Ca^{2+}]_i$ increase in presence and in absence of extracellular Ca^{2+}. Furthermore, CEINGE Cl 3 showed an $[Ca^{2+}]_i$ elevation when exposed to 10-30 ng/ml of Platelet Derived Growth Factor (PDGF). These results demonstrated that this immortalized brain-derived cell line displays both G protein-linked and tyrosine kinase-linked receptors on its plasmamembrane. (Supported by 40% and 60% MURST funds, CNR grants Progetto Finalizzato Biotecnologie to L.A. and G.F.D.R and A.I.R.C. grant to T.R.)

568.5

DIFFERENTIAL EFFECTS OF CORTICOTROPIN RELEASING FACTOR (CRF) ON cAMP SYNTHESIS IN THE BASOLATERAL AND CENTRAL NUCLEI OF THE RAT AMYGDALA.

T.D. Ely, P. J. Elliott* and C. D. Kilts. Emory Univ. Sch. of Med., Atlanta, GA 30322 and Glaxo, Inc., Ware, UK

Accumulating evidence indicates that the central (ACe) and basolateral (BLA) nuclei of the rat amygdala represent functionally distinct fields of corticotropin releasing factor (CRF)-mediated neurotransmission. We compared the influence of CRF on adenylate cyclase (AC) activity in the ACe, BLA and frontal cortex (FC). CRF (10*n*M-10μM) produced a concentration - and GTP - dependent increase in cAMP synthesis in homogenates of the FC. In contrast, a bimodal effect of CRF on cAMP synthesis in the ACe and BLA was observed with the maximal effect in the ACe and BLA being 1/3-1/2 that of the FC. In the ACe, CRF (1μM) produced a significant stimulation while higher and lower concentrations were without effect. In the BLA, CRF stimulated AC activity at low *n*M concentrations with the effect plateauing at 1μM. These data indicate a heterogeneity of amygdaloid CRF receptors at the level of signal transduction mechanisms which may underly the distinct effect of CRF on ACe and BLA neurons.(MH39967)

568.6

PHARMACOLOGICAL EVALUATION OF A STABLE HEXAPEPTIDE ANALOG OF NEUROTENSIN.

H. C. Akunne[1*], S. B. DeMattos[1], S. Z. Whetzel[1], D. L. Wustrow[2], M. D. Davis[1], J. X. He[2], W. L. Cody[2], L. D. Wise[2], T. A. Pugsley[1] and T. G. Heffner[1]. Departments of Neuroscience Pharmacology[1]/Chemistry[2], Parke-Davis Pharmaceutical Research Division of Warner-Lambert Company, Ann Arbor, MI 48105

Neurotensin (NT) is a tridecapeptide (pGlu-Leu-Tyr-Glu-Asn-Lys-Pro-Arg-Arg-Pro-Tyr-Ile-Leu) that displays a wide spectrum of biological actions both in the central and peripheral nervous system. Potential therapeutic applications of NT agonists include use in the treatment of psychosis, obesity and hypertension. Identification of small molecule ligands with high affinity to the physiologically relevant NT receptor site is desirable. The major signal transduction system for NT receptors in a number of different systems is the G-protein-dependent stimulation of phospholipase C leading to the mobilization of intracellular Ca^{2+} and stimulation of cGMP. We therefore studied both the binding and the functional actions of neurotensin analogs by quantitative measurement of cytosolic free Ca^{2+} concentration in HT-29 human colonic adenocarcinoma cells using the Ca^{2+}-sensitive dye, Fura-2/AM and by effects on cGMP levels in rat cerebellar slices. The metabolically stable NT8-13 analog, NTE (NMeArg-Lys-Pro-Trp-tLeu-Leu) from Eissi, NTE, plus other NT analogs and related peptides (neurotensin, NT8-13, xenopsin, neuromedin N, NT9-13, kinetensin and D-Trp^{11}-NT) bound to HT-29 cell membranes; and increased the mobilization of intracellular Ca^{2+} in these cells indicating NT receptor agonist properties. In addition, NTE increased cGMP levels in rat cerebellar slices, confirming the latter findings. The binding affinities to the HT-29 cell membrane preparation showed a good correlation to the EC50 values for the mobilization of $[Ca^{2+}]i$ ($r=0.85$). These results substantiate the *in vitro* NT agonist properties of the hexapeptide NT analog, NTE.

568.7

MELATONIN BINDING SITES IN RAT CIRCLE OF WILLIS ARTERIES ARE LINKED TO A G-PROTEIN. S. Capsoni, M. Viswanathan, A.M. De Oliveira and J.M. Saavedra*. Section on Pharmacology, Laboratory of Clinical Science, National Institute of Mental Health, Bethesda, MD 20892.

Melatonin is considered the most important hormone secreted by vertebrate pineal gland and is thought to be a biochemical transducer of photoperiodic variations in the environment. Autoradiography with 2-[^{125}I]iodomelatonin has revealed high affinity, specific melatonin receptors in rat arteries involved in thermoregulation, such as basal brain arteries forming the circle of Willis.
Our autoradiographic studies show that co-incubation with increasing concentrations of a non hydrolizable GTP analogue (GTP-gamma-S) (10^{-7}-10^{-2} M) provokes a dose dependent and a specific inhibition of 2-[^{125}I]iodomelatonin binding in rat arteries from the circle of Willis. This result suggests that, similarly to what has been described in mammalian pars tuberalis and brain cortex, putative melatonin receptors in rat cerebral arteries are coupled to second messengers by G-protein.

568.8

G-PROTEIN-MEDIATED ACTIVITIES OF CANNABINOID RECEPTORS IN CEREBELLAR GRANULE CELLS. S.R.Childers*, M.B. Roy and S. Stark, Dept. Physiol./Pharmacol. & Neurosci. Program, Bowman Gray Sch. Med., Wake Forest Univ., Winston-Salem, NC 27157.

Cannabinoid (Can) receptors are present in high density in cerebellum, and Can-inhibited adenylyl cyclase (AC) has been characterized in cultured cerebellar granule (CG) cells (M. Pacheco *et al.* [1993], Brain Res. **603,** 102). The present studies examined whether this system regulates glutamate release from these cells. Can agonists inhibited forskolin-stimulated cAMP levels in cultured CG cells by 50-60%. The potencies of Can agonists in inhibiting cAMP levels paralleled those in inhibiting AC, and in stimulating low K_m GTPase, in isolated cerebellar membranes. The $GABA_B$ agonist baclofen inhibited cAMP levels to a similar extent, and Can and $GABA_B$ effects on cAMP levels were non-additive, suggesting that these two $G_{i/o}$-linked receptors shared common effectors. Glutamate release was studied by preincubating cultured CG cells with [^{3}H]D-aspartate (D-asp), and stimulating release with 50 mM KCl, which released 8-9% of total radioactivity over 10 min. When cells were treated with the potent Can agonist WIN 55212-2 (1 μM) for 10 min before adding KCl, potassium-stimulated release of [^{3}H]D-asp was inhibited by approx. 40%. The specificity of this response was confirmed by the finding that the inactive stereoisomer, WIN 55212-3, had no significant effect on [^{3}H]D-asp release. These data suggest that cannabinoid receptors exist on terminals of cerebellar granule cells and negatively modulate glutamate release from these cells.

Supported by PHS grant DA-06784 from NIDA.

568.9

FATTY ACID COMPOSITION AND ACTIVATION OF INOSITOL PHOSPHATE AND ARACHIDONIC ACID METABOLISM BY PAF IN ASTROGLIAL AND IN NEUROBLASTOMA SK-N-BE CELLS. A. Petroni*, M. Blasevich, N. Papini, M.G. Marchini, S. Bergamaschi, S.Govoni and C. Galli, Institute of Pharmacological Sciences, University of Milan, via Balzaretti 9, 20133, Milan, Italy.

The activation of phospholipases leads to the formation of products such as Platelet-Activating Factor (PAF) and arachidonic acid (AA) metabolites, which are important mediators of physiopathological events in the CNS.
The aim of our study was first to characterize the lipid composition of astroglial and neuroblastoma SK-N-BE cells. Secondly, to study the pathways generating after phospholipase activation by PAF. Astroglial cells were obtained from 1 day old rat brain cortex, SK-N-BE cells were differentiated with 10μM retinoic acid and compared with the undifferentiated ones. The inositol phosphate pathway was evaluated by incubating the cells with 3[H]-myo-inositol for 18 hours. After PAF stimulation, the supernatants were extracted and analyzed by HPLC connected with a detector for the evaluation of labeled inositol phosphates. In astroglial cells maximal production of inositol phospathes and formation of AA-cycloxygenase metabolites was obtained at PAF concentration 10-8 M for 1 min stimulation,whereas in SK-N-BE inositol phosphate production and AA metabolism were less active. Protein kinase C (PKC) has been also investigated. Preliminary data have shown that in differentiated SK-N-BE soluble and particulate fractions, PKC enzyme activity was lower with respect to undifferentiated cells. Astroglial cells differently incorporated labeled fatty acids (FA) in PL classes. AA was mainly incorporated in diacyl-phosphatidyl-choline (PC) and in phosphatidyl inositol (PI). In undifferentiated SK-N-BE, AA was 7.5 %, and after differentiation it reached 14 %, suggesting either greater incorporation of the FA in membranes highly enriched with AA, formed after differentiation, or greater formation of AA from precursors.

568.10

BRADYKININ STIMULATES PHOSPHOLIPASE D IN PC12 CELLS BY A MECHANISM WHICH IS INDEPENDENT OF INCREASES IN INTRACELLULAR Ca^{2+}. J. Horwitz*, B. A. Passarello and M. Corso. Dept. of Pharmacology, Medical College of Pennsylvania, Philadelphia, PA 19129

Previously published work from this laboratory, done in PC12 pheochromocytoma cells, has suggested a possible role for Ca^{2+} in the regulation of phospholipase D. Therefore, the present experiments were designed to correlate phospholipase D activity with the actual level of intracellular Ca^{2+}. Phospholipase D activity was determined by measuring the production of [^{3}H]phosphatidylethanol in cells whose phospholipids had been prelabeled with [^{3}H]palmitic acid. Intracellular Ca^{2+} was measured with the fluorescent Ca^{2+} chelator, Fura-2. Ionomycin at a concentration of 10 μM caused a large unphysiological increase in intracellular Ca^{2+} and an increase in phospholipase D activity which was equal to or larger than that caused by bradykinin (10 μM). However, when the concentration of ionomycin (50 nM) was adjusted so that the increase in intracellular Ca^{2+} was comparable to that caused by bradykinin, there was no effect on phospholipase D activity. Agents such as carbachol, ATP, and thapsigargin also increased intracellular Ca^{2+} but had no effect on phospholipase D activity. In other experiments, the intracellular Ca^{2+} concentration was manipulated by preincubating the cells under two different conditions: 1) Ca^{2+}-free media plus EGTA (100 μM); 2) Ca^{2+}-containing media plus the intracellular Ca^{2+} chelator BAPTA/AM (80 μM). These preincubations blocked the bradykinin induced increase in intracellular Ca^{2+}. In contrast, these preincubations did not block bradykinin-stimulated phospholipase D activation. The response was attenuated by only 35%. These data suggest that physiological increases in intracellular Ca^{2+} do not mediate the effect of bradykinin on phospholipase D.

568.11

PHARMACOLOGIC CHARACTERIZATION OF MELATONIN RECEPTOR-MEDIATED PHOSPHOINOSITIDE HYDROLYSIS IN PIGEON BRAIN. U.L. Mullins and A.S. Eison*. CNS Special Projects, Bristol-Myers Squibb Co., Wallingford, CT 06492.

High densities of [^{125}I]-iodomelatonin binding sites are found in pigeon brain (Pang et al., 1990). Melatonin receptors are functionally linked to second messenger systems in other avian species (Dubocovich et al., 1991). The present study investigated the effects of several melatonin agonists on phosphoinositide (PI) hydrolysis levels in slices of ectostriatum, optic tectum, cerebellum, hypothalamus and pons medulla of the pigeon brain. White carneaux pigeons (*Colombia livia*) were sacrificed 4 hours after lights on (12:12 L:D cycle). Inositol monophosphate (IP_1) accumulations (in the presence of 10 mM lithium) were collected by ion exchange chromatography. Relative potencies of melatonin receptor-mediated IP_1 accumulation induced by agonists in ectostriatum were as follows: 2-iodomelatonin> 6-chloro-melatonin> N-acetylserotonin> melatonin> serotonin. While 10 nM melatonin induced a 1.5 fold increase in PI hydrolysis over basal levels, similar concentrations of 2-iodo-, 6-chloromelatonin, and N-acetylserotonin increased PI hydrolysis 2.4 fold, 2.1 fold and 1.9 fold, respectively. Melatonin-induced PI hydrolysis was blocked by N-acetyltryptamine, a melatonin antagonist, but not by ketanserin, a $5\text{-}HT_2/5HT_{1C}$ antagonist demonstrating that the effects seen were not a result of $5\text{-}HT_2$ or $5\text{-}HT_{1C}$ receptor stimulation. These data support the existence of a melatonin receptor in pigeon brain which is functionally linked to PI hydrolysis as a second messenger system.

568.12

CHOLECYSTOKININ OCTAPEPTIDE ELEVATES INTRACELLULAR CALCIUM IN CULTURED SYMPATHETIC NEURONS

H. Xian, R.M. Lynch and D.L. Kreulen*. Departments of Pharmacology and Physiology, Coll. of Med., Univ. of Arizona, Tucson, AZ 85724.

Previous studies have demonstrated that cholecystokinin octapeptide (CCK-8) mediates excitatory synaptic transmission in mammalian prevertebral sympathetic ganglia by regulating membrane ionic currents. CCK may activate phospholipase C cascade through CCK_A receptors in sympathetic neurons. Also CCK-8 could mobilize intracellular calcium, thereby generating an intracellular signal to modulate cellular activities including membrane responses. We examined this hypothesis utilizing calcium fluorescence microscopic imaging (Calcium imaging) and whole-cell voltage clamp techniques. Sympathetic neurons from guinea pig celiac ganglia were enzymatically dissociated and remained in culture for 3 - 15 days. Celiac neurons were loaded at 37°C with the Fluo-3 AM (3 μM; 50 minutes). In fifty-three percent of celiac neurons (n = 38), bath application of CCK-8 (0.3 to 1 μM) induced a significant increase in intracellular calcium. The response to CCK was either transient or sustained more than 10 minutes. On average calcium increased by thirty percent at the peak of the responses. This was approximately twenty-seven percent of the maximal response elicited by subsequent depolarization with 80 mM KCl. In whole-cell voltage clamp recording, all celiac neurons displayed L-type calcium current. Pulse application (1 sec) of CCK-8 (1 μM) did not increase the amplitude of the calcium currents in 7 cells tested. These results suggest that CCK-activated increase in intracellular calcium may be due to release from internal stores rather than influx through membrane calcium channels. (HL-27781 and DK-36289).

568.13

INVESTIGATION OF POTENTIAL INTERACTIONS BETWEEN SECOND MESSENGERS THAT MEDIATE SUBSTANCE P AND NOREPINEPHRINE STIMULATION OF GLYCOGENOLYSIS IN A HUMAN ASTROCYTOMA CELL LINE (UC11-MG). R.V.W. Dimlich*, S. Medrano, and E. Gruenstein. Depts. of Emer. Med. and Mol. Gen., Biochem., and Microbiol., University of Cincinnati College of Medicine, Cincinnati, OH 45267-0769.

This study demonstrated for the first time in astrocytes that substance P (SP) induces glycogenolysis that is mediated through an increase in $[Ca^{2+}]_i$. The glycogenolytic response was measured as a release of ^{3}H-glucose from glycogen. Changes in $[Ca^{2+}]_i$ were determined using video image analysis with fura-2. The effect of SP on [^{3}H]-glycogen and $[Ca^{2+}]_i$ levels was dose-dependent with a maximum effect observed at 10^{-8} M. The SP receptor antagonist CP-96,345 inhibited both $[Ca^{2+}]_i$ increase and glycogenolysis suggesting that the SP effect is mediated by an NK_1 receptor. Our previous data indicated that norepinephrine (NE) stimulates glycogenolysis through cAMP in this cell line. Having two neurotransmitters that stimulate the same physiological response, each through a different second messenger, provided a model for testing if cross-talk occurs between $[Ca^{2+}]_i$ and cAMP as second messenger systems regulating glycogenolysis in these cells. Subsequent experiments determined that, contrary to what has been determined for other physiological responses, the glycogenolytic effects of SP and NE in UC11-MG cells were additive and not synergistic at suboptimal doses. In addition, there was no evidence of interaction between cGMP and either $[Ca^{2+}]_i$ or cAMP in regulating glycogenolysis in these cells. The data of this study support the use of this human cerebral cortical astrocytoma cell line as a model for exploring potential interactions among neurotransmitters and corresponding intracellular second messenger systems and for studying the role of astrocytic glycogen in the CNS. [Supported by NS-25635 (RVWD), NS27814 (EG), and Ohio AHA (SW-90-09) (RVWD)].

568.14

DOWN-REGULATION OF MUSCARINIC RECEPTORS AND CARBACHOL-STIMULATED PHOSPHOINOSITIDE HYDROLYSIS IN SENESCENT RATS FOLLOWING ACUTE ADMINISTRATION OF CHLORPYRIFOS. W.R. Mundy[1*], T.R. Ward[1], S. Padilla[1] and T.M. Freudenrich[2]. [1]Neurotoxicology Division, U.S. EPA, RTP, NC 27711. [2]ManTech Environmental Tech., Inc., RTP, NC 27709.

Acute treatment with the organophosphate chlorpyrifos (CPF) results in prolonged inhibition of blood and brain cholinesterase activity (ChE). This work examined age-related differences in the response to ChE inhibition in F-344 rats. Male rats aged 5 months (young) or 26 months (aged) were injected s.c. with 250 mg/kg CPF or peanut oil vehicle (controls) and sacrificed 21 days later. CPF administration resulted in an 80% inhibition of ChE activity in whole blood from both young and aged rats. In control animals ChE activity was less in the frontal cortex of aged rats (36.4 nmol acetylcholine hydrolyzed/mg protein/min) compared to young rats (42.1 nmol acetylcholine hydrolyzed/mg protein/min). However, CPF treatment resulted in a similar 80% reduction in brain ChE activity in both ages. Scatchard analysis of ^{3}H-QNB binding was used to determine muscarinic receptor density (Bmax) and affinity (Kd) in the frontal cortex. Bmax was decreased 30% in both young and aged rats treated with CPF, with no change in Kd. The functional responsiveness of muscarinic receptors was measured in cortical brain slices as the accumulation of inositol phosphate (IP) following stimulation with the cholinergic agonist, carbachol. Carbachol (1-1000 µM) resulted in a concentration-dependent increase in IP accumulation. IP accumulation was decreased in CPF-treated rats, and this decrease was the same in both ages. These results show that a single treatment with CPF which results in a prolonged inhibition of blood and brain ChE can induce muscarinic receptor down-regulation and a functional decrease in muscarinic receptor signal transduction which was not different in young and senescent rats.

568.15

A RAPID ATTENUATION OF MUSCARINIC AGONIST-STIMULATED PHOSPHOINOSITIDE HYDROLYSIS PRECEDES RECEPTOR SEQUESTRATION IN HUMAN SH-SY-5Y NEUROBLASTOMA CELLS.
E.L. McEwen, D.M. Slowiejko and S.K. Fisher.* Neuroscience Lab. & Dept. of Pharmacology, Univ. of Michigan, Ann Arbor, MI 48104-1687

The relationship between the kinetics of muscarinic receptor (mAChR)-stimulated phosphoinositide (PPI) hydrolysis and receptor internalization has been determined for human SH-SY-5Y neuroblastoma cells. The addition of 1 mM oxotremorine-M (Oxo-M) to cells prelabeled to isotopic equilibrium with ^{3}H-inositol resulted in two kinetically distinct phases of PPI hydrolysis, as monitored by the release of a total inositol phosphate fraction (IP_t), in the presence of Li^+. From the rate of [^{3}H]IP_t formation, a PPI breakdown rate of 9.5%/min was obtained after 5s of Oxo-M addition. This initial phase of PPI hydrolysis was short-lived ($t_{1/2}$=14s) and after 60s the rate of inositol lipid breakdown had declined to a steady state level of 3.4%/min, which was maintained for at least 10 min. In the parent cell line, SK-N-SH, the rate of PPI hydrolysis observed after 5s of Oxo-M addition was also 2-3 fold greater than that obtained after 60s ($p<0.05$). Oxo-M addition to SH-SY-5Y cells also resulted in a 4-5 fold increase in the mass of $I(1,4,5)P_3$ within 5-10s. After 60s, the net formation of $I(1,4,5)P_3$ had declined to ~ 35% of that attained during the first few seconds of agonist addition and thereafter stayed constant for up to 5 min. Agonist occupancy of mAChRs on SH-SY-5Y cells also resulted in a rapid breakdown of phosphatidylinositol 4,5-bisphosphate (PIP_2:$t_{1/2}$=12s). Within 60s of agonist addition, however, a new steady state of [^{3}H]PIP_2 label was achieved and maintained for the next 10 min. In contrast to the rapid changes in receptor-stimulated PPI hydrolysis, no significant loss of cell surface mAChRs occurred before 5 min of agonist exposure. Thus a rapid, but partial, attenuation of PPI hydrolysis in response to mAChR activation occurs prior to mAChR internalization and may represent an important early adaptive response to chronic agonist stimulation. (NIMH 42652 and NIH 23831.)

568.16

BRADYKININ-STIMULATED PHOSPHOINOSITIDE TURNOVER AND PROSTACYCLIN PRODUCTION REGULATED BY PHORBOL ESTER AND CALCIUM IN CEREBRAL ENDOTHELIAL CELLS. J. Xu*, P.S. Zhang, S.A. Moore, C.Y. Hsu and E.L. Hogan. Neurology Dept, MUSC, Charleston, SC 29425

Bradykinin (BK) stimulated phosphoinositide hydrolysis and prostacyclin (PGI_2) production in murine cerebral endothelial cell (MCEC) culture in a dose-dependent manner. A short exposure of MCECs to phorbol 12,13-dibutryrate (phorbol ester), an activator of protein kinase C (PKC), inhibited BK-induced inositol phosphates (IPs). Staurosporine, a potent inhibitor of PKC, reversed phorbol ester's inhibition. Long-term pre-exposure of MCECs to phorbol ester for various times (2-18 hrs) demonstrates reduced inhibition of BK-induced IPs as they are enhanced at 4 hrs and rise to 7-fold over the basal level 18 hrs after exposure. BK-induced IPs were decreased by the removal of calcium from the incubation medium. Pre-exposure of MCECs to BK itself for 8 hrs diminished BK's IPs response by 35%. On the other hand, BK-stimulated PGI_2 production does not appear in the presence of phorbol ester on BK-PGI_2. Removal of calcium from the medium completely eliminated BK-stimulated PGI_2.

These results suggest that BK-linked phospholipase C is down regulated by PKC, is partially calcium dependent and is desensitized by BK receptor(s). BK-stimulated phospholipase A_2 is also down regulated by PKC and is calcium dependent. Long-term pre-exposure of MCECs to phorbol ester decreased PGI_2 production with increasing time of exposure. BK stimulation gradually recovered over 18 hrs. Supported by NIH grant NS11066.

568.17

CCK_B RECEPTORS MEDIATE PHOSPHATIDYLINOSITOL TURNOVER IN LATE PASSAGE RAT AR 4-2J CELLS. J.M. Morrone, K.G. Pratt, S. McLean* and S.H. Zorn. Pfizer Inc., Central Research Div., Department of Neuroscience, Groton, CT.

In AR 4-2J rat pancreatoma cells that express both CCK_A and CCK_B receptors, CCK-8 stimulates phosphatidylinositol (PI) turnover via CCK_A receptors (Pratt et al., Neurosci. Abstr. 18(1):452, 1992). However, recent studies suggest that with prolonged passaging of these cells the expression of CCK receptors and/or their coupling to PI turnover change with time. The present study was undertaken to characterize which CCK receptor is functionally coupled to PI turnover in late passage AR 4-2J cells. AR 4-2J cells prelabelled with [3H]-*myo*-inositol were exposed to the CCK receptor agonist pentagastrin (PG) in the presence and absence of selective CCK_A and CCK_B receptor antagonists. PG produced a dose-dependent stimulation of IP accumulation (EC50 = 0.38 nM), that was completely blocked by the selective CCK_B receptor antagonists CI-988, L-365,260 and LY-262,691 with Ki values of 2.4, 7.6, and 169 nM, respectively. The rank order of potency for blockade of PG-induced PI turnover was similar to that observed for inhibition of CCK_B receptor binding (IC50 values = 0.78, 8.1, and 90 nM, respectively). The CCK_A receptor antagonist L-364,718 inhibited the PG induced response with a Ki >100 nM, consistent with its low affinity for the CCK_B receptor (IC_{50} = 120 nM). Thus, in contrast to that observed in early passage cells, CCK_B receptors mediate agonist induced PI turnover in late passage AR 4-2J cells.

568.18

AUTORADIOGRAPHIC LOCALIZATION OF CARBACHOL-INDUCED PHOSPHOINOSITIDE TURNOVER IN DEVELOPING RAT NEOCORTEX.
M.L. Robinson*, M.D. Hartgraves and J.L. Fuchs. Department of Biological Sciences, University of North Texas, Denton, TX 76203.

Neurotransmitter receptor binding in the developing rat neocortex has been well studied, but little is known about the functional ontogeny of these receptors. This is the first developmental study to describe the distribution of phosphoinositide (PI) turnover using an autoradiographic method (Hwang et al., 1990) which allows resolution to cortical layers. PI turnover was examined in slices of somatosensory cortex from rats aged postnatal days 4-16. [^{3}H]CTP served as a precursor for the membrane-bound intermediate [^{3}H]CDP-diacylglycerol; lithium was added to block recycling of this intermediate. The ACh agonist carbachol was used to stimulate PI turnover. The slices were subsequently frozen and sectioned for autoradiography.

On P4, the upper cortical plate was moderately dense, while deeper layers were lightly labeled. By P10, labeling was dense in layers II through upper IV, negligible in deep IV through upper V, and moderate in deep V through VI. The difference between deep and superficial layers diminished by P16. At all ages, layer VIb was denser than VIa, and layer I was less dense than II-III. There was no apparent labeling in absence of carbachol. Agonist specificity is suggested by the observation that patterns induced by carbachol were quite different from those in adjacent slices exposed to the glutamate agonist ACPD. The laminar patterns of carbachol-induced PI turnover did not simply mirror those of muscarinic ACh receptor binding, although both receptor binding and PI turnover were lowest in layer IV to superficial V. These differences support the possibility of regional heterogeneity in the ontogeny of receptor coupling to second messengers systems. Supported by NIMH MH41865.

568.19

G-PROTEIN COUPLED RECEPTORS MEDIATE REDUCTION OF POTASSIUM CONDUCTANCES BY TRANS-ACPD AND MUSCARINIC AGONISTS IN CA3 PYRAMIDAL CELLS

Nathalie C. Guérineau, Beat H. Gähwiler, and Urs Gerber*
Brain Research Institute, University of Zürich, CH-8029 Zürich, Switzerland

Muscarinic agonists, acting at acetylcholine receptors, and trans-ACPD, acting at glutamate receptors, have been shown to elicit a variety of biochemical and electrophysiological responses involving the activation of G-proteins and are therefore termed metabotropic responses. However, it has never been established whether the receptors mediating the reduction of potassium conductances in mammalian CNS neurons are linked to G-proteins. We recorded from voltage-clamped CA3 pyramidal cells in rat hippocampal slice cultures using the whole-cell patch-clamp configuration in the presence of TTX. When cells were held at -50 mV, a 30 second bath-application of 1S,3R-ACPD (10 μM) or methacholine (MCh; 0.5 μM) activated an inward current associated with a decrease in gK. To determine if a G-protein is involved in mediating this response, cells were loaded with GDPβS (500 μM), a non-hydrolyzable analog of GDP, through the patch-pipette. After allowing 10 to 15 minutes for diffusion, both 1S,3R-ACPD and MCh no longer induced inward current in 9 of 11 cells. Conversely, when GTP (100 μM) or the non-hydrolyzable analog, GTPγS (250 μM), was included in the patch pipette the amplitude of ACPD- and MCh-induced currents was significantly enhanced: with GTP, 128% for ACPD and 69% for MCh; with GTPγS, 370% for ACPD and 196% for MCh. These results demonstrate that both the glutamatergic and the muscarinic receptor subtypes mediating the reduction in gK in hippocampal pyramidal cells are functionally coupled to G-proteins.

568.20

FURTHER STUDIES REGARDING THE CHANGES IN CARBACHOL-STIMULATED INOSITOL PHOSPHATE RELEASE DURING RETINAL DEVELOPMENT IN THE RAT.

P. Tandon[*1], A.C. Nostrandt[2], S. Willig[3], and S. Padilla[4], [1]CELMB,[2]Curr. in Toxicology, Univ. of NC, Chapel Hill, NC, 27514; [3]ManTech Environ. Ser.,RTP, NC 27709; [4]US Environ. Prot. Ag., RTP, NC, 27711.

Previous studies in our laboratory revealed a 5 fold increase in muscarinic receptor density in the pigmented rat retina between postnatal day (PND) 5 and adult which, curiously, does not correlate with the degree of carbachol-stimulated inositol phosphate (IP) release: carbachol-stimulated IP release peaks abruptly in the PND 10 retina and falls to adult levels by PND 15 (Tandon *et al.*, in press, Exp. Eye Res). To explore possible explanations for this dichotomy, we have been investigating the components of the second messenger cascade and the biochemical properties associated with the retinal muscarinic receptor. First, the phosphoinositide-specific phospholipase C activity of the retina during development does not parallel the developmental profile of the carbachol-stimulated IP release, and therefore does not explain the increased IP release at PND 10. In studies designed to explore the binding characteristics of the receptor, carbachol-stimulated IP release in the PND 10 retina was less sensitive to the muscarinic receptor blocker, atropine, than was the adult retina ($EC_{50}\cong$700nM for the PND 10 retina and 40 nM for the adult retina). These data indicate that some pharmacological aspects of the developing muscarinic receptor are unique from the adult receptor. Perhaps this is a partial explanation for the age-related differences in second messenger response to agonist stimulation in the retina.

BEHAVIORAL PHARMACOLOGY IV

569.1

BEHAVIORAL ACTIVITY OF NEUROSTEROIDS.

R.F. Ritzmann*, A.J. Glasky, and C.L. Melchior. Olive View/UCLA Medical Center and West Los Angeles Veterans Administration Medical Center, Los Angeles, CA 90073

The behavioral effects of low doses of the neurosteroids dehydroepiandrosterone (DHEA), pregnenolone (PE) and their sulfates (DHEAS and PS), which act as allosteric antagonists in the GABA system, were explored in adult male C57BL/6 mice. Subjects were placed in a Stoelting activity monitor for a 20 minute adaptation period, then injected with neurosteroid or vehicle and returned to the activity monitor for a 20 minute test of motor activity. Thirty minutes post injection subjects were tested for anxiety on an elevated plus maze. The number of entries onto the open arms was utilized as the index of anxiety. Reactivity was then assessed by measuring the acoustic startle response.

Motor activity was not altered by these drugs at doses below 0.1 mg/kg, except for PE which, at a dose of 0.01 μg/kg, increased motor activity. At doses of 0.5-20.0 mg/kg, DHEA produced a significant reduction of motor activity. On the elevated plus maze, PE at a dose of 0.01 μg/kg and PS at 1 and 10 μg/kg were anxiogenic. However, anxiolytic activity was found for DHEA at doses of 0.005-1.0 mg/kg, DHEAS at 0.05 and 0.5 mg/kg, and PS at 0.1 μg/kg. Decreased acoustic startle response occurred at 0.01 and 10 μg/kg PE and 0.1 μg/kg PS.

These data show that these neurosteroids have a variety of behavioral effects at very low doses. The differences between the compounds may reflect their different sites of action in the GABA system. The anxiolytic and anxiogenic activities observed support the suggested roles for neurosteroids in stress.

Supported by NIAAA AA08709 and the Veterans Administration.

569.2

INTRAVENTRICULAR INJECTION OF N$^{\omega}$-NITRO-L-ARGININE IMPAIRS LEARNING OF RATS IN A 14-UNIT T-MAZE.

D.K. Ingram, E. Spangler, D. Roberts, H. Kametani , and E.D. London*, NIA Gerontol. Res. Ctr., and NIDA Addiction Res. Ctr., NIH, Baltimore, MD 21224.

Peripheral injections of nitro-L-arginine compounds provided to laboratory rodents impair learning in a variety of tasks (Chapman et al. *Neuroreport* 3:567, 1992). These compounds presumably inhibit central activity of nitric oxide synthase (NOS) which retards nitric oxide (NO) generation that acts as a retrograde messenger to increase presynaptic glutamate release following postsynaptic stimulation of the glutamate receptor. Recent studies have questioned this hypothesis by observing no effects on performance in memory tasks with evidence of marked NOS inhibition (e.g., Barnes et al. *Neurosci Abs.* 18:1215, 1992). Using a 14-unit T-maze, we injected rats i.p. with N$^{\omega}$-nitro-L-arginine (NARG) to inhibit NOS activity (Ingram et al. *Neurosci Abs.* 18:1220, 1992) and observed impaired acquisition but not retention. Follow-up studies indicated that the NARG impairment may be related to vascular effects, since sodium nitroprusside, an NO generator causing marked vasodialation, could partially attenuate the NARG effect. Thus, to overcome possible peripheral vascular effects, we pursued studies in which NARG was injected i.c.v. Young (3 mo) male Sprague-Dawley rats were pretrained in 1-way active avoidance to a criterion (13/15 avoidances) in a straight runway. The next day about 1 hr before maze training, rats received injections of NARG (3 or 3.75 μg/μl) or vehicle (artificial CSF) via a cannula implanted into the left lateral ventricle about a week before training. During 15 trials, rats were required to locomote each of 5 segments within 10 s to avoid footshock (0.8 mA). Peformance variables included errors (deviations from correct pathway), runtime from start to goal, shock episodes and duration. Relative to controls, NARG-treated rats exhibited impaired performance in a dose-dependent manner. When treated with NARG during a retention test about 1 week later, rats showed no impairment. Therefore, central NARG treatment affected acquisition processes without evidence of nonspecific performance effects.

569.3

DISCRIMINATIVE STIMULUS (DS) EFFECTS OF NICOTINE IN HIGH ((HA) AND LOW (LA) ACTIVITY RATS.

J. James*, S. Robinson, J. Rosecrans,L. Hankansson, A. Nordberg, Dept. Pharmacol., VA Commonwealth Univ., Richmond, VA 23298 and Uppsala Univ., Uppsala, Sweden.

Previous research has shown that rats selected for differences in behavioral arousal (HA & LA rats) displayed a differential behavioral response (activity or habituation expts.) to nicotine; HA rats presented a more active forebrain serotonin system (5-HT). In the present study, HA rats again exhibited a more active 5-HT system (hippocampus). Dopamine (DA) turnover was faster in the HA rat (Striatum), but slower in the LA rat (hypothalamus; (metabolite/amine ratios). In contrast to previous work, nicotine elicited differential behavioral (VI-15 sec.) effects not related to HA or LA. Half of the rats in each activity group (Total N=32) elicited a biphasic pattern of responding (stimulation then depression), while the other half exhibited a dose-related depression of responding. A similar profile was noted in relation to tolerance and learning to discriminate nicotine. There were no differences in frontal cortex nicotine receptor binding between HA and LA rats. [Support-The German Research Council on Smoking and Health].

569.4

DISTANT ELECTROPHYSIOLOGICAL EFFECTS OF THE INTRACEREBRAL MICROINFUSION OF SALINE.

D.C. Smith*, E.J. Barea, S.E. Krahl and K.B. Clark. Dept. of Psychology and School of Medicine, Southern Illinois University-Carbondale, IL 62901.

The explicit purpose for using the technique of intracranial microinfusion is to determine the effects of *localized* application of a chemical. The results are interpreted in terms of the effects of these agents *at the specific location* where they were delivered. Electrophysiological research being performed in our lab suggests that the microinfusion of a small volume (0.5μl) of *saline* at, by literature standards, a slow to moderate rate (0.5μl/2 min) into the locus coeruleus (LC) was causing the distant release of norepinephrine (NE) onto cells in the dentate gyrus (DG) of the hippocampus (HPC). This result seriously questions the ability to localize the effects of intracerebral microinfusions.

The effects of microinfusing 0.5μl of 0.9% NaCl (pH=7.0) into the LC on the amplitude of the perforant path (PP)-DG evoked population EPSP (*p*EPSP) in Urethane anesthetized (1.5 g/kg) Long-Evans rats were studied. Both experimental (n=10) and control (n=9) animals had a guide cannulae implanted directly above the LC and were allowed to stabilize for 1 hr. Then a recording electrode was lowered into the granule cell layer of the ipsilateral DG, a stimulating electrode into the PP, and the position of these adjusted so as to maximize the amplitude of the evoked *p*EPSP. After a 1 hr stabilization period, a baseline *p*EPSP (average of 10 PP stimuli) was obtained, saline was microinfused through an injection needle that *did not* extend beyond the guide cannula, and then two additional average PP-DG *p*EPSP's were collected (immediately and 5 min postinfusion). Control animals had the injection needle inserted into the guide cannulae, but no injection was made). Immediately following the infusion of saline, the *p*EPSP was enhanced 5.3% over baseline, and by 5 min postinfusion was increased 12.6% ($p<.01$). These results are like those seen in the HPC when the LC is stimulated and suggest that the pressure from injecting a 0.5 μl volume at a relatively slow rate is stimulating the LC neurons sufficiently enough to cause the release of NE onto distant structures. Similar results have been obtained with medial septal microinfusions. We are currently examining these effects in unanesthetized animals.

569.5

S-ADENOSYLMETHIONINE AND METHIONINE INCREASE TAIL-FLICK LATENCY IN RATS. S.N.Young* and M.Shalchi. Department of Psychiatry and School of Dietetics and Human Nutrition, McGill University, Montreal, Canada H3A 1A1.

S-Adenosylmethionine (SAM) is an effective antidepressant with few side effects. As SAM increases brain serotonin and serotonin is involved in the gating of nociceptive afferents, we have studied the effect of SAM on tail flick latency to a thermal stimulus in the rat. Because methionine can raise brain SAM levels we also tested the effects of methionine. In a time course study rats were given 200 mg/kg SAM orally. Tail-flick latency was increased significantly 1.5 and 3 hours later. In a dose response study 200 and 400mg/kg but not 100mg/kg SAM increased tail flick latency significantly two hours after an oral dose. Methionine also increased tail flick latency with the peak effect occurring at 4 hours after oral administration. A dose response study revealed a significant effect of methionine at only 50mg/kg, a lower dose than that found with SAM. When methionine was added to rat chow to increase its level in the diet from 0.45% to 0.6% there was an increase in tail flick latency that reached significance after 21 days. Our results show that the tail-flick response which is a spinal reflex and is modulated in part by the activation of descending 5-HT systems is affected by SAM and methionine administration. SAM and methionine should be tested for their effects on clinical pain. In addition, because SAM is an antidepressant, and methionine shows a similar effect to SAM in the tail flick test, methionine should be considered for a possible antidepressant effect. (Supported by the Medical Research Council of Canada.)

569.6

EFFECTS OF DRUGS AND ^{60}CO IRRADIATION ON BRIGHTNESS DISCRIMINATION FOR RATS IN A CONTINUOUS Y WATER MAZE. J.L. Ferguson*, J.J. Burke and A.H. Harris. Department of Behavioral Sciences, AFRRI, Bethesda, MD 20889.

Male Long-Evans rats were trained to approach lights in a trianglar maze of water-filled alleys. At each apex a platform could be elevated to allow escape (correct answer) or to return the rat to the water (new trial). On odd trials (12/session) the light cue was put out 1 sec after the platform was lowered (positive offset); on even trials the light cue was put out 1 sec before (negative offset). Twenty minutes before each session rats were given (i.p.) haloperidol (0.1, 0.3 mg/kg), scopolamine (0.56, 1.0, 1.8, 3.0 mg/kg) and vehicle in 20 intervening sessions. Following the drug series the rats were exposed bilaterally to whole body gamma radiation (7.5 Gy ^{60}Co, 10 Gy/min) and tested at 2 hr, 5 hr and 1, 2 and 3 days. Haloperidol and scopolamine reduced the number of correct choices by 5-10%. Haloperidol (01. mg/kg) increased by more than 200% ($p<.05$) a measure of the rats' tendency to chose the alley that was correct on the prior trial. Following irradiation, at 2 hrs and at 3 days this measure of win-stay also increased by more than 200% ($p<.05$). These results relate to reports that ionizing radiation potentiates haloperidol-induced catalepsy and suggest a modified win-stay measure for the perseverative behavior sometimes observed in response to neurotoxins.

569.7

EFFECT OF ACETYLCHOLINESTERSE INHIBITION ON BEHAVIORS IS AGE DEPENDENT IN *APLYSIA*. M.Srivatsan* and B. Peretz. Dept.of physiology,Univ.of Kentucky Med.Centr. Lexington, Ky 40536.

Acetylcholinesterase (AChE) activity levels change with age in *Aplysia* (Peretz et al., Soc. Neurosci. Abstr. 18:583,1992; Srivatsan et al., J. Comp. Physiol.B,162:29-37). To determine the effect of varying AChE levels, we tested two behaviors before and during AChE inhibition. We chose siphon/gill withdrawal reflex (S/GWR) that is age dependant and respiratory gill pumping movement (GPM) that is age independent (Hallahan et al., Neurobiol. aging, 13:217-225,1992) to test in mature and old *Aplysia*. AChE activity levels were reduced by injecting a reversible specific AChE inhibitor BW284c51 into their hemocoel. The graded response of S/GWR to siphon stimulation of increasing water jet intensities and the rate of GPMs during 5 min. exposure to artificial sea water (ASW) of increasing acidity from pH7.8 to pH 3 were measured. Both behaviors were tested in the same animals with injection of filtered ASW (control) and two days later with injection of BW284c51 (expterimental). A significant age effect was present in the behavioral responses to AChE inhibition. In mature *Aplysia* AChE inhibition seemed to affect both their sensory and motor functions as shown by a significantly shortened duration of S/GWR for all the stimulus intensities with a loss of the graded response to intensities between 1.25 and 2.5 Kg/cm^2. Their GPM rates were reduced and they were unable to differentiate pH7.8 and pH7. By comparison old animals were not sensitive to AChE inhibition as shown by the S/GWR response being the same for ASW and BW284c51 injection. Also, their GPM rates were unaffected by AChE inhibition. The results show that S/GWR is more sensitive to reduced AChE levels than GPM and the effects of AChE inhibition on both behaviors is age dependent. The effects of AChE inhibition on the physiological properties of neurons will be reported.

569.8

CHRONIC SENSORY STIMULATION HAS AGE RELATED EFFECTS ON ACETYLCHOLINESTERASE ACTIVITY. B. Peretz* and M. Srivatsan. Dept. of Physiology, Univ. of Kentucky Coll. Med., Lexington, KY 40536.

Chronically applied sensory stimulation (CSS) results in improved motor neuronal function in the old adult *Aplysia* (Zolman & Peretz, Behav. Neurosci. 101:524, 1987). As a step toward understanding how CSS results in improved neural function, we investigated if AChE (acetylcholinesterase) activity was affected by chronically applied siphon stimulation. Since AChE was released by tissues with depolarization and its inhibition altered the response to siphon stimulation, we focussed on AChE (Peretz et al., Neurosci Abstr. 18:583, 1992; Srivatsan et al., J. Comp. Physiol. 162:29, 1992; Srivatsan & Peretz, Soc. Neurosci. Abstr. 1993). CSS, water jet stimulus, of 2.5 kg/cm^2 for 1s., was applied daily for four weeks to the siphon of freely behaving young, mature and old *Aplysia*. CSS in old animals resulted in significantly increased AChE activity in both the CNS and serum (cell-free hemolymph), 18% and 30% respectively compared to the control group, ($F(1,41)=5.6$, $p<0.025$); CSS significantly altered the response to varying the intensity of siphon stimulation, from 0.018 to 2.5 kg/cm^2, $F(4,112)=3.89$, $p<0.01$. In contrast, the effects of CSS on young and mature animals were not significant, yet it resulted in reduced AChE activity in the serum of -19 and -20%, respectively, and in the CNS of -19% and -2.5%, respectively. Behavioral changes were not correlated with changes AChE activity in the two age groups. The control groups of the three ages appeared to show effects associated with daily handling which may be related to decreased AChE activity. These results show that chronically applied siphon stimulation affects AChE activity, and age along with the CSS appear to determine the level of the activity. Long-term environmental changes as detected by the nervous system are expressed by at least one serum protein. Effects of CSS and AChE on neuron function will be reported.

569.9

DISTRIBUTION OF SUBSTANCE P IN BRAIN AND ITS ROLE IN AGGRESSIVE BEHAVIOR IN GRAY SHORT-TAILED OPOSSUMS *(MONODELPHIS DOMESTICA)*. B.H. Fadem*, K. Schubert, L. Taylor-Ali, J.M. Swann, M.B. Shaikh and A. Siegel., Laboratory of Limbic System & Behavior, Departments of Psychiatry and Neurosciences, N.J. Medical School and Graduate School of Biomedical Sciences, UMDNJ, Newark, N.J., 07103 and Department of Biology, Rutgers University, Newark, 07102.

It has recently been shown that substance P (SP) associated with limbic structures plays a central role in the onset of feline aggressive behavior. Since gray opossums display intense intraspecific aggression, the effects of systemic administration of the NK_1 antagonist, CP 96,345 on fight and threat behavior as well as the distribution of SP in brain were examined in this marsupial species. Preliminary behavioral analyses suggest that delivery of 4.0 mg/kg of CP 96,345 causes a decrease in threat and fight behavior in female but not in male gray opossums. Immunocytochemical analysis of brain revealed that SP positive neurons were located principally in the anterior aspect of the dorsomedial hypothalamus. In addition, SP fibers and preterminals were identified within the midbrain periaqueductal gray (PAG). The data suggest that descending fibers from the medial hypothalamus to the PAG mediate the expression of these forms of aggressive behavior in female gray opossums. [Supported by NSF grant BNS 8919601 (B.H.F.) and NIH grant NS07941 (A.S.)].

569.10

THE ATYPICAL ANTIPSYCHOTIC AGENT CLOZAPINE ANTAGONIZES THE MK-801 DISCRIMINATIVE STIMULUS CUE. T.A. Wettlaufer, F. Camacho, R.J. Fishkin, L.L. Kerman, A.T. Woods, & R. Corbett* Dept. Biol. Res., Neuroscience SBU, Hoechst-Roussel Pharm., Inc., Route 202-206, Somerville, NJ 08876-1258.

The non-competitive NMDA antagonists phencyclidine (PCP) and MK-801 induce a psychotomimetic state in man that closely resembles the positive and negative symptoms of schizophrenia. In addition, these compounds can produce a discriminative stimulus in rats and can generalize completely to each other. The atypical antipsychotic clozapine antagonizes MK-801-Induced Locomotion and Stereotypies (MK-801-ILS) in rats with an ED_{50} value of 1.1 mg/kg, i.p. The aim of the present investigation was to determine whether clozapine can antagonize the MK-801-induced PCP-like discriminative stimulus cue in rats trained to discriminate MK-801 from saline in a two-choice, discrete-trial avoidance paradigm. Four male Wistar rats were trained to discriminate MK-801 (0.075 mg/kg, i.p.) from saline. MK-801 was administered 60 min prior to the test sessions and the various doses of clozapine (0.31, 0.63, 1.25, 2.5 and 5.0 mg/kg, i.p.) were administered 30 min after the injection of MK-801. Clozapine dose-dependently antagonized the MK-801 discriminative stimulus cue in all four subjects. Furthermore, these doses of clozapine are significantly less than those shown to antagonize dopaminergic D_2 behaviors exhibited in the Climbing Mouse Assay (CMA: clozapine ED_{50} = 12.5 mg/kg, i.p.). Therefore, clozapine has a CMA/MK-801-ILS ratio of 11.2. These results suggest that clozapine antagonizes the MK-801 discriminative stimulus cue through mechanisms independent of dopamine D_2 antagonism.

569.11

BEHAVIORAL AND PHARMACOLOGICAL EFFECTS OF A PHENCYCLIDINE METABOLITE, (TRANS)-4OH-CYCLOHEXYL PCP.

A. Baba[1], T. Yamamoto[1], H. Yamamoto[1], T. Kawai[1], T. Suzuki[1], T. Moroji[1] and Y. Murashima[2]*. [1] Dept. of Psychopharmacology and [2]Dept. of Neurophysiology, Tokyo Inst. of Psychiatry, Tokyo. 156, Japan.

The psychologic responses of normal human subjects to phencyclidine (PCP) administration include both delirium, with clouded consciousness or vivid psychotic symptoms, and affective responses with no altered consciousness. Delirious states can be both acute or chronic, the latter being well beyond the drug half-life. The drug action may be particularly prolonged because of the formation of active metabolites. In order to better understand this drug action, we examined behavioral and pharmacological activities of a major metabolite of PCP, (trans)-4OH-cyclohexyl PCP. Intraperitoneal injection of (trans)-4OH-cyclohexyl PCP to mice caused an increase in locomotor activity in a dose dependent manner. In the in vitro binding experiments, (trans)-4OH-cyclohexyl PCP as well as PCP had relatively high affinity for the dopamine (DA) uptake site but not for sigma or NMDA/PCP receptor complex. These observations indicate that (trans)-4OH-cyclohexyl PCP may also play a role in psychotomimetic actions of PCP through its inhibitory effect on the DA uptake site.

569.12

THE EFFECTS OF SKF525A TREATMENT ON THE DELAYED ANTICONFLICT ACTION OF MK-801 IN RATS. Z.C. Xie* and R.L. Commissaris. Dept. Pharmaceut. Sci., Wayne St. Univ., Detroit, MI 48202.

In female rats, MK-801 (dizocilpine) exerts a delayed anticonflict (i.e., anxiolytic-like) effect (PBB 43:471-477, 1992). One explanation for the delayed onset of action for MK-801 treatment is that MK-801 is a 'pro-drug' and the anticonflict effects are caused by one or more of its hepatically-generated metabolites (2OH-, 8OH-MK-801). The present studies were designed to address this possibility. In the first experiment, the effects of MK-801 administered IP were determined in female rats following a range of pretreatment intervals (0.5-48 hr). As observed previously, 0.2 mg/kg MK-801 exerted anticonflict effects at pretreatment intervals of 14-32 hr, but not before 12 hr or after 32 hr. In the second experiment, the hepatic microsomal drug metabolism inhibitor SKF525A (50 mg/kg, IP, 12-hr pretreatment) had no effect on conflict behavior when administered alone, nor did it prevent the delayed anticonflict effect produced by a single dose of MK-801 (0.2 mg/kg) . In the third experiment, chronic treatment with MK-801 (0.2 mg/kg daily, administered IP immediately after testing) resulted in a robust anticonflict with no evidence of tolerance across three weeks of treatment. Administration of SKF525A did not prevent the anticonflict effect of this chronic MK-801 treatment. Together, these data are not consistent with the hypothesis that MK-801 exerts its delayed anticonflict effect via the formation of a P450-generated active metabolite. (Supported in part by MH 48171 to RLC).

569.13

A CONSTANT SPEED ROTOROD TASK IS MORE SENSITIVE TO DEPRESSANT EFFECTS OF DRUGS THAN AN ACCELERATING SPEED ROTOROD TASK IN RATS. M. C. Grace, M. D. Crago and J. V. Cassella.* Neurogen Corporation, Branford, CT 06405.

The rotorod task in rodents has long been employed as a measure of muscle relaxing or motor impairing effects of drugs. In some studies animals are placed on a rod revolving at a constant speed while in other cases animals are placed on a stationary rod that then accelerates. It might be argued that these are two distinct tasks that may be differentially sensitive to drug effects. This study examined the effects of sedative and muscle relaxant drugs in rats tested on both accelerating (rats were placed on the stationary rod which then accelerated linearly to 80 rpm over a 4 min period) and constant speed task (rotating at 20 rpm). All compounds showed greater deficits on the constant speed task than on the accelerating task. The sedative hypnotic Zolpidem (0.06 - 1.0 mg/kg, IV) and anxiolytics Diazepam (0.25 - 2.0 mg/kg, IV) and Alprazolam (0.03 - 0.5 mg/kg, IV) produced a much greater impairment in the constant task than in the accelerating task. The muscle relaxants Baclofen (0.25 - 4.0 mg/kg, IV) and Dantrolene (0.5 - 4.0 mg/kg, IV) produced very little impairment in the accelerating task while producing a much greater impairment in the constant task. The Diazepam and Alprazolam constant speed effects are comparable to the effects produced by these compounds in a locomotor activity task (a 15 minute test session in the dark Omni Digiscan Activity Monitor). Therefore, the magnitude of the depressant effect of drugs is dependent upon whether an accelerating or constant speed paradigm is employed.

569.14

DIFFERENTIATION OF SEDATIVE AND MUSCLE RELAXANT EFFECTS USING THE ACOUSTIC STARTLE RESPONSE AND LOCOMOTOR ACTIVITY MEASURE IN THE RAT. M. D. Crago, M. C. Grace, R.J. Primus* and J. V. Cassella. Neurogen Corporation, Branford, CT 06405.

Many behavioral paradigms are sensitive to the effects of sedative and/or muscle relaxant compounds in rats. While it is useful to know that a compound can depress certain behaviors, it is more informative to determine whether a compound is relatively more sedating or motor impairing (e.g. muscle relaxing). This study attempted to differentiate between sedative and muscle relaxant activity of drugs and compared the effects of these drugs in the acoustic startle paradigm and the locomotor activity test. The acoustic startle response was elicited by 20 stimuli at each of three intensities (85, 90, and 95 db; 50 ms duration) with each stimulus presented in a counterbalanced order every 30 seconds. Locomotor activity was assessed by an Omnitech digiscan system (infrared photo cells; animals tested under red light conditions; 62 db white noise; 15 minute test session). Rats were injected with either the sedative hypnotic Triazolam (0.0075 - 0.125 mg/kg,IV) or Zolpidem (0.03 - 2.0 mg/kg,IV) or the muscle relaxant Dantrolene (0.5 - 4.0 mg/kg,IV) or Baclofen (0.25 - 4.0 mg/kg,IV) 5 minutes prior to testing. All of these compounds reduced the amplitude of the acoustic startle response and decreased locomotor activity. However, the sedative hypnotics were much more potent in reducing locomotor activity than in decreasing startle amplitude while the muscle relaxants were relatively equipotent in reducing both behaviors. Therefore, the combined use of these paradigms could be used to determine the relative contribution of sedative or muscle relaxant properties of behavior-depressing compounds.

569.15

EVALUATION OF THE DISCRIMINATIVE STIMULUS EFFECTS OF THE NOVEL PUTATIVE SEDATIVE-HYPNOTIC CL 284,846. K. E. Vanover* and J. E. Barrett. American Cyanamid, Lederle Laboratories, CNS-Biology, Pearl River, New York, 10965.

CL 284,846, N-[3-(3-cyanopyrazolo[1,5-a]pyrimidin-7-yl)phenyl)]-N-ethylacetamide, is a novel non-benzodiazepine putative sedative-hypnotic with benzodiazepine-like sedative effects, but with less liability for the usually accompanying undesired side-effects. In an effort to further characterize its pharmacological activity CL 284,846 was established as a discriminative stimulus (DS). Rats (n=7) were trained to discriminate CL 284,846 (3.0 mg/kg, i.p., 30 min pretreatment) from saline in a two-lever, sucrose-reinforced drug discrimination paradigm. CL 284,846 functioned as a DS in all rats and showed a dose-related (0.03 - 10.0 mg/kg) increase in drug-appropriate responding up to the training dose and a dose-related decrease in response rate. Once this discrimination was established, similarities among the DS effects of CL 284,846 and known sedative and anxiolytic drugs were examined. The benzodiazepine agonist, triazolam (0.03 - 1.0 mg/kg) substituted for CL 284,846 only at doses which also decreased response rate, while the benzodiazepine partial agonist, Ro 17-1812 (0.1- 3.0 mg/kg) fully substituted for CL 284,846 with no effect on rate. Buspirone (1.0 - 10.0 mg/kg), a 5-HT_{1A} agonist, and pentobarbital (3.0 - 17.0 mg/kg), a barbiturate, failed to substitute for CL 284,846 up to rate-decreasing doses. A novel putative anxiolytic, CL 273,547 (3.0 - 56.0 mg/kg) was also tested and substituted in 3 of 5 rats at doses which also decreased response rate. Flumazenil (3.0 - 10.0 mg/kg, i.p.), a benzodiazepine antagonist, blocked the DS effects of CL 284,846 in 4 of 5 rats tested with no effect on response rate. Taken together, these results suggest that the DS effects of CL 284,846 are mediated via benzodiazepine receptors. CL 284,846, however, remains distinct from most sedatives in that its DS effects failed to generalize to pentobarbital. Further investigation is needed to clarify this distinction.

569.16

APPEARANCE OF CHOREIFORM MOVEMENTS IN MICE FOLLOWING ACUTE TREATMENT WITH HIGH DOSES OF CAFFEINE: AN ANIMAL MODEL FOR CHOREIFORM MOVEMENT. O. Nikodijevic, K. A. Jacobson, and J. W. Daly.* Lab. of Bioorganic Chemistry, NIDDK/NIH, Bethesda MD 20892.

Injection of caffeine at a dose of 35 to 70 mg/kg causes choreiform (dance-like) movements in NIH Swiss mice in a dose-dependent manner. This effect is less pronounced in mice that had ingested 1 g/kg caffeine in the drinking water for 7 days. The dopamine antagonist haloperidol decreased locomotor activity, and the L-type calcium channel blocker nitrendepine increased locomotor activity (effect follwing in chronic caffeine ingestion). Coadministration of haloperidol (1 or 3 mg/kg) and caffeine, or nitrendipine (50 or 100 mg/kg) and caffeine, markedly reduce the choreiform effect of caffeine. The A_{2a} selective adenosine agonist APEC, but not the A_1 selective agonist CHA, also diminishes choreiform movement. The data suggest involvement of dopaminergic and adenosine receptors as well as the calcium channel system in the appearance of choreiform movement. These animals could be used as a model for further investigation of the mechanism of choreiform movement as well as possible therapeutic approaches to certain choreas in humans related to disease states (e.g. Huntington's disease or Tourette syndrome) or side effects of drug treatment.

569.17

CAFFEINE DELAYS STARTLE HABITUATION IN HIGH CAFFEINE USERS E.J. Schicatano* and T.D. Blumenthal
Wake Forest University, Winston-Salem, NC 27109

Past studies have shown that caffeine delays startle reflex habituation in low caffeine users. The present experiment examined the effects of caffeine (4 mg/kg) on startle habituation in high caffeine users. Eyeblinks were measured in humans in a startle habituation paradigm in which 85 dB broadband noise stimuli were presented. Subjects were given a dose of caffeine (4 mg/kg), and then 24 hours later were administered either placebo or another dose of caffeine (4 mg/kg). In this respect, caffeine "withdrawal" was operationally defined as 24 hours without caffeine. The results demonstrated that caffeine delayed startle amplitude habituation. When comparing response amplitude in the first trial block for both caffeine and placebo conditions, no significant difference was demonstrated. Therefore, caffeine did not alter initial startle responding, instead, it affected plasticity of this response. Comparing first trial block response amplitude for high caffeine users given placebo, and low caffeine users given placebo (data from an earlier study), revealed a higher response amplitude for the high caffeine users (withdrawal condition). Thus, our data show that (1) Caffeine delays startle habituation in high caffeine users, and (2) withdrawal from caffeine in persons with a history of caffeine usage may render these individuals more sensitive to startle stimuli.

569.18

CHRONIC CLONIDINE TREATMENT EFFECTS ON CONFLICT BEHAVIOR AND FOOTSHOCK SENSITIVITY IN THE RAT. W.T. Repaskey and R.L. Commissaris*(SPON: T.J. Hill). Dept. Pharmaceutical Sciences, Wayne State University, Detroit, MI 48202.

It has been reported previously that chronic clonidine administration produces a robust anticonflict effect in rats over the course of several weeks of treatment (Behav. Pharmacol. 1:201-208, 1990). The present studies were designed to examine the possible relationship between the effects of chronic clonidine treatment on conflict behavior and sensitivity to footshock. Subjects were trained for conflict testing in the conditioned suppression of drinking (CSD) conflict task, a repeated measures modification of the Vogel acute conflict task. Subjects received either chronic clonidine (40 μg/kg) or chronic saline treatment twice daily, once immediately after behavioral testing and again 12 hours later. Punished responding in clonidine-treated subjects did not differ from saline-treated controls for the first week of chronic treatment, but was significantly greater than in controls by the second week of chronic treatment. For shock sensitivity determinations, the same subjects were given brief footshocks (0.08 - 0.35 mA; 500 msec duration) and were rated for the presence or absence of a response (e.g., twitch, jump, vocalization, etc.) by a trained observer who was blinded regarding both treatment condition and shock intensity. As expected, this procedure revealed a robust current intensity versus response function. Chronic clonidine treatment did not affect sensitivity to unavoidable footshock. Moreover, there was no correlation between footshock sensitivity and punished responding in the conflict task for either saline- or clonidine-treated subjects. These data indicate that the anticonflict effect of chronic clonidine treatment is not the result of a decrease in sensitivity to footshock. (Supported in part by MH #47181 to RLC).

HYPOTHALAMIC-PITUITARY-GONADAL REGULATION: NEUROPEPTIDES AND TRANSMITTERS

570.1

NOVEL TRIPLE-LABELING METHOD COMBINING DUAL-LABEL IN SITU HYBRIDIZATION DETECTION OF LHRH AND GALANIN mRNAs WITH IMMUNOCYTOCHEMICAL DETECTION OF NUCLEAR FOS PROTEIN. E. Hrabovszky[1], M. Vrontakis[2], D. Keisler*[3] and S.L. Petersen[1]. Depts. Anat. and Neurobiol.[1], and Ani. Sci.[3], Univ. Missouri, Columbia 65212 and Dept. Physiol., Univ. Mannitoba, Winnipeg[2].

Recently, a sensitive dual-label in situ hybridization technique was developed in this laboratory. Using this method, we simultaneously detected mRNAs encoding LHRH and the β_3-subunit of the $GABA_A$ receptor. Because of the well-known topographical and functional heterogeneity of LHRH neurons and our recent finding that only a subpopulation of LHRH neurons appears to express the β_3-subunit, we deemed it important to find a way to determine whether subpopulations of LHRH neurons expressing receptors or other peptides were those involved in producing the LH surge on proestrus. To accomplish this aim, we performed dual-label in situ hybridization histochemistry in conjunction with immunocytochemical detection of Fos protein, a putative marker of increased LHRH neuronal activity. For methodological validation, the present application of the new triple-labeling technique combines non-radioactive detection of LHRH mRNA using a digoxigenin-labeled cRNA probe, autoradiographic visualization of galanin mRNA hybridized to a ^{35}S-labeled cRNA probe and immunocytochemical localization of Fos protein in the cell nuclei of activated LHRH neurons. Methodological aspects of combined immunocytochemistry and in situ hybridization histochemistry, as well as applications of the new triple-labeling technique are discussed in detail.

570.2

GALANIN GENE EXPRESSION IN THE BED NUCLEUS OF THE STRIA TERMINALIS AND MEDIAL AMYGDALA INCREASES ACROSS PUBERTY BUT LACKS SEXUAL DIMORPHISM. B. Planas*, P.E. Kolb, M.A. Raskin and M.A. Miller. Department of Psychiatry and Behavioral Sciences, University o Washington, Seattle, WA 98195.

We have recently observed that galanin mRNA is co-expressed in the majority o vasopressin (VP) neurons of the bed nucleus of the stria terminalis (BNST) and th medial amygdala (AMe) of the male rat. We have shown that gonadal steroid regulate both VP and GAL gene expression in the BNST of adult male rats. In th BNST, both VP immunoreactivity and gene expression exhibit sexual dimorphism. Here, we have used in situ hybridization and quantitative autoradiography t determine whether GAL gene expression in the BNST and AMe is sexually dimorphic and whether the level of expression changes across puberty. Male and female Wistar rats (24 d, 90 d) were sacrificed and their brains removed. Brain sections were hybridized with a ^{35}S-labeled 680 bp cRNA probe complementary t GAL mRNA. The number of labeled cells (unilateral) and the average number o grains/cell were compared in four atlas-matched sections per region across groups. No sex differences in GAL gene expression were detected in either region. In the BNST, more neurons ($p<0.02$) were labeled in adult (male: 184±12, n=3; female: 190±8, n=5) compared to prepubertal rats (male: 128±8, n=5; female: 120±10, n=5). The average number of grains/cell was also higher ($p<0.001$) in adult rats (ad male: 89±4; ad female: 108±10; prep male: 62±2; prep female: 61±4). In the AMe, no age difference was observed in number of cells (ad male: 75±4; ad female: 72±7; prep male: 69±6; prep female: 67±2); however, the number of grains/cell was increased ($p<0.001$) across puberty in both sexes (ad male: 100±7; ad female: 107±7; prep male: 63±1; prep female: 56±3). A second experiment replicated the lack of a sex difference in the number of GAL mRNA expressing neurons in the adult BNST (male: 178±4, n=6; female: 171±9, n=6). These results indicate that GAL gene expression in these regions does not exhibit sexual dimorphism however, these pathways are activated across puberty.

570.3

COLOCALIZATION OF GLUTAMATE RECEPTOR SUBUNIT GluR1 mRNA AND GALANIN IN RAT PREOPTIC HYPOTHALAMUS. Z. Cao*, C. Ulibarri, and T.R. Akesson, Dept of Vet & Comp Anatomy, Pharmacology, & Physiology, Washington State University, Pullman, WA 99164.

Glutamate has been recently shown to play a hypothalamic role in the release of luteinizing hormone from the pituitary and there is evidence that this excitatory amino acid is active in circuits mediating sexual behavior. Galanin has also been recently implicated in the regulation of LHRH release; and in the preoptic area where this peptide is colocalized with estrogen receptors, galanin stimulates male sexual behavior. To identify sites where glutamate and galanin could interact to influence reproductive processes, we have used a double-labeling method combining immunohistochemistry and *in situ* hybridization. Two days after colchicine treatment, immunohistochemistry was used to visualize galanin immunoreactivity (GALir). The sections were then hybridized with GluR1 antisense RNA (gift of the Molecular Neurobiology Laboratory, Salk Institute) labeled with Dig-UTP that was detected after hybridization by anti-Dig alkaline phosphate conjugate. The subsequent enzyme-catalyzed color reaction with x-phosphate and nitroblue tetrazolium salt produced an insoluble purple/blue precipitate in and around the nuclei of neurons containing GluR1 mRNA.

Coexistence of GALir and GluR1 mRNA was observed in the diagonal band of Broca, the ventral subdivision of the lateral septum, the anteroventral and preoptic periventricular nuclei, the medial preoptic nucleus, and the bed nucleus of the stria terminalis. Proportions of doubly-labeled neurons were highest in the medial preoptic nucleus where 40-60% of the GALir cells expressed GluR1 mRNA and in the preoptic periventricular nucleus where colocalization represented 50% of the GALir cells. These findings are consistent with the possibility that glutamate and galanin have a synergistic influence on processes that regulate reproductive functions. Supported by HD22869.

570.4

MEDIAN EMINENCE IN VIVO RELEASE OF NPY AND LHRH BEFORE, DURING AND AFTER A PREOVULATORY LH SURGE IN EWES. J.P. Advis*, C.D. Conover, J.K. McDonald, J. Rabii and S. Bailey, Dept of Animal Sciences and Dept of Exercise Physiology, Rutgers Univ, New Brunswick, NJ 08903, Dept Anatomy / Cell Bio, Emory Univ, Atlanta, GA 30322, and Dept Biological Sciences, Rutgers Univ, Piscataway, NJ 08855.

Neuropeptide Y (NPY) might play a role, at the median eminence (ME) in the neuroendocrine control of preovulatory release of luteinizing hormone (LH) - releasing hormone (LHRH) and LH. Using a synchronized estrous cycle model and sampling ME push-pull cannula (PPC) perfusate and jugular blood at 10-min intervals for 6h, we studied: **a)** basal in vivo PPC release of NPY and LHRH, and plasma LH throughout the cycle; **b)** in vivo ME-LHRH release in response to increasing concentrations of NPY infused through the ME-PPC probe (0, 2, 6, 18 μM) in luteal and follicular ewes; and **c)** in vivo ME-LHRH release in response to increasing concentrations of NPY antiserum (As) infused through the ME-PPC probe (NRS no As, 1:1000, 1:100) during the onset of the synchronized preovulatory surge of LH. Basal in vivo release of both LHRH and NPY increased from luteal to surging ewes (mean ±sem (n), luteal vs surge, LHRH: 1.46±.43 (6) vs 18.3±1.22 pg/100 μl (6); NPY: 68.5±7.87 (6) vs 120±9.80 pg/100 μl (6); LH: 0.64±.04 vs 112±11.21 ng/ml (6)), predominantly due to increases in pulse amplitude ($P<0.05$). ME infusion of NPY increased LHRH and LH release in a dose-dependent fashion in follicular but not in luteal ewes, with the largest dose inducing surge-like levels of LHRH and LH in late follicular ewes ($P<0.05$). ME-infusion of NPY-As (1:100) decreased LHRH and LH release in late follicular ewes ($P<0.05$). Furthermore, infusion of NPY-As during the ascending phase of the LH surge stopped and even decreased LHRH and LH release for as long as the As was infused (2h). When ME-NPY-As infusion ceased, the arrested ascending preovulatory release of LHRH and LH resumed. Thus, ME-NPY secretion stimulated LHRH release during the preovulatory surge of LHRH and of plasma LH (supported by NJAES - Hatch 06108 & USDA 89-37240-4587 to J.P. Advis).

570.5

***C-FOS* IMMUNOREACTIVITY AFTER INTRACEREBRO-VENTRICULAR NEUROPEPTIDE Y INJECTION IN IMMATURE FEMALE RATS.** Fraley, GS *, G Torres, and DK Sarkar. Dept. VCAPP, Washington State University, Pullman, WA 99164-6520.

Neuropeptide Y (NPY), a member of the PP family, is known to be a strong modulator of hypothalamic function, particularly in food intake and reproduction. We have recently shown that NPY stimulates the onset of puberty in chicks and rats. In order to investigate the mechanism of NPY's action on the onset of puberty in rats we utilized the activation of the immediate early gene, c-fos, as an indicator of hypothalamic neural activation. Twelve immature Sprague-Dawley female rats had stainless steel cannulas implanted into the third ventricle at 24-days of age. During the afternoon of day 30, 10 μg of NPY was injected into the third ventricle of six animals and another six animals were injected with physiologic saline, the carrier agent for the NPY. This dose of NPY has been shown previously to initiate puberty within 4-5 days after injection. One hour after injection, the animals were perfused with heparinized physiologic saline followed by 4% paraformaldehyde and the brains removed and sectioned at 40 microns on a sliding microtome. Hypothalamic sections were stained immunocyto-chemically for c-fos gene product using a polyclonal primary antibody (Oncogene Science) following the ABC procedure (Vectastain). Results indicated a qualitative increase in c-fos immunoreactivity in the NPY injected animals as compared to controls within two major hypothalamic regions, the preoptic area (POA) and the paraventricular nucleus (PVN). We hypothesize that the NPY induction of puberty may either involve a direct stimulation of LHRH neurons within the POA or indirectly stimulate LHRH release via neurons within the PVN. Further colocalization studies of c-fos immunoreactivity will be needed in order to establish the neurotransmitters or neuromodulators involved with NPY to stimulate c-fos reactivity during the activation of reproductive function.

570.6

TYROSINE HYDROXYLASE mRNA IN THE MONKEY HYPOTHALAMUS: LOCALIZATION AND REGULATION BY STEROIDS. S.G. Kohama* and C.L. Bethea. Div. Reprod. Sciences, Oregon Regional Primate Research Center, Beaverton, OR 97006.

Regulation of hypothalamic dopaminergic neurons by chronic steroid stimulation was examined in spay rhesus monkeys by *in situ* hybridization (ISH) for tyrosine hydroxylase (TH). Spay monkeys were treated for 28 days with: empty capsule (control), estrogen implant (E), or E supplemented with progesterone (P) for the last 14 days of treatment (E+P). The first set of brains was fixed by bath immersion whereas the second set was perfused-fixed. Hypothalamic blocks were then processed for ISH. Sections (10 μm) were hybridized with 35-S labelled antisense rat cRNA probe. Specific labelling in hypothalamic nuclei and substantia nigra coincided with TH-immunostained neurons on adjacent sections or colocalized with ISH. Grain counts over labelled nuclei were obtained with computer imaging of darkfield illuminated sections. Although both sets of tissue had similar patterns of hybridization, the perfusion-fixed brains displayed more labelled cells. Preliminary grain counts of perfused tissue revealed little TH mRNA regulation by E or E+P in areas previously shown to have P receptors (PR) such as the periventricular and dorsal arcuate nucleus. However, grain number decreased in the ventral arcuate dopamine neurons after E or E+P treatment although little PR occurs in this population suggesting indirect effects of steroids. Supported by HD17269, HD18185.

570.7

MORPHINE EFFECTS ON TYROSINE HYDROXYLASE (TH) mRNA LEVELS IN HYPOTHALAMIC DOPAMINE NEURONS AND THEIR ROLE IN LHRH RELEASE. C.A.Barraclough*, J. Molnar and J-R He. Dept. Physiol.,Sch. Med, University of Maryland, Baltimore, MD 21201.

Morphine blocks icv NE-induced increases in LHRH mRNA and paradoxically, in these same rats, it amplifies LHRH release. We questioned whether altered hypothalamic dopamine (DA) secretion was involved in this phenomenon. Changes in TH mRNA levels in A15 to A12 DA neurons were used as an index of DA neuronal activity and used *in situ* hybridization histochemistry and quantitative image analysis methods. The data show that: (1) TH mRNA levels were not altered 1, 5 or 24 h after morphine in preoptic A15 or anterior hypothalamic A14 neurons; (2) TH mRNA levels in A13 cells increased significantly 1 ,5 and 24 h after morphine; (3) message levels in tuberoinfundibular DA neurons (TIDA) also were increased at 1 and 5 but not at 24 h after morphine; (4) Preoptic DOPAC levels in microdialysis samples increased by about 217% following either sc or direct morphine infusions into A13 cells; (5) icv DA (20 μg) did not affect LH release and it neither augmented nor suppressed NE-induced LH release. We propose that NE directly stimulates median eminence terminals to release LHRH and local TIDA neurons modulate this response. Morphine initially suppresses TIDA secretion [plasma prolactin (Prl) increases] but by 1 h a rebound in TIDA neuronal activity occurs and as PRL declines, TH mRNA levels increase in these cells. A15 to A13 dopamine neurons do not appear to be important modulators of LHRH neuronal responsiveness to NE. Supported by HD-02138.

570.8

TESTOSTERONE STIMULATES ROSTRAL AND MEDIOBASAL HYPOTHALAMIC GABA TURNOVER IN THE CASTRATE MALE RAT. D.R. Grattan* and M. Selmanoff, Department of Physiology, University of Maryland, School of Medicine, Baltimore, MD 21201-1559.

We recently determined that castration specifically decreased GABA turnover in the rostral and mediobasal hypothalamus, an effect that was only partially reversed by an implant of testosterone (T). This study aimed to confirm that T could stimulate GABA turnover in hypothalamus of the castrate rat, and to investigate whether pulsatile T replacement might stimulate GABA turnover more effectively than the constant levels provided by an implant. Animals were divided into 4 experimental groups: intact, 48 h castrate, 48 h castrate + T capsules (2 x 30 mm Silastic implants, 0.062" id, 0.125" od), 48 h castrate + T injections (100 μg/kg, 3x daily). GABA concentrations were measured in 5 microdissected brain regions, and in the anterior, posterior and intermediate lobes of the pituitary, either before, or 60 minutes after inhibition of the GABA degrading enzyme GABA transaminase by injection of aminooxyacetic acid (AOAA, 100 mg/kg ip). The rate of GABA accumulation in the tissue following injection of AOAA was used as an index of GABA turnover. In the diagonal band of Broca, the medial preoptic nucleus and in the median eminence, GABA turnover was significantly reduced by castration. There was no effect of castration on GABA turnover in the cingulate cortex, or in the pituitary gland. Both modes of T replacement increased GABA turnover to values not significantly different from intact controls, but neither treatment fully reversed the effects of castration. Hence, it is possible that another factor from the testes may also be involved in promoting GABA turnover in these regions. Alternatively, it may be that since even our pulsatile T replacement does not exactly mimic the normal pulsatile and circadian variations in plasma T, GABA turnover in these T-sensitive neurons in the rostral and mediobasal hypothalamus is also not fully reinstated. (Supported by NIH Grant HD21351)

570.9

GLUTAMATE AND GABA NEURONS INTERACT EXTENSIVELY IN NEUROENDOCRINE REGIONS OF THE FEMALE MONKEY BRAIN. K.K. Thind* and P.C. Goldsmith. Reproductive Endocrinology Center, Univ. California Sch. of Med., San Francisco, CA 94143-0556.

With their wide distributions, glutamate (Glu) and GABA neurons have extensive antagonistic effects throughout the brain. Since their direct interactions could play a pivotal role in governing CNS function, we performed double-label immunostaining for Glu and glutamic acid decarboxylase (GAD) in hypothalamic sections from two adult female cynomolgus monkeys. Ultrastructural analysis of 785 immunoreactive (-ir) profiles (63% Glu-ir, 28% GAD-ir, 9% Glu+GAD-ir) revealed strikingly consistent labeling of 2-4% somata (*SOM*), 65-80% dendrites (*DEN*), axons (*AX*), and 15-30% axon terminals (*AXO*) in the medial septum (MS), paraventricular nucleus (PVN), supraoptic nucleus (SON), arcuate-ventral hypothalamic tract (VHT1) and median eminence (ME). The three major types of labeled interactions showed regional differences in the processes involved:

-ir Contact	MS	PVN	SON	VHT1	ME
Glu/GAD	44.4%	50.0%	47.6%	50.0%	14.3%
Glu/Glu	27.7%	0.0%	23.8%	14.7%	33.3%
Glu/Glu+GAD	19.4%	16.6%	0.0%	23.5%	42.8%

Region	MS			VHT1		
Processes	*AXO/DEN*	*AX/DEN*	*DEN/DEN*	*AXO/DEN*	*AX/DEN*	*DEN/DEN*
Glu/GAD	31.2%	18.7%	43.7%	17.6%	0.0%	82.3%
Glu/Glu	80.0%	0.0%	10.0%	80.0%	0.0%	20.0%
Glu/Glu+GAD	85.7%	0.0%	14.3%	75.0%	0.0%	12.5%

Since Glu-ir and GAD-ir elements also synapse ubiquitously with other neuroendocrine systems, their extensive interactions seem to represent a logical regulatory network for controlling developmental and functional activity of numerous neuroendocrine systems. Supported by HD10907 and HD11979.

570.10

RELEASE OF GLUTAMATE AND ASPARTATE FROM THE PREOPTIC AREA DURING THE PROGESTERONE-INDUCED LH SURGE: IN VIVO MICRODIALYSIS STUDIES. D. W. Brann*, L. Ping, V. T. Wiedmeier and V. B. Mahesh. Dept. of Physiology and Endocrinology, Med. Col. of Georgia, Augusta, GA 30912

To examine the role of excitatory amino acid neurotransmitters in the regulation of LH and FSH secretion in ovariectomized (ovx) adult rats treated with estradiol and progesterone, we measured the release rates of glutamate and aspartate as well as the neurally inactive amino acid serine in microdialysis perfusate samples of the preoptic area (POA) of the hypothalamus collected at 30-min intervals during the LH surge induced by progesterone. The release rates of glutamate in the preoptic area increased at 1500h and 1600h and for aspartate at 1200h, 1300h, 1500h and 1600h in the progesterone treated estrogen-primed ovx rat as compared to estrogen controls. The increase in POA release rates of glutamate and aspartate in the progesterone treated rats occurred immediately prior to peak serum LH levels induced by progesterone. The neurally inactive neurotransmitter serine was unchanged by steroid treatment. Estradiol alone had no significant effect on the POA release rates. This study adds further evidence that excitatory amino acid neurotransmission is an important component in the neurotransmission line mediating gonadotropin surge expression in the female rat.

571.1

THROMBIN STIMULATES THE RELEASE OF INTRACELLULAR Ca^{2+} IN GT1-7 NEURONS. M.A. Javors,* T.S. King, X. Chang. Departments of Psychiatry, Pharmacology, and C&SB, University of Texas Health Science Center, San Antonio, Texas 78284.

Although best known for its effects on platelets, thrombin has been shown to bind to human brain and spinal cord and to produce intracellular biochemical effects through membrane surface receptors. Recently, immortalized GT1-7 neurons, which synthesize and secrete gonadotropin releasing hormone (GnRH), have been developed. The purpose of this study was to characterize thrombin-induced changes in cytosolic $[Ca^{2+}]$ in GT1-7 cells. GT1-7 neurons were loaded with fura-2 for the measurement of $[Ca^{2+}]_{cyt}$. Our results show that thrombin produced increases in $[Ca^{2+}]_{cyt}$ up to approximately 400nM in a concentration dependent manner between 0.025 to 0.2 units/ml. Chelation of extracellular Ca^{2+} with EGTA did not attentuate thrombin-induced $\Delta[Ca^{2+}]_{cyt}$. Indomethacin, an inhibitor of cyclooxygenase and prostaglandin synthesis, did not affect thrombin-induced $\Delta [Ca^{2+}]_{cyt}$. Hirudin, a specific inhibitor of thrombin's serine protease activity, partially inhibited thrombin-induced $\Delta [Ca^{2+}]_{cyt}$. These results suggest that a thrombin receptor on the surface membrane of GT1-7 neurons activates intracellular Ca^{2+} release, but not the entry of extracellular Ca^{2+}.

571.2

HOMOLOGOUS REGULATION OF THE GnRH RECEPTOR: EVIDENCE FOR TRANSLATIONAL CONTROL. M. Tsutsumi*[1], S. C. Laws[2], and S. C. Sealfon[3]. Fishberg Center in Neurobiology (1,2) and Neurology (2), Mt.Sinai Sch. of Med., New York, NY 10029 and Health Effects Research Lab. (3), US EPA, Research Triangle Park, NC 27711.

Gonadotropin-releasing hormone (GnRH) receptor number either increases or decreases in response to GnRH depending on its concentration and the duration of exposure. The molecular basis of this regulation could involve a combination of modulation of gene transcription, RNA processing, translation, or degradation. To investigate the underlying mechanisms, the homologous regulation of GnRH receptor binding using radioligand binding assay, mRNA levels using solution hybridization/ RNase protection assay and Northern blot analysis with the cloned mouse GnRH receptor (Tsutsumi et al.,Mol. Endo. 6: 1163, 1992), and mRNA activity using *Xenopus* oocyte based bioassay (Sealfon et al., Mol. Endo. 4: 1980, 1990) was studied in the mouse gonadotrope cell line, αT_3-1 cells (Windle et al., Mol. Endo.4: 597, 1990).

GnRH-A radioligand binding assay results show that treatment of αT_3-1 cells with 1 μM concentration of GnRH for 24 hours induce a significant decrease in the number of receptors. Despite changes in receptor number, cytosolic and total GnRH receptor mRNA levels were unchanged. In contrast, the measurements of mRNA activity in oocytes paralleled the changes observed in binding level. These data and the characterization of polysomes associated with GnRH receptor mRNA suggest that homologous regulation of GnRH receptor in this paradigm may occur at the level of translation. (Supported by NSF91-06877and Aaron Diamond Postdoctoral Fellowship).

571.3

REGULATION OF GnRH GENE EXPRESSION BY THE EXCITATORY AMINO ACIDS KAINIC ACID (KA) AND N-METHYL-D,L-ASPARTATE (NMA) IN THE RAT. James L. Roberts* and Andrea C. Gore. Fishberg Ctr. for Neurobiology, Mt. Sinai Med. Ctr., New York, NY, 10029.

The glutamate analogs NMA and KA are involved in the regulation of GnRH and LH release in mammals. Our laboratory and several others have demonstrated that GnRH cytoplasmic mRNA levels increase1 hour after NMA treatment. We also found that GnRH nuclear transcript levels remain unchanged under these conditions. In the present experiment we examined whether KA affects GnRH gene expression. Adult male rats were implanted with a jugular catheter. One to 2 days later, KA (2 mg/kg i.v.), NMA (14 mg/kg i.v.) or both substances were injected. Saline was injected as a control. Rats were decapitated 15 or 60 min later, the brains removed and the preoptic area dissected on ice and frozen. Cytoplasmic and nuclear RNA were extracted and assayed separately by RNase protection assay. As we previously found for NMA, KA increased cytoplasmic mRNA levels 1 hour after injection by about 30%. In the nucleus, no significant changes in GnRH primary transcript, processing intermediate or nuclear mRNA were observed following KA or NMA treatment. Therefore, excitatory amino acids regulate GnRH mRNA levels in the cytoplasm but not hnRNA levels in the nucleus. Since primary transcript levels presumably reflect GnRH gene transcription, and primary transcript levels do not change in the present study, these studies indicate that the regulation of GnRH gene expression by excitatory amino acids occurs at a post-transcriptional level, probably involving an increase in mRNA stability. We are currently examining the effects of administering KA and NMA together on GnRH gene expression. (Supported by NIH DK-39029).

571.4

REGULATION OF LHRH AND OXYTOCIN GENE EXPRESSION IN CNS SLICE-EXPLANT CULTURES: EFFECTS OF SECOND MESSENGERS. S. Wray, S. Key, S. Bachus* and H. Gainer. Lab of Neurochemistry, NINDS, NIH, Bethesda, MD 20892.

A wide variety of postnatal, differentiated neuroendocrine cells survive and express specific neuropeptide genes in organotypic slice-explant cultures. Utilizing this model system, we are examining the regulation of luteinizing hormone releasing hormone (LHRH) and oxytocin (OT) genes in primary neurons. This *in vitro* model allows us to determine the second messenger system(s) capable of directly affecting these neuropeptide genes and whether all cells within these given phenotypes respond similarly. In this study, we examined the signal transductive pathways in LHRH neurons and OT neurons, by application of 2nd messenger analogs, which could directly alter neuropeptide mRNA levels. Brain slices from pn day 5 rats were cultured as previously described (Wray et al., Neuroendo. 54:327-339, 1991). On the 18th day, experimental cultures were exposed to either: 1) 0.1uM PMA (phorbol ester), 2) 24uM forskolin, 3) 0.1uM PMA + 24uM forskolin, or 4) 24uM forskolin + 0.5 mM IBMX, for either 4, 8 or 24 hrs and then processed for *in situ* hybridization histochemistry. After exposure to emulsion, semi-quantitative, densitometric single cell analyses of LHRH and OT neuronal mRNA levels were done. Preliminary data indicate that forskolin increases LHRH mRNA after 8 hrs but that this increase is reversed after 24 hrs of forskolin exposure (in the presence or absence of IBMX). Further analysis of LHRH and OT neuronal mRNA levels is currently in progress.

571.5

THE ROLE OF NITRIC OXIDE IN THE STIMULATION OF LHRH RELEASE BY GLUTAMIC ACID. V. Rettori, A. Kamat and S.M. McCann*. Department of Physiology, The University of Texas Southwestern Medical Center, Dallas, TX 75235-8873.

The principal excitatory transmitter in the CNS is glutamic acid (GA). It is a physiologically significant stimulant of LHRH release. We discovered that norepinephrine-induced LHRH release is mediated by nitric oxide (NO). Consequently, we evaluated the role of NO in the stimulation of LHRH release by GA. Arcuate-median eminence fragments (A-MEs) from adult male rats were preincubated for 30 min in Krebs-Ringer bicarbonate glucose (KRBG) in a Dubnoff metabolic shaker (37°C, 95%O_2/5%CO_2). The A-MEs were then incubated with GA and/or other substances for 30 min. Media were assayed for LHRH by radioimmunoassay. Glutamic acid (10 mM) induced a highly significant release of LHRH which was blocked by the NO scavenger, hemoglobin (Hb, 20 μg/ml) which by itself had no effect on LHRH release. N^G-monomethyl-L-arginine (NMMA) (300 μM), a competitive inhibitor of NO synthase blocked the action of GA but had no effect by itself. Sodium nitroprusside (500 μM), which spontaneously releases NO, produced a highly significant increase in LHRH release which was blocked by Hb. The results show that GA releases LHRH via stimulation of NO release from NOergic neurons. Whether or not this is an action of GA directly on the NOergic neurons or on them, plus the LHRH terminal, remains to be determined. (NIH grants DK10073 and DK43900).

571.6

GONADOTROPIN-RELEASING HORMONE (GnRH) RELEASE AND EXCITATORY AMINO ACID RECEPTOR SUBTYPES IN MALE RAT DURING AGING. G. Aleppo, M.A. Sortino, U. Scapagnini and P.L. Canonico*. Institute of Pharmacology, University of Catania School of Medicine, Catania 95125, Italy.

Excitatory amino acids (EAAs) are largely recognized to be involved in the neuroendocrine regulation of the hypothalamo-pituitary-gonadal axis function by exerting their action directly at the hypothalamus. Excitatory neurotransmission participates to a variety of physiological and pathological processes also associated with aging. We have investigated the effect of different EAA receptor agonists on the release of GnRH from hypothalamic explants obtained from young (2-3 month-old) and aged (20-24 month-old) Wistar-Kyoto male rats. Basal release of GnRH did not differ in young and aged rats (32 ± 8 and 35.2 ± 5 pg/ml/30 min, respectively). All the hypothalami responded to stimulation of glutamate and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) to a similar extent, whereas the stimulation of GnRH secretion induced by N-methyl-D-aspartate (NMDA) was lower and that produced by kainate was more pronounced in hypothalami from aged rats (-70 and +161%, respectively). In addition, hypothalami from old rats, in contrast to young rats, when exposed to a maximal concentration of glutamate, were unable to respond to a subsequent challenge with glutamate. The present data suggest a differential involvement of the various glutamate receptor subtypes in the regulation of GnRH response to EEA during aging.

571.7

EFFECTS OF N-METHYL-D-ASPARTIC ACID (NMDA) ON LUTEINIZING HORMONE-RELEASING HORMONE (LHRH)-IMMUNOPOSITIVE NEURONS IN THE ARCUATE NUCLEUS OF MALE FERRETS. Y.P. Tang* and C.L. Sisk, Neuroscience Program and Department of Psychology, Michigan State University, East Lansing, MI 48824.

Intravenous injection of NMDA acutely stimulates LH release that is accompanied by a rapid, significant increase in expression of LHRH mRNA (Petersen et al., Endo. 129, 1679-81, 1991). The LH response is due to stimulation of neurosecretory activity of LHRH neurons projecting to the median eminence (ME). This study examined the effect of NMDA on LHRH neurons in the arcuate nucleus (ARC) of ferrets, since earlier studies indicated a functional heterogeneity within this population of neurons. In addition, to determine whether potential effects of NMDA might be selective to ME-projecting LHRH neurons, peripheral administration of the retrograde tracer Fluorogold (FG) was used to label neurons with axonal projections outside the blood brain barrier, e.g., the ME. Adult male ferrets received an iv infusion of either NMDA (10 mg/kg) or saline (SAL) on 2 consecutive days; an ip injection of FG (2.5 mg/kg) was given 10 min prior to each iv infusion. Ferrets were sacrificed 10 days after the second NMDA or SAL treatment. ARC LHRH cells were immunocytochemically identified using a rhodamine-tagged secondary antibody. The mean number of ARC LHRH neurons that did *not* contain FG was significantly larger in the NMDA group compared to the SAL group ($p<0.05$). This resulted in a significantly lower proportion of ARC LHRH neurons that were FG-labeled in NMDA-treated ferrets compared to SAL-treated ferrets (84.9±0.5% vs 94.1±2.2%; $p<0.05$). These results suggest that NMDA may stimulate LHRH synthesis in a subset of ARC neurons that do not project to ME, and provide additional evidence for heterogeneity among ARC LHRH neurons. Supported by HD26483 and HD00950.

571.8

ESTRADIOL AND ANDROGEN MODULATE LHRH RELEASE IN VITRO. Q. Li*, L. Tamarkin, P.L. Levantine, and M.A. Ottinger. University of Maryland, College Park, MD 20742.

In vitro release of LHRH was studied during steroid exposure. Longitudinal slices (1mm) of medial basal hypothalamus (MBH) taken from young adult male Japanese quail were placed in short term perifusion in Media 199. Both pulsatile and norepinephrine (NE; 10^{-6}M) stimulated chicken LHRH-I (cLHRH-I) secretion were monitored. Release of cLHRH-I from MBH occurred in a pulsatile manner (average number of pulses=2.09±0.13 pulses/hr over 9.25 hrs of incubation with an amplitude of 4.03±0.07 pg; n=6). cLHRH-I release significantly ($p<0.05$) increased to a peak of 22.89±1.06 pg with challenge by NE (15 min at hour 6 of perifusion). In a separate experiment, MBH slices were exposed to 17ß estradiol (E; 10^{-9}M), 5α-dihydrotestosterone (5α-DHT; 10^{-7}M)), or testosterone (10^{-7}M) during the perifusion. The effects of these steroids on NE stimulated cLHRH-I release revealed that short term E exposure significantly ($p<0.01$) potentiated NE-induced cLHRH-I release. Testosterone and 5α-DHT did not affect NE stimulated cLHRH-I release. Static pretreatment of MBH slices with E (10^{-9}M) for 14 hours prior to perifusion significantly ($p<0.05$) reduced the NE-induced cLHRH-I release. These results suggest that the avian hypothalamic LHRH-I pulse generating mechanism is located within the MBH. Further, these data provide evidence that E has dual effects on NE stimulated release relative to length of exposure.

571.9

EFFECTS OF ENDOTHELIN-1 AND-3, NOREPINEPHRINE, ANGIOTENSIN II, AND ARGININE VASOPRESSIN ON LHRH RELEASE IN MONKEY HYPOTHALAMI *IN VITRO*. A.M. Miller*, D. Mitsushima, K. Hartberg, and E. Terasawa. Reg. Primate Res. Ctr., Univ. Wisconsin, Madison, WI 53715

Previously we have found that norepinephrine (NE) and neuropeptide Y (NPY) stimulate *in vivo* release of LHRH in the rhesus monkey. Since both NE and NPY are vasoconstrictors, we have hypothesized that vasoactive substances, especially vasoconstrictors, are generally stimulatory to LHRH release. To test this hypothesis, we examined the effects of various vasoactive substances, i.e. NE, endothelin-1 and -3 (ET-1 & ET-3), angiotensin II (Ang-II), arginine vasopressin (AVP), and galanin (GAL) on LHRH release from monkey hypothalami *in vitro* using a perifusion system. Tissue comprised of the stalk-median eminence and a small portion of the MBH was obtained from the Tissue Distribution program of WRPRC. The tissue was bisected sagitally at the midline, and placed into separate perifusion chambers kept at 37 °C. A defined medium with 95% O_2 and 5% CO_2 was infused through the chambers at a rate of 50 µl/min, and perifusates were collected at 10-min fractions. The test substances at various doses were infused for 10 min at 60-90 min intervals, and 56 mM K+ was challenged at the end of experiment to test tissue viability. LHRH in perifusates was measured by RIA. Results: 1) ET-1 at doses of 10^{-6}, 10^{-7},10^{-8}, and10^{-9} M stimulated LHRH release in a dose responsive manner, with a peak occurring at 10-20 min after the initiation of ET-1 infusion, while ET-3 did not cause significant effects; 2) Ang-II at 10^{-6}M stimulated LHRH release with a peak at 10 min; 3) AVP at 10^{-5} and 10^{-7}M also stimulated LHRH release with a peak at 10 min; 4) NE at 10^{-7}M clearly increased LHRH release; 5) GAL (10^{-5} to 10^{-9}M) did not cause consistent results. These results indicate that all vasoconstrictors examined except ET-3 are stimulatory to LHRH release, while a non-vasoconstrictor, GAL, was not. The mechanisms of action of vasoactive substances on LHRH release remain to be determined.(Supported by NIH grants HD15433, HD11355 & RR-00167).

571.10

BLOCKADE OF ENDOGENOUS OPIATES INCREASES PULSE DURATION, AMPLITUDE, AND INTERPULSE SECRETION OF GnRH IN THE OVARIECTOMIZED EWE. D.B. Parfitt,* N.P. Evans, G.E. Dahl and F.J. Karsch, Neuroscience Program, Reproductive Science Program, and Deptartment of Physiology, University of Michigan, Ann Arbor, 48109.

Endogenous opioid peptides are postulated to inhibit gonadotropin-releasing hormone (GnRH) secretion. Previous studies, in which 10-min samples of pituitary portal blood were obtained from ovariectomized ewes, demonstrated that naloxone (an opiate receptor antagonist) increased the amplitude of GnRH pulses but did not alter GnRH pulse frequency. The present study employed rapid sampling of pituitary portal blood to characterize the effect of endogenous opiates on the shape, duration, and frequency of GnRH pulses. Ovariectomized ewes (n=4) were implanted with an apparatus for collection of pituitary portal blood. One week later, portal blood samples were collected at 1-min intervals for 4 hr immediately before and during a 4 hr naloxone infusion (1mg/kg, iv); jugular blood samples were obtained at 10-min intervals to monitor episodic luteinizing hormone (LH) secretion. Naloxone administration increased ($p<0.01$) GnRH pulse duration from 9.0±0.9 min/pulse to 16.2±1.6 min/pulse, and increased ($p<0.05$) the maximal GnRH value during a pulse from 14.6±3.0 to 27.5±5.2 pg/min. In addition, naloxone tripled ($p<0.05$) detectable GnRH secretion between pulses from 0.2±0.1 pg/min to 0.6±0.1 pg/min. Naloxone did not alter GnRH or LH pulse frequency, but an increase in the amplitude of LH pulses was observed after naloxone. These results suggest that opiate inhibition of GnRH secretion acts through a mechanism which limits the amplitude and duration of GnRH pulses, as well as restraining interpulse GnRH secretion in the ovariectomized ewe. (Supported by NIH HD18337; HD18258; MH14279).

571.11

ESTROUS CYCLE STAGE-DEPENDENT EFFECTS OF NEUROPEPTIDE Y (NPY) ON LHRH-STIMULATED LH AND FSH SECRETION FROM ANTERIOR PITUITARY FRAGMENTS *IN VITRO*. A.C. Bauer-Dantoin*, K.L. Knox, N.B. Schwartz and J.E. Levine. Dept. of Neurobiology & Physiology, Northwestern University, Evanston, IL 60208.

We recently demonstrated that NPY potentiates LHRH-stimulated LH secretion *in vivo*, and that these actions of NPY are exerted only under the endocrine conditions leading to preovulatory LH surges. The present experiments tested the hypothesis that NPY's facilitatory actions are exerted directly at the level of the anterior pituitary gland (AP) and depend on *in vivo* exposure of gonadotropes to the preovulatory endocrine milieu. Animals were sacrificed at 1600h on either metestrus (MET) or proestrus (PRO). APs were rapidly removed, cut into eighths, and placed into perifusion chambers. APs were perifused with M199 for a total of 8 hours, and perifusate samples were collected every five minutes. After thirty minutes of equilibration, APs received hourly pulses of LHRH alone (10^{-8} M), NPY alone (10^{-6} M), or LHRH + NPY. Basal runs consisted of perifusion with M199 for 8 hours with no peptide treatments. Calculations of total hourly LH and FSH reponses revealed that while LHRH significantly stimulated gonadotropin secretion from both MET and PRO pituitaries, NPY significantly enhanced LHRH-stimulated LH and FSH secretion only from PRO pituitaries, i.e. from tissue exposed to the endogenous endocrine milieu under which preovulatory gonadotropin surges are generated. NPY had no facilitatory effect on LHRH-induced gonadotropin secretion from MET APs. These results are consistent with our previous *in vivo* findings, and demonstrate that the facilitatory actions of NPY on LHRH-stimulated gonadotropin secretion *in vitro* are limited to the endocrine conditions under which preovulatory gonadotropin surges are generated. (HD 20677, HD 28048, DK 08513, HD 21921).

571.12

LOCALIZATION OF NEUROPEPTIDE-Y (NPY) IMMUNO-REACTIVE (ir) NEURONS WHICH PROJECT TO AREAS CONTAINING GnRH NEURONS. T.M. McShane*, P.M. Wise, and L. Jennes. Dept. of Physiology and Biophysics, and Dept. of Anatomy, Univ. of Kentucky, Lexington, KY 40536

NPY is reported to play an important role in the regulation of GnRH secretion. NPY-ir is widley distributed throughout the CNS, with cell bodies located in several areas including cortex, hippocampus, amygdala, brainstem, and a high density of perikarya in the hypothalamic arcuate nucleus. The medial septum-diagonal band complex (MSDB) contains GnRH cell bodies and receives innervation from NPY neurons, however, the source(s) of this innervation is unclear. We used retrograde transport of fluorescent tracers combined with immunofluorescence to identify the location of NPY neurons that project to the regions containing GnRH perikarya. Among several areas investigated, we detected colocalization of retrograde tracer and NPY-ir in neuronal cell bodies within the noradrenergic cell group A1 and amygdala. Our results indicate that GnRH-neuron containing regions receive NPY innervation from anatomically, and probably functionally, heterogeneous sources. Supported by NIH AG02224 to P.M.W. and HD24697 to L.J.

571.13

DISTRIBUTION OF VARIANT FORMS OF GnRH AND GTH IN THE BRAIN AND PITUITARY GLAND. L. Magliulo-Cepriano and M.P. Schreibman*, Graduate School-University Center and Brooklyn College Biology Department, C.U.N.Y., Brooklyn, New York 11210.

Immunoreactive (ir)-lamprey (l), mammalian (m), chicken II (chII), and salmon (s) gonadotropin-releasing hormone (GnRH) and ir-beta gonadotropin (GTH) I and II, have been localized in the brain and pituitary of Xiphophorus maculatus, the platyfish, at various stages of sexual development. Ir-GTH I was found in the pituitary of all stages examined. Ir-GTH II was seen only in pubertal and mature animals. Ir-sGnRH and -mGnRH were seen in the pituitary of all stages and in the brain of pubertal and mature animals. Ir-lGnRH was seen in the pituitary all stages studied but never in the brain. Ir-chIIGnRH was absent in all immature stages but seen in both the brain and pituitary of mature animals. Our results demonstrate that variant forms of GnRH and GTH are present at defined stages of development in specific regions of the brain and pituitary gland and suggest that different forms of GnRH and GTH regulate different aspects of reproductive system development and physiology. [Supported by NASA (NAGW-1704) and PSC-CUNY.]

571.14

PRIMARY STRUCTURE AND BIOLOGICAL ACTIVITY OF A THIRD GONADOTROPIN-RELEASING HORMONE FROM LAMPREY BRAIN

S. A. Sower*[1], Y.C. Chiang[1], S. Lovas[2] and J. M. Conlon[2]
[1]Dept. of Biochem.& Molec. Biol., Univ. of New Hampshire, Durham, NH, 03824 and [2] Dept. of Biomed. Scien., Creighton Univ. School of Medicine, Omaha, NE 68178

Previous studies have led to the identification of two molecular forms of gonadotropin-releasing hormone (GnRH-I and II) in the brain of the sea lamprey, *Petromyzon marinus*. We have now isolated a third molecular form of GnRH (GnRH-III) from the brain of this species that is different from GnRH-I and -II. The primary structure of GnRH-III is pGlu-His-Trp-Ser-His-Asp-Trp-Lys-Pro-Gly-NH_2. A synthetic decapeptide with this amino acid sequence was chromatographically identical to natural GnRH-III.

Intraperitoneal injection of synthetic lamprey GnRH-III (0.1 ug/g) produced a significant elevation of plasma progesterone (31% over basal values) in female lampreys that was comparable to that produced by the same dose of lamprey GnRH-I (36% over basal). The elevation in plasma estradiol produced by lamprey GnRH-III (244% over basal) was significantly less than the elevation produced by GnRH-I (322% over basal). We propose based on the biological activity of lamprey GnRH-III in these studies and the occurrence of this peptide during metamorphosis in lampreys, that both lamprey GnRH-I and -III are neurohormones involved in reproduction in lampreys.(Supported by NSF #DCB-9004332 and DCB-8904919 and the Great Lakes Fishery Commission to SAS).

571.15

ANATOMICALLY AND BIOCHEMICALLY DISTINCT POPULATIONS OF GnRH-IR NEURONS ARE ASSOCIATED WITH DIFFERENT NEUROTRANSMITTERS. T.L. Dellovade*, V. Alones, and E.F. Rissman
Dept. of Biology, NSF Center for Biological Timing, UVA, Charlottesville, VA 22903

The musk shrew (*Suncus murinus*) has three populations of GnRH-ir neurons which may be modulated by different neurotransmitter systems. The *most rostral* group is in the olfactory forebrain and contains the mammalian form of GnRH (mGnRH). Neurons containing GnRH-ir are present in the terminal nerve and ganglia (TN), the accessory olfactory bulb (AOB), and the taenia tecta (TT). Exposure to male chemical cues causes a 30-40% increase in the numbers of GnRH-ir cell bodies in this region. The AOB contains tyrosine hydroxylase (TH) neurons and fibers. The TN contains choline acetyltransferase (ChAT) and TH-ir fibers. The TT contains ChAT-ir fibers and is richly innervated by TH-ir fibers. The *second* major group of GnRH-ir neurons resides in the continuum between the vertical and horizontal limb of the diagonal band of Broca (DB), the medial septum (MS), preoptic area (POA), and is scattered in the hypothalamus (HT). These cells also contain mGnRH. GnRH-ir cell counts in this area vary relative to ovulation. This region is rich in ChAT-ir somata. The POA contains TH-ir cell bodies and fibers. The *most caudal* population of GnRH cells, residing in the linear caudal raphe (CLi), contains the chicken II form of GnRH (cGnRH II). These cells do not contain GnRH in any other mammal, however, a homologous population is present in several species of birds, amphibians and fishes. TH-ir cells and fibers are present in the central gray just dorsal to the GnRH-ir neurons. Also, TH-ir neurons and fibers reside in the CLi and ventral to it in the substantia nigra. TH-ir fibers appear to contact GnRH-ir cell bodies in the CLi. This work was supported by NSF grant BNS 9021226.

571.16

ENKEPHALINS MODULATE MEDIAN EMINENCE GnRH RELEASE THROUGH A DOPAMINERGIC MECHANISM : MORPHOLOGICAL EVIDENCE. F. Vandenbulcke, P. Ciofi, I. Dutriez, D. Deneux, J.C. Beauvillain*.
U156 INSERM, Place de Verdun, 59045 Lille cedex (France).

The presence of enkephalinase (neutral endopeptidase, NEP, E.C.3.4.24.11) in membranes of nerve endings in the rat median eminence (ME) (J.Neuroendocrinol.5(1993)205) suggests that neuropeptides, target for NEP, have paracrine and/or autocrine actions in this region. Although NEP is capable of hydrolysing *in vitro* a variety of regulatory peptides, *in vivo* studies indicate that in the brain this enzyme seems essentially implicated in the biological inactivation of enkephalins (ENK). The modulation of gonadoliberin (GnRH) release is one of the documented actions of ENK in the ME. However, it is at present unclear whether ENK act through dopamine (DA) endings, or directly on GnRH endings, or both.

As technical parameters and particularly the tissue fixation used to radioimmunodetect NEP with ^{125}I-labeled monoclonal antibodies are compatible with immunoperoxidase detection of GnRH and tyrosine-hydroxylase (TH), an ultrastructural pre-embedding double immunolabeling study was performed to determine if GnRH- and TH-containing nerve endings have NEP inserted within their plasma membrane, as visualized after radioautographic exposure of dipped, GnRH- or TH-stained ultrathin sections, by the presence of silver grains located over membrane appositions.

Results show that 60% TH-immunoreactive (ir) boutons display one or several grains over their membranes and that 27% GnRH-ir endings displayed one, but never more, silver grain, and this only in cases of apposition with other peroxidase-unstained nerve endings.

These observations are in favor of a location of NEP on TH-ir but not GnRH-ir nerve terminals and consequently provide morphological arguments 1) suggesting a paracrine and/or autocrine action of ENK on tuberoinfundibular DA nerve endings and 2) making it unlikely that the same opioid peptides act directly on GnRH periportal endings.

571.17

BINDING AND INTERNALIZATION OF FITC-LABELED NEUROTENSIN IN A SUB-POPULATION OF GnRH-CONTAINING NEURONS: A CONFOCAL MICROSCOPIC ANALYSIS. A. Beaudet*, K. Leonard, M.P. Faure, R.I. Weiner and G.C. Desjardins. Montreal Neurol. Inst. Montreal, Qué., Canada H3A 2B4 and Dpt. OBS/GYN, UCSF, CA 99143.

Several lines of evidence suggest that neurotensin (NT)- containing neurons in the rostral preoptic area mediate some of the stimulatory effects of estrogens on gonadotropin-releasing hormone (GnRH) secretion (Alexander et al., 1989). To determine whether this effect is exerted through a direct action of NT upon preoptic GnRH neurons, we have examined the binding and internalization of FITC- labeled NT (NT-FTC) to GnRH neurons: (1) in an immortalized GnRH producing cell line (Mellon et al., 1990) and (2) in slices of rat hypothalamus. GT1-7 cells were grown in DMEM for 10 days and incubated for 40 min. at 4°C and 37°C with 20 nM NT-FTC. All cells were then rinsed in buffer, fixed in paraformaldehyde and processed for GnRH immunocytochemistry using indirect immunofluorescence. Hypothalamic slices (350μm) were maintained at 35°C in oxygenated ringer, preloaded with 50nM of NT-FTC for 3 min, superfused with ringer for 30 min., fixed with 4% paraformaldehyde, and resectionned at 40 μm for GnRH immunostaining. All GT1-7 neurons showed intense GnRH immunostaining. A subset of these (30 %) concomitantly exhibited NT-FTC labeling. The latter was mainly pericellular in sections incubated at 4°C and present throughout the cytoplasm at 37°C, in keeping with the occurrence of temperature-dependant ligand internalization. A small proportion of GnRH-immunoreactive neurons in slices of the preoptic area similarly internalized NT-FTC, indicating that they also harboured NT receptors. Taken together, these results indicate that a sub-population of rat GnRH neurons express NT receptors and suggest that NT-mediated effects on GnRH secretion may be exerted directly.

571.18

LACK OF EXPRESSION OF SEROTONIN RECEPTOR SUBTYPE mRNAs IN GONADOTROPIN RELEASING HORMONE PRODUCING NEURONS OF THE RAT. D.E. Wright* and L. Jennes.
Dept. Anatomy and Neurobiology, Univ. of Kentucky Medical Center, Lexington, KY 40536-0084.

Serotonin is one of the neurotransmitters believed to participate in the regulation of gonadotropin-releasing hormone (GnRH) release at the level of the GnRH neuron. We therefor examined whether GnRH neurons produce mRNA encoding for the serotonin 1A, 1C, and 2 receptor subtypes using dual "in situ" hybridization. In the medial septum-diagonal band and rostral preoptic area, GnRH neurons were identified with digoxigenin-UTP labeled riboprobes. The number and distribution of GnRH mRNA-containing cells were similar to the GnRH neurons that were identified with a ^{35}S labeled riboprobe or with GnRH immunocytochemistry. Cells expressing serotonin 1A, 1C, and 2 receptor mRNA were detected with ^{35}S labeled riboprobes; all three receptor subtype riboprobes labeled select cells with distinct distributions in the medial septum-diagonal band and rostral preoptic area. However, no cells were detected that contain both GnRH mRNA and serotonin receptor mRNA in ovariectomized and ovariectomized-estrogen treated rats. In contrast, select GnRH neurons were detected that express mRNA for galanin. These results suggest that the effects of serotonin on GnRH release are not mediated by direct activation of serotonin 1A, 1C, and 2 receptors on GnRH neurons, but instead through other, yet unidentified serotonin receptor subtypes or through non-serotoninergic intermediary neurons. Supported by NIH HD24697.

571.19

SEX-STEROID-DEPENDENT CHANGES IN THE MORPHOLOGY OF LUTEINIZING HORMONE-RELEASING HORMONE NEURONS IN THE MALE SYRIAN HAMSTER. H.F. Urbanski* and N.M. Lew. Division of Neuroscience, Oregon Regional Primate Research Center, Beaverton, OR 97006.

The luteinizing hormone-releasing hormone (LHRH) neurons of Syrian hamsters show morphological changes during puberty and also in response to changes in photoperiod. The aim of the present study was to examine the extent to which perikaryal size of LHRH neurons is dependent upon the sex-steroid environment. Adult male Syrian hamsters were orchidectomized and received either empty or testosterone-filled Silastic capsules, subcutaneously. All of the animals, plus intact controls, were killed 10 days later. Their brains were fixed with 4% paraformaldehyde, sectioned coronally with a Vibratome (50 µm), and processed for immunocytochemistry using a monoclonal antibody to LHRH (HU4H). Detailed morphometric analysis of the immuno-positive perikarya, using an IBAS 2000 image analyzer, revealed a significant decrease in size associated with removal of the testes (ca. 15% for mean surface area and 8% for mean diameter). In contrast, the mean plasma levels of luteinizing hormone and follicle-stimulating hormone increased markedly (>700%). Moreover, development of these neuronal and endocrine post-castration events was significantly inhibited in the testosterone-treated animals. Taken together, these findings demonstrate the existence of a negative feedback relationship between sex steroids and LHRH-secreting neurons which is manifested at the perikaryal level. It is suggested that enhancement of LHRH secretion after orchidectomy leads to a significant depletion of neuropeptide reserves which can be detected within the neuronal cell body as a decrease in the extent of immuno-positive staining.

Supported by NIH grants HD-24312 and RR-00163

571.20

ULTRASTRUCTURAL CHARACTERISTICS OF GNRH NEURONS IN PREPUBERTAL MALE RATS. J.W. Witkin, M-T. Romero*, A-J. Silverman, Dept. Anat.& Cell Biol., Columbia Univ., New York, NY 10032

The attainment of sexual maturity is dependent upon the appropriate functional integration of the hypothalamic-pituitary-gonadal axis. We compared the ultrastructural characteristics of GnRH neurons in prepubertal males (S-D rats, 20-23 d.o.) with those in sexually mature animals. GnRH immunoreactivity was demonstrated (LR1 antibody) and tissues examined electron microscopically (as in Witkin,'92, J. Neuroendocrinol.4:428). Unlike sexually mature animals, in which the reaction product was characteristically scattered in the cytoplasm, in the prepubertals it was also heavily deposited in secretory granules. We quantified the synaptic input to a sample of 38 GnRH neurons from the preoptic area of 9 animals. The synaptic input to the GnRH neuronal soma was significantly less than that observed in adult rats, which itself is very sparse in comparison with other neurons in the same brain regions. Most strikingly, of the GnRH neurons examined, 6 had synapsing GnRH terminals on the cell body. This is in contrast to all of our earlier studies, in which although occasional GnRH-immunoreactive synapses were seen on GnRH dendrites, they were very rarely found on the GnRH soma. These findings suggest that the connectivity of the GnRH neuronal system alters with sexual development. AG05366 (JWW); DK42323 (AJS, JWW)

HYPOTHALAMIC-PITUITARY-GONADAL REGULATION: GONADOTROPINS, NEUROPEPTIDES, STEROIDS

572.1

GONADOTROPIC MODULATION OF ESTROUS AND HYPOTHALAMIC-PITUITARY-ADRENAL (HPA) AXIS FUNCTION IN THE LEW/N AND F344/N RAT. C.C. Smith, G. Cizza, M. Gomez, C. Greibler, P.W. Gold, and E.M. Sternberg*. Clinical Neuroendocrinology Branch, Nat'l. Inst. of Mental, Bethesda, MD 20892.

We have previously shown that LEW/N and F344/N rats' relative inflammatory disease susceptibility and resistance are associated with their relative HPA axis hypo- and hyperresponsiveness. In light of correlates linking gonadal hormone levels to HPA axis function, and a sexually dimorphic basis for differential stress and behavior, we examined the duration of estrous utilizing vaginal smears, and determined estradiol (E), progesterone (P), LH, and FSH plasma levels at the different phases of the estrous cycle in these two strains. LEW/N rats exhibit a trend towards a shorter estrous cycle relative to F344/N. Comparison of the duration of the component phases of estrous shows LEW/N rat metaestrous to be significantly longer than F344/N, while diestrous and estrous were significantly shorter. Proestrous was identical. Overall levels of E in LEW/N were significantly greater than in F344/N, however, only E in the estrous phase of the LEW/N cycle reached statistical significance compared to F344/N. Overall levels of P in the LEW/N rat were also significantly greater than in F344/N. The diestrous, proestrous, and estrous components of the LEW/N cycle had significantly greater P: metaestrous demonstrated a trend towards being greater. LH levels in the LEW/N rat were overall significantly greater than in F344/N, however, none of the component phases individually reached significance. FSH levels in the two strains did not differ. We conclude that the elevated levels of E and P observed in LEW/N rats could potentially affect gonadotropic and corticotropic systems at several levels. Direct E upregulation of GnRH could contribute to the elevated LH in LEW/N rats. Elevated E can also upregulate ACTH and cort through downregulation of MR and GR receptors. P can act stoichiometrically to competitively bind as an antagonist to MR and GR sites, also potentially upregulating ACTH and cort. However, the blunted ACTH and cort response in LEW/N rats suggests a failure of E and P to induce upregulation of ACTH and cort, and implies that gonadotropic actions in this strain are secondary to CRH hyporesponsivity.

572.2

CHARACTERIZATION OF CHRONIC ESTRADIOL TREATMENT IN VERVET MONKEYS. G.C. Desjardins*, J.R. Brawer, A. Beaudet and M.J. Meaney, Develop. Neuroendocrinology Lab., Douglas Hosp. Res. Center, Dept. of Psychiatry, McGill University, Montreal, Quebec, CANADA H4H 1R3.

Previous studies in the rodent have shown that chronic exposure to physiological levels of estradiol induced a series of hypothalamic-pituitary deficits which ultimately result in anovulatory polycystic ovaries (Brawer, 1990). Fundamental to this cascade, is the demonstration that 60% of hypothalamic beta-endorphin neurons degenerate in the 8 weeks following estradiol exposure (Desjardins, 1993). In order to investigate whether chronic estradiol exposure would result in similar effects in primates, 12 female vervet monkeys (St-Kitts, Behav. Sci. Foundation) were implanted with chronic release capsules containing 25mg of estradiol and yielding plasma estradiol concentrations of approximately 400 pg/ml. Estradiol (E_2)-implanted and cage-mate controls were sacrificed by anesthetic overdose and perfused with PBS. Ovaries from E_2-implanted animals displayed poor follicular development, occasional cysts and small atretic secondary follicles and were completely devoid of corpora lutea indicating a recent prolonged period of anovulation. In contrast, most controls exhibited the full range of normal follicular development, including large corpora lutea. Hypothalamic beta-endorphin concentrations were significantly reduced in the E_2-implanted animals as compared to controls (157±91 vs 806±356 pg/mg wet weight, respectively; n=3; $p<0.05$). In contrast, hypothalamic dynorphin concentrations were equivalent in both groups (8.32±0.17 vs. 11.93±3.4). Spearman's correlational analysis of beta-endorphin concentrations versus degree of ovarian pathology indicated that these were inversely related. These studies indicate that chronic E_2-exposure in vervet monkeys results in hypothalamic and ovarian changes consistent with the E_2-induced degeneration of beta-endorphin neurons previously documented in rodents.

572.3

INCREASED NEUROKININ B GENE EXPRESSION IN THE HYPOTHALAMUS OF LONG TERM OVARIECTOMIZED RATS. N.E.Rance* and T.R.Utton, Departments of Pathology, Anatomy and Neurology. University of Arizona College of Medicine, Tucson, AZ 85724.

Hypertrophy and increased gene expression of tachykinin neurons occurs in the infundibular (arcuate) nucleus of postmenopausal women (Rance et al., *J.Clin.Endo.Metab.*, 71:79,1990, Rance and Young, *Endocrinology*, 128:2239,1991). To test whether these changes could be secondary to ovarian failure, we used in situ hybridization to examine neurons containing neurokinin B (NKB) and substance P (SP) mRNAs in the rat arcuate nucleus after ovariectomy. We examined four groups: intact proestrus (PRO); intact diestrus (DD1); 2 month ovariectomized (OVX); and rats which were injected with estradiol valerate two months prior to sacrifice to produce constant estrous (CE). Computer microscopy was used to determine cell profile areas, the number of tachykinin neurons and autoradiographic grain density of labeled neurons. We report marked changes in NKB gene expression among the various groups. The number of neurons expressing NKB gene transcripts was significantly greater in OVX rats (16.9 ± 1.2 labeled neurons/arcuate section, mean ± SEM), than PRO (8.9 ± 1.2), DD1 (4.8 ± 1.2) or CE (2.9 ± 1.3) rats. In addition, the grain density of NKB neurons was almost doubled in the OVX group relative to all other groups. Finally, the mean profile area of NKB arcuate neurons was larger in conditions where blood levels of estrogen were low (DD1 and OVX) compared to PRO and CE rats. In contrast, the only change in SP arcuate neurons was atrophy in the CE rats. These data support the hypothesis that neuronal hypertrophy and increased NKB gene expression in the hypothalami of postmenopausal women is due to ovarian failure. (NIH AG-09214 and ADRCC R-029)

572.4

LHRH AND LH GENE EXPRESSION IN THE NEONATALLY ESTROGENIZED FEMALE RATS. E.S. SONG, S.H. CHO AND K. Kim*. Dept. of Molecular Biology, Coll. of Natural Sci., Seoul Nat'l Univ., Seoul 151-742 Korea.

The neonatal treatment of sex steroids such as estrogen induces sterility in female rats: Animals are characterized with tonic release of gonadotropin and fail to ovulate. Neonatal estrogenization is a useful model for analyzing interaction between LHRH and gonadotropin during the development. To delineate the mechanism underlying the neonatally induced sterilization, the present study examined LHRH biosynthesis in the hypothalamus and LH biosynthesis in the pituitary derived from the neonatally estrogenized (17β-estradiol, 10 µg, for 1-5 days after birth) sterile rats (ESR) at 50 days of age. LH mRNA levels were lower than those of intact rats, the amount of LHRH mRNA and LHRH content were significantly higher that those of control animals. During the perinatal period in which the LHRH neuronal system is organized, neonatal treatment with estrogen appears to modify the LHRH neuronal system. This study suggests that the sterility induced by estrogen is not due to aberrant synthesis of LHRH in the preoptic area but probably due to abnormal LHRH release from the mediobasal hypothalamus.

572.5

AROMATASE-IMMUNOREACTIVE CELLS ARE PRESENT IN THE MOUSE AND RAT BRAIN AREAS THAT EXPRESS HIGH LEVELS OF AROMATASE ACTIVITY. A. Foidart*, P. Absil, N. Harada and J. Balthazart, Lab. Biochemistry, Univ. Liège, B-4020 Liège, Belgium and Fujita Health University, Molecular Genetics, Toyoake, Aichi 470 11, Japan.

The aromatization of testosterone (T) into estradiol in the brain was discovered more than 20 years ago but it has been so far impossible to precisely localize the cells of the mammalian brain containing the enzyme aromatase which catalizes this important aspect of T metabolism. We recently designed an immunocytochemical technique which allows the visualization of aromatase-immunoreactive (ARO-ir) cells in the quail brain. In this species, a marked increase in the optical density of ARO-ir cells was observed in subjects that had been treated with the aromatase inhibitor, R76713 or racemic vorozole. This increased immunoreactivity, associated with a total blockade of aromatase activity (AA), is unexplained at present but it was used as a tool in the present study in which the distribution of ARO-ir material was reassessed in the brain of mice and rats that had been pretreated with R76713. As expected, the aromatase inhibitor increased the density of the immunoreactive signal. In both species, strongly immunoreactive cells were found in the lateral septal region, in the bed nucleus of the stria terminalis, in the central amygdala and in the dorso-lateral hypothalamus. A slightly lighter signal was also present in the medial preoptic area, in the nucleus accumbens, in several hypothalamic nuclei (e.g. paraventricular and ventromedial nuclei), in all divisions of the amygdala and in several regions of the cortex, especially in the cortex piriformis. A lighter label was also detectable in many other regions. These data demonstrate that, contrary to previous claims, ARO-ir cells are present in all brain regions that contain a high AA. These had not been detected before either because the techniques were not sensitive enough or the antibodies that were used had a different specificity. There is, in fact, a good correspondence between the distribution of AA and of ARO-ir cells in the mammalian brain as previously demonstrated in quail. The intensity of the immunostaining in mice and rats is however not always directly related to the level of AA which suggests that either different forms of aromatase are present in the brain or that the subcellular distribution of the enzyme in some brain regions (e.g. preferential localization in fibers and presynatic terminals) prevents it detection by the present technique.

572.6

AUGMENTED LH AND FSH SECRETORY RESPONSE TO A LOW DOSE OF GONADOTROPIN-RELEASING HORMONE (GnRH) IN OLDER VERSUS YOUNG MEN. A.D. Zwart, R.J. Urban, M.O. Thorner*, J.D. Veldhuis. Department of Internal Medicine, Division of Endocrinology and Metabolism, University of Virginia, Charlottesville, VA.

LH and FSH secretion is regulated by GnRH. As men age, there is an increase in level of gonadotropins, while testosterone slowly declines. We have assessed the dose-dependent response of LH and FSH to GnRH in older (10) and young (9) men, who received 5 iv boluses of GnRH (range 10 to 100 ug) 2 hours apart. LH and FSH were measured every ten minutes for 12 hrs including a 2 hr baseline. Deconvolution analysis was performed to estimate gonadotropin secretory burst mass, amplitude, duration and LH/FSH half-life. Mean $\pm$ SEM 2 hr baseline serum LH (IU/L) was not different in older vs young men (3.7 $\pm$ 1.7 vs 2.1 $\pm$ 0.3) while mean FSH was (5.9 $\pm$ 2.5 vs 3.8 $\pm$ 1.5, $P < 0.05$). Mean 2 hr LH responses were higher in older men ($P < 0.05$) for the 25, 50 and 75 ug doses. Mean 2 hr FSH responses were also higher for 10 and 25 ug GnRH. LH and FSH half-lives were not significantly longer in the older men. No difference was seen in gonadotropin pulse duration. The increase in LH mass from the 10 ug dose to the 25 ug dose was higher in older men (5.2 IU/L vs 0.78, $P<0.05$). The mass of FSH secreted in response to 25 ug was higher in older men (4.0 IU/L vs 1.6 in young men, $P<0.05$). FSH mass and amplitude increases secreted after 25 vs 10 ug GnRH also were higher in older men ($P<0.01$). We conclude that gonadotropin secretory responses to GnRH in older men show increased sensitivity specifically to low doses of GnRH with no evident differences in maximal release rates, LH or FSH half-life, or burst duration. Whether these changes are due to diminished gonadal feedback or altered hypothalamic pituitary control mechanisms in healthy aging is not known.

NEUROENDOCRINE REGULATION: CATECHOLAMINE AND GABA

573.1

EXPRESSION OF D2 RECEPTORS COUPLED TO POTASSIUM CURRENTS IN GH4C1 CELLS EXPOSED TO EGF. R. Gardette*, R. Rasolonjanahary, C. Kordon and A. Enjalbert INSERM U159, Paris

GH4C1 cells, a clonal cell line from a rat pituitary tumor, serve as a model to study the excitatory regulation of prolactin secretion. These cells however lack dopamine D2 receptors and are not suitable for exploring dopamine inhibition of prolactin secretion. Recently it has been shown that epidermal growth factor (EGF) is able to induce functional expression of D2 receptors in GH3 cells, a parental clonal cell line (Missale et al., Endocrinology 128, 1991).

We have undertaken a whole-cell patch-clamp study in order to check whether expression of D2 receptors coupled to potassium channels could be restored in GH4C1 cells exposed to EGF. Effects of dopamine on the non-inactivating voltage-dependent outward potassium current I_K were investigated both in control and in GH4C1 cells treated with EGF (10^{-9}M) for at least 4 days. The potassium current was not modified by EGF treatment by itself. In control cells, I_K current measured before and during dopamine application (100nM) was not changed. In contrast, dopamine markedly enhanced the potassium current in 69% of the EGF-treated cells (mean increase : 42$\pm$8%, n=11), without changing activation threshold. This effect was mimicked by the specific D2 receptor agonist bromocriptine (100nM) and blocked by sulpiride (1μM), a D2 receptor antagonist, indicating that the effect of dopamine was effectively due to the activation of D2 receptors.

These results bring further evidence that EGF-induced D2 receptors in clonal strains from rat pituitary tumors are functional and are coupled to the delayed outward potassium current I_K.

573.2

3-H-SPIPERONE BINDING TO RAT PITUITARY: MODULATION AFTER 6-HYDROXYDOPAMINE. L.C. Saland *, A. Samora, A. Apodaca, D. Ramirez. and D.D. Savage+. Depts. of Anatomy and Pharmacology+, University of New Mexico Sch. Med., Albuquerque, NM 87131.

D-2 dopamine (DA) receptors are found in rat pituitary (PIT), with binding of 3H-spiperone (SPIP) to proopiomelanocortin (POMC) cells in the intermediate lobe (IL), as well as to anterior lobe (DeSouza et al, '85, Endocrinol. 119: 1534). We examined SPIP binding to rat PIT after 6-hydroxydopamine (6-OHDA)-induced degeneration of nerve terminals to POMC cells. Male rats were injected with 6-OHDA as previously described (Saland et al, '91, Mol. Cell Neurosci. 2:418). Cryostat sections of PIT were incubated with 0.4nM SPIP (DuPont, S.A. 21.6 Ci/mM) in Tris/HCl buffer with or without excess haloperidol or ketanserin. Under these conditions, ketanserin had no effect on SPIP binding. One week after 6-OHDA, when fibers have degenerated, specific binding in the IL was reduced 60% compared to controls. No difference in SPIP binding was found three weeks after 6-OHDA. Increased release of DA during degeneration may produce a transient down-regulation of DA receptors on target cells. Support: NIH NS21256;GM08139 (LCS);AA06548 (DDS).

573.3

INTRACELLULAR MESSENGERS SUPPORTING PROLACTIN REBOUND SECRETION FOLLOWING DOPAMINE REMOVAL. K.A.Gregerson* and R. Chuknyiska, Dept. of Pediatrics, School of Medicine, UMAB, Baltimore, MD 21201.

Prolactin (PRL) release is tonically inhibited by dopamine (DA). Withdrawal of DA stimulates PRL release with transient secretory rates that exceed those observed prior to DA. Studies on the mechanism of this secretory rebound have demonstrated that both cAMP and inositol phosphates (IPx) levels increase following DA removal (Martinez de la Escalera and Weiner, 1988). In light of our findings that influx of extracellular Ca^{2+} is necessary for the PRL rebound, we have re-investigated the role of cAMP and IPx in this response. Cellular [cAMP] or IPx production was measured in pituitary cells from female rats. PRL release was also quantitated. Measurements were made either after a 20 minute incubation with test substances or 20 minutes after their removal. In the presence of 50nM DA, PRL release was significantly reduced (by 60%). Removal of DA doubled the rate of PRL release as compared to pre-DA levels. Cyclic AMP levels were also suppressed (by 50%) in the presence of 50nM DA and increased following DA removal, but only to pre-DA levels. Application of the K^+ ionophore valinomycin (10-9M), to hyperpolarize the cells, produced inhibition and, after its removal, rebound of PRL release, mimicking the effects of DA. However, it produced no changes in [cAMP]. Inclusion of either 50 mM K^+ or 100uM quinine with DA (to block DA-induced hyperpolarization) blocked inhibition and rebound of PRL, while cAMP responses to DA were unaffected. This dissociation of PRL release and [cAMP] indicates that cAMP plays little or no role in producing the PRL secretory rebound following DA removal. Incubation with DA (50nM) had no acute effect on IPx while removal of DA increased IPx production by 60-70%. The Ca^{2+} channel blocker, verapamil, blocked both the PRL secretory rebound and the increase in IPx production produced by DA removal. The data indicate that both PTL release and IPx production stimulated by DA removal are secondary to influx of extracellular Ca^{2+} and support the hypothesis that enhanced Ca^{2+} influx into lactotropes following DA removal is a primary mechanisms in producing PRL rebound secretion. [DK-40336]

573.4

EFFECT OF DOPAMINE REMOVAL ON CELLULAR Ca^{2+} IN RAT LACTOTROPE. M. -Y. Ho* and K. A. Gregerson. Dept. of Pediatrics, School of Medicine, UMAB, Baltimore, MD 21201

Dopamine (DA) tonically inhibits prolactin (PRL) secretion from lactotropes in the anterior pituitary. Removal of DA elicits a dramatic increase in PRL release to values above basal (pre-DA) rates. Our previous studies showed that lactotropes exhibited a period of increased Ca^{2+}-dependent spiking activity following DA withdrawal. We investigated the effect of DA removal on $[Ca^{2+}]_i$ during rebound secretion in single lactotropes isolated from female rats and identified by the Reverse Hemolytic Plaque Assay (RHPA). $[Ca^{2+}]_i$ was measured with fluorescent Ca^{2+} indicator fura2 1 to 3 days after cell dispersion at room temperature. Under unstimulated conditions, two distinct groups of lactotropes were observed: the first exhibited oscillatory $[Ca^{2+}]_i$ fluctuations probably due to spontaneous action potential and the second (quiescent cells) exhibited stable $[Ca^{2+}]_i$ levels ranging between 10 to 100 nM. Treatment with DA (100 nM) was found to affect $[Ca^{2+}]_i$ differently upon DA removal of the two groups of lactotropes. In cells with oscillatory Ca^{2+} fluctuations, DA resulted in a rapid decrease in $[Ca^{2+}]_i$ with the disappearance of the spontaneous Ca^{2+} fluctuations. Verapamil (Vp) can mimic the inhibitory effect of DA to some extent. Free Ca^{2+} was brought down by Vp to about two thirds of what was observed in the presence of DA. Upon DA (or Vp) removal, cells resumed oscillatory activities to about the same Ca^{2+} level as that observed prior to DA application. In quiescent cells, by contrast, DA exerted no effect on resting $[Ca^{2+}]_i$ but DA removal elicited a slow rise in $[Ca^{2+}]_i$ which eventually developed into oscillatory Ca^{2+} spikes at $[Ca^{2+}]_i$ levels much higher than observed before DA application. These findings are consistent with our previous report that application and removal of DA induced spontaneous Ca^{2+}-spiking activity in electrically quiescent cells. These data support the hypothesis that the rebound secretion of PRL is supported by the influx of extracellular Ca^{2+} after cells recover from DA-induced hyperpolarization. NIH DK-40336 and DK-02019.

573.5

CIRCADIAN CHANGES OF SERUM PROLACTIN LEVELS AND TUBEROINFUNDIBULAR DOPAMINERGIC NEURONS' ACTIVITIES IN OVARIECTOMIZED, ESTROGEN-TREATED RATS. Lee-Min Mai and Jenn-Tser Pan*, Inst. Physiol. and Dept. Anat., Natl. Yang-Ming Med. Coll., Taipei, Taiwan 11221, R.O.C.

The objective of this study was to correlate the changes of serum prolactin (PRL) levels with tuberoinfundibular dopaminergic (TIDA) neurons' activities during the afternoon of estrogen-treated rats. Rats were decapitated each hour from 1200 to 1900 h, and their serum PRL and median eminence DOPAC or DOPA levels were determined by RIA and HPLC-ECD, respectively. A prominent PRL surge started and peaked around 1400-1500 h, and remained significantly high till 1900 h. Using either DOPAC concentration or DOPA accumulation in the median eminence as indexes for TIDA neuron's activity, we found significant decreases starting from 1400 till 1900 h, which correlated nicely with PRL levels. In ovariectomized rats with no estrogen replacement, however, neither PRL level nor TIDA neuron's activity had any change during the afternoon. We further found that both changes in PRL level and TIDA neuron's activity were not present in suprachiasmatic nuclei-lesioned rats either. We conclude that the estrogen-induced PRL surge is circadian in nature which may originate from changes in suprachiasmatic and TIDA neurons' activities.

573.6

EFFECTS OF DOPAMINE, ENDOGENOUS OPIOID PEPTIDES AND THEIR ANALOGS ON HYPOTHALAMIC ARCUATE NEURON'S ACTIVITY IN BRAIN SLICES. Jing-Ying Lin, Julie Y.H. Chan* and Jenn-Tser Pan, Inst. Physiol., Natl. Yang-Ming Med. Coll., Taipei, Taiwan 11221, R.O.C.

Using extracellular single-unit recording technique in brain slices, we focused our recording on dorsomedial and ventrolateral side of the arcuate (ARC) nucleus where immunoreactive dopaminergic neurons are located, and compared slices obtained from ovariectomized or diestrous rats. Dopamine by itself inhibited 37% of 169 ARC neurons from ovariectomized rats. SKF-38393, a D_1-, and quinpirole, a D_2-receptor agonist also inhibited 31% (n=32) and 23% (n=39) of ARC neurons, respectively. Few neurons were excited by dopamine or its agonists, and most were not responsive. As for the effects of opioids on ARC neurons, most were inhibitory, viz., ß-endorphin inhibited 54.5% (n=33); DAGO, 62% (n=21); Met-enkephalin, 35.2% (n=54); DPDPE, 50% (n=8); dynorphin-A, 55% (n=11); and U-50488, 36% (n=39). Other neurons were not responsive. DAGO, dynorphin-A and U-50488 were also slightly more effective in inhibiting ARC neurons in slices obtained from diestrous rats. We conclude that dopamine and opioids mainly exert inhibitory effects on responsive ARC neurons through multiple receptor types, and ovarian hormones may have an effect on ARC neuron's responsiveness to opioids.

573.7

ACTIVATION OF DOPAMINE (D2) RECEPTOR HYPERPOLARIZES MEMBRANE POTENTIAL AND REDUCES [CA] IN SINGLE RAT MELANOTROPHS. A. K. Lee* Dept of Physiology and Biophysics, University of Washington, Seattle, WA 98195.

I have studied the effects of quinpirole, a dopamine (D2) receptor agonist on single rat melanotrophs of the intermediate pituitary. Dopamine inhibits secretion by melanotrophs. Melanotrophs were incubated with Indo-1 AM to measure free intracellular [Ca]. Nystatin perforated patch technique was used to measure and/or control membrane potential. The average resting membrane potential was -35 +/- 2 mV (n=17) and the average [Ca] was 183 +/- 11 nM (n=15). One uM quinpirole hyperpolarized the cell by 30 +/- 3 mV (n=17), and reduced [Ca] by 43 +/- 7 nM, independently of external Na. Clearly the [Ca] decrease did not depend on Na/Ca exchange. Furthermore 100 uM Cd also reduced [Ca]. When melanotrophs were voltage clamped between -60 and -40 mV, [Ca] increased. This confirms that melanotrophs have open voltage dependent Ca channels at their resting membrane potential. The calcium influx through these channels is probably responsible for basal secretion in melanotrophs. Dopamine could through hyperpolarization turn off these Ca channels and reduce [Ca], and thereby inhibit basal secretion. To determine if hyperpolarization is the only mechanism for reducing [Ca], melanotrophs were voltage clamped at -25 mV while 1 uM quinpirole was applied. Intracellular [Ca] still decreased by 19 +/- 5 nM (n=10). This decrease was seen in 8 ot 10 cells. Apparently dopamine could reduce intracellular [Ca] not only by hyperpolarization but also by other mechanisms such as direct modulation of Ca channels or activation of Ca ATPases.

Supported by PHS NRSA T32 GM07270 from NIGMS.

573.8

IMPAIRED CATECHOLAMINE SECRETION FROM THE PERFUSED ADRENAL GLANDS OF BB-WISTAR RATS FOLLOWING ACUTE HYPOGLYCEMIA. R. A. Wilke and C. J. Hillard*. Department of Pharmacology, Medical College of Wisconsin, Milwaukee, WI 53226.

A subgroup of insulin-dependent diabetic humans eventually lose the capacity to secrete catecholamines from their adrenal medullae. Although a similar defect has been described in the spontaneously diabetic BB-Wistar rat, the causative pathophysiologic mechanism has not yet been elucidated. We hypothesized that this defect was due to functional changes occuring within the adrenomedullary chromaffin cells, secondary to iatrogenic hypoglycemia experienced by these animals during the course of their maintenance on insulin. To test this hypothesis, we variously manipulated the blood glucose levels of BB-Wistar rats with exogenous insulin, perfused their adrenal glands *ex vivo*, and determined the catecholamine content of the perfusate fractions by fluorometric analysis. When adrenal glands were harvested from non-diabetic rats after 3 hours of insulin-induced hypoglycemia, their catecholamine secretory response to nerve stimulation or perfusion with ACh was significantly lower than the response of adrenals harvested from matched euglycemic control rats. Furthermore, the overall adrenomedullary catecholamine content of these hypoglycemic rats was also significantly reduced with respect to euglycemic controls. Together, these results demonstrate that exposure to severe acute hypoglycemia *in vivo* can significantly impair the secretory function of the adrenal medulla, and they suggest that iatrogenic hypoglycemia may have been responsible for the catecholamine secretory defect described previously in spontaneously diabetic BB-Wistar rats maintained on insulin.

573.9

ALPHA-2 ADRENERGIC RECEPTOR BINDING AND GALANIN CONTENT IN THE LOCUS COERULEUS OF ZUCKER DIABETIC FATTY (ZDFB/Drt) RATS. B. H. Hwang* G.-M. Wang and R. G. Peterson, Dept. of Anatomy, Indiana Univ., Sch. of Med. Indianapolis, IN 46202.

Non-insulin dependent diabetes mellitus (NIDDM) or type II diabetes is a common endocrine disorder and associated with a failure of pancreatic beta cells to meet an increased demand for insulin. However, its pathogenesis related to the central nervous system (CNS) is not understood. This study was thus aimed to explore how the locus coeruleus is associated with NIDDM. ZDFB obese (Obe) and their lean controls at 20-24 weeks of ages were studied. Animals were decapitated, and the locus coeruleus (LC) was cut with a cryostat into 14-µm sections. When sacrificed, Obe rats were very diabetic with high plasma glucose and low insulin, as compared with lean controls. One set of animals (n=5) was processed for alpha-2 adrenergic receptor binding (x1000 cpm/mg protein) using [^{125}I]-iodoclonidine. We found that alpha-2 adrenergic receptor binding was significantly lower in Obe rats (33.7 ± 3.2, n=5) than lean controls (66.0 ± 7.5, n=5). Another set of animals was processed for studying galanin content (x1000 cpm/mg protein) with a radio-immunohistochemnical assay. There was much more galanin content in Obe rats (95.6 ± 2.9, n=3) than in lean controls (64.3 ± 2.4, n=4). With severe diabetes characteristic of hyperglycemia, ZDFB obese rats had drastic changes in alpha-2 adrenergic receptor binding capacity and galanin content in the LC. This study suggests that CNS is closely associated with pathogenesis of NIDDM. Supported in part by NS-25087.

573.10

THE ROLE OF NITRIC OXIDE AND THE SYMPATHETIC NERVOUS SYSTEM IN MEDIATING THE EFFECTS OF ANGIOTENSIN II ON BLOOD FLOW TO CHOROID PLEXUSES OF THE RAT. A. Chodobski, J. Szmydynger-Chodobska, and C.E. Johanson, Program Neurosurg., Dept. Clin. Neurosci., Brown Univ. and R.I. Hospital, Providence, RI 02903.

We have previously shown that angiotensin II (AII) infused iv alters blood flow to choroid plexuses (CPs) with the peptide effects being dependent on both the AII dose and the anatomic location of CP. In the present study we investigated the role of the sympathetic nervous system in mediating the inhibitory effect of moderate AII doses (30 and 50 ng kg^{-1} min^{-1}) on choroidal blood flow by using the alpha-blocker, phentolamine (iv, 1 mg kg^{-1}). To characterize the choroidal vascular regulatory mechanisms operating during iv infusion of AII at higher dose (300 ng kg^{-1} min^{-1}) when choroidal blood flow was unchanged, we either blocked beta-receptors (propranolol, iv, 1 mg kg^{-1}) or inhibited the synthesis of nitric oxide (NO) by using NG-nitro-L-arginine methyl ester (L-NAME, iv, 0.1 mg kg^{-1}). Blood flow to CPs of the lateral (LVCP), third (3VCP), and fourth (4VCP) ventricles was measured in rats anesthetized with pentobarbital (ip, 50 mg kg^{-1}) and artificially ventilated. ^{123}I-*N*-isopropyl-*p*-iodoamphetamine was used as a marker. Phentolamine by itself did not alter choroidal blood flow, but it completely abolished the action of moderate doses of AII previously noted in LVCP and 4VCP. Propranolol by itself decreased blood flow to LVCP (from 3.19±0.07 to 2.54±0.18 ml g^{-1} min^{-1}, P<0.01), 3VCP (from 3.90±0.17 to 3.21±0.12 ml g^{-1} min^{-1}, P<0.02), and 4VCP (from 3.95±0.11 to 3.23±0.23 ml g^{-1} min^{-1}, P<0.02). AII infused 5 min after propranolol, further decreased blood flow to all CPs, but the changes were not significant. L-NAME by itself lowered blood flow to 4VCP (by 16%, P<0.05), but did not affect other CPs. AII administered 5 min after L-NAME caused an additional decrease in blood flow to all plexuses (by 18–26%, P<0.01-0.05). It is concluded that activation of alpha-receptors is involved in the inhibitory action of AII on choroidal blood flow observed with moderate peptide doses. It appears that the concomitant release of NO and perhaps the activation of beta-receptors result in no changes in choroidal blood flow with higher AII dose. Supported by NIH Grant NS 27601.

573.11

LACK OF CATECHOLAMINE MEDIATION OF INCREASED LH RELEASE DURING BLOCKADE OF KAPPA OPIOID RECEPTORS IN THE RAT MEDIAL BASAL HYPOTHALAMUS (MBH). S.Zhen and R.V.Gallo*
Dept. Phys. & Neuro., Univ. Connecticut, Storrs, CT 06269.

We have shown that kappa receptor blockade in the MBH with norbinaltorphimine (nBNI) stimulated LH release during midpregnancy. The literature indicates kappa receptor activation can decrease dopamine (DA) or norepinephrine (NE) release, and inhibiting NE synthesis blocks the increased LH release occurring in response to nonspecific opioid receptor blockade with naloxone. The goal of this study was to determine if DA or NE mediates the LH response to MBH kappa receptor blockade on days 13-17 of pregnancy in the rat. Push-pull perfusion + HPLC-ED was used to determine NE release in the MBH in response to CSF, CSF+nBNI (40ug/h), CSF + desipramine (DMI, a NE reuptake blocker, 10uM), or CSF+DMI+nBNI. CSF alone produced no change in LH release, and perfusate NE was undetectable. nBNI increased LH but perfusate NE remained undetectable. DMI increased perfusate NE but produced no change in LH release. nBNI+DMI increased LH secretion similar to nBNI alone, but produced no further change in MBH perfusate NE. Inhibition of NE synthesis with FLA-63 (25mg/kg), blockade of α-adrenergic receptors with phentolamine (5mg/kg), or DA receptor blockade with d-butaclamol (1mg/kg) did not affect the increased LH release occurring during MBH perfusion with nBNI. Thus neither NE nor DA mediates the increased LH release due to blockade of MBH kappa receptors.
Supported by NIH HD17728.

573.12

INVOLVEMENT OF BETA-ADRENERGIC RECEPTORS ON THE NATRIURESIS AND ATRIAL NATRIURETIC PEPTIDE (ANP) RELEASE INDUCED BY CENTRAL CHOLINERGIC ACTIVATION IN RATS. J.V. Menani, S.P. Barbosa, J. Antunes-Rodrigues, A.L.V. Favaretto, L.A. De Luca Jr.*, W.A. Saad, L.A.A. Camargo, J.E.N. Silveira and A. Renzi. Dept. of Physiology, School of Dentistry, UNESP, Araraquara, SP 14801-903 and Dept. of Physiology, School of Medicine, USP, Ribeirão Preto, SP 14049-900, Brazil.

In the present study we investigated the effect of previous treatment with propranolol (a beta-adrenergic antagonist) on the natriuresis, kaliuresis and ANP release induced by central cholinergic activation. Rats with stainless steel cannula implanted into the lateral ventricle were used. Previous intraperitoneal (IP) injection of propranolol (10 mg/kg b.w.) reduced the natriuresis (182 ± 35 vs 480 ± 58 uEq/2 h) and plasma ANP increase (107 ± 27 vs 266 ± 51 pg/ml) induced by intracerebroventricular (ICV) injection of the cholinergic agonist carbachol (7 nmol). No change in the kaliuresis, antidiuresis and pressor responses to ICV carbachol was observed after the treatment with propranolol. The present results suggest that a central mediation of natriuresis and ANP release may involve beta-adrenergic receptors and the sympathetic nervous system.
Research supported by FAPESP, CNPq and FUNDUNESP.

573.13

FURTHER EVALUATION OF LAMINA TERMINALIS CONTRIBUTIONS TO THE GABAergic INNERVATION OF THE PARAVENTRICULAR AND SUPRAOPTIC NUCLEI. B.L. Roland* and P.E. Sawchenko, The Salk Institute, La Jolla, CA 92037.

We previously identified intrahypothalamic cell groups that participate in the GABAergic innervation of the paraventricular (PVH) and supraoptic (SO) nuclei (*J Comp Neurol* 332:123, 1993). Because these local sources appeared to account for a small fraction of GABA projections to the magnocellular neurosecretory system, we evaluated whether cell groups of the lamina terminalis, i.e., the subfornical organ (SFO), median preoptic nucleus (MePO) and organum vasculosum (OVLT), might contribute substantially to this projection. In support of this, mRNAs encoding both the 65 and 67 kDa forms of glutamate decarboxylase (GAD) were localized in SFO, MePO and OVLT neurons identified in retrograde tracing studies as projecting to the region of the PVH (*Soc Neurosci Abstr* 18:823, 1992). In addition, injections of the anterograde tracer, PHA-L, centered in MePO or OVLT, labeled projections to the PVH and SO. Such anterogradely labeled terminals frequently co-stained for GAD-immunoreactivity. Attempts to evaluate these projections experimentally, by unilaterally transecting lamina terminalis outputs and comparing the GABAeric innervation on the ipsilateral and contralateral sides, provided limited support for the tract tracing data. Variations in post-lesion survival time over 3-25 days yielded minor reductions in the GABAergic innervation of the ipsilateral magnocellular nuclei, despite independent confirmation of the effectiveness of ablations by a lateralized reduction in salt-loading-induced immediate-early gene expression in magnocellular neurons, a phenomenon we have shown to depend on the integrity of descending lamina terminalis projections. It has been suggested that disruption of lamina terminalis projections to the hypothalamus may be compensated by synaptic remodeling (e.g., *Prog Neurobiol* 34:437, 1990). The discrepancy between the present anatomical and experimental data may be due to compensatory sprouting responses of as yet unidentified GABAergic neurons following interruption of lamina terminalis outputs.

573.14

INHIBITORY GABAERGIC INPUTS TO NEURONS IN THE SUBFORNICAL ORGAN IN VITRO. K. Inenaga*, L.-N. Cui, T. Nagatomo and H. Yamashita. Dept. of Physiology, Univ. Occupational & Environmental Health, Sch. of Med., Kitakyushu 807, Japan.

γ-Aminobutyric acid (GABA) is a widely accepted inhibitory neurotransmitter in the central nervous system. Recently Osaka et al.(1) have reported that inhibitory GABAergic interneurons intervene synaptic inputs to the SFO of cats and send tonic inhibition to the nucleus. For further investigation of the GABAergic inhibitory inputs to SFO neurons, intracellular recordings were made in rat brain slice preparations. Inhibitory postsynaptic potentials (IPSPs), which either were recorded spontaneously or were evoked by focal electric stimulation, were almost blocked by the $GABA_A$ antagonists bicuculline or picrotoxin. Following the application of bicuculline or picrotoxin, resting membrane potentials were depolarized by 4-8 mV. GABA and the $GABA_A$ agonist muscimol decreased membrane resistance and firing rate. The actions of muscimol persisted in the presence of TTX, suggesting that they were postsynaptic. The $GABA_B$ agonist baclofen also showed similar inhibitory effects but its action was smaller than that by muscimol. These results suggest that tonic and inhibitory GABAergic inputs exist in the SFO and are mainly mediated through $GABA_A$ receptors.

(1)Osaka et al. Brain Res. Bull. 29:581-587,1992

573.15

OXYTOCINERGIC NEURONS OF THE MAGNOCELLULAR SYSTEM ARE TONICALLY INHIBITED BY GABA IN VIRGIN BUT NOT LACTATING RATS. **J.Summy-Long*, S.Mantz and E. Koehler. Dept. of Pharmacol., M.S. Hershey Med. Ctr., Hershey, PA 17033.**

GABAergic fibers synapse on oxytocin (OT) neurons and these increase in number during lactation. Modulation of OT release by GABA was studied in conscious virgin (V;n=56) and lactating (L;n=47) rats injected (sc,15 ml/kg) with saline or 2.5 M NaCl (HS) for osmotic stimulation 2h before decapitation. CSF(5 ul), GABA(100 ug) or bicuculline(Bic; 100 ng) was given intracerebroventricularly(icv) 5 min (GABA) or 10 min (Bic) before decapitation. OT in plasma was quantified by RIA. Differences were determined by 2 way AOV & Newman Keuls t test. Osmotic stimulation increased (saline vs HS; p<0.01) plasma OT (pg/ml;x±SEM) in V(CSF: 11±1 vs 109±10) but to a lesser (p<0.01) extent in L rats (CSF:8±1 vs 36±4). Bic blockade of endogenous GABA action raised (p<0.01) plasma OT in unstimulated V (19 ±2) but not L (9±0.4) rats. GABA icv decreased (p<0.01) OT levels after HS in V (109±10 vs 68±6) and L (36±4 vs 20±4) animals while Bic had no effect (V 109±10 vs 106±7; L 36±4 vs 36±5). Thus, a GABAergic system inhibits release of OT in normally hydrated V animals that also are more active and tense than L rats. This inhibition was removed during osmotic stimulation when exogenously applied GABA attenuated OT release regardless of reproductive state. In L rats, OT release was not modulated by a Bic-sensitive endogenous GABAergic system.(Supported by RO1-HD25498).

574.1

FREE TISSUE CATECHOLAMINES OF RAT CAROTID BODY, IN VITRO, DURING MATURATION AND FOLLOWING CHRONIC HYPOXIA.

D.F. Donnelly*, T.P. Doyle., Department of Pediatrics, Yale University School of Medicine, New Haven, CT, 06510

Free tissue catecholamine levels (CAT) were estimated using Nafion-coated, carbon fiber electrodes placed in rat carotid bodies, in vitro. Four groups of carotid bodies from normal rats (1d (n=6), 2d (n=6), 10d (n=7) and 20-30d (n=11)), and 1 group from rats (CHR, 20-28d, n=9) placed in a hypoxic environment at birth (FiO_2 = 0.09) were studied. During superfusion with HEPES saline (PO_2≈150 Torr, 32-34°C), baseline CAT were significantly less in the normal 1-6d (0.55±0.06 μM) compared to normal 10d (1.26±0.24 μM) and normal 20-30d (2.59±0.35 μM). During 1 min hypoxia (PO_2≈0 Torr, at nadir), peak CAT was less in the 1d (1.46±0.42 μM) and 2d (2.6±0.5 μM) compared to the 10d (9.7±3.2 μM) and 20-30d (14.8±1.6 μM). Peak single-fiber nerve response also increased with age from 4.5±0.6 Hz in the 1d to 10.5±1.6 Hz in the 6d and 15.5±2.2 Hz in the 20-30d. CHR had elevated baseline CAT (14.4±1.3 μM) compared to similarly aged normal rats, and was relatively unchanged by hypoxia (15.6±2.2 μM). The magnitude of the change in nerve activity was approximately 40% of normal. We conclude that: 1) resting tissue catecholamine levels increase with age, 2) hypoxia causes an enhanced catecholamine release and the magnitude of release increases with post-natal age and 3) catecholamine levels of carotid bodies exposed to hypoxia after birth do not emulate the newborn.

574.2

LACK OF PARALLELISM BETWEEN THE EFFECTS OF HYPOXIA ON THE GLOMUS CELL pH_i AND CAROTID CHEMOSENSORY RESPONSES.

D. K. Ray, A. Mokashi, M. Katayama, F. Botrè and S. Lahiri*. Department of Physiology, University of Pennsylvania School of Medicine, Philadelphia, PA 19104, USA.

Effects of graded levels of superfusate PO_2 hypoxia on pH_i of dissociated and cultured glomus cells of rat carotid bodies were measured to test whether pH_i responses correspond to the well-known chemosensory responses to hypoxia. The dissociated and cultured glomus cells (3 days old), plated on polylysine coated cover-slips, were loaded with pH-sensitive fluoroprobe, carboxy-SNARF-1 (5 μM), and were superfused with pre-equilibrated Tyrode's solution at 37°C. Dual emission signals at 590 nm and 640 nm with excitation at 540 nm were recorded. Effects of four levels of pH_o (7.40-7.20) at a constant PO_2 and three levels of PO_2 (140-16 Torr) at a constant pH_o were studied. For calibration, the high K^+-nigericin method was used. The pH_i was 7.25 at pH_o of 7.40, and varied linearly with pH_o. Hypoxia (PO_2 = 49 Torr, 16 Torr) caused small and non-graded pH_i decreases (Δ pH up to 0.02), which fail to explain the well-known vigorous chemosensory responses to hypoxia. This lack of correspondence between pH_i response and the expected chemosensory activity indicates that pH_i does not mediate the chemosensory responses to hypoxia. (Supported in part by HL-43413-4 and HL-07027-18.)

574.3

ENDOGENOUS CARBON MONOXIDE (CO) AND CAROTID BODY SENSORY ACTIVITY.

N.R. Prabhakar[1]*, F.H. Agani[1], J.L. Dinerman[2] and S.H. Snyder[2]. [1]Departments of Medicine, Physiology and Biophysics, Case Western Reserve University, Cleveland, OH 44106, and [2]Departments of Neurosciences and Medicine, Johns Hopkins School of Medicine, Baltimore, MD 21205.

Recently, it has been reported that carbon monoxide (CO) is synthesized in mammalian neurons and may act as a neurotransmitter in the central nervous system (Science, 259: 381-384,1993). Carotid bodies are peripheral sensory organs that detect the changes in arterial oxygen. We examined whether CO is produced in the peripheral neuronal tissues, such as the carotid bodies, and assessed its possible effects on chemosensory activity. Experiments were performed on isolated perfused, superfused carotid bodies dissected from anaesthetized adult cats. Sensory discharge from the carotid body was recorded from clearly identifiable action potentials using conventional electrophysiological techniques. Zn-protoporphyrin IX (ZnPP-IX), is a potent inhibitor of heme oxygenase, an enzyme that catalyzes the formation of CO. We tested the effects of several doses of ZnPP-IX on chemosensory activity while perfusing the carotid bodies with normoxic medium (PO_2 = 146 mmHg; PCO_2 = 3 mmHg). ZnPP-IX (0.1, 0.3, 1, 3, and 10 μM) increased the chemosensory discharge in a dose-dependent manner (n = 6 carotid bodies). After switching the perfusion medium back to controls, sensory activity returned to initial values, suggesting that the effects of ZnPP-IX were reversible. These results suggest that carotid body synthesizes CO that seems to be inhibitory to the chemosensory activity. Supported by grants from NIH HL-45780 and HL-02599.

574.4

CONCURRENT, DISTRIBUTED ALTERATIONS IN BRAINSTEM NEURAL ASSEMBLIES DURING INDUCTION OF LONG-TERM ENHANCEMENT OF RESPIRATORY ACTIVITY *IN VIVO* BY CAROTID CHEMORECEPTOR STIMULATION.

K. F. Morris*, A. Arata, R. Shannon and B. G. Lindsey. Physiol. & Biophysics, Univ. South Florida Med. Ctr., Tampa, FL 33612.

Stimulation of carotid chemoreceptors results in long term enhancement (LTE) of respiratory activity (Millhorn et. al., Resp. Phys. 41:87). In extending our previous observations (Soc. Neurosci. Abst.17:103,18:829), we tested the hypothesis that correlated assemblies of medullary cardiorespiratory neurons exhibit persistent changes following the induction of LTE. The amplitude of integrated efferent phrenic nerve activity increased for >10 min. after single or multiple injections of 200 μl of CO_2 saturated saline solution via the external carotid arteries of 5, anesthetized, vagotomized, artificially ventilated cats. Spike trains were recorded simultaneously (max.=30) in the medullary raphe n., ipsilateral n. tractus solitarius, rostral and caudal ventral respiratory group (VRG) and contralateral VRG. Data were analyzed with cycle-triggered histograms, spike triggered averages of phrenic activity, cross-correlograms and the gravity method. Means of several parameters measured during pre- and post-stimulus respiratory cycles were compared (t-test). Firing rates of 51% of neurons increased, 27% decreased and 22% were unchanged. Inspiratory duration decreased (n=5), expiratory duration decreased (n=3) and peak heart rate increased (n=5). Of 12 neurons in one assembly (34 concurrently correlated pairs), 10 increased and 2 decreased firing rates. Neurons with opposite firing rate changes during and following induction of LTE had short-time scale correlations. The results support the hypothesis that the expression of LTE requires a change in the set point of distributed equilibrium-seeking brainstem networks with excitatory and inhibitory effective connectivity appropriate for gain control. Supported by NS19814.

574.5

BRAINSTEM PATHWAYS TO RESPIRATORY MUSCLES OF THE LARYNX IDENTIFIED BY TRANSNEURONAL LABELING WITH PSEUDORABIES VIRUS (PRV).

K.A. Gilbert*, R. Fay and R. Lydic, Departments of Anesthesia and Behavioral Science, Penn. State Univ., Col. of Med., Hershey, PA 17033.

Microinjection of carbachol into the medial pontine reticular formation (mPRF) of cat produces a REM sleep-like state and state-dependent hypotonia in the posterior cricoarytenoid (PCA) muscle of the upper airway (*FASEB J. 3*:1625, 1989). Since PCA primary motoneurons arise from the nucleus ambiguus (NA), which in the cat is ~9 mm from the mPRF, we hypothesized that the mPRF causes PCA muscle hypotonia via polysynaptic connections to NA. Injections of PRV were made into the PCA muscles of 3 rats (superior cervical ganglia removed) and 1 cat with survival times of 72-90 hrs in rat and 80 hrs in cat. In rat, dense primary labeling was observed in NA. Heaviest transsynaptic labeling was seen in nucleus of the solitary tract (NTS) and paratrigeminal nucleus. Moderate transsynaptic labeling was seen in laterodorsal tegmental (LDT)/pedunculo-pontine tegmental (PPT) nuclei, raphe magnus and obscuris, and gigantocellular reticular nucleus. Light transsynaptic labeling was seen in reticular nuclei of pontis oralis and caudalis and in central gray. In cat, primary labeling also was observed in NA, with lighter transsynaptic labeling in LDT/PPT, NTS, and gigantocellular, magnocellular, and lateral reticular fields of the pons and medulla. These results are consistent with previous studies concerning the descending inhibitory pathways from pontine reticular regions to spinal motoneurons. The present findings also suggest that additional pathways from the mPRF to NA may mediate state-dependent hypotonia in the PCA muscle.

Support by Dept. of Anesthesia, MH18825(KAG), DC00240(RF), & HL40881(RL)

574.6

PHASIC RESPIRATORY EVENTS OF RAPID EYE MOVEMENT (REM) SLEEP ARE SIMILAR DURING THE CHOLINERGICALLY-INDUCED REM SLEEP-LIKE STATE.

T.O. Leonard,* K.A. Gilbert, S.L.Shuman and R. Lydic, Dept. of Anesthesia, Penn. State Univ., College of Med., Hershey PA 17033.

Breathing during REM sleep is characterized by abrupt transitions between inspiration (I) and expiration (E) associated with bursts of muscle activity (EMG), eye movements (EM), or ponto-geniculo-occipital (PGO) waves. Microinjection of cholinomimetics into the medial pontine reticular formation (mPRF) causes a REM sleep-like state with similar respiratory phase switches. This study is quantifying these phasic respiratory events in cat (n=5) by comparing natural REM sleep (mPRF saline) and the REM sleep-like state caused by mPRF microinjection of carbachol (DCarb), bethanechol (DBeth), or neostigmine (DNeo). Respiratory phase switches (RPS) were defined as an irregular interruption in I or E. RPS were tabulated for 10 min in each state. Mean (±SD) RPS during REM following saline=21.67±5.51; DCarb=17.00±6.08; DBeth=8.33±6.51; and DNeo=7.33±1.53. Using permutations of RPS with and without EM, PGO, or EMG, 13 dependent measures were compared. There were no significant difference in RPS comparing natural REM sleep, DCarb, or DBeth. DNeo differed significantly from both saline and DCarb for the min devoid of RPS and for the total number of PGO bursts occurring with RPS. DNeo was significantly different from natural REM sleep for RPS alone. Thus, quantitative comparison of phasic respiration during natural and cholinergically-induced REM sleep-like state revealed more similarities than differences. Ongoing studies will evaluate the possible dose-dependency of these effects. *Support: Department of Anesthesia, HL-40881 (RL).*

574.7

MANIPULATION OF SECOND MESSENGER SYSTEMS IN THE PONTINE RETICULAR FORMATION: EFFECTS ON SLEEP AND BREATHING. S. L. Shuman, H. A. Baghdoyan, and R. Lydic*.
Dept. Anesthesia, Penn. State Univ.,Coll. Med., Hershey, PA 17033.

Microinjection of cholinomimetics into the medial pontine reticular formation (mPRF) of intact, unanesthetized cat causes a REM sleep-like state and state-dependent respiratory depression (*News Physiol. Sci.* 7:220, 1992). The mechanisms by which these changes occur are known to be G-protein-mediated, but the specific G-protein and second messenger system(s) involved are currently unknown. As a first step toward characterizing G-protein effects on sleep and breathing, microinjections (n=81) of 5-guanylyl-imidodiphosphate (GMP-PNP), cholera toxin, and carbachol were made into the mPRF of two cats. States of consciousness and breathing were then recorded for 2 hrs. Injections of cholera toxin or GMP-PNP had no immediate effect on the total time spent in REM sleep, latency to onset of REM, the total number of REM epochs, or the mean duration of REM epochs. Cholera toxin caused a delayed but long-term (mean=32.5 days) inhibition of the carbachol-induced REM sleep-like state. Return of REM sleep enhancement by carbachol occurred in a graded manner. GMP-PNP caused a significant decrease in respiratory rate during waking (-14.3%) and REM sleep (-13.8%) when compared to control. Cholera toxin significantly slowed respiration during waking (-26.5%) and non-REM sleep (-8.7%) but not during REM sleep (-5.9%). Thus, multiple types of G-proteins are likely to mediate pontine cholinergic control of sleep and breathing.

Support: Department of Anesthesia, MH-45361 (HAB) and HL-40881 (RL).

574.8

PHOSPHATE-ACTIVATED GLUTAMINASE AND GLUTAMIC ACID DECARBOXYLASE IMMUNOHISTOCHEMISTRY WITHIN THE INTERMEDIATE LATERAL TEGMENTAL FIELD OF THE RAT.
H.H. Ellenberger*. Department of Anatomy & Neurobiology, Dalhousie University, Halifax, Nova Scotia, Canada B3H 4H7.

The intermediate portion of the medullary lateral tegmental field (LTF) contains neuron populations critical for control of the cardiovascular and respiratory systems. Neurons utilizing excitatory or inhibitory amino acid neurotransmitters could provide an anatomical substrate within the intermediate LTF for generation of rhythmic and tonic drives essential for cardiopulmonary homeostasis, and provide for both sensorimotor integration and coordination of efferent control of the respiratory and cardiovascular systems.

The location of putative glutamatergic and GABAergic neuronal perikarya and terminal varicosities within the intermediate LTF were identified in the present study by immunohistochemical reactions for phosphate-activated glutaminase (PAG) and glutamic acid decarboxylase (GAD). Neuronal axons and terminal varicosities immunoreactive for PAG or GAD were distributed throughout the intermediate LTF, including the regions of the nucleus ambiguus, ventral respiratory group and the caudo- and rostroventrolateral reticular nuclei. Neuronal somata immunoreactive for PAG or GAD formed a more limited distribution within restricted portions of the intermediate LTF. These results provide anatomical evidence of a role for excitatory and inhibitory amino acid neurotransmission within cardiorespiratory neuron populations of the intermediate LTF.
Supported by the Medical Research Council of Canada.

574.9

CO_2 MODULATES PACEMAKER NEURONS IN THE MEDULLARY RAPHE AND PARAPYRAMIDAL REGION OF THE RAT *IN VITRO*.
G.B. Richerson*, Dept. of Neurology, Yale University & VAMC, West Haven, CT.

Central chemoreceptors constitute the major source of feedback for control of respiration in mammals, but it has been difficult to find evidence for chemoreceptors at the cellular level. I have used the amphotericin perforated patch-clamp recording technique in thin slices of the rat medulla to examine the response of neurons to changes in bath CO_2. Stable recordings were obtained from > 100 neurons for up to 6 hrs (R_{in} = 300MΩ to > 2 GΩ). In the medullary raphe and parapyramidal region between the inferior olive and the pons, most neurons spontaneously fired action potentials at a monotonously regular rate of 1-10 Hz. This repetitive firing was similar to that seen in beating pacemaker neurons in the dorsal raphe, with a linear ramp increase in E_m between spikes. Repetitive firing continued in the presence of TTX (1 μM) with a slower rate and broader, smaller spikes, but was abolished in Cd^{++} (100 μM). Most neurons in this region were not affected by changes in CO_2 between 3 and 9%. Some neurons (n = 15) were stimulated by CO_2. For example, increasing CO_2 from 5 to 9% increased the firing rate of one neuron from 4 to 7 Hz, and decreasing CO_2 from 5 to 3% decreased the firing rate from 2 to 0.4 Hz. Other neurons (n = 5) were inhibited by CO_2. When CO_2 was increased from 5 to 6%, the firing rate of one neuron decreased from 2.5 to 1.2 Hz, and when CO_2 was decreased from 5 to 4% the firing rate increased from 1 to 2 Hz. In both types of CO_2 sensitive neurons, the change in firing rate was associated with a change in slope of the linear ramp in E_m between spikes without a change in resting potential, suggesting that CO_2 modulates the pacemaker currents in these neurons. The high blood flow to this part of the medullary raphe and parapyramidal region, their projections to other respiratory nuclei, and the presence in this area of neurotransmitters known to have profound effects on breathing *in vivo* all suggest that these neurons are candidates for at least a subset of central respiratory chemoreceptors.

574.10

PROJECTIONS FROM RAPHE TO MEDIAL PONTINE RETICULAR FORMATION (mPRF) AND PARABRACHIAL NUCLEI (PN) DEMONSTRATED BY RETROGRADE FLUORESCENT LABELING.
D.B. Friedman*, L.H. Lee, and R. Lydic.
Dept. Anesthesia, Penn State Univ., Coll. Med., Hershey PA, 17033.

Pontine cholinergic neurotransmission plays a key role in regulating both sleep and breathing (*Am. J. Physiol. 264:*R544, 1993). Since cholinergic/monoaminergic interactions are important for sleep cycle control, monoamine-containing nuclei are also likely to contribute to state-dependent respiratory depression. The present study is examining the hypothesis that the cholinoceptive mPRF and respiratory-related PN receive input from the monoamine-containing raphe. The mPRF and PN of 10 cats were injected with 50 or 100 nl of the retrograde tracers True Blue (5%) or Fluoro-Gold (2%). After mPRF injections, retrograde labeling was observed in the raphe (inferior central magnus and obscurus, tegmental reticular, dorsal). Following PN injections, robust retrograde labeling was observed in the inferior central and central linear raphe. These data demonstrate projections from the raphe nuclei to cholinoceptive regions of the mPRF and to the PN. These projections are consistent with previous retrograde tracing studies and with a large body of physiological data showing that cholinergic/monoaminergic interactions are important for regulating arousal states and breathing. *Support: Department of Anesthesia, HL47749, HL40881(RL).*

574.11

IDENTIFICATION OF THE PROPRIOSPINAL RESPIRATORY NEURONS WHICH RELAY THE RAPHE MAGNUS-INDUCED RESPIRATORY INHIBITION IN CATS.
M. Aoki*, T. Imai, Y. Nakazono and H. Satomi. Dept. Physiology, School of Medicine, Sapporo Medical University, Sapporo 060, Japan.

We previously demonstrated that chemical as well as electrical stimulation of the medullary raphe magnus (NRM) induces marked respiratory inhibition in pentobarbital anesthetized cats. In the present study, we examined whether the propriospinal respiratory neurons in the upper cervical cord (C1-C2 segments) are involved in mediating the NRM-induced respiratory inhibition. We employed a combined technique of anterograde and retrograde labelings to identify the pathways. 1) anterograde tracing of raphe-spinal fibers by injecting biocytin (5%) into the NRM. 2) retrograde labeling of propriospinal respiratory neurons and the ventral respiratory group (VRG) neurons in the medulla by applying WGA-HRP to the phrenic nerve. Retrograde transynaptic labelings of neurons were detected in the ipsilateral C1-C2 segments and contralaterally in the VRG. A number of anterogradely labeled raphe-spinal fibers were distributed in the C1-C2 segments and the VRG. Some terminal fibers were observed to make apparent contacts with the dendrites of the retrogradely labeled propriospinal respiratory neurons. The present study provides anatomical evidence that the NRM-induced respiratory inhibition is in part mediated by the C1-C2 propriospinal respiratory neurons as well as the VRG neurons.

574.12

PERIODIC POLYPNEA DURING PREOPTIC/ANTERIOR HYPOTHALAMIC WARMING ACROSS SLEEP-WAKING STATES IN THE ADULT CAT AND KITTEN.
H. Ni*, J. Zhang, R.K. Harper and R.M. Harper. Brain Research Institute and Department of Anatomy & Cell Biology, UCLA, Los Angeles, CA 90024.

We compared respiratory responses to preoptic/anterior hypothalamic (POAH) warming in 6 unanesthetized, unrestrained adult cats and in 16 kittens (10 to 49 days) during sleep and waking states. Under surgical anesthesia, electrodes were placed for warming the POAH and for recording EEG, diaphragmatic and neck EMG. The POAH was warmed (0.5MHz, 84-190mW) bilaterally by two electrode pairs (1-2mm inter-tip distance, 1-2 mm bared tip, and 0.3 mm in diameter). In both adult cats and kittens, threshold warming was similar (2.8 ± 1.6^0C vs 2.8 ± 1.1^0C).

In adult cats, POAH warming elicited constant panting during QS. Warming during REM produced slightly faster breathing or a periodic, mild fluctuation in respiratory rate, but no panting. In the kitten, POAH warming also induced panting across all ages during QS, and a diminished response in REM. However, in contrast to the adult, during QS, kittens developed periodic episodes of panting interspersed with episodes of slower respiration (periodic polypnea). These results confirm the presence of thermal panting in the very young kitten in response to POAH warming, and suggest that sleep-state related thermoregulatory mechanisms are more readily disrupted during QS in the younger animal. *Supported by HD22506.*

574.13

***IN VITRO* ELECTROPHYSIOLOGICAL RESPONSES OF CAUDAL HYPOTHALAMIC NEURONS TO HYPOXIA AND HYPERCAPNIA: NEONATAL AND ADULT.** E.M. Horn* and T.G. Waldrop. Department of Physiology and Biophysics, Neuroscience Program, and College of Medicine, University of Illinois, Urbana, IL 61801.

Prior findings from this laboratory have shown that hypoxia and hypercapnia stimulate subpopulations of caudal hypothalamic neurons. Hypoxia stimulated 77% and hypercapnia stimulated 35% of the neurons studied in a brain slice preparation. The present goal was to determine if neurons in the neonatal caudal hypothalamus respond to hypoxia and hypercapnia in the same manner as in the adult. Brain slices (400-600 μm thick) containing the caudal hypothalamus were obtained from both neonatal (<12 days) and adult (>21 days) Sprague-Dawley rats. The slices were placed into an interface chamber and perfused with nutrient media equilibrated with 95% O_2/5% CO_2. The current responses to hypoxia (10% O_2/85% N_2/5% CO_2 for 90 sec.) and to hypercapnia (7% CO_2/93% O_2 for 3 min.) of voltage-clamped, caudal hypothalamic neurons were obtained with whole-cell patch recordings; extracellular recordings were performed on some neurons. The average resting membrane potential of neonatal neurons tended to be lower than adult neurons. Forty-five percent of the neonatal neurons were excited by hypoxia as indicated by a net inward current in the whole-cell patch recordings or by an increase in discharge frequency in the extracellular recordings. Hypoxia did not inhibit and hypercapnia did not affect any of the neonatal neurons tested. In contrast, eighty-five percent of the neurons from the adult caudal hypothalamus were stimulated and fifteen percent were inhibited by hypoxia. In addition, hypercapnia stimulated twenty percent and inhibited forty percent of the adult neurons. These preliminary findings suggest that both hypoxia and hypercapnia affect a higher percentage of neurons in the adult than in the neonatal caudal hypothalamus. These differences may contribute to the different respiratory responses elicited by hypoxia in the newborn as compared to the adult rat. (Supported by NIH 32864 and AHA).

574.14

N^6-L-PHENYLISOPROPYL ADENOSINE (L-PIA) DECREASES SLEEP-APNEAS IN RATS. D. Monti, D. W. Carley, J. Clancy, J. Trapp, R. Basner, E. Önal, M. Lopata, M. Radulovacki*. Departments of Pharmacology and Medicine, University of Illinois College of Medicine, Chicago, IL 60612

Spontaneous apneic events have been described in rats and compared to central apneas in man. In man, administration of adenosine stimulates respiration, presumably secondary to enhanced carotid body chemoreflexes. We tested the hypothesis that administration of L-PIA, an adenosine analog, decreases apneas in rats. We administered 0.3 mg/Kg, 1.0 mg/Kg and 1.5 mg/Kg of L-PIA to 9 adult male Sprague-Dawley rats implanted with EEG and EMG electrodes for sleep/wake scoring and placed in unrestrained body plethysmographs for respiratory monitoring. For each rat, sleep and respiratory activity were polygraphically recorded for 6 hours on 4 different days. Immediately prior to each recording, saline or drug was given by intraperitoneal injection. We defined apnea as cessation of respiratory effort for at least 2.5 seconds and expressed apnea indexes (AI) as apneas per hour. When apneas from all sleep stages were considered, the 2 highest doses of L-PIA were associated with equivalent and significant decreases in AI with respect to saline control (7.1 ± 1.6 - control; 2.5 ± .9 - 1.0 mg/Kg; 3.7 ± 1.6 mg/Kg - 1.5 mg/Kg: $p < .05$, repeated measures ANOVA). This L-PIA related decrease in AI was also observed when only slow wave sleep-related apneas were considered (9.2 ± 2.2 - control; 3.1 ± 1.0 - 1.0 mg/KG; 4.6 ± 2.1 - 1.5 mg/Kg: $p < .05$, for each dose with respect to control). Similar effects of L-PIA on non-slow wave sleep-related apneas were observed, but did not achieve statistical significance. We conclude that administration of an adenosine analog is associated with significant decreases in central apneas in rats. We speculate that a sleep-related decrease in respiratory drive contributes to central apneas, and may be opposed by stimulation of carotid chemoreflexes. Adenosine analogs may offer a viable therapeutic intervention in certain sleep-related breathing disorders.

574.15

RESPIRATORY, AUTONOMIC, AND EEG CORRELATES OF TRANSCENDENTAL CONSCIOUSNESS EXPERIENCES DURING TRANSCENDENTAL MEDITATION PRACTICE. F. T. Travis*. Maharishi International University, Fairfield, IA 52557

Periods of apneustic breathing up to 40 seconds have been reported during content-free self-awareness, called transcendental consciousness (TC), in Transcendental Meditation practice, suggesting changed respiratory control[1,2]. This study replicated and extended these findings. Of twenty TM subjects, five reported TC experiences. During these self-reported experiences: (1) respiration suspended for 5 to 16 sec's in four subjects, and breath volume abruptly decreased 40% in the fifth; (2) skin conductance levels decreased 0.75 μmohs over the 1.5 min prior to breath changes, followed by a 1.77 μmohs increase during periods of breath changes; (3) EEG patterns varied—increased frontal-central power and coherence in three subjects, central-parietal in one, and parietal in the other; with peak frequencies at 8-9.5 Hz in three subjects, 5.5-7.0 Hz in another, and 7-7.5 Hz in the last. Concomitant change in breath and skin conductance was not seen in subjects not reporting TC experiences.

Concomitant change in breath and skin conductance, which is also seen during orienting, could indicate orienting to the inner experience of transcendental consciousness. Breath and skin conductance are simple to measure, and could constitute an objective marker of this state.

References: 1) Farrow, & Hebert, Pychosom. Med., 1982, 44:133-153. 2) Kesterson & Clinch. (1989). Amer Physiol. Soc. 89:R632-R638

PAIN: PATHWAYS II

575.1

MICRONEUROGRAPHIC ASSESSMENT OF SENSITIVE AND INSENSITIVE C-FIBERS IN A HUMAN SKIN NERVE. Handwerker H.O.[2*], Schmidt, R.[1], Forster, C.[2], Schmelz, M.[2], Traversa, R.[1] and Torebjörk, H.-E[1]. [1]Department of Clinical Neurophysiology, University of Uppsala, Sweden and [2]Department of Physiology and Biocybernetics, University of Erlangen/Nürnberg, Germany.

In microneurography insensitive nociceptive or chemoceptive afferents have not been encountered with conventional search strategies. Here we have recorded from cutaneous fascicles of the peroneal nerve in healthy volunteers. Needle electrodes were inserted intracutaneously in skin sites from which C-fiber responses could be mechanically evoked. Electrical search stimuli were then used to recruit all C-fiber endings in the neighbourhood. The responses of single units to different types of natural stimuli or provocations of sympathetic reflexes were assessed by slowing of impulse conduction velocity in activated units. Though this search procedure is not entirely bias-free, it enabled us to characterize unit types which were missed in former microneurographic studies. Different C unit types are listed in the table. About 15% of the units were mechanically and heat insensitive (CM_iH_j). Treating the skin with mustard oil (allyl-iso-thiocynate, 100%) activated 3 of 7 CM_iH_j-units which became responsive to mechanical and heat stimuli after that treatment. We conclude that a significant proportion of mechanically and thermally insensitive C-fibers, which have previously been described in the skin of monkey and rat, also exist in human skin and that they may play an important role in pain and hyperalgesia evoked by chemical stimuli.

type	n (%)	c. v. (m/s)	mech. thr. (g)
CMH	49 (43)	0.99 ± 0.12	1.4 - 30
CM	35 (31)	0.86 ± 0.14	1.4 - 8.5
CM_iH_j	17 (15)	0.84 ± 0.24	
Symp.	12 (11)	0.76 ± 0.13	

Supported by Riksbankens Jubileums Fond, Sweden, Swedish Medical Research Council Grant no B 93-14x-5206-16B and the SFB 353

575.2

BIPHASIC MODULATION OF MORPHINE ON SUBSTANCE P RELEASED FROM DISSOCIATED DORSAL ROOT GANGLION NEURONS. H. Suarez-Roca and W. Maixner*. Sec. of Neurochemistry, Instituto de Investigaciones Clinicas, Univ. of Zulia, Apartado 1151, Maracaibo 4001-A, Venezuela. Dental Research Center, Univ. of North Carolina, Chapel Hill, NC 27514.

We have previously reported that morphine produces a concentration-dependent multiphasic modulation (inhibitions and facilitations) of substance P (SP) release from trigeminal nucleus caudalis slices by activating distinct populations of μ-,δ- and κ-opioid receptors (Suarez-Roca and Maixner, Brain Res. 579:195, 1992). In the present study, we have examined a wide range of morphine concentrations on K^+-evoked SP release from *dissociated* rat dorsal root ganglion (DRG) neurons in culture. Immunoreactive SP was measured in the media. Morphine produced biphasic effects on K^+-evoked SP release without affecting basal release. Low concentrations of morphine (30 nM) facilitated SP release whereas high concentrations (1 uM) suppressed release. These effects were abolished by opioid-receptor blockade with naloxone (30 nM). We conclude that an intact neuronal circuitry is not required for morphine to produce a bidirectional modulation of SP release and can act directly by stimulating opioid receptors located on primary afferents. Supported by DE08013 & TW00305.

575.3

ALTERATIONS IN THE NUMBER OF NADPH-DIAPHORASE-POSITIVE CELLS IN THE TRIGEMINAL NUCLEUS OF THE RAT FOLLOWING TMJ INSULT. A.L. Law, S.T. Meller, S. Murphy* and G.F. Gebhart. Dept. Pharmacology, Univ. of Iowa, Iowa City, IA 52242. The aim of this investigation was to evaluate the effect of tissue damage in the temporomandibular joint (TMJ) on changes in the number of NADPH-diaphorase-positive (NADPH-d+) cells in the trigeminal nucleus. Rats were divided into three groups: (1) 50 µl 20% mustard oil in left TMJ; (2) 50µl mineral oil in left TMJ; and (3) anesthesia only (sodium pentobarbital; 50mg/kg, i.p.). Rats were sacrificed at 6, 24 or 48 hours following TMJ insult or control. Rats were perfused with 2% paraformaldehyde and every tenth section (40µm) of brainstem including the trigeminal nucleus (n. caudalis, oralis, and interpolaris) were processed for NADPH-diaphorase using standard histochemical techniques. Sections from each experimental group were matched for experimenter-blinded counting. There were no differences in the number of NADPH-d+ cells between or within groups 2 or 3 at any of the time periods examined. In all groups, the majority of cells were in n. caudalis; within n. caudalis, a majority of cells were in the outer laminae (I and II). In group 1 there was an increase in the number of cells ipsilateral to the TMJ insult over the contralateral side. The major increase was found in the outer laminae of n. caudalis. While there was an increase at 6 and 24 hours, the largest increase in NADPH-d+ cells was found at 48 hours following TMJ insult. The results suggest that tissue insult within the TMJ is associated with ipsilateral increase in NADPH-d+ cells in the trigeminal nucleus, and that the increase is greatest at 48 hours.

575.4

PRETREATMENT WITH PERINEURAL CAPSAICIN (CAP) REDUCES SPINAL DYNORPHIN (DYN) LABELING AFTER THE CHRONIC CONSTRICTION INJURY (CCI). Y.-O. Lee[1] and K.C. Kajander[1,2]*. Depts of [1]Oral Science, and [2]Cell Biology & Neuroanatomy, and [2]Graduate Program in Neuroscience, University of Minnesota, Mpls, MN 55455.

Spinal DYN increases maximally ten days after the CCI (Bennett and Xie, 1988; Kajander et al., 1990). We investigated the effects of pre-treatment with perineural CAP on DYN A1-8-like immunoreactivity (DYN-LI) induced by the CCI. We also evaluated for a possible association between thermal hyperalgesia and DYN-LI. After baseline tests, using a hindpaw withdrawal method, rats (n=23) were assigned randomly to one of five groups. We applied perineurally around the left sciatic nerve one of the following solutions: group 1) 1.5% CAP in vehicle, group 2) vehicle, groups 3 & 4) 0.9% saline. Group 5 served as a testing control. Fourteen days after perineural treatment, the CCI was produced on the left sciatic nerve of the animals in groups 1-3, and a sham surgery was performed on the animals in group 4. Ten days after the CCI, animals (n=17) were sacrificed for immunocytochemistry of L4-L5 spinal segments. As compared with vehicle-pretreated rats, CAP-pretreated rats exhibited a smaller number of DYN-LI cells ($\bar{x}$±S.E. of 10 sections from each rat; 10.8±3.7 vs 5.1±1.0). A significant, positive correlation existed between the degree of hyperalgesia and the number of DYN-LI cells ($r=0.91$, $p<0.01$) in groups 2-5. CAP-pretreated rats did not develop thermal hyperalgesia. In conclusion, CAP pretreatment prevents development of hyperalgesia and reduces DYN labeling after the CCI. Increased DYN is positively correlated with thermal hyperalgesia in rats not treated with CAP. Thermal hyperalgesia may be related to increased DYN in spinal neurons. (Research supported by NIH grant NS29567)

575.5

EFFECTS OF ACIDIC FIBROBLAST GROWTH FACTOR (aFGF) ON THE PAIN RELATED BEHAVIOUR OF RATS WITH AN EXPERIMENTAL PERIPHERAL NEUROPATHY. J.M.A. Laird*, S. Boyce, S.A. Titmuss, F.D. Tattersall, K.A. Thomas+ and R.G. Hill. Neuroscience Research Centre, Merck, Sharp & Dohme Research Labs, Terlings Park, Harlow, U.K. and +Dept of Biochemistry, Merck Research Labs, Rahway, NJ 07065, U.S.A.

Loose ligation of one sciatic nerve in the rat produces a peripheral neuropathy characterised by symptoms indicative of abnormalities in pain sensation (Bennett & Xie 1988, Pain 33: 87). In this study we have examined the effects of i.v. administration of aFGF, a heparin-binding protein known to be neuronotrophic, on the development of these symptoms over the first 14 days.

Six groups of rats (n=12 per group) were anaesthetised with pentobarbitone (60 mg/kg i.p.). One group received sham operations. The remainder received sciatic nerve ligations, and in 4 groups, osmotic mini-pumps were implanted s.c. delivering either heparin vehicle, 0.03, 0.3 or 3 µg/kg/day aFGF i.v. The spontaneous behaviour and responses to noxious thermal and mechanical stimuli were examined at various times up to 14 days post-surgery (PS).

A dose-related effect of aFGF on heat hyperalgesia was seen at 7 days PS. The trend towards control (sham-operated) values was statistically significant ($p < 0.05$). Exploratory behaviour examined 3 days PS was significantly reduced in the nerve-injured rats, and this was partly reversed by 0.3 µg/kg/day aFGF (significantly different from vehicle group, $p < 0.05$). There was no effect on posture of the injured limb examined at 3 days PS, nor on mechanical hyperalgesia. These results show that administration of aFGF may prevent the development of some of the abnormalities of pain behaviour associated with an experimental peripheral neuropathy.

575.6

CHANGES IN SYNAPTIC ORGANIZATION OF THE SUPERFICIAL DORSAL HORN OF THE RAT AFTER EXPERIMENTAL PERIPHERAL MONONEUROPATHY. H.J.Ralston, III*, D.D.Ralston, J.Desmeules, and G.Guilbaud. Department of Anatomy, WM Keck Foundation Center for Integrated Neurosciences, University of California, San Francisco, Ca. 94143-0452 and Unite de Recherches, I.N.S.E.R.M., U161, Paris, France, 75014.

In a rat model of mononeuropathy (Bennet and Xie, '88), one sciatic nerve was ligated with four loose ligatures for 14 days producing behaviorally identified hyperalgesia and allodynia of the affected limb. All animals were cared for according to established protocols. Three days before euthanasia, the sciatic nerve was injected proximal to the ligature with 2µl of wheatgerm agglutinin horseradish peroxidase (WGA-HRP) or with choleragenoid-HRP. The anaesthetized animal was perfused intracardially with aldehydes and sections of lumbar spinal cord superficial dorsal horn (laminae I and II) reacted for the presence of HRP for electron microscopic (EM) examination. In comparison to the synaptic organization of the normal rat dorsal horn, that of the mononeuropathy model exhibits major changes. 1) Myelinated axons of varying diameters, but primarily small to medium size, degenerate. 2) The usual clusters of non-myelinated axons are diminished in number, although signs of degeneration in these axons are not evident. 3) Glomerular or "scalloped" shaped primary afferent terminals are greatly diminished in number and many of those that remain exhibit signs of continuing degeneration, such as oversized mitochondria, neurofilamentous hyperplasia, clustering of enlarged synaptic vesicles or increased electron density. The synaptic relationships of many of these degenerating terminals was preserved. 4) There appears to be a general reduction in overall synaptic contacts. 5) There is a notable reduction in normally abundant terminals in lamina II with dense-cored synaptic vesicles, believed to contain neuropeptides. Possible synaptic reorganization, in response to the peripheral nerve injury will be quantitatively described using EM immunocytochemical methods. Supported by NS 21445 from N.I.H. and from I.N.S.E.R.M., U161.

575.7

REGIONAL INCREASES IN BRAIN METABOLIC ACTIVITY INDICATIVE OF PAIN IN RATS WITH PERIPHERAL MONONEUROPATHY D.D. Price', J. Mao, and D.J. Mayer. Dept. of Anesthesiology, Medical College of Virginia, Richmond, Virginia 23298

Regional changes in brain neural activity were examined in rats with painful peripheral mononeuropathy (chronic constrictive injury, CCI) by using the fully quantitative [^{14}C]-2-deoxyglucose (2-DG) autoradiographic technique. CCI rats exhibited demonstrable thermal hyperalgesia and spontaneous pain behaviors 10 days after sciatic nerve ligation when the 2-DG experiment was carried out. In the absence of overt peripheral stimulation, reliable increases in 2-DG metabolic activity were observed in CCI rats as compared to sham-operated rats within extensive brain regions that have been implicated in supraspinal nociceptive processing. They included cortical somatosensory areas, cingulate cortex, amygdala, ventral posterolateral thalamic nucleus (VPL), posterior thalamic nucleus (PO), hypothalamic arcuate nucleus, central grey matter, deep layers of superior colliculus, pontine reticular nuclei, locus ceoruleus, parabrachial nucleus, gigantocellular reticular nucleus, and paragigantocellular nucleus. The increase in 2-DG metabolic activity was bilateral in most brain regions of CCI rats, but levels of 2-DG metabolic activity were reliably higher in the cortical hind limb area, VPL, and PO contralateral to the ligated sciatic nerve than in ipsilateral corresponding regions in CCI rats. In addition, patterns of increased neural activity found in the brain of CCI rats showed some similarities and differences to those found in the brain of rats exposed to acute nociception induced by noxious heat or formalin stimulation. Thus, these CCI-induced *spontaneous* increases in neural activity within *extensive* brain regions previously implicated in sensory-discriminative and affective-motivational dimensions of pain as well as centrifugal modulation of pain are likely to reflect brain neural processing of ongoing neuropathic pain. **Supported by PHS grant NS 24009.**

575.8

RVM NUCLEI THAT MEDIATE MORPHINE ANTINOCICEPTION FROM THE PAG. M.O. Urban* and D.J. Smith. Depts. Anesth. & Pharmacol., WVU-HSC, Morgantown, WV 26506-9134.

The rostral ventromedial medulla (RVM) is an important relay in mediating descending pain inhibition from the periaqueductal gray (PAG). The area contains the n. raphe magnus (NRM), n. reticularis paragigantocellularis lateralis (NRGL), and n. reticularis paragigantocellularis (NRG). The contribution of these nuclei in morphine induced antinociception (tail flick reflex) from the PAG was studied using lidocaine (4%) to provide a localized neural block. Male Sprague Dawley rats were implanted with four guide cannulae - one over the PAG and three in the RVM 1 mm apart mediolaterally with the center cannula over the NRM (R-C, -2.0 mm; Paxinos and Watson, 1986). PAG morphine (6 nmol) produced an antinociceptive response that was unaffected by either a single lidocaine (0.5 µl) injection into the NRM, bilateral injections into the NRGL, or triple injections into both sites. However, the response was completely inhibited by the administration of 1.0 µl lidocaine into either the NRM or bilateraliy into the NRGL. Triple injections of lidocaine (0.5 µl) 1 mm dorsal into the NRG also completely inhibited morphine's response, while single or bilateral injections into these sites had no effect. These data suggest that a large area of the RVM, containing primarily the NRG, mediates morphine antinociception from the PAG. The all or none nature of the block suggests that a significant portion of this area contains redundant neural circuits.

575.9

EXPRESSION OF FOS IN A1 AND C1 NEURONS IN RESPONSE TO NOXIOUS HEATING OF THE FOOT IN RATS. R.W. Blair* and S.L. Jones. Depts. of Physiology and Pharmacology, Univ. Oklahoma Health Sciences Center, Oklahoma City, OK 73190.

We previously showed that noxious heating of the foot evoked Fos in the ventrolateral medulla (VLM), with a contralateral predominance. The goal of the present study was to determine whether VLM neurons expressing Fos also were catecholaminergic. Rats were anesthetized with pentobarbital, and the left hindpaw was immersed in 55-60° water for 10 sec for 30 consecutive trials at 2 min intervals. Following a 2 hr survival time, the rats were perfused, and the medulla was processed, using double-label fluorescence immunohistochemistry, for Fos- and DBH-like immunoreactivity, or for Fos- and PNMT-like immunoreactivity. As we found before, there were more Fos-like immunoreactive (Fos-LI) neurons on the side contralateral to the stimulus. Of the A1 neurons in 2 rats, 46% on the contralateral and 42% on the ipsilateral side contained Fos-LI. Of the neurons expressing Fos, 40% on the contralateral and 63% on the ipsilateral side also contained DBH-LI. Of the C1 neurons in 2 rats, 12% on the contralateral and 5% on the ipsilateral side also contained Fos-LI. Of the neurons expressing Fos, 21% on the contralateral and 32% on the ipsilateral side also contained PNMT-LI. Thus, noxious heating of the foot causes the expression of Fos in a significant proportion of A1 and C1 neurons. (Supported by HL29618 and DA07196)

575.10

RESPONSES OF NEURONS IN CAUDAL VENTROLATERAL MEDULLA TO NOXIOUS VISCERAL STIMULATION IN THE RAT. K.A. Follett*, T. Ness, and T. Brennan. Depts. of Neurosurgery and Anesthesiology, Univ. of Iowa, Iowa City, IA 52242.

The role of the lateral reticular nucleus (LRN) in visceral nociception has not been investigated systematically. We evaluated responses of neurons in the region of LRN to noxious visceral stimulation in a rat model of visceral pain.

In Halothane-anesthetized rats, a microelectrode was placed stereotaxically into LRN to record the responses of single neurons to balloon distension of the colon (CRD) at pressures of 20, 40, 60, 80, and 100 mmHg. Neuronal activity was analyzed as peristimulus-time-histograms. In some animals, pentobarbital (15 mg/kg) was given following characterization of a neuron's responses to evaluate the effect of different anesthetic agents.

Twenty-four of 29 units studied were excited by CRD, 5 were inhibited. Responses were usually sustained after CRD termination. Phenylephrine-induced hypertension did not alter neuronal activity in instances in which CRD was accompanied by increases in blood pressure. All cells had cutaneous receptive fields (70% bilateral, 60% nociceptive specific, 40% nociceptive nonspecific). In 8 of 10 neurons studied, Pentobarbital administration abolished responses to CRD and cutaneous stimulation. Responses were facilitated in the other 2.

Neurons in the region of LRN respond to noxious visceral stimulation. This area may be involved in visceral nociception and may have a role in modulation of visceral nociception.

575.11

PARABRACHIAL AREA NEURONS RESPOND TO COLORECTAL (CRD) AND ESOPHAGEAL (ED) DISTENSION. M.B. Burton*, R.J. Traub, G.F. Gebhart. University of Iowa, Iowa City, IA 52242.

Studies have shown that the parabrachial area receives noxious cutaneous as well as cardiovascular/respiratory input. The aim of this study was to characterize the response of neurons in this area to noxious and non-noxious visceral stimuli: CRD and/or ED.

Pentobarbital-anesthetized rats were artificially ventilated. Mean arterial pressure (MAP) was monitored via the femoral artery. Tungsten electrodes were used for extracellular recordings in the parabrachial area. Spontaneously active neurons were characterized as to cutaneous receptive field, and responses to CRD and ED.

CRD-sensitive units were inhibited (75%) or excited (25%). 75% of units which responded to CRD also respond to ED. The spontaneous activity of CRD-sensitive units ranged from 0.5 to 18 Hz. In these animals CRD produced a strong depressor response, while ED produced a weak pressor or depressor response. Sodium nitroprusside (SNP; 0.1μg/kg i.v.) was administered to determine whether units responded to a change in MAP. Most cells did respond to SNP, however, some exhibited a greater response to ED than to SNP. A cutaneous receptive field was found for 20% of distension sensitive units.

In conclusion, parabrachial neurons respond to noxious visceral stimuli in both an excitatory and inhibitory manner, and receive convergent cutaneous (viscerosomatic) and visceral (viscerovisceral) inputs. Some of these neurons also respond to changes in MAP.

575.12

PREPROENKEPHALIN mRNA IN THE KÖLLIKER-FUSE NUCLEUS OF THE AWAKE RAT: VISUALIZATION IN PROJECTION NEURONS AND INCREASED EXPRESSION AFTER NOXIOUS CUTANEOUS STIMULATION OF DIFFERENT BODY SITES. O. Hermanson*, H. Ericson, D. Larhammar & A. Blomqvist. Dept of Cell Biology, Univ. of Linköping, Dept of Neuropharmacol., Astra Research Centre, Södertälje, and Dept of Medical Genetics, Univ. of Uppsala, Sweden.

Recent investigations with *in situ* hybridization have shown that neurons in the pontine Kölliker-Fuse nucleus (K-F) contain preproenkephalin mRNA (ppENK) (Hermanson et al., Soc. Neurosci. Abstr. 18:832, 1992). The purpose of this study was to investigate (i) if neurons in K-F with descending projections express ppENK, and (ii) if ppENK expression in the cell bodies of K-F is affected by nociceptive stimulation. Choleragenoid was injected into the spinal cord or ventrolateral medulla of male Sprague-Dawley rats. After immunohistochemical processing for the detection of retrogradely labeled neurons, the sections were hybridized with a ^{35}S-labeled cRNA probe to ppENK. A major part of the neurons retrogradely labeled from either target were found to express ppENK. Most of the double-labeled neurons were located in the middle third of the rostrocaudal extent of the ppENK-positive population. The effect of nociceptive stimulation on the ppENK expression in the K-F region containing the neurons projecting to the spinal cord or ventrolateral medulla was investigated in awake freely-moving rats that were pinched in the nape of the neck or the base of the tail. A third group of rats received non-noxious sensory stimulation (brush). A microcomputer image analysis system was used to quantify the number of neurons labeled by *in situ* hybridization. Cell bodies displaying a grain density >30 times background level over an area of >80 μm^2 were regarded as ppENK-positive. In both groups of stimulated rats, the number of cells expressing ppENK increased with 60% compared to the control group (ANOVA; $p<0.05$). The present findings demonstrate that the expression of ppENK in K-F neurons is increased by mechanical noxious stimulation and suggest that enkephalinergic K-F neurons are involved in descending regulation of autonomic functions.

575.13

A NOVEL LONG-LATENCY RESPONSE OF LOCUS COERULEUS NEURONS MEDIATED BY PERIPHERAL C-FIBERS: POSSIBLE SOURCE OF NORADRENERGIC DESCENDING PAIN MODULATION. H. Hirata* and G. Aston-Jones. Div. Behav. Neurobiol., Dept. Mental Health Sci., Hahnemann Univ., Phila., PA. 19102

Noradrenergic locus coeruleus (LC) neurons are phasically activated by a variety of stimuli including electrical foot shock (FS). The latencies of such responses (20-100 ms) in previous studies indicate rapid conduction velocities in afferent fibers (>2 m/s); FS responses indicative of C-fiber activation have not been documented. We report here a novel long-latency FS response of LC neurons and show that it is mediated by peripheral C-fibers.

In halothane anesthetized rats, extracellular recordings were obtained from individual LC neurons during FS stimulation. Stimulation with 0.5 or 2 ms pulses elicited responses 20 to 100 ms in latency, as previously observed. A new long-latency response (200-400 ms) prominently appeared with 5 ms FS stimuli. This late response was selectively abolished 42 min after application of capsaicin directly to the exposed proximal sciatic nerve. Thus, the late response resulted from peripheral C-fiber activation. The activation of LC neurons by C-fiber-mediated pain circuits may provide noradrenergic centrifugal feedback modulation of nociceptive processing, supporting previous proposals (Jones & Gebhart, 1986). The pharmacological mechanisms responsible for the late response are under study. Supported by PHS grant NS 24698.

575.14

TRANSNEURONAL LABELLING OF SMALL DIAMETER TRIGEMINAL GANGLION NEURONS AFTER PSEUDORABIES VIRUS (PRV) INJECTION INTO THE CENTRAL NUCLEUS OF THE AMYGDALA (CE). L. Jasmin*, K. Tarczy-Hornoch, H. Wang, J.D. Levine, A.I. Basbaum. Depts. of Anatomy, Medicine, Neurosurgery and Physiology and Keck Center for Integrative Neuroscience, UCSF, CA.

Although injection of the swine herpes virus PRV-Bartha into limb muscles results in very dense labelling of spinal cord projection neurons, motoneurons and interneurons, there is no labelling in the dorsal root ganglion (DRG), possibly because the virus enters latency in DRG cells (Rotto-Percellay et al. 1992). In the present study we demonstrate that when the virus is injected into the CNS, PRV-Bartha can be immunocytochemically detected in the DRG, implying lytic infection. Our studies focused on the pathway through which neurons of the CE receive nociceptive input. In previous studies we demonstrated that after injection of PRV into the CE, transneuronally labelled neurons can be identified in the lateral parabrachial nucleus and in marginal (lamina I) neurons of the medullary and spinal dorsal horn. In this study we specifically addressed the primary afferent input to the spino/trigemino-parabrachio-amygdaloid pathway.

The rats received a stereotaxic injection into the CE of 100 nl of PRV-Bartha (8 x 10^8 pfu) mixed with 40nm colloidal gold particles. The gold particles allowed precise localization of the virus injection sites. Using immunocytochemistry with antisera directed against PRV and with the ABC-DAB technique we identified PRV-infected neural and non-neural (satellite) cells in 50μm sections of the trigeminal ganglia. Twelve percent of the ganglion neurons were PRV-immunoreactive. Of these, 64% were small (20μm), 28% medium (30μm), and 8% large (60μm) diameter cells. The predominance of labelling in small diameter neurons (some of which we have shown to be substance P-immunoreactive) is consistent with studies that demonstrated that many neurons driven by the spino-trigemino-parabrachial pathway are exclusively responsive to noxious stimuli. The labelling of larger neurons suggests, however, that WDR neurons also contribute inputs to the amygdala. Our results also suggest that the route of infection of the DRG (i.e. central vs. peripheral) critically affects whether PRV becomes lytic or latent in the infected cells. Supported by NS 14627, DE/NIDA 08973 and the MRC (Canada).

575.15

PATTERN OF IMMEDIATE EARLY GENE EXPRESSION IN RAT BRAIN FOLLOWING A NOCICEPTIVE STIMULUS.

HB Gutstein,* WE Cullinan, RC Thompson, SJ Watson, and H Akil. Dept. of Anesthesiology and MHRI, University of Michigan, Ann Arbor, MI, 48109.

Recently, both noxious and stressful stimulation have been shown to induce changes in immediate early gene (IEG) expression in relevant neuronal systems. Few studies have examined the supraspinal components of this response, however, and the majority of this work has been done in anesthetized animals. The present study was undertaken to determine supraspinal patterns of IEG activation in awake animals after noxious stimulation, and ultimately, to differentiate the effects of the nociceptive stimulus from the effects of associatied stress.

Four male Sprague-Dawley rats underwent the injection of 0.05 ml of 2.5% formalin in the right hindpaw while awake, and were sacrificed by rapid decapitation 30 minutes later. Three animals served as unhandled controls. Brains were quickly frozen, then sectioned and subsequently fixed in paraformaldehyde. *In situ* hybridization histochemistry was then performed using a cRNA probe for c-fos.

Brain areas showing c-fos induction following this stimulus included: olfactory bulb, lateral septal nucleus, bed nucleus of the stria terminalis, hypothalamic paraventricular nucleus, dorsomedial hypothalamic nucleus, medial habenula, ventral posterior thalamic nucleus, and the hippocampus. Expression of c-fos mRNA was also detected in the cerebellum, posterior parietal and temporal cortices, as well as several limbic cortical regions (cingulate, orbitofrontal, and infralimbic).

These data suggest that awake noxious stimulation induces c-fos expression in many cortical and subcortical areas. Studies are currently underway contrasting these patterns of activation with those seen following non-nociceptive stress.

Supported by Foundation for Anesthesia Education and Research and DA 02265.

575.16

THE ROLE OF NOCICEPTIVE SUPERIOR COLLICULUS NEURONS IN APPROACH BEHAVIORS. P. Redgrave[1], M. Simkins[1], J.G. McHaffie[2], C.-Q. Kao[2], S.J. Goldberg[*3] and B.E. Stein[2] Depts. of Psychol. Univ. of Sheffield, U.K., and Physiology[2] and Anatomy[3], Medical College of Virginia, USA

Nociceptive neurons are widely distributed in deep superior colliculus (Stein & Dixon, Brain Res. 158:65-73, 1978) and, along with their low threshold neighbors, are organized into topographic maps, each biased for an expanded representation of the face (McHaffie, Kao, & Stein, J. Neurophysiol. 62:510-525, 1989). Presumably, a nociceptive-activated withdrawal system in the SC evolved in concert with a low-threshold-activated approach system to adapt the rodent face for exploration. Thus, nociceptive SC neurons can protect the face from potentially damaging stimuli by simultaneously inhibiting the tendency to approach and initiating withdrawal. However using antidromic techniques in the present study it was evident that many (22/56) nociceptive SC neurons which were maximally responsive to persistent stimulation had axons which exited the SC via the crossed tecto-reticulo-spinal (TRS) tract, through which approach (i.e., orientation) responses are evoked. Subsequent behavioral experiments showed that TRS transection interfered with movements directed only toward a persistent noxious stimulus without altering its apparent perception. These results suggest that approach (and removal by biting), rather than withdrawal, may be a more adaptive way of dealing with a persistent noxious stimulus than continued attempts to withdraw. Different qualities of the stimulus (innocuous vs noxious) or the duration of a noxious stimulus (transient vs persistent) may be used differentially to determine access to SC efferent projections associated with approach and withdrawal responses. Supported by a grant from the Wellcome Trust Fund.

575.17

ELECTROPHYSIOLOGICAL AND NEUROANATOMICAL STUDIES OF THE ROLE OF THE NEOSTRIATUM AND GLOBUS PALLIDUS IN NOCICEPTION. E.H. Chudler*, K. Sugiyama and W.K. Dong. Dept. of Anesthesiology, Univ. of Washington, Seattle, WA 98195.

Neuroanatomical experiments were performed to investigate the peptides contained within neurons in the neostriatum (CPu) and globus pallidus (GP) that project to the substantia nigra. Injections of the fluorescent tracer (DiI) into the substantia nigra retrogradely labeled neurons in the CPu and GP. When the same tissue sections were processed with immunocytochemical procedures, we found that some striatal and pallidal neurons that projected to the substantia nigra contained dynorphin and substance P.

The response properties of neurons in the CPu and GP to mechanical and thermal stimuli were studied in rats anesthetized with equithesin. Neurons responsive to noxious stimuli were concentrated along the border of the CPu and GP. Neuronal discharge elicited by noxious thermal and mechanical stimulation sometimes outlasted the duration of the stimulus. The receptive fields of nociceptive neurons were large and often bilateral. The trigeminal region was included in the receptive fields of almost all neurons. Wide-dynamic-range (WDR) neurons were able to encode a greater range of stimulus intensity than either nociceptive-specific (NS) or inhibited (INH) neurons in the CPu and GP. Nociceptive neurons in the neostriatum and globus pallidus may be involved with grading motor responses to noxious stimuli (WDR neurons) or signalling the occurrence of noxious stimuli and coordinating gross movements to noxious events (NS and INH neurons).

Supported in part by NIH grant NS29459 (WKD) and Univ. of Washington Graduate School Fund Award (EHC).

PAIN MODULATION: ANATOMY AND PHYSIOLOGY V

576.1

NOCICEPTIVE AND NON-NOCICEPTIVE THALAMIC RESPONSES IN BILATERAL PREFRONTAL DECORTICATED RATS. I. OMAÑA*, R. M. SANCHEZ-MORENO, AND M. CONDES-LARA. DEPARTAMENTO DE NEUROFISIOLOGIA, DIV. INVEST. NEUROCIENCIAS. INSTITUTO MEXICANO DE PSIQUIATRIA, MEXICO 14370 D.F. MEXICO.

THE PREFRONTAL AND ADJACENT CORTICES ARE INVOLVED IN PAIN TRANSMISSION. THESE AREAS ARE THE PRINCIPAL PROJECTION SITES FROM MEDIAL AND INTRALAMINAR THALAMIC NUCLEI. IN ADDITION, FRONTO-PARIETAL AND CINGULATE CORTICAL ABLATIONS REDUCE THE AUTOTOMY EXPRESSION IN DEAFFERENTED RATS. IN THIS STUDY IT WAS ANALYZED THE EFFECTS OF BILATERAL PREFRONTAL DECORTICATION ON THALAMIC EVOKED RESPONSES TO NON NOXIOUS AND NOXIOUS STIMULI. TWENTY TWO MALE ALBINO WISTAR RATS (230-270g) UNDER PENTHOBARBITAL ANAESTHESIA (40 mg/Kg I.P.) WERE DECORTICATED BY ASPIRATION. THALAMIC BILATERAL SIMULTANEOUS EXTRACELLULAR UNIT RECORDINGS WERE OBTAINED UNDER URETHANE ANAESTHESIA (1.5 mg/Kg). EIGHTY FIVE RESPONSIVE UNITS WERE RECORDED, 14 (16%) CELLS RESPONDED TO NON NOXIOUS MECHANICAL STIMULI (BRUSHING OR LIGHT PRESSURE), 25 (30%) CELLS RESPONDED TO NOCICEPTIVE MECHANICAL (PRESSURE WITH SERRATE FORCEPS), NOCICEPTIVE THERMAL (IMMERSING TAIL IN HOT WATER AT 50 oC DURING 30 s) OR MECHANO-THERMAL NOXIOUS STIMULATION. FORTY SIX (54%) CELLS DISPLAYED POLIMODAL RESPONSES ENCODING STIMULI FROM NON-NOXIOUS TO NOXIOUS INTENSITY. 27 (32%) NOCICEPTIVE NEURONS FAILED TO RESPOND ONCE OR TWICE TO NOXIOUS STIMULATION ; 10 (12%) CELLS DISPLAYED AFTERDISCHARGES (2 OR MORE MIN.) DRIVEN BY NOXIOUS STIMULATION; 6 (7%) CELLS EXHIBITED ENHANCED RESPONSES TO NON NOXIOUS AND NOXIOUS STIMULI. IN NORMAL RAT, THE INTRALAMINAR THALAMIC RESPONSES TO NOXIOUS STIMULATION CAN BE REPRODUCED IN A ORGANIZED FASHION. THE RESULTS SHOW THAT THESE CELLS DISPLAYED DISORGANIZED RESPONSIVENESS AFTER DECORTICATION.

PARTIALLY SUPPORTED BY CONACyT IOZ 0404M-91 AND IMP 3240.

576.2

NEURONS INTRINSIC TO THE AMYGDALA MEDIATE MORPHINE ANALGESIA IN THE FORMALIN TEST. **B.H. Manning[1*] and D.J. Mayer[2]. Depts. of [1]Anatomy and [2]Anesthesiology, Medical College of Virginia, Virginia Commonwealth University, Richmond, Virginia 23298**

Morphine produces analgesia in the rat paw formalin test in part through action at sites within the brainstem and diencephalon (Manning et al, 1993; Neuroscience, in press), but the analgesic efficacy of these sites appears to depend on ascending projections to a critical site(s) in the telencephalon. To test the possibility that neurons intrinsic to the amygdala are critical mediators of this analgesia, we induced focal lesions by bilaterally microinjecting N-methyl D-aspartate (NMDA; 0.25M in phosphate buffer, 0.2 μl injection volume) into the rostro-dorsal amygdaloid complex of the rat. Post-surgical seizures were controlled with diazepam (1 mg/kg i.p.). Following one week of recovery, the analgesic effect of morphine sulphate (7 mg/kg; s.c.) was assessed using the formalin test. Control groups were as follows 1) rostro-dorsal amygdala lesion/systemic saline 2) rostro-dorsal amygdala sham lesion/systemic morphine 3) rostro-dorsal amygdala sham lesion/systemic saline 4) adjacent areas lesion/systemic morphine 5) adjacent areas lesion/systemic saline 6) adjacent areas sham lesion/systemic morphine 7) adjacent areas sham lesion/systemic saline. Formalin pain was simultaneously rated using 1) the traditional rating scale and 2) flinching of the injured paw. None of the lesions reliably affected base-line pain scores. Lesions of the rostro-dorsal amygdala, however, reliably attenuated analgesia. Control lesions in adjacent areas had no effect. The results indicate that the amygdala is critical for the production of morphine analgesia in the formalin test, thereby contributing to a growing body of evidence suggesting that opioid control of prolonged pain requires structures higher in the neuraxis than those required for control of brief, intense acute pain.

576.3

NEURONAL BURSTING IN LATERAL AND MEDIAL THALAMIC NUCLEI IN THE NAIVE RAT. M. Lis-Planells; P.C. Rinaldi*. C. Posch; Department of Neurological Surgery, University of California Irvine, CA 92717.

Thalamic neuronal bursting has been identified in patients during stereotactic procedures for relief of chronic pain. It is unknown whether these spontaneously bursting neurons are found in the thalamus of normal humans. Deafferentation animal models have also shown neuronal bursting, particularly in the dorsal horn and lateral thalamic nuclei. This study characterizes spontaneous thalamic activity in naive rats in lateral thalamic nuclei (primary somatosensory relay nuclei - VPM, VPL) and medial thalamic nuclei (MD, CM, Cl, Re). Nine rats were sedated (chloral hydrate) and extracellular recordings were obtained from lateral thalamic nuclei (LThN) and medial thalamic nuclei (MThN). Peripheral receptive fields were tested bilaterally during recordings and single units were taped for computerized interval histogram and burst analyses. A total of 43 cells were analyzed (28 in LThN, 15 in MThN). Similar proportions of bursting activity were found in both LThN and MThN (60% & 61%, respectively). MThN neurons exhibited longer bursts (more spikes per burst), but durations of successive intervals between spikes were similar in both nuclear groups. In LThN 43% of the cells were responsive to pain and/or touch. In MThN most cells had no evoked responses and only one (7%) was responsive to pain. This study indicates that lateral and medial thalamic neurons exhibit spontaneous bursting activity in naive rats. The data support the concept that LThN and MThN show different responses to somatosensory stimulation but present similar electrophysiological properties. These analyses of cellular activity can form a basis on which to evaluate neural activity from the same nuclei in deafferentation and nociceptive animal pain models. They may also lead to further determining the role of neuronal plasticity in development of chronic pain.

576.4

INHIBITORY INFLUENCE OF NUCLEUS RAPHE OBSCURUS ON THE MIDBRAIN DEFENCE AREA. F.M. Semenenko, B.M. Lumb and T.A. Lovick* (SPON: Brain Research Association). Depts. of Physiology, University of Bristol, BS8 1TD and University of Birmingham, Birmingham B15 2TT, U.K.

Experiments have been carried out to investigate the influence of nucleus raphe obscurus (NRO) on the activity of neurones in the dorsal half of the midbrain periaqueductal grey (dPAG), the midbrain defence area.

In initial electrophysiological experiments in urethane-anaesthetized rats, selective stimulation of neuronal perikarya in NRO, by microinjection of an excitatory amino acid suppressed ongoing activity of 18/21 neurones tested in the dPAG. Iontophoretic application of 5-HT or the 5-HT_{1A} agonists 8, OH-DPAT and buspirone also inhibited these cells. Both the drug-induced and neuronally-evoked inhibitions were potentiated in the presence of a 5-HT reuptake blocker.

Projections from NRO to the PAG were investigated in a second neuroanatomical study using retrograde transport of coumarin or rhodamine-labelled latex microspheres. There was no evidence for a direct projection from NRO to the dPAG. However, significant numbers of neurones in NRO were found to project to the ventrolateral sector of the PAG.

We conclude that activation of neurones in NRO produces an inhibitory serotonergic influence on the excitability of cells in the dPAG. The effect appears to be mediated indirectly, probably via activation of inhibitory interneurones in the ventrolateral PAG.

576.5

COLUMNAR ORGANIZATION IN PAG: PHYSIOLOGICAL EVIDENCE FOR INTERCOLUMNAR INTERACTIONS. S.C. Chandler*, H. Liu, A.Z. Murphy, M.T. Shipley and M.M. Behbehani. Depts of Physiology and Biophysics, Anesthesia and Anatomy and Cell Biology. U. of Cincinnati College of Medicine, Cincinnati., OH 45267-0576.

Recent studies indicate that the periaqueductal gray (PAG) is organized into distinct longitudinal input and output columns. Forebrain afferents target dorsomedial, lateral and ventrolateral columns in PAG; these columns contain neurons that project to the rostral ventral medulla (RVM). In this study we assessed physiological interactions between the dorsomedial (PAGdm) and ventrolateral (PAGvl) columns in an *in vitro* slice preparation.

Electrical stimulation (20-50 Hz) was applied to a bipolar stimulation electrode in the PAGdm. In some experiments PAGdm was chemically activated by injection of 100 nl of 5 mM D,L homocysteic acid applied from a pipette glued to the stimulation electrode.

Electrical stimulation of PAGdm produced inhibition that lasted for as long as eight minutes in 13/61 cells (21%) in PAGvl. Naloxone, applied next to the recording site, blocked this inhibition in 46% of the cells tested. Stimulation of PAGdm produced an excitatory response that lasted between 33 and 68 seconds in 9/61 (15%) of the cells in PAGvl. Chemical stimulation of PAGdm produced the same response as electrical stimulation in 7/10 cells tested. Stimulation of PAGdm caused no change in the firing rates of 39 cells (54%).

We conclude that: (1) PAGdm modulates the activities of PAGvl neurons. (2) The inhibitory action of PAGdm on cells in PAGvl is mediated in part by activation of opioid receptors. These results support the hypothesis that there are significant physiological interactions among longitudinal input-output columns in PAG.

Supported by NIH grant #20643.

576.6

DORSAL RAPHE NUCLEUS EXERTS EXCITATORY AND INHIBITORY EFFECTS ON NEURONES IN THE DORSOLATERAL PERIAQUEDUCTAL GREY MATTER. V.V. Stezhka and T.A. Lovick. (SPON: Brain Research Association). Dept. of Physiology, University of Birmingham, Birmingham B15 2TT, U.K.

The influence of the dorsal raphe nucleus (DRN) on the activity of neurones in the dorsolateral periaqueductal grey matter (PAG) has been studied in coronal slices of rat midbrain maintained in vitro in an interface chamber.

Topical application to the DRN of 15-100nl droplets of 1-10mM D,L-homocysteic acid (DLH), to selectively activate neuronal perikarya, produced suppression (n=5) or facilitation (n=4) of ongoing activity recorded extracellularly from neurones in the PAG. Two further cells responded with a biphasic response and 7 were unaffected.

In a second study, iontophoresis of DLH into the DRN (800-1300nA from 5 barrels of a micropipette) was used to produce a more localised stimulus. This form of stimulation evoked dose-dependent inhibitory and excitatory responses in the PAG. In both series of experiments, iontophoretic application of serotonin (0-30nA) directly to the neurones in the PAG always inhibited ongoing activity.

We conclude that viable excitatory and inhibitory projections from the DRN to the dorsolateral PAG are present within the midbrain slice preparation. Furthermore, serotonin may be involved in mediating the inhibitory projection.

576.7

ACUTE MORPHINE CAUSES AN INCREASE IN ENKEPHALIN RELEASE DURING MICRODIALYSIS OF THE PERIAQUEDUCTAL GRAY. F.G. Williams*, M.M. Mullett, and A.J. Beitz. Department of Pathobiology, University of Minnesota, 1988 Fitch Ave., St. Paul, MN 55108

Opiate-induced antinociception in the periaqueductal gray matter (PAG) has been postulated to involve interneurons that employ GABA as their transmitter. In addition to this intrinsic PAG circuit, it is possible that endogenous opioids may be locally regulated as was observed in other regions of the nervous system. This study examined whether morphine altered the functional, dynamic release of enkephalin in the PAG of normal rats. Microdialysis probes containing Hospal acrylonitrile dialysis membrane (1mm length) were implanted in the ventrolateral PAG and perfused at 5 µl/min. Baseline dialysate enkephalin levels, measured by radioimmunoassay 3 to 5 hours after the start of dialysis, averaged 1.20 ± 0.22 pg/12 min. Following an IP morphine injection (12 mg/kgbw), enkephalin recovery increased significantly to 2.74 ± 0.69 pg/12 min ($p<.05$, 1 way ANOVA). Following a second IP morphine injection (12 mg/kgbw), extracellular enkephalin levels in the ventrolateral PAG declined to 1.33 ± 0.41 pg/12 min. This result suggests that endogenous opiates might be capable of amplifying enkephalin release in the PAG. Should the effect originate locally within the PAG, the results are consistent with the hypothesis that GABAergic neurons may exert a reciprocal tonic inhibiton of opiate-containing neurons. Supported by NIH grants NS28016, DA06687, and DE06682.

576.8

HYPOTHALAMIC PROJECTIONS TO THE ROSTROVENTRAL LATERAL MEDULLA: ROSTROCAUDAL ORGANISATION P.M. Hudson, F.M. Semenenko and B.M. Lumb (SPON: Brain Research Association). Physiology Dept., University of Bristol, BS8 1TD, U.K.

Hypothalamic integration of autonomic control and spinal processing of nociceptive inputs may be partly mediated via a relay in the rostroventrolateral medulla (RVLM). The present study used retrograde transport techniques to investigate the organisation of projections from the hypothalamus to rostrocaudal subregions of the RVLM.

Rats were injected with 50-150nl of fluorescent latex microspheres into a region of the RVLM under sodium pentobarbitone anaesthesia (60mg/kg i.p.). Animals were allowed to recover and left for 5-7 days, reanaesthetised and perfused transcardially with 4% paraformaldehyde. Serial 50 um sections were examined under a fluorescence microscope to view injection sites and labelled cells.

Injections of tracer into either the retrofacial or juxtafacial RVLM labelled neurones throughout an area of the hypothalamus between the dorsomedial nucleus and the preoptic area. However, there were marked differences in the patterns of labelling from these two regions. Injections into the juxtafacial region gave rise to widespread labelling. In contrast, labelled neurones were more restricted following injections into the retrofacial RVLM, and formed discrete groups in the lateral hypothalamic area and in a region ventral to the ventromedial hypothalamus. We suggest that the different patterns of somato-autonomic changes that can be evoked from the hypothalamus may be mediated via relays in different regions of RVLM.

Supported by The Wellcome Trust

576.9

CATECHOLAMINERGIC INPUT TO SEROTONIN NEURONS PROJECTING TO THE SPINAL CORD IN THE RAT VENTROMEDIAL MEDULLA OBLONGATA. M. Tanaka, H. Okamura, Y. Tamada, Y. Tanaka, S. T. Inouye* and Y. Ibata. Depts. of Anesthesiology and Anatomy, Kyoto Prefectural Univ. of Medicine, Kawaramachi-Hirokoji, Kmikyo-ku, Kyoto 602, *Int. Brain Funct., Mitsubishi Kasei Insti. of Life Sci.,Tokyo, Japan.

The midline of rostral ventral medulla (RVM) is the portion in which many serotonin neurons (nucleus raphe magnus and rostral nucleus raphe pallidus) and dense catecholamine (noradrenaline and adrenaline) fibers are distriuted. These serotonin neurons are known to project to the spinal cord and to be involved in pain modulation and cardiovascular pressure control. In this study, we have investigated the connection between spinally projecting serotonin neurons and catecholaminergic fibers in the rat RVM by light and electron microscopic immunocytochemistry.

First, light microscopic immunocytochemistry using triple labeling method revealed that serotonin-immunoreative(IR) neuron (visualized by avidin-biotin peroxidase complex and diaminobenzidine) which contains retrograde tracer (cholera toxin B subunit) injected in the cervical cord (labeled by FITC) was observed to be intimately surrounded by tyrosine hydroxylase (TH)-IR fibers (labeled by Texas Red). Second, Silver-gold intensified TH-IR axon terminals were detected to make synaptic contacts with serotonin-IR neuronal perikarya and dendrites by double labeling immunoelectron microscopy.

These results suggest that spinally projecting serotonin neurons concerned in pain modulation or cardiovascular control are directly regulated by catecholaminergic neurons at the level of RVM.

576.10

EFFECTS OF PERIAQUEDUCTAL GRAY (PAG) AND VENTROLATERAL ORBITAL (VLO) CORTEX STIMULATION ON NOCICEPTIVE NEURONS IN THE ROSTRAL VENTROMEDIAL MEDULLA (RVM) OF THE RAT. W.D. Hutchison*, L. Harfa and J.O. Dostrovsky. Dept. of Physiol., Univ. of Toronto, Canada M5S 1A8.

The On- and Off-cells of the RVM have been implicated in the modulation of nociception. The antinociceptive effects of PAG stimulation is thought to be mediated via a relay in the RVM (Vanegas et al., Brain Res. 321, 1984), but its effects on On- and Off-cells have not been studied extensively. In addition, a projection of VLO to PAG has been previously reported and therefore a possible role of VLO in modulation of RVM cells was investigated.

Recordings were made from 44 neurons in RVM and surrounding regions in barbiturate-anesthetized rats. Our studies confirmed the existence of the 3 types of neurons in RVM (On, Off, Neutral) on the basis of response characteristics to peripheral innocuous and noxious thermal and mechanical stimuli. Electrical stimulation of the PAG excited 16 and increased the response to noxious stimulation of one On-cell of the 29 tested. In contrast, PAG stimulation reduced the spontaneous firing of one Off-cell and the noxious stimulus-induced inhibition of 2 other of the 12 Off-cells tested. No effects were observed on the 3 Neutral-cells tested. In 4 experiments the VLO cortex was also stimulated but no significant effects were observed on the 12 On-cells and 3 Off-cells examined. Although the PAG-induced reduction of the inhibition of 2 Off-cells is consistent with the role of PAG and Off-cells in descending inhibition of nociception, the excitation of On-cells appears contradictory. Similar paradoxical effects on On-cells have been reported previously for PAG stimulation and for vagal afferent stimulation. It appears that VLO does not play a significant role in descending modulation of RVM nociceptive neurons. (Supported by MRC of Canada)

576.11

ULTRASTRUCTURAL EVIDENCE THAT SUBSTANCE P NEURONS MAKE SYNAPTIC CONTACTS ON NORADRENERGIC NEURONS IN THE A7 CELL GROUP THAT MODULATE NOCICEPTION. H.K. Proudfit* and M. Monsen. Dept. of Pharmacology, Univ. of Illinois at Chicago, Chicago, IL 60612.

We have demonstrated that substance P (SP) neurons in the ventromedial medulla project to noradrenergic neurons in the A7 catecholamine cell group. These SP neurons appear to produce antinociception by activating A7 neurons.

These experiments determined whether SP-containing axons make synaptic contacts on noradrenergic neurons in the A7 cell group. Pre-embedding immunocytochemistry combined with light and electron microscopic analysis was used to identify tyrosine hydroxylase (TH) antisera labeled with silver-intensified gold (SIG) and SP antisera labeled by the immunoperoxidase reaction.

TH labeling was found in perikarya, dendrites, and dendritic spines in the A7 region. SP labeled by SIG was found in axons and terminals. Some SP-labeled terminals made asymmetric synaptic contacts on TH-labeled dendrites and dendritic spines. SP-labeled terminals also contacted unlabeled dendrites and unlabeled terminals contacted TH-labeled dendrites and spines.

These results support the conclusion that stimulation of SP neurons produces antinociception by direct activation of noradrenergic A7 neurons. This work was supported by USPHS grant DA03980 from the National Institute on Drug Abuse.

576.12

PROJECTIONS OF NORADRENERGIC NEURONS IN THE A5 CATECHOLAMINE CELL GROUP TO THE SPINAL CORD AND THE VENTROMEDIAL MEDULLA DETERMINED BY DOUBLE RETROGRADE TRACING COMBINED WITH IMMUNOCYTOCHEMISTRY: IMPLICATIONS FOR A ROLE IN NOCICEPTIVE MODULATION. G.C. Newsom*, J.I. Choca, and H.K. Proudfit. Dept. of Pharmacology, Univ. of Illinois at Chicago, Chicago, Il. 60612

It is well established that activation of neurons in the ventromedial medulla (VMM) produces antinociception. In addition, VMM neurons are tonically inhibited by norepinephrine, the source of which appears to be the A5 cell group. These A5 neurons facilitate nociception because bilateral electrolytic lesions of the A5 group produce antinociception.

In contrast, activation of some A5 neurons appears to reduce nociception because electrical or chemical stimulation of A5 neurons produces antinociception which is not blocked by microinjection of tetracaine in the VMM, but is antagonized by intrathecal administration of yohimbine. It is likely that this antinociception is produced by A5 neurons that project directly to the spinal cord.

This apparent dual role of A5 neurons in modulating nociception was investigated using double retrograde tracing combined with immunocytochemistry. Rats received injections of Fluorogold in the spinal cord and rhodamine-labeled microspheres in the VMM. Brain stem sections were processed for tyrosine hydroxylase (TH) immunocytochemistry. Preliminary results show that some TH-immunoreactive A5 cells project to the spinal cord, some project to the VMM, and some project to both areas. Thus, there may be separate populations of A5 neurons that serve different functions. (Supported by USPHS grant DA03980 from the National Institute on Drug Abuse)

576.13

IDENTIFICATION OF ON- AND OFF-CELLS IN THE RVM OF UNANESTHETIZED DECEREBRATED RATS. R. W. Clarke, M. M. Morgan, and M. M. Heinricher. (Spon: Brain Research Association) Dept. Physiology and Env. Sci., Univ. Nottingham, Sutton Bonington LE12 5RD, U.K., and Dept. Neurology, Univ. California, San Francisco, CA 94143-0114.

The rostral ventromedial medulla (RVM) is involved in the descending modulation of nociception. Two classes of RVM neurons which underlie this modulation have been identified in rats anesthetized with barbiturate (Fields et al., 1983) or halothane (Morgan & Heinricher, 1992). "On-cells" become active and "off-cells" inactive immediately prior to nociceptive reflexes, and appear to be involved in the facilitation and inhibition of nociception, respectively. The object of the present study was to determine the characteristics of these neurons in unanesthetized, decerebrated rats.

Male Sprague-Dawley rats were anesthetized with halothane, placed in a stereotaxic frame, and decerebrated by suction to the pre- or mid-collicular level. Radiant heat sufficient to elicit a withdrawal reflex was applied to the tail and the responses of RVM neurons recorded. Rats were tested while lightly anesthetized (0.5-0.8% halothane) and in the absence of anesthestic.

On- (n=16) and off-cells (n=9) were found in unanesthetized decerebrated rats. Ongoing cell activity increased in 53% of the on-cells and in 89% of the off-cells when the anesthetic was removed. The on-cell burst and off-cell pause preceding the tail flick reflex were seen regardless of anesthetic level. Mean tail flick latency fell from 7.0 to 6.2 s 5 min after removal of halothane.

These data show that the characteristic features of on- and off-cells are not dependent on structures rostral to the midbrain. Moreover, these neuronal classes are not an epiphenomenon of anesthesia as has been suggested by others (Oliveras et al., 1989). [Supported by DA01949, DA05608, the Wellcome Trust and Guarantors of the Journal Brain (RWC)].

576.14

AN IN VIVO INTRACELLULAR STUDY OF NOCICEPTIVE MODULATORY CELLS IN THE RAT ROSTRAL VENTROMEDIAL MEDULLA (RVM) A. Zagon*, X. Meng and H. L. Fields. Dept. Neurology, Univ. California, San Francisco, CA 94143-0114.

Extrinsic factors which influence the activity of nociceptive modulatory cells in the RVM have been extensively investigated. Very little is known however, about the intrinsic electrophysiological properties of these cells. The present work studied the iatracellular properties of physiologically identified, nociceptive modulatory cells using in vivo, anaesthetized preparations.

The spontaneous activity of 40 cells with resting potential above 50 mV (input resistance 7-33 MOhm) was analyzed. "On", "Off" and "Neutral" cells in the RVM were characterized according to their activity during the tail flick test. 4-16 action potentials were collected preceeding each tail flick test and were averaged. The averaged action potentials were measured.

21 cells were distinguished by the presence of a short duration action potential (0.6 ms$\pm$0.02ms) and a biphasic afterhyperpolarization (AHP) with an early peak at 0.6 ms$\pm$0.03 ms latency to 4.0 mV$\pm$0.6 mV and a 6.7 ms$\pm$0.4 ms latency peak to 5.3 mV$\pm$ 0.4 mV. All of these cells were nociceptive modulatory: 17 On and 4 Off.

In 19 cells,(7 On, 3 Off, 9 Neutral) the action potentials were significantly longer (1.0 ms$\pm$0.1 ms, $p<0.001$) and were followed by an AHP of slower time course and larger amplitude: 2.3 ms$\pm$0.2 ms, 8.5 mV$\pm$0.7 mV. In 13 cases the AHP was biphasic, with a second peak at 11.4 ms$\pm$1.1 ms latency to 6.2 mV$\pm$0.6 mV. It is of interest that the average duration of the neutral cell action potential (n=9) was 1.2 ms$\pm$0.2 ms, which is significantly longer than the average for all On and Off cells (0.65$\pm$0.2ms, n=31, $p<0.001$).

Our data suggest that different subpopulations of RVM neurons can be characterized by differencies in their intrinsic electrophysiological properties.

Supported by DA01949 and Bristol-Myers Squibb Co.

576.15

EVIDENCE THAT DISTINCT CATECHOLAMINE CELL GROUPS FORM THE PRIMARY SOURCE OF NORADRENERGIC PROJECTIONS TO THE VENTROMEDIAL MEDULLA IN SASCO VERSUS HARLAN STOCKS OF THE SPRAGUE-DAWLEY RAT. J.I.Choca*, M.Monsen and H.K.Proudfit, Dept. of Pharmacology, University of Illinois at Chicago, Chicago, Il. 60612

Results from this laboratory have suggested that the A5 noradrenergic (NA) cell group constitutes the primary source of the NA projections to the ventromedial medulla (VMM). Nevertheless, evidence from other laboratories contradict our data and suggest that the majority of these fibers originate in the A6 cell group. The following experiments were performed to determine whether the neuroanatomical differences which occur across breeding stocks might account for the reported discrepancies.

Two groups of Sprague-Dawley rats (either Sasco or Harlan) received microinjections of rhodamine-labeled microspheres in the VMM, and brain stem sections were processed for tyrosine hydroxylase (TH) immunocytochemistry. These brain stem sections were examined for TH-immunoreactive cells which also contained the retrogradely-transported label (double-labeled cells). The results of these studies indicate that in the Sasco animals most of the double-labeled cells occur in the A5 NA cell group (46 $\pm$ 5%); whereas, in the Harlan animals most of the double-labeled cells occurred in the A6 NA cell group (45 $\pm$ 4%). These results demonstrate that neuroanatomical differences across breeding stocks can account for the reported discrepancies, and furthermore, they suggest a distinct physiological role for these cell groups in each stock. (Supported by USPHS grant DA03980 from the National Institute on Drug Abuse).

576.16

EFFECT OF PHENYLEPHRINE-INDUCED HYPERTENSION ON ROSTRO-VENTRAL MEDULLA NEURONAL ACTIVITY AND NOCICEPTION.
JC Crews*, MA Cahall, MM Behbehani. Dept. of Anesthesia; University of Cincinnati; Cincinnati, Ohio 45267-0531.

Phenylephrine-induced hypertension has been previously reported to effect the activity of nociceptive-responsive neurons in the RVM. This study examined the effect of pain and hypertension on the cell firing rate (CFR) of RVM *off* cells in barbiturate-anesthetized rats.

Animals were prepared for extracellular recording of RVM neurons. Neurons were characterized as *off* cells based upon the response to noxious heat applied to the tail. The CFR of RVM *off* cells in 10 of 12 animals increased in response to phenylephrine-induced hypertension. In the same neurons, pain produced by a tourniquet applied to the leg and inflated to 300mmHg for 60 mins caused a decrease in CFR, despite moderate nociception-related increases in BP. Further increases in BP induced by phenylephrine infusion increased the CFR of the RVM *off* cells despite continued nociceptive stimulation. In a separate group of 10 animals, phenylephrine-induced antinociception was measured by determination of tail flick latencies. In 7 of 10 animals, there was no change in tail flick latency during phenylephrine infusion as compared to baseline. In the other 3 animals, an antinociceptive effect was noted (%MPE=100).

In conclusion this study supports previously reported data indicating that phenylephrine-induced hypertension increases the CFR of RVM *off* cells. This response is maintained in nociceptive-stimulated animals. These results suggest that the hypertensive response to pain modulates descending inhibitory pain pathways. Interanimal variability in response to phenylephrine in this study may be related to barbiturate anesthesia. Supported by the Anesthesiology Young Investigator Award from the Foundation of Anesthesia Education and Research and the Burroughs Wellcome Fund.

PAIN MODULATION: PHARMACOLOGY IV

577.1

DEVELOPMENT OF A POLYMER-ENCAPSULATED CELLULAR IMPLANT FOR PAIN MODULATION. S.R. Winn*[1], S.D. Sherman[1], S. A. Morrison[1], J. Harvey[1], A. Lee[1], J. Sagen[2], J.P. Hammang[1], and E.E. Baetge[1]. [1]CytoTherapeutics, Inc. Providence, RI 02906; [2]Department of Anatomy & Cell Biology, University of Illinois at Chicago, Chicago, IL 60612

Intrathecal implantation of polymer-encapsulated bovine adrenal chromaffin (BAC) cells has been shown to be an effective method for delivering analgesic compounds to the rodent CNS (Sagen et al., *J. Neurosci.* 13(6): 2415, 1993). The immunologic isolatory capability of the encapsulating membrane allows cross-species implantation without immune suppression. Also, recently developed device designs allow implant retrieval if necessary.

The present studies evaluated enkephalin and catecholamine release from encapsulated neonatal-sourced BAC cells maintained *in vitro*. BAC cell-loaded capsules maintained in various serum-free media were repeatedly characterized for catecholamine release under basal, 20 µM nicotine-evoked, and 56 mM potassium-evoked conditions, with concurrent enkephalins measured. Results will be presented discussing the impact of serum-free conditions, matrix configurations, and membrane characteristics on the survival and function of BAC cell-loaded capsules. BAC cells thrive following encapsulation within appropriate 3 dimensional matrices and remain functional following retrieval from various implant sites in the CNS of xenogeneic hosts. In addition, studies are underway to evaluate appropriate cell lines and expression vectors for the production of stable cell lines capable of releasing catecholamines and enkephalin peptides. Development of a stable cell line releasing analgesic compounds would circumvent many issues related to primary tissues in transplantation treatment modalities.

577.2

Opioid peptide gene delivery to neurally-derived cells using herpes virus vectors. G. Davar*[1], W. Bebrin[3], R. Day[4], A. Dupuy[4], D.M. Coen[3], and X.O. Breakefield[1,2]. [1]Neurosci. Center, Mass. Gen. Hosp., [2]Neurosci. Prog., and [3]Dept. of Biol. Chem. and Molec. Pharm., Harv. Med. School, Boston, MA, USA; and [4]Lab. of Biochem. Neuroendocrin., Clin. Res. Inst. of Montreal, Montreal, Quebec, Canada.

Thymidine kinase (TK) negative herpes simplex virus (HSV) vectors were constructed which were designed to express the opioid peptide precursor prodynorphin. The full length coding sequence for rat prodynorphin cDNA from pFLRD3 (gift of Dr. J. Douglass, Vollum Inst.) was placed downstream of the Moloney murine leukemia virus long terminal repeat promoter into the HSV *tk* gene. Vero cells were cotransfected with this plasmid and infectious strain KOS HSV DNA. Virus progeny were selected with acyclovir. Two isolates were shown by Southern blot analysis to contain the expected prodynorphin gene insertion. These recombinant viruses were named *tk*LTRdynA and B.

The pituitary tumor cell line, AtT-20, was infected with either *tk*LTRdynB or a similarly constructed lacZ-containing HSV vector for 8-24hr prior to harvesting cells. Prodynorphin expression was determined by radioimmunoassay for the C-terminal fragment of prodynorphin or dynorphin A 1-17. Preliminary results are consistent with the expression of prodynorphin in the range of .12-.68 nanomoles per *tk*LTRdynB-infected cell. Experiments are underway to examine for the expression of prodynorphin in mouse trigeminal ganglion neurons infected with *tk*LTRdynB by corneal inoculation.

These data are evidence that expression of prodynorphin can be achieved in culture using a HSV vector. This vector may also provide a method for delivering prodynorphin to sensory neurons in vivo.

577.3

ROLE OF OPIATE RECEPTOR SUBTYPES IN MEDIATING ANTINOCICEPTION BY ADRENAL MEDULLARY TRANSPLANTS IN THE RAT SPINAL SUBARACHNOID SPACE. H. Wang* and J. Sagen. Dept. of Anatomy & Cell Biology, University of Illinois at Chicago, Chicago, IL 60612.

Our previous studies have shown that adrenal medullary tissue is a candidate for grafting in the spinal cord subarachnoid space in order to reduce pain. Results from our behavioral, biochemical, and morphologic studies have indicated that, in addition to catecholamines, opioid peptides released by chromaffin cells in the adrenal medulla play important roles in reducing pain sensitivity. Mu, delta and kappa opiate receptor subtypes have been implicated in the production of analgesia by opioids at the spinal level. In order to assess the role of specific opiate receptor subtypes in the antinociception produced by adrenal medullary transplants, rats were implanted with either adrenal medullary tissue or equal volumes of control striated muscle tissue into the subarachnoid space at the lumber enlargement. Pain sensitivity was assessed in these animals by use of the tail flick, paw pinch, and hot plate tests. Opiate antagonists were injected intrathecally, including the non-selective antagonist naloxone, naltrindole for delta receptor antagonism, nor-binaltorphine (norBNI) for kappa receptor antagonism, and ß-funaltrexamine (ß-FNA) for mu receptor antagonism. Dose-related reductions in antinociceptive potency were assessed before and following stimulation with nicotine. Results indicated that both the mu and delta antagonists significantly attenuated antinociception in adrenal medullary transplanted animals in a dose-related fashion, while the kappa antagonist produced only weak effects. However the analgesia could not be completely blocked by any of the selective antagonists, even at high doses, in contrast to the complete blockade by non-selective antagonist naloxone. Only a combination of mu, delta and kappa opiate receptor antagonists could completely block antinociceptive effects of adrenal medullary transplants. These findings suggest that all three spinal cord opiate receptor subtypes, particularly mu and delta, are involved in antinociception by adrenal medullary transplants. This transplanted chromaffin cells may release a combination of opioid peptides which interact with different host spinal opioid receptors to produce their analgesic effects (Supported by NS25054).

577.4

INTRACEREBROVENTRICULAR (ICV) INJECTION OF MORPHINE (MOR) MODULATES ACTIVITIES OF DORSAL HORN NEURONS (DHN) IN THE SPINAL CORD IN THE CAT. U.T. Oh*, T.S. Moon, T.K. Ha and K.H. Ko. College of Pharmacy, Seoul National University, Seoul 151-742, Korea

ICV infusion of MOR is used as a pain treatment modality in certain cancer patients. The analgesia resulting from ICV MOR was suggested to be mediated by supraspinal inhibitory control on nociceptive afferent process in the spinal cord. Yet, evidence that ICV MOR inhibits activity of DHNs in the spinal cord is controversial. Thus, in the present experiment, effects of ICV MOR on the neural activity of DHNs were re-evaluated.

Thirty-eight cats were anesthetized with alpha-chloralose (60 mg/kg) and paralyzed with pancuronium bromide. Recordings were made from 58 DHNs that include 11 projection neurons in L4 to S1 and C2 spinal segments. Somatic response of DHNs were examined in 56 cells: 34, 13, 5 and 1 cells were WDR, HT, LT and DEEP cells, respectively. To test effect of ICV MOR on activity of DHNs, MOR (50 - 200 ug) of 50 - 100 ul in volume was slowly infused in the cerebral aqueduct or the 3rd ventricle. ICV MOR inhibited spontaneous activity of 11 (25%) of 44 cells studied and excited 6 (13%) DHNs. Twenty-seven (61%) DHNs were not affected by the ICV MOR injection. The inhibition was maximal 400 sec after MOR injection, and average cell activity was reduced to 41 +- 8.7% of control during this time. Heat stimuli of 44, 46, 48 and 50 °C were applied to the skin to test effects of ICV MOR on the heat response of DHN. Among 22 cells tested, heat responses of 9 (41%) cells were excited while 6 (27%) were inhibited by ICV MOR. Heat responses of seven (32%) DHNs was not affected by the MOR injection. Naloxone (NAL) (1.6mg/kg, iv) reversed the effects of MOR in 5 out of 11 cases, exaggerated them in 5 cases, and did not affect in 1 case.

In summary, ICV MOR inhibited and excited spontaneous as well as noxious heat response of DHNs, and the MOR response in part but not always was reversed by NAL. These data suggest that inhibition of DHNs by ICV MOR may implicate neural substrate of analgesia produced by ICV MOR. Supported by RCNDD grant of Korea.

577.5

ENDOGENOUS OPIOID MODULATION OF C-FOS EXPRESSION IN THE RAT SPINAL CORD FOLLOWING NOCICEPTIVE AFFERENT ACTIVATION. C. Cheng*[1], V.L. Erickson, R. Stewart, L.A. Birder, M. Yoshiyama, J.R. Roppolo & W.C. de Groat Univ. of Pittsburgh, Pgh., PA 15261 & [1]Tri-Service General Hospital, Taipei, Taiwan.

A variety of behavioral and neurophysiological studies have suggested that endogenous opioid peptides can modulate nociception by their action within the central nervous system. This study examined the effect of naloxone (1mg/kg), an opioid antagonist, on the distribution of C-FOS positive neurons in the spinal cord of urethane (1.2gm/kg i.p.) anesthetized Wistar rats. Somatic nociceptive afferent activation of the L_4 spinal cord was produced by 0.2cc injection of 10% formalin into the hindlimb foot pad. Fifteen minutes prior to foot pad injection two groups of animals were given either naloxone (1mg/kg i.v.) or an equal volume of saline i.v. Two hours following foot pad injection the rats were perfused and spinal cord removed. Tissue was then processed for C-FOS (Cambridge antibody) and neurons with C-FOS positive nuclei (majority ipsilateral to injected foot pad) counted. Naloxone pretreatment produced a 48% increase (from control) in total C-FOS positive neurons in L_4 spinal cord. The medial dorsal horn, lateral dorsal horn and dorsal commissure all showed increases from control levels. The contralateral side of L_4 showed no increase in C-FOS. This study suggests that endogenous opioid mechanisms are involved in the inhibition of nociceptive neurons at the level of the spinal cord.

577.6

MORPHINE DOES NOT BLOCK NOXIOUS STIMULUS-EVOKED INCREASES OF FOS-LIKE IMMUNOREACTIVITY (FLI) IN LAMINAE I AND IIo TRIGEMINO-PARABRACHIAL NEURONS. H. Wang, L. Jasmin, K. Tarczy-Hornoch, J.D. Levine, H.L. Fields*, A.I. Basbaum. Depts. Anatomy, Medicine, Neurosurgery, Physiology, Neurology and Keck Center for Integrative Neuroscience, UCSF, CA.

In a previous study we demonstrated that unilateral molar tooth extraction, a moderately noxious stimulus, evokes a bilateral increase of FLI in neurons of the subnucleus caudalis (SubC) of the medullary trigeminal complex. This increase is found throughout the SubC but is concentrated in laminae I and IIo, which is the projection zone of small diameter primary afferents from the operated tooth. High doses of systemic morphine produced behavioral analgesia and largely abolished the increase in c-fos gene expression in laminae V-VIII of SubC. In contrast, morphine did not prevent fos expression in a large number of neurons in laminae I and IIo. The present study assessed whether the residual FLI is found in SubC nociresponsive relay cells, namely laminae I and IIo parabrachial projection neurons. The latter were retrogradely labelled after Fluoro-Gold (FG) injection into the right parabrachial nucleus. One week later, the rats were administered s.c. morphine (10 mg/kg; n=6) or physiological saline (n=4), 20 min before extraction of the two anterior maxillary molars. The surgery was performed under anesthesia with a mixture of 2% halothane and 50/50 O_2 and N_2O. After the animals awoke (5 min), their behavior was monitored for one hour, at which time they were perfused with 4% paraformaldehyde. A double immunocytochemistry protocol permitted visualization of immunoreactive Fos and FG, as black and brown reaction products, respectively. We found that morphine significantly ($p < 0.05$) reduced the total number of cells expressing Fos in laminae I + IIo ipsilateral to the tooth extraction. In contrast, there was no significant change in the number of double-labelled neurons, i.e., Fos-immunoreactive projection neurons ($p > 0.05$).

These results suggest that despite morphine analgesia, nociceptive messages are still relayed through SubC to higher brain centers, where they may trigger descending controls and/or influence the response to subsequent nociceptive inputs.
Supported by NS 14627, DE/NIDA 08973 and the MRC (Canada).

577.7

MORPHINE BLOCKS THE FORMALIN-INDUCED INCREASE IN DORSAL HORN NK-1 RECEPTOR mRNA EXPRESSION. K. E. McCarson* and J. E. Krause. Dept of Anatomy & Neurobiology, Washington University School of Medicine, St. Louis, MO 63110.

The tachykinin Substance P (SP) contained in small diameter primary afferent neurons and NK-1 receptors (NK-1R) located in the dorsal horn of the spinal cord have been implicated in the transmission of nociceptive information. DRG SP-encoding mRNA levels are increased by nociception, and we have shown that adjuvant or formalin-induced nociception increases NK-1R mRNA levels in the dorsal horn. Opiate agonists block pain-related behaviors and inhibit SP release from primary afferent neurons, and opiate antagonists potentiate pain-related behaviors during the late phase of the formalin test. This study determined the effects of opiate receptor ligands on the regulation of NK-1R mRNA levels in the lumbar dorsal horn during formalin-induced nociception. Rats were given a subcutaneous pretreatment of either Morphine (MS) or Naltrexone (NX) 20min before injection of formalin into the right hind paw. Saline pretreated animals receiving a sham injection served as controls. This resulted in four treatment groups; saline-sham, saline-formalin, MS-formalin, and NX-formalin. Pain-related behaviors were quantitated by counting hindlimb flinches 30-40 min after hind paw treatment. Spinal cord tissues were removed 6hr after hind paw treatment and total RNA was isolated from the dorsal quarters of the lumbar enlargements. NK-1R mRNA levels were quantitated using solution hybridization-nuclease protection assays. Formalin injection significantly increased both the number of flinches and NK-1R mRNA levels compared to controls. Pretreatment with MS blocked the formalin-induced increases in nociceptive behavior and NK-1R mRNA levels. NX pretreatment potentiated the number of flinches after formalin compared to saline pretreated controls, but NK-1R mRNA levels in these subjects were similar to those in saline-formalin subjects. The results of this study indicate that the analgesia produced by MS during the formalin test can block the formalin-induced upregulation of dorsal horn NK-1R mRNA. These and our previous studies demonstrate the plasticity of dorsal horn NK-1R mRNA expression, and the molecular mechanisms of this regulation are currently under examination.

577.8

EFFICACY AND AFFINITY OF MORPHINE AND ALFENTANIL IN THE RHESUS MONKEY WARM-WATER TAIL WITHDRAWAL. G.Zernig, E.R. Butelman, J. Lewis and J.H. Woods*. Dept. Pharmacol., U. Michigan, Ann Arbor, MI 48109.

The protoypical μ opioid agonists morphine and alfentanil were tested in a warm-water tail withdrawal antinociception assay at 50°C and 55°C. At 50°C, both agonists produced maximum antinociception (i.e. tail withdrawal latencies reached a 20 s cutoff). At 55°C, alfentanil was fully effective whereas morphine gave only 30% of the maximum response, indicating that morphine's effectiveness in this assay was lower than that of alfentanil. Dose response curves for either agonist were obtained at different times after administration of the irreversible μ opioid antagonist clocinnamox (0.1mg/kg s.c.). Analysis of these data according to Furchgott as modified by Black and coworkers showed that clocinnamox acutely decreased available μ opioid receptors by ≥ 88%. As expected, morphine's efficacy, e, was lower than that of alfentanil at both temperatures tested (means ± SEM): 50°C, 12±1 (n=9) vs 32±1 (n=8); 55°C, 3±1 (9) vs 8±1 (6); $p<0.0001$. The in vivo dissociation constant, K_A, was higher for morphine than for alfentanil. The respective log(mg/kg) values were: 50°C, 1.60±0.09 vs. -0.19±0.12 (40 vs. 0.7mg/kg); 55°C, 1.41±0.22 vs. 0.08±0.15 (26 vs. 1.2mg/kg; $p<0.0001$).

Supported by Austrian Science Foundation grant J0697-MED and USPHS grant DA 00254.

577.9

DELTORPHIN II AND DPDPE-INDUCED ANTINOCICEPTION IN INBRED MICE. G.I Elmer*,J.O. Pieper and S.R. Goldberg NIDA/Addiction Research Center, Behav. Pharm. & Genetics, Baltimore, MD, 21224.

Delta opioid receptor agonists are capable of modulating μ-agonist induced analgesia as well as producing analgesia when given alone. Recently, delta opioid subtypes have been proposed based upon differential antagonism and no cross tolerance between the delta agonists, DPDPE and deltorphin II. The purpose of this study was to investigate the degree of genetic variation in delta agonist induced analgesia and the degree of genetic covariation between the two proposed delta subtype selective agonists. Full dose-response curves for DPDPE and deltorphin II were determined in AKR/J, DBA/2J, BALB/cByJ, CXBH/ByJ and C57BL/6J mice. Each drug (0, 0.1, 0.3, 1.0, 3.0, 10.0 or 30.0 nmole) was given i.c.v. 10 min prior to antinociceptive testing on the hot-plate (55°C). Neither DPDPE nor deltorphin produced a fully efficacious analgesic effect in C57BL/6J mice (i.e. < 40% maximal effect). In the strains that did show an analgesic effect, the DPDPE ED_{50} varied by greater than 10-fold between the least and most sensitive strains. Conversely, genotype did not significantly affect deltorphin II-induced analgesia in these inbred strains. The ED_{50} for deltorphin II was approximately 1.52 nmole in these strains. The correlation between sensitivity to DPDPE- and deltorphin II-induced analgesia was not significant, r = .08. These results suggest a significant role of genotype in delta-agonist induced analgesia and support a pharmacological and genetic separation between the analgesic effects of DPDPE and deltorphin II.

577.10

SPINAL DELTA- BUT NOT MU-OPIOID RECEPTORS ARE INVOLVED IN INTRACEREBROVENTRICULAR ß-ENDORPHIN-INDUCED ANTINOCICEPTION IN MICE. [1]H.W. Suh*, [1]D. K. Song, [1]Y. H. Kim, and [2]Leon F. Tseng. [1]Dept. Pharmacol. Coll. Med., Hallym Univ., Chunchon, Republic of Korea, and [2]Dept. of Pharmacol. & Toxicol., Med. Coll. of Wisconsin, Milwaukee, WI, 53226, U. S. A.

The studies were designed to determine if delta- or mu-opioid receptors in the spinal cord are involved in intracerebroventricular (i.c.v.) administered ß-endorphin-induced antinociception. The antinociception was assessed by the tail-flick test in male ICR mice. The experimental approach was to use ß-Chloroaltrexamine (ß-CNA) a nonselective opioid receptor blocking agent which binds irreversibly both mu- and delta-opioid receptors, and a selective delta-opioid receptor agonist, DPDPE, to protect the bindings of delta-opioid receptors and a selective mu-opioid receptor agonist, DAMGO, to protect the bindings of mu-opioid receptors by ß-CNA. A combination of ß-CNA (1 μg) and DPDPE (10 μg) and ß-CNA and DAMGO (0.5 μg) given intrathecally (i.t.) for 24 hrs blocks the antinociception induced by i.t. administered DAMGO and DPDPE, respectively, while the effects of i.t. DPDPE and DAMGO, respectively, remained intact. Intrathecal pretreatment with ß-CNA or ß-CNA coadministered with DAMGO attenuated the inhibition of the tail-flick response induced by i.c.v. administered ß-endorphin. However, i.t. pretreatment with ß-CNA coadministered with DPDPE did not affect the inhibition of the tail-flick response induced by i.c.v. administered ß-endorphin. Intrathecal injection of ß-CNA alone did not affect the inhibition of the tail-flick response induced by i.c.v. administered morphine. The results indicate that delta- but not mu-opioid receptors in the spinal cord are involved in i.c.v. administered ß-endorphin-induced antinociception. The antinociception induced by i.c.v. morphine is not mediated by any type of opioid receptors.

577.11

THE SIGMA RECEPTOR LIGAND 1,3, di-*o*-tolylguanidine (DTG) DIFFERENTIALLY AFFECTS ACUTE AND TONIC FORMALIN PAIN.
B. Kest*, J. Mogil, W. Sternberg, R. Pechnick, and J.C. Liebeskind, Dept. of Psychology, University of California, Los Angeles, CA 90024.

We recently observed that the selective sigma ligand DTG produces antinociception on the tail-withdrawal test in mice. The tail-withdrawal test measures threshold for brief, phasic noxious heat. Since tests of nociception differ in stimulus quality, duration, and in their central substrates, results with DTG from the tail-withdrawal test may not be indicative of the role of sigma receptors in prolonged chemogenic pain. Therefore, male Swiss-Webster mice received DTG (10 mg/kg, IP) 15 minutes prior to administration of 20 µl of 5% formalin into the plantar surface of one hindpaw. Pain-related behavior (time spent licking the injected paw) was then continuously rated 0-10 min (acute phase) and 30-50 min (tonic phase). DTG produced significant antinociception in the acute phase, but increased pain scores relative to saline controls in the tonic phase. In a seperate group, DTG administered 45 min prior to formalin had no effect on acute pain. Thus, the longer latency between DTG administration and the onset of the tonic phase relative to the acute phase does not mediate the opposite effects produced by DTG on the two phases. The antagonism of DTG antinociception and hyperalgesia by both rimcazole (25 mg/kg, IP) and haloperidol (0.05 mg/kg, IP) administered prior to DTG supports a sigma receptor site of action in DTG's effects. Neither rimcazole nor haloperidol alone had any effect on the acute phase, but haloperidol alone decreased pain behavior in the tonic phase. These data suggest a modulatory role for sigma receptors in acute and tonic pain. Supported by an Unrestricted Pain Research Grant from the Bristol-Myers Squibb Company, NIH grant NS 07628 and NIMH Post-Doctoral Research Fellowship (BK) 5T32MH17140.

577.12

Analgesic effects of dynorphin A mediated by κ_1 and κ_3 receptors.
Dennis Paul* and Candice Jones, Dept. of Pharmacology, LSU Alcohol and Drug Abuse Center, and LSU Neuroscience Center, LSU Medical Center, New Orleans, LA 70112.

The dynorphin family of opioid peptides have been proposed as endogenous ligands for κ receptors. Using the mouse tail-flick assay, we assessed the possibility that dynorphin-A(1-13) (DYN-A) is an agonist at both the κ_1 and κ_3 receptor subtypes. Both i.c.v. and i.t. DYN-A produced analgesia dose-dependently. The non-selective kappa antagonist, quadazocine, was equally potent blocking i.c.v. and i.t. DYN-A analgesia (ED_{50}s=2.6 ng and 3.9 ng). In contrast, the κ_1 selective antagonist, nor-binaltorphimine, was 55-fold more potent as an antagonist of spinal (27 ng, i.t.) than supraspinal (1.5 µg, i.c.v.) DYN-A analgesia. This is consistent with a spinal κ_1 analgesic mechanism and a supraspinal κ_3 mechanism. ß-funaltrexamine did not alter i.t. or i.c.v. DYN-A analgesia, indicating that the analgesia was not μ receptor-mediated. In cross-tolerance studies, the κ_1 agonist, U-50,488 (6 mg/kg, s.c.), was cross-tolerant with spinal, but not supraspinal DYN-A analgesia. Conversely, the κ_3 agonist, naloxone benzoylhydrazone (70 mg/kg, s.c.) was cross-tolerant with supraspinal, but not spinal DYN-A analgesia. Together, these results suggest that DYN-A is an agonist at both κ_1 and κ_3 opioid receptors.

577.13

KAPPA-OPIOID RECEPTOR STIMULATION INHIBITS SUBSTANCE P (SP) RELEASE IN THE DORSAL HORN BOTH TONICALLY AND DURING A NOXIOUS THERMAL STIMULUS.
V. Zachariou and B.D. Goldstein*, Dept. of Pharmacol & Toxicol, Medical College of Georgia, Augusta, GA 30912.

The undecapeptide SP is found in high concentrations in the dorsal horn of the spinal cord, especially in laminae I and II, where the small lightly myelinated or unmyelinated Aδ- and C-fibers terminate. Activation of these fibers by noxious stimuli induces an increase in the release of immunoreactive SP in the dorsal horn, suggesting this peptide's role in the modulation of nociception. This study was undertaken to determine whether the κ-opioid peptide dynorphin (1-8) modulates the release of SP during the application of a nociceptive stimulus.

A push-pull canula was inserted into the dorsal horn of the lumbar enlargement in decerebrate/spinal transected rats. The spinal cord was perfused with artificial CSF, and SP levels were measured by RIA before and after the application of the nociceptive stimulus. All the drugs were applied through the perfusion apparatus.

Dynorphin (1µM) decreased the basal release of SP and inhibited the SP increase following a noxious thermal stimulus. The effect of dynorphin was reversed by the κ-antagonist, nor-binaltorphimine (Nor-BNI).

These data show that dynorphin acts at the kappa receptor to inhibit both basal release and nociception-induced release of SP from the primary afferent neuron in the dorsal horn.

577.14

EMD 61 753 IS A UNIQUE REPRESENTATIVE OF THE DIARYL-ACETAMIDES, A NOVEL CLASS OF K-OPIATE AGONISTS.
R. Gottschlich*, K. A. Ackermann, A. Barber, G.D. Bartoszyk, H.E. Greiner, M. Stohrer., Dept. of Preclinical Pharmaceutical Research, E. Merck, 6100 Darmstadt, Germany

All the known k-opiate agonists (e.g. U 50488, PD 117302, EMD 60 400) possess the structural feature of N-disubstituted *mon*oarylacetamides. In the frame of this study a series of novel substituted *di*arylacetamides was synthesized and evaluated biologically. k, µ and δ opioid binding was determined in the presence of [^{3}H] U 69593, [^{3}H] PL 017 and [^{3}H][D-Pen^{2,5}]enkephalin, respectively, with membranes from guinea-pig cerebellum (k) and rat cerebrum (µ and δ). The formalin test was carried out on male NMRI mice as described by Hunskaar and Hole, 1987.

EMD 61 753 (N-Methyl-N-[(1S)-1-phenyl-2-((3S)-3-hydroxypyrrolidin-1-yl) -ethyl] 2,2-diphenylacetamide) showed high affinity for k-opiate receptors wheras the affinities for µ and δ receptors were at least 100 x weaker. In contrast to the *mon*oarylacetamides, substitution of the aromatic rings in the *di*arylacetamides as well as other structural variations of the molecule did not improve activity. EMD 61 753 - unlike the centrally acting k-opiate ICI 197067 - showed more pronounced antinociceptive activity in the second ("inflammatory") phase of the formalin test (ID_{50} = 0.24 mg/kg s.c. and 3.8 mg/kg p.o.)

Thus, EMD 61 753 is a unique representative of a structurally novel class of k-opiate agonists. The data from the formalin test indicate that EMD 61 753 is biologically active after s.c. and p.o. application. Despite its lipophilic character EMD 61 753 seems to have limited access to the brain.

577.15

IN VIVO EFFECTS OF THE PERIPHERALLY-SELECTIVE, ORALLY-ACTIVE, κ-OPIOID RECEPTOR AGONIST EMD 61 753.
A. Barber, G.D. Bartoszyk*, R. Gottschlich, F. Mauler and C.A. Seyfried, Preclinical Pharmaceutical Research, E. Merck, 6100 Darmstadt, Germany.

EMD 61 753 (N-methyl-N-[(1S)-1-phenyl-2-((3S)-3-hydroxypyrrolidin-1-yl)-ethyl]-2,2-diphenylacetamide) is a novel κ agonist designed to have limited access to the CNS. It has potent, naloxone-reversible antinociceptive effects in tests of inflammatory pain (ID_{50} values in mouse formalin (2nd phase) and rat hyperalgesic pressure pain tests were 0.24 and 0.08 mg/kg s.c., respectively), and also inhibited neurogenic plasma extravasation produced by antidromic electrical stimulation of the rat saphenous nerve. EMD 61 753 is less effective in the rat writhing test (ID_{50} 6.3 mg/kg s.c.) and it reversed haloperidol-induced L-DOPA accumulation in the rat N. accumbens only at s.c. doses of and above 30 mg/kg. ID_{50} values after p.o. application in the formalin (2nd phase), hyperalgesic pressure pain and writhing tests were 3.8, 3.1 and > 200 mg/kg, respectively. The action of EMD 61 753 in the pressure test was completely antagonised by injection of 100 µg of the κ antagonist nor-BNI into the inflamed area, a dose of nor-BNI which produced no systemic inhibition of κ antinociception. Thus, EMD 61 753 may be able to produce peripheral inhibition of inflammatory pain at doses which do not elicit centrally-mediated antinociception and side effects (aversion, sedation, etc.).

577.16

PERIPHERAL OPIOID MECHANISMS MEDIATING ANALGESIA AND INFLAMMATION.
Y. Hong* and F.V. Abbott, Depts. of Psychiatry & Psychology, McGill Univ., Montreal, Quebec H3A 1A1.

The present study examined the effects of local injection of opioids on the different components of both pain and inflammation induced by injection of formalin. Opioid agonists (40 µl) were injected SC into the plantar surface of one rear paw 5 min prior to injection of 50 µl 1% formalin into the same site. The duration of pain behaviour were scored during 0-10 min (early phase) or 20-50 min (late phase). Extravasation was measured 10 or 50 min after formalin in separate rats by absorbance spectrophotometry following IV injection of Evans.

DAMGO (μ agonist; 1 µg) reduced behavioral pain only in the later phase, although extravasation in both phases was reduced. U50,488H (k agonist; 20 µg) decreased both components of the pain response, but extravasation was enhanced in the early phase and decreased in the late phase. These effects were not mimicked by systemic injection of the agonists, and were blocked by pretreatment with SC naloxone methiodide. DPDPE (δ agonist; up to 80 µg) did not produce any significant effects. The data indicate that peripheral opioid receptors modulate the pain and the inflammatory responses produced by formalin injection, and that the analgesic and anti-inflammatory effects are dissociable. The data also confirm that the early and later phases of formalin pain and inflammation reflect different physiological processes. (Supported by MRC)

577.17

OPIOID AGONISTS ON THE PAW FLICK TEST: MEASURES OF LATENCY AND TISSUE TEMPERATURE. P.E. Stewart and D.L. Hammond*, University of Chicago, Chicago, IL 60637

We recently observed that intrathecally-administered opioid agonists increased response latency in the uninflamed paw flick test to a lesser extent than in the tail flick test, even though baseline latencies were the same for both tests. This prompted an investigation of the relationships among stimulus intensity, response latency, tissue temperature, and the apparent efficacy of opioids. Sprague -Dawley rats were implanted with an intrathecal catheter. One week later, response latency was determined to one of four different heating rates applied to the plantar surface of a hindpaw. Response latency was inversely related to heating rate. Response latencies were then redetermined after i.t. injection of 0.3 μg DAMGO, a μ opioid agonist. The largest increase in response latency (16 sec) was obtained at the lowest heating rate, while the smallest increase in response latency (1.7 sec) was obtained at the highest heating rate. These data suggest that the efficacy of DAMGO is a function of stimulus intensity. However, measures of tissue temperature indicate that the withdrawal response occurs at the same tissue temperature (53 °C) for the three lowest heating rates and at 60 °C for the highest rate. Thus, it would appear that, in the paw flick test, the efficacy of DAMGO is not a function of stimulus intensity and measures of response latency may be misleading. Supported by PHS Grant DA 06736.

577.18

THE ANTINOCICEPTIVE TAIL-FLICK RESPONSE IS IMPERVIOUS TO CHANGES IN TAIL-SKIN TEMPERATURE. A.H. Lichtman*, F.L. Smith and B.R. Martin. Department of Pharmacology and Toxicology, Medical College of Virginia-Virginia Commonwealth University, Richmond, VA 23298.

There is recent concern that a drug induced elevation in tail-flick latency could be the result of a change in tail-skin temperature rather than an attenuation in pain perception. Therefore, the purpose of this study was to test whether tail-skin temperature has a relevant impact on the tail-flick response by evaluating the antinociceptive potency of morphine or Δ^9-THC in mice maintained at ambient temperature (23 ° C) or placed in heated cages (38 ° C). Although neither drug had an impact on tail-skin temperature, the tails of the subjects placed in the heated cages were 2 to 4 ° C warmer than those kept at ambient temperature. The ED50 values (with 95% confidence limits) of Δ^9-THC were 2.6 (0.8 to 8.7) mg/kg for subjects kept at ambient temeprature and 2.7 (2.0 to 3.8) mg/kg for mice placed in the heated cages. On the other hand, morphine was actually more potent in subjects kept at ambient temperature, 3.2 (1.2 to 3.1) mg/kg, than in those placed in the heated cages, 4.9 (3.6 to 6.7) mg/kg. If tail-skin temperature played a critical role in the tail-flick test, placement into the heated cages would have been expected to attenuate the antinociceptive potencies of each drug. These findings indicate that tail-skin temperature has a negligible influence on the antinociceptive tail-flick response. This research was supported by NIDA grants DA-03672, DA-05421, and DA-01647.

577.19

Characterization of Basal and Morphine-Induced Histamine Release in the Rat Periaqueductal Grey by In Vivo Microdialysis. K.E. Barke* and L.B. Hough, Dept. Pharmacology and Toxicology, Albany Medical College, Albany, NY 12184

The neurotransmitter histamine is thought to play a role in an endogenous system for pain relief. We have shown previously that antinociceptive doses of systemic morphine increase extracellular histamine levels in the periaqueductal grey (PAG). However, the cellular origin of basal and morphine-induced histamine release in the PAG was unknown. The neuronal origin of extracellular histamine in the PAG is important since histamine could be derived from other sources (i.e. mast cells), whereas pain relief mechanisms are thought to result from neuronal activity. Presently, treatment with alpha-fluoromethylhistidine (FMH, 100 mg/kg i.p.), the irreversible inhibitor of histamine synthesis, decreased basal histamine release in the PAG by 80 %. Also, morphine (12.8 mg/kg s.c.)-induced histamine release was completely abolished by this FMH pretreatment. These results indicate that histamine release in the PAG depends on rapid histamine synthesis. In addition, perfusion of the PAG with the sodium channel blocker, tetrodotoxin (10^{-6} M) decreased basal histamine release by 50%, suggesting that a portion of the extracellular histamine in the PAG is released by propagation of action potentials. Thioperamide (5mg/kg i.p.), an H_3 antagonist, increased histamine release in the PAG to 250% of baseline levels, suggesting that basal histamine release in the PAG is under the regulation of the H_3 autoreceptor. Taken together, these results suggest that basal and morphine-induced histamine release in the PAG has a neuronal origin (Supported by DA-03816, and DA-05460).

RETINA: FUNCTIONAL ORGANIZATION

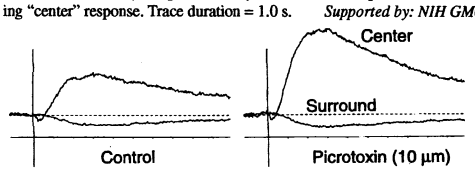
578.1

OPTICAL RECORDING OF LIGHT-EVOKED NEURAL ACTIVITY IN THE FROG RETINA

L. L. Crenshaw and D.M. Senseman*

Div. of Life Sciences, Univ. of Texas at San Antonio, San Antonio, TX, 78249

We have demonstrated the feasibility of using optical recording techniques to monitor light-evoked activity in the vertebrate retina. To minimize bleaching of photoreceptors during recording, we used the absorbance dye, RH155, which yields its largest extrinsic voltage-dependent signal in the near infrared. Traces shown below were obtained from an isolated frog retina stained with a 0.3 mg/ml RH155 solution for 45 min. The stained retina was imaged with an improved light detector system that allow 464 contiguous regions to be monitor simultaneously with a temporal resolution of 1.6 ms and a spatial resolution of 43 µm. A Grass Photostimulator generated controlled light flashes that were focused as small spots (200 µm dia) on to the retinal surface via the microscope's epi-illumination system. As shown below, spot illumination at 540 nm evoked a net depolarization of neural elements within the center of the flash while surrounding regions responded with a net hyperpolarization. Bath application of the GABA-antagonist, Picrotoxin (10 µm), preferentially increased the amplitude of the depolarizing "center" response. Trace duration = 1.0 s. *Supported by: NIH GM07717*



578.2

COMPONENT POTENTIALS OF THE DARK-ADAPTED CAT ERG J.G. Robson[1], L.J. Frishman*, and L. Du, College of Optometry, Univ. of Houston, Houston, TX 77204; [1]Physiological Lab, Cambridge, CB2 3EG, UK

We have studied how the amplitudes of both vitreal and intraretinal ERGs of the dark-adapted anesthetized cat depend upon stimulus intensity and are affected by dim steady backgrounds. Using TTX and glutamate-receptor antagonists we find that the response evoked by a brief ganzfeld flash delivering up to 10^4 quanta.deg^{-2} is the sum of at least four components. The contribution of each component (measured at a fixed time after each flash) appears to be proportional to flash intensity at low levels (voltage = intensity x sensitivity) but then saturates at some characteristic higher level (V_{max}). In order of their sensitivities the components are: a) a cornea-positive scotopic threshold response (pSTR) blocked by TTX (4-25 µM vitreal concentration); b) a slightly less sensitive negative scotopic threshold response (nSTR) also blocked by TTX; c) a negative scotopic response (nSR) having about one tenth the sensitivity and unaffected by TTX; d) the even less sensitive PII response. The pSTR and nSTR are both somewhat slower than the nSR. Although the sensitivities of the three most sensitive components are reduced in a similar way by backgrounds, the modelled V_{max} of the nSR is much more affected than that of the pSTR and nSTR. Complete suppression of these components requires application of a kainate-receptor antagonist (CNQX, 25 µM) as well as an NMDA-receptor antagonist (AP7, 200-400 µM). In intraretinal recordings the TTX-sensitive nSTR dominates the inner retinal response in the vicinity of the area centralis while the TTX-insensitive nSR prevails in the peripheral retina. Our findings suggest that the pSTR and nSTR are generated by spiking neurons, possibly Off- and On-center ganglion cells, while the nSR may originate from depolarizing amacrine cells. Supported by EY06671

578.3

CURRENT SOURCE DENSITY (CSD) ANALYSIS OF THE b-WAVE OF THE ELECTRORETINOGRAM.

X. Xu & C.J. Karwoski*, University of Georgia, Athens, GA 30602.

In some ways, the most important component of the ERG is the b-wave. This study attempts to gather information about the mechanism of origin of the b-wave. The CSD technique was used to locate the depths of its current sources and sinks in frog retina.

Our CSD profiles show a transient sink near the outer plexiform layer (OPL), and a slower, more sustained source at the inner limiting membrane (ILM). In the inner plexiform layer (IPL), there is a transient source, followed by a slower sink. This profile suggests for the b-wave an OPL sink and IPL source. The IPL sink and ILM source may underlie the M-wave. Pharmacological experiments using picrotoxin and barium support this view.

If the source-sink pair underlying the b-wave is due to K^+ spatial buffering through Muller cells, then the K^+ channels are relatively insensitive to Ba^{2+} (unlike the Muller cell K^+ channels underlying the M-wave, and unlike those in the pigment epithelial cells that underlie the c-wave). Another possibility is that bipolar cells directly generate the b-wave sink-source.

Supported by NEI grant EY-03526.

578.4

A COMPARISON BETWEEN CAT AND RABBIT PATTERN ELECTRORETINOGRAM (PERG) IN AN *IN-VITRO* PREPARATION

K.-H. Huemer* and R. Pflug Dept of General & Comparative Physiology, University of Vienna, A-1090 Vienna, Austria.

Retinae of rabbits and cats were isolated under dim red light, then put into a chamber and continuously superfused with oxygenated culture medium. Pattern-reversal electroretinograms were recorded using Ag-AgCl-electrodes in agar. Stimuli were directly projected onto the retina using the inverted ray path of a modified microscope. Recordings were averaged using AD-converter and computer. The averaging process was started after steady state-conditions were obtained.

This *in-vitro* method can be used to evoke PERGs in both species. The recordings had a much better signal-to-noise-ratio than *in-situ* recordings. The procedure allows a more precise and immediate analysis. In both species at low temporal frequencies (c. 2 Hz) a cornea-positive peak dominated the response showing features similar to luminance responses. We also found a second component of constant latency (not depending on temporal frequency) over the whole range examined (between 1.5 and 7 Hz).

The PERG is rather similar in both species. In cat the responses are slightly faster than in rabbit and the cornea-negative component is more pronounced. But these differences appear to be merely quantitative and the PERG generation sites are probably closely related in both species.

578.5

A PAF ANTAGONIST BLOCKS PAF-INDUCED GLUTAMIC ACID RELEASE FROM DISSOCIATED RETINAL CELLS AS WELL AS FROM RETINAL SYNAPTOSOMES.

N.G. Bazan*, P. Prouet, V.L. Marcheselli. LSU Eye Center and LSU Neuroscience Center, LSU Med. Ctr. Sch. Med., New Orleans, LA 70112

In experimental high intraocular high pressure, it has been shown that there is an increase in glutamic acid released from retinal perfusates. PAF is also released in the retina during ischemia. Very active phospholipases are present in the retinal release of polyunsaturated fatty acid. Overstimulation of glutamic acid releases is involved in retinal excitatoxicity. Under experimental conditions, BN-52021 blocks the [^{3}H]-glutamic acid release, induced by PAF on dissociated retinal cells and on retinal synaptosomes which have been previously loaded with [^{3}H]-glutamic acid. A 25% release of the total [^{3}H]-glutamic acid uptake is obtained when 10 nM PAF is injected into a perfusion cell, into which the loaded cells or synaptosomes have been placed. [^{3}H]-glutamic acid was measured on perfusates through an online radiochemical detector. 1 μM BN-52021 inhibits control levels of glutamic acid release. 40 mM KCl induced a more than 30% release, which cannot be blocked by BN-52021. The uptake of [^{3}H]-glutamic acid by dissociated cells is three times higher than in the homogenates, and is almost the same as the subcellular fractions enriched in synaptosomes. Our studies show that the second messenger PAF is a activator of glutamic acid release in the retina through a presynaptic site. PAF antagonists may be a useful therapeutic strategy to protect the retina in rapidly progressive forms of glaucoma. Supported by NIH grant EY05121.

578.6

REGULATION OF ELECTRICAL COUPLING AND NEURITE OUTGROWTH IN HORIZONTAL CELLS BY CALCIUM AND CON A

D.G. McMahon*, J.K. Crager and M.P. Mattson. Department of Physiology and Biophysics and Sanders-Brown Center for Ageing Research, University of Kentucky, Lexington, KY 40536

Teleost retinal horizontal cells display structural changes in response to light adapting signals, including changes in the density of gap junction channels at electrical synapses and elongation of dendritic processes called spinules. Recent results suggest that the Ca^{++}-activated enzyme PKC is involved in the structural plasticity of horizontal cells. In an effort to understand the role of cell calcium in these processes we have measured electrical coupling, neurite outgrowth and $Ca^{++}{}_i$ in horizontal cells from the retina of the giant danio (*Danio aequipinnatus*).

Cells were dissociated and maintained using standard techniques. Junctional conductance was measured using dual whole-cell patch clamp recording, $Ca^{++}{}_i$ by fura-2 imaging and cell morphology quantified by image analysis. In one set of experiments, cells were cultured in control (2.65mM) or low (0.125mM) Ca^{++} media. The proportion of cell pairs exhibiting electrical coupling was significantly lower in low Ca^{++} (6/21) versus control (14/21) and $Ca^{++}{}_i$ was also significantly lower in low Ca^{++} (36 ± 12 nM, N=26 vs 55 ± 14nM, N=36, $p<0.05$ t-test). In a substantial proportion of cell pairs, individual cells exhibited striking differences in $Ca^{++}{}_i$. In a second set of experiments, cells were cultured in the presence or absence of the lectin concanavalin A. Con A (2 μg/ml) increased the number of active growth cones/cell (2.5 ± 1.4, N=28 vs 1.1 ± 1.2, N=36) and the average cell perimeter (274 ± 63 μm vs 232 ± 80 μm), as well as increasing $Ca^{++}{}_i$ (144.1 ± 8.6 nM, N=90 vs 91.1 ± 10.8 nM, N=85, all $p<0.05$). The increases in growth cone number and $Ca^{++}{}_i$ both persisted in the presence of DNQX or nifedipine.

These results indicate correlations between reduced $Ca^{++}{}_i$ and reduced expression of coupling, and increased $Ca^{++}{}_i$ and increased neurite outgrowth.

578.7

DOPAMINERGIC LIGANDS DO NOT ALTER HORIZONTAL CELL RECEPTIVE FIELDS IN CAT AND RABBIT RETINAS.

R. Pflug and R. Nelson* Institut für allgemeine Physiologie, Universität Wien, Austria and Lab. of Neurophysiology, NINDS, NIH, Bethesda, MD 20892.

In fish and turtle retinas, D_1 receptor ligands uncouple horizontal cell (HC) gap junctions and reduce receptive field (RF) sizes. We have measured dopaminergic (DA) effects on HC RFs in mammalian retinas. In arterially perfused retina-eyecup of cat, or isolated rabbit retina, HC's were penetrated with sharp, KCL-filled, intracellular microelectrodes and responses to 200 µm slits of red light at different RF positions measured. RF space constants were calculated from conductive-sheet model curves. After addition of DA agonists and antagonists, space constants were remeasured at intervals (continuing up to 20 m). In rabbit DA agonists in the range of 70-700 µM slightly increased, whereas the D_1 antagonist SCH23390 slightly decreased, RF size. The D_2 antagonist sulpiride had no consistent effect. In cat DA itself slightly reduced RF size, while the unspecific DA receptor agonist apomorphine was without effect. None of the RF alterations exceeded 20%, a value similar to measuremental repeatability. Since A-type horizontal cells in cat and rabbit retinas are extensively coupled, changes in RF size should be substantial, but only small changes were observed. Even these might be secondary, perhaps reflecting alteration of receptor coupling.

578.8

NMDA RECEPTORS REGULATE HORIZONTAL CELL COUPLING IN THE FISH RETINA.

Y. Wang, K. Harsanyi and S.C. Mangel*. Dept. of Ophthalmology, Univ. of Alabama, Birmingham, AL.

The action of NMDA on cone horizontal cell electrical coupling was studied in intact, goldfish retinas, superfused with a Mg-free, bicarbonate-based Ringer's that contained D-serine (0.5 uM), a glycine analogue. Horizontal cell coupling was assessed by monitoring responses to small (0.4 mm diam) spots of light.

NMDA uncoupled horizontal cells in a dose-dependent manner, with threshold at 10 uM and saturation at 100 uM. Application of the NMDA antagonist, AP7 (50 uM), blocked the uncoupling action of NMDA (100 uM), as did prior application of SCH23390, a dopamine D1 antagonist, or prior treatment of the retinas with 6-hydroxydopamine, a procedure which destroys dopaminergic neurons. Addition of Mg (1 mM) partially blocked the uncoupling effect of NMDA at 100 uM and completely blocked it at 50 uM. The uncoupling effect of NMDA (50 or 100 uM) was also reduced, if it was applied without D-serine. Because flickering, but not sustained, light stimulation uncouples fish horizontal cells by increasing dopamine release, we tested whether NMDA receptors were involved. Prior application of AP7 blocked horizontal cell uncoupling due to flickering, but not sustained, light. These results indicate that flickering light releases dopamine and uncouples horizontal cells through activation of NMDA receptors.

Supported by NIH EY05102 and by RPB, Inc.

578.9

A - CURRENT OF LUMINOSITY AND CHROMATIC HORIZONTAL CELLS IS MODULATED BY NEUROTRANSMITTERS AND CALCIUM
A. Akopian[1] * and P. Witkovsky [1,2] , Depts. Ophthalmology[1] and Physiology & Biophysics[2], New York University Medical Center, New York, N.Y. 10016

Luminosity-type and chromatic-type horizontal cells (HC) were isolated from the Xenopus retina and studied by the whole cell version of the patch clamp technique. The A-current (Ia) of both HC types is prominent, (Akopian & Witkovsky, Soc. Neurosci. 352.16, 1992), particularly in the chromatic HC, and it helps shape the light response of the cell following the hyperpolarization evoked by a bright flash. The voltages for half inactivation and activation of Ia were, respectively -73 ± 3 mV and -20 ±2 mV. We found, however, that V half-inactivation was shifted significantly towards more positive potentials (-57 ±2 mV) when either GTP (100 μM) or GTPgammaS (100 μM) was added to the pipet solution. Ia activation was not affected by these substances.

The time constant of Ia inactivation varied from 27-34 mV in drug-free solution and was only slightly altered by the voltage level at which it was measured. Bath application of either glutamate (100 μM) or dopamine (20 μM) resulted in a 30-40% decrease in the time constant of Ia inactivation. The same effect was obtained with either the D1 agonist SKF 38393 (100 μM) or the D2 agonist, quinpirole HCl (20 μM). In addition, we found that in zero external calcium the peak Ia current was reduced by 30%. A comparable reduction was achieved by adding the dihydropyridine, nifedipine (10μM) to the bath, indicating that influx of Ca through L-type channels is implicated in the control of Ia. Collectively our data suggest that *in situ*, where HC's are exposed continuously to glutamate and dopamine, and where intracellular concentrations of calcium and second messengers are subject to modulation, the amplitude and response kinetics of a prominent intrinsic membrane current, Ia, also will be shaped by a variety of modulatory influences. Supported by EY 03570 to P.W.

578.10

A WIDE-FIELD BIPOLAR CELL IN THE RABBIT RETINA. C.-J. Jeon* and R. H. Masland, Harvard Medical School, Boston, MA 02114

We have studied a wide-field bipolar cell labeled in the rabbit retina by accumulation of biocytin. Biocytin was injected intraocularly. Two days later the retinas were isolated, fixed, and prepared as wholemounts. Biocytin was visualized by conventional immunohistochemical methods.

A subgroup of bipolar cells was found to accumulate intraocularly injected biocytin. They had 2-4 proximal dendrites and a wide (100 - 300 μm) dendritic spread. Their axons descended to layer 5 of the inner plexiform layer, where they had wide branching similar to that in the outer plexiform layer.

When groups of neighboring cells were examined, their dendrites were found to co-fasiculate: the distalmost dendrites of one cell ran alongside those of a neighbor. Typically, two adjacent cells were apposed only for one pair of dendrites, but cases of more were observed. Occasionally there were small bulb-like structures at the points where the dendrites joined together. The same pattern was present for the axonal processes in layer 5 of the inner plexiform layer, with more frequent cell-to-cell appositions. The cells are thus co-fasiculated with their neighbors in both the outer retina and the inner retina.

Supported by EY05747 and a Fight for Sight fellowship.

578.11

INTRACELLULAR RECORDING AND COMPUTER SIMULATIONS TO COMPARE ELECTRICAL AND DYE COUPLING IN NEURONS OF THE MUDPUPPY RETINA. T.J. Velte* and R.F. Miller. Dept. of Physiology, Graduate Program in Neuroscience, Univ. of Minnesota, Minneapolis, MN 55455.

We have studied dye coupling in mudpuppy neurons using Neurobiotin (Vector) as a cell tracer combined with intracellular recording in a perfused retina-eyecup preparation. A computer controlled light stimulus (Innisfree) was used to study receptive field properties through slit displacement techniques. The morphologies of stained cells were entered into the Eutectic Neuronal Reconstruction System which was connected to a Sun workstation and provided a means for detailed computer simulation studies. Computer simulations were carried out to model the spread of current through a single neuron or a small neural network. The cells were joined by a single resistor at locations on the cells where gap junctions were likely to be present. Modeling constraints varied the coupling resistance to match uncoupled versus heavily coupled paradigms.

This study has shown that Müller cells in the mudpuppy are extensively dye coupled. However, unlike previous studies in rabbit (Vaney, 91), neurons in the inner retina are at most coupled to a small number (1-2) of heterogeneous cells; yet most neurons in the mudpuppy are not dye coupled using this technique. When small neural networks were created from cells that exhibited dye coupling, current spread was maximized between cells with relatively few gap junctions (70), when a high membrane resistance (70,000 Ω cm^2) was used. This suggests that few gap junctions are needed to enable electrical coupling in these cells and may offer an explanation for the infrequent dye coupling observed in this study.

(Work supported by PHS Grant: EY-07133 to TJV and EY-03014 to RFM)

578.12

FUNCTIONAL CONSEQUENCES OF REGENERATIVE MEMBRANE AND COUPLING IN THE AII AMACRINE CELL OF CAT RETINA. R.G. Smith*, N. Vardi Dept Neurosci, Univ. of PA, Phila PA 19104-6058.

At scotopic intensities, the AII cell is hypothesized to transmit quantal photon signals, which could be obscured by noise due to anatomical convergence from 500 rods. This raises the question of whether the AII network possesses special noise-reduction features. A quantal photon signal appears in 5 AII cells due to anatomical divergence, and the AII is interconnected by gap junctions into a network. Furthermore, the AII cell contains Na and K channels and produces action potentials under electrical stimulation.

To explore the significance of the AII coupling and membrane properties for signal/noise issues, we simulated a square array of AII cells as single compartments containing fast activating, slow inactivating Na, and slow K channels. Each cell received a resting "dark" signal through a noisy synapse. The central 5 cells received in addition a simulated photon signal of about 5 mV. Without gap junctions, noise of 5-10 mV evoked intermittent action potentials 30 mV in amplitude and 50 msec in duration. Coupling the AII cells with 200 pS gap junctions reduced noise by a factor of 2 and eliminated noise-evoked action potentials. AII cells that received the simulated photon signal simultaneously with their neighbors produced action potentials, but when only one coupled AII received the photon signal, no action potential ensued.

This suggests that regenerative membrane properties and electrical coupling might function cooperatively in the AII cell to improve the signal/noise ratio of the quantal photon signal. Supported by MH48168.

578.13

FUNCTIONAL ARCHITECTURE OF HENLE'S LAYER IN PRIMATE FOVEA A. Hsu*, Y. Tsukamoto[1], R.G. Smith, and P. Sterling, Dept. of Neuroscience, Univ. Pennsylvania, Phila., PA 19104 and [1]Hyogo College of Medicine, 663 Japan.

Axons connecting cones in the foveal center to their terminals in the perifovea are numerous, creating a layer (Henle's layer) that occupies up to 46% of the retina's postreceptoral thickness. Since the layer must decrease retinal efficiency by scattering and absorbing light, we wondered what constrains the individual axons to be so thick (up to 1.8 μm).

We simulated an isolated foveal cone using a compartmental model consisting of an outer segment conductance (1 nS) coupled a cable approximating the length (380 μm) and taper of a cone axon, and terminating in a sphere (7 μm diam.) representing the synaptic terminal. A photovoltage was generated by a small decrease (1%) in the outer segment conductance and voltage transfer was computed by dividing the photovoltage in the outer segment by that at the terminal. When R_i=200 Ω-cm and R_m=25 kΩ-cm^2, voltage transfer was 80%. Tripling the axon diameter (5.4 μm) marginally improved transfer to 90%; reducing the diameter by 3-fold (0.6 μm) decreased transfer to 55%. For a thin axon (0.6 μm), increasing R_m to 150 kΩ-cm^2 restored the transfer to 80%. However, since the photovoltage is generated by a conductance change, not an ideal current source, increasing R_m also decreased the photovoltage. Adding a chloride conductance (max 0.9 nS) at the synaptic terminal linearly decreased the resting potential but affected neither the magnitude of the photovoltage nor its transfer.

Apparently, the dimensions of the cone axon are optimal for transferring photovoltages when R_m≈25 kΩ-cm^2. A thinner axon would decrease the photovoltage and decrease transfer. A thicker axon would only marginally improve performance and degrade the optics. Supported by EY08124.

578.14

MINIMUM RATE OF TRANSMITTER RELEASE AT A "ONE-BIT" SYNAPSE. R. Rao*[1,2], G. Buchsbaum[2], P. Sterling[1], Depts. Neurosci.[1] & Bioeng.[2], Univ. of Penn., Phila., PA 19104.

Neurons transfer signals by modulating quantal release of transmitter. Yet, how many quanta are required for any particular signal is unknown. At the mammalian rod synapse, both the signal and the transfer mechanism are identified. The signal is binary, representing the absorption (or not) of one photon; the transfer to the bipolar cell involves suppression of tonic release at the rod's single active zone. We calculated the minimum rate of tonic release required to reliably transmit this "one-bit" signal. Since the active zone is large (100 docking sites), the binomial statistics of quantal release become poisson, and the interrelease intervals should have an exponential probability density function whose decay depends uniquely on the release rate:

$$p(t) = \rho e^{-\rho t} \quad \text{where } \rho = \text{release rate}$$

Therefore, specifying a maximum allowable interval (T) and the probability of exceeding it (y) sets the shape of the distribution and hence the release rate that produces it:

$$\rho = -(\ln y)/T$$

We assumed for the bipolar cell a signal-to-noise ratio of 5 and a rod convergence of 20 (cat). Since the bipolar mpsc peaks and decays in time T, we used the shape of the mpsc (amphibian) and the rising phase of the bipolar cell light response (rabbit) to calculate T (~50ms). Taking the bipolar cell to have gaussian noise, we calculated y (~16%). It follows that transmission of the rod signal would require at least 40 quanta/s. If rod convergence were 100 (rabbit), the minimum rate would be 80 quanta/s. These release rates are at least an order of magnitude greater than what is generally reported at conventional active zones. To sustain this high rate for many hours (all night) may require a mechanism to facilitate reloading of the emptied docking sites. This may be the function of the large "ribbon" that serves as depot for ~700 vesicles.

578.15

BRAINACE: A DATABASE-HYPERMEDIA TOOL FOR NEUROSCIENTISTS

Frank H. Eeckman*[1], Richard M. Durbin[2] and Jean-Thierry Mieg[3]. [1] LLNL, Livermore, CA; [2] MRC, Cambridge, England; [3] CNRS, Montpellier, France;

BrainAce is an object-oriented database based on ACEDB, a database system originally developed for the C. elegans genome project (JTM and RD). ACEDB has been successfully adapted to become the database of choice for genomic data from other organisms such as Arabidopsis, human chromosomes, wheat, etc. Although originally written for Unix/X windows, a Macintosh interface is available (FE and RD) that is functionally identical to the Unix version.

ACEDB consists of a core data manager and specific application code. Data are stored in objects that are organized into classes. The objects have extendable structure, so that arbitrarily large amounts of information can be stored in them. That information may include annotations of various sorts, comments, and cross-references. The schema specifying data structures can be extended during the lifetime of a database. There is a browser mode and a general search facility to provide maximal flexibility. In addition, displays can be output in Postscript for laser printing, or as plain text for transfer to other sources. We are currently working to extend the display functions of ACEDB to the needs of specific retinal research groups.

578.16

PARTIAL CHARACTERIZATION OF A RAT RETINAL ~20 K MOLECULAR WEIGHT PROTEIN WHOSE PHOSPHORYLATION IS INHIBITED BY TAURINE: NATURE OF PHOSPHATE BOND AND EFFECT OF KINASE INHIBITORS. J.B. Lombardini*. Depts. of Pharmacology and Ophthalmology & Visual Sciences, Texas Tech Univ. Health Sciences Center, Lubbock, TX 79430.

Taurine (2-aminoethanesulfonic acid), is found in high concentrations in all mammalian tissues; however, there is little information concerning the function of taurine. The studies reported herein were designed to determine the nature of the phosphate bond in a retinal ~20 K protein whose phosphorylation is inhibited by taurine. Subjecting the ~20 K protein, after phosphorylation with [γ-^{32}P]ATP, to different treatments such as acetone, chloroform/methanol, NaOH (both 0° and 100°C), trichloroacetic acid (100°C), and pronase and ribonuclease A indicate that the phosphate moiety is incorporated into protein via a phosphoester bond. Digestion of the isolated ~20 K phosphoprotein with trypsin and 6 M HCl and analysis on 2-dimensional high voltage electrophoresis using cellulose plates demonstrate that serine and threonine residues are phosphorylated. Kinase activators and modulators such as cAMP, cGMP, phorbol ester, calmodulin, and Ca^{2+} have no effect on phosphorylation of the ~20 K protein. However, kinase inhibitors such as staurosporin and W-7 were found to be inhibitory. On the contrary, chelerythrine was determined to be a potent stimulator of phosphorylation. (Supported by the South Plains Foundation, Lubbock, TX)

578.17

AMACRINE CELL CIRCUITS PRODUCE CENTER-SURROUND BEHAVIOR IN MODELS OF CAT RETINAL GANGLION CELLS. M.A.V. Gremillion* and B.J. Travis. Los Alamos National Laboratory, Los Alamos, NM 87545.

Although center-surround(CS) behavior in retinal ganglion cells is well-characterized mathematically and empirically, the circuitry mechanism(s) which produce it are uncertain. We constructed a model of the retina that explores amacrine cell circuitry interactions contributing to ganglion cell behavior. Structural properties of the cat retina such as cell densities and distributions, arbor sizes, and connectivity patterns were accurately modeled. Realistic dynamics govern most individual cellular behavior.

Feedforward and feedback inhibitory circuits using both GABA-A and GABA-B receptors were examined. Spatial CS behavior (concentric center and surround domains resulting in characteristic area-response curves) was easily achieved with wide variations of both kinds of circuits and all parameters. In contrast, qualitative temporal behavior of ganglion cells as shown by the general shape of PSTH's (e.g. transient and sustained components, approximate duration of transients) was attained by only small subsets of circuit parameters. Circuits were then tuned to match transient and tonic firing rates and transient duration for individual experimentally recorded cells. GABA-B K+ conductances with time constants between 25 and 45 msec gave the best fit to PSTH's of X-ON ganglion cells, while shorter time-constant GABA-A conductances yielded PSTH's that fit Y-cell temporal behavior. CS behavior in these simulations resulted from the interaction of population parameters such as the densities and arbor sizes of the excitatory (cone bipolar) and inhibitory (amacrine cell) input populations, coupled to the effects of inhibitory conductance time constants. As the stimulus' spatial extent changes, the recruitment rate and relative weighting of the two input populations changes over both space and time.

RETINA: GANGLION CELLS II

579.1

SIMULATING THE MIDGET GANGLION CELL CIRCUIT FOR GAIN CONTROL. P. Sterling* and R.G. Smith. Dept. of Neuroscience, Univ. of PA 19104-6058.

The midget ganglion cell responds linearly to contrast. This must require special circuitry since the small cell would have a high input resistance (~2 GOhm) and thus would tend to saturate. Assuming a single channel conductance of 20 pS and 15 channels/synapse, 1 quantum of transmitter (saturating) would depolarize the membrane potential (Em) by at least 10 mV. A simulation with poisson-distributed quantal events having a 1 msec rise and 5 msec decay, 30 bipolar synapses and a release rate of 4/sec/synapse gave the midget ganglion cell a resting potential of -53 mV with 7 mV noise (rms). To produce spikes, Em must lie between -60 and -40 mV, but to code a signal within this range requires minimizing noise from quantal fluctuation and thus a higher total quantal rate.

To accommodate a high rate and maintain Em within the range appropriate for spiking seems to require an inhibitory conductance. Each quantum from the bipolar terminal that excites the ganglion cell also excites an amacrine process presynaptic to the ganglion cell (Calkins et al.'92). This synapse (presumed GABAergic) would cause a conductance similar in magnitude to the excitatory one and tend to clamp the ganglion cell near the Em of -50 mV required for spiking. When amacrine synapses were added to the simulation and the release rate was increased to 35/sec/synapse, noise was reduced to less than 2 mV.

This gain control circuit might resolve the contradictory needs to minimize cell size and quantal fluctuation while maintaining linearity. Supported by EY08124 and MH48168.

579.2

MOLECULAR AND PHYSIOLOGICAL CHARACTERIZATION OF A cGMP-GATED CHANNEL IN RETINAL GANGLION CELLS SENSITIVE TO NITRIC OXIDE. T. Leinders-Zufall*, I. Ahmad, F. Zufall and C. J. Barnstable. Departments of Neurology and Ophthalmology and Section of Neurobiology, Yale University School of Medicine, New Haven, CT.

cGMP is the major second messenger in vertebrate phototransduction, coupling the enzymatic steps of the tranduction cascade to cGMP-gated cation channels in photoreceptor cells. We now report that cGMP may also be an important regulator of the function of a subset of ganglion cells. Using in situ hybridization on adult rat retinal sections a photoreceptor-like cGMP-gated channel RNA has been detected in the ganglion cell layer. PCR analysis from a small pool of identified ganglion cells was carried out. Sequence analysis of cloned PCR fragments revealed two very homologous but different sequences one of which was identical to the rod photoreceptor cGMP-gated channel sequence. Using the whole-cell patch clamp technique we determined whether a functional cGMP-gated channel was expressed in individual ganglion cells. Internal perfusion of cGMP elicited in about 50% of the cells a non-desensitizing inward current of several hundred picoamperes due to the activation of nonselective cation channels. This current was completely but reversibly blocked by 3 mM external Cd^{2+}. Activation of the channels was highly specific for cGMP over cAMP and external removal of divalent cations increased the current through cGMP-gated channels. Since ganglion cells contain a soluble guanylate cyclase we tested whether nitric oxide (NO) could be a regulator of cGMP-gated channels. External application of the NO-donor sodium-nitroprusside (100 µM) resulted in the production of cGMP as measured by the induction of cGMP-gated channel activity. As a potential physiological source of NO within the retina we identified amacrine cells by using an enzyme stain for NADPH-diaphorase. We propose that nitric oxide is an important physiological messenger in the retina that regulates the activity of cGMP-gated cation channels in ganglion cells. This pathway is likely to be of major interest in other regions of the CNS as well. Supported by grants from the NIH.

579.3

Large and small synaptic events in On-β cells of the cat retina. Michael A. Freed* LNP, NINDS, NIH, Bethesda, MD.

Voltage noise was recorded from On-β cells under two conditions: darkness and illumination of the receptive field center with bars of bright red light (647 nm, 6 log(hν s^{-1} μm^{-2})) which caused a maximal depolarization. In either darkness or during illumination, there was negligible voltage noise due to photon absorptions. Negligible noise from bipolar cells was transmitted through their synapses upon the ganglion cell, since bipolar cells exhibit little voltage noise in bright light, and the synapses may fail in the dark. Action potentials were eliminated by depolarizing the cell. The voltage noise that remained showed evidence of large and small synaptic events.

Large depolarizing, events rose above the noise. These were fit by

$$f(t) = a(t/T)^{(k-1)} \exp[(k-1)(1-t/T)] \quad \text{(eq. 1)}$$

and took about 10 ms (T) to reach a peak voltage (a) of 600±50 μV (k=9). In one cell, the frequency of large events was increased by a bar illuminating the receptive field surround. In another cell, the bar was equally effective throughout the receptive field.

Large events caused the distribution of voltages to be skewed. When these events were deleted from the record, the remaining noise had a normal distribution, as if from randomly occurring small events. This noise increased with illumination. Difference spectra dropped off at high frequencies at a rate of $1/f^k$, where k equaled 18-20. These spectra were compatible with an event (eq. 1) which took about 20 ms (T) to reach a peak amplitude (a) of 27 μV.

The large events are intriguing and may be action potentials from neighboring ganglion cells filtered through small gap junctions between ganglion cells. The smaller events are probably due to release of quantal amounts of neurotransmitter at bipolar and amacrine cell synapses.

579.4

HOW NBQX ENHANCES NMDA CURRENTS IN RETINAL GANGLION CELLS. Weifeng Yu and Robert F. Miller* Dept. of Physiology, Univ. of Minnesota, MPLS, MN55455.

NBQX is the most selective non-NMDA antagonist yet available. We have previously shown that NBQX enhances NMDA-mediated synaptic currents in amphibian retinal ganglion cells (Coleman, Yu & Miller, Society for Neuroscience, #107.8, Vol.17, P256, 1991) in the presence of external Mg^{++}. In the present study, we have further analyzed this action of NBQX in retinal slice experiments, using both light and microhyperosmotic stimulation (MHS) of the IPL, to synaptically activate third-order neurons. Whole-cell recordings were obtained from ganglion cells. At 10μM, NBQX had no effect on exogenously applied NMDA at different holding potentials (-30mV to -70mV), making it unlikely that NBQX directly effects NMDA receptor sensitivity. However, when strychnine and picrotoxin are added to the bathing medium, light and MHS evoke larger excitatory currents, indicating the presence of substantial inhibitory currents in each neuron and, under these conditions, the NBQX enhancement of NMDA currents is eliminated. We then increased internal [Cl^-], to bring the Cl^- reversal potential to 0mV, giving inhibition and excitation the same reversal potential. If NBQX simply blocked postsynaptic inhibition, we would expect to see an NBQX-mediated decrease in NMDA current, but instead we saw an increase. This latter experiment suggests that postsynaptic, algebraic interactions between inhibition and excitation are not solely responsible for NMDA enhancement by NBQX. From these experiments, we conclude that NBQX effectively blocks both non-NMDA excitation and amacrine-mediated inhibition. However, this inhibitory action must exist at both post- and presynaptic sites and when eliminated, enhances release of glutamate at the presynaptic terminals as well as post-synaptic NMDA currents through the elimination of inhibition.

579.5

CHOLINERGIC INNERVATION OF RABBIT RETINAL ON-CENTER DIRECTIONAL GANGLION CELLS. C. Brandon*, Dept. of Cell Biology and Anatomy, Chicago Medical School, North Chicago, IL 60064.

A homogeneous population of rabbit retinal ganglion cells (GC's) projects to the medial terminal nucleus (MTN) of the accessory optic system (1,2); these cells almost certainly have ON-center, directionally-selective (DS) receptive fields (3). ON-DS GC's respond strongly to cholinergic agents (4), but morphological evidence for such input is conflicting (5,6). This study was undertaken to identify cholinergic synaptic inputs onto these presumed directional cells.

A mixture of FITC- and RITC-labeled latex microbeads was injected into the rabbit MTN (2); one week later, bead-labeled retinal cells were impaled and filled with Lucifer Yellow (LY). Neighbouring starburst cells (DAPI-labeled) were also injected and filled with LY. LY-filled cells were visualized in wholemount by LY immunocytochemistry.

The dendrites of LY-filled MTN-GC's stratify at about 65-70% depth in the IPL, the same depth as the proximal cholinergic stratum. In 50-micron cross-sections, these processes lie precisely within the proximal ChAT-immunoreactive sublamina. In planar views, the distal dendrites of LY-filled starburst cells and MTN-GC's are precisely co-stratified, with many contact sites marked by (probable synaptic) varicosities in the cholinergic dendrites. Synapses *en passant* are common. In fortuitous cases, the entire cholinergic arbor is contained within the GC arbor, so that the total number of synaptic contacts may be roughly estimated: each cholinergic amacrine makes synaptic contacts at about 50 sites on a given MTN-GC. The extensive overlap of cholinergic amacrines (ca. 20-70-fold) means that MTN-GC's receive an extremely dense cholinergic innervation. Supported by USPHS EY-05601.

(1) Oyster *et al.*, J. Comp. Neurol. *190*, 49 ('80); (2) Buhl & Peichl, *ibid 253*, 163 (1986); (3) Simpson *et al.*, Prog. Brain Res. 50; (4) Masland & Ames, J. Neurophysiol. *39, 1220* ('76); (5) Famiglietti, J. Comp. Neurol. *324*, 322 ('92); (6) Vaney *et al.*, Neurobiol. of Inner Retina ('89).

579.6

MORPHOLOGICAL AND PHYSIOLOGICAL IDENTIFICATION OF THE RABBIT RETINAL GANGLION CELL THAT PROJECTS TO THE SUPRACHIASMATIC NUCLEUS. D.S. Tjepkes, F.R. Amthor and D.C. Tucker*. Dept. of Psychology and NRC, Univ. of Alabama at Birmingham, Birmingham, Alabama 35294.

The retina has previously been shown to have a projection to the suprachiasmatic nucleus (SCN). SCN neurons have been shown to have a maintained firing rate that either has a sustained increase or decrease in its firing rate in response to increased illumination (Groos and Mason, *J.Comp.Phys.*, 1980; Meijer *et.al.*, *Brain Res.*, 1986). The goal of this research is to physiologically and morphologically identify the retinal ganglion cells that mediate these responses, which are likely to play a role in the photic entrainment of circadian rhythms.

Fluorescently labelled latex beads were stereotaxically injected into the suprachiasmatic nuclei of rabbits. The retinas containing retrogradely labelled ganglion cells were mounted in a fluorescence microscope and superfused with oxygenated Ames medium. The activity of the labelled cells was examined with extracellular electrodes while visual stimuli were projected through the microscope condenser. The cell was then intracellularly impaled with a pipette electrode and injected with HRP and processed to allow morphological examination.

It is hard to restrict injections to the SCN because of its proximty to the optic chiasm. Nevertheless, the first cells labelled exhibit a sustained rate of firing that increases with an increase in general illumination, with no sign of an antagonistic surround. These cells responded poorly to small flashing spots. These responses are consistent with a role of luminance coding. The cell's morphology is characterized by a small somata and very fine dendrites that are locally bistratified.

This research was supported by NEI grant EY05070.

579.7

NONLINEAR CONTRIBUTIONS TO THE STEP RESPONSES OF CAT RETINAL GANGLION CELLS. J.F. Cox* and M.H. Rowe, Program in Neurobiology, Ohio University, Athens, OH 45701.

Retinal ganglion cells typically respond to the abrupt contrast reversal of a stationary grating with a sudden increase or decrease in firing rate, depending on the configuration of the grating relative to the cell's receptive field. We have characterized the timecourses of such positive and negative step responses by examining filtered peristimulus time histograms of firing rate, collected following sudden contrast reversals. Positive step responses generally begin with a rapid rise in firing rate that is followed by a more gradual decline. However, in many Y-cells and Off-center X-cells the decline is interrupted by a second excitatory component that produces a hump in the step response profile. Thus, there are two components to many step responses, an early component and a late one.

Both components were evident at spatial frequencies that were well above the spatial resolution of the surround mechanism, ruling out any possible contribution of the surround. The late component was exhibited in both positive and negative step response profiles, indicating that it is the result of a rectifying mechanism. Furthermore, the late component does not vary with spatial phase. Thus, the late component of the step response has characteristics similar to those of the nonlinear rectifying subunits, previously described for Y-cells. When we subtracted the estimated contribution of this mechanism from the overall response profiles, the resulting profiles can be readily fit to a linear model (Shapley & Victor, J Physiol 318: 161, 1981) of center mechanism dynamics.

579.8

LOCAL AND NON-LOCAL RECTIFIED RESPONSES OF PHASIC RETINAL W-CELLS OF THE CAT. **M.H. Rowe* and J.F. Cox. Neurobiology Program, Ohio University, Athens, OH 45701-2979.**

We have examined the receptive field profiles of cat retinal W-cells using narrow bars briefly presented at various locations within the receptive field. Each stimulus sequence consisted of 1000-10000 individual stimuli of fixed duration, e.g., 50 msec, presented with no interstimulus interval. The location and polarity (brighter or darker than background) of all stimuli within a sequence were randomized, and 2-dimensional (space-time) response profiles were generated by reverse correlation of the cell's spike train with the stimulus sequence (Jones and Palmer, J. Neurophysiol., 58: 1987). All 28 cells studied to date had receptive fields consisting of one or more excitatory discharge zones, which we will refer to as either bright or dark depending on the polarity of the effective stimulus. Only 5 of these cells had receptive fields consisting of a single discharge zone. In 12 cells, two discharge zones, one bright and one dark, were present. In most of these cases, the two discharge zones were not in spatial register, indicating that they were produced by separate inputs. The remaining 11 cells had 2 or more (up to 5) discharge zones of common polarity (bright or dark). Nine of these 11 cells also had at least one discharge zone of opposite polarity, and discharge zones of opposite polarity were rarely in spatial register.

In 21 of the 28 cells, an additional mechanism was present that produced inhibition in response to both bright and dark bars at all stimulus locations. This mechanism appeared to have a spatial extent of at least 24 degrees, and to show no spatial structure within it's boundaries. This non-local mechanism presumably corresponds to the suppressive field described previously.

Supported in part by RO1 EY08038.

579.9

PUPILLARY LIGHT REFLEX IN RCS RATS: AGE-RELATED CHANGES AND COMPARISONS WITH VISUAL ACUITY.

S.J.O. Whiteley, M.J. Young, P.J. Coffey†, and R.D. Lund* Dept. of Anatomy, University of Cambridge, Cambridge CB2 3DY, U.K. †Dept. of Psychology, University of Sheffield, Sheffield S10 2UR, UK.

The Royal College of Surgeons (RCS) rat has been extensively studied in recent years as an animal model of human retinal degeneration and for studying replacement therapies using transplantation. We are studying the behavioural consequences of this retinal degeneration, expanding previous studies on the pupillary light reflex (PLR) using our pupillometric system to characterise these changes, and comparing these observations with acuity measurements.

Pigmented RCS animals from 3 age groups (4, 8, and 12 months old) were tested for both direct and consensual PLR. Age matched normal rats (Long Evans) served as the control group. Animals were lightly anaesthetised with halothane/nitrous oxide, and the pupil illuminated with infra-red light. The stimulus consisted of a fiber optic light source with a series of neutral density filters, and was controlled with a computer activated electronic shutter. The pupil was viewed through a CCD camera, and the data collected with an ISCAN pupillometer at 33 Hz. The data from these tests were then analysed for a number of parameters, including latency and amplitude of response, as well as rate of constriction. Following testing, animals also underwent a series of behavioural choice tests to measure visual acuity.

Animals tested up to age 12 months still possessed a vigorous PLR. Latency of response increases with age, with approximately a 50% increase between the ages of 4 and 8 months, although the latency of all age groups was substantially longer (~100%) than that seen in age-matched controls (Long-Evans). An interruption in the early phase of the pupillary response wave form was often seen at 4 months. Amplitude of response was less than controls at all times, but did not show a systematic reduction with age. These observations are being correlated with changes in morphological parameters and acuity measures during the course of degeneration.

Funded by grants from MRC and Cambridge Foundation

579.10

EFFECTS OF QUADRANT-DIRECTED RETINAL STIMULATION ON THE PUPILLARY LIGHT REFLEX IN HUMANS.

W.B. Pickworth*, M. Butschky and M.W. Holden, NIDA, Addiction Research Center, Baltimore, MD 21224

The differential effects of Maxwellian and full field retinal stimulation suggested that separate neural mechanisms mediate measures of the light reflex (Pickworth et al, SN Abs, 1992). In the present study, quadrant-directed, Maxwellian stimulation was used to determine whether different retinal loci contribute equally to measures of the light reflex. Six drug-free volunteers were dark-adapted for 30 min prior to the study. The light reflex was evoked in five distinct conditions of ambient light (A) and a patch (P, or no patch, NP) over the contralateral eye: A=0, NP; A=30,P; A=30, NP; A=60,P; A=60, NP. In each of the conditions stimuli were delivered to: full field and to nasal and temporal superior and inferior quadrants. The stimulus was 40 units above the ambient level. Prestimulus diameter was significantly increased by decreasing A and after P. Constriction amplitude and velocity were significantly larger after stimulation of the nasal inferior quadrant. These findings indicate that the light reflex is differentially influenced by the retinal quadrant stimulated, whereas pupil size is determined by ambient light and patch condition. These data extend the concept that different neuronal mechanisms are involved in tonic and phasic aspects of the light reflex and that the differentiation may reflect dissimilar contributions from the retinal quadrants.

579.11

SIMULATION OF THE SIGNALS OF GANGLION CELLS IN THE HUMAN FOVEA TO HYPERACUITY TASKS.

T. Wachtler, C. Wehrhahn* and B. B. Lee#. MPI für biologische Kybernetik, Tübingen and #MPI für biophysikalische Chemie, Göttingen, Germany.

We have simulated the discharges of retinal ganglion cells to hyperacuity stimuli in the human fovea. The image of the stimuli (a two-line vernier and a two-dot vernier) on the retina was computed due to the optical transfer properties of the human eye. The image was sampled by an array of cones similar to that found in the human fovea. Excitation of ganglion cells was determined by the spatial properties of their receptive fields. These were estimated on the basis of measurements of receptive fields in the parafovea of macaque monkeys. Considering the statistics of ganglion cell firing behavior we can predict the vernier thresholds of an observer exploiting the information in the signals of the ganglion cells. The results of the simulations show that the parvocellular-system cannot supply sufficient information to detect a vernier offset. In order to achieve hyperacuity thresholds of the range determined in human observers receptive field sizes of M-cells and the optical transfer function of the eye must be restricted to a limited range of variation. The 75% threshold for the detection of the relative localization of two lines or two dots then corresponds to a signal difference of one spike in two M-cells.

579.12

PHARMACOLOGY OF LIGAND-GATED CURRENTS OF CAT GANGLION CELL TYPES IN A RETINAL SLICE PREPARATION.

E.D. Cohen* and G.L. Fain Jules Stein Eye Institute, UCLA, Los Angeles, CA 90024-7008

We have examined the receptor pharmacology of ligand-gated currents of on- and off- α and β ganglion cells in a retinal slice preparation. Cat retinal slices were prepared by a procedure similar to Edwards et al. (Pflugers Arch. 1989 414:600-612) using HCO_3^- and HEPES-buffered solutions. Ganglion cells were voltage clamped in HEPES-buffered Ringer at -70mV. Agonists and antagonists were bath applied. Both on- and off- α and β types showed a similar distribution of receptors and pharmacology. In Mg^{2+}-free Ringer containing 200μM Cd^{2+}, the EAA agonist NMDA (200μM) elicited a large inward current. Application of 200μM AP7 totally blocked the NMDA-induced response. In 1mM Cd^{2+}, kainate (10-30μM), AMPA (10-70μM) and quisqualate (0.1-30μM) all induced inward currents. These responses were all blocked by the quinoxaline 10μM CNQX, though for quisqualate at concentrations of ≤3μM. Bicucculine MeCl (20μM) blocked the inward currents evoked by 200μM GABA on all cells. Baclofen, at (10-30μM) had no effect on voltage-gated Ca^{2+} or K^+ currents. Glycine (200μM) also elicited an inward current, and these currents were blocked by 1μM strychnine. Application of ACPD or L-APB had no effect. Supported by EY01844

AUDITORY SYSTEM: COCHLEA

580.1

THE MAMMALIAN COCHLEA EXPRESSES mRNA FOR SUBUNITS OF THE L-TYPE CALCIUM CHANNEL.

G. E. Green and D. G. Drescher*, Lab. of Bio-otology, Depts. of Otolaryngology and Biochemistry, Wayne State Univ. Sch. of Med., Detroit, MI 48201.

Dihydropyridine-sensitive, L-type calcium channels are composed of the subunits α_1, α_2/δ, B, and sometimes γ. The α_1 subunit forms a functioning channel pore while the remaining subunits modify channel properties. In the cochlea, dihydropyridine-sensitive calcium channels are thought to play important roles in hair cell function, including control of neurotransmitter release and modulation of outer hair cell length. Hair-cell calcium channels possess unusually rapid kinetics of activation and deactivation, properties which may be necessary for conveying phase information of sound. In the present work, we utilized reverse transcription and the polymerase chain reaction (PCR) to detect expression of mRNA encoding calcium channel subunits in the cochlea of the 16 day-old CBA_J mouse. PCR primers for each subunit class were derived from a pair of conserved sequences flanking a variable region of appropriate reference cDNA. Cochlear mRNA was reversed-transcribed and resulting cDNA was amplified by PCR under the following conditions: 94 °C for 45 s, 53 °C for 1 min, and 72 °C for 1.5 min, with an extension of 2 s/cycle, for 40 cycles. PCR products ranged in length from 400 to 1000 base pairs. A comparison of banding patterns for amplified cDNA from the cochlea with patterns for similarly amplified cDNA from brain, heart, skeletal muscle and lung demonstrated that the cochlea possesses a distinctive set of ß subunit variants. The cochlea was also found to express mRNA for the α_1 and α_2/δ, but not the γ subunits. The present results suggest that the unusual kinetic properties of the hair-cell calcium channel may be reflected in the characteristic set of calcium channel subunits found within the cochlea.

Supported by NIH Grant DC00156.

580.2

ASSOCIATION OF MINERALOCORTICOID BINDING SITES (TYPE I RECEPTORS) WITH COCHLEAR BLOOD VESSELS.

P.K. Sinha, W.S. Quirk and D.Z. Pitovski*. Department of Otolaryngology-Head and Neck Surgery, Wayne State University School of Medicine, Detroit, MI 48201.

Classical physiology has confined the action of mineralocorticoids to the modulation of ion exchange in the distal tubules of the kidney. However, studies have shown significant evidence for a direct action at the blood vessel level as inferred by the presence of mineralocorticoid (Type I) receptors in vascular tissue (Funder et al., *Endocrinology*, 125: 2224-2226, 1989; Stumpf, *Experientia*, 46: 13-25, 1990). We have now localized mineralocorticoid binding sites (Type I receptors) to cochlear blood vessels by light microscopic autoradiography.

Microdissected lateral wall tissue from the cochleae of male Hartley guinea pigs were incubated with [^{3}H]-aldosterone (60 nM) and a 500-fold excess of RU-28362 (30 μM), a specific glucocorticoid agonist to prevent low affinity binding to glucocorticoid (Type II) receptors. Specific binding was determined by introducing a competitive control containing an additional 2000-fold excess of cold aldosterone (120 μM). Tissues were then rinsed, fixed, dehydrated and embedded for sectioning. Sections were covered with autoradiographic emulsion and allowed to expose in light-tight boxes. Autoradiograms were developed and examined for grain densities with a computer-aided image analysis system.

Our results demonstrate a high density of silver grains associated with the capillary network of the lateral wall of the cochlea. The presence of specific mineralocorticoid binding sites (putative Type I receptors) suggests that mineralocorticoids play a role in the physiology of cochlear microvessels and may be a novel modulator of cochlear blood flow. (Supported by NIH CIDA DC 00046 to D.Z.P.)

580.3

CLONING NEURONAL RECEPTORS FROM THE SPIRAL GANGLION OF THE RAT. A.F. Ryan*[1,2] and A. Stein[1]. Division of [1]Otolaryngology and [2]Department of Neurosciences, UCSD Medical School and VA Medical Center, La Jolla, CA 92093.

There is considerable evidence that glutamate is the neurotransmitter between cochlear hair cells and spiral ganglion neurons. In a previous study, we found that of the several forms of non-NMDA glutamate receptors, only GluR2 and GluR3 could be detected by in situ mRNA hybridization in spiral ganglion neurons (Ryan et al., NeuroRep. 2: 643, 1991). To determine whether other non-NMDA glutamate receptor subunit isoforms are also expressed at levels undetectable by hybridization histochemistry, the spiral ganglion was isolated by rapid microdissection from the inner ears of young adult rats. mRNA was extracted and reverse transcribed. The resultant cDNAs were amplified by polymerase chain reaction (PCR) with probes specific for the 3' ends of GluR1-5 mRNAs. All five subunit isoforms were detected in the spiral ganglion with PCR. The results suggest that while GluR2 and GluR3 may be the most numerous non-NMDA subunit isoforms in the ganglion, other non-NMDA glutamate receptors are also expressed. They may contribute to spiral ganglion glutamate pharmacology by participating in hetero-oligomeric receptors.

Supported by grant DC00139 from the NIH/NIDCD and by the VA Research Service.

580.4

MOLECULAR ANALYSIS OF GLUTAMATE RECEPTOR EXPRESSION IN COCHLEAR AND VESTIBULAR GANGLION CELLS. A.S. Niedzielski* and R.J. Wenthold, Laboratory of Neurochemistry, NIDCD, NIH, Bethesda, MD 20892.

Ionotropic glutamate receptors are believed to mediate neurotransmission at the hair cell/eighth nerve synapse. On the basis of pharmacological and structural profiles of cloned receptor subunits glutamate-gated ion channels are presently categorized as being either AMPA, kainate or NMDA receptors. The AMPA receptors have been designated GluR1-4, the kainate receptors are classified as KA1&2 and GluR5-7, and the NMDA receptors are NMDAR1 and NMDAR2a-d. In situ hybridization, DNA amplification by PCR and immunocytochemistry were used to determine glutamate receptor subtype expression in the cochlear and vestibular ganglia of the rat.

The AMPA receptors GluR2-4, but not GluR1, were detected in inner ear neurons by in situ hybridization with subunit-specific oligonucleotides. Immunocytochemical studies using subunit-specific antibodies confirmed the presence of GluR2-4 in the cochlear ganglion. In addition, in situ hybridization and PCR analysis of cochlear and vestibular neurons detected high levels of mRNA for the NMDAR1 receptor and low or undetectable levels of mRNA for NMDAR2a-c. These results suggest that the neurons of the inner ear coexpress specific AMPA and NMDA receptor subtypes at the hair cell/afferent nerve synapse. Coexpression of AMPA, kainate and NMDA receptors may contribute to the unique pharmacology and physiology of the eighth nerve and may play a role in pathological conditions associated with hearing and balance disorders.

580.5

EVIDENCE FOR THE EXPRESSION OF A GLUTAMATE/ASPARTATE TRANSPORTER IN RAT COCHLEA. H.S. Li, A.S. Niedzielski, K.W. Beisel, H. Hiel, R.J. Wenthold and B.J. Morley*. Boys Town Nat'l Research Hospital, Omaha, NE 68131 and Neurochemistry Laboratory, Nat'l Institute of Deafness and Other Communication Disorders, Bethesda, MD 20892.

It is nearly certain that the neurotransmitter in the cochlear inner hair cells (IHCs) is an excitatory amino acid. Although there is substantial evidence to indicate that the neurotransmitter is glutamate, the data are not unequivocal. Unlike the brain, IHCs are not in juxtaposition to glial cells and therefore the mechanisms controlling glutamate neurotransmission may be unique. In preliminary studies using a rat cochlea cDNA library and cDNA synthesized from rat organ of Corti RNA we have found evidence for a proposed glial type of glutamate/asparate transporter (GLAST) in the cochlea. For these studies, DNA from a rat cochlea, unidirectional cDNA library constructed in the ZAP XR vector was screened by polymerase chain reaction (PCR) using degenerate primers complementary to the conserved sequences in the coding region of the glutamate transporter family. PCR amplification products were then sequenced. The resulting sequence demonstrated that the PCR amplification products were identical with that of GLAST. In a subsequent study, cDNA was synthesized from total RNA isolated from rat organ of Corti and was amplified by PCR using specific primers. Studies are in progress to localize the expression of GLAST in the cochlea.

580.6

DEMONSTRATION OF THE A_1 ADENOSINE RECEPTORS IN RAT COCHLEA. V. Ramkumar[1], R. Ravi[1], M. Wilson[1], T. Gettys[2], C. Whitworth[1] and L. P. Rybak*[1]. [1]Depts. of Pharmacology and Surgery, SIU School of Medicine, Springfield, IL 62794 and [2]Dept. of Medicine, Duke Univ. Medical Center, Durham, NC 27710.

Adenosine (A_1) receptors (A_1ARs) are found in a variety of tissues in the body where their physiological roles have been identified. In the cochlea, the existence of these receptors has not been described previously. Membranes prepared from rat cochleas demonstrate high affinity and saturable binding of [^{125}I]APNEA, an A_1AR agonist, with B_{max} and K_d values being 40.5 $\pm$ 0.5 fmol/mg protein and 1.28 $\pm$ 0.03 nM, respectively. Various adenosine analogs competed for [^{125}I]APNEA binding sites with an order of potency indicative of these sites being the A_1AR. Covalent labeling of the receptor protein by the agonist photoaffinity probe ([^{125}I]AZPNEA) identified a 36 kDa receptor protein, labeling of which was inhibited by theophylline, an antagonist of the A_1AR. [^{125}I]APNEA binding was significantly reduced following pretreatment of membranes with pertussis toxin, suggesting the interaction of these receptors with the G_i and/or G_o proteins in cochlear membranes. Activation of the A_1AR with R-PIA led to inhibition of forskolin-stimulated adenylyl cyclase activity. These data indicate the presence of an important inhibitory receptor in the peripheral auditory system which may play a role in modulating auditory functions.

580.7

ALTERATIONS IN COCHLEAR ELECTRICAL POTENTIALS BY INTRACOCHLEAR ATP AND ANALOGS. S.G. Kujawa, M. Fallon and R.P. Bobbin*. Kresge Hearing Research Lab., LSUMC, New Orleans, LA 70112

Several lines of evidence implicate a neuromodulator role for ATP in the cochlea. Previous experiments (Bobbin, et al., ARO Abstr. 16, 102, 1993) demonstrated reversible alterations in sound-evoked electrical and otoacoustic responses from the cochlea following intracochlear application of ATP. *In vitro*, outer hair cells are depolarized by ATP. The purinergic receptor mediating these effects remains unclear. Thus, we tested several ATP analogs for their effects on cochlear potentials.

Drugs (ATP, adenosine, 2-methylthio ATP, ATP-α-S, ATP-γ-S) were dissolved in artificial perilymph (3 μM-1 mM) and applied by perfusing the perilymph compartment of the guinea pig cochlea at 2.5 μl/min for 10 min. The compound action potential of the auditory nerve (CAP), cochlear microphonic (CM) and summating potential (SP) were recorded from an electrode in basal turn scala vestibuli. Potentials were evoked by 10 kHz tone bursts varied in intensity from 8-98 dB SPL in 6 dB steps.

CAP was suppressed and its latency increased, SP was suppressed at low and increased at high intensities and CM was essentially unchanged following perfusions. All effects were dose-responsive. The order of potency was: ATP-γ-S $\gg$ ATP > ATP-α-S > adenosine > 2-methylthio ATP. Results suggest that ATP, through activation of P2 purinergic receptors, may play a neuromodulatory role in shaping the cochlear response to sound.

(Supported by NIH DC00722, DC00379, & DC00007).

580.8

EMBRYONIC FORMS OF FIBRONECTIN mRNA ARE EXPRESSED TRANSIENTLY DURING POSTNATAL DEVELOPMENT OF THE RAT COCHLEA. N.K. Woolf*, D.V. Jacquish, F.J. Koehrn and V.L. Woods. Depts. of Otolaryngology and Medicine, UCSD Medical Center and the VA Medical Center, La Jolla, CA 92093.

We previously reported the reappearance of alternately spliced forms of fibronectin with embryonic mRNA patterns in the postnatal rat cochlea: fibronectin mRNA synthesis was detected in the organ of Corti at one week postpartum, but not at birth or two weeks postpartum. In order to clarify the relationship between fibronectin gene expression and the ontogeny of the inner ear, patterns of fibronectin synthesis were examined within the organ of Corti at two day intervals, from embryonic day 16 through two weeks postpartum. In situ hybridization with segment-specific probes revealed embryonic forms of fibronectin mRNA were expressed briefly after birth in the mesothelial cells lining the basilar membrane. Postnatal synthesis of the embryonic-type of fibronectin coincided temporally with a period for significant auditory nerve fiber growth, nerve-hair cell synaptogenesis and organ of Corti morphogenesis. The results suggest a functional role for fibronectin in postnatal cochlear development.

Supported by NIH DC139, DC386 & VA Res. Serv.

580.9

PRESENCE OF NEUROACTIVE SUBSTANCES IN THE POSTNATAL COCHLEA. D.D. Simmons*, H.D. Moulding, and H. Adi Klein. Dept. of Biology and Brain Research Institute, UCLA, Los Angeles, CA 90024-1606.

During development, neurons undergo specific chemical and structural changes as they attempt to reach their target cells. The present study focuses on specific immunocytochemical changes in the postnatal cochlea of hamster which may be related to efferent neurons originating from the superior olivary complex in the brainstem. Antibodies against calcitonin gene-related peptide (CGRP), growth associated protein (GAP-43), synaptophysin (SYN), and nerve growth factor receptor (NGFR) were used to probe for the presence of such substances histochemically as well as with western blots. CGRP was identified within the cochlear nerve at postnatal day (P) 4 but not underneath hair cells until P6. GAP-43 was detected both within the nerve and underneath hair cells by P4. SYN was never detected within the nerve but was found underneath hair cells by P5. There is also evidence for NGFR immunoreactivity around P6. It is possible that these data correlate with the progression of efferent development in the cochlea. At the earlier ages, there are at least two separate efferent populations: those still growing in the nerve and those with mature synapses under hair cells. Efferents containing CGRP may be the last to innervate the hair cells. Our data are consistent with the hypothesis that distinct efferent populations sequentially innervate the developing cochlea.

(Supported by grants from the NIDCD and the UCLA Academic Senate)

580.10

POSTNATAL DEVELOPMENT OF HAIR CELLS AND THE ORGAN OF CORTI IN THE HAMSTER. J.A. Kaltenbach*, and P. Falzarano, Dept. of Audiol., Dept. of Otolaryngol., Wayne State Univ., Detroit, MI 48201

A morphometric analysis of the developing organ of Corti and its component hair cells was carried out in an age-graded series of Syrian golden hamsters using the scanning electron microscope. The purpose was to establish a quantitative framework that would provide insight into the rules and principles by which the mammalian cochlea attains its adult proportions. This study examined the postnatal development at two day intervals spanning the period from birth to 22 days after birth. Our analysis included measures of cochlear length, hair cell numbers, as well as measures of hair cell sizes in each of 5 sectors along the cochlear spiral. Our results demonstrate several fundamental principles of cochlear development: 1) The full 2 1/4 turns seen in the adult cochlea are already present at birth, but the cochlea continues to elongate for the next 12-14 days; 2) Development of hair cells in the apex generally lags that in the base. This is apparent in that basal turn hair cells are already differentiated at birth while those in the apex become differentiated postnatally. 3) Growth in cochlear length occurs mainly by increases in cell size rather than in cell numbers; while hair cells do increase in numbers during the first 10 days of cochlear growth, this increase involves addition of hair cells to pre-existing regions of the cochlear apex only. Moreover, the full complement of hair cells is established 4 days before the full size of the cochlea is attained; in contrast, cochlear elongation involves growth in cell size at all positions along the cochlear spiral. 4) The period of hair cell growth exceeds the period of organ of Corti growth and appears to be possible by decreases in intercellular spacing, primarily in the apical region of the cochlea; 5) Inner and outer hair cell neighbors remain constant at different ages indicating that the spatial relationships between the two hair cell populations is preserved as the cochlea grows.

580.11

DEVELOPMENT OF EFFERENT CONTACTS IN THE POSTNATAL RAT COCHLEA. **L.L. Bruce* and M.A. Christensen. Dept. Biomedical Sci., Creighton Univ., Omaha, NE 68178.**

The development of functional efferent contacts in the maturing cochlea was studied with DiI. DiI moves transcellularly when the plasma membranes of two cells are in direct contact allowing the identification and study of newly formed contacts. DiI was placed in the contralateral or midline brainstem of perfused rats 0-22 days of age (P0-22) and incubated at 37°C for 6 wks. Labeled profiles appeared in the cochlea in a sequence comparable to that reported for efferents in previous studies. However, transneuronal labeling of the spiral ganglion and cochlear hair cells also occurred in an age dependent pattern. At P0 numerous spiral ganglia and inner hair cells (IHCs) were transneuronally labeled. By P2 most IHCs and occasional outer hair cells (OHCs) in rows 1 and 2 were labeled. By P22 only spiral ganglia were transneuronally labeled. Fewer labeled hair cells and spiral ganglia were labeled after contralateral implants (which directly labeled only medial olivocochlear axons) than midline implants. Electron microscopic analysis revealed densely labeled profiles (efferents) directly contacting both IHCs and afferents with limited areas of membrane labeling including the plasma membrane across from the efferent contact, tight junctions and endoplasmic reticulum. This indicates that efferents including medial olivocochlear fibers grow into the cochlea, rapidly make contacts with nearby afferents and IHCs and later with OHCs. During maturation these specialized junctions with hair cells but not with afferents are lost.

Supported by NIH grant P50-DC00215.

580.12

α-DIFLUOROMETHYLORNITHINE (DFMO) INHIBITS COCHLEAR FUNCTION AND POLYAMINE METABOLISM IN DEVELOPING RATS. C. Henley#, C. Whitworth†, L. Rybak†, B. Lonsbury-Martin*, #Dept. of Otorhinolaryngology, Baylor College of Med., Houston, TX 77030; † Depts. of Otolaryngology and Pharmacology, Southern Illinois U. Sch. of Med., Springfield, IL 62702; *Dept. of Otolaryngology, U. Miami Sch. of Med., Miami, FL 33101.

DFMO is an inhibitor of ornithine decarboxylase (ODC), a key enzyme in polyamine synthesis. Cochlear ODC activity increases during the maturation of hearing in neonatal rats (Henley, Hear Res 55:45-49,1991), and cochlear distortion-product otoacoustic emissions (DPOAEs), a measure of outer hair cell function, are significantly altered by DFMO (Henley, et al., J Cell Biochem, 14F:22, 1990). The purpose of this study was to correlate biochemical changes in cochlear polyamine metabolism with functional losses produced by DFMO. Rats were treated with DFMO (500 mg/kg/day) or saline during the period of increasing polyamine synthesis (days 1-10). ODC was determined by measuring the decarboxylation of ornithine and polyamines (putrescine, spermidine, spermine) were quantified by HPLC in cochlear tissues (stria vascularis, organ of Corti, cochlear nerve). Cochlear function was assessed using DPOAEs, click-evoked compound action potentials (CAP) and endocochlear potentials (EP). DFMO inhibited ODC in all cochlear tissues. DFMO depleted putrescine and spermidine in the organ of Corti and cochlear nerve which correlated with significant physiological deficits in DPOAEs and CAPs. In contrast, DFMO did not deplete polyamines in the stria vascularis nor did it affect EPs. Thus, biochemical effects of DFMO on putrescine and spermidine correlated well with functional deficits.

(Work supported in part by NIDCD/NIH Grants DC 00656, DC 00321 and ES03500. DFMO provided by Marion Merrill Dow, Cincinnati, OH).

580.13

AXONEMAL FORMS AND THE MORPHOGENETIC ROLE OF THE KINOCILIUM IN AUDITORY HAIR CELLS. H.M. Sobkowicz*, S.M. Slapnick and B.K. August, Dept. of Neurology, Univ. of Wisconsin, Madison, WI 53706 USA

Axonemal patterns in the auditory cilium ("kinocilium") were examined in 165 cross-sections. The study was done on the developing organ of Corti in the intact mouse and in culture, and on regenerating hair cells following mechanical injury to the organ in culture. The prevalent form (49%) of the auditory kinocilium consists of 9 + 0 microtubules. The 9 + 2 (single) form, previously thought to characterize the organelle, occurs only in about 6%. In the immature kinocilium, one of the peripheral doublets tends to move inward to form the modified 8 + 1 (double) configuration, resulting in an irregular microtubular distribution. The kinocilium is transitory, and it desintegrates around the tenth postnatal day. Exceptions are post-traumatic regenerating hair cells that reform their kinocilia during repair of their cuticular plates and regrowth of the stereocilia. Regenerating kinocilia repeat the initial developmental patterns.

During regeneration of the cuticular plate, the cytoplasmic area adjacent to basal bodies displays pericentriolar fibrous densities, growth vesicles, and microtubules, all surrounded by concentrating actin filaments. The growth vesicles may spread across the entire apical part of the cell. Pericentriolar bodies are associated with microtubules, evidently leading their nucleation. Microtubules appear to influence the alignment of actin filaments and participate in the formation of the filamentous matrix of the cuticular plate. We postulate that the enigmatic auditory kinocilium plays a morphogenetic role in the differentiation and regeneration of cuticular plates and stereocilia.

(NIH Grant R01 DC00844)

580.14

DEGENERATIVE PROCESSES ASSOCIATED WITH NEURONAL DEATH IN THE AGING SPIRAL GANGLION. M. L. Feldman*, Dept. of Anatomy & Neurobiology, Boston University School of Medicine, Boston, MA 02118.

The events leading to the death of spiral ganglion (SG) neurons were studied in an age-graded series of rat cochleas. Since these cells provide the entire auditory input to the CNS and exhibit marked age-related cell loss (Keithley & Feldman, '79), neuronal death in this locus is significant in understanding hearing loss in old age.

In contrast to age-dependent changes such as lipofuscin accumulation and axonal hypermyelination which have not been able to be clearly related to cell death, two frequently observed changes, affecting both Type I and II SG cells, appear to be lethal. Both may first appear in young adult animals. The first change involves the progressive enlargement of a cytoplasmic membrane-bound vacuole of unknown origin. This eventually compresses the nucleus and most of the perikaryon to the point where they occupy only a thin peripheral crescent under the perisomatic Schwann cell sheath, with further compression destroying the soma. The second change involves irregular vacuolization of the Schwann cell sheath, particularly within the innermost lamellae. This is followed by shrinkage of the soma, accompanied by distortion of the somal and sheath contours, and, eventually, disintegration of the neuronal plasma membrane. The cell body then degenerates and the sheath lamellae become severely disrupted. In the absence of an exogenous phagocytic cell, the neuronal debris probably is engulfed by the Schwann cell, forming a large lipofuscin deposit. The final marker of the former neuron is a thin shell formed by attenuated, spidery Schwann cell processes or the naked basal laminae extending from these processes. Further degenerative processes occur late in the lifespan. These include the formation of perinuclear skeins of packed microtubules and the development of multiple, irregular, non-membrane bound cytoplasmic lacunae. [Supported by NIH/NIA grant AG-06217]

580.15

DISTORTION PRODUCTS AT THE BASILAR MEMBRANE OF THE COCHLEA: DEPENDENCE ON STIMULUS FREQUENCY AND INTENSITY AND EFFECT OF ACOUSTIC TRAUMA. L. Robles*, M. A. Ruggero#* and N. C. Rich#. #Dept. of Otolaryngology, Univ. of Minnesota, Minneapolis, MN 55455, U.S.A., and *Depto. de Fisiología y Biofísica, Fac. de Medicina, Univ. de Chile, Santiago, Chile

Basilar membrane (BM) vibrations in response to two-tone stimuli contain distortion products (DPs) of cochlear origin (Robles et al., 1991, *Nature* 349: 413-414). We have further investigated the generation of DPs in the chinchilla cochlea using a laser velocimeter focused on glass microbeads placed on BM sites with characteristic frequency (CF) of 8-10 kHz. For primary tones (frequencies: f_1 and f_2) presented at equally-low levels, the magnitudes of the DPs ($2f_1$-f_2 or $2f_2$-f_1 = CF) decreased rapidly with increasing f_2/f_1; at high stimulus levels, DP magnitudes decreased slowly for small f_2/f_1 but fell precipitously at higher f_2/f_1 ratios. With increasing stimulus level, $2f_1$-f_2 DP phases tended to lag for large f_2/f_1 but changed little, or showed small leads, for small f_2/f_1. With fixed f_2/f_1 and constant (moderate) level of one primary tone, the $2f_1$-f_2 DP magnitude was a nonmonotonic function of the level of the other primary tone: it first increased at a faster-than-linear rate, peaked at f_1 levels 5-10 dB > f_2 levels and decreased at higher levels. Exposure of the ear to certain intense tones with frequency higher than CF induced substantial reductions (22-37 dB) of the magnitudes of the $2f_1$-f_2 (= CF) DP, while affecting the $2f_2$-f_1 (= CF) DP and responses to CF tones to a much smaller extent (7-13 dB). These results strongly resemble psychoacoustical findings and indicate that $2f_1$-f_2 DPs originate near the BM sites of their primary frequencies and propagate to locations with CF = $2f_1$-f_2. [Supported by NIH Grants DC-00110 and DC-00419, and FONDECYT (Chile) Grant 92-0976].

580.16

RESISTANCE TO NOISE-INDUCED HEARING LOSS IN THE MONGOLIAN GERBIL. F.A. Boettcher* and M.A. Gratton. Department of Otolaryngology, Medical University of South Carolina, Charleston, SC 29425.

The auditory system develops resistance to the ototraumatic effects of noise as a noise exposure is repeated. A model of resistance to noise-induced hearing loss (NIHL) will be described for the gerbil using the auditory brainstem response (ABR), distortion product otoacoustic emissions (DPOAE) and cochlear morphology. Young adult gerbils were exposed to an octave band of noise centered at 4 kHz on a 25% duty cycle. The average threshold shift at 8 kHz was 33 dB following the first 6-hour exposure, but was only 5 dB following the 12th daily exposure. ABR amplitudes were reduced and latencies were increased after the first exposure, but were near baseline levels following the 12th exposure. DPOAE amplitudes were reduced at 4-10 kHz on the first day of exposure but were similar to controls on the 12th day of exposure. Alterations in cochlear ultrastructure and biochemistry will be compared to physiological results in order to examine mechanisms of resistance to NIHL.
Work supported by NIH-DC-00422.

AUDITORY CORTEX I

581.1

DESCRIBING SCALP POTENTIAL DATA IN TERMS OF EQUIVALENT DIPOLE COMPONENTS WITH PHYSIOLOGICALLY PLAUSIBLE PARAMETERS: APPLICATION TO THE AUDITORY P50. H. L. Gillary*. Dept. of Physiology, Univ. of Hawaii, Honolulu, HI 96822.

A new procedure has been developed to describe segments of multi-channel scalp electrical potential data in terms of potentials generated on a homogeneous spherical volume conductor by parametric equivalent dipole current sources expected to mimic the real sources. It has been applied to an epoch of 14-channel band-pass filtered (10-50 Hz) auditory evoked potential data. The epoch of average-referenced data was segmented at time points of minimum variance (along channels) and dipole components (DCs) fit, using a simplex algorithm to minimize residual variance, to a tapered subepoch comprised of a segment corresponding to the P50 wave flanked by contiguous segments. Initially an 8-parameter DC with fixed location and orientation and a Gaussian time-dependent magnitude function was fit to each of the 3 segments, and subsequent DCs added by fitting each to the segment of the residual with maximum variance and then refitting all to the whole subepoch. Additional parameters were then added to DCs with peak latencies in the P50 segment to allow rotation and a more flexible magnitude function. The P50 segment was thus represented as the sum of a) DCs with peak latencies within it (primary DCs), b) the tails of DCs with peaks in adjacent segments, and c) a residual. When using 3 DCs with peaks in the adjacent segments, the respective residual variances for the P50 segment for 1) an 8-parameter primary DC, 2) a 10-parameter primary DC with linear rotation, or 3) 2 8-parameter primary DCs, were 11.5, 11.3, and 4.4 %. For all 3 cases, the ratio of the mean powers of summed primary DCs to summed tails was about 3.7. The above procedure can be adapted to longer subepochs of data. It is also amenable to modifications involving more realistic head and current source models and other minimization algorithms, and shows promise as a general means to unravel scalp potential data into parametric components that represent separable physiological sources in real heads.

581.2

MIDLATENCY AUDITORY EVOKED RESPONSES: A COMPARISON OF MEASUREMENT TECHNIQUES. R.J.Erwin*. Dept. of Psychiatry, Univ. of Penn., Philadelphia, PA 19104.

Five techniques for measuring the P1 component of the auditory evoked potential were contrasted using data obtained from 15 normals at the Cz recording site with click stimuli presented at 3 stimulus rates (1/sec, 5/sec and 10/sec). Both mandible reference and current source density derived (CSD) data obtained using 1-300 Hz and 10-300 Hz bandpasses were examined. The measurements were: 1) Nb to P1 peak to peak amplitudes; 2) prestimulus baseline to P1 peak amplitudes; 3) Integrated amplitudes based on a signed mean of amplitudes; 4) Integrated amplitudes based a principal component analysis (PCA) latency window; 5) PCA factor scores. Overall, allowing for scaling differences, most of the methods resulted in comparable results across stimulus rates (amplitudes diminished at faster rates) with some exceptions. For the CSD visually integrated measures, the amplitudes did not vary across rates, possibly due to the difficulty in determining boundaries. Both integrated and baseline to peak methods resulted in greater P1 amplitudes for the 1-300 Hz mandible data sets across rates and for the 10-300 Hz mandible data set at the 10/sec rate. These differences might be due to component overlap from preceding stimuli. Overall the findings suggest that a number of methodological factors need to be considered in the determination of P1 component amplitude.

581.3

CHANGES IN AUDITORY EVOKED POTENTIALS DURING SLEEP AND WAKEFULNESS IN THE RAT. Meneses-Ortega, S.*[1], Brailowsky S.[1], and Knight R.T.[2], [1]Instituto de Fisiología Celular and Facultad de Psicología, U.N.A.M., México, D.F., [2]Dept. of Neurology, Univ. of Calif., Davis, VAMC, Martínez, Calif. 94553, U.S.A.

Event-related potentials (ERPs) can assess the interrelationship between information processing and arousal level. The present study examined the effects of behavioral state on auditory evoked potentials. Experiments were carried out on male Wistar rats in which recording electrodes were placed over frontal and occipital cortex, and on the vertex. The reference electrode was placed on the far frontal sinus. Amplifiers were set at a bandpass of 1-300 Hz. Auditory ERPs were recorded in a sound attenuated chamber in response to clicks (square wave pulses, 0.1 msec duration, 50 dB above brainstem auditory evoked potentials threshold level). EEG and behavioral states were continuously monitored and recordings were made during waking (immobile and active) and during sleep (slow and REM sleep phases). Brainstem auditory evoked potentials (BSAEP, 0-5 msec) were unaltered by behavioral state. However, all middle and late components of the auditory evoked potentials, except for a negativity appearing at 17 msec latency (i.e. N17), were differentially affected by the behavioral state: during quiet waking a P30 component had maximum amplitude, whereas during slow wave sleep the N50, N80 and P130 components increased in amplitude. During REM sleep all components were reduced or abolished. No changes in the latency of the components were found. These findings demonstrate that the responsiveness to acoustic stimuli is modulated during sleep.

Supported in part by DGAPA-UNAM and the NINDS

581.4

BINAURAL VERSUS MONAURAL AUDITORY EVOKED POTENTIALS IN RAT NEOCORTEX

S. DI* and D. S. BARTH *Department of Psychology, University of Colorado, Boulder, Colorado 80309 USA*

High spatial resolution epicortical recording techniques and numerical modeling were used to investigate laterality effects on the middle latency auditory evoked potential (MAEP) complex.

Our data confirm previous reports that auditory stimulus laterality has a consistant effect on the amplitude, timing, and spatial distribution of the MAEP complex. The earliest temporal components (P1a, P1b and N1) show the greatest sensitivity, and are absent during ipsilateral stimulation. The later positive slow wave (P2) is present at the same amplitude during all stimulation conditions. Generation of the P2 appears to be independent of prior activation of areas 36 and 41 reflected in the early components, suggesting its generation by a more diffuse thalamocortical pathway, possibly from the medial division of the medial geniculate. Serial versus parallel activation of rodent auditory cortex is discussed in the context of laterality sensitive MAEP components.

581.5

THE RAT P1 AUDITORY MIDDLE LATENCY EVOKED POTENTIAL. H. Miyazato, R. D. Skinner*, E. Garcia-Rill, N. Reese and B. Hendricks. Department of Anatomy, University of Arkansas for Medical Sciences, Little Rock, AR 72205

The human P1 auditory middle latency potential is characterized by rapid habituation, sleep state dependence and is blocked by the cholinergic antagonist scopolamine. The P1 is abnormal in autism, Alzheimer's disease, schizophrenia and narcolepsy. Our aim is to identify and characterize the P1 potential in the rat in order to use this animal as a possible model for one or more of these diseases. Adult rats were implanted under anesthesia with skull screws for recording at the vertex bilaterally (1 mm lateral of the midline) and in the frontal sinus for reference. Rarefied click stimuli (64 trials) were used to average evoked auditory responses in alert rats in a sound attenuating chamber with background white noise. Thresholds for the early auditory evoked potentials (BAERs) was 50-55 dB and 70-75 dB for the P1 middle latency potential. The amplitude of the P1 potential was dependent on the frequency of stimulation (negative exponential). Using the averaged response at 0.2Hz as 100%, the averaged P1 amplitude was attenuated by 36% at 0.5Hz and by 81% at 2Hz frequency of stimulation. In a paired click paradigm (5 sec between trials), the amplitude of the averaged P1 response to the second click showed a linear relationship with the interstimulus interval (ISI). At ISIs of 1.0, 0.5 and 0.1 sec, the averaged response amplitude was 65, 44 and 10%, respectively, of the amplitude of the averaged P1 response to the first click. The cholinergic antagonist scopolamine reduced the P1 response in a dose dependent manner. Following i.p. injections of 5, 10 or 20 mg/kg, the amplitude of the averaged P1 response returned to within 1 standard deviation of the averaged control value (saline injection) at 45, 78 and 157 minutes, respectively. The latency of the P1 response in the rat peaked at 13 msec compared to 25 msec in the cat and 60 msec in the human. The characteristics of this potential (rapid habituation, state dependence and cholinergic modulation) in the rat match those evident in the cat and human at a longer latency.

Supported by NIH grants 21981 and 20246.

581.6

SURFACE EVOKED POTENTIALS MEASURED WITH A NOVEL MICRO-ELECTRODE ARRAY ON THE FERRET PRIMARY AUDITORY CORTEX.

A.L. Owens, S.A. Shamma*, T.J. Denison. Institute for Systems Research and Dept. of Electrical Engineering, University of Maryland, College Park, MD 20742.

In order to elucidate the general principles that the primary auditory cortex (AI) uses to process sound stimuli, we have mapped the response areas in AI with a single tungsten electrode. Though quite useful in recording electrical activity, the method limits the amount of available data to the point of insertion. Hence, numerous insertions of the electrode are required to fully map the spatial properties of the cortical response areas. In addition, the time involved adds an unknown variable as the condition of the animal changes.

To circumvent these problems, we have developed a multi-electrode array using the techniques of silicon IC technology. A 5 X 5 matrix of gold electrodes with 210 μm spacing between elements has been fabricated. Leads exit the array and travel 5 cm down a polyamide shank to a series of gold contact pads which provide a junction to external preamplifiers. The gold electrodes, leads, and contact pads are sandwiched between two layers of polyamide, which serves as a flexible insulator. Small holes are opened in the polyamide to access the electrodes and contact pads. When placed on the surface of the cortex, the flexible probe conforms to the shape of the brain and allows for the recording of cortical activity over a 1E+6 μm^2 area.

The micro-electrode array is used to generate surface maps of the following evoked potential parameters: best frequency for response, latency of response peak, excitatory bandwidth, and sensitivity to frequency sweep direction. Our results indicate that maps of the spatial distribution of the these parameters correlate well with data from unit clusters. These parameters are important for modeling sound processing at the cortical level.

581.7

INHIBITION OF DISCHARGE COUPLED TO A DESCENDING PATHWAY IN THE AUDITORY SYSTEM OF CATS.

M. Piesman, E. Chao, E. Gruen, C. Woody, E.G. Zotova*. UCLA Med. Ctr., Los Angeles, CA 90024.

Recordings of single unit activity were obtained from 457 neurons of the auditory system of conscious cats given 1 ms binaural click stimuli. A temporally related reduction of discharge was disclosed at three functionally related areas of the auditory pathway. Of 66 neurons studied in the secondary auditory cortex, 46 neurons (70%) showed inhibition of discharge during the 48-56 ms latency period after click. The posterior ectosylvian region of the auditory cortex showed a significant decrease in firing in the 40-56 ms post stimulus period, having 27 (64%) out of 42 cells with reduction of activity. In the inferior colliculus, a smaller but still significant percentage of cells (22%, 16 out of 72 cells) had decreased unit activity in the period 56-76 ms after click. Inhibition of discharge was measured by comparing numbers of cells with decreased firing in the experimental periods versus pre and post click control periods. The primary auditory cortex and the dorsal and ventral cochlear nuclei were also analyzed for comparable periods of inhibition, but none were found. These findings and the latency periods at which they were found led us to conclude that the inhibition was traveling in a descending direction.

581.8

SPATIAL FREQUENCY FILTERS IN CAT AUDITORY CORTEX.

B. M. Calhoun, C. E. Schreiner*. W.M. Keck Center of Integrative Neuroscience and Coleman Laboratory, UCSF, San Francisco, CA 94143.

Vocalizations and many other naturally occurring sounds are characterized by broad-band spectra with non-uniform spectral envelopes. We are currently investigating the processing of complex signals by cortical neurons and are using broad-band stimuli to determine receptive field properties.

One of the most distinguishing aspects of sounds is their spectral envelope. To allow a systematic approach for studying influences of spectral envelopes on the response characteristics of cortical neurons, 'ripple spectra' were generated. This type of stimulus has four main advantages: 1) it can be easily described by its parameters, 2) it has several characteristics in common with visual gratings used to study the spatial frequency processing in the visual system, 3) like vowels, it has a spectrum displaying several frequency regions with increased spectral energy (formants) separated by regions of decreased spectral energy, and 4) the ripple stimulus is similar to a single 'spatial frequency' in the spatial domain of the basilar membrane. By varying the ripple density, the 'spatial' filtering characteristics of the neuron can be determined. The standard stimulus characteristics used are: a harmonic series (f_0 = 50 to 200 Hz) with a 6 dB/octave decline of the component amplitudes (120 to 255 components) as carrier; a bandwidth of 3 octaves; the spectral envelope of the signal is represented by a sinusoid on a logarithmically scaled frequency axis; the frequency of the envelope sinusoid is referred to as ripple density (ripples/octaves); the modulation depth of the envelope (ripple depth) is linear on a dB scale.

The ripple transfer function is found by systematically varying the ripple density for two different envelope phases (0 and 90 degree). The resulting complex ripple transfer function is usually bandpass. A 'best ripple density' can be defined that is usually between 0.6 and 4 ripples/octave. The fourier transform of the complex ripple transfer function is a spatial function similar to the excitatory/inhibitory receptive field obtained with two-tone stimuli. This new method for obtaining filter functions of cortical neurons takes into account the interaction of more complex spectral features. By comparing spatial frequency filtering qualities of neurons obtained with narrow-band and broad-band stimuli, some filtering properties of central auditory neurons can be more thoroughly characterized. (Work supported by ONR Grant N00014-91-J-1317)

581.9

ORGANIZATION OF PRIMARY AUDITORY CORTEX EVIDENT IN RESPONSES TO RIPPLED COMPLEX SOUND STIMULI.

S.A. Shamma, H. Versnel* and N.A. Kowalski. Institute for Systems Research and Department of Electrical Engineering, University of Maryland, College Park, MD 20742.

We hypothesize that the primary auditory cortex (AI) encodes the profile of the acoustic spectrum by performing a *local* Fourier-like transformation, much the same way as in the primary visual cortex (VI) (De Valois and De Valois, Spatial Vision, Oxford Press, New York, 1990). In VI simple cells are tuned to the frequency and phase of sinusoidal visual patterns and from the frequency tuning curve, the receptive field (space domain) of a cell can be predicted by reverse Fourier transformation. In an analogous manner, we recorded responses of AI units to broadband sound complexes with sinusoidally modulated spectral profiles (ripple) in the ferret. The ripple frequency (expressed in cycles/octave), and the ripple phase were varied.

The results show that AI neurons are highly selective to ripple frequency and phase. Most cells are tuned to frequencies between 0.2 and 2 cycles/octave. The reverse Fourier transforms of the ripple tuning curves closely correspond to excitatory and inhibitory response areas determined with two-tone stimuli (Shamma, Fleshman, Wiser and Versnel, J. of Neurophysiol. 69: 367-383, 1993). This correspondence can be quantified in terms of best ripple frequency and phase. The spatial mapping of these parameters along the isofrequency axis is also described.

581.10

REPRESENTATIONS OF NATURAL AND SYNTHETIC VOCALIZATIONS IN THE PRIMARY AUDITORY CORTEX OF AN ADULT MONKEY.

X. Wang, R. Beitel*, C.E. Schreiner and M.M. Merzenich. Keck Center for Integrative Neuroscience and Coleman Laboratory, UCSF, San Francisco, CA 94143-0732.

As the first step in a series of experiments designed to determine the neural representations of communication sounds and the cortical plasticity that creates, modifies and sustains them, we studied responses of neuronal populations in the primary auditory cortex (AI) of anesthetized common marmosets (*Callithrix*), a New World monkey with a complex vocal repertoire, to behaviorally important natural vocalizations and their synthetically-created variations. Experimental stimuli included three specific vocalizations frequently produced by each monkey and a conspecific companion, and synthetic variations of these three vocalizations, in which the temporal or spectral features of these natural vocalizations were systematically altered. The distributed cortical representations of natural and synthetic time- or frequency-modified stimuli were then determined, using microelectrode mapping techniques in field AI.

Natural vocalizations evoked neuronal discharges that were synchronized much more closely to stimulus events -- and to the responses of other engaged cortical neurons -- than did synthetic vocalizations. Such preference for the time signature of natural vocalizations was observed for nearly all units that responded to these complex stimuli. Correspondingly sharp selectivity to spectral features was also recorded in some but not all studied hemispheres. Recorded response selectivity was marked by sharp continuum boundaries. Equally strong preference was recorded for the vocalizations of a socially important conspecific, e. g., a companion or mate.

These results suggest that: 1) Species-specific vocalizations are represented by the activities of distributed neuronal populations in AI. 2) The selective representations of specific natural vocalizations -- e. g., the highly specific representations of the vocalizations of a particular conspecific companion -- can emerge by the operation of plastic mechanisms in adult monkeys. 3) The specificity of these representations may reveal operation of mechanisms accounting for the categorical discrimination of communication sounds. (Work supported by the Coleman Fund, HRI, NIH Grant NS-10414 and ONR Grant N00014-91-J-1317)

581.11

SPATIAL REPRESENTATION OF PERIODICITY PITCH IN THE HUMAN AUDITORY CORTEX

[1]G. Langner*, M. Sams, [1]P. Heil, L.K. McEvoy, R. Hari, and A. Ahonen
Low Temp.Lab., Helsinki Univ.Technol., 02150 Espoo, Finland
and [1]Zoological Inst., TH-Darmstadt, 6100 Darmstadt, Germany

Neurons in the inferior colliculus of cats respond selectively to the modulation period of amplitude modulated sound and the preferred modulation periods are mapped orthogonally to the tonotopic gradient (Langner and Schreiner, 1988, *J. Neurophysiol.* 60, 1799 - 1840). However, the topographic representation of periodicity in the cortex is unknown as well as the representation in the human auditory system. Therefore, we utilized magnetoencephalography to study the responses of the human cortex to periodicity pitch stimuli.

Neuromagnetic fields were recorded from 5 subjects with a 122-channel neuromagnetometer which covers the whole head. The acoustic signals were composed of harmonics and had pitches from 50 Hz to 400 Hz. To avoid spectral cues as far as possible, all signals were synthesized with flat spectral envelopes and cutoff-frequencies of 400 and 5000 Hz. The 500-ms signals were presented monaurally.

The sounds elicited a 100-ms deflection (N100m) followed by sustained field (SF) in the supratemporal auditory cortex. The source of SF moved as a function of pitch about 10 mm rostro-caudally. Since spectral cues in the sounds were minimized, this may indicate that periodicity pitch is mapped in the human auditory cortex.

581.12

TEMPORAL PITCH MIGHT BE ALREADY PLACE-CODED TOGETHER WITH PLACE PITCH IN THE PRIMARY AUDITORY CORTEX OF THE JAPANESE MONKEY.

H. Riquimaroux*, T. Takahashi and T. Hashikawa, Neural Systems Lab., Frontier Research Program, Inst. of Physical and Chemical Research (RIKEN), Wako, Saitama 351-01, Japan.

The present study has examined whether the temporal pitch known as the missing fundamental is co-place-coded with the place pitch in the auditory cortex. Adult Japanese monkeys (*Macaca fuscata*) were used. All surgeries were aseptically performed with anesthesia. A chamber was placed for chronic recordings on the left temporal part of the skull. The stimuli used were white noise bursts, tone bursts and bursts of a combination of higher harmonics of a low frequency. They were presented to the animal's right ear. Unit recordings were made with glass-coated Elgiloy electrodes from the primary auditory cortex (AI). During recordings, the animal was anesthetized with a mixture of nitrous oxide and oxygen, supplemented by ketamine and xylazine injections. PST (post-stimulus-time) histograms were analyzed. The present data have confirmed previously reported tonotopicity, low frequency anteriorly while high frequency posteriorly, in the Japanese macaque's auditory cortex when the recording time window was short (< 20 msec) and the stimulus was at threshold levels. In the low frequency area (< 500 Hz), the same neuron responds both to the best frequency (BF) and to combinations of successive higher harmonics which create a temporal periodicity identical to the BF, but not to these higher harmonics themselves. The findings suggest that the temporally-coded pitch in the periphery appears to be already place-coded together with the place pitch by the same neuron to produce an identical pitch at AI. Thus, this evidence agrees well with previous psychoacoustical findings.

AUDITORY CORTEX II

582.1

NEURONAL RESPONSES TO FREQUENCY MODULATED SOUNDS IN THE POSTERIOR AUDITORY AREAS OF THE CAT'S CORTEX.

B. Tian* and J.P. Rauschecker. Laboratory of Neurophysiology, NIMH, Poolesville, MD 20837, U.S.A.

Frequency modulated (FM) sounds are important components of auditory communication signals in many different species, including birds, bats, and primates. In addition, FM sweeps are the auditory equivalent to moving light stimuli in the visual domain, which are most effective in driving visual cortical neurons. Last year (Tian and Rauschecker, *Soc. Neurosci.* 1992), we reported about the responses to FM sounds of neurons in the cat's anterior auditory field (AAF). This year we have extended the same approach to the study of the posterior auditory cortical areas.

Extracellular single unit recording was performed in areas PAF, VPAF, and DPAF of cats under halothane anesthesia. Neurons were assigned to different areas on the basis of histological track reconstructions (Reale and Imig, 1980). Most neurons (n=47) were recorded from area PAF. Of all neurons in the posterior auditory areas 24% (16/71) did not respond to pure-tone stimulation. However, virtually all of these same neurons (15/16=94%) responded well to FM sweeps with rates of 4 to 600 Hz/ms. In contrast to the anterior areas, only 27% of the units (18/66) responded best to the higher FM rates (high-pass neurons). About 60% of the neurons (39/66) showed band-pass characteristics with a preference for a certain range of FM rates, 8 % (5/66) were low-pass units. Cells were considered FM-direction selective, if the response in one direction was at least twice as large as the response in the reverse direction. Almost one half of the cells (32/66) in the posterior areas showed FM-direction selectivity, when this criterion was applied.

While the anterior auditory areas of the cat's cortex seem to specialize in the processing of fast-changing transient sounds, neurons in the posterior cortical areas are more often band-pass tuned to the rate of frequency modulation. This suggests an even higher degree of specialization in the process of feature extraction for the purpose of auditory communication.

582.2

NEURAL PROCESSING OF FREQUENCY MODULATED (FM) SWEEPS IN CAT PRIMARY AUDITORY CORTEX (AI).

J.R. Mendelson*[1], C.E. Schreiner[2], K.L. Grasse[3], M.Sutter[4], [1]Div. Life Sciences, Univ. of Toronto, Scarborough,Canada; [2] Dept. Otolaryn., UCSF Medical School, San Francisco, CA, [3]Dept. Psych., York Univ., North York, Canada, [4] Dept. Organismal Biology, Univ. of Chicago, Chicago IL

FM sweeps are an essential feature of naturally occurring auditory sounds such as communication signals and sound sources that move relative to an observer. We have previously shown that neurons in cat AI often manifest a preference for a particular *speed* (i.e., rate of change of frequency over time) and/or *direction* (i.e, changing from a low to a high frequency, or vice versa) of FM sweep. In addition, we have shown that these responses are systematically distributed along the dorsoventral extent of AI. To further examine FM sweep selectivity in AI, we have investigated the functional relationship between unit responses to FM sweeps, tone bursts and click stimuli along the dorsoventral axis of AI. To this end, single and multiunit activity were recorded from cat AI. Log FM sweeps (0.2-64.0 kHz) were presented in two directions at three different speeds. We found a number of response properties that were significantly correlated with preferred direction and/or speed of FM sweep. The results showed that direction selectivity was significantly correlated with integrated excitatory bandwidth (Q10 dB and Q40 dB), nonmonotonicity, and latency. Preferred speed was significantly correlated with integrated excitatory bandwidth, threshold, nonmonotonicity, click response and binaural response type. The functional relationships observed between these response parameters may reveal additional neural mechanisms underlying FM sweep selectivity in auditory cortex.

582.3

PROPERTIES OF VIRTUAL-SPACE RECEPTIVE FIELDS OF NEURONS IN CAT PRIMARY AUDITORY CORTEX.

J.F. Brugge*, R.A. Reale, J.E. Hind, J.C.K. Chan, A.D. Musicant and P.W.F. Poon Dept. Neurophysiology and Waisman Center, Univ. Wisconsin, Madison WI 53706.

We synthesized a set of signals (clicks) for earphone delivery whose waveforms and amplitude spectra, measured at the eardrum, mimic those of sounds arriving from a free-field source. The complete stimulus set represents 1800 sound-source directions, which together surround the head to form a 'virtual acoustic space' for the cat. Under barbiturate anesthesia neurons in AI cortex exhibit sensitivity to the direction of a sound in virtual acoustic space. The aggregation of effective sound directions forms a virtual space receptive field (VSRF). At 20 dB above minimal threshold, VSRFs fall into one of several categories based on spatial dimension and location. Most VSRFs were confined to either the contralateral (59%) or ipsilateral (10%) hemifield. Seven percent spanned the frontal quadrants and 16% were omnidirectional. Eight percent fit into no clear category and were termed 'complex'. The results are in essential agreement with free-field studies. The size, shape, and location of VSRFs remained stable over many hours of recording. VSRFs are shaped both by spectral information extracted by the cochlea and auditory nerve and by excitatory and inhibitory interactions of activity arriving from the two ears. Response strength and timing are not uniform throughout the VSRF. These features may encode additional directional information.

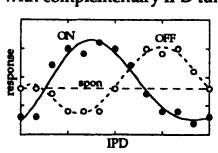
(Supported by NIH Grants DC00116 and DC00398)

582.4

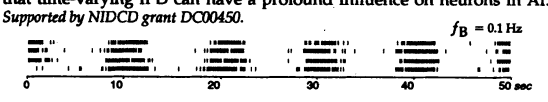
INTERAURAL DELAY TUNING IN CAT AI UNDER STATIC AND TIME-VARYING CONDITIONS.

M.N. Semple* and M.W. Spitzer. Dept. of Anatomy & Neurobiology, Univ. of California, Irvine, CA 92717.

Most acoustic environments give rise to ongoing fluctuations in interaural phase disparity (IPD), a primary cue for sound localization. We studied single-unit responses to IPD in primary auditory cortex (AI) of lightly-anesthetized cats, under static and time-varying (IPD_d) conditions. Dichotically presented tones were calibrated for amplitude and phase; IPD_d was created with binaural beats or by phase-modulating the signal at one ear. Static stimuli often elicited ON- and OFF-responses with complementary IPD tuning (Fig.1). Most statically-tuned cells (and some that were uninfluenced by static IPD) were highly responsive to IPD_d. Under some conditions many neurons responded vigorously throughout a portion of each IPD cycle, maintaining much higher response rates than could be elicited statically. At low beat frequencies (f_B) such responses were sustained for several *seconds* (Fig.2). In contrast, only 1-2 spikes were elicited in a given cycle with $f_B > 2$ Hz, i.e., at very high rates of simulated motion (> 720°/s). With IPD sweeps restricted to depths of 45°, responses could often be elicited beyond the limits of the statically determined IPD tuning curve. These results reveal that time-varying IPD can have a profound influence on neurons in AI.



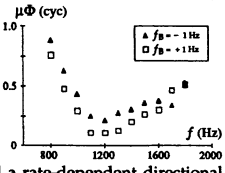
Supported by NIDCD grant DC00450.



582.5

HETEROGENEOUS EFFECTS OF FREQUENCY, LEVEL AND DYNAMIC STIMULUS PARAMETERS ON INTERAURAL DELAY TUNING IN CAT AI. M.W. Spitzer* and M.N. Semple. Dept. of Anatomy & Neurobiology, University of California, Irvine, CA 92717.

Interaural phase disparity (IPD) is but one of many stimulus attributes that affect the discharge of neurons in low-frequency primary auditory cortex (AI). To assess IPD tuning of individual neurons across a broad range of stimulus conditions, we presented static and time-varying IPD stimuli, dichotically, to lightly-anesthetized cats. We systematically varied tone frequency (f), sound pressure level (SPL), modulation rate (or beat frequency, f_B) and direction of simulated motion. For some units sensitivity to IPD was evident throughout the entire response area, whereas for others it was restricted to a limited portion of the response area. In a few cases the relation between mean phase ($\mu\Phi$) and f was a linear function whose slope could be used to infer a *characteristic delay*. More typically $\mu\Phi$ was a complex function of f, sometimes consisting of two linear sub-components (see Fig.). The $\mu\Phi$ vs f curve was shifted as a function of SPL for some neurons, but more often it was unaffected. We commonly observed a sytematic vertical shift of the $\mu\Phi$ vs f curve when the direction of modulation was reversed. Some neurons only responded to modulation in one direction, or showed a rate-dependent directional preference. The heterogeneous effects of f, SPL, modulation direction and rate on delay-tuning suggest a subdivision of IPD-sensitive cortical neurons into multiple response classes. *Supported by NIDCD grant DC 00450.*



582.6

COMPARISON OF AZIMUTH TUNING OF DIFFERENT CLASSES OF SINGLE UNITS DISTINGUISHED BY UNILATERAL EAR OCCLUSION IN PRIMARY AUDITORY CORTEX (AI) OF BARBITURATE ANESTHETIZED CATS. F.R. Samson, P. Barone, J.C. Clarey, and T.J. Imig*. Dept. of Physiol., Kansas University Med. Ctr., Kansas City, KS 66160-7401.

Azimuth-sensitive neurons (n=131, BFs 5 - 24kHz) were identified by their responses to noise bursts that varied in azimuth and over a broad range of sound pressure levels. Two main classes of neurons were distinguished using unilateral ear occlusion (plugging) that simulates monaural stimulation. Monaural directional (MD) cells (27/131) remained azimuth sensitive and most exhibited relatively minor changes in azimuth tuning with unilateral ear plugging, whereas binaural directional (BD) cells (53/131) became insensitive to azimuth, unresponsive, or exhibited substantial changes in azimuth tuning. Fifty-one cells were excluded from the analysis as their responses were unreliable. Ear plugging revealed that BD cells may exhibit excitatory/inhibitory (EI cells, 23/53), facilitatory (predominantly binaural or PB cells, 13/53), or a mixture of facilitatory and inhibitory (FI cells, 17/53) binaural interactions. MD, EI and FI cells exhibited lateral azimuth preferences. A large majority of MD and EI cells exhibited contralateral preferences, whereas FI cells were evenly split between contralateral and ipsilateral preferences. EI and FI cells were significantly more broadly tuned in azimuth than MD cells. PB cells responded preferentially to azimuths near the midline. Supported by NIDCD grant # DC00173.

582.7

MULTIPLE REPRESENTATIONS OF VELOCITY INFORMATION IN THE AUDITORY CORTEX OF THE MUSTACHED BAT. H. Teng* and N. Suga. Department of Biology, Washington University., St. Louis, MO 63130.

Constant-frequency components (CF_{1-4}) of biosonar pulses emitted by the mustached bat, *Pteronotus parnellii*, carry information of relative target velocity. In the auditory cortex of this species, the CF/CF area contains neurons specialized for the extraction of velocity information from certain combinations of CF components in pulse-echo pairs. Recent studies with anatomical tracers by J. Olsen and D. Fitzpatrick show that the CF/CF area projects to a small area, named the DIF area, which is located at the posterior bank of the dorsal portion of the fossa for the sylvian artery. The aim of this study was to examine the response properties of DIF neurons, and to compare these with the properties of CF/CF neurons.

Single-unit responses were recorded from the DIF and CF/CF areas with tungsten-wire electrodes. DIF and CF/CF neurons were sharply tuned to a particular combination of the pulse CF_1 and the echo CF_n (n=2 or 3) in frequency. They were suited for extracting velocity information. They were not suited for processing range information since they were broadly tuned to time intervals between the pulse CF_1 and the echo CF_n. There was no significant difference in sharpness of frequency or delay tuning between them. However, DIF neurons were different from CF/CF neurons in the following three aspects. (1) The best velocity was about 4 m/s faster for DIF neurons than for CF/CF neurons. (2) Most DIF neurons showed stimulus-locked responses only up to 20 paired stimuli/s, while most CF/CF neurons showed such responses up to 40 stimuli/s. (3) The best CF_1 amplitude and threshold for facilitation were about 20 dB higher for DIF neurons than for CF/CF neurons. Therefore, DIF neurons are suited for processing high relative velocities when the bat is emitting loud pulses at low repetition rats, whereas CF/CF neurons are suited for processing velocity information in the late search and approach phases of insect pursuits. (Work supported by NIDCD research grant DC00175)

582.8

FACILITATION OF CORTICAL DELAY-TUNED NEURONS IN THE FM-FM AREA IS NOT MEDIATED BY NMDA WITHIN THE CORTEX. A. Tanahashi, N. Suga*, Department of Biology, Washington University, St. Louis, MO 63130

The FM-FM area in the auditory cortex of the mustached bat, *Pteronotus parnellii*, receives a projection from the dorsal division of the medial geniculate body (MGBd) and is specialized for the representation of target distances between 7 and 310 cm. FM-FM neurons show a facilitative response to a combination of FM components in pulse-echo pairs. Each FM-FM neuron is tuned to a particular echo delay and echo amplitude. Neurons in the MGBd also show facilitative response. Their facilitative response greatly depends upon NMDA (N-methyl-D-aspartate) (Butman, 1992). The aim of the present study was to examine whether the responses of cortical FM-FM neurons simply reflect the responses of thalamic delay-tuned neurons or are enhanced by an additional NMDA mediated facilitation in the cortex.

In four unanesthetized mustached bats, single unit activity was recorded from 20 FM-FM neurons with multi-barreled carbon filament microelectrodes. APV (D-2-amino-5- phosphonovalerate), an antagonist of the NMDA receptor, was iontophoretically applied at the recording site. APV were applied with an electric current of -50 nA for 10 min and neuron activity was recorded before, during and after the application. This amount of APV was sufficient to suppress completely a burst of discharges evoked by an NMDA locally applied through the multi-barreled electrode. However it did not change delay-tuned facilitation of any of the 20 neurons studied. Our results indicate that NMDA is not related to facilitative responses observed in the FM-FM area.
(Work supported by a research grant NIDCD DC00175)

582.9

Tonotopic Organization Of Temporal Response Patterns Reflecting The Voice Onset Time (VOT) Phonetic Parameter In Primary Auditory Cortex (A1). M. Steinschneider*, C.E. Schroeder, J.C. Arezzo and H.G. Vaughan, Jr., Albert Einstein College of Medicine, Bronx, NY, 10461.

Delineating the spatio-temporal distribution of cortical speech-evoked activity encourages a neurophysiologic framework for understanding speech perception. We investigated the relationship between temporal response patterns encoding VOT with the tonotopic organization of A1 by examining the current source density and multiunit activity evoked by the syllables /da/ and /ta/ and by tone bursts in 2 awake monkeys.

Two temporal response patterns encode VOT. In the first, responses are time-locked to stimulus and voicing onset. Responses to the latter speech segment are usually present for VOTs of 40 and 60 msec (/ta/) and markedly diminished for a VOT of 20 msec (/da/). These responses correlate with the perceptual boundary and occur in low best-frequency locations. In contrast, the second pattern contains a component time-locked to stimulus onset followed by activity phase-locked to the syllable periodicity. This pattern does not correlate with the perceptual boundary and occurs at higher best-frequency sites.

We conclude that temporal patterns encoding VOT are segregated on the basis of the cortical tonotopic organization. The first pattern reflecting the perceptual boundary mirrors similar responses in the auditory nerve (Sinex et al.,1991), suggesting that the response is generated in the auditory periphery and transmitted to the cortex. This neural pattern helps reconcile hypotheses emphasizing temporal versus spectral aspects of VOT encoding. (supported by DC00657, MH06723 and the J.S. McDonnell Foundation)

582.10

CORTICAL NEURAL NETWORKS IN TONONOPICAL ORGANIZATION OF GUINEA PIG AUDITORY CORTEX REVEALED BY OPTICAL IMAGING AND ANALYSIS. K.Fukunish*, N. Murai, H. Uno, Advanced Research Laboratory, Hitachi, Ltd., Hatoyama, Saitama, 350-03 JAPAN.

Previously, the spatio-temporal neural activity evoked by clicks and pure tones in the guinea pig auditory cortex were observed using a 128-channel optical recording system with voltage-sensitive dye by the authors. The functional module were found in the dynamical neural traversing in the cortical field to click responses. In addition, the spatial relations between the responses to clicks and tones were discussed.

Here, the neural network structures of the tonotopical organization in the guinea pig primary auditory cortex are studied by observing evoked responses to the tone bursts. The temporal neural activities in the tonotopical patterns are analyzed by time series analysis using MAR (multivariable autoregressive) model. The results reveals the existence of synchronized oscillatory neural activities of about 30 Hz in the response regions to the tone bursts. The oscillatory sources can be obtained by decomposing the power spectral density to its contribution at each region by this method. The distribution pattern of the dominant sources (the cortical region) of the neural oscillation are identified. The existence of the mutual neural connections in the cortical field are found along the tonotopical band from the dorsal to the ventral. On the other hand, the oscillation may be propagated from the source region to the cortical region in the caudal or the rostral direction with one way neural connections. Thus neural networks of the tonotopicity in the cortex can be estimated by spatio-temporal neural observation and analysis.

582.11

TONOTOPIC ORGANIZATION OF GUINEA PIG AUDITORY CORTEX DEMONSTRATED BY INTRINSIC SIGNAL OPTICAL IMAGING THROUGH THE SKULL. J. S. Bakin*, M. C. Kwon, S. A. Masino, N. M. Weinberger, and R. D. Frostig, Center for the Neurobiology of Learning and Memory, and Department of Psychobiology, University of California, Irvine, CA 92717

Optical imaging of intrinsic signals provides an activity-dependant high spatial resolution image of the functioning cortex. Here we report the first successful intrinsic signal optical imaging of primary auditory cortex (AC). Moreover, imaging was obtained through a thinned but intact skull.

The AC of sodium pentobarbitol anesthetized adult male Hartley guinea pigs was imaged during random presentation of binaural tone bursts of several frequencies at various intensities. Immediately following several replications of the optical findings, the AC was mapped electrophysiologically (tungsten microelectrode, ≈1MΩ). For each cortical site 1) neural responses to the identical auditory stimuli used during imaging were determined, and 2) receptive fields (RF) were characterized by presenting an isointensity series of tone bursts (100ms on, 900ms off, 10ms rise/fall time) using a frequency range appropriate for the given units (selected from a range of 0.5 to 40.0kHz) and repeated across a large intensity range (0 to 90 dB).

Optical imaging revealed a tonotopic frequency organization. This organization was confirmed by determination of RFs; i.e., at a given site, optical and electrophysiological responses were evoked by the same frequency. These results provide the basis for application of optical imaging to the functioning of auditory cortex, including, but not limited to, its modification by learning and other related processes.

Supported by The Beckman Foundation (RDF) and the Office of Naval Research N00014-91-J-1193 (NMW).

AUDITORY SYSTEM: CENTRAL ANATOMY—MIDBRAIN, THALAMUS, AND CORTEX

583.1

TWO POPULATIONS OF COMMISSURAL PROJECTIONS CONNECTING LIKE REGIONS OF THE INFERIOR COLLICULUS IMMUNOSTAIN FOR DIFFERENT AMINO ACID TRANSMITTERS. D.M. Caspary* and R.H. Helfert, Depts. of Pharmacology and Surgery, SIU School of Medicine, Springfield, IL 62794-9230

Tract tracing combined with immunocytochemistry was performed to identify neurotransmitters that may be used by the commissural projections between the inferior colliculi (IC). Three chinchillas received injections of the anterograde tracer biocytin (BCT) in the central nucleus of the left IC (CIC). After perfusion, brainstem sections through the IC were collected and the tracer was visualized using a metal-intensified, avidin-biotin-peroxidase method prior to osmication and embedment in plastic resin. Serial thin sections were cut from portions of the right CIC and external nucleus of the IC (EIC) containing BCT-labeled boutons, and collected on 400-mesh grids for electron microscopy. Alternate sections were immunolabeled for the inhibitory amino acid γ-aminobutyric acid (GABA) and the excitant amino acid glutamate using post-embedding immunogold methods.

The BCT injections resulted in the bilaterally symmetrical labeling of boutons in the IC. This labeling was confined to distinct laminae in the CIC and EIC. Two populations of BCT-labeled synaptic terminals were observed contralaterally in both the CIC and EIC. One population contained pleomorphic vesicles, formed symmetric synapses with their targets, and exhibited an intense immunoreactivity for GABA. The other formed asymmetric synapses, possessed round vesicles, and contained heavy immunogold labeling for glutamate. These results suggest that specific areas in both the CIC and EIC receive both inhibitory (GABAergic) and excitatory (glutamatergic) input from corresponding regions in the contralateral IC.

Supported by the Deafness Research Foundation and NIDCD DC00151.

583.2

AGE-RELATED CHANGES IN THE SYNAPTIC ORGANIZATION OF THE INFERIOR COLLICULUS OF THE FISCHER 344 RAT. R.H. Helfert*[1,2], T.J. Sommer[1], C. Jeffery[3], L.F. Hughes[1,4], and D.M. Caspary[1,2]; Depts. of [1]Surgery, [2]Pharmacology, [3]Microbiology and Immunology, and [4]Psychiatry, SIU School of Medicine, Springfield, IL 62794-9230

The numbers and distribution of both GABA- and non-GABA-immunoreactive synaptic terminals was assessed in the central nucleus of the inferior colliculus (CIC) of five 3-month-old (Y) and five 28-month-old (O) male Fischer 344 (F-344 rats). Thin sections were obtained from the right CIC from each animal and immunolabeled for GABA. Montages were constructed from electron micrographs taken of portions of the CIC from each animal contained in three randomly selected grid squares. After adjusting the field to more accurately reflect neuropil, each terminal was measured and categorized based on immunoreactivity to GABA and type of postsynaptic target.

There were significant age-related decreases in the number and density of synaptic terminals in the CIC (Y: $\bar{x}$ #=246.33, O: 174.33, $P<.01$; Y: $\bar{x}$ density =13.57/100 μm², O: 10.22/100 μm², $P<.05$). However, the sizes and ratio of GABA+ and GABA- terminals did not differ as a function of age group. There was a significant age-related decrease in the density of both terminal types on small (<0.5 μm)- and medium (0.5-1.5 μm)-caliber dendrites (Y: $\bar{x}_{small}$= 0.65/100 μm², O: 0.50/100 μm², $P<.01$; Y: $\bar{x}_{medium}$=0.53/100 μm², O: 0.36/100 μm², $P<002$). A loss of both GABA+ and GABA- neurons occured in the aged group (Y: $\bar{x}$ #=123.16, O: 87.17, $P<.02$), but their numerical declines were at equivalent rates. These results suggest that there is an age-related loss of both GABAergic and nonGABAergic synaptic terminals in the CIC of F-344 rats, and that this loss may be related to both a reduction in the population of target neurons and changes in the dendritic organization of surviving target neurons.

Supported by NIDCD DC00151 and the SIU Central Research Committee.

583.3

EFFECTS OF AGING ON $GABA_A$ AND GLYCINE RECEPTOR BINDING IN THE INFERIOR COLLICULUS AND COCHLEAR NUCLEUS OF THE FISCHER 344 RAT: A QUANTITATIVE AUTORADIOGRAPHIC STUDY. J.C. Milbrandt*[1], R.L. Albin[2], and D.M. Caspary[1]. [1]Dept. of Pharmacology, SIU School of Medicine, Springfield, IL 62702. [2]Dept. of Neurology, Univ. of Michigan, Ann Arbor, MI 48109.

Previous studies have demonstrated an age-related loss of both GABA and glycine in the brainstem of the Fischer 344 rat. To assess possible postsynaptic deficits, we used quantitative receptor autoradiography to determine changes in the binding profiles of the $GABA_A$ receptor and the strychnine-sensitive glycine (GLY) receptor were evaluated in three age groups of Fischer 344 rats: 3, 20, and 26 months. $GABA_A$ receptor binding was localized and quantified in three primary subdivisions of the inferior colliculus (IC): dorsal cortex (DCIC), external or lateral cortex (ECIC), and central nucleus (CIC). GLY receptor binding was examined in two subdivisions of the cochlear nucleus (CN) that receive major glycinergic inputs: dorsal cochlear nucleus (DCN) and anteroventral cochlear nucleus (AVCN).

$GABA_A$ receptor binding was assessed in the IC using ^{3}H-muscimol, ^{3}H-flunitrazepam, and ^{3}H-TBOB. $GABA_A$ receptor binding for all three ligands was significantly higher in the DCIC compared to the CIC and ECIC. In each subdivision of the IC, no significant age-related changes in receptor binding for the $GABA_A$ receptor ligands were observed.

The glycine receptor was localized in the CN using ^{3}H-strychnine. Binding was significantly higher in DCN compared to AVCN. An age-related decrease in ^{3}H-strychnine binding was observed in AVCN and DCN for the 20 month and 26 month groups compared to the 3 month group. This age-related loss of GLY receptor binding in the CN, implying a loss of inhibition, could result in a decreased efficacy of complex signal processing.

(This research was supported by NIH DC00151)

583.4

OUTPUTS OF A FUNCTIONALLY-CHARACTERIZED REGION OF THE INFERIOR COLLICULUS OF THE CBA MOUSE MODEL OF PRESBYCUSIS. M.A. Armour, R.D. Frisina, J.P. Walton, W.E. O'Neill and D.G. Flood* Otolaryngology Div., Surgery, Physiology & Neurology Depts., U. Rochester Sch. of Medicine & Dentistry, Rochester, NY 14642.

The CBA mouse has proven to be a useful animal model for behavioral and neurophysiological studies of presbycusis - hearing loss with age. However, there is little known about the anatomy of the central auditory system of the CBA. Therefore, initial investigations of the normal anatomy of young adults will serve as a basis for discovering the neuroanatomical changes underlying the behaviorally and physiologically measured age-related hearing loss of this strain. To further these goals, single unit responses to pure tones and temporal features of sounds were mapped in the central nucleus of the inferior colliculus (ICC) of 9 awake, tranquilized CBA mice ranging in age from 1.5 to 16 months. An anterograde and retrograde tracer (HRP, Sigma Type XII) was iontophoresed into the 16-20 kHz isofrequency lamina of the ICC, at the conclusion of a single-unit mapping experiment. Following a 24 hr survival time, the mice were anesthetized, intracardially perfused with heparinized saline, and fixed with glutaraldehyde/paraformaldehyde. Three sets of coronal sections were cut at 60 um. One set was reacted with DAB and counterstained with cresyl violet, the other two were reacted with TMB and one counterstained with safranin-O. Major ipsilateral outputs (labeled fibers and boutons) were observed in dorsal cortex of the IC (DC), brachium of IC (BIC), nucleus of BIC, all 3 major divisions of the medial geniculate body of the thalamus (MGB), and dorsal and ventral divisions of the ventral nucleus of the lateral lemniscus. Contralateral outputs were observed in a laminar pattern in the ICC, in patchy clusters of the DC, BIC, and all 3 divisions of MGB but to a lesser extent than ipsilaterally and not in all animals. A bilateral projection to the superior colliculus and periaquaductal gray was seen in some mice. We are currently contrasting these findings with those in other species and older animals.

[Supported by NIH-NIA Grant #: P01 AG 09524 & the International Center for Hearing & Speech Research - RICHS, Rochester, NY]

583.5

Dextran-labeled Axons from the Cochlear Nucleus and their Contacts Visualized on Lucifer Yellow-Filled Projection Neurons in the Inferior Colliculus. D. L. Oliver, G. E. Beckius, and E-M. Ostapoff*, Dept. of Anatomy, University of Connecticut Health Center, Farmington, CT 06030-3405.

We have developed a method that combines tracing neural circuits and studying the cellular innervation patterns of identified neurons. Neurons in the inferior colliculus take up fluoresc ently-labeled latex microspheres injected into the medial geniculate body, and axons contacting those neurons are labeled by injections of rhodamine- and biotin-labeled dextran into the cochlear nucleus. Both the pre- and postsynaptic components are visualized with fluorescent optics in a fixed slice, where the dendritic morphology is revealed by intracellular injection of biotinylated Lucifer Yellow compounds. ABC histochemistry permanently preserves both components of this circuit.

This paper reports on 39 neurons from the inferior colliculus whose axons projected to the medial geniculate body. Twenty of these neurons received axonal contacts directly from the cochlear nucleus. Disc-shaped and stellate cells were identified, based on their dendritic morphology and the orientation of the dendrites relative to the dextran-labeled afferents. These data show that two cell types receive contacts from the cochlear nucleus and are part of the CN-IC-MGB circuit. Funded by NIH grants R01-DC00189, R01-DC00127, and P01-DC01366.

583.6

INPUTS TO COMBINATION-SENSITIVE NEURONS IN THE MEDIAL GENICULATE BODY OF THE MUSTACHED BAT. Jeffrey J. Wenstrup* and Carol D. Grose. Dept. of Neurobiology, Northeastern Ohio Universities College of Medicine, Rootstown, OH 44272.

This study used retrograde tracers (WGA-HRP, Fluoro-Gold, cholera toxin B subunit, and dextran-conjugated rhodamine) to examine sources of input to regions in the medial geniculate body (MGB) showing combination-sensitive responses to the bat's biosonar signals. Tracer-filled, micropipette electrodes recorded combination-sensitive, single and multiple unit responses in the MGB, then placed a single iontophoretic tracer deposit. Areas showing each major type of combination-sensitive response received tracer deposits, including CF/CF areas and FM-FM areas with both short and long best delays.

Three major inputs were found: the inferior colliculus (IC), the auditory cortex, and the thalamic reticular nucleus. In the midbrain, labeled cells were common in the central and external nuclei of IC and in the pericollicular tegmentum. In the central nucleus (ICC), most labeled cells were in regions representing higher frequencies, i.e., the higher harmonic element to which combination-sensitive neurons respond. Very few neurons were labeled in regions tuned to frequencies between 25-32 kHz, whether in CF/CF or FM-FM experiments. Auditory cortical labeling in layer VI was centered in the more dorsal regions where combination-sensitive neurons have been recorded. A few experiments showed a broader distribution of cortical label, but it is uncertain whether this included 25-32 kHz regions of tonotopically organized auditory cortex. Retrogradely labeled cells in the thalamic reticular nucleus were limited to its dorsal and caudal parts, just ventral to the MGB. Within MGB, retrogradely labeled cells were rarely found beyond the deposit site.

These results support our previous work suggesting that ICC does not send direct, low-frequency input to combination-sensitive regions of the MGB; nor does this input appear to originate from other MGB regions. Strong candidates include the external nucleus of IC and pericollicular tegmentum, the thalamic reticular nucleus, and, possibly, auditory cortex. (Supported by USPHS grant DC00937.)

583.7

GABAergic AXOSOMATIC ENDINGS PREFERENTIALLY TARGET NON-GABAergic NEURONS IN THE CAT MEDIAL GENICULATE BODY. J.A. Winer*, S.K. Khurana, J.J. Prieto, and D.T. Larue. Department of Molecular and Cell Biology, University of California, Berkeley, California 94720-2097.

The density of axosomatic GABAergic endings on GABA-positive and -negative neurons was studied with postembedding immunocytochemistry. The number and spatial arrangement of such endings has important implications for understanding how GABAergic cells—either intrinsic or extrinsic—exert inhibitory and disinhibitory effects in the auditory thalamus.

We counted GABA-immunostained axonal endings (puncta) contacting ventral division perikarya in immunostained, deplasticized 1-1.5 µm-thick sections. The ventral division was identified in adjoining semi-thin sections stained with toluidine blue. One hundred GABA-positive and 100 immunonegative, mid-nucleolar neurons were drawn under oil immersion, and the GABAergic endings contacting their perikaryal membranes were counted.

Immunonegative neurons received six times more somatic endings/µm than GABAergic neurons. The same pattern was evident in thicker sections immunostained for GABA or GAD. This suggests a discrete convergence of axosomatic inhibition onto presumptive thalamocortical relay neurons. This pattern aligns them with cortical pyramidal neurons, which likewise receive many GABAergic axosomatic endings, and it suggests a common principle of inhibitory input to auditory forebrain projection cells. In contrast, many hindbrain GABAergic or glycinergic auditory neurons also receive GABAergic and/or glycinergic axosomatic endings, suggesting that disinhibition is more prevalent in the brain stem. Since some of these hindbrain cells are projection neurons, perhaps all such cells receive a more robust axosomatic inhibition than do local circuit neurons.

Supported by United States Public Health Service grant R01 NS16832-13.

583.8

EVIDENCE FOR DESCENDING PROJECTIONS FROM THE MEDIAL GENICULATE BODY TO THE INFERIOR COLLICULUS IN THE CAT. S. Paydar, D.T. Larue*, and J.A. Winer. Department of Molecular and Cell Biology, University of California, Berkeley, California 94720-2097.

For a study of the projections of the inferior colliculus to the medial geniculate body, we injected HRP-WGA or HRP into various subdivision of the inferior colliculus and perfused the animals 18 hours later. Besides the anterograde granular transport in various medial geniculate nuclei and the orderly arrangement of brain stem and commissural input to the inferior colliculus, we found retrogradely labeled neurons in the medial division of the medial geniculate body, in the nucleus of the brachium of the inferior colliculus, and in peripeduncular and adjoining posterior intralaminar regions. Many were elongated multipolar neurons with long, sparsely branched and widely ramifying dendrites. They resembled cells associated with the posterior intralaminar system or the midbrain tegmentum more closely than they did thalamocortical relay neurons or Golgi type II cells.

The comparatively short survivals, the modest size of the injections, and the granular nature of the intracellular reaction product each suggest that transneuronal transport or direct damage to the medial geniculate body cannot explain these results. A similar pattern was observed in the rat, but not in the mustached bat.

No conceptual framework is available yet to place this finding in a functional context. As a working hypothesis, we suggest that geniculocollicular projections could provide rapid feedback to lemniscal and extralemniscal inferior colliculus subdivisions. Such input would be parallel to, and independent of, the massive corticocollicular projections. It could rapidly convey important limbic and integrative influences to the midbrain for postural adjustments or motor responses to sound.

We are grateful to J.C. Adams for suggesting the possibility of this pathway. We thank Dr. K.D. Games for access to her experiments. Supported by United States Public Health Service grant R01 NS16832-13.

583.9

A STEREOLOGICAL ANALYSIS OF PARVALBUMIN-CONTAINING NEURONS IN AUDITORY NEOCORTEX. C.B. Smelser and N.T. McMullen*. Department of Anatomy, University of Arizona College of Medicine, Tucson, AZ 85724.

Parvalbumin (PV) is a calcium-binding protein present in GABA-ergic cells in the cerebral cortex and in thalamic relay neurons. Within the auditory forebrain, parvalbumin immunocytochemistry (PVi) labels both the cells and neuropil of the ventral MGB and delineates the primary auditory cortex (AI). In the present study, the terminal-like labeling of PVi puncta within laminae III/IV was used to define the rostral and caudal limits of the auditory cortex. PVi (SWANT #235, 1:10K) was performed on 40 um thick frozen sections obtained from young rabbits. Serial coronal sections through the entire temporal pole and medial geniculate body were processed. We used a modification of the Cavalieri method, the fractionator and disector to obtain an unbiased estimate of neuron number within AI as well as the percent of neurons which contained PV. To estimate neuron density, a grid composed of $0.01mm^2$ squares was superimposed over the ten selected sections from each animal with the aid of an image combining computer microscope. A single square within each layer in all sections was randomly selected for neuron counting at 400x. The mean volume of the auditory cortex was 26.9 mm^3. The total number of neurons in rabbit AI was 1.48×10^6 with a mean neuronal density of $53.9 \times 10^3/mm^3$. The mean neuronal density of PV neurons was $4960/mm^3$ indicating that approximately 9.2% neurons in AI contain PV. The percentage of PVi nonpyramidal neurons within each layer was as follows: lamina I: 3.9%, lamina II: 2.6%, lamina III: 5.5%, lamina IV: 10.2%, lamina V: 6.6%, lamina VIa: 6.6%, lamina 6b: 5%. Novel findings were the presence of PVi neurons within lamina I and lightly-labeled PVi pyramidal neurons in lamina VIa. (Supported by the NIH, ADCRC, DRF and Whitehall Foundation)

583.10

INTRINSIC AND EXTRINSIC PROJECTIONS OF AAF NEURONS WITHIN THE IPSILATERAL AUDITORY CORTEX IN CAT. H. Ojima[1,2]*, G. Hollrigel[2] and L.M. Kitzes[2] Neural Systems Laboratory, FRP, Institute of Physical and Chemical Research (RIKEN), Wako, Saitama, Japan[1]; Dept. of Anatomy and Neurobiology, University of California Irvine, Irvine CA 92717[2]

Projections from the anterior auditory field (AAF) to other auditory cortical areas and intrinsic projections within AAF were studied and related to the tonotopic organization of target zones. After injections of PHA-L or neurobiotin into both deep and superficial layers of single AAF loci, the distributions of anterogradely labeled fibers and terminals were reconstructed in the tangential plane. The projections and tonotopic maps were super-imposed using lesions made during the mapping procedure.

There were mainly two streams of labeled fibers. One was oriented roughly anteroposteriorly and provided terminal fibers with boutons to the dorsal posterior area (DP) and dorsal parts of primary auditory cortex (AI). The other was oriented obliquely in a posteroventral direction and provided terminals to the secondary auditory field (AII) and ventral parts of AI. In each field, labeled terminal fibers formed several discrete patches along these streams. The density of fibers and terminals was much greater in one or two patches. In AI, the frequency representation of these high density patches corresponded to that at the injection site.

Intrinsic projections within AAF from the injection site are oriented between the dorsal tip of the anterior ectosylvian sulcus and the suprasylvian sulcus, roughly parallel to the isofrequency coutours in the field.

583.11

MULTIPLE CORTICAL AUDITORY FIELDS IN THE FERRET DEFINED BY THEIR ARCHITECTONICS AND THALAMO-CORTICAL CONNECTIONS. Angelucci, A., Clascá, F., and Sur, M*. Dept. of Brain & Cognitive Sciences, MIT, Cambridge, MA 02139.

In striking contrast with the auditory cortex of the cat, a single auditory field (AI) has been mapped electrophysiologically in the ectosylvian gyrus of the ferret *(Mustela putorius)*. We have sought to define the extent and subdivisions of the cortical region receiving projections from the medial geniculate body (MGB), using multiple neuroanatomical techniques. Adult ferrets received small injections of retrograde tracers (Fluorogold, Fast Blue, Fluororuby and WGA-HRP) in the cortex or iontophoretic deposits of PHA-L in the MGB. Series of brain sections adjacent to those used to study the labeling were stained for Nissl substance, acetylcholinesterase, cytochome oxidase or myelin, in order to define cortical fields and thalamic nuclei.

We have found that in addition to AI, at least one other field is present in the posterior ectosylvian gyrus. Both fields receive heavy projections from the ventral and medial divisions of MGB. PHA-L injections show that axons arising from small cell groups in these divisions reach widespread zones of the cortex. The cortex of the ventral bank of the suprasylvian sulcus is also auditory, as revealed by its dense connections with the dorsal and medial divisions of MGB and the lateral division of the posterior nuclear complex. These data indicate the presence of multiple auditory cortical fields in ferrets as well, and represent a first step in clarifying the location of these fields and their thalamocortical associations.

Supported by: NIH EY07719, and MEC 92EX-30550728 to F.C.

583.12

DIRECT PROJECTION FROM THE PRIMARY AUDITORY CORTEX TO THE NUCLEUS SAGULUM, SUPERIOR OLIVARY COMPLEX AND COCHLEAR NUCLEUS IN THE ALBINO RAT.

M. Feliciano*, E. Saldaña and E. Mugnaini. Lab. of Neuromorphology, The University of Connecticut, Storrs, CT 06269-4154.

The auditory cortex (AC) is the startpoint of descending auditory pathways. It has generally been accepted that the descending projections of the AC do not extend beyond the inferior colliculus (IC) and, therefore, the cortical influence on lower auditory nuclei is necessarily conveyed by the IC. In the last 60 years, several isolated accounts reported that the ablation of the AC resulted in degenerating fibers in the lateral lemniscus (LL), superior olivary complex (SOC) and cochlear nuclei (CoN), thus challenging the previous tenet (e.g. Metler, 1935, J. Comp. Neurol. 63:25; Zimmerman et al. 1964, J. Comp. Neurol. 123:301). In an attempt to confirm the existence of direct cortical projections to subcollicular auditory nuclei, and to determine the trajectories, topography, morphology and possible targets of these projections, the anterograde tracer *Phaseolus vulgaris* -leucoagglutinin (PHA-L) and biotinylated dextran (BDA) were iontophoretically injected at different locations within the primary auditory cortex (Te1) of adult albino rats. Terminal anterograde labeling was found: 1) in the ipsilateral nucleus sagulum, horizontal cell group (which separates dorsal and ventral nuclei of the LL), and rostral and medial paralemniscal regions; 2) in different structures of the bilateral SOC, notably in the ventral nucleus of the trapezoid body and the lateral superior olive; and 3) bilaterally, in the granule cell domains surrounding the ventral CoN, and in the dorsal CoN. These findings demonstrate that indeed a direct projection from Te1 to subcollicular auditory nuclei exist and suggest that the neocortex may have a direct influence in the processing of sound at the initial stages. Supported by PHS grant NIDCD# 1805-01, and D.G.I.C.Y.T. of Spain, PB90-0523-CO2-01.

583.13

CONNECTIONS OF FIVE AUDITORY CORTICAL FIELDS WITH THE STRIATUM AND THE AMYGDALA IN THE CAT. J.J. PRIETO* AND J.A. WINER. Department of Histology, University of Alicante, 03080-Alicante, Spain (J.J.P.), and Department of Molecular and Cell Biology, University of California at Berkeley, Berkeley, CA 94720-2097, USA (J.A.W.).

Connectional studies of the cat auditory cortex and basal forebrain in the cat are fragmentary and incomplete. We studied the afferent and efferent connections of cortical areas with the striatum and the amygdaloid nuclei. WGA-HRP was injected in AI, AAF, P, AII and temporal (Te) cortex, and the sections were processed with tetramethylbenzidine.

All fields but Te project to the caudate and putamen. In addition, AI and AII also send axons to the pallidus and the claustrum. Injections in AI, AAF, AII and P labeled many cells retrogradely in the putamen, pallidus and claustrum. On the other hand, Te cortex received only a tiny projection from the putamen and the globus pallidus. The caudate nucleus did not project to any area studied.

The basomedial amygdaloid nucleus projected to all areas but Te. AII and P also had reciprocal connections with the lateral amygdaloid nucleus. Temporal cortex, however, had strong reciprocal connections with the lateral, basolateral and central amygdaloid nuclei.

These findings suggest that there is both parallel processing of auditory information by some basal forebrain nuclei, and a segregation of input in other centers. Such projection patterns imply that, even among the tonotopically organized cortical fields, there are discrete patterns of corticoamygdaloid and corticostriatal connectivity. This supports the idea that these fields participate in different facets of auditory and limbic function. Supported by United States Public Health Service Grant RO1 NS16832-13, and DGICYT grants PB 91.0752 and PB 91.0754 from the Spanish Government.

583.14

METABOLIC MAPPING OF THE AUDITORY SYSTEM OF MICE USING QUANTITATIVE CYTOCHROME OXIDASE HISTOCHEMISTRY.

A. Cada* and F. Gonzalez-Lima. Dept of Psych and Inst. for Neurosci, Univ of Texas, Austin TX 78712 USA

We measured the metabolic capacity of the primary auditory system with a quantitative histochemical method based on an image analysis system calibrated with internal standards of known cytochrome oxidase activity determined spectrophotometrically (Gonzalez-Lima & Garrosa, Neurosci Lett 122:251, 1991). Staining quality among nuclei was also assessed using the Wong-Riley's qualitative scale (Trends Neurosci 12: 94, 1989). Generally, the most peripheral nuclei had the highest activity and the greatest percentage of cell body staining. Higher nuclei demonstrated less activity and most of their activity resided in the neuropil. The cochlear nuclei and the superior olivary/ trapezoid body complex had the highest activity collectively, while the trapezoid body nucleus was the single structure with the most activity. This nucleus exhibited prominently active cell bodies with little evidence of dendritic or axon terminal staining. The anterior portion of the ventral cochlear nucleus and the ventral half of the ventral lateral lemniscus had lower activities and consisted of medium neuropil with less cell body staining. The central and external divisions of the inferior colliculus displayed an active dorsal-to-ventral band in each subdivision. These activity patterns may correlate with the frequency range of vocalizations for these animals. The dorsal inferior colliculus, medial geniculate body, and auditory cortex exhibited the least activity overall. These areas contained light to medium neuropil staining with less than 1% of active cell bodies. The possible functional significance of the variations in cytochrome oxidase activity among auditory structures is presented. (Supported by RO1 MH43353).

583.15

ANATOMICAL ORGANIZATION OF AUDITORY EVOKED POTENTIALS IN THE RAT: AN HRP STUDY. B. Brett, S. Di, J. Rudy*, D. Barth. Dept. of Psychology. University of Colorado, Boulder, 80309.

The neurogenesis of cortical components of the auditory evoked potential (AEP) in the rat is not well established. Recent research in our lab using high resolution mapping of epicortical potentials and numerical analysis has suggested that these components may correspond to the activation of parallel fiber pathways which originate in subdivisions of the medial geniculate nucleus of the thalamus.

The object of the present study was to obtain anatomical validation of the neurogenesis of these components. Click evoked AEP's were mapped with an array of 64 platinum electrodes on the surface of the cortex. The electrodes were configured in a 3.5 x 3.5^2 mm matrix, with a distance of .5 mm between each electrode. The array was positioned to cover both primary (area 41) and secondary (area 36) regions of auditory cortex and provided a high resolution representation of the AEP complex. Temporal components were isolated using isopotential maps which depict the areas of maximal activity over the cortex during amplitude peaks. Horseradish peroxidase (Sigma VI, 10 %) was injected into the location of maximal activation for each component of the AEP. Survival time was approximately 48 hours, then the subjects were perfused. The brains were removed and the medial geniculate nucleus (MGN) was sliced on a cryostat. The slices were then reacted with tetramethylbenzidine (TMB) and counterstained with neutral red.

Our results indicate that the shortest latency biphasic sharp waves localized over area 41 are generated by a fiber pathway which originates in the ventral division of the MGN. The longer latency monophasic sharp waves localized over area 36 are generated by a fiber pathway which originates in the dorsal MGN. The biphasic slow waves distributed over most of auditory cortex are generated by a third parallel fiber pathway which originates in the medial MGN.

583.16

CEREBRAL LATERALITY IN HOMOSEXUAL MALES. **M.Reite*, J. Sheeder, D. Richardson, P. Teale University of Colorado Health Sciences Center Denver, Colorado 80262**

The 100 msec latency auditory magnetic evoked field component M100 is generated in Heschl's gyri on the superior aspect of the superior temporal gyri. M100 localization appears to be a sensitive indicator of cerebral laterality. Significant interhemispheric sex differences are found in M100 localization, with sources further anterior in the right hemisphere of normal males compared to normal females. This study examined interhemispheric symmetry of M100 source location in 8 medically healthy homosexual males, and 8 age and education matched heterosexual males. MEG fields evoked by auditory tone pips were recorded from left and right hemispheres in response to contralateral ear stimulation. Source estimates were based on a modified Gauss-Newton method of least squares minimization, and referenced to bony landmarks using a sonic digitizer system capable of ± 1.0 mm accuracy, and worst case operational accuracy of ± 3.0mm. Sources in the normal males were significantly further anterior in the right hemisphere (t= -5.2, p=.0001). Sources in the homosexual males did not exhibit significant interhemispheric asymmetry, being similarly located on both hemispheres (t= -1.6, p=.13). In addition, source location variance was significantly higher in the homosexual subjects. These findings suggest a possible anatomic and/or functional difference in the superior temporal gyrus of at least some homosexual male subjects. Supported by USPHS MH47476 and MH46335.

584.1

CYCLIC-AMP INCREASES A MEMBRANE CONDUCTANCE IN RAT TASTE CELLS. M. Scott Herness* Indiana University School of Medicine, Muncie, IND. 47306.

Cyclic nucleotide-gated ion channels have been reported in many sensory systems. Second messenger systems utilizing cyclic nucleotides are thought to be important in gustatory transduction. This report suggests promising evidence for the occurrence of cyclic nucleotide-gated channels in gustatory receptor cells. Inside-out patches, excised from dissociated rat taste receptor cells and bathed in symmetrical buffered saline solutions responded with an increase in conductance when bath solution was replaced with buffered saline containing concentrations of cAMP ranging from 10 - 500 μM. Signals were filtered with a four-pole Bessel filter at 2 kHz (-3db) before being digitized with a sampling rate of 20 mHz. This conductance increase was reversible when rinsed. A current voltage plot, constructed by calculating amplitude histograms, yielded a conductance of 30 pS. More frequent transitions appeared to occur at negative potentials than at positive potentials. In at least one patch conductance was also elicited by cGMP. Discrete single unit events have not been observed.

Supported by NIH DC00401.

584.2

Expression of mRNA for amiloride-sensitive sodium channel in the rat taste tissues. X.J. Li*, P. M. Hwang, S. Blackshaw and S.H. Snyder. Department of Neuroscience. Johns Hopkins Medical School. Baltimore, MD 21205.

Although experiments to date indicate that salty taste involves interaction of sodium with a passive, apical Na^+ channel that can be blocked by the diuretic drug amiloride, the molecular structure and localization of this channel in the tongue remain unknown. We report the expression of amiloride-sensitive Na^+ channel gene in the taste tissues of rat tongue. Utilizing the polymerase chain reaction(PCR) technique with primers based on the sequence of a recently cloned amiloride-sensitive epithelia Na^+ channel (Canessa et al Nature 361, 467.1993), we have identified the PCR products encoding this sodium channel from rat taste tissues including taste buds in the circumvallate papillae. We have also isolated the identical cDNA for this channel from a rat taste cDNA library, which was constructed from the circumvallate papillae of rat tongue. Reverse transcription-PCR assay and Northern blot indicate that mRNA for amiloride-sensitive Na^+ channel is present in the rat taste, olfactory and skin tissues, although it is most abundant in the lung, distal colon and kidney. The preliminary *in situ* hybridization studies show that the amiloride-sensitive Na^+channel is distributed in the taste papillae as well as the tongue epithelium. Further characterization of its localization in the tongue is underway. The existence of epithelia Na^+ channel in the taste tissues indicate an involvement of this channel in the salty taste transduction.

584.3

NERVE-DEPENDENT GLYCOCONJUGATES ARE PRESENT IN TASTE BUDS AND ON THE BOUNDARIES BETWEEN TASTE BUDS. Q. ZENG, Z. SHAO and B. OAKLEY* DEPT. OF BIOLOGY, UNIV. OF MICHIGAN, ANN ARBOR, MI 48109.

We screened 12 lectins on rat tongue sections to obtain information on carbohydrate expression in taste buds and the surrounding gustatory epithelium. After fixation in acid-alcohol or 4% paraformaldehyde, cryostat sections of rat tongue were treated with biotinylated lectins followed by an avidin-biotin-peroxidase complex and diaminobenzidine. Four lectins had interesting selective staining patterns: *Bauhinia purpurea* (BPA), *Helix pomatia* (HPA), *Lotus tetragonolobus agglutinin* (LTA) and *Ulex europaeus agglutinin* (UEA-I). UEA-I bound to the surface of fungiform papillae and to cells of its taste bud. HPA reacted with more than half of vallate or foliate elongated taste cells. LTA reacted with less than a third of elongated taste cells. Double-stained tissue sections suggest that keratin 18 and carbohydrates recognized by LTA may be co-expressed, because many LTA-positive cells were members of the subset of cells with keratin 18-like immunoreactivity. BPA selectively stained the boundaries between taste buds, but not the elongated taste cells. In electrophysiological recording, summated taste responses of the gerbil chorda tympani nerve were unaffected by exposure of the tongue to 200μg/ml BPA, or HPA or LTA. Gustatory denervation eliminated UEA-I BPA, HPA, and LTA reactivity of elongated taste cells and the BPA reactivity of cells between taste buds. Evidently, carbohydrate expression of lectin-positive cells comprising the boundaries between taste buds and as well as the elongated cells within taste buds is nerve-dependent.

Supported by NIH Grant DC-00083

584.4

MERKEL-LIKE BASAL CELLS IN AMPHIBIAN TASTE BUDS ARE HOMOLOGOUS WITH TYPE III CELLS IN MAMMALIAN TASTE BUDS. Dae-Joong Kim, Rona J. Delay & Stephen D. Roper* Dept. of Anatomy and Neurobiology, Colorado State University, Fort Collins CO 80523 and the Rocky Mountain Taste and Smell Center, Denver CO 80262

A number of investigators have shown there is a subpopulation of taste bud cells that contain serotonin (5HT). In amphibia, these cells are small oblate basal cells, thought to be related to cutaneous Merkel cells and hence termed Merkel-like basal cells. In contrast, mammalian 5HT-containing cells are elongate and resemble receptor cells. Ciges *et al* ('76) and Takeda & Suzuki ('83) concluded that serotonergic cells in mammalian taste buds were Type III cells, believed by some to be the gustatory receptor cells. We have conducted an immunocytochemical survey of taste buds from *Necturus*, hamster, rabbit, rat and mouse, seeking to identify 5HT-containing cells and to draw comparisons among the different species. Basal cells in *Necturus* were intensely immunopositive for 5HT and formed a distinctive ring at the peripheral base of the taste bud. We found some taste cells in rabbits stained only faintly with antisera to 5HT. Taste cells in rats, mice and hamsters were not immunopositive for 5HT. Antisera to tryptophan hydroxylase, a key biosynthetic enzyme for serotonin, did not react with mammalian taste buds, although it produced intense immunostaining in known serotonergic nuclei in the brain. However, after pretreating animals with 5HTP or injecting L-tryptophan, taste cells even in mammals other than rabbits were immunopositive for 5HT. 5HT-immunoreactive cells were elongate and were disposed to the peripheral boundary of taste buds, identical to how Royer & Kinnamon ('91) described type III cells in their EM analysis of rabbit taste buds. Based on the similarity in immunostaining for 5HT and upon their identical dispositions within the taste bud, we postulate that amphibian Merkel-like basal cells and mammalian type III cells are homologous and that they may mediate the same neuromodulatory function in taste buds (see abstract by Ewald & Roper). Supported by NIH grants DC00374, AG06557 and DC00244.

584.5

MODULATION OF RECEPTOR CELL FUNCTION BY BASAL CELLS IN *NECTURUS* TASTE BUDS. Douglas A. Ewald* & Stephen D. Roper Dept. of Anatomy & Neurobiology, Colorado State U., Fort Collins CO 80523 and the Rocky Mountain Taste & Smell Center, Denver CO 80262

Basal cells, which comprise only 10% of the total cell population of *Necturus* taste buds, are involved in the majority of morphologically identifiable synapses in the taste bud (Delay & Roper, J. Comp. Neurol. **277:** 268, 1988). In thin slices of lingual epithelium that contain taste buds, focal chemical stimulation (140 mM KCl) of the apical tips of taste receptor cells elicits receptor potentials in taste receptor cells and postsynaptic responses in basal cells (Ewald & Roper, J. Neurophysiol. **67:** 1316, 1992). Merkel-like basal cells in the *Necturus* taste bud contain serotonin (5-HT; Delay, Taylor, & Roper, submitted). In simultaneous intracellular recordings from a basal cell and a receptor cell, we have tested whether direct depolarization of basal cells elicits synaptic responses in receptor cells. No synaptic responses were observed during single electrical depolarizations applied to basal cells (up to 1-sec duration, to ~0 mV). However, repetitive basal cell stimulation (5/min.) produced long-term effects on receptor cells in 11 out of 23 experiments. Specifically, repeated basal cell stimulation increased receptor cell input resistances (mean ± S.D. = +27 ± 11%) and receptor potentials elicited by chemosensory stimulation (+19 ± 5%) 1 min. after a 1 min. stimulation. Receptor cells were also hyperpolarized (2-10 mV) by repetitive basal cell stimulation. Identical stimulation of pairs of receptor cells did not produce these effects. All of the effects of repetitive basal cell stimulation were mimicked by applying 100 μM 5-HT to the bath. These findings suggest that basal cells, when repetitively depolarized, can enhance the electrotonic propagation of receptor potentials from the apical (chemosensitive) tips of receptor cells to the basal (synaptic) regions. We propose that 5-HT, released from basal cells, mediates these modulatory effects on taste bud function. Supported by NIH DC01238.

584.6

STIMULATION OF THE GERBIL'S GUSTATORY RECEPTORS BY THE SUPER SWEETENER 4-CYANOPHENYL-CYCLONONYL GUANIDINE ACETIC ACID (SUCRONONIC ACID)R. CRUZ, L. SOMENERAIN, W. JAKINOVICH JR.,* Dept. of Biol. Sci., Lehman College and the Graduate School, City Univ. of New York, Bronx, NY 10468 J.M. TINTI, and C. NOFRE Universite Claude Bernard, Lyon, France.

The purpose of this study was to figure out how the gerbil responds to the artificial sweetener 4-CYANOPHENYL-CYCLONONYL GUANIDINE ACETIC ACID (sucrononic acid). First, the gerbil's chorda tympani nerve responses were obtained to sucrononic acid and sucrose. As in the human, sucrononic acid (threshold = 1 x 10^{-8}M) was observed to be a more effective stimulant than sucrose in the gerbil (threshold = 1 x 10^{-3}M). Next, conditioned taste aversion studies were conducted and it was observed that gerbils trained to avoid sucrononic acid generalized an avoidance to sucrose, but not NaCl, HCl or quinine. These results show that to the gerbil sucrononic acid tastes like sucrose as it does in the human. (Supported by R01-NS2538-01 of NINCDS,NIH and S06 RR08225 of MBRS,NIH)

584.7

NEURAL RESPONSES OF THERMAL-SENSITIVE LINGUAL FIBERS TO BRIEF MENTHOL STIMULATION. Robert F. Lundy Jr. and Robert I. Contreras*. The Florida State University, Dept. of Psychology, Tallahassee, FL, 32306-1051.

Electrophysiological responses to anterior tongue stimulation (10 s) with menthol and several standard taste solutions were obtained from 45 thermal-sensitive lingual fibers in rats. Cold (6 °C, 15 °C) water significantly increased and 45 °C warm water significantly decreased the discharge rate of single fibers adapted to room temperature water (22-24 °C). Weak menthol concentrations (0.0128 mM, 0.064 mM, 0.128 mM) elicited a response in more fibers (91%) during the first 2 s of stimulation compared to the first and second 5 s of stimulation. In contrast, stronger menthol concentrations (0.64 mM, 1.28 mM) increased discharge frequency throughout stimulus duration. Contrary to prior observations, a significant increase in discharge frequency was not evident during the ensuing period after menthol off-set. Most taste solutions (NaCl, glucose, quinine-HCl, citric acid) significantly suppressed neural discharge frequency during the first 2-5 s of stimulation, but not during the second 5 s of stimulation. Unlike prior observations, strong menthol concentrations increased the discharge frequency of thermal-sensitive lingual nerve fibers during brief stimulus presentation. Possible explanations for the highly variable response of thermal-sensitive fibers to weak menthol concentrations are discussed.

584.8

RESPONSE OF GENICULATE GANGLION CELLS TO TASTE NERVE INJURY. S.T. McGlathery, B.G. Manion and M.C. Whitehead*. Department of Surgery, Division of Anatomy, UCSD, La Jolla, CA 92093.

Damage to peripheral taste nerves results in degeneration of central axonal endings in the nucleus of the solitary tract (NST) (McGlathery and Whitehead, Soc. Neurosci. Abstr., '92). Degeneration of central endings after nerve injury in the gustatory system resembles that in other sensory systems (e.g. trigeminal, vestibular, auditory). In this study, fluorescent labelling was used to determine whether the central degeneration results from ganglion cell death. Fast blue (0.4%) was injected bilaterally (60 µl per side) into the tongues of 5 golden hamsters. The chorda tympani was transected unilaterally 7 days post-injection, and the animals allowed to survive an additional 13-14 days. Fink-Heimer staining of the NST showed heavy degeneration on the experimental side, verifying the nerve cut in each case. Geniculate and trigeminal ganglia were sectioned and Fast blue-labelled cells counted. Numbers of labelled cells in experimental geniculate ganglia (221 ±133) did not differ significantly from those in control ganglia (240 ±81). Consistency of tongue injections, between sides, was evaluated by counting labelled trigeminal ganglion cells which did not differ significantly between experimental (1508 ±539) and control sides (1680 ±533). We conclude that the degeneration of central axonal endings after nerve transection results from a transganglionic process. Geniculate ganglion cells survive nerve section and could, therefore, regenerate nerve fibers both centrally and peripherally. (Supported by NIH Grants DC00452 and DC01901).

584.9

EFFECT OF PROTEIN PHOSPHORYLATION AND CALMODULIN INHIBITORS ON CHEMORECEPTOR FUNCTION IN THE RABBIT CAROTID BODY. J. Chen*, L. He, B. Dinger and S. Fidone. Dept. of Physiol., Univ. of Utah Sch. of Med., Salt Lake City, UT 84108.

Previous investigations have implicated protein kinases and calmodulin in the regulation of neurotransmitter release, and the modulation of specific ion channel conductances. In the present experiments we have investigated the effects of an inhibitor of calcium/calmodulin dependent protein kinase II, KN-62, and a calmodulin inhibitor, trifluoperazine (TFP), on ^{3}H-catecholamine release (^{3}H-CA synthesized from ^{3}H-tyrosine) and carotid sinus nerve (CSN) activity evoked by hypoxia or nicotine (100 µM) from rabbit carotid bodies superfused in vitro. TFP (10 µM) inhibited ^{3}H-CA release evoked by hypoxia (69% ± 7% inhibition; $\bar{X}\pm$ SEM, $p<0.0005$) and nicotine (47% ± 10%, $p<0.025$). TFP likewise inhibited CSN activity evoked by these stimuli. In contrast to these findings, KN-62 (10 µM) did not alter hypoxia-evoked ^{3}H-CA release or CSN discharge, but this drug did significantly inhibit nicotine-evoked ^{3}H-CA release (48% ± 2%; $p<0.05$) and chemoreceptor activity. In preliminary experiments ^{3}H-CA release and CSN activity evoked by either hypoxia or nicotine were decreased in the presence of H-7 (300 µM), an inhibitor of cyclic nucleotide protein kinases and protein kinase C. Our data indicate that protein phosphorylation and calmodulin activity play significant roles in chemoreceptor activation, and further, that specific signal transduction pathways are mediated by selected protein kinases. Supported by USPHS grants NS12636 & NS07938.

584.10

NITRIC OXIDE (NO) MEDIATES EFFERENT NEURAL INHIBITION OF CAROTID BODY CHEMORECEPTORS. Z.-Z. Wang*, B. Dinger, L.J. Stensaas and S.J. Fidone. Dept. of Physiology, Univ. of Utah of Utah Sch. of Med., Salt Lake City, UT 84108

Previous anatomical studies revealed an extensive plexus of NO synthase (NOS)-immunoreactive axons arising from the carotid sinus nerve (CSN), and neurophysiological experiments indicated NO is a potent inhibitor of chemoreceptor discharge. The present study demonstrates that N^G-nitroarginine methylester (L-NAME, 0.1 mM), which blocks NO synthesis in the carotid body, is able to reversibly block the increase of cGMP which occurs in vascular smooth muscle and type I cells following CSN stimulation. In in vitro superfused and vascularly perfused carotid bodies in which chemoreceptor activity was recorded from axons split from the CSN, electrical stimulation of the CSN induced rapid inhibition of the steady-state chemoreceptor discharge and increase in cGMP formation in blood vessels; both were reversible by 0.1 mM L-NAME. In contrast, in the in vitro superfused cat carotid body, CSN stimulation did not alter the steady-state chemosensory discharge, but attenuated the dynamic chemoreceptor response to hypoxia following 5 min of CSN stimulation. This delayed inhibition paralleled a delayed cGMP production in type I cells and was also reversible by L-NAME. These data thus reveal two distinct NO-mediated efferent neural pathways that inhibit carotid body chemoreceptors. (Supported by USPHS grants NS12636 and NS07938)

584.11

PARAMECIUM CHEMORECEPTION Of IMP AND GLUTAMATE Wan Qing Yang and Judith Van Houten* Dept. of Zoology, University of Vermont Burlington, VT 05405-0086

Paramecium is repelled by inosine monophosphate (IMP), as measured by T-maze assays. Two purine nucleotides, AMP and GMP, interfere with *Paramecium's* behavioral response to IMP, while CMP and cAMP do not, indicating that the IMP-induced response is purine nucleotide-specific. Glutamate is an attractant in T-maze assays and inhibits *Paramecium's* response to IMP, but not vice versa. Glutamate analogs (NMDA, KA and QA) inhibit the response to glutamate, but not the response to acetate, indicating that the inhibition is specific and that the glutamate receptor in *Paramecium* might be ionotropic.

^{3}H-glutamate binding to whole cells saturates by 60 min and the Kd is ~103 µM. The 5'-ribonucleotides IMP, GMP and AMP all partially displace glutamate binding, but CMP and cAMP do not. These results are consistent with the T-maze assays. QA displaces glutamate binding, while NMDA, L-AP4, ACPD and KA displace only at higher concentration or not at all. Combinations of ligands with IMP in glutamate binding studies suggest that AMP and GMP may share a binding site with IMP and that QA may share a different glutamate site.

Intracellular cAMP RIA measurements show that IMP decreases cAMP level by 50%, while glutamate increases it about three fold, compared to the controls. Therefore, cAMP may be a second messenger for IMP and glutamate stimulation or adaptation.

This work was supported by NIH and the VCCC.

585.1

BRAINSTEM CONNECTIVITY OF THE HAMSTER CHORDA TYMPANI DEMONSTRATED WITH BIOTINYLATED DEXTRANS. A.P. Knox* and M.A. Barry. Department of BioS ıure and Function, UCONN Health Center, Farmington, CT 06030-3705.

The connectivity of the chorda tympani branch of the facial nerve (CT) of adult-male golden Syrian hamsters was investigated with biotinylated dextran (BD) (10 kDa, Molecular Probes). The chorda tympani was exposed in the middle ear and crystals of BD were applied to the cut end of the nerve. Survival times varied from 2-15 days. The ABC reaction (Vector, standard kit) was utilized to reveal transported BD in transverse 50 μm brain sections.

Multiple fascicles of transganglionic (anterograde) labeled fibers entered the rostral medulla and extended caudally to the level of the area postrema, where terminals were found in the commissural nucleus. Retrograde label was confined to the preganglionic parasympathetic cells of the superior salivatory nucleus. Terminal label was extremely dense at the rostral pole of the solitary nucleus (NST), but was also very dense at caudal levels of the rostral NST where it overlapped with gustatory projections of nerve IX. The appearance of most fibers and terminals was similar at 2 and 15 days, but by 15 days some vesiculated fibers were seen. The persistence of apparently the majority of terminal fibers 15 days following nerve section shows that most fibers do not undergo complete transganglionic degeneration, and that the loss of acetylcholinesterase staining in the NST after CT damage (Barry and Frank, Exp. Neurol. 115:60, 1992) is not due primarily to a loss of fibers. For anterograde transport, BD was superior to other tracers such as HRP, Cholera toxin, and WGA-HRP because of high sensitivity, rapid transport, temporal stability, and particularly the "Golgi-like" character of the label.

Supported by NIH DC-00853 and 5P01-DC00168-12.

585.2

IN VITRO WHOLE CELL RECORDINGS FROM THE GUSTATORY ZONE OF RAT NUCLEUS OF SOLITARY TRACT DEMONSTRATE IMMATURE INTRINSIC PROPERTIES IN NEURONS AT THREE WEEKS POSTNATAL. H. Bao, R.M. Bradley and C.M. Mistretta*, School of Dentistry, Univ. of Michigan, Ann Arbor, MI 48109.

Second order taste neurons in the rostral portion of rat nucleus of solitary tract (NST) continue to mature morphologically through about 65 days postnatal and demonstrate increases in response frequencies to chemical stimuli to about 60 days after birth. In initial attempts to learn when intrinsic electrophysiological properties mature, we have used whole cell recordings in the rostral portion of gustatory NST in two groups of rats: P15 (15-16 days postnatal; n = 9 cells) and P20 (18-22 days postnatal; n = 16 cells). Recordings were made from *in vitro* slices of the brainstem, and data on postnatal rats were compared with adult data previously reported (Bradley and Sweazey, J. Neurophysiol., 1992). Resting membrane potentials averaged about -47 mV across P15, P20 and adult groups, whereas input resistance averaged 446, 500 and 336 MΩ, respectively --- a developmental decrease. Membrane time constants (fast, slow) also decreased, from longer values at P15 and P20 (5, 32; 5, 31 ms) to shorter (2, 21 ms) in adults. Action potential amplitudes were similar across groups (80; 75; 75 mV). However, spike numbers in response to a 1500 ms, 100 pA depolarizing pulse increased from 29 at P15, to 54 and 50 in P20 and adult animals. Of the four intrinsic firing patterns reported in adult NST, typical examples of patterns II and III were apparent in P15 and P20 neurons. Cells that could be categorized with pattern I also were observed, but the patterns were very irregular and appeared immature. Neurons with pattern IV were not observed. In summary, intrinsic electrophysiological properties of rat NST cells are not yet mature at postnatal day 20, indicating a rather lengthy maturation period for these characteristics. Supported by NIH Grant DC00456.

585.3

CELL TYPES IN THE NUCLEUS OF THE SOLITARY TRACT? AN HISTORICAL PERSPECTIVE. L. Schweitzer*, X. Zhang, Z. Jin, H. Dulger and W.E. Renehan Dept. of Anatomical Sciences and Neurobiology, Univ. of Louisville Sch. of Med., Louisville, KY 40292 and Div. of Gastroenterology, Henry Ford Health Sci. Ctr. Detroit, MI 48202

A major emphasis in the field of neuroanatomy has been to determine the relationship between the structure of neurons and their function. The end product of many of these endeavors has been to establish "cell types" within functional subdivisions of the brain. Cell types have provided a useful construct for organizing data and exploring the mechanisms underlying the reception, processing and transmission of information in the nervous system. Efforts to determine morphologic cell types in the rostral aspect of the nucleus of the solitary tract (NST) have been constrained by the neuroanatomical methods used. The first studies employed Nissl stains and thus features like somatic cross-sectional area and form factor were used as the defining characteristics with which to type cells. Shortly thereafter, Golgi impregnation and retrograde HRP tract-tracing techniques were employed and dendritic branching patterns and axonal projections gained favor as the characteristics of choice. Most recently we have employed intracellular physiology/neurobiotin injection and three-dimensional computer reconstruction paired with a statistical technique known as cluster analysis to investigate cell types in the NST. Cluster analysis allows us to assess the utility of individual features of cellular morphology, and thus certain cell typing strategies, for grouping cells. How these cell types relate and the value of features previously employed for defining cell types will be reviewed and compared to our own results.

Supported by NIH-NICDC Grant #01074.

585.4

BIOPHYSICAL PROPERTIES OF MORPHOLOGICALLY CHARACTERIZED NEURONS IN THE ROSTRAL NUCLEUS TRACTUS SOLITARIUS (rNTS). M.S. King* and R.M. Bradley, Univ. of Michigan, Ann Arbor, MI 48109.

We have determined the biophysical properties of morphologically characterized neurons in the rat rNTS. Whole-cell recordings were made with biocytin-filled pipettes in a brain slice preparation of the rat medulla. 58 neurons were reconstructed using the Eutectic Neuron Tracing system and the neurons were separated into three morphological groups (ovoid, multipolar and elongate) as described by other investigators using the Golgi technique. Most neurons (49%) were classified as ovoid, while 35% were multipolar and only 16% were elongate. The passive membrane properties of the three groups were similar, except that the input resistance of the ovoid neurons was highest. While a high percentage (42-56%) of all three types of neurons responded to a 1200 ms, 100 pA depolarizing current pulse with a repetitive spike train, 31% of the ovoid neurons responded with a short burst of action potentials and 44% of the elongate neurons showed a delay in the onset of the spike train following a hyperpolarizing prepulse. Less than 16% of the multipolar neurons demonstrated either of these firing characteristics. Stimulation of the solitary tract in the brain slice caused EPSPs, IPSPs and mixed responses in rNTS neurons. These synaptic inputs were not specific to a morphological type. The responses of the three types of neurons to substance P, GABA and NMDA were also tested. A high percentage (57-100%) of each type responded to one or more of these neurotransmitters. Since the inputs and outputs of the three morphological groups are similar, they may not represent functionally-distinct groups. Instead, subpopulations of these three groups, which display different firing patterns, may subserve different functional roles. The same input in these subpopulations would produce different outputs. (Supported by NIH DC00288).

585.5

EFFECTS OF NEED-FREE SODIUM APPETITE ON TASTE RESPONSES IN THE NUCLEUS OF THE SOLITARY TRACT OF RATS. R. Tamura and R. Norgren* Dept. Behavioral Science, College of Medicine, Penn State Univ., Hershey, PA 17033

Intracranial injection of renin rapidly induces intake of hypertonic saline in rats that are otherwise in sodium balance. It is known that need-induced sodium appetite alters gustatory responses both on the periphery and in the nucleus of the solitary tract (NST). To determine whether these changes in responsiveness were due to the appetite itself or to sodium imbalance, gustatory neurons in the NST were tested with a battery of sapid stimuli after renin injections into the third ventricle. The rats were chronically prepared, but lightly anesthetized during the recording procedure. The effectiveness of the renin injections was assessed by measuring intake of 0.5M NaCl on the day following the recording session. Eighty-five gustatory neurons were tested; 46 after renin injections, 39 after vehicle injections. Except for the 2 hypertonic concentrations saline, neuronal responses to sapid stimuli did not differ significantly between the 2 conditions. When the rats were treated with renin, the average responses to 0.3 M and 1.0 M NaCl were 74% and 70%, respectively, of those after vehicle injections. A similar tendency was evident for the subsample of NaCl-best neurons, but the effect was smaller. In some respects, these results resemble the effects produced by dietary sodium restriction, but are opposite those produced by acute sodium loss. Supported by MH 43787 and MH 00653.

585.6

THE INFLUENCE OF GUSTATORY CORTEX ON TASTE CELLS OF THE SOLITARY NUCLEUS OF THE HAMSTER. H. Liu,* M. M. Behbehani and D. V. Smith. Depts. Otolaryngology and Physiology, Univ. Cincinnati Coll. Medicine, Cincinnati, OH 45267.

In this study we determined the influence of electrical stimulation of the gustatory (insular) cortex on the ongoing spontaneous discharge of taste responsive-cells in the NST in urethane-anesthetized hamsters. The sensitivities of 13 single neurons to three basic taste stimuli (0.032 M NaCl; 0.1 M KCl and 0.1 M sucrose) presented to the anterior tongue were determined. Of the 13 cells, 5 were NaCl-best, 4 were sucrose-best and 4 were KCl-best. Microinjection of D,L-homocysteic acid (DLH; 50 nl, 10 mM) into insular cortex excited 2 cells (1 NaCl-best; 1 sucrose-best), inhibited 5 cells (2 NaCl-best, 1 KCl-best and 2 sucrose-best) and had no effect on 6 cells (3 KCl-best, 1 sucrose-best and 2 NaCl-best). The onset of DLH-evoked responses ranged from 3 to 28 seconds; the duration of responses ranged from 1 to 8 min. Low-frequency, single pulse electrical stimulation of insular cortex had no effect on the NST cells sampled. Trains of high-frequency (100 Hz, 100 μA, 10 sec) electrical stimulation produced results similar to the DLH stimulation of the insular cortex except for one cell which was unresponsive to train stimulation but inhibited by chemical stimulation. In 4 cells (2 NaCl-best and 2 sucrose-best) bicuculline methioide blocked the inhibitory response evoked by both high frequency and DLH stimulation of the insular cortex. It is concluded that 1) insular cortex neurons have a synaptic influence on NST cells; 2) high-frequency train stimulation or chemical stimulation of the insular cortex is more effective in altering the firing pattern of NST neurons than single-pulse stimulation, and 3) inhibitory responses are possibly mediated by a GABAergic system. Further experiments will address the organization of this descending system and its specific influences on gustatory-elicited responses. Supported by DC-00066 and DC-00353.

585.7

GUSTATORY-ELICITED EXPRESSION OF C-*FOS* IN BRAINSTEM NUCLEI. M.I. Harrer, M. Dinkins, J.B. Travers, and S.P. Travers*. Depts. of Oral Biology and Psychology, Ohio State University, Columbus, OH 43210

Understanding the topographic organization of the gustatory system has been impeded by the difficulties inherent in using neurophysiological techniques to study small neurons in these small nuclei, coupled with problems of adequately stimulating the entire taste bud population. We have begun to study the organization of brainstem gustatory regions using immunocytochemical techniques to detect the expression of *c-fos* after taste stimulation in awake, behaving rats. Water-deprived rats were stimulated with alternate deliveries of 1.0M sucrose and 0.03M quinine HCL, separated by distilled water rinses, delivered though intraoral cannulae during a 30-45 min period. These stimulations elicited behavioral ingestion and rejection. At 1-2 hrs following stimulation, rats were deeply anesthetized with pentobarbital (150 mg/kg), perfused, and brainstem tissue processed using standard immunocytochemical techniques. Taste stimulation produced Fos-like immunoreactivity (Fos-LI) in the rostral division of the nucleus of the solitary tract (rNST), the subjacent reticular formation, and the medial and ventral lateral subnuclei of the parabrachial nucleus. Labelled NST nuclei formed a continuous distribution that spanned the rNST, but was limited to the medial half of the central subnucleus, i.e., the region overlapping neurophysiological gustatory responses. Interestingly, Fos-LI was not marked in the lateral half of the central or the lateral subnuclei, regions responsive to oral somatosensory stimulation. Thus, this approach appears to provide a sensitive, specific method for studying the functional activation of first- and higher-order neurons participating in gustatory processing.

Supported by NIH DC00416 and DC00417.

585.8

TIME COURSE OF DISCRIMINATION OF TASTE STIMULI IN THE PARABRACHIAL PONS OF THE RAT. P.M. DiLorenzo*, G.S.Hecht, and S. Monroe. Dept. of Psychology, SUNY at Binghamton, Binghamton, NY 13902.

At the core of traditional analyses of the electrophsyiological responses to taste is the response measure. This consists typically of the number of spikes that occur during some arbitrary interval of time when a tastant is on the tongue. However, when activity is summed over time, the dynamic aspects of the responses are lost. Moreover, the critical interval of measurement is problematical. Despite evidence that a taste stimulus can be identified within 200 msec of contact with the tongue, virtually all theories of taste coding are based on response measures taken over 3-5 sec of response. We recorded the electrophysiological responses of 38 neurons in the parabrachial nucleus of the pons (PbN) in urethane anesthetized rats to a range of concentrations of representatives of the four basic taste qualities: NaCl, HCl, quinineHCl and sucrose. We then analyzed the pairwise differences in across neuron patterns of response as they were elaborated over the time course of the response. By changing the interval over which the response was measured, it was possible to chart the degree of difference between 2 across neuron patterns over time. These differences were largest and occurred earliest (peak about 750 msec) in comparisons of the pattern of response to sucrose with that evoked by all other tastants. The discrimination of different types of tastants appeared to be accomplished within different time frames over the first 3 sec of response. When tastants were presented at near threshold concentrations, responses to all tastants were most discriminable within the first 500 msec of response. These data suggest that different judgements regarding taste discrimination may have different time frames and that the appropriate interval for analysis of PbN taste responses may depend on the particular taste quality.

This work was supported by a grant from the Whitehall Foundation to P.M.D.

585.9

CONDITIONED TASTE AVERSIONS ARE RETAINED AFTER LESIONS OF THE PARABRACHIAL NUCLEI. P.S. Grigson*, T. Shimura, and R. Norgren. Dept. Behavioral Science, College of Medicine, Penn State Univ., Hershey, PA 17033

In phase I, 42 rats were given 15 min access to 0.3 M alanine (conditioned stimulus; CS) followed by an ip injection of the unconditioned stimulus (US), either 0.15 M LiCl (1.33 ml/100 g b.w.) or saline. Following 3 CS-US pairings, all rats that received LiCl demonstrated a strong conditioned taste aversion (CTA) to alanine. In phase II, the saline and LiCl injected animals were divided into 3 groups that received either (1) electrophysiologically-guided bilateral electrolytic lesions of the nucleus of the solitary tract (NST), (2) electrophysiologically-guided bilateral ibotenic acid lesions of the parabrachial nucleus (PBN), (3) or appropriate control procedures. In phase III, retention of the preoperatively acquired CTA was evaluated in a 2-bottle, 15 min test during which the rats had simultaneous access to dH20 and 0.3 M alanine. In Phase IV, all rats were retrained in a CTA using 0.1 M NaCl as the CS and LiCl as the US. As demonstrated previously, the results showed that PBN lesions, but not those in the NST, disrupted postoperative acquisition of a CTA. When acquired preoperatively, however, both groups of rats demonstrated full retention of the CTA after their lesions. Supported by DC 00047, DC 00240, and MH 00653.

585.10

CHEMOSENSORY BRAIN MECHANISMS OF LEARNING IN GOLDFISH R.J. Arcement*and D.J. Ingle. Columbia Univ.-Teachers College, N.Y., NY 10027 & M.I.T., Cambridge, MA 02139

This study examined the effects of single and multiple ablations of the gustatory and olfactory brain centers on chemosensory discrimination ability. Surgery included ablations of the olfactory tract (OT), telencephalon (T), facial lobe (FL) and vagal lobes (VL). Goldfish were trained to discriminate between two extracts (diluted 1:100) of raw flesh; bluefish vs beef. This was done by habituating fish to one extract while rewarding for snapping at the source of the second extract. Critical tests for discrimination compared the amount of time a fish spent at each extract tube. After goldfish were trained to selectively approach either an extract of fish or beef, ablations were made of either OT, T, FL, VL, or combinations of these regions. We found that ablation of OT or FL alone or combined removal of FL plus OT or FL plus VL did not abolish this chemosensory discrimination capability. Only when all three centers were removed (FL plus VL, then later OT) was that capability abolished in one group and much reduced in two others. These results indicate that input via either OT or VL is sufficient for this particular discrimination between food extracts. Furthermore, even with both gustatory lobes removed, near total ablation of T does not abolish this olfactory discrimination. Presumably OT projections to the hypothalamus mediates this residual ability, a finding which extends the earlier work of Wright and Harding in the mouse (Science, 1982, 216, 322-323).

BASAL GANGLIA AND THALAMUS VI

586.1

RODENT VENTRAL STRIATUM: 5'-NUCLEOTIDASE, CELL CLUSTERS, AND INFRALIMBIC CORTICAL INNERVATION. M.G. Laubach* and D.J. Woodward. Dept. of Physiology and Pharmacology, Bowman Gray School of Medicine, Wake Forest University, Winston-Salem, NC 27157.

The goal of this study was to describe the distribution of 5'-nucleotidase (5'NT) within the ventral striatum and to compare terminal fields of deep and superficial layer projections from the infralimbic cortex with respect to 5'NT. Biotin-dextran was iontophoresed in infralimbic cortex and terminals were visualized using standard methods. Sections were counter-stained for Nissl substance. The distribution of 5'NT was determined on adjacent sections using the method of Schoen and Graybiel ('92). Additional brains were stained for 5'NT, calbindin, substance-P, and leu-enkephalin. Three-dimensional surface images of 5'NT were reconstructed from serial sections in four brains using the Biographics Workstation. In the core, 5'NT is localized to several contiguous patchy structures surrounding the anterior commissure which are in register with leu-enkephalin positive zones and are continuous with the 5'NT zones of the dorsal striatum. In the shell, 5'NT is concentrated in a longitudinal band along the septal border, extending from the rostral pole of the ventral striatum to the bed nucleus of the stria terminalis. The ventral portion of this zone is coincident with an area of high cell density. A second 5'NT zone along the shell/core border is coincident with an area of high cell density and is most evident at caudal levels. The infralimbic cortical innervation was denser in the shell than in the core. In the shell, infralimbic fibers from the superficial layers tended to avoid the 5'NT-rich cell clusters. By contrast, fibers from the deep layers densely innervated the 5'NT-rich cell clusters. Thus, this study extends the principles proposed by Gerfen '89 to the infralimbic cortex and the shell of the ventral striatum.

(MH 44339, DA 02338, & AFOSR 90-0146 to DJW and DA 07246 to MGL)

586.2

GLUTAMATE AND ASPARTATE INPUTS TO THE RETROSPLENIAL GRANULAR CORTEX (RSg) AND THE ANTERIOR THALAMIC NUCLEI (ATN) IN THE RAT.

A. Gonzalo-Ruiz*, A.R. Lieberman and J.M. Sanz. *Dept. of Anatomy, School of Physiotherapy, Soria, Spain and Dept. of Anatomy, UCL, London, England.*

We have examined the organization and transmitter-related characteristics of projections to the RSg from the ATN by retrograde labelling with WGA-HRP and glutamate (Glut)/aspartate (Asp)-immunohistochemistry. Iontophoretic injections of WGA-HRP were placed into different parts of the RSg in adult albino rats anaesthetized with Nembutal (45mg/Kg). The animals were reanaesthetized 1-2d later, and perfused with fixative. Brains were sectioned coronally at 50µm using a Vibratome. The sections were reacted sequentially for the histochemical detection of WGA-HRP (using DAB as chromogen) and the immunocytochemical localization of either Glut or Asp, using the ABC method. After injections located in superficial layers (I-III) of the dorsal and ventral RSg, Glut+HRP and/or Asp+HRP containing neurons (possible non-overlapping populations) were present in the ipsilateral anterodorsal (AD) and anteroventral (AV) subnuclei of the ATN. All such injections also produced, in ipsilateral AD and AV, significant numbers of neurons which were only HRP+. Thus the thalamocortical projections from ATN may be heterogeneous with respect to transmitter content. Injections of WGA-HRP confined to deep layers (V-VI) of the dorsal and ventral RSg produced anterograde. HRP (and light Glut or Asp) labelling predominantly in the ipsilateral AD and AV. The small varicosities seen with the light microscope in ATN lie within, and presumably form synaptic contacts mainly in, the neuropil. Some labelled terminals also lie close to neuronal cell bodies. Ultrastructural studies combining HRP labelling and Glut/Asp-immunohistochemistry will be required to confirm that such terminals contain Glut and/or Asp and to assess their synaptic organization within the ATN; the possible transmitter heterogeneity of the ATN-RSg projections also calls for further investigation. (Supported by DGICYT PM92-0139).

586.3

SPATIAL DISTRIBUTION OF CHEMICALLY-IDENTIFIED INTRINSIC NEURONS IN RELATION TO PATCH AND MATRIX COMPARTMENTS OF RAT NEOSTRIATUM. Y. Kubota* and Y. Kawaguchi, Laboratory for Neural Systems, Frontier Research Program, RIKEN, Wako, Saitama 351-01, Japan

The spatial distributions and dendritic branching patterns of chemically identified subpopulations of striatal intrinsic neurons, defined by immunoreactivity for choline acetyltransferase (ChAT), neuropeptide Y or parvalbumin, were studied in relation to patch and matrix compartments of rat neostriatum.

ChAT- and parvalbumin- immunoreactive cells and fibers showed an uneven pattern of distribution in the striatum. Parvalbumin immunoreactivity was denser in the neuropil of lateral and caudal parts than of the medial part. Neuropeptide Y immunoreactivity was uniform. Certain neuropeptide Y cells (about 20%) were also immunoreactive for calbindin D_{28k}, indicating that at least a small population of calbindin D_{28k} immunoreactive cells are medium aspiny cells.

Cells immunoreactive for ChAT, neuropeptide Y or parvalbumin showed basically similar distribution patterns in relation to the patch and matrix compartments. Most stained cells were located in the matrix, but some were located at the borders of patches and a few were inside patches. Most primary dendrites of stained cells in the matrix or patches remained confined to these compartments, but cells on the borders invariably extended dendrites into both compartments. The striatal intrinsic neurons form chemically differentiated neuronal circuits within the matrix and the patches and those whose dendrites cross the borders may contribute to associational interconnections between the two compartments, unlike the spiny projection neurons whose dendrites are confined to one or other compartment.

586.4

GABAergic AXONS IN THE RAT VENTRAL FOREBRAIN: A LIGHT AND ELECTRON MICROSCOPIC STUDY. H.T. Chang*, Q. Tian, and P. Herron, Department of Anatomy & Neurobiology, The University of Tennessee, Memphis, College of Medicine, 875 Monroe Ave., Memphis, TN 38163

The ventral forebrain region, including the ventral striatum (VS), the ventral pallidum (VP), and the substantia innominata (SI), has been shown to be an important link between the basal ganglia, the limbic system, as well as the basal forebrain cholinergic system. Although previous studies have shown that this region is richly innervated by GABAergic fibers, little is known with respect to the relative densities of GABAergic to non-GABAergic axon terminals in this region. To address this issue, we have developed a specific rabbit antiserum to GABA and used a postembedding immunocytochemical reaction to analyze the quantitative distribution of GABA-like immunoreactive axon terminals in the rat VS, VP and SI. We found that 9.5% of the axon terminals in VS, 85.5% in the VP, and 64.8% in the SI are GABAergic. These results are consistent with previous findings that a majority of inputs to VS are excitatory, and that a majority of inputs to VP are inhibitory. Moreover, our result shows that GABA may play an important role in the SI where both the cholinergic and the non-cholinergic neurons reside within a neuropil innervated by many different noncholinergic fibers.

(This study was supported by NIH Grant AG05944.)

586.5

THE CORTICO-PALLIDAL PROJECTIONS IN THE RAT: AN ANTEROGRADE TRACING STUDY WITH BIOTINYLATED DEXTRAN AMINE. A. Naito, H. Nakanishi* and H. Kita Dept. of Anatomy and Neurobiology., Col. of Medicine., Univ. of Tennessee Memphis, Memphis, TN 38163.

As a result of studies on the corticofugal pathways in the rat using an anterograde tracing technique, we have found a direct cortical projection to the globus pallidus (GP). Biotinylated dextran amine (BDA, 2%) dissolved in 0.01 M phosphate buffer (pH 7.2) was pressure injected into various cortical areas of 34 male Sprague-Dawley rats (280-390 g). After a survival period of 5-8 days, the rats were fixed with 4% paraformaldehyde and the brains were sectioned on a Vibratome. The sections were processed for visualization of BDA containing fibers utilizing the ABC-method. A number of BDA-labeled thin fibers with small boutons were found in the ipsilateral GP after BDA injections into the frontal agranular cortex. However, only a few fibers were labeled after an injection into other cortical areas. Injection of BDA in the medial and the lateral cortical areas resulted in labeling in the medial and the lateral regions of the GP, respectively. Injection of BDA in the rostral and the caudal cortical areas resulted in labeling in the ventral and the dorsal GP regions, respectively. A number of labeled boutons were also observed in the entopeduncular nucleus and the substantia innominata after BDA injections into the rostoral part of the frontal cortex. These findings indicate; 1) that the cortex projects to the ipsilateral GP, 2) that the main projection arises from the frontal agranular cortex, and 3) that the cortico-pallidal projections are topographically organized.

(supported by the USPHS Grants NS-25783 and NS-26473, and the Human Frontier Science Program Grant)

586.6

IDENTIFIED TARGETS OF THE PALLIDOSTRIATAL PROJECTION IN THE RAT. B. D. Bennett, S. Bacon* and J. P. Bolam, MRC Anatomical Neuropharmacology Unit, Department of Pharmacology, Oxford University, Oxford, United Kingdom.

The globus pallidus (GP) is known to project to the striatum and it has been demonstrated that lesions of the GP produce a decrease in NADPH-diaphorase (NADPH-d) staining in the striatum (Staines and Hincke '91 Soc. Neurosci. Abst. 17, 456). These findings imply that the pallidostriatal projection may exert a direct effect on NADPH-d cells. The object of the present study was to test directly whether GP cells form synaptic contact with NADPH-d cells of the striatum.

Rats received iontophoretic deposits of PHA-L in the GP. Sections of the striatum were processed to reveal the anterogradely transported tracer and then incubated to reveal NADPH-d-containing structures. In some sections the NADPH-d activity was revealed first. In the light microscope, anterogradely labelled fibres were sparse but were observed in many areas of the striatum. These fibres gave rise to clusters of large boutons which were sometimes observed to form baskets around unlabelled perikarya. The proximal dendrites of NADPH-d cells were seen to be apposed by labelled terminals. Correlated light and electron microscopy confirmed that the anterogradely labelled terminals apposed to the dendrites of NADPH-d cells formed symmetrical synaptic contact with these neurones. The PHA-L injections in the GP also resulted in the retrograde labelling of some striatal projection neurones. Although the pallidostriatal terminals had a distinct morphology, the possibility cannot be excluded that some of the labelled boutons arose from collaterals of the retrogradely labelled spiny cells.

Thus, in addition to spiny neurones (Kita et al. '91 Soc. Neurosci. Abst. 17, 453) NADPH-d cells are one of the synaptic targets of pallidostriatal neurones. The identity of other classes of neurones postsynaptic to the pallidal fibres in the striatum is under investigation.

586.7

AXON COLLATERALS OF PARS RETICULATA PROJECTION NEURONS SYNAPSE ON PARS COMPACTA NEURONS. M. Damlama*, J.P. Bolam[1], and J.M. Tepper, Center for Molecular and Behavioral Neuroscience, Rutgers University, Newark, NJ USA 07102 and [1]MRC, Anatomical Neuropharmacology Unit, Oxford University, Oxford, U.K.

Nigral dopaminergic (DA) neurons in pars compacta have been suggested to receive inputs from GABAergic pars reticulata (PR) neurons as well as from neostriatum and globus pallidus. Although electrophysiological evidence implies such a local nigral interaction, synaptic contacts have not yet been demonstrated. Furthermore, since inputs could arise from PR interneurons and/or projection neurons, the identity of the PR neurons projecting to the pars compacta DA neurons needs to be determined.

Adult male Sprague-Dawley rats were anesthetized with urethane (1.3 g/kg i.p.). Intracellular recordings were obtained with micropipettes filled with 3% biocytin in 1 M potassium acetate from PR projection neurons identified by antidromic activation from thalamus and/or superior colliculus at short latency (0.6-3.4 ms). Following identification, neurons were intracellularly filled with biocytin. Serial sections through the substantia nigra were processed to reveal biocytin using DAB as a chromogen and TH immunocytochemistry using BDHC as a chromogen.

Antidromically identified PR projection neurons possessed medium sized polygonal to fusiform cell bodies. The axon usually issued from the cell body and formed extensive collaterals within a few hundred μm of its origin. Collaterals exhibited intermittent clusters of varicosities, both in PR and pars compacta, and had long stretches with no varicosities. Some of the varicosities were in close apposition to DA dendrites. In the electron microscope the varicosities were identified as large (~ 2 μm) synaptic boutons that contained several mitochondria, and pleomorphic vesicles. The boutons formed symmetric synapses. Individual labeled collaterals were observed to form multiple synaptic contacts with single neurons in the pars compacta. Supported by MH 45286 (JMT) and the MRC (JPB).

586.8

GABAERGIC INHIBITION OF NIGROSTRIATAL DOPAMINERGIC NEURONS BY SELECTIVE ACTIVATION OF PARS RETICULATA PROJECTION NEURONS D. R. Anderson, W. Li and J. M. Tepper*, Center for Molecular and Behavioral Neuroscience, Rutgers, The State University of New Jersey, Newark, NJ USA 07102

The spontaneous activities of substantia nigra pars compacta dopaminergic (DA) neurons and a population of pars reticulata (PR) non-DA neurons are reciprocally related. This reciprocity has been postulated to be due to inhibition of dopaminergic neurons by a specific type of PR interneuron (Grace & Bunney, *Brain Res.333*:271, 1985). However, it is possible that reciprocity exists between DA neurons and GABAergic PR projection neurons, mediated by axon collaterals of the latter. Therefore, we examined the response of DA cells to selective activation of PR projection neurons achieved by antidromic stimulation of their projection sites *in vivo*.

Adult male Sprague-Dawley rats were anesthetized with urethane, and bipolar stimulating electrodes were implanted in the anterior-lateral neostriatum, thalamus, and superior colliculus. Extracellular single unit activity of antidromically identified DA nigrostriatal neurons was recorded by conventional means, and responses to single pulse stimulation (0.25 - 1 mA) of thalamus or superior colliculus were analyzed with peristimulus time histograms.

Forty one of 44 DA neurons were inhibited at short latency by antidromic activation of PR from thalamus (mean onset 1.8 ms; duration of inhibition 52.1 ± 8.3 ms; inhibition 44 ± 5%) or tectum (mean onset 0.8 ms; duration of inhibition 38.9 ± 5.1 ms; inhibition 32 ± 5%). Bicuculline methiodide (20 mM) filled microelectrodes completely blocked tectal- or thalamic-induced inhibition of DA neurons (n=13) and caused a dramatic increase in burst firing, even in the absence of increased spontaneous firing rate. These data demonstrate that PR projection neurons inhibit pars compacta dopaminergic neurons through a monosynaptic $GABA_A$ synapse and that tonic GABAergic input, acting through a $GABA_A$ receptor and most likely originating from extrastriatal sources, serves to suppress burst firing in DA cells *in vivo*. Supported by MH 45286.

586.9

SOMATOSTATIN, NADPH-DIAPHORASE AND STRIATAL COMPARTMENTALIZATION IN THE RAT. W.J. RUSHLOW*, N. RAJAKUMAR, K. ELISEVICH, C.C.G. NAUS and B.A. FLUMERFELT. Dept. of Anatomy, University of Western Ontario, London, Canada, N6A 5C1.

Heterogeneity is now a widely recognized feature of the striatum. Consistent with this concept, we have previously demonstrated that the somatostatin (SOM) neuron population is composed of several smaller subpopulations based on the distribution of various proSOM-derived peptides. Neurons that contain SOM 28, SOM 14 and SOM 28(1-12) are found predominantly in the matrix compartment of the ventral striatum while neurons that contain only SOM 28 are more numerous in the patch compartment and dorsolateral striatum. SOM fibre labelling is most abundant in the ventromedial striatum and least dense in the patch compartment and dorsolateral striatum. However, this pattern of fibre distribution has been determined using antisera directed against SOM 14, SOM 28(1-12) or other SOM antibodies that cross-react with these peptides. Unfortunately, these antisera do not label the entire population of SOM neurons. NADPH-diaphorase labelled fibres, in contrast, are homogenously distributed across the striatum including the patch compartment. These fibres are morphologically identical to SOM fibres and retrograde tracing studies indicate that there is no external source providing NADPH-diaphorase labelled fibres to the striatum. Given that SOM 28 is 100% colocalized with NADPH-diaphorase in the perikarya, these results suggest that neurons that contain SOM 28, SOM 14 and SOM 28(1-12) innervate the matrix compartment while neurons that contain only SOM 28 innervate the patch compartment and the dorsolateral striatum. Supported by MRC (B.A.F.), NSERC (C.C.G.N.) and Huntington's Society of Canada (W.J.R.)

586.10

SEGREGATED CONNECTIONS AND POSSIBLE FUNCTIONAL HETEROGENEITY OF STRIOSOMES OF THE RAT STRIATUM. B.A. Flumerfelt, N. Rajakumar and K. Elisevich*, Dept. of Anatomy, University of Western Ontario, London, Ontario, Canada, N6A 5C1.

Striosomes receive inputs from the prefrontal cortex and project to dopaminergic neurons of the substantia nigra. However, these connections are mostly restricted to striosomes situated in the medial part of the striatum. Recently, we described projections from striosomes in the lateral part of the striatum to neurons of the entopeduncular nucleus (EPN) that project to the lateral habenula, thereby indicating an involvement of striosomes in the regulation of serotoninergic neurons of the dorsal raphe (J. Comp. Neur. '93, 331:286). In the present study injections of fluorogold into the lateral part of the striatum in adult rats resulted in labeling of neurons in sensorimotor and frontal association (Fr2) areas, suggesting a possible connection between these areas and striosomes that project to the EPN. Placement of the anterograde tracer, biotinylated dextran, into the Fr2 region in combination with calbindin immunolabelling showed a preferential projection to striosomes while fibers traced from the primary sensorimotor area avoided the striosomes. Combined anterograde tracing from the Fr2 region and retrograde tracing from the EPN resulted in overlapping labeling which indicated a possible involvement of the Fr2 region in the control of serotoninergic neurons via striosomes in the lateral part of the striatum. Similar injections of tracers into the prefrontal cortex and substantia nigra pars compacta demonstrated an overlap of labeling within the striatum. This suggests a functional heterogeneity of striosomes: the medial striosomes are involved in the control of nigral dopamine neurons and receive inputs from the prefrontal cortex; the lateral striosomes are involved in the control of serotoninergic neurons of the dorsal raphe and receive inputs from the frontal association cortex. [Supported by MRC Canada]

586.11

TOPOGRAPHICAL ORGANIZATION OF THE CORTICOSTRIATAL PROJECTION IN THE RAT. K. Elisevich, N. Rajakumar, A.W. Hrycyshyn* and B.A. Flumerfelt, Department of Anatomy, University of Western Ontario, London, Ontario, Canada, N6A 5C1.

The topographical organization of the corticostriatal projection in the rat is not conclusive, as most studies have employed either degeneration or retrograde tracing methods. In the present study, projections from the primary motor (Fr1), sensory (Pa1), forelimb (FL) and hindlimb (HL) areas, the prefrontal cortex (PF) and association areas of frontal (Fr2), parietal (Pa2), temporal (Te2) and occipital (Oc2) cortices were traced in adult rats employing the anterograde tracers biotinylated dextran (BD) and/or rhodamine dextran (RD) in combination with calbindin immunolabeling. The results revealed that the Pa1, FL, HL and Fr1 projections are extensively overlapped. The Pa1, FL and HL fibers were sparse and confined to the calbindin-poor area of the dorsolateral striatum (CPDS) while those of Fr1 were dense and occupied the dorsolateral quadrant of the striatum. The PF fibers occupied the medial half of the striatum and overlapped extensively with the Te2 and Oc2 fibers which occupied the ventromedial and dorsomedial areas, respectively. The Fr2 fibers were seen in the lateral half of the striatum other than the CPDS, overlapping extensively with Fr1 and Pa2 fibers which occupied the central region. Although the corticostriatal projections were bilaterally symmetrical, the density of fibers varied greatly. The Fr1, Fr2 and PF areas provided substantial inputs to the contralateral striatum whereas the other projections were sparse. The present study indicates that the CPDS receives converging inputs from primary sensorimotor areas whereas the remainder of the striatum receives inputs from association and prelimbic cortices that converge in an orderly manner.
[Supported by MRC Canada]

586.12

THE EARLY-FORMING PROJECTIONS OF THE SUBSTANTIA NIGRA IN THE MUTANT MOUSE *WEAVER*. B. Martin and S. Roffler-Tarlov*, Program in the Neurosciences, Tufts Univ. Sch. of Med., Boston, MA 02111.

Weaver is an autosomal recessive mutation in mouse. The nigrostriatal system of weaver is affected by arrested differentiation and death of dopamine-containing neurons in the substantia nigra after the first postnatal week. The early-forming dopamine islands present in the neonatal weaver's caudoputamen degenerate several weeks later leaving tyrosine hydroxylase (TH)-weak ghosts. In order to examine the striatal cell clusters that are targets of the dopamine island fibers and to provide retrograde labelling of nigral cells in the midbrain, the right rostral striatum of 2 day-old weaver and wild-type pups was infused with fluorescent dye (Fluoro-Gold, .1 µl of a 4% solution) (Snyder-Keller, Neurosci. Let. 91, 1988, 136-141). The injected pups were perfused on postnatal day 7. The brains were cut into a series of transverse sections that were examined for localization of Fluoro-Gold and TH immunoreactivity. Islandic deposits of Fluoro-Gold that corresponded to regions of TH-positive staining were found in the caudoputamen of both weaver and wild-type mice. The disposition and numbers of cells in the midbrain that contained Fluoro-Gold also were equivalent in 7 day-old weaver and wild-type mice. Fluoro-Gold labelling appeared both in populations of TH-positive neurons spared in the adult weaver and in cell groups that die. The results indicate that projections from both vulnerable and spared neurons in weaver's substantia nigra have reached target regions in the neonatal period and that the normal clustering of islandic targets takes place in weaver neonates. NIH NS20181

586.13

ORGANIZATION OF THE PROJECTIONS FROM SUBSTANTIA NIGRA TO HIPPOCAMPAL FORMATION IN THE RAT. A. Gasbarri*, R. Innocenzi, E. Campana and C. Pacitti, Department of Science and Biomedical Technology, Laboratory of Human Physiology. Collemaggio, 67100 L'Aquila, Italy.

Anterograde, retrograde tracing experiments and degeneration studies following midbrain lesions showed that the hippocampal formation (HF) receives projections from ventral tegmental area and substantia nigra (SN), which contain the dopaminergic (DA) neurons of the A10 and A9 respectively. The ascending projections from the substantia nigra (SN-A9) to the hippocampal formation were investigated in the rat by means of tracing techniques. Iontophoretically injected *Phaseolus vulgaris*- leucoagglutinin (PHA-L) into SN resulted in labeling primarily in subiculum and adjacent CA1 field of caudal HF. Very few scattered labeling was found in the rostral HF. The distribution of SN hippocampally projecting neurons was also examined by injecting retrograde fluorescent tracers (Fluoro Gold, Fast Blue and Nuclear Yellow) in different hippocampal areas. The results of these experiments show that the caudal HF receives afferents from medial SN, pars compacta (SNc), whereas very few SNc cells appear to project to the rostral HF. It is established that central dopaminergic system in the basal ganglia, in particular the mesostriatal and the mesolimbic DA pathways, arising from the mesencephalic A9 and A10 cell groups, play a role in the control of locomotor activity. It is also generally accepted that nigral projections are involved in the control of locomotor activity. It could thus be postulated that the retention of an acquired locomotor behavior might involve the nigro-hippocampal pathway.

586.14

NIGRAL INNERVATION OF CHOLINERGIC AND NON-CHOLINERGIC CELLS IN THE RAT MESOPONTINE TEGMENTUM: A DOUBLE LABEL EM STUDY. I. Grofova* and M. Zhou, Department of Anatomy, Michigan State University, East Lansing, MI 48824.

Pontomesencephalic tegmentum surrounding the superior cerebellar peduncle contains a prominent component of cholinergic neurons which are believed to control motor functions as well as behavioral states such as wakefulness and sleep. The core of this region is the pedunculopontine tegmental nucleus (PPN) which receives a major afferent input from the pars reticulata of the substantia nigra (SNR). However, it remains unclear whether the nigral afferents distribute evenly to both cholinergic and non-cholinergic components of the nucleus. We have addressed this issue in experiments involving anterograde labeling of nigral fibers with PHA-L combined with ChAT immunohistochemistry at both light and electron microscopic levels. The double labeled light microscope preparations showed an extensive overlap between the PHA-L labeled nigral fibers and the cholinergic cells in the PPN. However, the densest patches of nigral terminal varicosities were not in register with groups of cholinergic cell bodies. In the electron microscope, the majority of nigral terminals synapsed on unlabeled dendrites and cell bodies while synapses on ChAT positive dendrites and cell bodies were quite rare. These results strongly suggest that the nigral input to the mesopontine tegmentum is preferentially related to the non-cholinergic neurons. Supported by NIH NS 25744.

586.15

EFFERENT CONNECTIONS OF THE RETRORUBRAL FIELD (A8): A PHA-L STUDY IN THE RAT. S. J. SHAMMAH-LAGNADO*, M. COSTA, M. GOTO, A. PERAÇOLI and N. S. CANTERAS. Inst. Biomed. Sci., Univ. São Paulo, São Paulo, SP, 05508-900, Brazil.

Deposits of *Phaseolus vulgaris* leucoagglutinin (PHA-L) were placed in the retrorubral field (RRF), defined as containing the A8 dopaminergic cell group, in 20 rats. RRF fibers ascend mainly via the medial forebrain bundle and, besides providing a massive input to the striatal complex (caudate putamen, *fundus striati*, accumbens, olfactory tubercle, and amygdalostriatal transition area), project to many other structures, among which the ventral pallidum, the substantia innominata, the anterolateral and anteroventral areas of the bed nuclei of the stria terminalis, the anterior basomedial and the medial part of the central amygdaloid nuclei, the lateral preoptic and the lateral and dorsal hypothalamic areas, the zona incerta, the centromedial, parafascicular (medial part), mediodorsal (caudal pole), ventromedial and midline thalamic nuclei, the central gray substance, the ventral tegmental area, the substantia nigra compacta, the interfascicular, rostral linear, caudal linear and dorsal nuclei of the raphe. Descending RRF fibers distribute to several reticular districts, the pedunculopontine and dorsolateral tegmental nuclei, the parabrachial area, and the nucleus of the solitary tract. Thus, RRF projections are in many respects similar to those of the ventral tegmental area and appear closely related to viscerolimbic circuitry. (Supp. FAPESP grant 91/0450-5)

586.16

DEVELOPMENT OF THE RAT NEOSTRIATAL SYNAPTIC NEUROPIL N.A. Sharpe*, and J.M. Tepper. Aidekman Research Center, Center for Molecular and Behavioral Neuroscience, Rutgers University, Newark, NJ USA 07102.

Neostriatal neurophysiology and neuroanatomy undergoes significant postnatal developmental changes in the rat. We have previously shown that corticostriatal synapses are distributed differently at P14 than in adults. In the present study, the distribution of asymmetric neostriatal synapses in rats was compared at ages P10 and P21.

At the light level corticostriatal afferents labeled with biocytin terminated in clusters in P10 rats. This type of clustering was not seen in P21 or adults rats. At P10 the neostriatal neuropil appears immature with little myelination apparent and loosely packed synaptic vesicles in presynaptic terminals. The P21 neuropil appears similar to that in the adult with capsular fibers showing a relatively high degree of myelination and boutons relatively densely packed with synaptic vesicles. The total synaptic density at P10 (12.6 ± 0.9 synapses/ 100 μm^2) was less than at P21 (20.4 ± 1.3 synapses/100 μm^2) rats, $df = 20$, $t = -2.1$, $p < 0.05$. Most asymmetric synapses at P10 terminated on dendrites (67%) while at P21 relatively few asymmetric synapses were found on the dendrites (20%) with most synapses ending on spine heads (63%). The absolute axospinous synaptic density at P10 (2.1 ± 0.5 synapses/100 μm^2) was also lower than at P21 (12.6 ± 1.1 synapses/100 μm^2), $df = 20$, $t = -4.0$, $p < 0.01$. The axodendritic synaptic density at P10 (8.4 ± 0.7 synapses/μm^2) was slightly but significantly less than that at P21 ($11.9 + 0.8$ synapses/ 100 μm^2; $df = 20$, $t = 2.2$, $p < 0.05$). Comparison of synaptic vesicle size in axodendritic and axospinous presynaptic terminals revealed that vesicles were significantly larger at P10 (43.5 nm $\pm$ 7.4 nm, 40.6 nm $\pm$ 5.9 nm respectively) than at P21 (37.6nm + 6.9 nm, 34.3 nm $\pm$ 4.2 nm respectively) for both axodendritic ($df = 97$, $t = 4.0$, $p < 0.01$) and axospinous ($df = 106$, $t = 5.1$, $p < 0.01$) synapses. These data support previous anatomical and electrophysiological studies, indicating that in the first few postnatal weeks excitatory synapses in neostriatum are relatively undeveloped and that by P21 the neuropil has many of the ultrastructural attributes of the adult neostriatum. Supported by NS 30679.

586.17

MORPHOLOGICAL DEVELOPMENT OF RAT NEOSTRIATAL MEDIUM SPINY NEURONS INTRACELLULARLY LABELED WITH BIOCYTIN F. Trent* and J.M. Tepper, Center for Molecular and Behavioral Neuroscience, Rutgers The State University of New Jersey, Newark, NJ USA 07102

As part of an ongoing investigation of the postnatal development of the neostriatum, intracellular recordings were obtained in urethane anesthetized Sprague-Dawley rat pups ranging in age from postnatal day 6 (PD6) to PD40 and in adults. Using micropipettes filled with 3% biocytin or 2% neurobiotin in 1M potassium acetate, cells were filled by injecting 1-3 nA depolarizing current at a 50% duty cycle for 3-10 minutes. Cells were visualized using DAB and camera lucida reconstructions of representative examples from each age group were drawn from serial sections under a 60X or 100X oil immersion lens.

Although the maximal dendritic extent, mean number of proximal dendrites and dendritic tips were not age-dependent, spine density increased dramatically through PD29 with a sharp rise during the the 3rd postnatal week. Prior to PD15, dendrites were thin, highly varicose and essentially aspiny, although cells bore sparsely distributed spines on both the soma and proximal dendrites that disappeared by the end of the 3rd week. Growth cones were not detected on either axonal or dendritic tips. Mean somatic cross-sectional area remained constant over development.

The local axon collateral field was as extensive in the youngest recovered neuron (PD8) as in adults. As is frequently observed in adults this collateral plexus was often positioned asymmetrically with respect to the parent neuron. In most cases the main axon could be traced through the internal capsule verifying that the neurons possessing thin and heavily varicose aspiny dendrites observed in young pups were indeed medium spiny neurons. The morphological development of the axonal system precedes the simultaneous appearance of the long-lasting hyperpolarization following cortical stimulation and the increase in spine density by 2 weeks. These data suggest that this hyperpolarization is independent of the axon collateral system, but is dependent on the development of spines and presumably axospinous inputs, consistent with the idea that the hyperpolarization is a disfacilitation. Supported by NS30679.

586.18

DIRECT CORTICONIGRAL PROJECTIONS IN THE RAT. Y. Shinonaga[1], M. Takada*[2], and N. Mizuno[2]. [1]Department of Anatomy and Cell Biology, Faculty of Medicine, University of Toronto, Toronto, Ontario, Canada M5S 1A8. [2]Department of Morphological Brain Science, Faculty of Medicine, Kyoto University, Kyoto 606, Japan.

Earlier anatomical and physiological studies have failed to establish unequivocally the presence of direct corticonigral projections, mainly due to the technical limitations at the times. Accordingly, no detailed systematic studies on direct projections from the cerebral cortex to the ventral midbrain tegmentum (VMT) have been available at all. Thus, employing both more sensitive retrograde and anterograde tracers, cholera toxin and *Phaseolous vulgaris*-leucoagglutinin, we made an attempt to examine how the corticofugal projectioins to the VMT are topographically organized in the rat. Cells of origin of these projections were located in extensive cortical fields including the prelimbic, infralimbic, lateral and medial precentral, agranular insular, orbital and anterior cingulate cortices. Terminal distribution of these cortical fibers were simplified into five different areas in the VMT, including the ventral tegmental area, medial, central and lateral zones of the substantia nigra, and retrorubral field. Furthermore, some of the fibers were passing throught the VMT to reach the deep layers of the superior colliculus, periaqueductal gray and midbrain reticular formation. These results indicate that the direct corticofugal projections to the VMT are more extensive and well organized than have hitherto been considered in the rat. These cortical fibers may play an important role to provide another access rout to the VMT and these cortical information may influence directly upon the VMT. This work is partially supported by the Ministry of Education, Science and Culture of Japan.

BASAL GANGLIA AND THALAMUS VII

587.1

CORTICOSTRIATAL PROJECTIONS FROM THE LATERAL SUPRASYLVIAN CORTEX OF THE CAT DEMONSTRATED WITH BIOCYTIN AND CALBINDIN IMMUNOHISTOCHEMISTRY. J.G. McHaffie[1,2]*, K. Hoshino[1], M. Norita[1], and B.E. Stein[2]. Dept. Anatomy, Niigata University School of Medicine, Niigata 951, JAPAN[1] and Dept. Physiology, Medical College of Virginia, Richmond, VA 23298[2].

We have demonstrated recently (McHaffie et al. Prog. Brain Res., 1993) that regions along the lateral bank of the lateral suprasylvian (LS) sulcus, which modulates the activity of neurons in the deep laminae of the superior colliculus (SC), also projects to the striatum (ST). Presumably, these fibers form the first neuronal link in an 'indirect' projection that links the cortex with the SC. However, it has not yet been established if these corticostriatal fibers terminate on output neurons in the matrix of the ST. In the current experiments, we used the anterograde tracer biocytin in combination with calbindin immunohistochemistry, which specifically stains GABA-ergic output neurons in the matrix that project to the substantia nigra, pars reticulata or the globus pallidus. Injections of biocytin into the lateral bank of LS resulted in label in both the ST and the SC. In the ST, labeled fibers and terminals were found in the dorso-caudal aspect of the head of caudate (CA) and in the caudal part of the putamen (Put). In the CA, terminals were concentrated in the matrix, often in close apposition to calbindin-positive soma; few terminals were seen in the calbindin-negative 'patches'. In the Put, terminals were concentrated in a region adjacent to the external capsule. While these data demonstrate that corticostriatal fibers from LS terminate predominately in the matrix of the ST as delimited by calbindin immunohistochemistry, it remains to be determined with electron microscopic techniques if these terminals make direct contact with calbindin-positive output neurons. Supported by NSF grant INT-9211589 and NEI grant EY06562.

587.2

INTRINSIC EXCITATORY AND INHIBITORY CIRCUITS INTERCONNECT DIFFERENT FUNCTIONAL REGIONS OF THE CAUDATE NUCLEUS OF THE CAT. James S. Wilson*, Dept. Anat., Howard Univ. Col. Med., Washington, D.C., 20059.

Based on its cortical afferents, the neostriatum can be divided into different functional zones which include a dorsolateral motor region, an intermediate associational area and a medial limbic area. The purpose of this study was to determine if these different areas are interconnected by intrinsic circuits. An intrastriatal stimulating electrode was placed in the limbic area of the caudate (Cd) nucleus. An intracellular recording electrode was angled at 45° such that all functional areas of the Cd nucleus was traversed in a single electrode pass. In 42 neurons recorded in four cats, we found that 1) a single site of intrastriatal stimulation produced responses in all functional areas of the Cd nucleus, 2) an initial EPSP was recorded in 100% of the responsive neurons (40), and 3) the closer the recorded neuron was to the site of stimulation, the shorter was its response latency and larger was the amplitude of the EPSP. In addition, in 21 neurons, IPSPs were also recorded following the EPSPs. Because our recording electrode ran perpendicular to intrastriatal fiber bundles and because responses were recorded outside of the topographic area stimulated, we conclude that intrinsic excitatory and inhibitory circuits interconnect different functional areas of the Cd nucleus. In light of past experiments, we suggest that intrinsic circuitry can recruit assemblies of neurons not defined by extrinsic-afferent connections alone resulting in a stimulus evoked synchronization of neuronal activity.

587.3

FINE STRUCTURE OF EXTERNAL PALLIDAL TERMINALS WITHIN THE MONKEY THALAMIC RETICULAR NUCLEUS. C. Asanuma.* Laboratory of Neurophysiology, National Instute of Mental Health, NIH Animal Center, Poolesville, MD 20837.

The external pallidal projection to the squirrel monkey thalamic reticular nucleus (TRN) was examined in the electron microscope following injections of WGA-HRP into the external segment of the globus pallidus and immunohistochemistry for GABA. In brief, vibratome sections through the brains were processed for anterogradely transported WGA-HRP, and blocks of tissue through the TRN processed for electron microscopy. Thin sections were immunohistochemically stained for GABA directly on the grids with colloidal gold.

The most common GABAergic synaptic profiles within the monkey TRN neuropil are large boutons packed with many pleomorphic vesicles as well as mitochondria. These boutons stain densely for GABA, are always presynaptic, and establish *symmetric* synaptic contacts upon the somata and dendrites of TRN neurons. This GABAergic terminal type thus displays several features of the previously described F terminals in monkey dorsal thalamic nuclei and F1 terminals in the cat lateral geniculate and perigeniculate nuclei. A small number of GABAergic profiles which participate in serial synapses was also found within the TRN neuropil. These profiles are in receipt of synapses and establish symmetric synaptic contacts upon TRN dendrites. The GABA staining within these profiles is weak and they contain fewer vesicles and mitochondria than the F terminals. The external pallidal terminals, labeled with HRP, arise from thinly myelinated axons, and resemble F terminals; viz. they are large, packed with many pleomorphic vesicles and mitochondria, are always presynaptic, stain densely for GABA, and establish *symmetric* synapses. The pallidal terminals are frequently presynaptic to the somata and proximal dendrites of TRN neurons. HRP reaction product is never seen in profiles which participate in serial synapses.

These observations indicate that the external pallidal terminals within the monkey TRN resemble F terminals and establish *symmetric* synaptic contacts frequently upon the somata and proximal dendrites of TRN neurons. The fine structure and cellular relationships of other GABAergic synaptic profiles within the TRN, arising from both extrinsic and intrinsic sources, remain to be detailed.

587.4

THE (VENTRAL) PALLIDOSTRIATAL PATHWAY IN THE MONKEY: EVIDENCE FOR INTEGRATION OF BASAL GANGLIA CIRCUITS. W.P.J.M. Spooren*, E. Lynd-Balta, S. Mitchell, & S.N. Haber. Dept of Neuro. & Anat., Univ. of Rochester, Rochester NY 14642, U.S.A.

Current theories suggest that the basal ganglia consist of functionally segregated circuits which form parallel pathways from cortex via striatum to pallidum and substantia nigra and via the thalamus back to the cortex. However, this hypothesis incorporates an idea of unidirectional flow of information through the basal ganglia which lacks consideration of important feedback information. For instance, it has been shown that the pallidum projects back to the striatum, i.e. the pallidostriatal pathway, suggesting a bi-directional flow of information between the striatum and the pallidum. The pallidostriatal pathway has been investigated to some extent in the rat and the cat. However, relatively little is known about this pathway in the monkey. Understanding of the organization of this pathway in the monkey is important for the integration of this connection into theories on basal ganglia function. Accordingly we studied the pallidostriatal pathway in the monkey with special emphasis on the striatal projections of the ventral pallidum. Small circumscribed injections of both retrograde (HRP & Lucifer yellow (LY) and anterograde tracers (PHA-L, LY& tritiated amino acids) were made into various regions of the ventral and dorsal pallidum as well as into the striatum. The results demonstrate that the pallidostriatal pathway is an extensive pathway in the monkey. It is organized in a topographic manner preserving a general, but not strict medial to lateral and ventral to dorsal organization. The terminal arrangement of pallidostriatal fibers is widespread such that non-adjacent pallidal regions send fibers to the striatum that show considerable overlap in the striatum suggesting convergence of terminals from different segregated pallidal regions. Moreover, the pallidostriatal pathway is found to have a reciprocal but also a large non-reciprocal component to the striatopallidal pathway. Thus segregated pallidal regions converge in the striatum. On the basis of these data it is concluded that segregation of so-called cortico-basal ganglia-cortical pathways is maintained in the striatopallidal direction but not in the pallidostriatal direction. Supported in part by PHS grants: MH45573 and NS22511.

587.5

SHELL AND CORE IN THE PRIMATE VENTRAL STRIATUM IDENTIFIED WITH ANTIBODIES AGAINST CALBINDIN. G.E. Meredith[1] A. Pattiselanno[1], H.J. Groenewegen*[1] and S.N. Haber[2]. [1]Department of Anatomy, Faculty of Medicine, Vrije Universiteit, Amsterdam, The Netherlands and [2]Department of Neurobiology and Anatomy, University of Rochester Medical Center, Rochester, N.Y. 14642.

Shell and core subdivisions, which are recognized in nucleus accumbens of the rat ventral striatum, differ in the distributions of various neurochemicals and receptors and in their patterns of connectivity. In particular, the shell has been most closely associated with inputs from the limbic system. To explore whether comparable striatal regions exist in primates, we immuno-reacted striatal sections of marmoset and Rhesus monkey brains for calbindin and stained adjacent sections for Nissl. In the ventral striatum of both primates, a medial and ventral zone can be distinguished; in the Rhesus monkey, this zone is consistently poor in calbindin-immunostaining whereas in the marmoset, it appears more inhomogeneous. In both species, the cell density is lower than the more central zone. In the rat ventral striatum, a medial and ventral calbindin-poor zone which is also low in cell density, i.e. the shell, extends into the rostralmost parts of the striatum, whereas in both primates, this zone first appears a few millimeters caudal to the striatal rostral pole. As in the rat, this region in the primate extends into the caudalmost nucleus accumbens. For both primates, the staining and cytoarchitectural characteristics suggest a "shell" identity for the region. When compared to the rest of the striatum, however, the full extent of the primate shell appears to be proportionally smaller than that of the rodent. In both primates a rich but unevenly immunostained region, the "core", lies central to the shell. It is not possible to discern a clear border between the core and the ventral caudate nucleus or the putamen.

587.6

HIGHLY RESTRICTED INPUTS TO STRIOSOMES FROM PREFRONTAL CORTEX IN THE MACAQUE MONKEY. F. Eblen* and A.M. Graybiel, Dept. of Brain & Cogn. Sci., MIT, Cambridge, MA 02139.

We have previously demonstrated that the caudal orbital cortex is a major source of striosomal afferents in macaque monkey, whereas the remaining orbitofrontal cortex and nearby convexity strongly innervate the matrix. To continue our investigation of the prefronto-striatal projection, we made intracortical deposits of anterograde tracers (L-[35S]-methionine and WGA-HRP) in various areas of medial prefrontal and dorsolateral prefrontal cortex of 6 adult cynomolgus monkeys (*Macaca fascicularis*). Tracer distributions were studied in relation to striosome/matrix compartments demonstrated by stains for enkephalin and acetylcholinesterase.

The results indicate that most medial prefrontal areas, and dorsolateral prefrontal cortex, project predominantly to the striatal matrix. Only medial prelimbic cortex immediately rostral to the tip of the corpus callosum projected to striosomes. Striosomes labeled were mainly in the caudate nucleus; farther ventrally, enkephalin-rich zones, apparently matrix, were also labeled. Striosomes were never labeled in the dorsolateral caudate nucleus and most of the putamen. The projection fields from most prefrontal areas partially overlapped, with dorsolateral prefrontal cortex projecting the most laterally and prelimbic cortex medially along the ventricular wall.

The present findings are reminiscent of the results obtained with orbital injections. Only proisocortex labeled striosomes, the more dorsal striosomal projection was accompanied by ventral matrix labeling but the nucleus accumbens was spared. The sets of striosomes in the ventromedial caudate nucleus and parts of the ventral matrix targeted by posterior orbital and precingulate cortex may comprise a "prelimbic" area of the primate striatum.

Supported by NIH Javits Award NS25529 and DFG (Eb 127/1-1).

587.7

ORGANIZATION OF CHEMICAL COMPARTMENTALIZATION IN THE HUMAN STRIATUM. D.J. Holt*, A.M. Graybiel, and C.B. Saper. Comm. on Neurobiology, Univ. of Chicago, Chicago, IL 60637, Dept. of Brain and Cognitive Science, MIT, Boston, MA 02139, and Dept. of Neurology, Harvard Medical School/Beth Israel Hospital, Boston, MA 02115.

We recently demonstrated a pattern of cholinergic innervation of the human striatum that extends the traditional "striosome-matrix" model by recognizing the presence of three compartments with distinct levels of innervation. We now compare the compartmental segregation of other chemically-defined fiber systems, as defined by immunocytochemical staining of adjacent sections for choline acetyltransferase (ChAT) vs. met-enkephalin, tyrosine hydroxylase, or calbindin. The boundaries of the cholinergic compartments were largely respected by the other three markers, but the relationships of staining intensities were often quite complex. For example, previous studies have often assumed that striosomal and matrix compartments are identical, whether defined by cholinergic or calbindin staining. We found that, in the putamen and nucleus accumbens, regions representing the lighter staining compartments in ChAT sections were often calbindin-rich. The reversal of relative staining intensities suggests that the varying combinations of different chemical markers in the striatal compartments allow regionally specialized, transmitter-specific processing.

587.8

THE ORGANIZATION OF TEMPORAL LOBE LIMBIC INPUTS TO THE VENTRAL STRIATUM IN MACAQUE MONKEYS. D.P. Friedman*, J.P. Aggleton, [+], R.C. Saunders[#], & S. Vinsant. Bowman Gray School of Medicine, Wake Forest University, Winston-Salem, NC 27157, [+]Dept.Psychology, University of Durham, Durham, UK, and [#]Clin Brain Disorders Branch, NIMH, Bethesda, MD.

The projections to the ventral striatum from the hippocampus, rhinal sulcus, and amygdala were examined using both anterograde and retrograde tracers. The patterns of these inputs were then related to the distributions of neurochemical markers revealed by immunohistochemistry.

The hippocampal inputs arise primarily from the subiculum and adjacent regions of CA1. They terminate throughout the rostro-caudal extent of nucleus accumbens (NAS), but are almost exclusively restricted to the medial and ventral portions of this structure. Only light projections were found in the striatal portion of the olfactory tubercle. The entorhinal and perirhinal regions had a very similar pattern of NAS terminations, but they also provide an input to the olfactory tubercle.

Inputs from the amygdala, by contrast, are densest in the olfactory tubercle. A lighter projection to NAS, which arises primarily in the basal nucleus, is most dense in the caudal and ventral portions of this structure. Whereas there is considerable overlap between the amygdala, rhinal, and hippocampal inputs, there are differences. In particular, the distribution of the hippocampal and rhinal inputs appears to correspond closely to the calbindin-poor regions in the NAS. Furthermore, these projections also mapped onto a less cell-dense portion in medial and ventral accumbens that may correspond to the so-called "shell" region. The amygdala projections were more widespread and were not confined to the calbindin poor zone. *Supported by NIDA 07955.*

587.9

THE CINGULOSTRIATAL PROJECTION IN THE MONKEY: RETROGRADE TRACING STUDY. K.Kunishio* and S.N.Haber, Dept. of Neurobiology and Anatomy, Univ. of Rochester, Rochester, NY 14642

The ventral portion of the striatum receives input from structures related to the limbic lobe, and the dorsolateral region receives input from sensorimotor cortex. As part of a larger study to understand the organization of limbic-related striatal input, we studied the organization of the cingulostriatal projections in the monkey using the retrograde tracers such as Lucifer yellow(LY) and horseradish peroxidase conjugated to wheat germ agglutinin(HRP-WGA) that were injected into the different regions of the ventral and dorsolateral striatum. Routine immunocytochemistry was performed on 50μm sections. The rostroventral part of the caudate nucleus receives input from the rostral part of the anterior cingulate cortex. The ventral part of the putamen receives input from both anterior and posterior cingulate cortex. Projections to the core of nucleus accumbens are derived from the anterior cingulate cortex (areas 25, 24a, 24b, and 24c). However, labeled neurons in area 24c are found mainly in the medial portion of the lower bank of the cingulate sulcus. The shell of the nucleus accumbens receives fibers from areas 25, 24a, and 24b. In contrast, dorsolateral striatum receives projections from areas 24c and 23c, mainly from the lateral portion and the fundus of the cingulate sulcus. The results indicate that the lateral portion and the fundus of areas 24c and 23c project to the dorsolateral striatum, and the medial portion projects to the ventral striatum. It is possible that areas 24c and 23c are associated with the different functions such that the lateral portion and the fundus of each area are related to the motor function, whereas the medial portion is associated with limbic related function. Supported in part by MH45573

587.10

POST-ROLANDIC CONNECTIONS OF THE MEDIAL PULVINAR IN THE RHESUS MONKEY. D.F. Siwek* and D.N. Pandya, E.N.R.M. Veterans Administration Hospital, Bedford, MA, 01730

Retrograde (HRP) and anterograde (RLAA) neuronal pathway tracers were injected into the thalamus of 5 adult rhesus monkeys to examine the pattern of the connections between the medial pulvinar (Pul_m) and the cerebral cortex. The results showed that an injection of HRP that encompassed the medial pulvinar, labeled neurons in the inferior parietal lobule (areas Opt, PFG), superior temporal gyrus (TS1, TS2, TS3), superior temporal sulcus, STS (areas TPO_{1-3}, IPa, PGa, OAa) and Insula. Additionally, labeled neurons were found on the medial surface (areas 23, PGm, and retrosplenial cortex) and in the parahippocampal gyrus (areas TF, TH, and TL and presubiculum). A similar injection of RLAA showed a nearly identical pattern of labeling indicating that these projections are reciprocal. When the injection was limited to the central portion of Pul_m, label was observed only in the poly-modal cortex, areas $TPO_{2\&3}$, within STS and in area TS1 and TS2 to a slight extent. When a similar injection extended dorsally in Pul_m, the pattern of labeling in the STS was the same, but label also occurred in retrosplenial cortex, area 23, Presubiculum, TL and TH.

These data show that whereas neurons in the central portion of Pul_m are reciprocally connected with the polymodal cortex of the STS, thalamic cells lying in more peripheral locations within the Pul_m are connected with post-Rolandic parasensory association areas of the temporal and parietal cortices. *(Supported by NIH grant 16841 and the ENRM VA Hospital, Bedford, MA, 01730)*

587.11

THE RETICULAR THALAMIC NUCLEUS (RTN) PROJECTS CONTRALATERALLY TO THE CENTROMEDIAN/PARAFASCICULAR COMPLEX (CM/Pf) IN PRIMATES, BUT NOT TO THE PARAFASCICULAR NUCLEUS (PF) IN RODENTS. L.-N. Hazrati*, D.Pinault, and A.Parent Lab. of Neurobiology, University Laval, Québec, Canada, G1J 1Z4

Retrograde and anterograde tracing studies have been conducted to investigate the contralateral RTN projection to the CM/Pf in squirrel monkeys and to Pf area in rats. In monkeys, injections of the fluorescent retrograde tracer true blue (TB) into CM/Pf area labeled RTN cells on both ipsilateral and contralateral sides. However, the labeled cells were less numerous (1/5) contralaterally than those seen on the ipsilateral side. Anterograde tracing with biocytin in monkeys lead to the labeling of very few RTN fibers that crossed the midline and arborized in the contralateral thalamus. These observations demonstrated that the RTN projections to contralateral thalamus exists in squirrel monkeys, but are not very extensive. In Sprague-Dawley rats, we did not observe any labeling in the contralateral RTN following TB injection in the Pf. However, in these experiments a heavy labeling of cell bodies was observed on the ipsilateral side. In addition, a few cells were seen in the contralateral side, specifically in an ill-defined area that borders the RTN and zona incerta (ZI). The use of cholera-toxin as retrograde tracer combined to parvalbumin (PV) and calbindin (CaBP) immunohistochemistry demonstrated that these few cells were located in the CaBP-rich ZI and were not in the PV-rich RTN. Anterograde tracing experiments with biocytin in rats, did not lead to the labeling of any RTN fiber on the contralateral side, thus confirming the results obtained with retrograde labeling technique. In summary, this study has revealed the presence of a discrete contralateral RTN projection to the CM/Pf area in squirrel monkeys, whereas the contralateral projection to Pf could not be confirmed in rodents. The negative results in rodents do not preclude the possible existence of contralateral RTN projections to other thalamic nuclei, as reported previously. Nevertheless, we believe that contralateral projections of RTN should be reviewed for each of the thalamic nuclei in various species so as to delineate the functional significance of such contralateral projections.

587.12

THE THALAMIC RETICULAR NUCLEUS (TRN) IN THE MONKEY: A CYTOARCHITECTONIC AND MORPHOLOGICAL STUDY. *J. Lübke*, Department of Human Anatomy, University of Oxford, Oxford OX1 3QX, UK.*

The monkey TRN is a sheet of GABAergic neurons which surrounds most of the dorsal thalamus. The main body of TRN consists of 5-8 layers of cells bordering from rostral to caudal, the lateral part of the anteroventral, ventrolateral and ventrobasal nuclei and the lateral geniculate nucleus. The continuity of the TRN with the zona incerta is obvious in coronal sections. In a region assigned to the perireticular nucleus in cats only a few scattered small cells could be observed in the adult monkey TRN. To investigate whether neurons differ morphologically in certain sensory parts (sectors) of the TRN I have injected neurons intracellularly with Lucifer yellow using a fixed slice preparation. Neurons in the caudal (visual) and intermediate (somatosensory) sectors of the TRN have a fusiform, elongated morphology. Neurons have round, ovoid or elongated somata ranging in area between 625-900 μm^2. In general, 3-7 primary dendrites, often with a very thick initial segment, emerge from the two poles of the soma, which then run paralell to the two borders of the nucleus for up to 500 μm. Only a few, shorter dendrites run perpendicular to the plane of the nucleus. The dorso-rostral part (motor, limbic) of the TRN contains some neurons with a multipolar morphology, but they are intermingled with fusiform neurons. Some of the smooth 1st order dendrites give rise to 2nd order dendrites (7-12) which branch into shorter 3rd order dendrites (2-5). Spine-like protusions and/or hair-like processes as well as dendritic varicosities are mainly found on 2nd and 3rd order dendrites. In contrast to reported heterogeneity of TRN neurons in somatostatin and calbindin-immunoreactivity in the cat and monkey and the expression of calbindin in a discrete population of rat TRN neurons during development we have found no basis for classification of monkey TRN neurons according to their dendritic morphology. *Supported by the Wellcome Trust.

587.13

RETICULAR THALAMIC NUCLEUS INPUT TO THE NUCLEI OF THE MONKEY THALAMUS: LIGHT AND ELECTRON-MICROSCOPIC STUDY. H. Yi, I.A. Ilinsky*, and K. Kultas-Ilinsky, Dept. of Anatomy, Univ. of Iowa Coll. Med. Iowa City, IA, 52242.

The reticular nucleus of the thalamus (RNT) is believed to regulate the flow of information between the thalamus and cortex. Yet, its synaptic relationships with thalamocortical projection neurons (PN) and local circuit neurons (LCN) are largely unknown, especially in primates. In this study, iontophoretic injections of WGA-HRP or PHA-L were made at different RNT locations, and labeled fibers and terminals were traced in limbic and motor thalamic nuclei. Additionally, postembedding immunocytochemistry with anti-GABA antibody was performed in WGA-HRP labeled tissue.

The results demonstrate that single RNT fibers travel distances of several millimeters posteriorly from the injection site, giving off occasional short thin branches terminating with enlargements. In none of the nuclei studied were RNT boutons seen to form pericellular baskets; instead, they appeared to be distributed randomly in the neuropil. EM analysis confirmed that RNT terminals made a few synaptic contacts at all levels of somadendritic membrane of PN as well as LCN. Internuclear differences in the ratio of RNT contacts on PN and LCN, and the overall bouton distribution pattern and density were noted suggesting functional differences in the mode of RNT interaction with different thalamic nuclei. Supported by NSF Grant 9109065.

587.14

SYNAPTIC RELATIONSHIPS OF CEREBELLAR AFFERENT TERMINALS IN THE MONKEY THALAMUS STUDIED IN SERIAL SECTIONS AND 3D RECONSTRUCTIONS. A. Mason, S. Beck, H. Yi, K. Kultas-Ilinsky* and I.A. Ilinsky, Dept. of Anatomy, Univ. of Iowa, Coll. of Med., Iowa City, IA 52242.

Previous EM studies of cerebellar afferent terminals to the monkey thalamus have demonstrated their engagement in a variety of complex synaptic relationships with dendrites of projection (PN) and local circuit neurons (LCN). However, the analysis of single ultrathin sections at random did not allow to appreciate the real spatial organization of these synaptic arrangements.

In this study WGA-HRP injections were made in dentate nuclei bilaterally. Labeled cerebellar boutons (CB) in the ventrolateral thalamic nucleus were analyzed both quantitatively and qualitatively in serial ultrathin sections. Then the outlines of CB and associated structures were digitized, aligned and reconstructed into 3-dimensional images using Silicon Graphics hardware and Alies Studio software.

Our results indicate that a single CB contacts no more than 2 PN dendrites but up to 5 vesicle containing LCN dendrites. The number of synaptic contacts established on each PN dendrite by a single CB can be as high as 12, whereas on a single LCN dendrite it is usually 1, or 2 at most. Dendro-dendritic contacts between LCN and PN dendrites contacted by the same CB were found but infrequently. In contrast, dendro-dendritic synapses between LCN dendrites receiving synapses from the same CB were significantly more frequent.

These results further illustrate the complexity of thalamic circuits processing cerebellar information prior to its transmission to the cortex. Supported by RO 1 NS 24188.

587.15

GLUTAMATERGIC INPUTS FROM THE PEDUNCULOPONTINE NUCLEUS TO THE MIDBRAIN DOPAMINERGIC NEURONS IN PRIMATES: A LIGHT AND ELECTRON MICROSCOPIC STUDY. A. Charara*, Y. Smith and A. Parent. Centre de Recherche en Neurobiologie, Hôpital de l'Enfant-Jésus, Québec, Canada, G1J 1Z4.

The present study was undertaken to investigate the pattern of synaptic innervation of the midbrain dopaminergic neurons by the ascending fibers from the pedunculopontine nucleus (PPN) in primates. For this purpose, adult squirrel monkeys (*Saimiri sciureus*) were used, and the anterograde transport of *Phaseolus vulgaris*-leucoagglutinin (PHA-L) was combined with immunohistochemical techniques for the visualization of tyrosine hydroxylase (TH) and the calcium binding protein calbindin D-28K (CaBP) at both light and electron microscopic levels. At the light microscopic level, injections of PHA-L in the PPN were seen to produce a dense anterograde labeling of fibers and axonal varicosities that arborized profusely throughout the rostrocaudal extent of the substantia nigra pars compacta, ventral tegmental area and retrorubral area, but much less so in the substantia nigra pars reticulata. The anterogradely labeled varicosities were often closely apposed on the surface of dendritic shafts and perikarya that displayed TH or CaBP immunoreactivity. At the electron microscopic level, the PHA-L-immunoreactive terminal boutons were found to be heterogeneous in size (ranging from 0.5 to 1.5 µm in maximum diameter), contained numerous densely packed round electron lucent vesicles, a few dense core vesicles and 1-2 mitochondria. These terminals formed asymmetric synapses predominantly with dendritic shafts displaying TH immunoreactivity. Moreover, postembedding immunogold techniques demonstrated that some of these terminals expressed glutamate immunoreactivity.

Our findings provide the first ultrastructural evidence for the existence of a glutamatergic input from the pedunculopontine nucleus to the midbrain dopaminergic neurons in primates. This excitatory brainstem input may play a crucial role in the functional organization of the basal ganglia, in both normal and pathological conditions. {supported by the MRC and FRSQ}

587.16

CORTICAL AND SUBCORTICAL INPUTS TO THE CENTROMEDIAN-PARAFASCICULAR THALAMIC COMPLEX IN PRIMATES. P.-Y. Côté*, A. Charara, and A. Parent. Centre de Recherche en Neurobiologie, Hôpital de l'Enfant-Jesus, Québec, Canada, G1J 1Z4.

Recent studies in our laboratory documented the relationship between the centromedian-parafascicular thalamic complex (CM-Pf) and the basal ganglia in primates. From these studies emerged the concept that the thalamus actively participates in information processing through the basal ganglia. The aim of the present study is to examine the cortical and subcortical afferents to the CM-Pf complex, using the retrograde tracers WGA-HRP and cholera toxin B subunit. After injections centered upon the CM-Pf complex, retrograde cell labelling occured in both cortical and subcortical structures. At cortical levels, retrogradely labelled neurons were found principally in cingulate, parietal, temporal, frontal and prefrontal cortices. Cortical labelled cells were large, polymorphic and distributed according to a specific laminar pattern. Most labelled neurons occured in layer V, where they displayed a pyramidal shape, whereas smaller, oval neurons were found principally in layer VI. At subcortical levels, labelled cells were encountered in the following structures : reticular thalamic nucleus, internal pallidal segment, basal accessory amygdaloid nucleus, substantia nigra, and brainstem nuclei including the pedunculopontine nucleus, laterodorsal tegmental nucleus, central and dorsal raphe nuclei, and locus coeruleus. Labelled cells also occured contralaterally in the rostrodorsal half of the reticular thalamic nucleus, in the anterior and middle regions of the cingulate cortex and in brainstem nuclei.

These findings indicate that the major inputs to the CM-Pf complex come from a highly heterogeneous set of structures among which the ipsilateral cerebral cortex, reticular thalamic nucleus and brainstem are the most prominent. The results of our current studies should help to refine the emerging concept suggesting that CM and Pf are respectively associated with the "motor" and "associative" aspects of basal ganglia function via two distinct, parallel circuits.

[Supported by MRC of Canada and FRSQ].

587.17

DIGITIZED ANATOMY CORRELATED WITH [18F]fluoro-L-DOPA-PET IN MONKEY.

A. W. Toga*, W.P. Melega, M.J. Raleigh, S.C. Huang, D.C. Yu, M.E. Phelps, B. Quinn, B.A. Payne, and K. Ambach

Laboratory of Neuro Imaging, Dept of Neurology, Pharmacology and Psychiatry, UCLA Sch. of Med. Los Angeles, CA. 90024

Interpretation of functional anatomic scans is hampered by low resolution and limited structural detail. Mapping functional or metabolic data upon its anatomic counterpart helps accurately identify the site action. We have been able to improve delineation of regions of interest of [18F]fluoro-L-DOPA (FDOPA) PET data by mapping onto higher resolution digital anatomic data from the same animal.

An adult male monkey (vervet) received a pre-amphetamine control FDOPA scan followed by 10d of chronic amphetamine treatment (incremental dose from 4mg/kg/d to 18mg/kg/d; i.m.). A post-drug FDOPA scan was obtained at one week and one month and the animal immediately sacrificed. The head was rapidly frozen in liquid nitrogen and cryosectioned on a modified PMV cryomacrotome. High resolution color images (1024^2, 24-bit per pixel) were digitized directly from the cryoplaned blockface at 300 micron intervals (Quinn *et al.*, Soc. Neurosci., 1992). Anatomical images were reconstructed into a three dimensional (3D) volume that served as a reference template for interpretation of PET data. Pre- and post-drug FDOPA scan data were mapped to this anatomic data using a global matching transformation. Significant reductions of [18F] activity were identified in striatal structures. Higher resolution digitized anatomy and multimodality mapping enabled us to be more specific in the localization of sites of action and appreciate greater detail in metabolically active dopaminergic structures.

SPINAL CORD AND BRAINSTEM V

588.1

A COMPARISON BETWEEN PROJECTIONS OF ORBITAL CORTEX AND AMYGDALA TO THE MEDULLARY LATERAL TEGMENTAL FIELD IN THE CAT. B. F. M. Blok, L. J. Mouton* and G. Holstege, Dept. Anatomy, Groningen, Netherlands.

Recently a new concept was presented to divide into the motor system in three subsystems (Prog. Brain Res., vol. 87, 1991, p. 398). A basic motor system exists containing premotor interneurons, and a somatic motor system controlling proximal and distal muscles. The third system is the emotional motor system (EMS) with a medial component determining the gain setting of motoneurons, and a lateral component involved in eliciting specific emotional behavior. The orbitofrontal cortex, the central nucleus of the amygdala (CA) and the bed nucleus of the stria terminalis (BNST) are thought to be part of the lateral component of the EMS. The question arises what the relation is between these parts of the lateral components. In 4 cats 10-50 nl WGA-HRP was injected into the medullary lateral tegmental field (LTF). Retrogradely labeled cells were observed bilaterally in the orbital gyrus (OG), and ipsilaterally in the CA and the lateral part of the BNST (BNSTL). After a large injection in dorsal parts of the LTF at the level of the facial nucleus retrograde labeling was observed in lamina V in the lateral bank of presylvian sulcus, the orbital gyrus and coronal gyrus. The CA and the BNSTL were extensively filled with retrogradely labeled cells. After a small injection in the LTF dorsal to the facial nucleus retrogradely labeled cells were observed only in lamina V of the ventral part of the lateral bank of the presylvian sulcus and in the medial part of the OG. Labeled neurons were present in the central parts of CA and in the most ventral and lateral parts of BNSTL only. The results suggest that the medullary LTF receives afferents from the OG, the CA and the BNSTL in a topically organized manner. A major difference between the projections of the OG and the CA and the BNSTL to the LTF is that the OG projects bilaterally, and the CA and BNSTL ipsilaterally. The similarity between the OG, CA and BNSTL projections to the medullary LTF suggests that all three areas are involved in similar emotional motor systems, but the bilaterality of the OG projections suggests a more specific projection system originating in the orbital gyrus.

588.2

AFFERENTS TO THE NUCLEUS MAGNOCELLULARIS OF THE MEDULLA. Y.Y. Lai,* J.R. Clements and J.M. Siegel. Dept Psychiat, UCLA and Neurobiol Res, VAMC, Sepulveda, CA 91343, Dept Vet Anat, Texas A&M Univ, College Station, TX 77843.

The nucleus magnocellularis (NMC) had been found to relate to both motor and cardiovascular activities. NMC glutamate receptors are involved in motor activation and inhibition, while both glutamate and acetylcholine receptors participate in cardiovascular regulation. This experiment was designed to identify the afferent projections to the NMC. Fifty naloliters of 2.5% WGA-HRP was injected into the NMC. After 2 days, cats were sacrificed and their brainstems processed with TMB, NADPH-d and glutamate immunohistochemistry. Heavy concentrations of WGA-HRP neurons were found in nuclei reticularis pontis oralis and caudalis, Edinger-Westphal, Kolliker-Fuse, and paragigantocellularis; moderate concentrations in periaqueductal gray, mesencephalic reticular formation, retrorubral, paralemniscal tegmental field, cuneiformis, subcoeruleus and parvocellularis; light concentrations in pedunculopontine (PPN), trigeminal sensory, gigantocellularis, vestibular complex, lateralis reticularis, and medullae oblongatae centralis. Double-labeled WGA-HRP/glutamate and WGA-HRP/NADPH-d neurons could be found throughout the brainstem nuclei. However, no cholinergic neurons (LDT/PPN neurons with NADPH-d staining) double labeled with WGA-HRP.

588.3

BURST ACTIVITY IN THE LATERAL MEDULLARY RETICULAR FORMATION OF THE TURTLE. *L. N. Eisenman*, R. Sarrafizadeh, and J. C. Houk*. Department of Physiology, Northwestern University Medical Center, Chicago, IL 60611-3008.

It is believed that burst activity in the cerebellorubral network is supported by positive feedback transmitted through recurrent excitatory connections. Recent anatomical studies (Sarrafizadeh et al. Neurosci. Lett. 149:59, 1993) have demonstrated the existence of the lateral reticular nucleus (LRN) in the lateral medullary reticular formation (LMRF) of the turtle. To further understand bursting in the cerebellorubral network, we initiated extracellular recording studies of the cells in the LMRF.

The in vitro brainstem-cerebellum was prepared from freshwater pond turtles (Chrysemys picta), and stimulating electrodes were placed in the spinal cord (SC) and cerebellar cortex (CB). Recordings were made from 31 cells at depths from 300 to 1000 μm from the ventral surface. Electrode tracks and lesions suggest that we were recording in the region of the LRN. The cells show little spontaneous activity so they were located by their responses to single pulses or trains to either SC or CB. Short latency responses to single pulses to the SC were recorded from 6 cells with latencies varying between 2.5 and 40 ms, and 16 cells burst to 100 Hz SC stimulation. 10 cells showed short latency responses to CB stimulation with latencies varying between 4 and 30 ms, and 19 cells burst to 100 Hz stimulation of CB. Bursts outlasted the stimulus by as much as several seconds and achieved frequencies up to 50 Hz.

Since these cells displayed activity similar to that seen in red nucleus (RN), we attempted simultaneous recording. In four cases we isolated single units in the contralateral RN while recording from LMRF. In our best case both cells burst simultaneously in response to 100 Hz train stimulation of SC. The cells displayed similar bursting behavior where the firing was initially vigorous and then slowly tapered off. In some trials, the bursts were not tightly linked. The variability of the responses may result from differing levels of activity in the multiple recurrent pathways affecting each of the cells. Simultaneous RN and putative LRN bursts with similar temporal patterns support the recurrent network concept.

588.4

SOME NEW OBSERVATIONS ON THE RUBROSPINAL TRACT IN THE CAT, LIGHT- AND ELECTRONMICROSCOPICAL STUDIES. Suus Brugman, R. Vos, V. van der Horst*, H. de Weerd and G. Holstege, Dept. Anatomy, Groningen, Netherlands.

In 5 cats 10-20 nl WGA-HRP was injected in a large portion of the caudal red nucleus and immediately adjacent areas. In all 5 cases labeled fibers were observed to descend contralaterally in the dorsolateral funiculus throughout the length of the spinal cord. Some labeled fibers were also found s2in the ipsilateral dorsolateral funiculus, and in one case a few continued into the sacral cord. Contralaterally, labeled rubrospinal fibers were observed to terminate in laminae V to VII and in two cases in laminae III and IV, but only at the level C8-T1. In all 5 cases labeled fibers were found among motoneurons in the dorsolateral part of lamina IX at the transition level C8-T1. In one of the 5 cases the WGA-HRP red nucleus injection was combined with a retrograde HRP injection in the intrinsic forepaw muscles. The C8 and T1 segments were processed for EM, according to the TMB method of Ralston and Milroy (1991). In semithin sections anterogradely labeled fibers were observed around retrogradely labeled motoneurons. In EM sections heavily labeled terminals with axodendritic and axosomatic synaptic contacts, and many rounded vesicles were found in the intermediate zone. In the area of the labeled motoneurons several labeled terminals were found making contacts with labeled dendrites and somata. A relatively small number of mainly rounded vesicles were present in these terminals. Very rarely some flattened, coated and dense core vesicles were observed. The number of rounded vesicles in these terminals appeared smaller than in the terminals in the intermediate zone and much smaller than in the rubromotoneuronal terminals in the monkey (Ralston et al.; N.S. Lett., 95:102). The EM-results indicate that direct, probably excitatory rubro-motoneuronal projections exist in the cat. The paucity of the rounded vesicles in the terminals suggests that the rubro-motoneuronal projections are less concentrated in the cat than in the monkey.

588.5

CONNECTIVITY PATTERNS OF SINGLE LAST-ORDER PAD MEDIATING INTERNEURONS WITH TWO BRANCHES OF THE SAME GROUP I FIBER. J.N. Quevedo*, J.R. Eguibar, I. Jiménez and P. Rudomin. Dept. of Physiol. CINVESTAV and ICUAP, México 07000.

In the anesthetized cat, we have analyzed the connections of single last-order interneurons mediating PAD with two intraspinal branches of the same afferent fiber. A pair of stimulating micropipettes was introduced into the intermediate nucleus, with tips separated 0.5-5 mm, to produce antidromic responses in both branches. PAD in each branch was detected as a reduction of the intraspinal threshold. Last-order PAD mediating interneurons were directly activated through the same micropipettes used for excitability testing. At short conditioning-testing stimulus intervals (1.5-2 ms) the PAD evoked by conditioning microstimulation (μS) is monosynaptic (MS-PAD). In 10/14 of fibers, μS with a low strength (below that required to antidromically activate the proximal branch of the afferent fiber), produced MS-PAD in the proximal but not in the distal branch. When the strength of the intraspinal stimulus was increased, the MS-PAD elicited in the proximal branch grew by 2-3 discrete steps, suggesting recruitment of additional interneurons. Some (but not all) of these interneurons appeared to be connected also with the distal branch (n=2). In one fiber μS applied through one micropipette produced MS-PAD in the distal branch but not in the proximal branch, whereas μS applied through the other micropipette produced MS-PAD in both branches. This observation discards the possibility that the effect of μS was due to a non-synaptic mechanism (i.e., local responses). Our results suggest a) that some of the PAD mediating interneurons make selective contacts with a single branch while others may have contacts with at least two branches of the same afferent fiber; b) that a single branch can be target of more than one interneuron and c) that the effects produced by interneurons synapsing with one branch can be rather discrete and may not spread to the other branch. This arrangement could be the basis for the selectivity in the control exerted by the motor cortex on PAD-mediating interneurons shown in the accompanying presentation. Partly supported by NIH NS09196 and CONACyT 039-N9107.

588.6

DIFFERENTIAL CONTROL EXERTED BY THE MOTOR CORTEX ON THE SYNAPTIC EFFECTIVENESS OF TWO INTRASPINAL BRANCHES OF THE SAME GROUP I AFFERENT FIBER. J.R. Eguibar*, J.N. Quevedo, I. Jiménez and P. Rudomin. Dept. of Physiol. CINVESTAV and ICUAP, Mexico 07000.

We have shown previously that stimulation of the motor cortex has rather selective effects on the PAD of group I muscle afferents (Neurosci. Abstr., 17:1024,1991). We now report the effects of cortical and sensory nerve stimulation on the PAD of two intraspinal branches of the same single afferent fiber in the anesthetized cat. To this end two micropipettes were introduced in the intermediate nucleus region of the lumbar spinal cord. Antidromic responses of afferent fibers were recorded from a gastrocnemius or semitendinosus fine nerve filament. Collision tests were used to ensure that the antidromic responses were produced by activation of two intraspinal branches of the same afferent fiber. Stimulating pulses were delivered in alternation through each micropipette and stimulus current was automatically adjusted to produce antidromic firing with a constant probability (0.5) in each branch. Altogether we analyzed the effects of conditioning inputs on two branches of 11 Ia and 2 Ib fibers. PBSt conditioning stimulation (4 shocks, 300 Hz, > 2xT) reduced the intraspinal threshold of both branches to about the same extent. However, in 5/13 fibers, PBSt stimulation with low strengths (< 1.4 x T), produced larger PAD in one branch than in the other branch. Differential inhibition of PAD elicited in two branches of the same Ia fiber was also obtained by stimulation of the motor cortex (8 shocks, 700 Hz) or cutaneous nerves (n=4). The asymmetry of the cortical and cutaneous actions depended on the magnitude of the background PAD. In one Ib fiber we observed that cortical stimulation could reduce the intraspinal threshold of one branch practically without affecting the threshold of the other branch. Our observations suggest that two branches of the same afferent fiber can be the targets of different sets of PAD mediating interneurons and that these interneurons have rather selective connections with the intraspinal terminals of the fibers. This arrangement could be the basis for a differential control of presynaptic inhibition exerted by segmental and supraspinal inputs. Partly supported by NIH NS09196 and CONACyT 039-N9107.

588.7

CHANGES IN POLYSYNAPTIC INPUTS TO MOTONEURONS DURING A REVERSIBLE COLD BLOCK OF THE DORSOLATERAL CAT SPINAL CORD. J.F. Miller*, K.D. Paul, W.Z. Rymer and C.J. Heckman. Physiology, Northwestern Univ. & Veterans Administration, Lakeside Hospital, Chicago IL 60611

A reversible cold block of the dorsal thoracic spinal cord of the cat was employed to study the effects of dorsolateral descending pathways on synaptic inputs to hindlimb motoneurons. This technique allows intracellular recordings of synaptic potentials to be obtained before and during the block in the same cell. Two afferent input systems have been studied thus far: the ipsilateral cutaneous sural nerve and the contralateral tibial nerve. Both nerves generate a mixture of excitation and inhibition in extensor motoneurons.

In the precollicular decerebrate preparation prior to cold block, the excitatory components of these inputs are dominant. Prolonged electrical stimulation (1s, 50-100 Hz) of either nerve resulted in a powerful reflex excitation of the cat medial gastrocnemius (MG) muscle. However, during the cold block, the reflex excitation was either markedly reduced or, in 2 of 6 experiments, actually converted to net inhibition. Similarly, intracellular recordings of the synaptic potentials elicited by these two inputs in MG motoneurons before and during cold block have shown that the inhibitory components of the PSPs were substantially increased (10-80%) in 6 of 8 cells. This increased potency of the inhibitory pathways may partially account for the changes in recruitment seen in surgical lesions of the dorsolateral quadrants (Powers and Rymer, *J. Neurophysiol.* 45: 1540, 1988).

Supported by NIH grant NS28076 and VA Merit Reviews.

588.8

EXCITATION OF LUMBAR MOTONEURONS BY THE MEDIAL LONGITUDINAL FASCICULUS IN THE *IN VITRO* BRAINSTEM SPINAL CORD PREPARATION OF THE NEONATAL RAT M.K. Floeter* and A. Lev-Tov[1]. Lab of Neural Control, NINDS, NIH, Bethesda, Maryland, 20892 and [1]Department of Anatomy, Hebrew University Medical School, Jerusalem, Israel

The excitation of lumbar motoneurons by reticulospinal axons traveling in the medial longitudinal fasciculus (MLF) was examined in the newborn rat. Injections of the tracer DiI into the MLF of 6 day old rats labeled a small number of fibers within the lumbar enlargement. Using *in vitro* preparations of the brainstem and spinal cord of rats 1-6 days postnatal, the MLF was stimulated and intracellular recordings were made from lumbar motoneurons. MLF stimulation excited motoneurons through long latency pathways in most motoneurons and through both short (<40 ms) and long latency connections in 16 of 40 motoneurons studied. Short and longer latency components of the excitatory response were evaluated using mephenesin to reduce activity in polysynaptic pathways. Paired-pulse stimulation of the MLF revealed a modest temporal facilitation of the short latency EPSP at short inter-stimulus intervals (20-200ms). Trains of stimulation at longer interstimulus intervals (1-30 seconds) resulted in a depression of EPSP amplitude. The short latency excitation from the MLF was reversibly blocked by CNQX, an antagonist of non-NMDA glutamate receptors, except for a small CNQX-resistant component.

588.9

PROJECTION PATTERNS OF SINGLE PONTINE RETICULOSPINAL AXONS IN THE CERVICAL AND LUMBAR ENLARGEMENTS IN THE CAT. K. Matsuyama, Y. Kobayashi and S. Mori*, Department of Physiology, Asahikawa Medical College, Asahikawa, 078, JAPAN.

In this study, we examined the projection patterns of single reticulospinal axons in the cervical and lumbar enlargements (CE and LE) of the cat spinal cord. For this purpose, we injected an anterograde neural tracer, Phaseolus vulgaris leucoagglutinin (PHA-L, 5% solution, 0.4 μl) into the left pontine reticular formation in one cat (P1.5, H-3.5, L1.5). After a survival period of 8 weeks, the animal was perfused and both the brain and the spinal cord were immediately removed. Serial transverse sections (50 μm) of the brainstem and the spinal cord (C1 to T2 and L4 to S2) were cut on a cryotome and were stained according to a PHA-L immunohistochemical procedure. In this example, pontine reticulospinal axons, including their collaterals and terminal fibers, could be traced as far as the sacral level. We were able to fully reconstruct the collateral branchings of three axons descending in the left ventral funiculus of the CE and a further three axons in the LE. In the CE, each axon gave off a minimum of one, and a maximum of four collaterals in each segment. In the LE, the maximum number of collaterals was two, and in some segments no collateral branching was observed. All collaterals reconstructed from two axons in each enlargement projected only to the left (ipsilateral) gray matter. Collaterals from the remaining axon in each enlargement projected to both the left and the right gray matter. In addition, collaterals from any one axon always showed similar termination patterns in the different segments.

588.10

EXCITATORY POSTSYNAPTIC POTENTIALS EVOKED BY VENTROLATERAL FUNICULUS STIMULATION IN NEONATAL RAT MOTONEURONS IN VITRO. M. Y. Wang* and R. Zhu. Cell Electrophysiology Lab, Wannan Med. College, Wuhu, Anhui 241001, China.

Intracellular recordings were made from 25 antidromically identified motoneurons(MNs) in transverse thoracolumbar spinal cord slices(500 μm) from neonate rats(7-16 days). In 20 MNs, a depolarization potential(EPSP) was evoked by ventrolateral funiculus stimulation. When recorded at resting potential, the mean latency, time-to-peak, amplitude, half-decay time and duration of 1.2 ms, 2.7 ms, 14 mV, 5 ms and 33 ms,respectively, was obtained from the EPSPs elicited by suprathreshold stimuli. The EPSPs were graded and also the amplitude was membrane potential-dependent with mean reversal potential of -8 mV. The constant latency of EPSPs was observed at 0.1 -5 Hz stimuli, while the amplitude was eliminated by stimuli of > 20 Hz. Low Ca/high Mg solution attenuated but Mg-free solution enhanced the EPSPs, which were depressed by kynurenic acid (0.5-1 mM) and partially by ketamine(50-100 μM), APV(1-10 μM) or DNQX(1 μM). The results suggest that MNs are possibly excited by the descending fibers via releasing excitatory amino acids.
(Supported by China NNSF No.38970295)

588.11

THE SYNAPTIC CONNECTIONS BETWEEN VENTROLATERAL FUNICULUS AXONS AND α-MOTONEURONS IN THE NEONATAL RAT SPINAL CORD. M. Pinco* and A. Lev-Tov, Dept. of Anatomy, The Hebrew University Medical School, Jerusalem, Israel.

Stimulation of ventrolateral funiculus axons (VLF) in the *in vitro* spinal cord preparation of the neonatal rat, elicited compound PSPs in lumbar α-motoneurons. Bath application of specific blockers of excitatory and inhibitory amino acid receptors, resolved a non-N-methyl D-aspartate (non-NMDA) and an NMDA receptor mediated, short- and long-latency EPSPs, as well as $GABA_A$-and glycine-mediated IPSPs. The short-latency EPSPs elicited by VLF stimulation in the presence of the polysynaptic blocker mephenesin, the NMDA receptor blocker 2-amino-5-phosphonovaleric acid (APV), and the glycine and $GABA_A$ receptor blockers strychnine and bicuculline, did not exhibit the prolonged synaptic depression which is characteristic of EPSPs elicited during low-frequency activation of dorsal root afferents. Double pulse and tetanic stimulation induced facilitation and potentiation of EPSP elicited by VLF stimulation, and at the same time caused a severe depression of EPSPs produced by dorsal root afferents. High frequency stimulation of VLF axons in low-calcium Krebs saline exhibited an enhanced potentiation, reaching maximal values of 600- 800% above the pretetanic control, and a substantial post-tetanic potentiation (PTP). Stimulation of dorsal root afferents under the same conditions resulted in 30-60% EPSP potentiation followed by PTP. It is suggested that synapses formed by propriospinal and reticulospinal tract fiber (which are the main descending constituents of the VLF, and are known to develop at an early embryonic stage) on lumbar α-motoneurons, are functionally specialized, and their high level frequency potentiation helps to maintain efficacious segmental and inter-segmental connectivity in the embryonic and the neonate mammalian spinal cord.

588.12

MONOSYNAPTIC INNERVATION OF OROFACIAL MOTOR NEURONES BY NEURONES OF THE PARVICELLULAR RETICULAR FORMATION. D. Mogoseanu, A.D. Smith* and J.P. Bolam, MRC Anatomical Neuropharmacology Unit, Mansfield Road, Oxford, OX1 3TH, United Kingdom.

The parvicellular reticular formation (PcRt), one of the subcortical premotor regions, projects to the cranial motor nuclei that innervate the orofacial muscles. In order to determine whether the PcRt has the potential to directly influence the orofacial motor neurones a combined anterograde and retrograde study was carried out in the rat.

Rats received injections (10 μL) of cholera toxin B conjugated to horseradish peroxidase (CB-HRP) in the masticatory muscles or facial muscles and stereotactic injections of biocytin in the PcRt. After 24h they were anaesthetised, perfuse-fixed and sections of the trigeminal motor nucleus (TMN) or facial motor nucleus (FMN) were incubated to reveal the retrogradely transported CB-HRP and the anterogradely transported biocytin. The sections were then examined by light and electron microscopy.

The sections of the motor nuclei contained many retrogradely labelled neurones interspersed among fibres and terminals that were anterogradely labelled with the biocytin from the PcRt. In the electron microscope the retrogradely labelled neurones were seen to receive synaptic input from many terminals. The biocytin labelled terminals, in both the TMN and FMN had similar morphologies: they were 1-2μm in diameter, contained several mitochondria and were packed with many round vesicles. In the TMN anterogradely labelled terminals formed both symmetrical and asymmetrical synapses with both retrogradely labelled or unlabelled dendrites and perikarya of neurones. In the FMN the anterogradely labelled terminals were observed to form mostly asymmetrical synapses with both retrogradely labelled or unlabelled perikarya and dendrites.

It is concluded that the neurones of the PcRt that project to the orofacial motor nuclei form direct synaptic contact with motor neurones that project to orofacial muscles. This synaptic organisation represents a route whereby the PcRt can influence orofacial movements directly.

588.13

DISTRIBUTION OF GABA-IMMUNOREACTIVE PREMOTOR NEURONS FOR THE TRIGEMINAL MOTOR NUCLEUS IN THE RAT. O. TAKAHASHI*, T. SATODA AND T. UCHIDA. Dept. Oral Anatomy II, School of Dentistry, Hiroshima University, Hiroshima 734, Japan

It appears to be established that the pontine and medullary reticular formation are one of the relay nuclei among the descending pathways for jaw movement. This study was undertaken to clarify GABAergic projection from these nuclei including the parvocellular reticular nucleus (PCRt), one of key structures of the mastication control system, to the trigeminal motor nucleus (Vm). After iontophoretic injection of cholera toxin B subunit (CTb) into the Vm, a substantial number of retrogradely-labeled neurons were found predominantly in the contralateral supratrigeminal nucleus (sV) and bilateral PCRt. In this area, CTb-positive neurons were distributed throughout the rostrocaudal extent. Sequential double immunofluorescence histochemistry for GABA and transported CTb revealed that the vast majority of sV and PCRt neurons retrogradely labeled with CTb injected into the Vm showed GABA-like immuno-reactivity. It is conceivable that the GABAergic projections from the PCRt to the Vm may constitute a link in the intrinsic control system for jaw movement.

588.14

ANATOMICAL CHARACTERISTICS OF PREMOTOR INTERNEURONS IN RAT SPINAL CORD. B. Jiang*, K. E. McKenna and W. Z. Rymer, Department of hysiology , Northwestern University, Chicago, IL 60611.

Previous work has focused on the neural mechanisms underlying impaired reflex responses following incomplete spinal cord injury, such as the clasp-knife reflex. Our neurophysiological data has shown that enhanced responsiveness of inhibitory interneurons in the intermediate nucleus of the spinal cord, which were activated by free nerve endings in muscle and tendon, is responsible for these abnormalities. The present study seeks to identify the anatomical location and characteristics of these interneurons.

A retrograde transneuronal tracer, pseudorabies virus (PRV) was injected (3 μl) into the left medial gastrocnemius muscles (MG) of rats. Four to five days later the rats were perfused. Tissue sections (50 μm) of the spinal cord were processed for immunohistochemical detection of PRV. Fluoro-Gold was also used in another set of rats to confirm the location and morphology of the MG motoneuron pool.

As expected, PRV labelled α-motoneurons were found in the lamina IX of segments L4/L5 ipsilateral to injection site. Labelled interneurons were seen throughout the dorsal, intermediate and ventral cord of segments L2-L6. However, the majority of labelled interneurons were observed in the lamina V, VI, VII and X. Most of interneurons were labelled ipsilaterally, but in a few cases they were labelled bilaterally. This study anatomically demonstrated the location of spinal interneurons involving the control of the MG motoneurons. Ongoing work seeks first to identify the neurotransmitters of these interneurons, and then to investigate whether these interneurons receive free nerve ending mechano-receptor input from muscle.

Supported by NIH grants NS28076-03 and 1T32HD07418-01.

588.15

SPINAL PREMOTOR INTERNEURONS IN BEHAVING MONKEYS: RESPONSE PATTERNS & OUTPUT LINKAGES TO MUSCLES. S.I. Perlmutter* & E.E. Fetz, Physiology & Biophysics, and Regional Primate Research Center., U. of Washington, Seattle, WA

Activity of 210 task-related C7-T1 spinal neurons was recorded in 2 macaques generating isometric ramp-and-hold flexion-extension torques about the wrist. Spike-triggered averages (STA) of EMG from 12 forearm muscles detected post-spike effects for 54 neurons. 8 were classified as motoneurons based on location and characteristic STA features, and 46 as premotor interneurons (PM-INs). Single-pulse microstimuli in the cord produced wide-spread output effects and identified the shortest latency for post-spike effects. Broad STA peaks or troughs, often beginning before the trigger, were common, and are attributed to synchronous firing of other premotor neurons. 63% of PM-INs had output effects only in flexors, 13% only in extensors, 24% in both (7 cofacilitation; 1 cosuppression; 3 reciprocal). Most had divergent effects, on average in 54% of non-redundant coactivated muscles. Response patterns of PM-INs were tonic (46%), phasic (13%), phasic-tonic (13%), decrementing (11%), unmodulated (9%), and phasic inhibition (7%). PM-INs had more tonic and fewer phasic responses than other interneurons. 70% of PM-INs had output effects consistent with their task-related modulation. Pyramidal tract input was clearly present for 10/16 PM-INs, a higher proportion than for other interneurons. PM-INs exhibit many properties previously seen in other premotor populations (e.g. rubromotoneuronal cells also include unmodulated and bidirectionally active cells, and can cofacilitate antagonists [Cheney et al., Prog Br Res 87:213]), but the overall profile of response types and linkages to muscles of PM-INs differed from premotor cells in motor cortex, red nucleus and dorsal root ganglia. [NS12542, NS09189]

LIMBIC SYSTEM V

589.1

ULTRASTRUCTURAL LOCALIZATION OF NEUROPEPTIDE Y-LIKE IMMUNOREACTIVITY IN THE LATERAL NUCLEUS OF THE AMYGDALA. C. R. Farb* and J. E. LeDoux. Center for Neural Science, NYU, NY, NY 10003.

The lateral nucleus of the amygdala (AL) has been implicated as the site of formation of emotional memories. Memory processing in the AL is thought to involve glutamate as a neurotransmitter. Neuropeptide Y (NPY), which is present in AL (McDonald 1989), might also play a role in synaptic transmission, possibly by inhibition of glutamate release (Colmers, 1988). NPY-like immunoreactivity (NPY-LI) in AL was examined at the electron microscopic level. NPY-LI was seen in perikarya, dendrites, axons and axon terminals. The majority of terminals with NPY-LI formed symmetric (inhibitory?) synaptic junctions on small, unlabeled dendrites. Often these dendrites were simultaneously contacted by other unlabeled terminals making asymmetric (excitatory?) synapses. Labeled terminals also formed symmetric synaptic junctions with perikarya and proximal dendrites. A small number of labeled terminals formed asymmetric synaptic junctions on dendritic spines. Frequently, labeled terminals were closely apposed to unlabeled terminals. Unlabeled terminals often made asymmetric synaptic contacts on NPY-labeled dendrites. Post-embedding studies revealed that some, but not all, NPY-immunoreactive terminals were also immunoreactive for GABA. These results suggest that NPY and GABA, either separately or together within the same neuron, might play a role in inhibition and modulation of synaptic transmission in AL, possibly through both pre- and postsynaptic mechanisms. Supported by MIH38774 & MH46516.

589.2

GABAERGIC PROJECTIONS FROM THE INTERCALATED CELL MASSES (ICMs) TO THE CENTROMEDIAL (CM) AMYGDALOID COMPLEX AND BASAL FOREBRAIN (BF). [1]D. Paré*, [2]Y. Smith, and J.-F. Paré. [1]Dept. Physiol., Fac. Medicine, Univ. Laval and [2]Neurobiol. Res. Ctr., Enfant-Jésus Hospital, QUÉ., CANADA.

The ICMs are dense clusters of small GABAergic cells interposed between the basolateral (BL) and CM nuclear groups. Up to now, the ICMs have been largely ignored in anatomical studies of the amygdaloid complex. These experiments were thus undertaken to identify some of their targets with tract tracing methods in the cat.

Intra-amygdaloid projections of the ICMs: Small iontophoretic injections of cholera toxin (CTx) were performed in the central medial (n=2), central lateral (n=2), medial (n=2), BL and/or lateral (n=4) and basomedial (n=1) nuclei as well as in the ICMs (n=2). Moderate to massive retrograde labeling was seen in the caudal ICMs after CTx injections in the central and medial nuclei. Evidence for a light projection to the BL nucleus and other ICMs was also obtained. Extra-amygdaloid projections of the ICMs: WGA-HRP deposits were placed in numerous cortical areas (n=3) and dorsal thalamic nuclei (n=28), in stria terminalis and anterior commissure nuclei (n=3) as well as in lateral and preoptic hypothalamic areas (n=4). Very few retrogradely labeled cells were seen in the ICMs following these injections. In contrast, massive labeling was found after injections involving the substantia innominata and diagonal band. Futhermore, most labeled intercalated cells were also GABA-immunoreactive. These results were confirmed by iontophoretic injections of PHA-L in the ICMs.

Considering that the CM complex and BF constitute two of the main output stations of the amygdala, the ICMs have the necessary connections to play a pivotal role in gating extra-amygdaloid influences.

Supported by MRC grants MT-11562 and MT-11237.

589.3

DISTRIBUTION OF DOPAMINERGIC FIBERS IN THE CENTRAL AMYGDALOID NUCLEUS AND BED NUCLEUS OF THE STRIA TERMINALIS OF THE RAT. L. J. Freedman*[1] and M. D. Cassell[1,2]. [1]Neuroscience Program and [2]Department of Anatomy, University of Iowa, Iowa City, IA 52242.

The distribution of dopaminergic fibers in the principal components of the central extended amygdala (Central amygdaloid nucleus (Ce), substantia innominata, and bed nucleus of the stria terminalis (BNST)), was studied using immunocytochemistry against tyrosine hydroxylase, dopamine β-hydroxylase and dopamine. Dopamine and tyrosine hydroxylase immunoreactive fibers were found most densely distributed in the dorsolateral subdivision of the BNST and the medial part of the lateral division of Ce. Smaller numbers of dopaminergic fibers were found in the rest of the central extended amygdala. In contrast, dopamine β-hydroxylase fibers were virtually absent from the dorsolateral bed nucleus of the stria terminalis and lateral part of the central amygdaloid nucleus, but were distributed in a moderate density in the medial part of Ce, dorsal substantia innominata and posterolateral BNST. Our results show that dopamine fibers are most concentrated over those regions of the central extended amygdala with large numbers of GABAergic neurons whose projections remain within the central extended amygdala, while noradrenergic fibers are most heavily concentrated over those regions containing a large proportion of brainstem projection neurons. That dopamine fibers are concentrated over regions with GABAergic medium spiny neurons suggests that those regions might be organized as a striatal parallel.

589.4

INTRAAMYGDALOID CONNECTIONS OF THE RAT BASOLATERAL AMYGDALA. C-J. Shi* & M.D. Cassell. Dept. of Anatomy, Univ. of Iowa, Iowa City, IA 52242.

The amygdala functions to integrate sensory inputs with appropriate behavioral and somatic reactions. While the extended central and medial amygdala appear to contribute to the expression of a variety of reactions through their hypothalamic and brainstem projections, the basolateral amygdala seems to be the major recipient of sensory information from cortex and thalamus. The question therefore arises as to what are the pathways that sensory infomation uses to access and converge on the extended central and medial amygdala. To address this question, the intrinsic amygdaloid connections of the basolateral amygdala in rat were investigated using the biocytin anterograde tracing method. The results show that the lateral nucleus gives few direct projections to the extended amygdala, but heavy innervation of the posterior basomedial and posterolateral cortical nuclei and moderate labeling in the basolateral nucleus. The basolateral nucleus (BL), mainly its very caudal part, projects massively to the extended central amygdala, including all parts of the central amygdaloid nucleus (Ce), dorsal substantial innominata and lateral part of the bed nucleus of stria terminalis (BNST), but not to the extended medial amygdala. The anterior basomedial nucleus projects both extended central and medial amygdala except posteriodorsal part of the medial amygdaloid nucleus and posteriomedial part of the BNST. The posterior basomedial nucleus mainly projects to the extended medial amygdala as well as the lateral capsular part of the Ce. The morphology of neurons that project to the extended amygdala was evaluated with intracellular staining techniques. Only class I and II neurons within the BL provide these intraamygdaloid connections. The data suggested sensory information is carried through polysynaptic intrtaamygdaloid pathways before accessing output nuclei. Supported by NIH NS25139.

589.5

COMPARATIVE ANATOMY OF THE PRIMATE AMYGDALA: A HISTOCHEMICAL AND IMMUNOCYTOCHEMICAL STUDY.
D.R. Brady*. Lab. of Neurosci., NIA/NIH, Bethesda, MD 20892.

The amygdala is a subcortical structure occupying a strategic position in the circuitry of the limbic system, linking neocortical sensory input with neural systems associated with emotions and vegetative status. The basolateral division of the primate amygdala has developed phylogenetically in tandem with the expansion of neocortical association areas, with the greatest increase observed in human. On-going studies of the amygdaloid complex are directed toward identifying unique biochemical features of these nuclei. Coronal sections through the amygdaloid complex of new and old world monkeys, gorilla, chimpanzee and human were histochemically stained for the nitric oxide synthase marker NADPH-diaphorase (NADPH) and immunocytochemically reacted with antibodies to: calcium-binding protein, parvalbumin (PV); a lectin forming pericellular nets around PV neurons, *Wisteria floribunda* (WF); a non-phosphorylated neurofilament protein that identifies cortico-cortical projection cells, SMI32; neuropeptide Y (NPY) and somatostatin 1-28 (SOM). The morphology of NADPH, PV, NPY and SOM was similar between species, consisting of stellate, multipolar and fusiform cells that were aspiny or sparsely spiny. While NPY and SOM were observed in all amygdaloid nuclei, few NADPH cells stained in the central nucleus and the majority of PV and WF labeled cells occupied lateral (L) and basal (BL) nuclei. Labeled fibers mimicked the cellular distribution for each antibody. SMI32 labeled medium to large pleomorphic neurons in L, BL and accessory basal (AB) nuclei, with the greatest density in BL and L nuclei. Our results suggest that L, BL and, AB amygdaloid nuclei, those connectionally related to neocortex, share similar cytochemical characteristics that may relate to their phylogentic development.

589.6

ORGANIZATION OF CORTICAL INPUTS TO THE LATERAL NUCLEUS OF THE MONKEY AMYGDALA
L. Stefanacci* and D.G. Amaral. The Salk Institute and Group in Neurosciences, UCSD, La Jolla, CA 92037.

The amygdaloid complex is a cytoarchitectonically heterogeneous structure that has been implicated in the mediation of affective responses to perceived stimuli. In an effort to chart the sources of cortical sensory information to the amygdala, we have begun a comprehensive examination of inputs to the lateral nucleus of the amygdala in *Macaca fascicularis*. We placed discrete injections of the retrograde tracers Fast blue and Diamidino yellow into different rostrocaudal and dorsoventral levels of the lateral nucleus in 19 monkeys. In general, retrogradely labeled cells were identified in insular, temporal, and prefrontal cortical areas after all injections. Labeled cells were observed in hitherto unreported sources of inputs to the lateral nucleus such as the parahippocampal and posterior inferior temporal cortices. The projections to the lateral nucleus were topographically organized. Prefrontal areas, for example, originated stronger projections to more caudal regions of the lateral nucleus than to more rostral regions. The caudal insula and area 10 of the prefrontal cortex originated stronger projections to more dorsal regions of the lateral nucleus than to more ventral regions. Area TE, or rostral inferotemporal cortex, projected most heavily to rostral and dorsal parts of the lateral nucleus. We also observed that the caudal ventral lateral nucleus received significantly fewer cortical inputs than any other part of the nucleus. These results demonstrate that the lateral nucleus receives inputs from a wider variety of cortical regions than previously thought and that these inputs are differentially distributed to different portions of the nucleus.

589.7

LOCALIZATION OF NEUROPEPTIDE Y AND SOMATOSTATIN-LIKE IMMUNOREACTIVITY IN NEURONS OF THE MONKEY AMYGDALA.
A.J. McDonald*, F. Mascagni and J.R. Augustine. Dept. of Cell Biology and Neuroscience, Univ. of South Carolina Sch. of Med., Columbia, S.C. 29208

Neurons in the monkey amygdala exhibiting neuropeptide Y (NPY) and somatostatin (SOM) immunoreactivity were identified using ABC immunohistochemistry. Co-existence of the two peptides was demonstrated using two-color immunoperoxidase and adjacent section methods. Numerous NPYir neurons were observed in the basolateral and superficial amygdaloid nuclei. A moderate number of NPYir neurons was seen in the medial subdivision of the central nucleus but only a few neurons were observed in the lateral subdivision. Numerous SOMir neurons were stained in all major amygdaloid nuclei and always outnumbered NPYir cells. Approximately 90% of NPYir neurons also exhibited SOMir; the percentage of SOMir neurons that exhibited NPYir varied in different nuclei. In the superficial amygdaloid nuclei, the medial subdivision of the central nucleus, and most portions of the basolateral nuclei, the predominant cell type stained with both the NPY and SOM antibodies was a spine-sparse nonpyramidal neuron. In the dorsomedial subdivision of the lateral nucleus most peptide-positive neurons had spiny dendrites. This study demonstrates that specific cell populations in the primate amygdala contain NPY, SOM, or both peptides. Most peptide-positive neurons in the basolateral and superficial amygdaloid nuclei appear to be local circuit neurons. The finding of neurons with spiny dendrites in the dorsomedial portion of the lateral nucleus suggests that these cells may be functionally different from peptide-positive neurons in other portions of the basolateral amygdala. The lateral subdivision of the central nucleus is distinguished from other amygdaloid nuclei by containing a large population of SOMir neurons that do not exhibit NPYir. Supported by NIH Grant NS19733.

589.8

PREFRONTAL CORTICAL INNERVATION OF THE BASOLATERAL NUCLEUS OF THE AMYGDALA: AN ELECTRON MICROSCOPIC STUDY.
M.B. Reed, A.J. McDonald and F. Mascagni*. Dept. of Cell Biology and Neuroscience, Univ. of South Carolina School of Med., Columbia, SC 29208.

Prefrontal cortex projections to the basolateral nucleus of the amygdala (ABL) in the rat were investigated using the Phaseolus vulgaris leucoagglutinin (PHA-L) anterograde tract tracing technique. Iontophoretic injections of PHA-L were made into the prelimbic, infralimbic and agranular insular cortices. After a two week survival, animals were perfused and 75-micron-thick sections were cut with a vibratome. The avidin-biotin peroxidase immunohistochemical technique was used to demonstrate PHA-L labeled fibers and terminals in the ABL. Electron microscopic evaluation revealed that labeled terminals contained round synaptic vesicles and made asymmetrical synapses. Virtually all labeled terminals made synaptic contacts with dendritic spines; a few contacts were seen with small dendrites. These findings indicate that prefrontal inputs target spiny pyramidal neurons in ABL; these neurons have projections to the ventral striatum and prefrontal cortex. The morphology of the synapses (Gray's Type I) suggests that they are excitatory. Prefrontal inputs may interact with other specific neuronal elements that target dendritic spines of ABL neurons. These putative interactions might play a role in synaptic plasticity in the amygdala. (Supported by NIH Grant NS-19733).

589.9

THE CAT'S LATERAL AMYGDALOID NUCLEUS AS A SENSORY-LIMBIC RELAY.
Llamas, A.*, Clascá, F., Román-Guindo, A., and Mateo-Sierra, O.
Dept. of Morphology, Sch. of Med., Autónoma Univ., Madrid
SPAIN

The lateral amygdaloid nucleus (L) is the main gateway for neocortical and thalamic inputs to the amygdala. In turn, L sends heavy projections to some neocortical areas as well as to the entorhinal cortex (EC), basal and cortical amygdaloid nuclei (ABC), and, in cats, to hippocampal sector CA1 (Llamas et al., Soc. Neurosci. Abstr. 471, 1991). We have investigated the spatial overlap between these multiple afferents and efferents within L, using axonal tracers in adult domestic cats. Afferents from auditory, gustatory and visual thalamus end in separate portions of L. Perirhinal and association auditory cortex project massively to the medial and posterior portion of L. Sylvian ("insular") neocortex projects to a more restricted anterior and dorsal zone of L. Some of this projections are not reciprocal. Amygdalo-neocortical connections arise from dorsolateral parts of L. Projections to EC arise from central and medial parts of L, throughout the anteroposterior extent of the nucleus. Neurons projecting to ABC lie ventrolaterally in the anterior third of L and laterodorsally in its posterior third. Efferents to CA1 arise from a central and dorsal portion of L. Connections with CA1, EC and ABC are non-reciprocal. Thus, sensory inputs and outputs to limbic targets are to a great extent segregated within L. These findings highlight the pivotal position of intranuclear connections within L for sensory-limbic interactions through the amygdala.
Support: DGICY+PB88-0170; FIS 93/0197

589.10

AMYGDALOID PROJECTIONS TO THE VENTROMEDIAL NUCLEUS OF HYPOTHALAMUS: A RETROGRADE LABELING STUDY USING CHOLERA TOXIN B SUBUNIT.
G. Rajendren* and R.L. Moss. Dept. Physiol., The Univ. Texas Southwestern Med. Ctr., Dallas, TX 75235-9040.

The projections of various subdivisions of the amygdala into the ventromedial nucleus of hypothalamus (VMH) were studied in 6 ovariectomized rats unilaterally injected (50 nl) with cholera toxin (CTX) B subunit into the VMH. Ten days after the injection of the tracer, the females were sacrificed and the brains were processed immunocytochemically. In two rats the injections were localized at the dorsal part of the VMH, and the injection extended into the dorsomedial nucleus of hypothalamus in one of these two rats. In another two rats the injections were confined to the lateral part of the VMH. In the fifth rat the injection covered the entire extent of the VMH. In the sixth rat the injection covered the lateral edge of the VMH and parts of the lateral hypothalamus. In all the six rats, the maximum density of CTX labeled cells was found in the ipsilateral medial amygdala, the anteroventral and the posteroventral subdivisions of the ipsilateral medial amygdala showing intense staining. Scattered CTX-filled cells were observed throughout the anterodorsal and posterodorsal subdivisions of the ipsilateral medial amygdala. The anteroventral subdivision of the contralateral medial amygdala was also labeled in all 6 rats. In all six rats, cells labeled with CTX were observed in the ipsilateral posterior basomedial nucleus, anterolateral amygdalohippocampal area, posteromedial amygdalohippocampal area and the subiculum. These structures contralateral to the injection were not labeled.

The present studies provide a more detailed picture of the amygdaloid projection into the VMH. Supported by NIH Grant MH41784.

589.11

CONNECTIONS OF THE ADULT RAT PREFRONTAL CORTEX REVEALED BY INTRACEREBRAL INJECTION OF A SWINE ALPHA HERPESVIRUS. L.W. Enquist*[1], P. Levitt[2] and J.P. Card[3]. [1]Dupont Merck Pharmaceutical Co., Wilmington, DE, [2]The Medical College of Pennsylvania, Philadelphia, PA, and [3]The University of Pittsburgh, Pittsburgh, PA.

Alpha herpesviruses are transported through the nervous system in a circuit specific pattern following injection into peripheral nerves and their targets. We have examined the behavior of two strains of a swine alpha herpesvirus (pseudorabies virus, PRV) injected into the medial prefrontal cortex of adult rats. Wild type (PRV-Be) and attenuated (PRV-Ba) virus were both transported from the site of injection in a pattern entirely consistent with the known connectivity of the prefrontal cortex. However, the two strains of virus exhibited differences in the direction of viral transport from the injection site. PRV-Be virus was transported in both the anterograde and retrograde direction and ultimately infected neurons in all areas known to either project to or receive projections from the prefrontal cortex. In contrast, the attenuated strain of virus was only transported retrogradely from the injection site, even at substantially longer post inoculation intervals. With advancing survival, both strains of virus also passed transneuronally to infect circuits of neurons in a predictable temporal sequence in patterns consistent with retrograde viral transport. The data demonstrate that intracerebral injection of different herpesvirus strains can be effectively used to analyze aspects of neuronal circuitry. Furthermore, the well characterized genetic differences present in the two strains of virus used in this analysis provide a basis for examining the factors which influence specific uptake and transport of PRV in the central nervous system.

589.12

PHA-L ANALYSIS OF ASCENDING PROJECTIONS FROM THE POSTERIOR HYPOTHALAMUS IN THE RAT. R.P. Vertes*, A.M. Crane, L.V. Colom and B.H. Bland. Center for Complex Systems, Florida Atlantic Univ., Boca Raton, FL 33431, Div. of Neuroscience, Baylor College of Medicine, Houston, TX 77030, and Dept. of Psychology, Univ. of Calgary, Calgary, Alta., Canada T2N 1N4.

Regions of the posterior hypothalamus (PH), particularly the supramammillary nucleus (SUM), are thought to serve as an important relay between the brainstem and septum-hippocampus involved in the generation of the hippocampal theta rhythm. We previously showed (Vertes, J. Comp. Neurol. 326:595, 1992) that the SUM projects densely to the dentate gyrus of the hippocampus (HP) as well as to several structures intimately connected with the HP including nucleus reuniens (RE) of the thalamus, medial and lateral septum, entorhinal cortex and endopiriform nucleus. The aim of the present report was to examine ascending projections from regions of the PH adjacent to the SUM and to compare them with SUM projections.

Single injections of PHA-L were made into regions of the PH in 35 male rats, and patterns of projections analyzed. We found that injections in the PH dorsomedial/dorsolateral to SUM produced moderate to dense labeling in the following structures: the periventricular gray, the periventricular nucleus of thalamus (Th), the fields of Forel/zona incerta, the dorsomedial hypothalamic area, RE, mediodorsal nucleus of Th, lateroposterior nucleus of Th, medial and lateral preoptic region, bed nucleus of stria terminalis, substantia innominata, and medial and lateral septum. In contrast to SUM projections, the PH is the source of few, if any, projections to the HP, entorhinal cortex or endopiriform nucleus.

The findings indicate that the PH can influence the HP indirectly through projections to RE and the septum but not directly through monosynaptic projections to the HP.

Supported by NIMH grant MH45075.

589.13

DISCHARGE PROPERTIES OF NEURONS OF THE SUPRAMAMMILLARY NUCLEUS AND MAMMILLARY BODY: RELATIONSHIP TO HIPPOCAMPAL THETA RHYTHM. B. Kocsis*, R.P. Vertes and J.S. Thinschmidt. Center for Complex Systems, Florida Atlantic University, Boca Raton, FL 33431

The supramammillary nucleus (SUM) and the mammillary body (MB) are intimately connected with the hippocampal formation (HF) -- the SUM via ascending projections primarily to the dentate gyrus and the MB via descending HF to MB projections through the fornix. We proposed that the SUM may serve as an important link from the pontine reticular formation to the septum-hippocampus in the generation of the theta rhythm. Kirk and McNaughton (Neuroreport 2:723, 1991) recording multi-unit activity described a population of SUM cells that fired synchronously with the theta rhythm of the HF. Alonso and Llinas (J. Neurophysiol. 68:1321, 1992) recently identified rhythmically bursting cells in the MB in the guinea pig slice that they suggest may be related to theta.

Extracellular single unit recordings were taken from the SUM, MB and surrounding regions of the diencephalon in urethane anesthetized rats. Hippocampal EEG activity was recorded with stainless steel electrodes implanted in the dorsal hippocampus. 170 neurons were recorded in 40 rats. Of these, 29 cells discharged synchronously with the theta rhythm (theta cells); 141 showed no relationship to theta (non-theta cells). Twenty of 29 theta cells were histologically localized to the SUM/MB (13 in SUM, 7 in MB); 83 of 141 non-theta cells were located outside of the SUM/MB. Eleven of 20 theta cells were "complex spike" cells -- 6 in SUM, 5 in MB. The results show that a large percentage of SUM/MB neurons discharge synchronously with theta, whereas, few if any diencephalic cells outside of the SUM/MB exhibit this characteristic. Theta-related SUM/MB neurons may be involved in the generation of the theta rhythm and/or transfer of theta rhythmicity from the HF to other parts of the CNS.

589.14

PROJECTIVE **SEPTAL NEURONS ARE IMMUNOREACTIVE FOR THE ESTROGEN-SYNTHESIZING ENZYME AROMATASE. ARE THERE "ESTROGENERGIC" NEURAL PATHWAYS IN THE BRAIN?** R.L. Jakab*[1], N. Harada[2], and F. Naftolin[3]. [1]Sect. Neurobiol. and [3]Dept. Ob/Gyn, Yale Univ., Sch. Med., New Haven, CT 06510, U.S.A.; and [2]Molec. Genetics, Fujita-Gakuen Health Univ., Sch. Med., Toyoake, AICHI 470-11, Japan.

Mapping studies of several research groups have concluded that the distribution of neurons displaying aromatase-immunoreactivity (AR-IR) and the areas exhibiting AR enzyme activity do *not* completely overlap in the mammalian brain. For instance, in the rat anterior hypothalamic area (AH) which has high enzyme activity, we have detected AR-IR only in axons and not in somata. Conversely, in the lateral septum where enzyme activity is low, AR-IR neurons are abundant (Jakab et al., J. Steroid. Biochem. 1993, 44:481-498). This and similar discrepancies found in other brain regions suggest that the action of the AR-IR neurons is, at least partly, manifested in their *projection target areas.* In the present study, we tested this hypothesis focusing on the septo-hypothalamic pathway.

Experimental: To verify whether AR-IR lateral septal neurons project to the AH, small amounts (0.05-0.1 μl) of the retrograde tracer horseradish peroxidase (HRP; 30% in saline) were stereotaxically injected to this hypothalamic area of adult rats. After two-day survival, large populations of lateral septal neurons were found to contain HRP granules. Light and electron microscopic immunostaining demonstrated AR-IR in many of these retrogradely-labeled cells.

Conclusion: This finding supports the notion that brain-derived estrogen may not exclusively act locally, but may affect remote areas via neural pathways.

(Supported by a Grant from the Tourette Syndrome Association to R.L.J., and by NIH grant HD13587 to F.N.)

589.15

SEROTONERGIC SYNAPTIC INPUT TO GABAERGIC SEPTOHIPPOCAMPAL NEURONS IN THE RAT: A PRE-EMBEDDING TRIPLE-LABEL ELECTRON MICROSCOPIC STUDY. Takashi Honda* and Kazue Semba. Dept. of Anat. & Neurobiol., Dalhousie Univ., Halifax, N.S., B3H 4H7 Canada.

The hippocampus is known to receive projections from GABAergic and cholinergic neurons in the medial septum-diagonal band region. To understand the transmitter-specific regulation of the activity of GABAergic septohippocampal neurons, wheatgerm agglutinin conjugated with 10 nm gold (WGA-G) was injected into the hippocampus. Parvalbumin, a marker for GABAergic septal neurons, was then immunohistochemically visualized with PAP and DAB methods, and serotonin visualized with an immunogold (1 nm) method, followed by silver intensification for both gold labels. In the medial septum, serotonin-immunoreactive axon terminals labeled with small granules contained clear round vesicles and a few dense-cored vesicles. They made synaptic contacts with cell bodies that were labeled for both WGA-G (large granules) and parvalbumin (diffuse electron-dense label). In addition, many serotonin-positive axon terminals made synaptic contacts with unlabeled dendrites and somata. These results provide a morphological basis for synaptic regulation of GABAergic septohippocampal neurons by serotonin.

Supported by the Medical Research Council and Scottish Rite Charitable Foundation of Canada.

589.16

CABP-D28K AND NADPH-DIAPHORASE COEXISTENCE IN THE GUINEA PIG LATERAL SEPTUM. O. Doutrelant, P. Ciofi* and P. Poulain. INSERM U 156, Pl. de Verdun, 59045 Lille (France).

The lateral septum (LS) is recognized as an important integratory unit receiving a variety of afferents. Its mediolateral part (LSml) contains a well-defined group of neurons identifiable by their somatic spines. In the rat, these LSml somatospiny neurons contain calbindin D-28K (CaBP) and receive excitatory glutamatergic hippocampal projections. In the guinea pig, we have shown that some LSml somatospiny neurons are CaBP-positive and also receive an enkephalinergic innervation from the perifornical hypothalamus.

The aim of the present study was to determine whether CaBP coexists with NADPH-diaphorase (ND), an enzymatic cofactor implicated in the nitric oxide synthesis, CaBP and ND being know to protect neurons against excitotoxicity. Light microscopic double-immunostaining for CaBP and met-enkephalin combined with ND histochemistry was performed on cryostat frontal sections cut throughout the LS of female guinea pigs.

Our results show that 1) CaBP and ND are present on delimited territories of the LS, 2) overlapping occurs in discrete territories, including the LSml, 3) coexistence of CaBP and ND is observed for some neurons in the LSml, and 4) enkephalinergic fibers contact CaBP, ND or CaBP-ND neurons.

Our data show a selective pattern in the topographical distribution of CaBP cells, ND cells and enkephalinergic fibers suggesting specific physiological functions for these different populations of neurons.

589.17

PROPOSED SUBDIVISION OF THE MEDIAL SEPTAL COMPLEX OF THE MACAQUE MONKEY BASED ON CYTOARCHITECTONIC, CHEMOARCHITECTONIC AND CONNECTIONAL DATA. H. M. Stroessner-Johnson* and D.G. Amaral. The Salk Institute, La Jolla, CA 92037; UCSD, Group in Neurosciences, La Jolla , CA 92037.

The medial septal complex (MSC) has traditionally been subdivided into two regions: the medial septal nucleus (MSN) and the nucleus of the diagonal band of Broca (DBN). Currently, there is no consensus on a precise, anatomically defined border between these two regions. Morphometric analyses of the MSC in young and aged monkeys has prompted us to reinvestigate the organization of the MSC.

We have analyzed Nissl, myelin, and immunohistochemical preparations using antibodies directed against choline acetyltransferase, glutamate decarboxylase, calbindin and parvalbumin. In addition, we have used preparations (n=31) from our laboratory's library of retrograde dye (Fast blue and Diamidino yellow) injections into levels spanning the rostrocaudal extent of the hippocampal formation for our analysis of the distribution of projection cells in the septohippocampal pathway.

Based on cell size, orientation, packing density, presence of immunolabeled cells and fiber density, and distribution of retrogradely labeled cells after dye injections into the hippocampal formation, we have identified as many as seven subdivisions in the MSC. For example, the MSN-Dorsal region does not contain cholinergic cells or retrogradely labeled cells but does contain GABAergic cells. The MSN-Intermediate group contains a moderate density of small to medium sized oval, multipolar and fusiform cholinergic cells whereas the MSN-Ventral magnocellular group has a very high density of medium to large sized multipolar cells. There are three subdivisions associated with the NDB- (Ventral parvocellular stream, Dorsal parvocellular stream, and Magnocellular stream), three associated with the MSN- (Dorsal, Intermediate, and Ventral magnocellular) and one in the transitional area (MSN/DBN- Lateral).

589.18

Expression of c-fos in the mamillary body following injections of muscimol into the dorsal and ventral tegmental nuclei of Gudden in the rat. I.Shim* and D.Wirtshafter, Dept. of Psych., University of Illinois at Chicago, Box 4348, Chicago, IL 60680.

In a previous study, we have shown that the projections from the ventral tegmental nucleus of Gudden (VTN) to the medial mamillary nucleus, and from dorsal tegmental nucleus (DTN) to the lateral mamillary nucleus, contain a substantial GABAergic component. Since GABA appears to act as an inhibitory transmitter, these findings raised the possibility that reducing the activity of cells in the VTN and DTN would result in a disinhibition of the cells in the mamillary body. The present study examined this possibility by studying the expression of Fos, the protein produced by the c-fos proto-oncogene, in the mamillary body following injections of the GABA agonist muscimol into VTN or DTN. The expression of Fos has been used to map functionally and anatomically related neural pathways.

Unilateral injections into the VTN produced a significant increase in Fos immunoreactivity in the ipsilateral medial mamillary nucleus. In contrast, injections into the DTN resulted in a great increase in lateral mamillary nucleus. These results strongly suggest that projections from tegmental nuclei to mamillary body are inhibitory, and are in agreement with other studies demonstrated that the VTN and DTN project to the medial and lateral mamillary nuclei, respectively.

589.19

DIENCEPHALIC ORIGINS OF MELANIN-CONCENTRATING HORMONE IMMUNOREACTIVE (MCH-ir) PROJECTIONS TO THE LATERAL PART OF THE MEDIAL MAMMILLARY NUCLEUS (MMN). **V.C.G. FURLANI[1] AND J.C. BITTENCOURT*[1,2].** (1) Dept. of Anatomy ICB/USP and (2) Nucleus of Neurosciences and Behavior, Institute of Psychology/USP, Sao Paulo, SP - BRAZIL - CEP 05389-970

The MMN is subdivided by pars lateralis, pars basalis, pars medialis, pars medianus and pars posterior. The lateral division (pars lateralis) of the MMN is constituted by a group of small cells between the medial division of MMN and the lateral mammillary nucleus. The MMN receives afferents mainly from dorsal and ventral subiculum, medial pre-frontal cortex, ventral tegmental nucleus, nucleus centralis superior (pars compacta), entorhinal medial cortex, medial septal area, tuberomammillary nucleus, ventral and dorsal pre-mammillary nuclei, supramammillary nucleus and dorsolateral hypothalamic area. The mammillary nuclei have received a considerable attention for the relationship with association procedures and spatial memory, the elaboration of emotion expression and possibly act on the aggressive behavior specific-specimen. Moreover, it can announce in the sexual behavior, regulation of the autonomic activities, the beginning of the attack activity and the defense behavior. Bittencourt et al (1992) described an extensive MCH/NEI-ir fibers on the MMN, and MCH/NEI-ir cells on the dorsolateral hypothalamic area, zona incerta, olfactory tubercle and pontine reticular formation. In order to determine the origins of the MCH/NEI-ir innervation of MMN, we have combined immunohistochemical and neuronal fluorescent retrograde tracer methods (5% solution of True-Blue in distilled water, crystalline deposits in the MMN). We have found condensed groups of double-labeled (MCH/NEI-ir) neurons in the following regions: dorsolateral hypothalamic area and tuberal subdivision of tuberomammillary nucleus.

SUPPORTED BY: FAPESP AND CNPq (BRAZIL).

589.20

IDENTIFICATION OF AFFERENTS TO THE SEROTONINERGIC NEURONS OF THE DORSAL RAPHE NUCLEUS USING FLUORESCENT TRACT TRACING IN THE RAT **Roger P. Dilts* and Margaret C. Boadle-Biber Dept. of Physiology Medical College of Virginia-VCU, Richmond, VA 23298-0551**

The purpose of this study was to identify the afferent connectivity of the ascending serotoninergic neurons of the dorsal raphe nucleus. Fluorogold (50-100 nl, 2% in saline) was pressure injected using a 31 ga. injection cannula. Six to 21 days after the injections animals were sacrificed by lethal injection and perfused with Ca^{++}-free Tyrode's buffer followed by 4% paraformaldehyde and 0.01% glutaldehyde in phosphate buffered saline (PBS). Brains were bi-sected and post-fixed for 2 hours in 4% paraformaldehyde/PBS, transferred to 20% sucrose/PBS and serial sectioned from +4.0 mm to -13.00 mm respective of Bregma at 40 microns using a freezing stage microtome. Sections were mounted and counter stained using a 0.02% solution of Neutral Red in 70% ethanol buffered with imidazole to pH 7.2-7.4. Injections which included the dorsal raphe and extended into the surrounding regions were compared to injections into the surrounding regions which excluded the dorsal raphe. Neurons were identified using a Zeiss Axioskop equipped with filter sets 00, 17 and 18 at 50-400x magnification and charted with reference to Paxinos and Watson ('86). Neurons labelled with fluorogold following injections into the dorsal raphe were identified in the prefrontal cortex, claustrum, as well as in regions immediately associated with the medial forebrain bundle including; the horizontal limb of the diagonal band, anterior amygdala area, the perifornical region of the lateral hypothalamus, parafascicular nucleus of the thalamus, supramammillary nucleus, posterior hypothalamus, ventral tegmental area, substantia nigra and central gray. Sparsely labelled neurons were identified throughout the pons and medulla including the parabrachial complex and locus coeruleus. Neurons labelled by injections which excluded the dorsal raphe nucleus were found in similar regions but with a different topography. This study provides evidence that the serotoninergic neurons within the dorsal raphe nucleus receive a characteristic pattern of innervation from the telencephalon and diencephalon. Supported by NIH grant # NS14090 to M.C.B.B.

'86 Paxinos,G. and Watson,C. The Rat Brain in Stereotaxic Coordinates 2nd Ed. Academic Press, Inc. San Diego, CA U.S.A

589.21

DOES THE LIMBIC SYSTEM AFFECT STRIATAL FUNCTION VIA THE NIGROSTRIATAL PATHWAY?

B.H. Turner*. Dept. of Anatomy, Howard University College of Medicine, Washington, DC 20059.

Anatomical studies show that diverse limbic structures provide the major afferents to the dopaminergic A8, A9, and A10 cells. To investigate a functional role of this circuit, the intracellular responses of caudate (Cd) neurons to stimulation of the preoptic area and frontal cortex of anesthetized cats was recorded. It was found that a single or multipulse stimulation of the preoptic area produced a response in Cd neurons, characterized by a long latency, slowly depolarizing potential. This response disappeared with the administration of the dopaminergic neurotoxin, MPTP, while leaving the corticostriate response unaffected. It was also found that intracellular injection of current into Cd neurons caused a train of action potentials, the frequency of which almost doubled with concurrent preoptic stimulation. We conclude that limbic stimulation affects striatal function via the nigrostriatal pathway.

590.1

MORPHOLOGICAL SPECIALIZATION WITHIN THE SOMATOSENSORY CORTEX OF THE STAR-NOSED MOLE (*Condylura cristata*). K.C. Catania*, J.H. Kaas, R.G. Northcutt, and P.D. Beck. Neurobiology Unit, Scripps Institution of Oceanography and Dept. of Neurosciences, School of Medicine, University of Calif., San Diego, La Jolla, CA 92093-0201, and Dept. of Psychology, Vanderbilt University Nashville, TN 37320.

The cortex of the star-nosed mole was examined in what corresponds to the somatosensory face region of other small mammals. Microelectrode recordings revealed the location of the appendages of "star" of the mole. The cortex was then flattened and sectioned parallel to the pia. Subsequent staining with cytochrome oxidase (CO) revealed a series of divisions within the somatosensory face region. Two cytoarchitectonically distinct areas were observed to contain distinct subdivisions based on cytochrome oxidase staining. S1 was identified based on location, response to tactile stimulation of the star, and its architectonic appearance. Within S1 we found 11 CO dense stripes or bands separated by sharp lines of low CO staining. We believe these stripes correspond to the 11 contralateral appendages of the "star" of the mole. A second more posterior set of stripes may correspond to the second somatosensory area, S2. These subdivisions may reflect a general principle of brain organization such that sensory inputs distinguished by spatial and transduction factors are segregated from each other in central sensory representations. Supported in part by NIH NS24869 and GM08107.

590.2

INTERMODAL CONNECTIONS IN THE CEREBRAL CORTEX OF THE OPOSSUM. S. Martinich* and C.E. Rocha-Miranda. Department of Neurobiology, IBCCF°, UFRJ, Rio de Janeiro, RJ 21949, Brazil

It has been claimed that the non-specialized features of the opossum cortex reflect a basic plan of mammalian brain organization emerged early in evolution. One question arising is how polysensory integration, which demands complex networks in the more evolved Eutheria, is achieved in a cortex with a more limited number of areas. We have addressed this issue by analyzing the connections of different cortical fields after injections of WGA-HRP and fluorescent tracers.

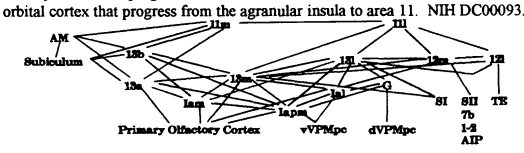
Our results stress three points: 1) The auditory cortex projects ipsilaterally to all the visual areas and contralaterally at least to the striate area and PSpl, a peristriate region adjoining the visual and auditory primary areas; PSpl, which receives subcortical afferents from the pretectum and a superficial portion of the medial geniculate nucleus, is the main visual target of the auditory cortex. 2) The visual area with densest projections from somatosensory cortex is PSa, a peristriate region bordering the parietal cortex, of which thalamic afferents include the lateroposterior, posterior and ventrolateral nuclei. 3) One field of the Frontal Lobe (F4) can be defined as a rostral region densely interconnected with contralateral sensory cortex; the cortical connections of F4 involve visual, auditory, somatosensory and besides other frontal regions of both hemispheres; subcortical centers connected with F4 include the thalamic dorsomedial and ventromedial nuclei, paraventricular nuclei and mesencephalic and pontine reticular formations; no connections of F4 with the superior colliculus or pretectum have been observed.

Thus, cross-modality and interhemispheric integration circuits in the opossum cortex may use as anatomical substrate direct connections among lower order sensory areas, which receive distinctive subcortical inputs, as well as a loop with a restricted region of the frontal lobe. (Supported by CNPq and FINEP).

590.3

LIMBIC AND SENSORY HIERARCHIES IN THE ORBITAL CORTEX OF THE MACAQUE. S. T. Carmichael* and J. L. Price. Dept Anatomy . & Neurobiology. Washington University Scholl of Medicine. St. Louis, MO 63110

The connections of the orbital cortex were examined with small injections of axonal tracers. Sensory inputs from visual, somatosensory, olfactory, gustatory and visceral sites terminate in lateral and posterior orbital areas. Limbic inputs from the amygdala are directed to posterior-medial and posterior-lateral orbital areas. while the hippocampus projects only to medial orbital areas. Cortical connections within the orbital cortex associate these limbic or sensory inputs into either a medial or lateral orbital network. The medial orbital network receives inputs from olfactory and visceral-related posterior orbital areas and from the amygdala and hippocampus and involves two medial subdivisions of area 13 and area 11m. The lateral orbital network receives input from olfactory and visceral-related posterior orbital areas as well as gustatory, and somatosensory and visual cortical areas and converges on two lateral subdivisions of area 13 and area 11l. There are only sparse interconnections between the two networks. A quantitative analysis of the laminar position of the cells of origin of these connections indicates that, in both networks, projections from posterior areas to more orbital areas originate predominantly from layer V, while projections from more rostral areas to posterior areas originate predominantly from layer III. Together, the laminar pattern and sensory and limbic progressions can be used to construct two hierarchies in the orbital cortex that progress from the agranular insula to area 11. NIH DC00093.



590.4

DISTRIBUTION OF NADPH DIAPHORASE IN THE PREFRONTAL CORTEX OF THE RHESUS MONKEY. S.M.Dombrowski and H.Barbas*. Boston University and School of Medicine, Boston, MA 02215.

Nicotinamide adenine dinucleotide phosphate (NADPH) diaphorase has been shown to colocalize with nitric oxide synthase and generate a novel neuronal messenger, nitric oxide (NO). Evidence suggests that NO has a major role as a messenger molecule in white blood cells, blood vessels, and neural tissue where its role is not yet clear. In this study we examined the distribution of NADPH diaphorase positive neurons in the prefrontal cortex of the rhesus monkey. The highest density of positive neurons was observed in limbic cortices: primary olfactory areas, agranular (PAll), and dysgranular (Pro, 13, 24, 25, 32) areas. In contrast, the density of positive neurons was lower in the eulaminate cortices (areas 9, 10, 12, 46, and 8). Many positive neurons were found in layers V and VI, fewer in layers II and III, rarely seen in layer I, and there was no evidence of any in layer IV. Positive neurons were also observed in the white matter, but their densities followed the opposite trend than in the corresponding overlying cortices. The results suggest that the distribution of NADPH diaphorase positive neurons is correlated with the degree of laminar differentiation and cell density of prefrontal cortices. Thus, NADPH diaphorase positive neurons are most widely distributed in limbic cortices which have a low laminar definition and cell density, and are comparatively sparse in eulaminate cortices which have high cell density. In view of the above results and the fact that NO can act as a neurotoxic agent, we suggest that NO may have a role in shaping the cortical architecture during development.
(Supported by NIH grant NS24760)

590.5

PREFRONTAL CORTEX IN HUMANS, APES AND MONKEY: IMAGE ANALYSIS OF AREA 10. K. Semendeferi[1], A. Schleicher[4], E. Armstrong[3], K. Zilles[4], and G. W. Van Hoesen[2]*. Depts of Anthropology[1], Anatomy[2] and Neurology[2], Univ. of Iowa, Iowa City, IA 52242, A.F.I.P[3], Washington D.C. 20306, Institute for Brain Research[4], H.- H. University, 4000 Duesseldorf 1, Germany.

The prefrontal cortex has long been considered important to human evolution. Comparative studies of humans, apes and monkeys can trace the evolution of cortical areas by investigating changes in their organization and size. To accomplish this, prefrontal areas need to be defined. Visual inspection of Nissl or myelin material has failed to produce a generally accepted map in apes and humans. Computer imaging and advanced statistical techniques now provide quantitative and reproducible signatures of cortical areas. Such techniques were applied to Brodmann's area 10 and to surrounding cortical areas (9, 46, 11) of the human, common chimpanzee, pygmy chimpanzee, gorilla, orangutan, and macaque cortex.

The Gallyas cell stain was used to provide a strong optical contrast. Section thickness was 20um. The slides were digitized with a resolution of 20um per pixel and the Grey-Level Index (GLI) method was used to show the area-percentage of stained perikarya versus neuropil and to demonstrate interareal and interlaminar differences in cell packing density. We generated more than 650 profiles from the pial surface to the white matter at various locations in the cortex. The profiles reveal variation in density of perikarya through the cortex and are directly comparable across species and cortical areas.

Throughout the frontal pole, human and gorilla profiles have consistently lower GLI values than the rest of the species. This indicates decreased cell packing density of perikarya and increased neuropil space. Further statistical manipulation of the mean profiles (Mahalanobis technique, which is based on distances between profiles) is being done to create clusters of profiles of Area 10 across primates.

Supported by the Leakey Foundation Grant QM54, NS 14944 and PO NS 19632.

590.6

INTRINSIC CIRCUITS OF HUMAN PREFRONTAL CORTEX. M.F. Kritzer* and P.S. Goldman-Rakic. Section of Neurobiology, Yale University, New Haven, CT. 06510.

The prefrontal cortex of humans and monkeys has long been ascribed with complex cognitive functions. Intrinsic connections likely contribute to these functions, and local prefrontal circuits have recently been studied in macaques (Kritzer & Goldman-Rakic, Neurosci. Abstr. 18: '92; Lund et al., Cerebral Cortex 3, '93). We now report similar intrinsic circuits in the prefrontal cortex of man.

To reveal circuits in human brain, we placed diI crystals in particular layers of post-mortem prefrontal cortex (area 10) taken from 4 normal humans; tissue was kept in 10% formalin for 2-6 months prior to vibratome sectioning. Label was documented with a fluorescence microscope and image-grabbing software and assigned to specific layers in counterstained material. Antero- and retrograde label was always observed; the distribution of label, however, differed markedly for crystals placed in specific layers. Thus, crystals in layers II/III produced widespread (~3mm) and patchy label in supragranular layers, with a narrow column of label crossing remaining layers. In contrast, crystals in layer IV produced more uniform label arrayed within ~2mm-wide columns which traversed all layers. Finally, crystals in layer V yielded lateral labeling in supra- and infragranular layers, while label from crystals in layer VI spread 4-6mm in this same layer, and conformed to a narrow column in remaining layers. On a layer-by-layer basis, these circuits strongly resemble those of macaques. Thus, the comparable and unique higher order functions mediated by homologous regions of prefrontal cortex in human and non-human primates are supported by similar intrinsic circuitry. MH44866-05.

590.7

AXOSOMATIC INPUTS TO PREFRONTAL CORTICAL PYRAMIDAL NEURONS PROVIDING ASSOCIATIONAL OR CALLOSAL PROJECTIONS IN MONKEY. D.S. Melchitzky*, S.R. Sesack, and D.A. Lewis. Depts. of Behav. Neuroscience & Psychiatry, Univ. Pittsburgh, Pittsburgh, PA, 15213.

Pyramidal cells, the major class of cortical excitatory neurons, can be divided into different classes based upon the target region of their axonal projection. Local circuit neurons are critical regulators of the activity of pyramidal neurons through primarily inhibitory synaptic inputs. However, little is known about how inputs from local circuit neurons differ among populations of pyramidal neurons in the highly differentiated prefrontal cortex of primates. In this study, WGA-HRP was injected into area 9 of monkey (*Macaca fascicularis*) prefrontal cortex. Retrogradely labeled pyramidal neurons furnishing callosal or associational projections were then identified in layers III and V of areas 9 and 46. The morphological features of callosally-projecting neurons in layer III (smooth or slightly indented nuclei, abundant cytoplasm and organelles, sparse Nissl substance) were comparable to those previously described in callosal neurons located in other regions and species. Obvious differences between callosal and associational neurons in these characteristics were not detected. The somatic membrane of callosal and associational cells was contacted by axon terminals that: (1) contained primarily pleomorphic vesicles and formed symmetric axosomatic synapses; (2) were morphologically similar but did not exhibit synaptic specializations in the single sections analyzed; or (3) contained primarily spherical vesicles and formed asymmetric synapses on adjacent dendritic targets. Preliminary examination suggests that callosal and associational neurons in the monkey prefrontal cortex receive a similarly dense axosomatic input. However, quantitative assessment of a larger cell sample is needed to confirm this qualitative impression.

590.8

POSTNATAL DEVELOPMENTAL CHANGES IN DENDRITIC SPINE DENSITY ON LAYER III PYRAMIDAL NEURONS IN MONKEY PREFRONTAL CORTEX. S.A. Anderson[1]*, J. Classey[2], D.A. Lewis[1] and J.S. Lund[2]. [1]Depts. of Psychiatry and Behav. Neurosci., University of Pittsburgh, Pittsburgh, PA, 15213 and [2]University of London.

Normal cortical development in primates appears to involve an initial overproduction of synapses, followed by synapse elimination until adult levels are achieved. Previous electron microscopy studies in primary sensory and motor cortical regions have demonstrated that these changes in synaptic density involve primarily asymmetric, presumably excitatory synapses, the majority of which are located on the dendritic spines of pyramidal neurons. The timing of synapse reduction remains unclear, but may occur largely during adolescence. However, the timing and extent of changes in synaptic input to subpopulations of pyramidal cells has not been determined. In this study, the rapid Golgi technique was used to identify pyramidal neurons located in mid-layer III of areas 9 and 46 of monkey (*Macaca mulatta*) prefrontal cortex. Tissue was examined from neonatal, adolescent, and adult animals; some of the adolescent animals were characterized endocrinologically, and were known to be either prepubertal or pubertal. Neurons were reconstructed using the Eutectics Neuron Tracing System. Preliminary observations revealed that spine density increased substantially during the first months of life, then declined markedly during adolescence, with most of the reduction occurring after the onset of puberty. For example, on the apical dendritic tree, spine density decreased 30% between prepubertal and adult animals. While additional studies are necessary to confirm these findings, they may be relevant to the hypothesis that schizophrenia can result from an abnormality in the process of synapse elimination during adolescence.

590.9

CALRETININ MAY DEFINE THALAMOCORTICAL CONNECTIONS BETWEEN THE HUMAN LIMBIC THALAMUS AND CINGULATE CORTEX. B.A. Vogt*[1], E.A. Nimchinsky[2], J.H. Morrison[2] and P.R. Hof[2]. [1]Dept of Physiology and Pharmacology, Bowman-Gray Sch of Med, Winston-Salem, NC 27103, and [2]Fishberg Res Ctr for Neurobiology, Mount Sinai Sch of Med, New York, NY 10029.

An antibody to the calcium-binding protein calretinin (CR) was used to analyze the distribution of this protein in the human cingulate cortex and its associated thalamic nuclei. Although the distribution of CR-immunoreactive (ir) interneurons did not vary appreciably in the rostrocaudal axis of the cingulate cortex, the anterior cingulate cortex (ACC) was characterized by an intensely CR-ir neuropil. Layer I, in particular, was broad and intensely labeled. Midline and intralaminar thalamic nuclei, including the centrolateral and parafascicular nuclei, which project to this area in the primate were strongly CR-ir. Area 23 of the PCC was characterized by a thin, intense band of CR-ir fibers in layer Ia. Neurons in the lateroposterior and medial pulvinar nuclei which project to PCC in the primate were similarly CR-ir. Areas 29 and 30 in PCC were characterized by a prominent band of CR-ir neuropil staining in layer II-IV. Cells of the laterodorsal and anterodorsal nuclei, which project to areas 29 and 30 in the primate, were CR-ir. There are large numbers of CR-ir fibers in the cingulum bundle, which directly enter layer I of ACC and at more caudal levels penetrate the cortex to reach the superficial layers. These data support the notion that thalamocortical projections might be responsible in part for CR immunoreactivity in the cingulate cortex and indicate that CR may be a potent tool for studying select neuronal systems in the human cerebral cortex.

590.10

PROJECTIONS FROM THE ANTEROMEDIAL NUCLEUS OF THE THALAMUS TO THE LIMBIC AND VISUAL CORTICES. T. van Groen* and J.M. Wyss. Dept of Cell Biology, University of Alabama, Birmingham, AL 35294

Previous studies indicate that the anteromedial (AM) nucleus of the thalamus primarily projects to the anterior cingulate cortex (area infraradiata; IR); but, our retrograde tracing studies suggest that many AM axons extend beyond this cortical area. In this study we have used anterogradely and retrogradely transported tracers to characterize the projections of AM. Injections into the rostral part of AM reliably label a terminal field extending from ventrocaudal IRα to rostral IRβ cortices. In addition to the IR projections, rostral AM injections densely label axons and terminals in area 18b and rostral "retrosplenial granular a" cortex (Rga) and less densely label terminal fields in ventral subicular, caudal entorhinal and perirhinal cortices. In contrast, injections into caudal parts of AM consistently label terminal fields in dorsal IRα, caudal IRβ, and precentral agranular (Preag) cortices. Thus, AM projections display a general topographical organization, e.g., rostral parts of AM project to rostral parts of IRβ, whereas caudal parts of AM project to caudal parts of IRβ. Further, rostral parts of AM project to caudal limbic cortex (i.e., Rga, subicular and entorhinal cortices); the mediocaudal parts of AM project to Preag but not to posterior limbic cortex, and the medioventral parts of AM project to area 18b. Axons of AM terminate primarily in layers I and IV in most cortical areas, but the AM projections to the caudal entorhinal and perirhinal cortices terminate in the deep layers (i.e., layers V-VI). These results demonstrate that AM projects to diverse cortical areas, and to different laminae in each area. This suggests that the projections of AM to each cortical area are functionally distinct.

590.11

INTRINISIC ELECTROPHYSIOLOGY AND MORPHOLOGY OF NEURONS IN PERIRHINAL CORTEX J.M. Beggs* and E.W. Kairiss. Department of Psychology and Center for Theoretical and Applied Neuroscience, Yale University, Box 11A, Yale Station, New Haven, CT 06520.

Studies of cortical neurons have sought to classify cells on the basis of their intrinsic membrane properties and morphology. We have extended this effort in two ways. First, we have examined rat perirhinal cortex, which is a transitional form of cortex situated between one-layered archicortex of the hippocampus and six-layered neocortex. Second, we have directed our statistical analyses to see if cells were grouped into distinct classes (as previous studies have suggested), or whether there was a continuous distribution of cell characteristics.

Intracellular recordings were made from over 90 cells from an *in vitro* preparation of rat areas 35 and 13. Single-electrode current clamp recordings with intracellular electrodes were used to study membrane responses to hyperpolarizing and depolarizing current steps. Analysis of the resulting membrane voltages included a variety of features: action potential width, rate of rise and fall, decay time constant, spike frequency adaptation, and first interval as a function of injected current. In addition, latencies and amplitudes of the after-hyperpolarizations (AHPs) and depolarizing afterpotentials (DAPs) were measured. Distributions of these features were unimodal to a first approximation, and no single variable or group of variables could be found to partition the data set into distinct classes. Thus, there is a continuum along features with "regular spiking" (RS) and "intrinsically bursting" (IB) cells representing extremes. Another type of cell was infrequently recorded and showed distinctly higher firing rate and low spike frequency adaptation, similar to "fast spiking" (FS) cells found in other studies.

Injection of 5% carboxyfluoroscein into some of the recorded cells revealed that those with regular spiking responses were pyramidal with small apical dendrites, while those with bursting responses were also pyramidal with large apical dendrites. Preliminary results with fast spiking cells indicate that they have stellate morphology. (Supported by NIH and Yale University)

590.12

SYNAPTIC PHYSIOLOGY OF NEURONS IN PERIRHINAL CORTEX E.W. Kairiss* and J.M. Beggs. Department of Psychology and Center for Theoretical and Applied Neuroscience, Yale University, Box 11A, Yale Station, New Haven, CT 06520.

Perirhinal cortex and amygdala are known to have reciprocal projections (McDonald & Jackson, *J. Comp. Neurol.*, 262:59-77, 1987) and have been implicated as necessary for expression of fear-potentiated startle in rats (Rosen *et al, J. Neuroscience*, 12:4624-33, 1992). To study interactions between these areas, we have developed an *in vitro* preparation designed to maintain their connectivity. We are interested in the following issues: Are there monosynaptic connections between these areas that can be contained in the slice? Can plasticity be induced at these connections? What cell types receive and send these projections?

Our preliminary results indicate that stimulation of the lateral nucleus of the amygdala (50μA) can elicit postsynaptic potentials (psps) in cells recorded intracellularly in perirhinal cortex. These psps had a latency of 4.85 +/- 0.32 ms (mean +/- SD), and followed consistently at this latency when stimulated at 50, 100, and 200Hz. Knife cuts in the slice to determine the pathway of these fibers reveal that they are not passing through the angular bundle. Together, these data suggest that there is a monosynaptic connection that can be contained in the slice from the lateral nucleus of the amygdala to perirhinal cortex via the external capsule.

To determine whether cells in perirhinal cortex support synaptic plasticity, high frequency stimulation (50, 100, 200Hz) was applied from the underlying white matter and from intracortical sites. In 8 experiments where such stimulation was given, long-term potentiation was obtained in 2/8 cases, long-term depression was obtained in 2/8 cases, and no change was observed in 4/8 cases. The incidence of LTP and LTD reported here is similar to that reported for neocortex proper.

This *in vitro* preparation affords the opportunity to study the physiology and pharmacology of cortico-limbic plasticity. These studies pave the way for more detailed experiments that will focus on the interactions between higher-order cortex and the amygdala. (Supported by NIH and Yale University)

591.1

SYNCHRONIZATION OF SLEEP RHYTHMS IN MULTI-SITE THALAMIC AND CORTICAL RECORDINGS. M. Steriade* and D. Contreras. Lab. of Neurophysiol., Laval University, Quebec, Canada G1K 7P4.

Sleep spindles (7-14 Hz) are generated by synaptic operations within the reticular thalamic (RE) nucleus and are modulated by inputs from thalamocortical (TC) and cortical cells, whereas delta potentials (1-4 Hz) arise from the interplay of two intrinsic currents of TC cells. A recently described slow (<1 Hz) cortical oscillation is reflected in RE and TC neurons and assists in grouping thalamic-generated rhythms within slowly recurring sequences (Steriade et al., J. Neurosci., 1993a-c). Here we report data on RE-, TC-, and cortical-cells' synchronization by means of multi-site, simultaneous intra- and extracellular recordings in anesthetized cats.

(a) Synchronization among RE cells, and between RE and TC cells, was demonstrated by crosscorrelograms (CCs) of spindle oscillations evoked by cortical stimulation. That synchronization is due to synaptic linkages between RE and RE & TC neurons was shown by the shift predictor test. (b) Rhythmic spike-bursts at delta frequency occurred spontaneously in RE neurons and could also be triggered by depolarizations from a holding V_m of -90 mV. Simultaneously, target TC cells displayed IPSPs that were closely time-related to RE-cell's spike-bursts. This suggests that, besides the intrinsically generated delta rhythm, an oscillation with a similar frequency may arise through inhibitory synaptic coupling from RE to TC cells. (c) CCs indicated that the slow rhythm was synchronized between RE and neocortical cells, between various RE neurons recorded at a distance of ~ 1-1.5 mm (with in-phase or 180° out-of-phase relations), and between an intracellularly recorded RE cell and an adjacent neuron recorded across the membrane. Most TC cells were also synchronized at the slow rhythm with cortical and RE neurons, in phase or with a delay of 100-200 ms. The coherence between cortical and thalamic cells varied in parallel with the synchronization state of the EEG. These data indicate that various sleep rhythms, previously viewed as generated by properties of different structures, are united by interacting thalamic and corticothalamic networks.

Supported by MRC grant MT-3689.

591.2

SYNCHRONIZATION OF THE SLOW (≈0.3 Hz) OSCILLATION IN CORTICAL NETWORKS. F. Amzica* and M. Steriade. Lab. of Neurophysiol., Laval University, Quebec, Canada G1K 7P4.

A slow oscillation (<1 Hz) of cortical and thalamic neurons was recently described under various anesthetics as well as in brainstem-disconnected animals and during natural quiescent sleep (Steriade et al., 1993a-c, J. Neurosci., 13: in press). Intracellular recordings showed that this oscillation arises from cortical networks even in the absence of the thalamus and has a pivotal role in grouping the thalamic-generated sleep spindles (7-14 Hz) and delta potentials (1-4 Hz) within the ≈0.3 Hz rhythm.

Here we report data on the synchronization of this slow oscillation, resulting from multi-site (up to 6 channels) simultaneous extra- and intracellular recordings of cortical neurons (areas 6, 4, 5, 7, 21, 19, 18 and 17) in cats maintained under ketamine-xylazine anesthesia. Correlation and spectral analyses were performed from spikes, focal or intracellular waves, and EEG components. There was a close temporal relation between spikes' and waves' oscillatory patterns in 75% of neuronal groups.

The repartition of the slow oscillation over different areas revealed that in 40% of cases the rhythm appeared with the same weight in FFTs from all areas. In the remaining 60% of cell-groups, a clear prevalence of this rhythm was observed in visual areas 21 & 18 and suprasylvian areas 5 & 7 over pericruciate areas 6 & 4. (*a*) Multiple recordings within the same area, with tracks separated by 1-2 mm, showed that in 80% of cell-groups the depolarizing envelopes and/or spike-trains in caudal foci preceded those in more rostral sites, with time lags of 5-35 ms. (*b*) With simultaneous recordings from distant areas, a preferred sequential pathway was observed in ≈60% of triple or more numerous recordings from posterior, middle suprasylvian, and pericruciate areas. Time lags in distant recordings ranged from 10 to more than 100 ms. Long delays could well have involved inhibition-rebound sequences in thalamic neurons projecting back to the neocortex. These data suggest that the slow rhythm is distributed through short- and/or long-range synaptic linkages across intracortical and corticothalamic networks.

Supported by MRC grant MT-3689.

591.3

CELLULAR SUBSTRATES OF EEG BURST-SUPPRESSION PATTERNS. R. Curró Dossi*, F. Amzica, D. Contreras and M. Steriade. Lab. of Neurophysiol., Laval University, Quebec, Canada G1K 7P4.

The EEG pattern of burst-supression (BS) is defined as transient sequences of high-voltage slow waves with intermingled sharp waves, alternating with periods of depressed background activity or complete flatness. While this aspect was observed under a variety of experimental and clinical conditions, there is no available data as to the associated neuronal events.

We have recorded intra- and extracellularly cortical neurons (from areas 17 & 18, 5 & 7, and 4 & 6) with identified thalamic or callosal projections (n=42), as well as reticular thalamic (RE) and thalamocortical (TC) neurons from various dorsal thalamic nuclei (n=48), under different types of anesthesia. BS was induced by the administration of the same or other anesthetics upon an already synchronized EEG. (*a*) All tested neocortical cells entered BS pattern. The V_m hyperpolarized by 8-10 mV as a precursor sign of EEG-BS, the cells displayed sequences of phasic depolarizing events appearing exclusively during the EEG wave-bursts, and the periods of EEG flatness were associated with a complete electrical silence of neurons. (*b*) At variance with cortical cells, only 70% of RE and 60% of TC cells displayed an activity closely related to the EEG-BS. Those thalamic neurons that deviated from the EEG-cell correspondence were mainly TC elements that, during flat EEG periods, discharged rhythmic spike-bursts within the delta frequency range (1-4 Hz). It is known that this is an intrinsic oscillation of TC cells, arising at a V_m more negative than -75 mV. With further deepening of the EEG-BS pattern (silent periods of >30 s), thalamic cells ceased firing during blackout phases of the EEG. Single shocks applied to the thalamus or cortex could replace the periods of electrical silence by apparently normal EEG and neuronal activity.

Supported by MRC grant MT-3689.

591.4

INTRALAMINAR NUCLEI AND THE WHERE OF AWARENESS. J.E. Bogen.* Dept. of Neurol. Surg., Univ. of So. Calif., Los Angeles, CA 90033

How certain neural mechanisms (Mc) momentarily endow with conscious awareness (C) percepts represented elsewhere is more likely to be solved when structures essential to Mc are identified. The loss of C with bilateral thalamic lesions involving the intralaminar nuclei (ILN) contrasts with retention of C after large cortical ablations depriving C of specific contents. A role of ILN in the perception of poorly differentiated (i.e., not cortically computed) sensations (e.g., crude pain) is suggested by their afference of directly ascending pathways. A role for ILN in awareness of cortical activity is suggested by their widespread afference from cortex, a property shared with striatum. A role for ILN in volition is suggested by their heavy projection to striatum. Unlike striatum, ILN also project widely to almost all neocortex, enabling an effect on ideation; this last property is in common with other structures (e.g., locus coeruleus) but none of them have the same direct cortical afference. And passage through n. reticularis (NRT) of ILN efferents to cortex could impact the attention selective action of NRT. These five anatomical facts are supportive of the inference from clinical observation.

591.5

VISUAL AND SPATIAL INFORMATION PROCESSING IN HUMAN PARIETAL ASSOCIATION CORTEX: USING MEG.
S. Murakami*, S. Kuriki[1], O. Ohshiro[1], M. Fujii, R. Fukatsu and N. Takahata. Dept. of Neuropsychiatry, Sch. of Medicine, Sapporo Medical University, Sapporo 060, Japan and [1] Research Institute for Electronic Science, Hokkaido University, Sapporo 060, Japan

The neural functin of the parietal association cortex(PAC) is known to be related to the visual spatial cognition. We reported the magnetic field responses in PAC to visual stimulation (Neurosci. Res. 16 (1993):225). In this study, we investigate the influence of the eye position to the magnetic field responses to retinal visual stimulation for the purpose of analyzing the neural mechanism of visual spatial cognition in PAC. The subjects for experiments were studied in a magnetically shielded room to record the responses to visual stimulation with a 37-channel SQUID magnetometer system (BTi), from PAC. Two LEDs were set, one as a visual stimulation and the other as a gazing point,which was used for the eye position change in orbita. The stimulation LED was lit for 0.5 sec at random intervals of 1-1.5 sec and located 3 degrees away from the gazing point. The magnetic field responses to visual stimulation, were averaged from 256 sweeps. Main magnetic field responses peaking at 140-180 msec after stimulating LED onset were recorded in all subjects. A change in the magnetic field responses with the eye position, which was changed by gazing LED, was not found in the latency but found a little in the amplitude of the main responses. The estimated current dipole sources of the main responses were located in the almost one region of PAC, but the direction of the current was different.

591.6

AUDITORY AND VISUAL REPRESENTATIONS IN HUMAN PREFRONTAL CORTEX. [1]J.M. Clarke*, [2]P. Chauvel, [3]J.-M. Scarabin and [2]E. Halgren. [1]Dept. Psychology, Univ. North Texas, Denton, TX, 76203; [2]INSERM CJF90-12 & [3]INSERM U335, Univ. Rennes I, 35033 Rennes, France.

Electrophysiological findings of auditory and visual representations in the prefrontal cortex were apparent from intracerebral 'depth' recordings of focal, sensory-evoked spike-wave complexes in an epileptic patient. In addition to clinical monitoring, the patient participated in behavioral evoked potential studies involving auditory and visual discrimination tasks. Inspection of evoked-potential recordings from different medial-to-lateral sites revealed overlapping, but non-identical evoked-spike topographies for the two sensory modalities (located approximately at the intersection of Broadmann's areas 9 & 46). Maximal sensory-evoked spike responses were localized 7mm more laterally for auditory than for visual stimuli, and had an earlier latency for auditory presentations (M=83ms following stimulus onset) than for visual ones (M=115ms). Effects of sensory habituation were seen; evoked spikes were less frequent following repeated presentations of an unchanging tone than when tones alternated in pitch, or when a tone followed an omission in stimulus presentation. Visual hemifield effects were found, with greater prefrontal responsiveness to presentations in the contralateral visual hemifield. These results are consistent with electrophysiological findings in animals indicating overlapping auditory and visual representations in prefrontal cortex. [Supported by INSERM, USPHS (NS18741), VA, Univ. Rennes I].

591.7

SYNAPTIC INHIBITION AND DIS-INHIBITION IN RAT AUDITORY THALAMUS.

B. HU*, D. Mooney, V. Senatorov. *Loeb Research Institute, Ottawa Civic Hospital, Ottawa, Ontario, Canada. K1Y 4E9*

Synaptic inhibition and disinhibition are important regulatory mechanisms underlying thalamocortical information processing. Using a newly-developed in vitro explant preparation, we have examined the synaptic events underlying cell inhibition in the auditory thalamus.

Electrical stimulation of the brachium of the inferior colliculus (BIC) elicited an early EPSP followed by a biphasic IPSP in the ventral (n=24) or dorsal (n=11) divisions of the medial geniculate body (MGB). The early phase of the IPSP was chloride-dependent. It had a reversal potential of -63±2 mV and was fully blocked by the $GABA_A$ receptor antagonists picrotoxin or bicuculline (20 μM respectively). The occurrence of the second phase of the IPSP required a higher intensity of stimulation. The second phase reversed its polarity around E_k (-84±2 mV) and was partially antagonized by the $GABA_B$ receptor blocker phaclofen (30 μM).

Bath application of muscarine (10-20 μM; n=12) consistently and reversibly blocked both $GABA_A$ and $GABA_B$ components of the evoked IPSPs. This prominent disinhibition was nevertheless quasi-complete (with ~15% residual IPSPs) and invariably associated with a 25-40 percent increase of membrane resistance. Muscarine lacked a direct antagonistic effect on hyperpolarizations evoked by GABA applied exogenously, nor did it affected glutamatergic synaptic potentials.

We conclude that inhibition in rat MGB involves chiefly GABA receptor activation. Muscarine suppresses GABAergic IPSPs likely via inhibition of local GABAergic interneurons (McCormick and Pape, 1988). *Supported by Parkinson Foundation of Canada, Ontario Mental Health Foundation and MRC of Canada.*

591.8

MULTISENSORY CONVERGENCE AND INTEGRATION IN RAT CORTEX. R. Ramachandran[1], M.T. Wallace[2], H.R. Clemo*[2] & B.E. Stein[2]. Departments of Biomedical Engineering[1] & Physiology[2], Medical College of Virginia/Virginia Commonwealth University, Richmond VA 23298

A grid of electrode penetrations (n=81) was made across occipital, temporal, and parietal cortices in 26 adult rats. Recordings were made from 843 neurons, and although the traditional divisions of these cortices into visual, auditory, and somatosensory domains was apparent, multisensory neurons (n=93) were encountered within each region of cortex. The incidence of multisensory neurons was highest near the borders between 'unimodal' regions, with the multisensory convergence patterns matching the modalities represented in the border zones. In addition, a focus of multisensory neurons was found in rostral parietal cortex, near the border with motor cortex. Regardless of a neuron's location or modality convergence pattern, it could be shown to integrate information from the different sensory modalities. Thus, stimuli that originated from similar points in sensory space resulted in response enhancement, whereas spatially disparate stimuli either failed to produce an interaction or resulted in response depression. Multisensory interactions were multiplicative and could take place within a wide (up to 400 msec) temporal window. The spatial, temporal, and multiplicative features governing these interactions are similar to the principles governing multisensory interactions in other species and in subcortical as well as cortical areas. Thus, many of the principles of multisensory integration appear to supersede structure, function and species. Supported by NIH grant NS 22543.

591.9

INTEGRATION OF SENSORY SIGNALS BY ASSOCIATION CORTEX IN THE MILLISECOND RANGE.

Kiyohiko Nakamura*. Grad. Sch. of Information Sci.
Univ. of Electro-Communications, 1-5-1 Chofugaoka, Chofu
Tokyo 182, Japan.

The cerebral cortex is capable of processing different sensory signals in a few hundred milliseconds to produce correct response. Previous work has presented a mechanism of cortical circuits, *temporal competition*, which is an optimal parallel processing using firing timing of several milliseconds. Latency of cortical response to sensory stimuli varies with sensory modality in the range of tens of milliseconds, however. This report presents a hypothetical mechanism which anables the cortical circuits to integrate neural signals of different latencies. The mechanism assumes a long-term potentiation (LTP) of cortical cells coactivated by multi-modal inputs. A test of the mechanism by computer simulation of a model circuit is presented. The mechanism suggests: (1) intracellular processes of the LTP may hold effects of inputs of short latencies, (2) the LTP produced by coactivation of inputs may determine onset of the temporal competition among the inputs of different latencies, and (3) after the onset, the competition may restrict firing to cells activated first. It follows from these that the cortical processing of temporal competition is capable of integrating sensory signals of different latencies in several milliseconds.

591.10

PREDICTION OF THE EEG BY A NOVEL NEURAL NETWORK SUGGESTS CHAOTIC DYNAMICS IN THALAMOCORTICAL CIRCUITRY. G. Jandó, G. Buzsáki* & R. M. Siegel. CMBN, Rutgers University, Newark, NJ 07102.

A two-layer linear network, a four-layer standard back-propagation feed-forward network and a novel highly interconnected linked associator network were trained to predict different states of the electroencephalogram recorded from the neocortex. The latter network, termed as linked autoassociator, consisted of one input and one output layer and four hidden layers of autoassociator units. All the units in the hidden layers received signals from the input layer and the previous hidden layer. The fourth hidden layer signaled to the first hidden layer. The autoassociators units had sigmoidal input-output characteristic and a time dependent capacitance. Connections between layers, the capacity and non-linear function were optimized using simulated annealing. In all networks the input signals were time delayed values of the EEG based on Takens theorem which states that time-delayed signals are a complete representation of the dynamics of a multi-element system. The output neuron predicted the value of the EEG 10 msec forward. Thus, the network was trained to extract predictive components from the EEG signal. The linked autoassociator was based upon the hippocampal network. In particular, the direct inputs to the hidden layer units corresponded to the feed-forward perforant path connection, while the autoassociator corresponded to CA3. The feedback corresponds to the subiculum-entorhinal intracortical connection.

The training sets for the three types of networks consisted of sinusoids, known chaotic data (Duffing equation), oscillatory states of EEG (e.g. spindling, slow wave sleep) and desynchronized EEG. The first two sets were used to test that the networks could learn known dynamics. As expected, the linear network could only learn the sinusoid. Although both the feed-forward and the linked autoassociator could learn the Duffing chaotic data, the spindling and the slow wave sleep, the performance of the linked autoassociator was superior. None of the networks were able to learn the desynchronized EEG. Once the networks learned the temporal characteristics of the different types of signals, the learning was turned off and the networks were used to explore the encapsulated dynamics. This was done by feeding the network's predicted values back into the input of the network. These artificially generated signals had the same wave shape and frequency components of high voltage spike and wave spindles.

Further exploration of long time series of the artificial signals using standard analysis drawn from chaos theory (e.g. phase plot, Poincaré sections) reveal a striking similarity to data from other natural chaotic systems. This suggest that the neural signals are truly chaotic (i.e. aperiodic yet have a strong predictive component) and that it may be possible to represent the dynamics of certain EEG patterns by a simple deterministic set of equations. Supported by ONR N00014-93-1-0334 and NS-27058.

591.11

NEURONAL AND SLOW WAVE ACTIVITY IN CEREBRAL CORTEX PRECEDING SPONTANEOUS AND EVOKED ELEVATIONS OF CEREBRAL BLOOD FLOW. E.V. Golanov* and D.J. Reis. Div. of Neurobiol., Cornell Univ. Med. Coll., New York, NY 10021.

We sought to determine in rat if elevations of cerebral blood flow (rCBF) in cerebral cortex appearing spontaneously or evoked from rostral ventrolateral medulla (RVL) or fastigial nucleus (FN) were associated with local neuronal events. Rats were anesthetized with 1.6-1.75% isoflurane, rCBF measured by laser-Doppler flowmetry and EEG recorded. At rest, rCBF spontaneously fluctuated (4-6/min) with each flow wave preceded by a EEG burst reflected simultaneously across cortex. Signal averaging revealed that bursts consisted of triphasic positive-negative-positive complexes followed, 1.4±0.4 sec later, by a transient elevation of rCBF. Identical burst-rCBF complexes were evoked after 33 or 48 msec by single shocks of RVL or FN, respectively. The polarity of the triphasic complex was reversed in cortical laminae IV-V. Evoked responses were inhibited for 30-40 msec by a preceding spontaneous burst-rCBF complex. Spontaneously discharging (1.2±0.6 Hz) neurons, comprising 9.1% of active neurons, were recorded extracellularly in laminae IV-V which invariably discharged (2-4 spikes) 25±3 msec after spontaneous or evoked burst-wave responses and during the negative component of the triphasic complex. We conclude: (a) elevations in cortical rCBF elicited from FN and RVL or appearing spontaneously with EEG bursts share a common electrophysiological signature, a triphasic vascularly-associated complex; (b) this complex reflects activity of neurons in laminae IV-V; (c) their discharge appears to predict associated elevations in rCBF. They may represent cortical neurons acting as a common final pathway in transducing intrinsic neuronal signals into cortical vasodilation not coupled to metabolism.

592.1

SEASONAL PATTERNS OF NEUROGENESIS IN THE HIPPOCAMPUS OF ADULT FOOD-CACHING BIRDS AND THEIR POSSIBLE RELATION TO SPATIAL LEARNING.

A. Barnea*, F. Nottebohm. The Rockefeller University, N.Y., 10021.

Recent evidence suggests that the avian hippocampus plays an important role in spatial memory and that it is functionally related to food caching behavior (Krebs *et al.*, 1989). Black-capped chickadees (*Parus atricapillus*) store food items during fall and winter in a scattered distribution within their home range and use memory to retrieve them. We used free ranging adult chickadees to test the hypothesis that there is a seasonal peak in the incidence of hippocampal adult neurogenesis which correlates with food storing behavior. Birds were captured and injected intramuscularly with a single dose of 50 µCi of 3H-thymidine. These birds were released and then recaptured six weeks later. At that time birds were killed and their brains were processed for autoradiography and histology. Different birds were injected during August, September, October, December, February, and March.

The great majority of labeled hippocampal neurons was found in a 350 um thick layer of the tissue dorsal to the dorsal wall of the lateral ventricle. These neurons were relatively large (mean nuclear diameter of 11.4 um±1.17, n=308). Labeling index, determined by the percentage of labeled hippocampal neurons, differed between times of year: it peaked at the end of summer and became less significant as winter gave way to spring. These results correlate with the occurrence of food-storing behavior which starts in early autumn and decreases as winter progresses. We suggest that new neurons become available shortly before they are needed to encode new food storing information and that the neurons and the information they hold are replaced, perhaps at yearly intervals.

592.2

SEASONAL CHANGES IN TELENCEPHALON VOLUME IN A FOOD-STORING BIRD (BLACK-CAPPED CHICKADEE *Parus atricapillus*).

T.V. Smulders, A.D. Sasson, and T.J. Devoogd*. Department of Psychology, Uris Hall, Cornell University, Ithaca, NY 14853.

Like many members of the Paridae, black-capped chickadees scatter-hoard food-items. Anecdotal field data suggest that this behavior is more frequent in fall and in winter than it is in spring and in summer. Because the avian hippocampus is involved in the spatial memory for stored food items (Sherry & Vaccarino, 1989; Krebs, 1990), we decided to look for seasonal changes in the volume of the hippocampus, in parallel with the seasonal changes in behavior. Our hypothesis predicts a larger hippocampus in the food-storing season.

Birds were caught at 4 different times of the year and hippocampal volumes from Nissl-stained tissue were compared among the groups. Hippocampal volume and overall telencephalon volume differ significantly with season. However, both are larger when average temperature is higher. The effect on the hippocampus can be totally accounted for by the effect on the telencephalon as a whole. Therefore, we find no specific effect on hippocampal volume, related to seasonal food-storing activity. The relation between temperature and telencephalon size could be explained by either a direct effect of temperature on the brain, or through food-availability, which parallels temperature over the season (Goutis & Winkler, 1992). Alternatively, both could be due to a lagging effect of daylength. *Supported by MH-48926 and a Philips award from the Belgian-American Education Foundation.*

592.3

MORE HIPPOCAMPAL NITRIC OXIDE SYNTHASE IN FOOD-STORING THAN IN NON-STORING BIRDS.

A.D. Székely and J.R. Krebs (SPON: Brain Research Association) Departments of Pharmacology and Zoology, University of Oxford, Oxford OX1 3PS, United Kingdom

Nitric oxide (NO) has been hypothesised to be involved in the induction of long-term potentiation (LTP), which is a putative cellular basis of memory in the mammalian hippocampus. Neurones releasing NO can be detected by NADPH-diaphorase histochemistry.

In birds, the hippocampal formation (HP) is the morphological equivalent of the mammalian hippocampus. Functionally this region is involved in the processing of memory associated with spatial behaviour. Long-term enhancement of synaptic response has also been observed in the avian HP. To study the distribution of NADPH-diaphorase staining, the birds (4 marsh tits and 4 blue tits of either sex) were anaesthetised and perfused through the heart. Coronal sections of 50 µm were reacted in the presence of NADPH and nitro blue tetrazolium. In both species, the staining was seen in neurones and in strongly labelled neuropil which revealed circumscribed brain areas including the neostriatum, archistriatum, lobus parolfactorius, nucleus of the diagonal band and the nucleus rotundus. In HP, a few elongated neurones were stained, situated mainly in the vicinity of the lateral ventricle and along the lateral border. In the HP of marsh tits, the level of diffuse neuropil staining was substantially higher, highlighting the entire structure, than in the blue tits where the HP could not be distinguished from the surrounding regions. The higher level of labelling found in the HP of food-storing birds suggests a specific role for NO in hippocampal processing of spatial information.

Supported by the Royal Society.

592.4

HIPPOCAMPAL LESIONS IMPAIR SUN COMPASS LEARNING IN HOMING PIGEONS.

V.P. Bingman* and T.J. Jones. Department of Psychology, Bowling Green State University, Bowling Green, OH 43403.

The hippocampal formation of birds and mammals is a brain structure known to be critical for spatial memory and the learning of environmental maps. But how is a spatial map learned and what is the role of the hippocampal formation in the learning process? The ability to use the sun to define directions in space, or sun compass, is perhaps the most ubiquitous, naturally occurring spatial orientation mechanism found in the animal kingdom. In addition to guiding movement, the sun compass of birds may serve as a directional reference system that enables animals to learn about the spatial distribution or location of other stimuli in the environment. Thus, the sun compass may play a critical role in map learning. We report that homing pigeons with hippocampal lesions are unable to use the sun compass to learn the location of a food reward. The results indicate that the importance of the hippocampal formation in map learning may be related to its participation in a neural process in which information from a directional reference system, in this case the sun compass, is used to register the location of other stimuli that are normally components of some type of map.

592.5

EFFECT OF HIPPOCAMPAL LESIONS ON PLACE LEARNING IN PIGEONS.

T. Fremouw,* P. Jackson-Smith, C. P. Shimp, and R. P. Kesner. Dept. of Psychology, Univ. of Utah, Salt Lake, UT 84112.

We studied how the hippocampus affects pigeons' (Columba livia) performance in a dry land version of the Morris water maze when searching for a single hidden food location. The maze was a 2m-diameter circular arena with eight foodwells drilled in the floor. The entire floor was covered with sawdust which concealed the eight foodwells. Intramaze cues consisted of pictures on the inner wall of the arena. Extramaze cues consisted of pictures on the room walls, a door, and shelving. Hippocampal and control pigeons were pretrained to search for food hidden in all eight of the foodwells. After the pigeons learned to search for food, a single food location was chosen (one of the eight foodwells) and, thereafter, food was placed only in that hole. Four trials per day were then given, with each trial lasting a maximum of four minutes. If a bird did not find the food within that time the food location was uncovered and the bird was allowed to eat a small portion of the food there. Hippocampal birds learned the food location significantly slower than controls. However, by day 20 hippocampal and control birds were performing at similar levels. On day 21 a new food location was chosen. Again, hippocampal birds learned the location significantly slower than the controls. Finally, a probe trial was conducted in which the maze was rotated 180 degrees. The performance of both groups of pigeons was unaffected by this rotation suggesting that both groups used extramaze cues. Hippocampal lesions in pigeons produce learning deficits in a place learning task that are similar to those produced by hippocampal lesions in rats.

592.6

IMPAIRED COLOR-REVERSAL LEARNING IN PIGEONS AFTER LESIONS OF THE HYPERSTRIATUM VENTRALE.

L. Chaves and W. Hodos*. Dept. of Psychology, University of Maryland, College Park, MD 20742.

Lesion studies of color-reversal learning in pigeons show that an impairment results when (1) the tectofugal visual pathway is damaged at either the thalamic level (nucleus rotundus) or the telencephalic level (ectostriatum), or (2) the thalamofugal visual pathway is damaged at the telencephalic level (the visual Wulst). An impairment does not result, however, when the thalamic source of thalamofugal input to the visual Wulst (OPT) is damaged. These results suggest that in pigeons, the Wulst maintains a role in color-reversal learning by receiving visual information routed from the tectofugal pathway via other visual areas in the telencephalon such as the hyperstriatum ventrale (HV). In the present study, medial and lateral regions of HV were ablated. Pigeons were trained post-operatively to discriminate two colors presented simultaneously. After reaching criterion, they were required to perform a series of discrimination reversals in which the positive and negative stimuli were interchanged. An impairment in color-reversal learning resulted when lateral HV was damaged in conjunction with damage to the frontothalamic tract (FT), which carries ascending visual input from OPT to the visual Wulst. No deficits were observed when either lateral HV or FT were damaged alone. Lesions of medial HV resulted in impaired performance of a color-discrimination task (i.e., original learning), but did not affect discrimination reversal. These findings suggest that the visual Wulst requires input from both the tectofugal pathway (via lateral HV) as well as from the thalamofugal pathway in order for its role in color-reversal learning to be maintained.

592.7

MEMORY IN THE CHICK: MULTIPLE CUES, DISTINCT BRAIN LOCATIONS. T.A. Barber (Patterson)*. Department of Psychology, Dickinson College, Carlisle, PA 17013.

Biochemical, electrophysiological, and morphological changes in the intermediate medial hyperstriatum ventrale (IMHV) of chicks trained on a one-trial passive avoidance task suggest that the IMHV is an important site of memory storage. However, post-training lesions of the IMHV are not amnestic (Patterson, Gilbert and Rose, 1990).

The effects of post-training IMHV lesions in a two-color discrimination task were examined. Lesions impaired discrimination between aversive and non-aversive beads but did not impair ability to peck non-aversive or novel beads, indicating that IMHV lesions disrupt the association between color and the aversive taste.

These data suggest that the IMHV processes and/or stores the association of color and aversion. Post-training IMHV lesions do not "impair" retention; chicks can successfully avoid the aversive bead on the basis of other components such as the shape or size. This interpretation explains why significant alterations occur in the IMHV after training, because they are involved with forming the color-specific aspect of the multiple representations of the aversive bead.

These results suggest that memory is comprised of different forms of representation that are processed and stored in different areas of the chick forebrain and even simple associations can be stored in the brain in the form of multiple, dispersed representations.

592.8

BILATERAL L-MAN LESIONS DISRUPT SONG CONSOLIDATION AT SONG ONSET IN ADULT MALE WHITE-CROWNED SPARROWS. S. Benton, P. Marler*, D.A. Nelson and T.J. DeVoogd. Neurobiology & Behavior, Cornell Univ., Ithaca, NY 14853; Dept. Zoology, Univ. Calif., Davis, CA 95616.

The function of nucleus l-MAN in adult male songbirds is incompletely understood. Lesioning l-MAN during song learning in juvenile birds disrupts learning, while the same lesions made in singing adults produce no immediate deterioation in stereotyped song. However, when l-MAN is lesioned in adult canaries on a short-day light cycle, the birds produce aberrant songs when put on long-days (Suter et al., 1990; Hill et al., 1992). We have made bilateral l-MAN lesions in adult (3 yr. old) male white-crowned sparrows (*Zonotrichia leucophrys oriantha* and *Z. l. nuttalli*) on short-days. White-crowned sparrows are sensitive-period learners that maintain a stable song pattern through adulthood. Substantial unilateral to complete bilateral lesions have minimal effects on song structure but appear to affect other aspects of song consolidation. When put on long-days, lesioned males appear to experience a prolonged plastic period of up to two months before the onset of full song. This plastic phase is characterized by periods of no song, low volume song, and periods during which song elements are embedded among call notes. These results are consistent with the hypothesis that l-MAN may modify song consolidation via NMDA receptors in RA. It has been suggested that input from l-MAN may selectively reinforce and refine the output from RA through post-synaptic NMDA receptors (Mooney, 1992). This mechanism could account for refinements made to established song during the plastic period experienced at song onset each spring. *Supported by HD21033.*

592.9

DEVELOPMENT OF SONG SYSTEM NUCLEUS HVC IN JUVENILE MALE ZEBRA FINCHES DEPRIVED OF SONG. C. E. Collins*, E. Wallhäusser-Franke, R.P. Clower and T. J. DeVoogd

Department of Psychology, Uris Hall, Cornell Univ., Ithaca, NY 14853

Songbird species learn aspects of their song. In many of these species, this learning occurs in a juvenile sensitive period. Songs heard during this interval are used as models for the song that the young bird will later produce. If the juvenile does not hear song, the sensitive period for acquiring a model may be prolonged (Eales, '85, '87). Several labs have noted neural events that occur in phase with song learning that could provide a substrate for the learning. The present study manipulates the opportunity for song learning and searches for consequent ultrastructural changes in HVC, a song-related brain area.

Zebra finch pairs raised young in a sound proof room. Four to five days after the young hatched, the adult male was removed. At independence the young were housed together. At 30 or 55 days, males were perfused and song-related area HVC was prepared for electron microscopy. Serial section analyses were conducted using the unbiased disector stereological method (Sterio, '84). Preliminary results suggest that at 55 days the number of synapses in HVC is higher in song deprived than in control birds. Thus, learning a song may be associated with the loss of synapses, or preventing this form of learning may selectively preserve synapses, in accord with recent theories on synapse selection (Scheich, et al, '91). *Supported by HD-21033.*

592.10

CLASSICAL CONDITIONING ENHANCES THE EXPRESSION OF THE IMMEDIATE EARLY GENE ZENK IN RESPONSE TO CONSPECIFIC SONG Erich D. Jarvis, Claudio Mello and Fernando Nottebohm*. Lab. of Animal Behavior, The Rockefeller University, New York, N.Y. 10021

A classical conditioning paradigm was developed to see if induction of the immediate early gene ZENK in response to playbacks of conspecific song (Mello et. al., 1992) could be influenced by context. Canaries were placed into a plexiglass box with a metal bar floor and no perches. They learned to avoid a mild (200uA) shock to their feet delivered at the end of each song stimulus by fluttering and thus breaking contact with the metal floor. The stimuli used were short (1.8-4.0 sec) fragments of canary song presented once every 30 sec. At the end of 45 min. birds trained in this manner were responding correctly and avoiding shock 80-90% of the time. They were then killed and their brains prepared for in-situ hybridization with canary ZENK, an immediate early gene encoding a transcription factor. Results were quantified by densitometric analysis of X-ray film autoradiograms. Pairing song with shock avoidance training doubled the level of ZENK expression in two telencephalic auditory regions, the caudomedial neostriatum (NCM) and the mediocaudal hyperstriatum ventralis (HVMC), as compared with the effectiveness of presenting song alone or in random association with shock. We infer that the up-regulation of ZENK expression in NCM-HVMC by exposure to conspecific song is influenced by the context in which the song occurs.

STRESS: NEUROCHEMISTRY

593.1

AMYGDALA LESIONS BLOCK PSYCHOLOGICAL STRESS-INDUCED METABOLIC ACTIVATION OF THE DOPAMINE, NOREPINEPHRINE, AND SEROTONIN SYSTEMS IN THE RAT PREFRONTAL CORTEX: A BEHAVIORAL, NEUROENDOCRINE, AND NEUROCHEMICAL STUDY. LE Goldstein, AM Rasmusson, BS Bunney, RH Roth* Yale Medical School, Depts. Psychiatry and Pharmacology, New Haven, CT 06510

When exposed to threatening stimuli, rats exhibit a species-specific defense reaction which includes freezing behavior, ultrasonic vocalization, and elevation of serum corticosterone. Exposure to psychological stress is also known to selectively activate dopamine, serotonin, and norepinephrine utilization in the anteromedial prefrontal cortex (mPFC). Neuroanatomical site(s) providing afferent control of the stress activation of these mPFC biogenic amine systems is at present unknown. Using a conditoned stress model in which rats are trained to fear a sub-startle threshold tone previously paired with footshock, the present study demonstrates that: (1) conditioned stress-induced metabolic activation of the mPFC dopamine, serotonin, and norepinephrine systems persists for at least two weeks after training, and (2) combined post-training N-methyl-D-aspartate (NMDA) excitotoxic lesions of the basolateral and central nuclei of the amygdala block metabolic activation of these biogenic amine systems. In addition, amygdala lesions abolished conditioned stress induced freezing behavior, ultrasonic vocalization, and elevation of serum corticosterone. Pre-training NMDA lesions of the amygdala also completely blocked ultrasonic distress vocalizations during the conditioning period. In contrast, NMDA excitotoxic lesions of the mPFC do not block the behavioral or adrenocortical responses to conditioned stress. In fact, lesion of the mPFC potentiates conditioned ultrasonic distress vocalization. These data provide evidence of amygdalar afferent control of the mPFC monoaminergic system responses to conditioned stress. In addition, these experiments suggest that the mPFC may modulate the subcortical circuitry coordinated by the amygdala. Supported by USPHS grant MH 14092.

593.2

EFFECTS OF CHRONIC SOCIAL STRESS ON SEROTONIN RECEPTOR BINDING. C.R. McKittrick*, D.C. Blanchard, R.J. Blanchard, B.S. McEwen, and R.R. Sakai. Rockefeller University, New York, NY 10021, University of Hawaii, 2430 Campus Rd., Honolulu, HI 96822 and University of Pennsylvania, Philadelphia, PA 19104.

Mixed-sex rat colonies (5 male, 2 female) housed in a visible burrow system for 14 d have been used to determine the effects of chronic social stress on brain 5HT receptors. The male rats quickly form dominance hierarchies consisting of one dominant (DOM) and four subordinates; the subordinates can be further subdivided into stress responsive subordinates (SRS) and nonresponsive subordinates (NRS) based on analyses of corticosterone responses to 1 hour of novel restraint stress. Glucocorticoids and stress have been shown to affect binding to various 5HT receptor subtypes. We have found that 5HT1A receptors are downregulated in several areas of hippocampus in colony-housed animals as compared to individually-housed age- and weight-matched controls (CON). In CA1, 5HT1A binding is significantly decreased in DOM and SRS animals (↓24% and 19%, respectively, compared to CON), while there is no corresponding change in the NRS. However, in CA3, binding is decreased in all colony animals compared to CON (DOM:↓22%; SRS:↓24%; NRS:↓18%). In contrast, only SRS have significant decreases in binding in CA2 (↓20%) and the dentate gyrus(↓18%). The chronic social stress also affects 5HT2 receptors, as binding is increased in layer IV of parietal cortex of both SRS (↑64%) and NRS (↑72%). The changes observed in both receptor subtypes are consistent with those seen following CORT or ACTH administration. *[Supported by: NARSAD (RRS), NSF BNS9111524 (DCB) and MH41286(BMc)]*

593.3

THE EFFECT OF CHRONIC SOCIAL STRESS ON GENE EXPRESSION IN THE RAT HIPPOCAMPUS. H.M. Chao*, D.C. Blanchard, R.J. Blanchard, B.S. McEwen, and R.R. Sakai. The Rockefeller University, New York, NY 10021, University of Hawaii, Honolulu, HI 96822 and University of Pennsylvania, Philadelphia, PA 19104

The housing of rat colonies in a visible burrow system, provides an effective method by which the behavioral and neuroendocrine effects of social stress can be examined. Subordinate rats have been shown to exhibit elevated plasma corticosterone relative to the dominant rat in the colony. Moreover both dominant and subordinate rats in this system have higher basal corticosterone levels than singly housed controls.

Previous studies have indicated that glucocorticoids negatively regulate the expression of the mRNAs encoding the glucocorticoid receptor (GR), the mineralocorticoid receptor (MR) and the growth-associated protein, GAP-43. This study examines the effect of social stress, and the resulting sustained increase in steroid levels, on gene expression. Our results indicate that GR, MR and GAP-43 mRNAs are decreased in subordinate rats relative to controls, in the CA1 region of the hippocampus. No statistically significant differences in mRNA expression are observed in other hippocampal subfields. The dominant rats, which have levels of circulating glucocorticoids intermediate between control and subordinate rats, exhibit intermediate levels of these mRNAs. These findings suggest that the stress of group housing elicits changes in hippocampal gene expression consistent with those previously demonstrated by surgical or pharmacological manipulation of adrenal steroid levels. [Supported by NSF BNS9111524 (DCB) and NARSAD (RRS)]

593.4

EVIDENCE FOR THE INVOLVEMENT OF GABA AND DOPAMINE IN THE SOUND STRESS INDUCED ACTIVATION OF THE ASCENDING SEROTONINERGIC NEURONS OF THE DORSAL RAPHE NUCLEUS
Karl C. Corley *, Roger P. Ditts and Margaret C. Boadle-Biber Dept. of Physiology, Medical College of Virginia-VCU, Richmond, VA 23298-0551

We have previously demonstrated that sound stress increases the activity of tryptophan hydroxylase (E.C. 14.12.16.4) (TrpH), the rate limiting enzyme in serotonin biosynthesis, using both an *ex vivo* and *in vivo* assay to monitor the accumulations of the enzymatic product, 5-hydroxytryptophan (5-HTP). In an attempt to delineate some of the pharmacology and neurotransmitters mediating the increased enzyme activity produced by stress we have studied the effects of drugs (I.P.) known to affect serotonin, GABA and dopamine neurotransmission, respectively. The serotonin uptake inhibitor, fluoxetine (8.0 mg/kg) blocked the *ex vivo* stress induced increase in TrpH. Consistent with these findings, the increase in 5-HTP produced by sound stress *in vivo* is blocked by the administration of the 5-$HT1_A$ agonists , gepirone (1.0 mg/kg) and 8-OH-DPAT (0.1 mg/kg) with similar effects *ex vivo*. Administration of the 5-HT_2 antagonist nefazadone (100 mg/kg) also blocked the stress induced activity in TrpH measured *ex vivo*. The benzodiazepine, diazepam, dose dependently attenuated and blocked (0.25-1.0 mg/kg) stress induced TrpH activity. Administration of the typical neuroleptic, haloperidol (0.5 mg/kg) or the atypical neuroleptic, clozapine (10 mg/kg), blocked both the *in vivo* and *ex vivo* increases in TrpH activity produced by sound stress. The actions of fluoxetine, as well as, the 5-$HT1_A$ agonists, are consistent with our hypothesis that sound stress increases the firing and metabolic activity within the serotoninergic neurons of the dorsal raphe nucleus. These data also support a role for GABA and dopamine in mediating the effects of sound stress. Supported by NIH grant #NS14090 to M.C.B.B.

593.5

NEUROTENSIN BLOCKS THE SOUND STRESS INDUCED ACTIVATION OF THE ASCENDING SEROTONINERGIC NEURONS OF THE DORSAL RAPHE NUCLEUS M.C. Boadle-Biber*, M. Novitzki and R.P. Ditts Dept. of Physiology , Medical College of Virginia-VCU, Richmond, VA 23298-0551

In this study we investigated the effects of the tridecapeptide, neurotensin (NT), on tryptophan hydroxylase (EC 1.14.16.4) (TrpH), the rate limiting enzyme in serotonin biosynthesis. Rats (125 -150 g), were implanted with 27 ga. guide cannulae directed towards the lateral ventricles (A/P -1.0, M/L 1.5, D/V 3.2 mm from bregma), fitted with 31 ga. arbitrators, and allowed to recover for at least three days. Animals were subsequently habituated to handling for two days and on the third day were left untreated or infused with either 0.5 ul saline or 0.3 - 10 nmols of NT in 0.5 ul saline. Five minutes after the infusion, rats were injected with the amino acid decarboxylase inhibitor, m-hydroxyhydrazine (I.P. 100 mg/kg), and placed into the sound stress chambers. Rats were either exposed to one-half hour of random 110 dB sound or no sound (control) and subsequently decapitated. Brains were rapidly removed, frozen and subsequently analyzed by HPLC-EC. Results were analyzed using random ANOVA with $p < 0.05$ and post-hoc analysis. Sound stress significantly increased the accumulation of 5-HTP within the midbrain (473.5 ± 35.5; mean ± S.E.M.) compared to controls (277 ± 14.4) as well as in the terminal fields of striatum, cortex and hippocampus. NT at all doses blocked the sound stress induced increase in 5-HTP within the midbrain, as well as within all the terminal fields. Ten nmol of NT significantly reduced 5-HTP values below controls in both sound and sham treated midbrain (111 ± 9 & 158 ± 7, respectively). Neither stress nor NT affected TrpH activity in the hindbrain. These results suggest a role for NT in modulating the ascending serotoninergic systems of the dorsal raphe nucleus. This work is supported by NIH grant #NS14090 to M.C.B.B..

593.6

IS THE STRESS-INDUCED ACTIVATION OF DOPAMINE RELEASE IN THE PREFRONTAL CORTEX MODULATED BY EXCITATORY AMINO ACIDS?
H.P. Jedema and B. Moghaddam*, Department of Psychiatry, Yale University School of Medicine, VA medical Center 116A/2, West Haven, CT 06516.

The dopaminergic projection to the prefrontal cortex (PFC) is known to be activated by mild stress. The excitatory amino acid (EAA) systems in the PFC have recently been shown to be activated by restraint, swimming, as well as pharmacological stressors. Similar to dopamine, the stress-induced activation of EAAs is much more profound in the PFC than regions in the basal ganglia such as striatum and nucleus accumbens. It has been hypothesized that the stress-induced activation of dopamine in the PFC may be due to an initial increase in the release of EAAs which will then release dopamine by acting on EAA receptors that modulate dopamine release in this region. We have studied the effect of EAA antagonists on the stress-induced release of dopamine in the PFC. Thus, NMDA or non-NMDA antagonists (AP5 and CNQX respectively) were locally perfused into the PFC through the microdialysis probe while the response of extracellular levels of dopamine to a 20 minute exposure to restraint was assessed. This stressor increased the dopamine levels in the PFC by nearly 100%. Perfusion of 50 μM CNQX or 200 μM AP5 during restraint did not lead to a decrease in this response. Currently, we are investigating the effect of higher concentration and longer time of perfusion of these antagonists to ensure that the EAA receptors were sufficiently blocked prior to the stress procedure. Nevertheless, considering the present data, it remains unclear whether the stress-induced activation of dopamine in the PFC is dependent on the activation of EAA systems. Supported in part by MH-48404 and Department of Veterans Affairs Centers for PTSD and Schizophrenia.

593.7

EFFECT OF A PHARMACOLOGICAL STRESSOR ON THE EXTRACELLULAR LEVEL OF EXCITATORY AMINO ACIDS IN THE PREFRONTAL CORTEX AND STRIATUM. M. Karreman, B. Moghaddam*, Department of Psychiatry, Yale Univ. Sch. of Med., VA Medical Center, West Haven, CT 06516.

Previous studies from our laboratory have shown that exposure to restraint and swimming stress increases the neuronal release of excitatory amino acids in several regions of the rat brain. The stress-induced increase in the excitatory amino acid levels was shown to be more profound in the prefrontal cortex as compared to other regions in the basal ganglia. In the present study we wished to determine whether pharmacological stressors have similar effects on the extracellular levels of excitatory amino acids. Thus, we used the technique of intracerebral microdialysis to assess the effect of the anxiogenic β-carboline FG 7142 (20 mg/kg, i.p.) on the extracellular levels of excitatory amino acids in the prefrontal cortex and striatum. FG 7142 significantly increased the extracellular level of glutamate in the prefrontal cortex, whereas there was no significant change in the extracellular glutamate level in the striatum. Pretreatment with the benzodiazepine agonist, diazepam (5 mg/kg, i.p.), blocked the effect of FG 7142 in the prefrontal cortex. Diazepam alone did not cause a significant change in the extracellular level of aspartate or glutamate in the prefrontal cortex; however, it increased the extracellular glutamate level in the striatum. This study provides direct evidence that anxiogenic drugs such as FG 7142 preferentially increase the extracellular level of glutamate in the prefrontal cortex as compared to the striatum, and that this effect can be reversed with diazepam.

593.8

DOPAMINE RELEASE IN THE NUCLEUS ACCUMBENS DURING SOCIAL STRESS: AN *IN VIVO* MICRODIALYSIS STUDY. J.W. Tidey*, C.A. Cohen, R. Kream and K.A. Miczek. Department of Psychology, Tufts University, Medford MA 02155 and Department of Anesthesiology, Tufts University School of Medicine, Boston MA 02111.

Paradoxically, exposure to both aversive and reinforcing events increases mesocorticolimbic dopamine (DA) release. The present study utilizes an ethologically valid model, namely exposure to an aggressive male rat, to examine the effects of social stress on DA, DOPAC and HVA release using *in vivo* microdialysis in freely moving rats. Following 6 20-min baseline samples, each subject is placed in the empty home cage of a rat which had previously attacked and defeated it. Forty minutes later, the aggressive stimulus rat is returned to its home cage; samples are collected while the subject is physically protected with a wire mesh enclosure but can see, hear and smell the stimulus rat. One hour and forty minutes later, the subject is returned to its home cage and samples are collected until dialysate levels return to baseline. Preliminary results suggest that placement of the subject into the cage of an aggressive stimulus rat results in heightened DA and DOPAC release relative to controls placed in a clean novel cage; DOPAC levels remain elevated after DA levels return to baseline. Increased DA release is correlated not with motor hyperactivity but with defensive vocal and postural responses, suggesting that mesocorticolimbic DA may be important in initiating biologically significant behavior.

593.9

ACTIVATION OF NOREPINEPHRINE RELEASE IN CORTEX AND HYPOTHALAMUS BY NITROPRUSSIDE INFUSIONS.

Gennady N. Smagin, Artur H. Swiergiel, R. Don Brown*, Adrian J. Dunn. Department of Pharmacology, Louisiana State University Medical Center, Shreveport, LA 71130-3932.

Intravenous infusion of sodium nitroprusside (NP) decreases blood pressure and results in a hemodynamic stress that accelerates the firing rate of noradrenergic neurons in the locus coeruleus (LC). We tested whether NP increased the release of norepinephrine (NE) in the medial prefrontal cortex (PFM) and medial hypothalamus (MHT) as measured by microdialysis and HPLC. Rats were anesthetized with pentobarbital and urethane and microdialysis probes were inserted into the PFM and/or the MHT. NP infused into the femoral vein at a dose of 10 μg/30 μl/min for 15 min rapidly decreased blood pressure by 48%. During NP infusion the concentrations of NE in the cortical and hypothalamic microdialysates were elevated by 41% ($p < .001$) and 67% ($p < .05$) of the pre-infusion value, respectively. After infusion, PFM NE rebounded to 127% (ns) and MHT NE to 133% ($p < .05$). MHT MHPG increased by 51% ($p < .05$) but PFM MHPG and concentrations of DOPAC, 5-HIAA and HVA from PFM or MHT were not significantly affected by the infusion.

We conclude that hemodynamic stress activates NE neurons projecting to the PFM and MHT. We presume that the increase in cortical NE release is due to activation of the LC and may reflect a behavioral aspect of the stress. The hypothalamic response may be involved in the autonomic regulation of blood pressure.

Supported by NINDS (NS 27283) and AFOSR (F49620-93-1-0125DEF).

593.10

COLD STRESS EXPOSURE ALTERS HIPPOCAMPAL CHOLINERGIC NEUROTRANSMISSION.

M.J. Stillman*[1], B. Shukitt-Hale[1], A. Levy[2], and H.R. Lieberman. Military Performance and Neuroscience Division, United States Army Research Institute of Environmental Medicine, Natick, MA 01760-5007, [1]GEO-CENTERS, INC., Newton Centre, MA 02159, and [2]IIBR, Ness Ziona, ISRAEL.

Few *in vivo* investigations have been conducted to assess the effects of stress on the cholinergic system. This study examined the time course of the effect of cold stress on hippocampal cholinergic neurotransmission. Hippocampal extracellular acetylcholine (ACh) and choline levels were evaluated using *in vivo* microdialysis in male Fischer 344 rats before, during, and following 80 min cold stress. Measurements were taken in two groups of rats immersed in a water bath: normothermic restrained (N-REST) rats (37 °C water), and cold restrained (C-REST) rats (20 °C water). Results were also compared to normothermic freely moving (N-FREE) rats. C-REST rats displayed decreased ACh levels relative to both N-REST and N-FREE rats at the onset of cold exposure. By the end of cold exposure and following removal from cold, ACh levels approximated baseline values. N-REST rats had levels similar to N-FREE rats, except for a marked increase in ACh following removal from 37 °C water. C-REST rats displayed a gradual elevation in choline levels during cold stress, whereas N-REST and N-FREE rats both displayed gradual decreases during the microdialysis session. These findings indicate that cholinergic neurotransmission can be influenced by the application of and removal from an acute stressor.

593.11

STRESS-INDUCED ALTERATIONS IN PERIPHERAL BENZODIAZEPINE RECEPTORS IN RATS:CONTRIBUTIONS OF GENDER AND ACTIVITY.

R. Drugan*, S. Luczak, H. Oh, R. Ferland and P. Holmes. Dept. of Psychology, Brown University, Providence, R.I. 02912

There are two distinct types of benzodiazepine receptors. The central benzodiazepine/GABA receptor complex (BGRC) is located in brain and is involved in actions of minor tranquilizers and the pathophysiology of anxiety. The peripheral benzodiazepine receptor (PBR) is found in both brain and peripheral tissue and is thought to be involved in steroid synthesis and energy metabolism.

Similar to the BGRC, the PBR in both brain and peripheral tissue can be rapidly altered by stress. Our laboratory has examined the cause for these changes and have targeted the renin-angiotensin system as an important factor. A sexual dimorphism in the renal PBR response to stress is evident and differential reactivity of the renin-angiotensin system appears to be critical. Recent microdissection studies in kidney also reveal a differential regional distribution of stress effects in males and females.

Since the PBR is located on the outer mitochrondrial membrane, the possibility of activity/metabolic influences during stress may be critical. We employed both experimental and pharmacological tools for increasing activity independent of stress and examined PBR alterations. D-amphetamine administration and forced running produced changes opposite to stress in cardiac and olfactory bulb PBR, respectively. Supported by a PHS grant MH 45475 to RCD.

593.12

EFFECT OF COLD STRESS ON NEURONAL EXCITABILITY AND LTP IN THE DENTATE GYRUS OF FREELY MOVING RATS

J.M. Sarvey*, T. Southard[1], S.T. Ahlers[2] and C.R. Bramham[1]. [1]Department of Pharmacology, Uniformed Services University, and [2]Naval Medical Research Institute, Bethesda, MD.

Exposure to relatively mild cold (4°C) can impair working memory. The effect could result from a disruption in hippocampal function at the level of neuronal transmission, at the level of synaptic plasticity, or both. Rats were chronically implanted for unilateral stimulation of the perforant path and recording of evoked field potentials in the dentate hilus. Animals were handled and then habituated on 3 consecutive days to a standard recording chamber and to an adjacent experimental chamber. Baseline field potentials, input-output curves and paired-pulse tests were obtained in the standard chamber. Rats were then transferred to the cold chamber (4°C) where tests were repeated, and after a 90 min exposure, high-frequency stimulation (HFS) was applied to the perforant path and field potentials were monitored for 2h. A control group received identical treatment except the temperature in the experimental cage was 23°C. All field potentials were collected when the rat was awake and motionless. HFS evoked LTP of the EPSP slope and population spike and there was no difference between groups in the magnitude or time course of LTP. However, there was a 25% reduction in population spike amplitude and an enhancement in paired-pulse facilitation upon transferring rats from the standard to the experimental cage in both cold and control groups. The results suggest that cold exposure does not affect the capacity for synaptic modification in the dentate gyrus as assessed by LTP induction.

593.13

GLUCOSE RESTORES THE ACQUISITION OF RESPONSE CHAINS DISRUPTED BY COLD AIR EXPOSURE IN RATS.

J. Schrot, S.T. Ahlers. Naval Medical Research Institute, Bethesda, MD 20889, and Joel L. Davis. Office of Naval Research, Arlington, VA 22217.

Exposure to cold environments disrupts memory processes. These decrements can be reversed by glucose administration. The interaction of cold and glucose on the acquisition of behavior has been less well studied. In this study, a repeated acquisition procedure was used to investigate the effect of glucose (50 or 100 mg/kg, ip) administered preceding exposure to air temperatures of 2 or 22°C on the acquisition of four-member response sequences in rats. The results indicate that error and timeout responding increased during cold exposures. A biphasic response pattern of acquisition followed by performance was observed during control sessions. Acquisition was characterized by frequent error responses while performance was characterized by extended runs of errorless sequence completions interspersed with occasional error responses. Exposure to cold air disrupted this pattern, the onset of the performance phase was delayed and the magnitude of the error bursts increased. The administration of glucose at doses of 50 or 100 mg/kg preceding cold air resulted in an overall pattern of responding that more closely resembled control responding. Similar results were observed of timeout responses. The repeated acquisition procedure contains elements of both working and reference memory. Working memory is requisite for acquiring the novel sequence of lever presses each day, while reference memory is invoked by the timeout stimulus. Cold exposure disrupted both working and reference memory aspects of the procedure. Glucose administration reversed the effects of cold exposure on both aspects of the procedure. These results are consistent with findings that glucose facilitates memory consolidation and they support the hypothesis that glucose enhances memory encoding.

593.14

PERIPHERAL β-1 ADRENORECEPTORS ARE INVOLVED IN STRESS-INDUCED HYPERCHOLESTEROLEMIA IN RATS.

F. X. Brennan, Jr.*, C. L. Cobb, L. H. Silbert, L. R. Watkins, & S. F. Maier. Psychology Department, University of Colorado, Boulder, CO, 80309.

Increases in plasma cholesterol are now accepted as a significant risk factor for the development of Coronary Heart Disease (CHD). Plasma cholesterol levels may become elevated as a result of exposure to stressful stimuli. We have recently reported that rats exposed to three 2 hr sessions of inescapable tailshock, one session per day for three successive days, show a robust 40% increase in total plasma cholesterol. Other data has shown that rats given injections of epinephrine show comparable elevations in total plasma cholesterol. The purpose of the following experiments was to investigate whether catecholamines were involved in the stress-induced rise in cholesterol, by using adrenergic receptor antagonists.

In experiment 1, propranalol (25 mg/kg, s.c.), a standard beta-adrenoreceptor blocker, was injected prior to each of the daily stress sessions. Propranalol attenuated the cholesterol increase in stressed rats, but increased the cholesterol levels of nonshocked animals. This finding is in line with some data that suggests that in certain situations, propranalol can itself produce increases in plasma cholesterol. We therefore, in Experiment 2, examined whether the same dose of propranalol injected prior to the final stress session only would eliminate the stress-induced increase in cholesterol. Results indicated that propranalol attenuated the increase in cholesterol in stressed animals, while producing no increase in nonshocked animals.

Propranalol antagonizes both β-1 and β-2 adrenoreceptors, and also readily crosses the blood-brain barrier. Available data implicate peripheral β-1 adrenoreceptors as relevant for cholesterol metabolism. We therefore, in Experiment 3, injected atenolol (10 mg, s.c.), a peripherally restricted β-1 specific antagonist. Results indicated that atenolol blocked the cholesterol increase induced by stress, while having no effect in the absence of stress. The experiments support the idea that peripheral β-adrenoreceptors, particularly β-1 receptors, are involved in stress-induced increases in plasma cholesterol.

593.15

DIFFERENTIAL REGULATION OF RAT BRAIN C-FOS EXPRESSION IN RESPONSE TO ACUTE VS REPEATED STRESS. K.R. Melia*, A.E. Ryabinin@, R. Schroeder, M.C.Wilson, and F.E. Bloom. The Scripps Research Institute, La Jolla, CA 92037. @TSRI and P.K.Anokhin Institute of Normal Physiology, Moscow, Russia 103009.

Acute stress activates the hypothalamic-pituitary-adrenal (HPA)-axis increasing serum levels of corticosterone. In contrast, repeated presentation of a homotypic stressor results in habituation of this response. To elucidate the neural systems which regulate activation and habituation of the HPA-axis, we examined the pattern of CNS c-fos expression, as a marker for altered neuronal activity, in response to acute (2h) vs repeated (2h/day for 4 or 9 days) restraint stress. On day 1 restraint stress increased plasma levels of corticosterone and c-fos mRNA in the cortex, hippocampus, hypothalamus and septum, but not the amygdala or brainstem. On day 4 stress-induced increases in plasma corticosterone and CNS expression of c-fos mRNA were significantly smaller than on day 1, and were completely habituated by day 9. The inability of restraint to induce c-fos mRNA in repeatedly stressed rats is not due to a general nonresponsivity of the c-fos gene since exposure to a novel stressor (20 m swim stress) could induce c-fos in restraint habituated rats. The induction of c-fos mRNA in the CNS in response to acute restraint stress, and the complete habituation of this response in repeatedly restrained rats, is not secondary to changes in corticosterone levels since the same pattern of induction and habituation was seen in adrenalectomized rats.

These results support the hypothesis that differential responding of the HPA-axis to acute vs repeated stress is due to changes in the activity of neural structures which innervate CRF neurons in the paraventricular nucleus of the hypothalamus. Understanding the neural systems which regulate this axis may provide insight into the biological basis of those psychiatric disorders which are characterized, in part, by dysregulation of the HPA-axis.*(Partially supported by NARSAD and MacArthur Fnd. Depression Network (KRM).*

593.16

TYROSINE INCREASES HYPOTHERMIA-INDUCED NOREPINEPHRINE (NE) RELEASE IN RAT HIPPOCAMPUS ASSESSED BY IN VIVO MICRODIALYSIS. S. Luo[1], E.T.S. Li[*1] and H.R. Lieberman. U.S. Army Research Institute of Environmental Medicine, Natick, MA 01760; [1] Nutritional Sciences, University of Toronto, Ontario, Canada M5S 1A8.

Previous studies have shown that hypothermia-induced behavioral depression is reversed by the NE precusor, tyrosine. To examine the mechanisms of action of tyrosine in cold stress-induced behavioral depression, extracellular levels of NE were monitored in the hippocampus of male Fischer rats. Two experiments were performed. First, NE release was determined in hypothermic rats. Animals with implanted dialysis probes (n=6) were restrained and immersed in a water bath (17°C) for about 20 min. until a core body temperature (T_c) of 30°C was obtained. The control group (n=6) consisted of restraint and immersion in a 36°C water bath for 20 min, which did not alter normal T_c. Second, the effects of tyrosine on NE release in hypothermic rats were determined. Twelve naive rats with implanted dialysis probes were given either tyrosine (400 mg/kg, ip) or saline prior to hypothermia. In both experiments, dialysate samples were collected every 10 min ranging from 30 min before to 50 min after immersion in water, and the samples were immediately analyzed by high performance liquid chromatography with electrochemical detection. Restraint and hypothermia increased NE efflux to 204% and 329% of baseline, respectively ($p < 0.05$). Tyrosine administration enhanced the NE release of hypothermic rats compared to saline treatment ($p < 0.05$). The results indicate that extracellular release of NE increases during acute hypothermia and that of tyrosine's beneficial behavioral effects may be mediated by the noradrenergic system.

593.17

EFFECT OF UNILATERAL OR BILATERAL DEPLETION OF PREFRONTAL CORTICAL DOPAMINE ON STRESS ULCER DEVELOPMENT IN RATS. R.M. Sullivan* and H. Szechtman. Dept. of Biomedical Sci., McMaster Univ., Hamilton, Ontario, CANADA, L8N 3Z5.

The mesocortical dopamine (DA) system in rats is known to be activated by a variety of stressors, and may do so in an asymmetric manner. The present study investigates the functional significance of this system in the ability to cope with aversive situations. Sixty-six male Sprague-Dawley rats were assessed for direction of amphetamine-induced rotation and distributed evenly among groups. Rats then received vehicle (n=21) or 6-hydroxydopamine infusions in left, right or bilateral cortex (n=15/group), following desipramine pretreatment. Three injection sites/side were used (12 ug 6-OHDA/side), aimed at the most dense DA innervation (0.2-3.0 mm ant. to bregma). Fourteen days later rats were food-deprived (24 h) and placed in Plexiglas restrainers at 4° C for 3 h. They were then sacrificed, stomachs examined microscopically for gastric mucosal lesions, and brain regions dissected out for biochemical analysis. Mean total gastric pathology differed significantly across groups ($F_{3,62} = 3.22$, $p = 0.029$). Left and bilateral lesions aggravated ulcer development relative to controls, but marginally failed to reach significance, while right lesion effects were more pronounced ($t = -2.89$, $p < 0.01$). An additional group of operated but unrestrained shams showed no pathology. The results suggest that right prefrontal cortical DA may be preferentially involved in affording protection against the pathological consequences of stress exposure. (Supported by NSERC)

593.18

HABITUATION AND SENSITIZATION OF PLASMA CATECHOLAMINE RESPONSES IN AGED RATS. T.R. Mabry*, P.E. Gold, and R. McCarty. Department of Psychology, University of Virginia, Charlottesville, VA 22903.

This study examined age-related alterations in habituation and sensitization of plasma CA responses in chronically stressed (CS) animals. Fischer 344 male rats (ages 3 and 22 months) were restrained in individual Plexiglas tubes for 30 minutes per day for 26 days. Age-matched controls (HC) were handled but not restrained. After the last restraint session, all animals were prepared with chronic tail artery catheters. Two days after surgery, the CS animals were again restrained. Blood samples were collected under basal conditions and at intervals during and after restraint. The next day, the CS rats were exposed to a novel stressor: immersion in 25° C water for 15 minutes. Blood samples were collected under basal conditions and at 0, 15, 30, and 45 minutes post-immersion. HC rats received both experimental treatments in a counterbalanced order. Plasma samples were later assayed for content of norepinephrine (NE, pg/ml) and epinephrine (EPI, pg/ml) as a measure of sympathetic-adrenal medullary activity. Data for plasma catecholamines were analyzed by computing an area under the curve (AUC, pg/ml x minutes) for each animal. The AUC provides a measure of each animal's catecholamine response profile which includes the period during and immediately following stressful stimulation. For both restraint stress and water immersion, there was a significant age-related difference in overall NE and EPI AUCs, with older rats having significantly higher response profiles. For restraint, stress history did not affect AUC measures within age groups. For water immersion, stress history was a strong predictor of potentiation of NE and EPI AUCs. Plasma levels of NE and EPI remained significantly elevated throughout the cold water immersion sampling period for both CS age groups when compared to handled controls. These data are consistent with the view that aged rats display exaggerated sympathetic-adrenal medullary responses to stressful stimulation. These exaggerated physiological responses may underlie in part the inability of aged animals to adapt to or, in some cases, to survive significant environmental challenges. Supported by NIA (AG 07648).

NEUROPEPTIDES AND BEHAVIOR: VASOPRESSIN, NPY, NEUROTENSIN, AND OTHERS

594.1

A PHYSIOLOGICAL ROLE FOR VASOPRESSIN IN FEAR CONDITIONING. J.D. Stoehr* and W.G. North. Department of Physiology, Dartmouth Medical School, Lebanon, NH 03756.

We have previously shown that homozygous Brattleboro rats display deficits in the conditioned freezing paradigm. These deficits may be due, in part, to inappropriate emotional and autonomic responses to novel stimuli. If this is so, normal Long Evans rats treated with vasopressin, or with vasopressin antagonists, should exhibit respective enhancements, or deficits, in this conditioning paradigm. Long Evans rats injected with the V_1 antagonist 2-(O-methyl)-tyrosine-AVP introcerebroventricularly (ICV, 3 ng) or peripherally before testing (sc, 2 µg) displayed significantly lower levels of freezing ($p < 0.05$), while rats treated peripherally (sc, 1 µg) or ICV (10 ng) with intact AVP displayed significant elevations in freezing when compared with vehicle-treated controls ($p < 0.05$). The data therefore imply that both central and peripheral vasopressin may be involved in the expression of appropriate behavioral responses to conditioned fear.

594.2

THE ROLE OF SEPTAL VASOPRESSIN INNERVATION IN PATERNAL BEHAVIOR IN PRAIRIE VOLES (*MICROTUS OCHROGASTER*). Z.X. Wang*[1], C. F. Ferris[2], M. Bamshad[1], and G. J. DeVries[1]. [1]Neurosci. Behav. Prog., Dept. of Psychol., Univ. of Mass., Amherst, MA 01003. [2]Physiol. Dept., Univ. of Mass., Med. Ctr., Worcester, MA 01665.

Monogamous male prairie voles (*Microtus ochrogaster*) show an increase in paternal behavior three days after being paired with females. This increase is correlated with a decrease in density of central vasopressin-immunoreactive (AVP-ir) projections of the bed nucleus of the stria terminalis (BST) and the medial amygdaloid nucleus (MA) (Bamshad et al., 1992). Here we present the results suggesting that the AVP pathways are activated in male prairie voles by cohabitation with females, and are implicated in paternal behavior. In a first study, males that were paired with females for three days had more AVP mRNA labeled cells in the BST, more grains per labeled cell, and a higher level of testosterone than sexually naive males. Females did not show differences in their AVP mRNA expression. This suggests that in males the AVP pathways are activated by mating, and that the earlier observed decrease in AVP-ir fiber density in the lateral septum may be related to an increase of AVP release. In a second study, castration not only reduced AVP expression in the projections of the BST and MA, but also reduced paternal behavior of male prairie voles. This effect could be reversed when castrated voles were treated with testosterone. Although this suggests that paternal behavior as well as AVP expression in the BST and MA are enhanced by testosterone, it does not necessarily mean that testosterone influences paternal behavior by acting on AVP projections of the BST and MA. In a third study, septal injections of AVP enhanced spontaneous paternal behavior of sexually naive males while the injection of $V1_a$ antagonist, $d(CH_2)_5Tyr(Me)AVP$, reduced paternal behavior. This suggests that septal AVP indeed enhances paternal behavior of prairie voles by a $V1_a$ receptor-mediated mechanism.

594.3

EFFECT OF WATER TEMPERATURE ON THE PORSOLT SWIMMING TEST IN RATS. D. Jefferys* and J.W. Funder. Baker Medical Research Institute, Melbourne, Australia 3004.

When rats and mice are forced to swim under conditions from which they are unable to escape they become progressively more immobile over a 15 min test period, and remain immobile for ~70–80% of the 5 min retest period next day. This pattern of acquisition and retention was seen when mice were swum at 20° or 25°C; at 30° or 35° retention was impaired, and adrenalectomy impaired retention at 25° but not 20°C (1). The present studies examine the effect of water temperature on swimming responses in the rat, to explore commonalities and possible differences with mice. Rats swum at 30°C show patterns of acquisition and retention identical to 25°C. At both these temperatures the antiglucocorticoid RU486, and the κ-selective opioid antagonist MR2266, were without effect administered alone, but together impaired retention to the low levels seen in adrenalectomized rats. At 20°C, acquisition was very low (15–25% immobility), with retention rates higher (35–40%), both substantially lower than in rats swum at 25°C, and uninfluenced by administration of thyroxine, dexamethasone, ketocyclazocine or glucose. We conclude that substantial differences exist between mice and rats in terms of the temperature dependence of the Porsolt swimming test, and that the failure of acquisition of the immobile response at 20°C in rats reflects the operation of pathways other than the glucocorticoid/opioid/thyroid hormone mechanisms subserving this behavioral response at higher temperatures.

1. Peeters B et al. Physiol. Behav. 51, 127, 1991.

594.4

FAMOTIDINE TREATMENT INDICATES THAT BLOCKADE OF THE *POEF* EFFECT BY GASTRIC VAGOTOMY IS LIKELY DUE TO A DISRUPTION ONLY OF AFFERENT PROCESSES. T.M. Robinson, P. Abbott and M.B. Kristal*. Behavioral Neuroscience Program, Dept. of Psychology, University at Buffalo, Buffalo, NY 14260.

Ingestion of placenta or amniotic fluid by rats has been shown to enhance opioid-mediated analgesia such as that produced by morphine, vaginal stimulation, and late pregnancy. The active substance in placenta and amniotic fluid (POEF for *P*lacental *O*pioid-*E*nhancing *F*actor) apparently has no analgesic effect by itself. We recently showed that the POEF effect in rats requires (a) peroral administration of POEF (in placenta or amniotic fluid), (b) an intact gastric vagus nerve, and (c) the central actions of opioids. To determine whether vagal involvement depended on afferent information arising from gastric receptors, or on an efferent effect on digestion (was POEF manufactured or activated during digestion?), we examined the amount of enhancement of vaginal-stimulation-induced analgesia that occurred after placenta ingestion in rats that had received orogastric infusion with either vehicle or with 5 or 10 mg/kg famotidine, a potent H_2-receptor antagonist that severely reduces gastric secretory activity. Rats that ingested placenta showed significantly more antinociception than did rats fed a control substance, but famotidine had no effect on POEF activity. These results suggest that gastric secretions are not required for POEF to be effective, and that vagal *afferent* pathways are involved.

Supported by NSF grant BNS88-19837 awarded to M.B.K.

594.5

INFLUENCE OF AMBIENT TEMPERATURE ON THE EFFECTS OF NPY ON BODY TEMPERATURE AND FOOD INTAKE. F. B. JOLICOEUR*, S. M. BOUALI, D. J. McGRATH, D. P. MÉNARD, A. FOURNIER[1] AND S. ST-PIERRE[1]. Depts of Psychiatry and Pharmacology, University of Sherbrooke, Sherbrooke, Qué, Canada, J1H 5N4 and [1]INRS-Santé, pointe claire, Qué, Canada.

The functional relationship between food intake and thermoregulation is well known. Since control of body temperature in the *milieu interne* depends in a large part on ambient temperature in homeothermic animals, food intake is thus related to temperature of the *milieu externe*. Because thermoregulation and food consumption are interrelated and because thermoregulation processes are influenced by ambient temperature, we examined the effects of neuropeptide Y (NPY) on both body temperature and food intake in various thermal environments following ICV administration of 20 µg. Results reveal that the prominent effects of NPY on body temperature and food intake in relatively thermoneutral environments are drastically altered at more extreme ambient temperatures. NPY produced hypothermia in animals placed at 4, 12 and 21°C, and actually increased body temperature in animals subjected to 30 and 38°C temperature. On the other hand, in comparaison to ambient temperatures of 12 and 21°C, ambient temperatures of 4 and 30°C significantly reduced the stimulatory effect of NPY on food consumption. Moreover, at 38°C, the effect of NPY on food intake was totally abolished. These data demonstrate that ambient temperature has a critical influence on central actions of NPY.

Supported by the Medical Research Council of Canada, Grant PG 11125

594.6

EFFECTS OF PERTUSSIS TOXIN ON FEEDING AND BODY TEMPERATURE CHANGES INDUCED BY NPY AND NPY2-36. S. M. BOUALI*, A. FOURNIER[1], S. ST-PIERRE[1] AND F. B. JOLICOEUR. Depts of Psychiatry and Pharmacology, University of Sherbrooke, Sherbrooke, Qué, Canada, J1H 5N4 and [1]INRS-Santé, pointe claire, Qué, Canada.

Many effects of NPY have been attributed to a decrease in the activity of adenylate cyclase. Pre-treatment with pertussis toxin (PTx) has been shown to inhibit many pharmacological effects of NPY including increased feeding following administration in the paraventricular nucleus (PVN). In the present study, we examined the influence of PTx pre-treatment on the effects of NPY on food consumption and body temperature following administration in the preoptic (POA), perifornical (PeF), lateral hypothalamus (LH) and PVN. The effects of the same pre-treatment on the actions of NPY2-36 were also studied since we have found that this fragment produced opposite effects on body temperature to that of NPY when injected in the POA, whereas the effects on food intake were similar (Bouali et al.1992, Soc Neurosc 18:895). In each region, PTx was administered three days prior to the injection of NPY and NPY2-36 in doses equal to those of the peptides for producing a significant effect on body temperature and food intake. The results shows that the effects of NPY and NPY2-36 on food intake, after administration in the PeF, are not affected by PTx pre-treatment. Interestingly, the effects of NPY on body temperature following administration in the POA were completly inhibited by PTx whereas the effects of NPY2-36 were partielly affected. These results are important as they provide evidence that, in the POA at least, the receptors mediating the hypothermic effects of NPY could be biochemically different from those mediating the hyperthermic effect of NPY2-36. Furthermore, these results raise the intriguing possibility that these variable effects of PTx pre-treatment depend on the site where NPY is administered since the PTx inhibition of NPY-induced feeding occur when the peptide was injected in the PVN and LH. Finally, it appears that the receptors mediating the thermal and feeding responses induced by NPY and NPY2-36 posses different mechanisms of coupling to their effectors. Supported by the Medical Research Council of Canada, Grant PG 11125.

594.7

EFFECTS OF NEUROTENSIN ON BEHAVIORS INDUCED BY INTRA-STRIATAL AND INTRA-ACCUMBENS ADMINISTRATION OF N-n-PROPYLNORAPOMORPHINE (NPA). D.J. McGRATH*, S.M. BOUALI, D.P. MENARD, AND F.B. JOLICOEUR. Depts. of Psychiatry and Pharmacology, University of Sherbrooke, Sherbrooke, Qué., Canada, J1H 5N4.

Neurotensin has been shown previously to affect hyperactivity but not stereotypy induced by various, peripherally administered, dopamine stimulating drugs, thus pointing to a specific action on mesolimbic dopaminergic processes. To further investigate this selective influence we have examined, in rats, the effects of intracerebroventricular administration of 0.9 to 15.0 µg neurotensin (NT) on behaviors produced by either bilateral intra-striatal or bilateral intra-accumbens injections of 20 µg NPA, a potent and selective dopamine D_2 receptor agonist. At both injection sites NPA produced hyperactivity and stereotyped behavior consisting mostly of continuos sniffing. These results were somewhat unexpected considering the traditional assumption that the nucleus accumbens and striatum control hyperactivity and stereotypy respectively. NT was found to more potently reduce the hyperactivity induced by intra-striatal than by intra-accumbens injection of NPA. Furthermore NT reduced stereotypy elicited by injections of NPA at both sites. From these results it can be concluded that : a) dopaminergic stimulation of both the striatum and the nucleus accumbens can elicit hyperactivity and stereotypy, pointing to more complex control mechanisms for these behaviors and b) that NT can not only antagonize hyperactivity but also stereotypy elicited by injections of NPA into both nucleus accumbens and striatum; and furthermore that NT's antagonism of hyperactivity is more potent when this hyperactivity is elicited from striatal dopaminergic stimulation.

Supported by the Medical Research Council of Canada.

594.8

EFFECT OF SR 48692 A NON PEPTIDE NEUROTENSIN RECEPTOR ANTAGONIST ON BEHAVIOURAL AND NEUROCHEMICAL CHANGES INDUCED BY NEUROTENSIN. R. Steinberg, M. Fournier, P. Brun, M. Poncelet, J. Souilhac, J.P. Terranova, D. Gully, G. LeFur* and P. Soubrié. Sanofi Recherche, F-34184 Montpellier, France.

Unilateral microinjection of neurotensin (NT) in the ventral tegmental area (VTA) of the rat (2.5 µg/0.5 µl) or in the mouse striatum (10 pg/1 µl) produced behavioural excitation illustrated by contralateral circling. Given orally, SR 48692 (IC50 = 1nM on ^{125}I NT specific binding to guinea pig brain) significantly reduced these rotations according to a triphasic dose-effect relationship. Inhibition occurred at 0.05 and 0.1 mg/kg, intermediate doses producing no significant antagonism and at doses ≥.5 mg/kg, a second phase of antagonism was observed. Blockade of amphetamine-induced locomotion in rats by NT applied in the nucleus accumbens was dose-dependently reduced by SR 48692 (from 0.1 to 1 mg/kg). Applied in the VTA, NT (2.5 µg/0.5 µl) increased DOPAC/DA ratios measured by HPLC and NT (1 pg/65.nl) stimulated DA efflux detected by in vivo electrochemistry in the nucleus accumbens. Neither of these biochemical changes were affected by SR 48692 (0.1, 1, 10 mg/kg).

These results indicate complex interactions between NT and mesolimbic and nigrostriatal DA systems. More particularly, the differential ability of SR 48692 to affect NT-evoked behavioural vs biochemical changes support the concept of neurotensin receptors heterogeneity.

594.9

TASTE REACTIVITY TO ISOTONIC SALINE FOLLOWING CENTRAL INJECTION OF NK3 TACHYKININ AGONISTS IN RATS. C. Polidori*, R. Seely[1], R. Ciccocioppo, M. Massi and G. De Caro. University of Camerino, MC, Italy and [1] University of Pennsylvania, Philadelphia, PA, U.S.A.

Previous studies have shown that central injection of NK3 tachykinin (TK) agonists inhibit salt intake in rat. To further describe the mechanism of this inibition the taste reactivity test was used in the present study. Replete male rats (325-350 gr) were fitted with a cannula in the third cerebral ventricle, for TKs injection, and a small intraoral tube to deliver 1 ml of 0.9% NaCl solution over a period of 1 minute. The animals were injected with [Asp5,6,MePhe8] substance P 5-11, and Suc-[Asp6,MePhe8] substance P 6-11 both selective NK3 agonists at the dose of 100 and 30 ng/rat. The results have showed that both doses of the two TKs diminshed the number of ingestive responses and reduced the latency to onset of passive dripping. On the other hand, the number of aversive responses remained low following injection of the two peptides and was similar to that of controls. These finding are in keeping with the reported effect on NaCl intake of NK3 agonists in. Further studies, employing also non preferred of NaCl concentrations, are needed to fully describe the effects of TK on salty taste evaluation.

594.10

COHABITATION WITH MALES DECREASES BRAIN LHRH IMMUNOREACTIVITY (LHRH-IR) IN FEMALE PRAIRIE VOLES INDEPENDENT OF SYSTEMIC ESTROGEN LEVELS. O.C. Hnatczuk* and J.I. Morrell. Inst. Animal Behavior, Rutgers Univ, Newark, NJ 07102.

In female prairie voles (Microtus ochrogaster), male contact increases blood estradiol (E_2) level and induces sexual receptivity. Male urine applied to the nose of a female increases posterior olfactory bulb LHRH content and serum LH levels. We investigated the importance of E_2 and cohabitation with males for the induction of female sexual receptivity. Naive, ovariectomized (ovex), and ovex low E_2 treated females were housed with a male for up to 70 hours and observed for sexual receptivity, or isolated for 70 hours and briefly tested for sexual receptivity with a stud male. While all isolated females were non-receptive, cohabitating naive and low E_2 females were receptive. After testing, females were perfused and brain LHRH-IR examined. The anatomical distribution of LHRH-IR was similar in all experimental conditions and resembled other rodent species, with the greatest concentration of LHRH-IR in the olfactory bulb and tubercle, the diagonal bands of broca, and the septal, preoptic and rostral hypothalamic nuclei. Although the arcuate contained many LHRH-IR fibers, few LHRH-IR neurons found in this area. Male cohabitation reduced LHRH-IR across the different estrogen conditions. We infer that cohabitation with a male induces the release of LHRH, initiating a subsequent physiological or behavioral response in the female. (HD 22983-JIM)

594.11

HYPOTHALAMIC INSULIN CHANGES IN RELATION TO PERIPHERAL INSULIN INFUSION AND FEEDING AS REVEALED BY MICRODIALYSIS. K. Gerozissis, M. Orosco, A. Pelé, C. Rouch and S. Nicolaidis.* Neurobiologie des Régulations, URA 637 CNRS, Collège de France, 11, place Marcelin-Berthelot, 75231, Paris CEDEX 05, France.

Insulin was measured by a sensitized radioimmunoassay in microdialysates from the hypothalamic nuclei VMH-PVN of freely moving, freely feeding Wistar and lean (FaFa) or obese (fafa) Zucker rats.

Basal hypothalamic immunoreactive insulin levels were similar in Wistar and lean Zucker rats (57 ± 0.4 and 64 ± 8 pg/ml respectively) but significantly lower (38 ± 5 pg/ml) in obese animals. When 0.5 U of insulin was infused i.v., hypothalamic insulin doubled in the obese rats but increased less in the lean Zucker and Wistar rats. Furthermore, hypothalamic insulin was increased in relation to meals in the Wistar and the obese Zucker rats, although more dramatically in the latter.

These data raise again the question of the origin (central or peripheral) of brain insulin in the present microdialysates. The already observed relatively short latency and the high amplitude of response in the insulin resistant obese Zucker rat, favor the idea that, at least part of the peptide comes from a central origin.

594.12

GONADOTROPIN RELEASING HORMONE (GnRH) MESSENGER RNA (mRNA) EXPRESSION IN A FISH WITH TWO CLASSES OF ADULT MALES. C. M. Foran, M.A. Marchaterre*, T. R. Myers, A.H. Bass and D. A. Myers. Sect. Neurobiology and Behavior & Dept. Physiology at NYS College Veterinary Medicine, Cornell Univ. Ithaca, N.Y. 14853

The plainfin midshipman, *Porichthys notatus*, has three classes of sexually mature adults: type I males, type II males and females. Each morph has distinct reproductive tactics and neuronal traits (Bass, TINS 15:139, 1992). Since GnRH is considered to regulate the expression of adult reproductive behavior among vertebrates, GnRH gene expression is being studied in midshipman to identify molecular and cellular events that determine the expression of morph-specific behaviors. The cDNA for GnRH in midshipman was previously cloned and sequenced (Grober, Bass and Myers; Soc. Neurosci., 1992). Based on this sequence, oligonucleotide probes (40mers; ^{33}P-labelled) were generated for use with *in situ* hybridization histochemistry to identify GnRH-mRNA transcripts in the brain. Tissue was sampled from adult (6 Type I males, 2 Type II males, 7 females) and juvenile male (2) morphs. Adult tissues included those from both reproductively active and inactive individuals. GnRH-mRNA positive perikarya were identified in the olfactory bulb (OB), the ganglion of the nervus terminalis (NT) which is localized ventrally at the junction of the olfactory bulb and telencephalon, the magnocellular (PM) and parvocellular (PP) regions of the preoptic area, and the ventrolateral thalamus (VT). GnRH expression in NT was consistently high in all animals, suggesting it is functionally distinct and not related to reproductive status. Elsewhere, levels of expression varied among the morphs or between breeding and non-breeding states. Support from NSF.

594.13

EFFECT OF AT-1 AND AT-2 ANGIOTENSIN II (AII) RECEPTOR ANTAGONISTS ON DIPSOGEN-INDUCED FOS-LIKE IMMUNOREACTIVITY (FLI) IN RAT BRAIN. **B.-H. Li*, N.E. Rowland and G. Smith.** Psychology, Univ. of Florida, Gainesville FL 32611-2065.

Administration of AII to rats either intravenously (iv) or intracerebroventricularly (ic) causes drinking and induction of prominent FLI in subfornical organ (SFO), median preoptic (MnPO/OVLT), paraventricular hypothalamic (PVN), and supraoptic (SON) nuclei. The FLI is similar by both routes except for preferential activity in posterior SFO after iv AII and in anterior SFO after ic AII. Administration of the AT-1 antagonist, losartan (ic), abolished AII-induced FLI in SFO, MnPO and PVN, with a partial block in SON. Administration of the AT-2 antagonist, PD123319 (ic), did not affect AII-induced FLI in MnPO and SFO, but produced partial blocks in SON and PVN. This suggests that the AT-2 antagonist PD 123319 has either direct or indirect actions in PVN and SON. We will consider these data in relation to the antidipsogenic actions of both antagonists. IC injection of the cholinergic agonist and dipsogen, carbachol, induces FLI in the same regions as AII; the effect of the AT-1 and AT-2 antagonists are under investigation. [Support: NIH (AG10014) and Am. Heart Assn. (FL)]

594.14

MIF-1 acts as an opiate antagonist in the tail-flick test with lizards. **G. W. Reed, W. L. Nores, L. Raibstein, A. J. Kastin, G. A. Olson*, and R. D. Olson. Department of Psychology, Univ. of New Orleans, New Orleans, LA 70148**

Previous research suggests that MIF-1 (Pro-Leu-Gly-NH_2) may act as an opiate antagonist. Five doses of MIF-1, (0.00001, 0.00005, 0.0001, 0.0005, and 0.001 mg/kg), each combined with 10.0 mg/kg of morphine sulfate, a control dose of morphine sulfate (10.0 mg/kg), and control solutions of saline and diluent were injected IP in 256 lizards (Anolis carolinensis). Tail-flick latencies were obtained 15 min. after injection in a diagram-balanced design. The data were analyzed with a Blocks by Dose independent groups ANOVA. Results indicated a significant main effect for Blocks, with each block having quicker latencies than the previous one, $F(3, 224) = 3.35$, $p<.05$. Tukey's HSD test indicated that the first block had significantly longer latencies than the fourth block, but that none of the other comparisons were significant. The main effect for Dose was also significant, $F(7, 224) = 2.32$, $p<.05$. Tukey's HSD test indicated that the morphine control group had significantly longer latencies than all other groups, but that none of the other comparisons were significant. These results suggest that all doses of MIF-1 were able to block the effects of morphine. These findings further suggest that peptides may be active at lower doses than previously expected, and that all of the doses tested in this study appeared equally efficacious.

594.15

HRT, BRAIN AND BEHAVIOR: EPIDEMIC WITHOUT EPIDEMIOLOGY. H. Stowell*, Milledgeville GA 31061.

In 1991 US Govt. Accounting Office estimated 2.3 million American women to be purchasing via physicians "noncontraceptive estrogens". They failed to distinguish reversible, postsurgical & acute (≤2 years) Hormone Replacement Therapy (HRT) from irreversible chronic HRT in Women Otherwise Naturally Menopausal (HRT-WONM). In 1993 a Salk Institute report on hormonal chemoprevention of cancer described HRT-WONM as subject to "ever-changing practices of prescription" & suggested that its gonadal steroid combinations may produce circulating levels of non-SHBG-bound steroids approaching "that associated with the normal ovulatory cycle" [1,2]. WHO has no epidemiology of HRT-WONM, referring to "national Medical Associations" for desired data. Pilot research since 1991 confirms wide variability of steroid combinations, dose, dose-scheduling (daily, monthly or 6-monthly), dose route (oral vs. non-oral), & duration (10 year max to lifelong), but such data are informal. Current conventions of patient confidentiality (including a pair-bonded spouse) prevent a valid epidemiology. But current data from neurobiology of steroids, brain peptides & the mesolimbic dopamine system suggest strong effects of HRT-WONM on psychosexual & affiliative behaviors; effects supported by anecdotal evidence during the past 2 decades.

1. GAO/PEMD-92-12 Appendix IV, Dec. 1991.
2. Henderson BE et al. Science 259 (1993) 633-8.

DRUGS OF ABUSE: ETHANOL AND BENZODIAZEPINES—TOLERANCE, DEPENDENCE, WITHDRAWAL

595.1

FAWN-HOODED (FH) RATS VOLUNTARILY DRINK ENOUGH ALCOHOL TO DEVELOP TOLERANCE.

Amir H. Rezvani, D. Overstreet, A. Ejantkar, D. Janowsky* and O. Pucilowski. Skipper Bowles Center for Alcohol Studies and Dept. of Psychiatry, Univ. of North Carolina Sch. of Med., Chapel Hill, N.C. 27599.

Recently, we reported that FH rats, which possess a genetic serotonin impairment, exhibit a high preference for alcohol. To further characterize this strain of alcohol preferring rats, FH rats were given either free access to food, a solution of 10% (v/v) alcohol and tap water or only food and water for three months. Then, rats were withdrawn from alcohol and about six hrs. later were injected (ip) with a dose of 1.5 g/kg alcohol (16% v/v). Change in body temperature was measured one hr after alcohol injection. In a separate experiment, FH rats were given only one hr access to alcohol every morning and their blood alcohol levels (BAL) were determined 30 mins. after exposure. To determine if FH rats regulate their alcohol intake, rats were injected either with saline or different doses of alcohol 15 mins. before one hr alcohol exposure and their alcohol intake was measured at the end of one hr session. Our findings show that a) FH rats drank high enough alcohol (5.3±1.3 g/kg/day) to develop tolerance to its hypothermic action, b) in a limited access paradigm, they drank 1.14±0.06 g/kg/hr alcohol and their BAL reached 81± 4.4 mg/dl, and c) pre-administration of different doses of 10% (v/v) alcohol reduced the alcohol intake dose-dependently in the limited access paradigm. In conclusion, FH rats prefer alcohol for its pharmacologic but not taste-related effects, and they drink enough to develop tolerance to its hypothermic action.

595.2

A GENETIC COMPARISON OF TOLERANCE TO ETHANOL AND ANXIETY DURING WITHDRAWAL. T. K. Booker, A. Gonzales, A.C. Collins*. IBG, Univ. Co. Boulder, CO 80309.

The ethanol (EtOH)-sensitive LS mice develop more tolerance to EtOH and cross-tolerance to nicotine (Nic) than do the EtOH-resistant SS mice following 1-2 weeks of high-dose, EtOH-containing liquid diet treatment. This treatment also evoked substantial weight loss which may be a confound. Therefore, LS and SS mice were treated for 6 months with a low dose of EtOH (15% v/v in water), and were tested for sensitivity to EtOH and Nic as well as withdrawal anxiety. This method produced peak blood EtOH levels of 60-80 mg/dl, but did not elicit changes in EtOH metabolism or weight loss. The LS developed tolerance to EtOH as measured by the sleep-time, Y-maze activities, and temperature tests. SS mice showed tolerance to EtOH only for the sleep-time test which agrees with short-term high-dose EtOH treatment results. The LS mice showed increased anxious behavior in the mirrored chamber test as withdrawal progressed and the SS exhibited less. These results support the notion that initial sensitivity to EtOH influences the development of EtOH tolerance and severity of withdrawal. Neither LS or SS developed tolerance to Nic following low dose treatment unlike the results obtained following 1-2 weeks of high-dose EtOH treatment. High dose treatment evoked weight loss and a change in the volume of distribution of Nic. Perhaps low-dose EtOH did not result in tolerance to Nic because weight changes did not occur. Supported by AA-06391 and DA-00116.

595.3

SENSITIVITY AND TOLERANCE TO THE SEDATIVE EFFECTS OF ETHANOL IN THE HIGH- AND LOW-ALCOHOL-DRINKING (HAD AND LAD) RATS. J.M. Murphy*, W. Dyr, W.J. McBride, L. Lumeng, and T.-K. Li. Inst. Psychiat. Res. and VAMC, Indiana U. Sch. Med., and Dept. Psychology, Purdue Sch. Sci., IUPUI, Indianapolis, IN, 46202-4887.

The HAD and LAD lines of rats have been selectively bred for preference and nonpreference of a 10% (v/v) ethanol (E) solution, respectively, when food and water are available ad lib. The present study investigated the effects of 1, 2 and 3 g E/kg (i.p.) on the loss of righting reflex (LR), the duration of sleep time (regain of righting, RR), and the blood alcohol content (BAC) at RR in the HAD and LAD rats. Adult males (n=8/line) were housed with ad lib food and water. Each rat received the three E doses given in ascending order, separated by three weeks. A second 3 g/kg dose was tested 24 h after the first 3 g/kg injection. All rats had a shorter interval to LR as the E dose increased ($p<0.05$), but HAD and LAD rats did not differ at any dose. The time to RR increased with dose. The HAD rats showed significantly ($p<0.05$) longer time to RR than LAD rats at the 3 g/kg dose (395±37 vs 256±18 min) and BACs for HAD rats were significantly lower than for LAD rats (244±19 vs 172±22 mg%). When retested with 3 g/kg 24 h later, the HAD rats again showed a significantly longer RR than LAD rats (279±35 vs 210±16 min) but, relative to the first injection, only the HAD rats significantly decreased the duration of RR and increased BAC at RR (217±12 mg%), indicating that tolerance to the first dose of E had developed. The LAD rats are less sensitive than the HAD rats to an initial exposure to high-dose E. However, with E exposure, it appears that the HAD rats develop tolerance and approach the same level of sensitivity as exhibited by the LAD rats. (Support: Kosciuszko Fdn., AA08553).

595.4

Na+ CHANNEL ANTAGONISTS RESTORE DOPAMINERGIC FIRING IN ETHANOL-WITHDRAWN RATS.

Marco Diana, Marco Pistis, Annalisa Muntoni & Gianluigi Gessa. "B.B. Brodie" Dept. of Neuroscience, Univ. of Cagliari, Italy.*

We have previously shown that DA firing of the mesolimbic system is drastically reduced in rats withdrawn from chronic ethanol administration. This effect is accompanied by a reduction in DA release in the nucleus accumbens and by a prolongation of the refractory periods of the same neurons. Since the absolute refractory period (ARP) should reflect opening-closing of the Na+ channels, we tested different Na+ channel antagonists as they promote closing of the Na+ channels in order to verify if they would, by decreasing the length of the ARP, restore DA firing. Male Sprague-Dawley rats were made dependent on ethanol and upon cessation of chronic ethanol administration (12-14 hrs after last ethanol administration) were surgically prepared for extracellular recordings of antidromically-identified meso-accumbens DA neurons. Although ARP could not be measured after administration of the Na+ channel antagonists as they decrease conduction velocity, spontaneous DA firing, burst firing and spikes/burst were potently stimulated by iv administration of bupivacaine (0.5-5.0 mg/kg) and lidocaine (5-20 mg/kg) in ethanol withdrawn rats. The effect of the drugs in saline-treated control rats was much milder and significantly different. The results are consistent with an alteration in Na+ channels kinetic of DA neurons in ethanol dependent rats. Further experiments using intracellular recordings will be required to confirm this possibility.

595.5

EFFECT OF NMDA ANTAGONIST (KETAMINE) ON CHRONIC TOLERANCE TO ETHANOL: IMPORTANCE OF INTOXICATED PRACTICE J.M. Khanna*, G.S. Morato, A. Chau, G. Shah and H. Kalant. Addiction Research Foundation of Ontario and the Department of Pharmacology, University of Toronto, Toronto, Canada M5S 1A8.

We have recently shown that NMDA antagonists ((+)MK-801 & ketamine) inhibit the development of rapid and chronic tolerance to ethanol (E). The present experiments examined the possible importance of intoxicated practice for the effect of ketamine (K) on tolerance to E. Two groups of rats received daily practice (11 days) on the tilt-plane test under the influence of E, preceded by either K or saline (S). Two more groups had daily practice followed by E (preceded by either K or S). Two other groups had practice preceded and followed by K or S without E. All groups were tested under E on days 5 and 12. Tolerance occurred in both groups that received S + E, but it developed later in those that did not practise under E. No tolerance was seen at either time in the practice group receiving K + E, but K did not prevent tolerance in the non-practice group. These results suggest that NMDA antagonists will block only a learned component of tolerance and not that component acquired purely by pharmacological exposure. (Supported in part by RO1 AA08212-04)

595.6

SUBCHRONIC $5HT_{1C/2}$ ANTAGONISM BY RITANSERIN PARTIALLY REVERSES ETHANOL WITHDRAWAL-INDUCED ANXIETY.
C.J. Wallis, S.M. Rezazadeh* and H. Lal Department of Pharmacology, Texas College of Osteopathic Medicine, Fort Worth, TX 76107.

It has been suggested that the "anxiety-like" symptoms measured during ethanol withdrawal (EW) may be partially due to a disturbance in 5HT receptor activity (Lal, et al, Alcohol 8:467, 1991). In the present studies, we investigated the efficacy of ritanserin, a potent $5HT_{1C/2}$ antagonist, to modify an EW symptom in two animal models of anxiety: the elevated plus-maze (EPM) and the pentylenetetrazol (PTZ) discrimination paradigm. Long-Evans hooded rats were given a nutritionally balanced liquid diet containing 4.5% ETOH for 10 d (Lal et al., JPET 247:508, 1988). Twelve h after removal of the ETOH diet, rats were tested in the EPM. We observed a significant reduction in the open-arm activity and the number of total arm entries indicative of EW. Acute ritanserin (0.04-5 mg/kg, ip, 30 min) had no effect on EW induced "anxiety-like" behavior on the EPM. Subchronic ritanserin (0.04-0.64 mg/kg, ip, bid 12 hrs) administered concurrently with the last five (5) days of ETOH diet produced an inverted U-shaped dose related increase in the time spent on the open arms of the EPM (0.32 mg/kg optimal) and partial reversal of the EW-induced reduction in total arm entries. In animals trained to discriminate between saline and PTZ (an anxiogenic drug), EW generalized to PTZ, indicating an EW-induced PTZ-like interoceptive discriminative stimulus (EW-IDS). Neither acute nor chronic ritanserin blocked the EW-IDS, but acute ritanserin (0.32 mg/kg, 1h) did reduce the ED_{50} for PTZ discrimination (from 5.55 mg/kg to 2.18 mg/kg). These data support the hypothesis that chronic ritanserin only partially blocks ethanol withdrawal-induced "anxiety". Supported by NIAAA grants AA06890 and AA09567.

595.7

CUE-INDUCED CRAVING IN ALCOHOLICS: CSF MONOAMINE PREDICTORS AND PHARMACOLOGIC MODULATION. I. Petrakis, E. Webb, N. Cooney, L. Trevisan, H. Kranzler, L.P. Karper*, D.S. Charney, J.H. Krystal. Alcoholism Research Center, VA Medical Center,West Haven, CT 06516

5-HT systems have been implicated in alcoholism, but there is no published data on the effects of 5-HT modulations upon craving for alcohol elicited in alcoholics by exposure to alcohol. This study evaluated tryptophan depletion effects on cue-induced craving for alcohol in alcoholics. METHODS: In an ongoing study, alcoholics (n=8) 1-3 months post-detoxification completed a lumbar puncture one day prior to exposure, but not consumption, of alcohol. Patients exhibiting at least a 20% increase in craving completed two additional cue-exposure tests. Placebo or active typtophan depletion sessions were separated by a week. Depletion was achieved by giving an amino acid load devoid of trypotophan that increased utilization of remaining plasma tryptophan. Placebo depletion included tryptophan in the amino acid load. RESULTS: Preliminary results indicate a significant correlation between CSF MHPG levels and subsequent cue-induced craving. 5-HIAA and HVA showed non-significant correlations. Tryptophan depletion appeared to reduce cue-induced craving for alcohol. IMPLICATIONS: These preliminary data indicate that CSF monoamine metabolite levels may predict reactivity to alcohol cue exposure. They also suggest that 5-HT systems contribute to cue-induced craving in a tryptophan-dependent fashion.

595.8

APPARENT LACK OF ACUTE TOLERANCE DEVELOPMENT TO ETOH'S DISCRIMINATIVE STIMULUS ATTRIBUTES.
K.L. Goulden, R.I. Briscoe, B.D. Youngblood, D.V. Gauvin and F.A. Holloway*, Psychobiology Labs, Dept. Psychiatry & Behav. Sci., Univ. of Okla. Hlth. Sci. Cntr., Oklahoma City, OK 73190

Twenty male S-D rats were trained to discriminate between 1.0 g/kg ETOH and saline (SAL) in 10 min sessions under an FR-10 schedule of food reinforcement. Group 1 was injected i.p. 15 min prior to the sessions with SAL or ETOH, when blood levels were on the *ascending* limb of the BAC (152.2 ± 11 mg/dl). Group 2 was injected i.p. 60 min prior to the sessions with SAL or ETOH, when blood levels were on the *descending* limb of the BAC (79.3 ± 9 mg/dl). The number of training sessions to criterion discriminative performance (STIC) did not differ between groups. Various doses of ETOH were tested at the group's training pretreatment interval and in 15 min increments from 15 min to 180 min after injections. The group mean ED50's for the response-choice measure, when ETOH was administered at each groups' pretreatment interval, was not significantly different (Group 1: 0.49 ± 0.05 g/kg; Group 2: 0.57 ± 0.07 g/kg; T(18)=0.85, n.s.). Additionally, the groups' median effective time interval (METI) did not differ (Group 1: 110.2 ± 8.9 min; Group 2: 120.7 ± 8.8 min; T(18)=0.83, n.s.). The data from the present study suggest that tachyphylaxis or acute tolerance did not develop to the interoceptive subjective states associated with ETOH administration in the rat, across the ascending and descending limbs of the BAC.

595.9

SLOW RELEASE SILASTIC CAPSULES (SC) FOR CHRONIC ADMINISTRATION OF BENZODIAZEPINES (BZ). **X. Jing, E. Wala, W.R. Martin, J.W. Sloan*, Dept. of Anesthesiology, Coll. of Med., Univ. of Ky., Lexington, KY 40536.**

Release of diazepam (DZ), nordiazepam (ND), oxazepam (OX), halazepam (HL), flunitrazepam (FN) and lorazepam (LZ) from S/P medical grade Silastic tubing (.058 id x .077 od) 7 cm (A) or 11 cm (B) long and from Silastic Medical Grade Rx-50 tubing (.078 id x .090 od) 11 cm long (C) was determined in vitro. SC filled with different BZ were placed in 20 ml of saline and shaken at 37°C for 1 or 4 weeks. Concentrations of BZ were determined in saline every day by HPLC. Rate of release (mg/day) from SC (A) varied between BZs: DZ (0.9), FN (0.2), HL (0.1), ND (0.04) and OX (0.005). From longer SC (B) ND and OX were released faster than from SC (A). From SC with a thinner wall (C) all BZ were released with higher rate than from SC (B). Rats were implanted with SC (A) (90 mg/x1cap/week) or (90 mg/x2cap/week), SC (B) (180 mg/2cap/week) or (180 mg/x3cap/week) and SC (C) (180 mg/x2cap/week) or (270 mg/x2cap/week) and then stabilized on DZ for 4 weeks. Plasma levels of DZ and its metabolites were determined at weekly intervals. After the second implantation plasma levels of DZ were stable. With SC (A) stabilization plasma levels of DZ were related to the amount of implanted DZ. Despite the differences in in vitro release of DZ from SC (B) and (C) plasma levels of DZ are not different. Preliminary data indicate that in vitro and in vivo releases of DZ from SC are not totally in agreement. Supported by NIDA grant DA02195.

595.10

TOLERANCE TO THE ANTICONVULSANT EFFECT OF DAILY DIAZEPAM INJECTIONS IS FACILITATED BY PREVIOUS DIAZEPAM INJECTIONS BUT NOT DIAZEPAM INJECTIONS PRECEDED BY CONVULSIVE STIMULATION.
T.E. Kippin*, L.E. Kalynchuk, and J.P.J. Pinel. Dept. of Psychology, Univ. of British Columbia, Vancouver, B.C. Canada. V6T 1Z4

Mana et al. (1991) found that the development of tolerance to the anticonvulsant effect of bidaily diazepam on convulsions in amygdala-kindled rats is contingent upon the occurrence of convulsive stimulations during the periods of diazepam exposure. Subsequently, Mana and Pinel (1987) found that the critical factor in the dissipation of this contingent tolerance was the occurrence of convulsive stimulations in the absence of drug. Accordingly, we predicted that a drug-alone condition would produce tolerance more rapidly than a drug-after condition because in the latter condition each trial would reduce any tolerance that might have developed. During the 15-trial treatment phase, amygdala-kindled rats received either a daily injection of diazepam (2.5 mg/kg) 1 hr before a stimulation, diazepam 1 hr after a stimulation, diazepam with no stimulation, or saline with and without stimulation. At the end of the treatment phase only the diazepam-before rats displayed significant tolerance to diazepam's anticonvulsant effect. All rats were then given a series of daily diazepam-before-stimulation treatments in order to compare the rate at which tolerance developed. The diazepam-only group acquired tolerance significantly faster than did the diazepam-after and saline groups. These results support the drug-effect theory of tolerance, and they suggest that savings measures and drug-only control groups should be included in studies of contingent tolerance.

595.11

TOLERANCE TO THE ANTICONVULSANT EFFECT OF DIAZEPAM IS FACILITATED BY AN ASCENDING-DOSE REGIMEN IN AMYGDALA-KINDLED RATS. L.E. Kalynchuk*, C.K. Kim, T.E. Kippin, and J.P.J. Pinel. Dept. of Psychology, Univ. of British Columbia, Vancouver, B.C., Canada. V6T 1Z4.

In clinical practice, anticonvulsant therapy is often initiated at a low dose and then incrementally increased until a therapeutically effective dose is reached. On the basis of the drug-effect theory of tolerance, we predicted that this ascending-dose regimen would promote tolerance development in a manner akin to shaping. Accordingly, we recently found that such a regimen facilitates the development of tolerance to pentobarbital's anticonvulsant effect in amygdala-kindled rats. The purpose of this experiment was to assess the effect of an ascending-dose regimen on the development of tolerance to diazepam's anticonvulsant effect. During the 20 trials of the tolerance-development phase, amygdala-kindled rats received either a constant high dose (10 mg/kg), a constant low dose (1 mg/kg), an ascending-dose regimen (starting at 1 mg/kg and increasing by .2 mg/kg increments to 3 mg/kg), or saline. Diazepam was administered by i.p. injection once every 48 hr, and each injection was followed 1 hr later by a convulsive stimulation. On the tolerance test, all rats received diazepam (3 mg/kg) 1 hr before a convulsive stimulation. The ascending-dose rats displayed significantly more tolerance to diazepam's anticonvulsant effect than did the high-dose or the low-dose rats. In addition, both the ascending-dose and the high-dose rats displayed a withdrawal effect after the cessation of diazepam. These results support the drug-effect theory of tolerance and provide further evidence that ascending-dose regimens facilitate the development of tolerance to anticonvulsant drug effects.

595.12

THE EFFECT OF CONVULSIVE STIMULATIONS ON THE DISSIPATION OF TOLERANCE TO THE ANTICONVULSANT EFFECT OF DIAZEPAM. C.P. McIntyre, L.E. Kalynchuk, T.E. Kippin, and J.P.J. Pinel*. Dept. of Psychology, Univ. of British Columbia, Vancouver, B.C., Canada. V6T 1Z4.

Mana and Pinel (1987) reported that the experience of convulsive activity in the absence of anticonvulsant drugs is a critical factor in the dissipation of tolerance to the anticonvulsant effect of ethanol. The purpose of this experiment was to extend this finding to diazepam and to assess the rate at which tolerance declines in rats that receive convulsive stimulations in a drug-free state. Tolerance was induced in amygdala-kindled rats by exposing them to 25 bidaily tolerance-development trials in which diazepam (2.5 mg/kg) was injected 1 hr prior to a convulsive stimulation. The rats were then divided into nine groups. Three groups received no treatment other than bidaily handling for one trial, three trials, or seven trials. Three other groups received an injection of saline 1 hr before bidaily convulsive stimulations for one trial, three trials, or seven trials. The final three groups received bidaily convulsive stimulations 1 hr before an injection of diazepam for one trial, three trials, or seven trials. Tolerance did not dissipate in rats that received neither diazepam nor convulsive stimulations over the retention interval. In contrast, tolerance dissipated gradually in the rats that received stimulations during the retention interval, even if they received diazepam after each stimulation. These results confirm previous findings that the occurrence of convulsive activity in a drug-free state is a key factor in the dissipation of tolerance to anticonvulsant drug effects, and they provide systematic evidence about the time-course of the dissipation--tolerance declined gradually over the 16-day retention period.

595.13

LACK OF TOLERANCE TO MIDAZOLAM'S DISRUPTION OF AN OPERANT DURATION DIFFERENTIATION BY RATS. S. Das, S.C. Fowler, J.A. Stanford and M.C. Wilson*. Depts. of Psychol. and Pharm., Univ. of Miss., University, MS 38677

Thirsty rats were trained to maintain forelimb force on a silent, isometric, force-sensing operandum for a minimum of 3 s to receive a water reward. Separate groups were treated daily with 10 or 30 mg/kg midazolam (orally) either 30 min before or after 15-min sessions for 28 d. Rats dosed pre-session earned significantly fewer rewards than rats dosed post session, and this impaired performance, in the form of increased frequency of premature response terminations, continued throughout the 28 d (i.e., tolerance was not complete). Withdrawal from midazolam produced an improvement in performance in the groups previously dosed pre-session, but these same groups persisted in receiving significantly fewer rewards than the post-session rats after 14 d of withdrawal. The persistence of impaired performance long after drug removal suggests a role for learning processes in the maintenance of this behavioral deficit. Supported by DA05253.

595.14

RO 15-4513 INCREASES NITROUS OXIDE WITHDRAWAL SEIZURE FREQUENCY IN MICE. L.K.Vaughn*, D.K.Marsee. Marquette Univ., Milwaukee, WI 53233.

We have previously shown that nitrous oxide (N_2O) produces its anxiolytic effect by acting at the benzodiazepine (BZ) receptor (Quock et al., Psychopharmacology 107:310, 1992). It is not known, however, whether BZ receptors are involved in N_2O dependence and withdrawal. We tested the hypothesis that BZ receptors are involved in withdrawal from N_2O by examining whether Ro 15-4513, a partial BZ receptor inverse agonist which has been shown to exacerbate ethanol withdrawal seizures (Lister & Karanian, Alcohol 4:409,1987), and flumazenil, a BZ antagonist, could increase N_2O-induced withdrawal seizure (WS) frequency. Male, Swiss Webster mice, 10-12 wk old, were exposed to a 75% N_2O: 25% O_2 atmosphere for 90 min during which they were injected i.p. with either vehicle or one of 4 doses of Ro 15-4513 (0.3 - 10 mg/kg) (80 min after placement in N_2O) or vehicle or one of 3 doses of flumazenil (5-20 mg/kg) (55 min after placement in N_2O). 10 min after removal from N_2O, the mice were tested for frequency of WS evoked by handling. WS frequency increased from 45% in vehicle-injected mice (n=20) to 80 - 90% in Ro 15-4513 injected mice (n=10 - 20). Ro 15-4513 or vehicle produced no seizures when injected into mice exposed for 90 min to a control gas mixture of 75% N_2: 25% O_2. Flumazenil did not produce a statistically significant change in seizure frequency from a vehicle-injected rate of 21% (n=14) to 20% at 5 mg/kg (n=15), and 0% at 10 and 20 mg/kg (n=15). Treatment with Ro 15-4513 significantly increased the frequency of WS and suggests that BZ receptors are involved in N_2O-induced withdrawal seizures. (Supported by NIH Grant DE-09998).

595.15

CHRONIC BRETAZENIL (B) PRODUCES TOLERANCE TO THE RATE-DECREASING EFFECTS OF CHLORDIAZEPOXIDE (C), MIDAZOLAM (M) AND ABECARNIL (A), BUT NOT RU32698 (R). M.E. Bronson*. Auburn Univ. School of Pharmacy, Dept. of Pharmacal Sciences, Auburn Univ., AL 36849-5503.

The effects of C, B, M, A and R on fixed interval 1 minute responding were examined in 18 rats before and during chronic treatment with water (W), C or B. C and B were administered in a final dose of 30 mg/kg/day. Dose effect curves for B were redetermined weekly in the chronic B group and in 1/2 of the chronic W rats, while C was tested weekly in the other rats. All dose effect curves were cumulative and were preceded by a water injection (baseline). B (1-132 mg/kg) had no effect on responding before or during chronic B, whereas tolerance to C (3.2-56 mg/kg) developed by week 7. When all rats were tested with C at 8 wks, there was tolerance to C in all groups except the chronic W group that received weekly B. When B was tested at 9 wks. there was no change in response rates in any group. When rats received M (3.2-56 mg/kg) on wk. 10, there was tolerance in both the chronic B & C groups but not in the chronic W groups. In contrast, when A (0.1-32 mg/kg) was tested at wk. 11 there was tolerance in all groups. Tolerance to R (3.2-56 mg/kg) was also evident on wk. 12 in all groups except the chronic B group. When the dose-effect curve for C was redetermined at wk. 13, there was a further rightward shift in the chronic B group but not in the other groups. Interestingly, when the B dose-effect curve was redetermined on wk. 14, B produced an increase in responding in the chronic C group; and in the weekly B group, the highest dose of B now eliminated responding. Two wks. after the chronic regimen was terminated, redetermination of the C dose-effect curve showed loss of tolerance to the rate-decreasing effects of C in all groups but the chronic C group. Baseline rates in the chronic C group were increased more than 100% and a high (cumulative 56 mg/kg) dose was required to return responding to normal baseline, a finding that may indicate prolonged abstinence withdrawal. Furthermore, B has been shown to precipitate withdrawal in animals treated chronically with full benzodiazepine agonists, and the increased responding produced by B in chronic C rats at 14 wks. may represent a mild form of precipitated withdrawal. Supported by USPHS grant DA06637.

595.16

INCREASED ANXIETY AND SENSITIVITY TO CONVULSANTS FOLLOWING PRECIPITATED WITHDRAWAL FROM CHRONIC BENZODIAZEPINE TREATMENT IN MICE.

G.H. Jones*, D.N. Stephens, H. Schneider and B.J. Cole. Department of Neuropsychopharmacology, Schering AG, Müllerstrasse 170-178, D-1000 Berlin 65, Germany.

Chronic treatment with standard benzodiazepine (BZ) anxiolytics can lead to serious withdrawal symptoms following cessation of treatment. As this withdrawal can limit the clinical use of BZ's it is important to develop procedures in animals to predict the relative severity of these symptoms.

We compared the behavioural syndrome following precipitated withdrawal from chronic treatment with diazepam (DZ) or alprazolam (ALPR). Groups of mice (n=10 per group) were injected i.p. once a day for 10 days with either vehicle, DZ or ALPR. On day 11, 24 hours after the last drug treatment, all subjects received an i.p. injection of 25 mg/kg flumazenil (Ro 15-1788) to precipitate withdrawal. 5 min later the mice were tested in the mouse elevated plus-maze. Each test lasted 5 min. Immediately afterwards the mice were tested for their susceptibility to pentylenetetrazole (PTZ)-induced seizures. Withdrawal from equivalent doses of DZ and ALPR (based on tests of anxiolytic activity), significantly reduced the time spent in the open arms by approx. 30% (i.e. increased anxiety), but only DZ withdrawal increased the susceptibility to PTZ-induced seizures (by 37%).

These results show that it is possible to measure apparent increases in anxiety during withdrawal from BZ's using the mouse plus-maze test and that the level of anxiety is a more sensitive measure of withdrawal from BZ's than the proconvulsant effects. Furthermore, the results from these experiments suggest that different BZ's may produce different withdrawal syndromes.

595.17

INCREASED SEVERITY OF WITHDRAWAL SEIZURES IN MICE FOLLOWING REPEATED ETHANOL WITHDRAWAL EPISODES. H.C. Becker* and R.L. Hale. VA Medical Center and Medical University of South Carolina, Charleston, SC 29401

We have previously demonstrated that the severity of withdrawal seizures is exacerbated in adult C3H mice that have experienced multiple cycles of ethanol (EtOH) withdrawal in comparison to mice that have been withdrawn from EtOH a single time, even when total EtOH exposure is equated across groups. This study was designed to examine whether the differential withdrawal response is related to different rates of EtOH elimination following withdrawal from chronic EtOH exposure in inhalation chambers. One multiple withdrawal group (MW_{1-4}) was tested after each of four cycles of 16 hr EtOH vapor separated by 8 hr periods of abstinence; another multiple withdrawal group (MW_4) was tested only during the fourth withdrawal cycle; a single withdrawal group (SW) was tested during each cycle, but only received EtOH on the fourth cycle; and a control group (C) was repeatedly tested, but never received EtOH. Blood EtOH levels following the last 16 hr bout of intoxication were 116-147 mg/dl for all EtOH-exposed groups. The rate of EtOH elimination after the final cycle did not differ in MW and SW groups. The withdrawal response intensified with each cycle in the MW_{1-4} group and mean areas under the 7 hr curve for handling-induced convulsions after the fourth cycle for MW_{1-4}, MW_4, SW, and C groups were 5±.9, 8±1.3, .2±.2, 0±0, respectively. These data support the "kindling" hypothesis of EtOH withdrawal and indicate that the potentiated withdrawal response after repeated episodes of withdrawal is not due to an alteration in the rate of EtOH elimination. Supported by VA Medical Research Service and NIAAA.

595.18

PRECIPITATED ABSTINENCE (PA) FOLLOWING INTRAVENOUS (IV) ADMINISTRATION OF FLUMAZENIL (FL) TO DIAZEPAM (DZ) DEPENDENT RATS. **E.P. Wala, W.R. Martin* and J.W. Sloan. Dept. of Anesthesiology, Coll. of Med., Univ. of Ky., Lexington, KY 40536.**

Rats implanted with silastic capsules containing DZ (1 x 90 mg/cap/week) had plasma levels of DZ and metabolites equal to about 6 ug/ml. After 5 weeks of stabilization on DZ rats were administered FL (dissolved in DMSO) or DMSO (bolus IV into tail vein) weekly in doses 10, 20 and 40 mg/kg (latin square) and observed for signs of PA for 10 min prior and 40 minutes after FL. Tonic-clonic (T-C) seizures, whose incidence was dose-related, emerged rapidly. Precipitated Abstinence Scores (PAS) were significantly greater at 5 min after 40 and 20 mg/kg of FL than after DMSO alone. Twitches and jerks (TJ), writhing, arched back, tachypnea, ear-twitching and head-bobbing were observed. Behavioral activation was also dose-related. Other rats were implanted with 2 guide cannulae, cortical (Co) and 2 hippocampal (H) electrodes and stabilized for 2 weeks on Dz (3 x 180 mg/cap/week). Total plasma levels of DZ and metabolites were about 17 ug/ml. Rats were administered IV FL (40 mg/kg) and DMSO control after completing 4 weeks of microinjections of FL or DMSO into the brain. ECoG and signs comprising the PAS were recorded simultaneously. Clonic (C) and T-C seizures and TJ were observed early and late after FL. ECoG indicated H convulsive activity. These data indicate that in rats IV FL evokes signs of abstinence which are similar to signs seen after focal H microinjections of FL as well as other signs. Supported by NIDA grant DA02195.

DRUGS OF ABUSE: OPIOIDS AND OTHERS—MISCELLANEOUS

596.1

EFFECTS OF SIGMA LIGANDS ON PHENCYCLIDINE-INDUCED EEG AND BEHAVIOR IN RATS. D.F. Sisson*, L.R. King, S. Teferi, and J.E. Moreton, Dept. Pharmacol. & Toxicol., Univ. MD Schl. of Pharmacy, Baltimore, MD 21201.

Phencyclidine (PCP) binds with high affinity to two distinct receptor classes. One class is within the ion channel of the NMDA receptor, where PCP acts as a non-competitive antagonist by blocking the ion channel. PCP also binds to sigma receptors. The physiological significance of sigma receptors is not well defined.

This study examined the interactions between PCP at a dose of 1.25 $mg \cdot kg^{-1}$ iv and several sigma receptor ligands delivered icv in 5 µl distilled water. The sigma ligands and doses were as follows: 20 µg DTG, 2 µg DuP-734, 8 µg ifenprodil, 20 µg (+)-pentazocine, and 4 µg (+)-3-PPP. In these experiments, an initial 5 µl, icv injection of distilled water was administered, followed in 45 min by an icv injection of a sigma ligand, followed in 5 min by an iv injection of PCP. EEG was recorded continuously during this period. Behavior was scored on a five point rating scale for locomotion, ataxia and stereotypy 15 min after the injection of distilled water, 4 min after the injection of a sigma ligand, 1 min after the injection of PCP and every 5 min thereafter for 40 min. Power spectra of 1 min epochs of EEG coincident with the behavioral scorings were determined by FFT.

At 1 min post-PCP injection, DTG enhanced PCP-induced locomotion and stereotypies, pentazocine enhanced stereotypy, ifenprodil decreased locomotion, ataxia and stereotypy, and DuP-734 and 3-PPP reduced locomotion and stereotypy. DTG, and pentazocine enhanced the PCP-induced increase in EEG power in the delta, theta and beta bands, ifenprodil increased delta-band power only, DuP-734 decreased high frequency beta, and 3-PPP decreased low frequency beta power.

DTG and pentacozine inceased, while DuP-734 and 3-PPP reduced, a subset of PCP-induced EEG and behavioral effects. The effects of ifenprodil were best explained as a general sedative effect. This work was supported by NIDA Grant DA03173. DuP-734 was a gift from The Du Pont Merck Pharmaceutical Company.

596.2

COMPARISON OF THE EFFECTS OF COMPOUNDS WITH SELECTIVE ACTIVITY AT PCP/NMDA AND SIGMA RECEPTORS AND DOPAMINE REUPTAKE SITES ON THE HYPOTHALAMO-PITUITARY-ADRENAL AXIS IN THE RAT. R. N. Pechnick*[1], R. E. Poland[2] and B. De Costa[3]. [1]Department of Pharmacology, LSU Medical Center, New Orleans, LA 70119, [2]Division of Biological Psychiatry, Harbor-UCLA Medical Center, Torrance, CA 90502, and [3]Laboratory of Medicinal Chemistry, NIDDK, Bethesda, MD 20892.

Phencyclidine (PCP) binds to PCP/NMDA and sigma receptors and also inhibits the reuptake of monoamines. Aside from its behavioral effects, PCP stimulates the pituitary-adrenal axis in the rat, causing the release of ACTH and corticosterone. However, the mechanism(s) by which PCP produces these effects is not known. The s.c. administration of the PCP analog N-[1-(2-thienyl)cyclohexyl]piperidine (TCP), which has a high degree of selectivity for the PCP/NMDA binding site, increased plasma levels ACTH and corticosterone. The acute administration of 1,3-di-O-tolylguanidine (DTG), a highly selective sigma receptor ligand, also increased plasma levels ACTH and corticosterone. In contrast, N-[1-(2-benzo(b)thiophenyl)cyclohexyl]piperidine (BTCP), a PCP analog that is a potent dopamine reuptake inhibitor but has low affinity for the PCP/NMDA binding site, did not affect plasma levels of ACTH and corticosterone. These results indicate that whereas interactions with either PCP/NMDA or sigma binding sites might stimulate the hypothalamo-pituitary-adrenal axis, inhibition of dopamine reuptake probably is not involved in the activation of this axis by PCP. (Supported by NIDA grant DA-04113)

596.3

NMDA-LIKE ACTIVITY MEDIATES NALOXONE-INDUCED CONTRACTIONS OF ISOLATED GUINEA PIG ILEUM (GPI) AFTER INCUBATION WITH MORPHINE. R.Yu. Yukhananov* and A.A. Larson. Department of Veterinary Pathobiology, University of Minnesota, St. Paul, MN 55108 U.S.A.

The ability of competitive and noncompetitive antagonists of NMDA to inhibit opioid withdrawal supports a role of NMDA receptors in opioid dependence. In support of this, acute administration of morphine increases the behavioral response to intrathecally injected NMDA plus naloxone in mice. The GPI contains a glutamate receptor of the NMDA-type. To further characterize the role of NMDA receptors in acute opioid dependence, we examined naloxone-induced withdrawal contractions of the isolated GPI following incubation with morphine. Glutamate induced contractions with an ED50 of 24.6 µM. Morphine (5-500 nM) dose-dependently inhibited contractions of the GPI induced by glutamate (10-500 µM) with a Ke of 10.2 nM. Naloxone prevented the inhibitory action of morphine on glutamate-induced contractions with a Ke of 19.3 nM. Naloxone also antagonized the inhibitory effect of morphine on electrically-induced contractions of the GPI through receptors with similar affinity (Ke=17.9 nM). Following continuous exposure to morphine (10-50 min), naloxone (0.1-4 µM) induced contractions of the GPI. Competitive (AP5, CPP) and noncompetitive (MK-801) antagonists of NMDA reduced naloxone-induced contractions at doses which also inhibit the contractile effect of glutamate. Incubation with morphine also decreased the ED50 for glutamate by 3.2 fold. The present data show that activation of NMDA receptors is required for naloxone-induced contractions of the GPI following morphine. (Supported by NIDA 04090, 04190 and 00124)

596.4

PCP-INDUCED DA RELEASE IS POSITIVELY CORRELATED WITH [^{3}H]TCP, BUT NOT [^{3}H]GBR 12935 BINDING, IN STRIATUM FROM TWO INBRED MICE STRAINS. A.L. Jewell*, J.M. Carney, and L.P. Dwoskin, Colleges of Pharmacy and Medicine, University of Kentucky, Lexington, KY 40536.

Previous studies from our laboratory have demonstrated a positive correlation of phencyclidine (PCP)-induced locomotor stimulation with endogenous dopamine (DA) release from striatal and frontal cortex, but not olfactory tubercle, slices from DBA/2J and C57BL/6ByJ inbred strains. PCP produced greater effects in the DBA/2J than C57BL/6ByJ strain in both assays. To determine if this strain-related, differential responsiveness to PCP is correlated with differences at the DA transporter or PCP receptor, the current studies determined binding parameters for [^{3}H]GBR 12935 and [^{3}H]TCP in striatum, frontal cortex and olfactory tubercle homogenates from these inbred strains. No strain difference for [^{3}H]GBR 12935 affinity (Kd) or number of binding sites (B_{max}) in striatum or olfactory tubercle was observed. However, the striatum exhibited a 2.6-fold higher B_{max} compared to olfactory tubercle, and binding in frontal cortex was not detectable. [^{3}H]TCP binding in striatum revealed a significantly higher B_{max} (2691±92 and 1916±145 fmol/mg prot) for DBA/2J than C57BL/6ByJ strain, respectively. Olfactory tubercle exhibited a lower B_{max} (2351 ± 135 and 3094±527 fmol/mg prot) for the DBA/2J than C57BL/6ByJ, respectively. No differences were observed in frontal cortex. Therefore, the differential, strain-related responsiveness to PCP in the DA release assay is positively correlated with [^{3}H]TCP binding in the striatum, but not in frontal cortex and olfactory tubercle. (Supported by grants from NIDA DA 07219 and UK Research Center on Drug and Alcohol Abuse).

596.5

THE ROLE OF SEROTONIN IN THE DISCRIMINATIVE STIMULUS EFFECTS OF MU- AND KAPPA-OPIOIDS. Kelly R. Powell*, Mitchell J. Picker and Linda A. Dykstra, Department of Psychology, University of North Carolina, Chapel Hill, NC 27599.

The effects the 5HT-1A agonists, 8-OH-DPAT, buspirone and NAN-190, and the 5HT-2 antagonist, ketanserin, were evaluated in rats trained to discriminate the mu-opioid agonist, morphine, or the kappa-opioid agonist, U50,488, from saline. In U50,488-trained rats, U50,488 dose-dependently increased drug-lever responding. When administered alone, 8-OH-DPAT, buspirone and NAN-190 partially substituted for the U50,488 discriminative stimulus whereas ketanserin produced predominantly saline-lever responding. The opioid antagonist, naltrexone, failed to antagonize the effects of 8-OH-DPAT, buspirone and NAN-190 suggesting that these effects are not opioid-mediated. When administered in combination with U50,488, 8-OH-DPAT and buspirone, but not ketanserin, attenuated the discriminative stimulus effects of the training dose of U50,488. In contrast, 8-OH-DPAT, buspirone and ketanserin all potentiated the discriminative stimulus effects of lower doses of U50,488 . In morphine-trained rats, morphine dose-dependently increased drug-lever responding. 8-OH-DPAT, buspirone and NAN-190 all produced a moderate amount of morphine-appropriate responding however, these effects were not dose-dependent nor were they consistent across animals. Ketanserin produced predominantly saline lever responding. When administered in combination with morphine, 8-OH-DPAT attenuated the discriminative stimulus effects of the morphine training dose and potentiated the effects of lower doses of morphine. Buspirone and ketanserin failed to alter the morphine dose-effect curve. These results suggest that 5HT is involved in the discriminative stimulus effects of U50,488, but may not be involved in morphine's discriminative stimulus effects. (Supported by DA 02749; DA 00033; F31 DA 05537)

596.6

ACUTE ADMINISTRATION OF THE 5-HT$_2$/5-HT$_{1C}$ AGONIST 2, 5-DIMETHOXY-4-IODOAMPHETAMINE (DOI) TO SOCIALLY HOUSED STUMPTAIL MACAQUES PRODUCES BEHAVIORAL EFFECTS SIMILAR TO THOSE OBSERVED WITH HALLUCINOGENS. D.C. Jolly*, L.A. Tonkovich, J.E. Young, J.M. Davis, and R.F. Schlemmer, Jr. Department of Pharmacodynamics, University of Illinois at Chicago, and Research Department, Illinois State Psychiatric Institute, Chicago, IL 60612.

Effects on social and solitary behavior of stumptail macaques (Macaca arctoides) may indicate therapeutic or toxic effects of psychotropic drug candidates. In monkeys, hallucinogens such as d-lysergic acid diethylamide (LSD) and 5-methoxydimethyltryptamine (5-MeODMT), reduce social interaction and induce both body shakes and myoclonic spasms of the limbs (limb jerks). The involvement of serotonin (5-HT) receptor subtypes in hallucinogen induced behavioral changes was investigated in monkeys. The behavioral effects of DOI, a 5-HT$_2$/5-HT$_{1C}$ agonist and hallucinogen were assessed in a monkey social colony consisting of 4 adult females and one adult male. During daily 60 minute sessions, a blind observer recorded social and solitary behaviors of socially housed monkeys. Observations made in the 9 sessions prior to drug administration served as a baseline. The 4 females each received 5 acute doses of DOI (0.1 to 1.0 mg/kg), in a crossover design. DOI was given once per week, 15 minutes before each observation session. DOI reduced social interaction, in that treated monkeys exhibited less social grooming. The data suggested that DOI treated monkeys showed increased spatial separation from other monkeys, and increased visual scanning (checking). Treated monkeys showed a dose-dependent reduction in self grooming. Also DOI induced limb jerks, and increased body shakes. These behavioral findings support the contention that stimulation of 5-HT$_2$/5-HT$_{1C}$ receptors is involved in the mediation of hallucinogen induced changes in social and solitary behavior of monkeys.

596.7

NEUROANATOMICAL ANALYSIS OF HIPPOCAMPUS FROM MARIJUANA-EXPOSED RHESUS MONKEYS. M. Halks-Miller*, M.-Y. Yao, J. Arjomand, C. Rebert & G. Pryor, Dept. of Neuroscience, SRI International, Menlo Park, CA 94025.

Investigators have reported neuroanatomical changes in the brains of both rats and monkeys after exposure either to marijuana (MJ) smoke or to THC. The present studies were designed to use unbiased stereologic methods to assess putative structural changes in rhesus monkey brain after long-term exposure to MJ smoke. Our previous studies of the cerebellum from these subjects (Soc. Neurosci. Abst. 1992, **18:** 1601) did not reveal any pathological or morphometric alterations after exposure to MJ. This study focuses on the hippocampus, a brain area that is rich in cannabinoid receptors and has been reported to show alterations in synaptic and neuronal morphology in monkeys and rats, respectively, after exposure to MJ.

Fifteen male rhesus monkeys were implanted with an array of intracerebral glass electrodes and divided into three groups for exposure to either placebo smoke (P), moderate MJ smoke (M), or high MJ smoke (H) for one year. Three untreated control monkeys were also included for study. All animals were sacrificed by per cardiac perfusion with an aldehyde fixative approximately six months after the last exposure to MJ. A selected level of the hippocampus was removed and embedded for light and electron microscopy (EM). The hippocampus was measured to estimate the volume of the dentate gyrus, CA3 & CA1. The number of neurons in different hippocampal layers and their volumes were also measured. The number of synapses in each layer was estimated from electron micrographs using the unbiased dissector method (Gundersen et al. 1988, APMIS **96**: 881) on pairs of serial sections. Synaptic number was then normalized to number of neurons in the relevant cell layers. The three test and the untreated control groups were compared by analysis-of-variance (ANOVA) methods for possible marijuana-induced changes in the neuroanatomical parameters described above. These tests failed to reveal any differences among the marijuana-treated and control groups. These studies do not support the premise that chronic exposure to marijuana smoke leads to permanent structural changes in primate hippocampus.

596.8

FUNCTIONAL EXPRESSION OF A CANNABINOID RECEPTOR USING THE BACULOVIRUS SYSTEM. D.A. Dove Pettit, V. Showalter, M.E. Abood*, and G.A. Cabral. Dept. of Microbiology/Immunology and Pharmacology/Toxicology, Virginia Commonwealth University, Richmond, VA 23298.

The baculovirus expression system was employed to express a rat cannabinoid receptor that was identified in a cerebral cortex cDNA library (Matsuda, Nature 346:561). The coding sequence for the receptor was inserted into a baculovirus transfer vector. Co-transfection of *Spodoptera frugiperda* (Sf9) cells with the recombinant transfer vector and linearized wild-type baculovirus DNA (AcNPV) allowed for the production of a cannabinoid receptor recombinant virus (SKR6-AcNPV). SKR6-AcNPV was plaque purified (3X) and was used to infect Sf9 cells. Northern analysis of SKR6-AcNPV-infected Sf9 cells revealed novel hyper-production of a 3.7 kb transcript. To assess novel viral protein production, SKR6-AcNPV-infected Sf9 cells (MOI 20) were metabolically labeled with ^{35}S-Met. Limited expression of all viral proteins was observed. Transmission electron microscopy of infected Sf9 cells (MOI 20) revealed the presence of large vacuoles in the cytoplasm, loss of membrane integrity, and granular peri-nuclear membrane deposition, suggestive of glycoprotein accumulation. Collectively, these data suggest that high multiplicity infection of Sf9 cells with SKR6-AcNPV alters cellular integrity and limits recombinant viral protein expression. However, lower multiplicity infection of Sf9 cells (MOI of 5) revealed novel recombinant viral protein expression. Furthermore, radioligand binding studies using ^{3}H CP55,940, a potent cannabinoid analog, revealed greater than 50% specific binding in crude P2 membrane preparations from SKR6-AcNPV-infected Sf9 cells (MOI 5). Uninfected Sf9 cells did not specifically bind CP55,940. Scatchard analysis of the saturation plot obtained determined the Bmax to be equivalent to that observed in P2 preparations from rat brain. These data support the functional expression of the cannabinoid receptor in the baculovirus system. (Supported by DA-05832, DA-05274, F31-DA-05518)

596.9

DELTA-9-TETRAHYDROCANNABINOL SUPPRESSION OF LUTEINIZING HORMONE SECRETION IN THE MALE RAT: EFFECTS OF N-METHYL-D,L-ASPARTATE. L.L. Murphy* and J. Ogan, Department of Physiology, Southern Illinois University School of Medicine, Carbondale, IL 62901.

Marijuana and its primary psychoactive cannabinoid constituent, delta-9-tetrahydrocannabinol (THC), have pronounced inhibitory effects on reproductive function in humans and experimental animals, presumably through the inhibition of pituitary gonadotropin secretion. There is abundant evidence that THC exposure can inhibit the secretion of luteinizing hormone (LH) in intact and gonadectomized animals. Activation of the excitatory amino acid receptors with N-methyl-D,L-aspartate (NMA) has been shown to stimulate LH secretion in a number of animal models. In the present study, we examined the effects of NMA and MK-801, a noncompetitive NMA receptor antagonist, on pulsatile LH secretion and determined the effects of NMA on LH release in animals pretreated with an inhibitory dose of THC. Adult male rats (250-300g) were orchidectomized and 2 weeks later were implanted with atrial cannula for 10 min interval blood samples and drug administration. A stimulatory dose of NMA (20 mg/kg b.w.) was administered 10 min after pretreatment with THC (0.5 mg/kg b.w.), MK-801 (0.1 mg/kg b.w.) or vehicle. Results indicated that within 20 min both MK-801 and THC significantly suppressed pulsatile LH secretion in the castrate male rat for 40 and 60 min, respectively. Moreover, NMA effectively stimulated LH release 10 and 20 min after its administration. Whereas NMA reversed the suppression in LH induced by MK-801, THC pretreatment prevented the ability of NMA to stimulate LH release. Thus, THC appears to block NMA-induced LH secretion in the castrated male rat. Whether THC elicits this action through a direct effect at NMA receptors or alters neurotransmitter or neuropeptides which may mediate the action of NMA on LH-releasing hormone neurosecretory neurons remains to be determined. (Supported by NIDA grant DA 05452).

596.10

ENDOGENOUS CANNABINOID ACTIVITY IS RELEASED FROM RAT BRAIN SLICES BY A DEPOLARIZING STIMULUS. D.M. Evans*, J.T. Lake, and A.C. Howlett. Dept. Pharm./Phys. Sci., St. Louis Univ. Sch. of Med., 1402, S. Grand Blvd., St. Louis, MO 63104

As previously reported by this laboratory, an endogenous factor capable of inhibiting the specific binding of the radiolabelled cannabinoid agonist [^{3}H]CP-55940 to its receptor can be released from nerve terminals in response to an influx of Ca^{2+} induced by an ionophore (Evans et al. (1992), J. Neurochem. 58; 780-782). In the present report, we provide evidence that the endogenous ligand for the cannabinoid receptor can be released in response to a depolarizing stimulus (75mM K^{+}) in the presence of extracellular Ca^{2+}. K^{+}-evoked release was not observed in the absence of extracellular Ca^{2+}, and was reduced by the specific calcium channel blockers verapamil and ω-conotoxin. Efflux of cannabinoid receptor binding activity was maximal at short incubation times and was potentiated by inclusion of the protease inhibitors, captopril and thiorphan. Fractions from a semi-purified sample of the effluent inhibited adenylyl cyclase activity. These results suggest that the endogenous cannabinoid may be a peptide.

Supported by DA03690 and DA06913.

596.11

EFFECTS OF PROPRANOLOL PRETREATMENT ON DELTA-9-TETRAHYDROCANNABINOL-INDUCED SUPPRESSION OF LUTEINIZING HORMONE IN OVARIECTOMIZED RATS. B.A. Adrian*. and L.L. Murphy Department of Physiology, School of Medicine., Southern Illinois University. Carbondale, IL 62901.

Delta-9-tetrahydrocannabinol(THC), the primary psychoactive constituent of marijuana, potently, but transiently, inhibits luteinizing hormone(LH) secretion when administered to ovariectomized(OVX) rats. The current study was designed to determine if this effect of THC is mediated through inhibitory noradrenergic mechanisms by attempting to block the THC-induced inhibition of LH with the beta-adrenergic receptor antagonist propranolol. Four week OVX rats were pretreated with propranolol (0.5, 3 or 6 mg/kg in saline, iv) 10 min. before the start of the experiment. Ten minute serial blood samples were collected for 1 hour before animals received THC (0.5 mg/kg,iv, or vehicle (10% propylene glycol in 1% Tween 80-saline) and continued for 2 hours after treatment. Propranolol treatment alone did not have an effect on basal LH levels. The administration of THC significantly depressed plasma LH when compared to pre-THC treatment levels. Pretreatment with the 0.5 and 6 mg/kg doses of propranolol did not alter the ability of THC to suppress LH secretion. However, pretreatment with 3 mg/kg propranolol effectively prevented the suppression of LH by THC. Thus, the actions of THC on LH secretion can be prevented by blocking beta-adrenoreceptors with propranolol. However, high doses may produce nonspecific interactions that mask this effect. (Supported by NIDA DA 05452)

596.12

ANDROGENIC-ANABOLIC STEROID REGULATION OF TACHYKININ- AND ENKEPHALIN-mRNA EXPRESSION IN THE RAT STRIATUM. C. S. Menard*, L. R. Lucas, C. Y. Lee, G. P. Dohanich, and R. E. Harlan. Dept. of Anatomy, Neuroscience Training Program, and Dept. of Psychology, Tulane Univ. Sch of Med., New Orleans, LA 70112.

Both motor abnormalities and addiction have been reported by athletes who abuse high levels of androgenic-anabolic steroids (AAS). In this study, expression of substance P/neurokinin A (SP/NKA) mRNA and preproenkephalin (PPE) mRNA was detected by *in situ* hybridization on coronal brain sections (20 µm) obtained from male rats treated daily for 2 weeks with either sesame oil vehicle or high levels of AAS (a cocktail of 2 mg/kg testosterone cypionate, 2 mg/kg nandrolone decanoate, and 1 mg/kg boldenone undecylenate). AAS treatment resulted in increased expression of PPE mRNA in the dorsolateral and ventromedial aspects of the caudal caudate/putamen (CPu). In contrast, AAS resulted in decreased expression of SP/NKA mRNA in the caudal CPu. These results are consistent with previous reports of dopamine-induced PPE up-regulation and SP down-regulation in this region. Interestingly, moderate increases in androgen receptor immunoreactivity in the caudal CPu also were found in animals similarly treated with AAS. Collectively, these data may indicate one brain region where AAS modify central nervous system functioning. Since the effects of AAS treatment on PPE and SP/NKA mRNA expression, as well as androgen receptor immunoreactivity, were variable across other regions of the striatum, AAS-induced modifications of these peptide systems may be complex and region-specific. Supported by DA06194 (REH) and DA 05521-01 (CSM).

596.13

ANABOLIC STEROIDS AND BRAIN REWARD. A.S. Clark*, R.C. Lindenfeld and C.H. Gibbons. Dept. of Psychology, Dartmouth College, Hanover, NH 03755.

Anabolic steroids are synthetic androgen-like compounds which are taken in high doses by athletes with the intention of enhancing muscular appearance, strength and/or athletic performance. Recent research indicates that steroid dependence develops with chronic anabolic steroid use in some individuals. This evidence led us to explore the extent to which anabolic steroids influence brain stimulation reward using the rate-frequency paradigm of intracranial self-stimulation. Adult male Long-Evans rats were implanted with electrodes in the medial forebrain bundle and tested for self-stimulation until stable rate-frequency curves were established. The anabolic steroid methandrostenolone (Dianabol) or oil vehicle were administered over a two week period, and rate-frequency responding monitored on a daily basis. No marked changes in the rewarding efficacy of the brain stimulation or alterations in motor/performance factors were observed in animals after short-term treatment with anabolic steroids. In a second experiment, animals which received a single injection of amphetamine (0.5 mg/kg, i.p.), displayed a curve-shift to the left of approximately 0.2 log units. Rate-frequency responding to amphetamine will be determined in the same rats following chronic treatment with anabolic steroids. These studies may reveal the neural substrates underlying anabolic steroid abuse.

596.14

ANABOLIC STEROID ADMINISTRATION INCREASES VULNERABILITY TO AN ANIMAL MODEL OF ANOREXIA NERVOSA. P.F. Aravich*, J.J. Choi & T.S. Rieg. Eastern Virginia Medical School, Norfolk, VA 23501; Veterans Affairs Med. Ctr., Hampton, VA 23667; Governor's School for Science & Technology, Hampton, VA 23666.

Anabolic steroid abuse and anorexia nervosa (AN) are significant health concerns for competitive female athletes. Recent data from our laboratory (e.g., Rieg et al., this meeting) indicate that relative adiposity is inversely related to vulnerability to a rat model of AN. Since anabolic steroids increase lean body mass and decrease relative adiposity, it was predicted that they would increase susceptibility to the weight-loss syndrome. The syndrome incorporates two features of AN: restricted feeding (1.5 h/day food access) and exercise (22.5 h/day running wheel access). Three groups of female rats were injected daily with 0, 1 or 10 mg/kg (sc) of nandrolone phenylpropionate (NPP). Drug administration occurred before and during the weight-loss protocol. It was found that there was a dose-related increase in susceptibility to the syndrome, defined as fewer days to lose 25% of original body weight. This effect was not related to changes in terminal food intake or wheel running. As expected with NPP, relative kidney weight increased. Finally, NPP mitigated the syndrome's increase in relative adrenal weight but exacerbated its decreases in relative spleen and thymus weights. It was concluded that NPP increases susceptibility to the weight-loss syndrome and promotes immune organ atrophy. The possibility that anabolic steroid abuse may be a risk factor for AN in competitive female athletes and that it may worsen immunological function in the disorder should now be determined.

596.15

ANABOLIC STEROIDS: ENHANCEMENT OF THE BEHAVIORAL ACTIONS OF TETRAHYDROCANNABINOL IN THE MOUSE RING-IMMOBILOITY MEASURE. D.R. Compton.* Department of Pharmacology and Toxicology, Medical College of Virginia-Virginia Commonwealth University, Richmond, VA 23298.

Anecdotal evidence suggests adverse psychological and physiological effects of high dose, chronic anabolic steroid abuse. Polydrug abuse is also common, so possible pharmacological interactions between anabolic steroids and Δ^9-tetrahydrocannabinol (THC; the active component of marihuana) are potentially of great importance.

Male ICR mice were pretreated with testosterone propionate (i.p.) or vehicle (sesame oil) one hour before administration of various doses of THC (i.v.). Catalepsy was assessed 1.5 hr following THC treatment using the ring-immobility procedure. Preliminary data indicated the effect of THC was increased from 27% to 44% by testosterone pretreatment, and immobility maintained (38%) up to 3 hr postinjection, a time at which the normal effects of THC have diminished to vehicle control levels. Dose-response analysis suggests the shift in potency of THC is a parallel shift. Anabolic steroids were evaluated in ligand binding assays, and did not bind to either the THC receptor or to the dopamine transporter, both implicated in the production of catalepsy.

In conclusion, pretreatment with anabolic steroids increases the potency and time course of action of THC. The mechanism of action of this phenomenon is unknown. However, it may be possible to use this indirect measure of steroid activity as a marker for further evaluation of the adverse effects of chronic high dose anabolic steroid abuse. (Supported by NIDA grant DA 07502.)

596.16

NITRIC OXIDE MODULATES OPIOID TOLERANCE IN LC NEURONAL ACTIVITY IN VITRO. D.A. Highfield*, S.J. Grant, R.S. Revay. Depts. Psychology, Biology and Neuroscience Prog., Univ. Delaware, Newark, DE. 19716.

Recent studies have shown that inhibition of nitric oxide synthesis attenuates analgesic tolerance and diminishes precipitated withdrawal in chronic morphine treated animals. Noradrenergic neurons in the locus coeruleus (LC) exhibit cellular correlates for both opioid tolerance and withdrawal. We therefore examined the effects of blocking nitric oxide synthesis with N$^{\omega}$-nitro-L-arginine (N-Arg) during chronic morphine treatment on the activity of LC neurons.

Rats were implanted with morphine pellets (75mg Morphine base, NIDA) for 7 days. Subjects were also given injections of 10 mg/kg N-Arg twice daily i.p. Sham pellets and saline injections were used as controls. Single unit extracellular recordings were obtained from LC neurons in brain slices. A dose response curve and ED50 for the selective µ agonist DAMGO was obtained for each cell. Changes in the ED50 across treatment groups were used to determine the interaction of chronic morphine and inhibition of nitric oxide synthesis.

As expected, chronic morphine induced substantial tolerance to DAMGO challenge, indicated by an increase in the ED50. In animals given N-Arg along with morphine, the ED50 of DAMGO challenge was intermediate between the chronic morphine alone group and the sham treated animals. These results support a **modulatory** role for nitric oxide in the development of tolerance to chronic opiate treatment in LC neurons.

Supported by the State of Delaware, and ICI Pharma.

596.17

COMPARATIVE EFFECTS OF MK-801 AND N^G-MONOMETHYL-L-ARGININE (NMMA) ON MORPHINE ABSTINENCE SYNDROME. George A. Matwyshyn, Sanjay N. Thorat, Marc Barjavel, K.P. Gudehithlu* and Hemendra N. Bhargava, Dept. Pharmacodyn., Univ. Ill., Chicago, IL 60612.

NMDA receptor appear to be involved in opiate tolerance process possibly by modifying the formation of nitric oxide (NO). The effects of NMDA receptor antagonist, MK-801 and the inhibitor of NO synthase, NMMA on morphine abstinence syndrome have been compared in male Swiss - Webster mice rendered dependent on morphine by s.c. implantation of a morphine pellet containing 75 mg of morphine base for 3 days. Six hours after pellet removal, mice were injected i.p. with vehicle or MK-801 (0.03, 0.1 and 0.3 mg/kg). Thirty min later the animals were injected with NTX (50 µg/kg, s.c.). MK-801 had no effect on the stereotyped jumping behavior, body weight loss, body temperature or the formation of fecal boli. NMMA (2 and 4 mg/kg ip) or its vehicle injected 15 min prior to NTX (50 µg/kg, s.c.) blocked the stereotyped jumping response but did not affect the other withdrawal signs. It is concluded that inhibition of NO synthase, inhibits hyperactivity responses observed during withdrawal but the NMDA receptor antagonist has no effect on the morphine abstinence syndrome (Supported by NIDA grants DA-02598 and RSDA K02-0130).

596.18

EFFECT OF N^G-MONOMETHYL-L-ARGININE ON TOLERANCE TO U-50,488H IN THE RAT. Hemendra N. Bhargava* and George A. Matwyshyn, Dept. Pharmacodyn., Univ. Ill, Chicago, IL 60612.

The effect of N^G-monomethyl-l-arginine (L-NMMA)(2-8 mg/kg, i.p.) on the development of tolerance to U-50,488H, a k-opiate agonist, was determined in the rat. Male Sprague - Dawley rats were made tolerant to U-50,488H by twice daily injections of the drug (25 mg/kg, ip) for 4 days. This treatment resulted in the development of tolerance to the analgesic and hypothermic effects of U-50,488H as evidenced by the decreased responses ($AUC_{0\text{-}300\,min}$) to a 25 mg/kg dose of U-50,488H in chronically drug injected rats. L-NMMA (4 and 8 mg/kg, ip) injected 10 min prior to each injection of U-50,488H inhibited the development of tolerance to the analgesic but not to the hypothermic effect of U-50,488H. A dose of 2 mg/kg of L-NMMA although appeared to inhibit the tolerance to the analgesic action, the effect was not statistically significant. L-NMMA did not affect the body weight gain of rats injected with U-50,488H. It is concluded that the blockade of nitric oxide synthase results in the inhibition of tolerance to the analgesic effect of k-opiate agonist selectively (Supported by a grant DA-02598 and a Research Scientist Development Award K02-DA-00130 from the National Institute on Drug Abuse).

596.19

EFFECT OF TOLUENE ON EVOKED RESPONSES IN THE HIPPOCAMPAL SLICE PREPARATION. N. Shinsky, B. M. Potter,* and G. T. Pryor. Neuroscience Department, SRI International, Menlo Park, CA 94025.

Toluene (T), an ubiquitous industrial solvent, is subject to human abuse because of its euphoric effect. It has pharmacologic properties resembling those of other abused substances such as barbiturates and alcohol. Although studied extensively for its toxicologic potential, the mechanisms underlying its acute CNS effects have not received much attention. To begin to identify these mechanisms, we used the hippocampal slice preparation to examine T's effect on neurotransmission.

Population excitatory postsynaptic potentials (EPSPs) and population spikes (PSs) evoked by stimulation (0.1 Hz) of Schaffer collaterals in the *stratum radiatum* were recorded from pyramidal cells of area CA1 of transverse hippocampal slices from the right hemisphere of adult male Swiss-Webster mice (28-32 g). The corresponding left hippocampus was analyzed for norepinephrine (NE) content using HPLC-EC. Administration of 1 mg/kg of T (i.p.) 10 min before sacrifice did not affect the *in vitro* hippocampal response compared with control mice. Also, no significant difference in hippocampal NE level was observed. Applied *in vitro* at concentrations of 50, 150, 350 µM, T increased the amplitude and slope of the EPSP in a dose-dependent manner. When intensity of the stimulation was sufficient to evoke PS, T usually increased the amplitude of the descending limb and decreased the amplitude of the ascending limb of the PS. These results suggest that T causes a disturbance in K^+ currents, presumably in I_A and I_C. It is known that several neurotransmitter receptors such as NE, DA, adenosine, and GABA, by being coupled to the same second messenger system or G protein, have an effect on two types of K^+ channels. Therefore, our results suggest that T may affect K^+ conductance by modulating neurotransmitter release, or by interacting specifically with receptors linked to the ion channel, second messenger systems, or with the ion channel itself.

596.20

EXAMINATION OF MOTIVATED BEHAVIOR AND ITS CORRELATES IN HUMANS IN NATURAL SETTINGS. R. F. Mucha*, R. Weiss, G. Mutz and E. Stephan. Inst. of Med. Psychology and Behav. Neurobiology, U. of Tuebingen, 7400 Tuebingen* and Inst. of Psychology, Univ. of Cologne, 5000 Cologne 1, Germany.

Environmental cues can be used as conditioned stimuli for conditioning produced by such motivationally relevant events as application of addictive substances, natural reinforcers and pain. An important question is how laboratory data from humans compares to what is expressed in their natural enviroment. To approach this, we carried out studies on smoking in light to moderate smokers as a test model. They carried a small computer during their daily activities (Kölner Vitaport System) which measured continuously simple psychophysiological and behavioral data. We developed an activity monitor to discriminate general activities typical of smokers: standing, walking and sitting. Without consideration of these activities, there was a clear decrease in heart rate as result of smoking a cigarette. Through application of the sensor it was possible to address the question why laboratory studies indicate that smoking a cigarette produces a significant increase in heart rate. Also presented will be data on procedures for monitoring the the onset of smoking behaviour, including activation of an event recorder by the subject that smoking will occur, the activation of a switch indicating that the cigarette package is being removed and use of a inhalation sensor on a cigarette holder. (aided in part by DFG Bi 195/24-1)

DEVELOPMENTAL DISORDERS OF THE NERVOUS SYSTEM II

597.1

ALTERATION OF MATERNAL RETRIEVING BY NEONATAL UNDERNUTRITION IN THE RAT. P. Carrillo,* C. Torrero, M. Regalado, and M. Salas. INE-Universidad Veracruzana, Xalapa, Ver.; IIB-Univ. Nal. Aut. de México. México, D.F. 04510.

The maternal retrieving (MR) is initiated by picking up the pup by a fold of skin on its dorsal or lateral surfaces and transporting it back to the nest. Neonatal undernutrition in the rat results in long-term alterations of maternal nest building, nursing time and pup retrieval. Because little is known about the effects of neonatal undernutrition upon MR we characterize the response during the lacta ting period. Undernutrition was performed by daily removal of half of the litter (n=4) to an incubator for 12h from postnatal days 1-24. Control dams were obtained from litters adjusted to 8 pups at birth and left undisturbed. Weaning was performed at 25 days of age. Subjects were kept in a - room maintained at 24°C, 12h dark/12h light cycle, and water and food (Purina chow) ad lib. Maternal retrieving of dams was assessed in their own maternal cage by measuring the - latency of the response, the body area (head, neck, body and pelvis zones) where pups were carried, and if pups voca lizations occurred. Data show that neonatally underfed mothers were slower, spent less time in retrieving and do not exhibit preferences for carrying pups in a particular body area; moreover pups retrieved by underfed dams vocalize - more than controls. Data suggest that neonatal food deprivation of environmental cues related with the undernourishing procedure, interferes with the MR of newborns.

Supported by DGAPA. UNAM. IN-20091.

597.2

PAW PREFERENCE IN MICE WITH TOTAL AND PARTIAL CALLOSAL AGENESIS INDUCED BY PRENATAL IRRADIATION. **A.C. Manhaes, E. M. Caparelli-Daquer, S. L. Schmidt *. Departamento de Fisiologia, Universidade do Estado do Rio de Janeiro, RJ, Brazil; Instituto de Biofisica UFRJ; and Department of Psychology, University of Alberta, Edmonton, Alberta, Canada.**

The hypothesis that the development of the corpus callosum (CC) plays a role in the establishment of the direction of behavioral lateralization was tested by studying paw preference in mice with callosal defects induced by ionizing radiation. It is well known that exposure of pregnant mice at embryonic day 16 (E16) affects the CC in a dose-dependent manner: Three Gy produces total agenesis whereas a total dose of 2 Gy causes not only total but also partial callosal agenesis. Here we studied paw preference in adult male mice with callosal defects due to exposure to different doses of gamma irradiation (n=48, total dose of 2 Gy; n=26, 3Gy; n=93, nonirradiated controls), reasoning that directional laterality might also be disturbed in a dose-dependent manner. Every behavioral session consisted of 25 reaches of food and for most mice repeated measurements were done at least three times. Most mice maintained their initial paw preference throughout all subsequent testings and, thus, paw preference was considered a stable measurement of behavioral asymmetry. After the behavioral testings the animals were perfused and their brains were embedded in albumin-gelatin, frozen, cut, and stained with Cresyl-violet. The callosal area was measured with the aid of the Nissl-stained sections. The CC and the cortical extension were affected in all irradiated animals. When the cortical extension was taken into account, the callosal remnant of some animals in the 2 Gy group was considered analogous to a "normal" callosal size. The analysis of directional laterality indicated a tendency for right paw use in the 2Gy group (60%) that was markedly increased in the 3 Gy group (95%). In the 3Gy group, directional laterality was significantly different from chance level as opposed to an absence of such difference in normal controls(49%). In the 3 groups, most mice presented a significant individual paw preference. The present data are consistent with a previous study on paw preference in a strain of mice that present some animals with callosal defects. The dose-dependent effect indicates that the early absence of the corpus callosum disrupts the normal pattern of directional asymmetries, leaving intact individual asymmetries.

597.3

NEUROENDOCRINE STRESS HORMONES DURING THIRD TRIMESTER OF ADOLESCENT PREGNANCY. F.M. Crinella*, A. Chicz-DeMet, B. Simon, E.M. DeMet, C.K. Cho and J.E. Lee. State Dev. Res. Inst., Costa Mesa, CA 92626, Dept. Psychiatry & Human Behavior, UC Irvine, CA 92717 and Psychiatry Service, VAMC Long Beach, CA 90822.

Increases in stress-related peptide hormones have been reported during pregnancy. The present study examined plasma β-endorphin (βE), ACTH, and cortisol in 52 adolescent mothers (range 12 to 17 yr) during the third trimester (TTRI). Mean values for βE (49.0±24.1 pg/ml), ACTH (35.4±13.5 pg/ml), and cortisol (26.4±10.2 μg/dL; SD) generally agree with previously reported studies in adult mothers. Significant correlations were obtained between βE and ACTH during early TTRI (26-32 gestational weeks, GW; $r=0.463$, $p<0.02$) and late TTRI ($r=534$, $p<0.02$). Cortisol and ACTH were significantly correlated during early ($r=0.595$, $p<0.001$), but not late TTRI (33-39 GW; $r=0.402$, $p=0.079$). No significant mean differences were found for these three biochemical stress indices in early and late TTRI. Within late TTRI, however, ACTH ($r=0.424$, $p<0.06$), but not βE or cortisol, tended to correlate with GW at birth. Rising ACTH levels during the TTRI of pregnancy may be indicative of either elevated maternal anxiety as the delivery date approaches or may be due to the release of placental hormones in response to fetal stimulation.

597.4

HPA AXIS AND DEPRESSION IN ADOLESCENT PREGNANCY. A.Chicz-DeMet*, B. Simon, J. Wiger, T. Kim, and E.M. DeMet. Dept. Psychiatry & Human Behavior, UC Irvine, CA 92717, State Dev. Res. Inst., Costa Mesa, CA 92626 and Psychiatry Service, VAMC Long Beach, CA 90822.

Disturbances in the hypothalamic-pituitary-adrenal (HPA) axis are associated with stress and depression. Depression in adolescent mothers has been proposed as a potential risk factor for child cognitive development in later years. The Child Depression Inventory (CDI) and Symptom Checklist 90 Revised (SCL-90R) self-report questionnaires were administered to 52 expectant mothers (age 15.5±1.4 yr, SD) during the third trimester (TTRI) of pregnancy to assess depression, anxiety, and stress. Plasma β-endorphin (βE), ACTH, and cortisol were studied in maternal blood samples. As expected significant correlations were obtained between βE and ACTH ($r=0.688$; $p<0.001$), and ACTH and cortisol ($r=0.524$; $p=0.001$). Mothers with non-optimal birth outcome ($N=3$) had increased depression scores on both the SCL-90R (standardized difference = 0.84; $p=0.17$) and CDI (standardized difference = 0.72; $p=0.13$). Depression ratings, however, were not associated with increases in stress hormones in high risk pregnancies or in the total sample. The results support a putative role of depression as a risk factor in pregnancy, but fail to confirm a relationship between depression and stress in this small sample.

597.5

LYMPHOCYTE PBR AND DBI MODIFICATIONS IN CHILD NEUROPSYCHIATRIC DISORDERS. C.Ferrarese*[1,2], M.Perego[1], C.Marzorati[1], M.Molteni[1], G.Moretti[1], N.Pecora[2], M.Frigo[2], G.Bianchi[2], R.Riva[2] and L.Frattola[2]. Scientific Institute "E. Medea", 22040 Bosisio Parini, Como([1]) and Dept. of Neurology, Univ. of Milan, Osp. San Gerardo, 20052 Monza([2]), ITALY.

Peripheral benzodiazepine receptors (PBR) and their endogenous ligand diazepam binding inhibitor (DBI) are present in lymphocytes, where they modulate immunologic functions. PBR modifications have been previously described in lymphocytes of anxious patients. We now investigated possible modifications of PBR and DBI in lymphocytes of children with various neuropsychiatric disorders.

80 children of the Scientific Institute "E. Medea", with diagnosis of epilepsy (50) affective disorders (23) and Down's syndrome (7) where compared to 10 age-related normal children for the levels of PBR and DBI in lymphocytes. Mononuclear blood cells were separated with a ficoll gradient; PBR density (Bmax) and affinity (Kd) were investigated by the binding of the specific ligand [^{3}H] PK 11195 and DBI levels were determined by a specific radioimmunoassay (RIA).

Both PBR and DBI levels of Down patients were similar to the controls; on the contrary, DBI levels were significantly elevated in lymphocytes of untreated epileptic patients, while PBR density was increased only after anticonvulsant therapy. Two different groups were present in children with affective disorders: one with PBR levels similar to controls, one with PBR levels significantly decreased. DBI levels were also changed in same patients, without correlation with PBR changes. PBR and DBI modifications in affective disorders were not related to DSM III diagnosis, treatment or degree of mental retardation.

PBR and DBI modifications in epileptic patients might explain immunologic alterations in this disorder. PBR decrease in children with affective disorders may be linked to anxiety, which is often masked and difficult to assess in children.

597.6

RISK PERIOD FOR INTRAVENTRICULAR HEMORRHAGE IS ATTRIBUTABLE TO PERINATAL INDUCTION OF GERMINAL MATRIX MICROVASCULAR MATURATION. Laura R. Ment*, William B. Stewart, Martin J. Asis, Miri F. Einat, Charles C. Duncan, Joseph A. Madri. Depts Pediatrics, Neurology, Surgery, Biology and Pathology, Yale University, New Haven, CT 06510.

Intraventricular hemorrhage (IVH) has been attributed to increased cerebral blood flow to the immature germinal matrix (GM) microvasculature. The risk period for IVH is the first 4 - 5 postnatal days and is independent of gestational age in preterm infants of less than 34 weeks. The newborn beagle pup provides a good model for IVH; the risk period for IVH in the beagle pup is the first 3 - 4 days. We used the newborn beagle pup model to study the hypothesis that the risk period for IVH is secondary to the rapid perinatal induction of maturation of the GM microvasculature. We isolated germinal matrix microvascular endothelial cells (BBMEC) from PND 1 beagle pups by differential centrifugation. BBMEC were cultured in DM high glucose medium with penicillin and streptomycin; low-passage BBMEC were placed in three dimensional culture. By day 6, BBMEC formed abundant tube-like structures which stained with the endothelial cell markers Bandeiraea and Factor-VIII related antigen. Northern blotting and hybridization analysis of 10 ug of RNA from day 1 and day 6 BBMEC cultures demonstrated increased amounts of SPARC (secreted protein, acidic, rich in cysteine, (O.D. 0.7 vs O.D. 0.84, day 1 vs day 6) and decreased amounts of alpha smooth muscle actin (O.D. 0.94 vs O.D. 0.71, day 1 vs day 6), consistent with endothelial cell differentiation. BBMEC were placed in coculture with fetal rat brain astrocytes (1:1 ratio, total cell count 80,000/ml). The extracellular matrix proteins laminin and collagen V were detected by day 6 using immunofluorescence. Electron microscopy demonstrated endothelial tight junctions at day 4. We conclude that the risk period for IVH in preterm neonates may be secondary to the rapid development by the GM microvasculature of the traditional characteristics of the blood brain barrier: basement membrane proteins and endothelial cell tight junctions.

597.7

MALE PREVALENCE FOR READING DISABILITY IS FOUND IN A LARGE SAMPLE FREE FROM ASCERTAINMENT BIAS J. Liederman* and K. A. Flannery, Psych Dept, Boston University, Boston, MA 02215.

It is widely believed that males are more vulnerable to neurodevelopmental disorders than females. This notion has been recently challenged in a highly publicized paper by Shaywitz, Shaywitz, Fletcher and Escobar (1990). They argued that male vulnerability for one neurodevelopmental disorder, reading disability, was an artifact of ascertainment biases rather than a true gender difference. The Shaywitz et al. (1990) paper was based on a longitudinal sample of about 400 children whereas the current paper analyzed 16, 888 cases from the National Collaborative Perinatal Study. Results indicated that when children had a history of having been referred to special classes there was a 2.6 to 1 bias in favor of boys, but when classification of reading disability was based strictly on reading performance, (by two different classification methods) there was still at least a 2 to 1 bias in favor of boys. Analyses demonstrated that this gender bias for reading disability was not due solely to "bad behavior" in boys.

597.8

BALLOON CELLS IN DYSPLASTIC HUMAN CEREBRAL CORTEX ARE LABELLED BY IMMUNOHISTOCHEMICAL MARKERS FOR BOTH NEURONAL AND ASTROCYTIC INTERMEDIATE FILAMENTS. M. J. De Rosa*[1], R.S. Fisher[1,2,4], and H. V. Vinters[1,3,4]. [1]Brain Research Institute, Departments of [2]Anatomy and Cell Biology and [3]Pathology and Laboratory Medicine and [4]Mental Retardation Research Center, UCLA Sch. of Med., Los Angeles, CA 90024.

We have defined two aberrant cell types in resections of human cerebral cortex performed in the treatment of intractable childhood epilepsy involving cortical dysplasia. Hypertrophic neurons are large cells of apparently neuronal morphology and phenotype with coarse neurofilamentous cytoplasmic inclusions. Balloon cells are distended in appearance and contain a glassy, eosinophilic cytoplasm. We have, by immunohistochemistry, demonstrated colocalization of both neuronal and astrocytic elements within these cells suggesting that they may be an undifferentiated cell type. In this study, we attempt to further define the balloon cells' lineage with antibodies directed against neuronal (SMI 31 and 34 to phosphorylated neurofilaments and neurodegenerative tangles, respectively) and astrocytic (glial fibrillary acidic protein) intermediate filaments and an antibody against galactocerebroside (GC), a marker of oligodendrocyte differentiation. We have demonstrated balloon cell colocalization of SMI 34 but not SMI 31 with GFAP. Our initial studies suggest the balloon cells are not immunoreactive with the antibody to GC. Conclusion: The balloon cell appears to be an inappropriately differentiated cell type, perhaps a stem cell, which has ignored the oligodendrocyte lineage and expressed a split phenotype consistent, in some respects, with either a neuronal or astrocytic lineage.

597.9

ABNORMAL CLASSICAL EYEBLINK CONDITIONING IN AUTISM. L.L. Sears, P.R. Finn & J.E. Steinmetz*. Prog. in Neural Science and Dept.of Psychology, Indiana University, Bloomington, IN 47405.

Eleven subjects with autism (age range = 7 to 22) and age- and IQ- matched controls were classically eyeblink conditioned using a 450 ms tone conditioned stimulus (CS) coterminating with a 100 ms air puff unconditioned stimulus (US). Subjects with autism conditioned at a significantly faster rate than controls and exhibited a more rapid extinction of the conditioned response (CR). The magnitude of the difference in learning and extinction rates between matched subjects varied with age, suggesting developmental learning abnormalities in autism. Subjects with autism also exhibited an abnormal topography of the CR consisting of short latency, large amplitude responses. The rapid acquisition and extinction of the poorly-timed CRs may reflect developmental abnormalities in the cerebellum and hippocampus, as previously reported in autism (e.g. M. Bauman, Pediatrics, Suppl.,1:791, 1991), since these brain areas are involved in classical eyeblink condtioning (e.g. Steinmetz et al., J. Neuroscience, 11:4403, 1992). The abnormalities in classical conditioning seen in autism suggest an ability to rapidly associate paired stimuli but with impairments in modulation of learned responses depending on processing of certain contextual information.

597.10

THE GENE FOR FAMILIAL DYSAUTONOMIA IS LINKED TO CHROMOSOME 9 AND SHOWS STRONG LINKAGE DISEQUILIBRIUM WITH D9S58. A.Blumenfeld[$*], S.A.Slaugenhaupt[$], D.E.Lucente[$], F.B.Axelrod[#], C.B.Liebert[$], C.Maayan[+], M.Monahan[$], J.A.Trofatter[&], J.L.Haines[$], X.O.Breakefield[$] and J.F.Gusella[$]. [$]Molecular Neurogenetics Unit, MGH and Harvard Med. Sch., Boston, MA 02129. [#]NYU Med. School, New York, NY 10016. [+]Hadassah Univ. Hosp., Jerusalem, Israel.

Familial dysautonomia (DYS), the Riley-Day syndrome, is an autosomal recessive disorder characterized by developmental loss of neurons from the sensory and autonomic nervous system. It is limited to the Ashkenazi Jewish (AJ) population, where the carrier frequency is 1 in 30. We have mapped the DYS gene to the chromosomal region 9q31-q33 by linkage with ten DNA markers in twenty-six families. The maximum lod score of 21.1 with no recombinants was achieved with *D9S58*. This marker also showed strong linkage disequilibrium with DYS, with one allele present on 73% of all affected chromosomes compared to 5.4% of control AJ chromosomes (X^2=3142, 15 d.f. p_0.0001). The other nine markers distributed within 23 cM proximal or distal to *D9S58*, also yielded significant linkage to DYS. To define flanking loci, the phase of the markers was determined in dysautonomia families. Recombination events in the DYS families confirm the marker order based on reference pedigrees and define *D9S53* and *D9S105*, that were mapped 10cM apart, as the closest flanking markers for the familial dysautonomia gene.

597.11

EXPRESSION OF MULTIPLE DYSTROPHIN ISOFORMS IN THE BRAIN POSTSYNAPTIC DENSITY (PSD) OF THE *MDX* MOUSE AND DUCHENNE MUSCULAR DYSTROPHY PATIENT. K. Wu*[1,2], T.W. Kim[1,2], Y.Y. Huang[3], T. Byers[4], B.A. Sieber[1,2] and J.B. Black[1,2]. [1]Dept. of Neurosci. & Cell Biol., UMDNJ/RWJ Med. Sch., Piscataway, N.J. 08854; [2]Grad. Program in Physiol. and Neurobiol., Rutgers-The State Univ. of N. J., Piscataway, N.J. 08854; [3]Div. of Neurosci., NYSPI, New York, N.Y. 10032; [4]Dept. of Physiol. & Biophys., Indiana Univ., Indianapolis, IN. 46202.

Duchenne muscular dystrophy (DMD) is a fatal X-linked inherited disease affecting one in 3500 boys. Cognitive impairment is a common feature of DMD, but underlying mechanisms are unknown. DMD is characterized by a defect in dystrophin that has been detected recently in brain. We have previously found that the 427 kilodalton (kDa) brain dystrophin is a component of the PSD and that this synaptic protein is absent in the *mdx* mouse, an animal model of human DMD (Kim et al., *Proc. Natl. Acad. Sci. USA*, 1992). Recently, multiple novel spliced mRNAs generated from a single DMD gene were detected in brain. To begin defining potential functions of the protein products of these multiple mRNAs, we sought to localize dystrophin isoforms within the complex cortical synaptic structure. We focused on the PSD, since abundant evidence suggests that the density is crucial to synaptic function. We report here that an antibody against the C-terminal domain of dystrophin (anti-D11) recognized three distinct region-specific proteins with apparent molecular weights of 73, 71 and 69 kDa in purified PSD from rat cerebral cortex. The three proteins were differentially expressed during cortical development, suggesting role(s) in synaptic maturation and function. In contrast to the 427 kDa dystrophin, the multiple dystrophin isoforms were present at normal levels in cortical PSDs from *mdx* and human DMD brains. The *mdx* mutation does not affect expression of the various dystrophin isoforms, suggesting that they potentially subserve different, perhaps compensatory, synaptic functions.

597.12

PRENATAL AMPHETAMINE EXPOSURE ALTERS THE NUMBER OF CELLS IN THE ENTORHINAL CORTEX OF THE RAT. C. L. Lawrence, M. Lyon[◆], and W. O. McClure.* Dept.of Biological Sciences, Univ. Southern California, Los Angeles 90089-2520, and [◆]Dept. Psychiatry and Behavioral Sciences, Univ. of Arkansas for Medical Sciences, Little Rock AR 72205.

A substantial amount of evidence suggests that at least some of the schizophrenia(s) are related to errors in fetal development. To test the fetal development hypothesis we have exposed rat pups *in utero* to dopaminergic agonists and have examined the behavior and neuroanatomy of these animals when they matured to young adulthood. Because of the known anomalies in the organization and number of cells in the parahippocampal gyrus of human schizophrenics, we were particularly interested in examining the entorhinal cortex of treated rats. Twenty-three pregnant Wistar rats were injected subcutaneously with d-amphetamine sulfate (5 mg/kg) or saline on specified days. Three "windows" of pregnancy were examined: I, 11-14 d; II, 15-17 d; and III, 18-20 d. Pups at 45-50 days of age were sacrificed by cardiac perfusion. Horizontal sections from -7.3 mm to -3.9 mm ventral to bregma were mounted and stained for cells with neutral red. Counts of cells were taken in the entorhinal cortex at each of 10 - 19 stations along the dorsal-ventral (DV) axis. Treatment with amphetamine had no effect upon the number of cells in either window II or III. In contrast, treatment with amphetamine increased the number of cells in window I by 21% ($p < 0.05$). The amphetamine-induced increase was evenly distributed over all DV levels. In addition, the number of cells in control animals in window I was decreased by 28% ($p = 0.004$) when compared to controls in windows II and III. The data suggest that *in utero* treatment can alter the neuroanatomy of adult rats, and indicate that certain aspects of human schizophrenia can be replicated in an animal model. Supported by the NIH and the Hedco Foundation.

597.13

MATERNAL ANTIBODIES ACCESS THE NEONATAL CENTRAL NERVOUS SYSTEM. R.H. Fabian*[1,3], A.L. Welch[2,3], Y.C. Chen[2,3], C.E. Hulsebosch[2,3]. [1]Dept. Neurol., [2]Dept. Anat. Neurosci., and [3]Marine Biomed. Inst., U. Tex. Med. Branch, Galveston, TX 77555.

To test the hypothesis that maternally derived antibodies have access to the developing neuraxis of the neonate, purified rabbit IgG, prepared by caprylic acid precipitation, was injected intraperitoneally into adult female rats immediately following parturition. The neonates were allowed to nurse and were sacrificed at 6, 12, 24, 36, 48 and 72 hours post-injection (N=5 per time point) by perfusion with saline followed by 4% paraformaldehyde. The neonatal neuraxis was removed and immunohistochemically stained for rabbit IgG. The antibody concentration, as determined by density of reaction product, reached a peak at 48 hrs. In a separate group of neonates (N=5), treated as above, the pups were sacrificed at 48 hrs. by perfusion with saline and the neuraxis (brain and spinal cord) was dissociated and extracted. Rabbit IgG was immunoprecipitated and identified by Western blot. The majority of rabbit IgG remained in an intact form in the neonatal brain tissue. We conclude that maternal IgG has access to the developing neonatal central nervous system via lactation and may have an effect on CNS development. Supported by the Amyotrophic Lateral Sclerosis Association, NIH, Bristol-Myers Squibb.

598.1

SPONTANEOUS AND STIMULATION-INDUCED SYNCHRONIZED AFTER-DISCHARGES IN THE ISOLATED CA1 OF KAINATE-TREATED RATS.
C. L. Meier*, F. E. Dudek†. Sandoz Research Institute, CH-3001 Bern, Switzerland; †Department of Anatomy and Neurobiology, Colorado State University, Fort Collins, Colorado 50283, U.S.A.

A subcutaneous kainate injection in rats (18 mg/kg, mean body weight: 200 g) causes neuronal damage in hippocampal areas leading to persistant reduction of functional inhibition and subsequent hyperexcitability (J. Neurophysiol. 68:2120,1992). In area CA1 (isolated with knife cuts), this hyperexcitability is characterized electrophysiologically by a partial loss of the Schaffer stimulation-evoked IPSP and a prolongation of the evoked EPSP, which gives rise to a short burst of action potentials. When $GABA_A$ receptor-mediated inhibition was blocked by bicuculline in this previous study, afterdischarges of synchronized bursts following the initial Schaffer stimulation-evoked burst were only seen rarely (1 out of 21 slices [5%] from 13 kainate-treated animals). We performed a second series of experiments in younger rats (mean body weight: 100 g), and the exposure to the neurotoxin was prolonged by giving multiple injections of kainate (10x5 mg/kg, s.c.) over 10 hr. When this group was tested several months after the kainate injections, the probability for synchronized afterdischarges of bursts in the isolated CA1 was markedly increased. In normal Ringer solution, afterdischarges of multiple synchronized bursts occured either spontaneously or upon stimulation of the Schaffer collaterals in 2 out of 56 slices [4%] from 2 out of 20 animals [10%]. Following blockade of the remaining inhibition by bicuculline, however, afterdischarges were observed more frequently (12 out of 53 slices [23%] from 5 out of 18 animals [28%]). The occurence of prolonged afterdischarges of synchronized bursts in CA1 of kainate-treated rats (in the absence of input from CA3) suggests that recurrent excitatory connections may form in CA1 after kainate-induced damage to CA3.

598.2

AGE-RELATED EPILEPTOGENIC EFFECTS OF CORTICOTROPIN RELEASING HORMONE ON EVOKED FIELD RESPONSES IN THE ISOLATED CA1 PYRAMIDAL CELL LAYER OF RAT HIPPOCAMPAL SLICES.
B.N. Smith* and F.E. Dudek Dept. of Anat. and Neurobiol., Colorado State Univ., Fort Collins, CO 80523.

Corticotropin releasing hormone (CRH) has been proposed to be involved in the generation of massive infantile spasms (Baram, T.Z., Ann. Neurol. 1993, 33:231). However, no correlate to this epileptogenic effect of CRH has previously been described *in vitro*. Possible age-dependent epileptogenic effects of CRH were investigated using extracellular field potential recordings from the CA1 pyramidal cell layer in 400-600 μm thick hippocampal slices from adult and juvenile (postnatal day 5-21) rats. Bath application of CRH (0.2 μM) to slices from adult animals resulted in a reversible 6 ± 3% (mean ± SEM; percentage of normalized control response) increase in population spike amplitude following stratum radiatum stimulation. Similar CRH application to slices from young animals resulted in a significantly larger increase in population spike amplitude (16 ± 2%; $p<0.04$). In 10-30 μM bicuculline methiodide, stratum radiatum stimulation resulted in interictal-like bursts of population spikes beginning at 8 days of age. Application of CRH to these slices lengthened evoked bursts by 25 ± 8% in adults and 75 ± 16% ($p<0.04$) in juvenile animals and also increased the number of spikes elicited per stimulus (32 ± 10% in adults; 96 ± 12% in juveniles; $p<0.005$).

The present findings suggest that CRH may be more effective in the augmentation of epileptiform activity in the CA1 region of young animals than in adults, supporting the proposition that centrally released CRH may play a role in the development of some types of pediatric epilepsy. Supported by NINDS Postdoctoral Fellowship #NS09289 (B.N.S.) and NIH grants #HD05958 and #NS16683 (F.E.D.).

598.3

EFFECT OF HYPOXIA ON HIPPOCAMPAL PYRAMIDAL CELLS AND INTERNEURONS FROM IMMATURE RATS.
J.Owens, Jr., C.A. Robbins,and P.A. Schwartzkroin*. Depts. of Neurological Surgery and Physiology/Biophysics, Univ. of Washington, Seattle, WA 98195

The immature CNS manifests a well documented, but poorly understood, heightened seizure susceptibility. Hypoxia, a clinically important epileptogenic injury, differentially affects the adult and the developing nervous systems (Ann. Neuro. 29:629-637). To investigate the pathogenesis of hypoxic injury, Sprague Dawley rats were exposed on days P8, 9, and 10 to fifteen minutes of gradually decreasing O_2 concentration (minimum 4%). During the treatment, all animals showed behavioral signs of seizure activity, including wet dog shakes and clonic jerks. Preliminary morphological investigations using cresyl violet as well as the Gallayas silver degeneration stain have failed to reveal any consistent difference between treated and control animals one week after the last exposure. Hippocampal slices were prepared from experimental and age-matched littermate control animals at one day, five days, and one week after the third treatment and intracellular recordings were obtained under current clamp. In preliminary analyses, no statistically significant difference in pyramidal cell or interneuron membrane properties has been identified (CA3 n=53, CA1 n=20, interneuron n=7) between treated and control animals. However, input resistance of CA3 pyramidal cells from treated slices appears somewhat elevated one day after the last hypoxic episode, and returns gradually to control levels by one week post-treatment. (Supported by NIH grants NS15317, NS20482, and GMO7266)

598.4

Electrographic seizures (EGSs) in juvenile rat hippocampal slices in normal ACSF.
L.S.Jones* and J.H.Hamilton, Anatomy, U. South Carolina Sch. of Med., Columbia, SC 29208.

Hippocampal slices from juvenile (J) rats (21-28 days) were used to develop a model of in vitro EGSs in normal ACSF. We report here that with standard neurophysiological techniques, 625 μM slices from J rats (ave 25 days) can be stimulated tetanically to produce EGSs that, once initiated, could continue for several hours (ave. 2 hrs 13') without requiring further stimulation. The stimulation protocols tried were modifications of the stimulus train induced bursting (STIB) paradigm for interictal-like burst induction (Brn. Res. 344:296). The most successful protocol subjected slices initially to a less intense tetanus (designed to produce long-term potentiation [LTP]) via the Schaffer collaterals (CA2-3) followed 15 min later by the STIB protocol through the same electrode. Extracellular recordings were in s.pyr. of CA3 and CA1. Slices from J rats tended to give high numbers of afterdischarges (ADs) in response to the first STIB tetanus (> 40) and 6 out of 10 of these slices produced EGSs. The slightly older rats (31.5 days) gave many fewer ADs after the first STIB tetanus (14.9) and never produced EGSs, but produced the expected, interictal-like bursts usually seen with STIB. Since the EGSs resembled those produced in 0-Mg^{++}, but continued for much longer and had a more consistent, organized appearance, we also tried evoking the EGSs by lowering the Mg^{++} slightly (from 1.2mM to .7-.9mM) prior to and during the STIB; this also resulted in EGS production in J rats and the EGSs *continued* after the slices were returned to 1.2mM Mg^{++}.

Work supported by NS27903.

598.5

NEURAL NETWORK OF ABSENCE SEIZURES IN LETHARGIC (*lh/lh*) MICE: USE OF GABA-B AUTORADIOGRAMS, EEG RECORDINGS AND MICROINJECTIONS.
D.A. Hosford*, F-H. Lin, Z. Cao, D. Kraemer, and A. Huin. Duke & Durham V.A. Medical Centers, Durham, N.C. 27705.

In previous studies we showed that $GABA_B$ receptors are required for absence seizures in *lh/lh* mice, and we found increased numbers of $GABA_B$ binding sites in neocortical membranes of *lh/lh* compared to coisogenic (+/+), nonepileptic littermates (*Science* 257:398, 1992). In this study we determined the neural network of absence seizures in *lh/lh* mice by identifying neuronal structures which satisfied 3 criteria: i) autoradiographic enhancement of $GABA_B$ binding in homologous structures of *lh/lh* compared to +/+; ii) ability of the structure to generate seizures, recorded with bipolar electrodes; iii) ability of the structure to regulate seizures, after microinjection of $GABA_B$ agonists or antagonists.

Slide-mounted brain sections from male, 8-week-old mice (n = 8 pairs) were incubated either: i) with 80 nM [^{3}H]-GABA and 40 μM isoguvacine, with (total) or without (nonspecific) 200 μM baclofen, for 45 min at 4°C; or ii) with 200 nM [^{3}H]-baclofen, with or without 200 μM baclofen, for 5 min at 24°C. Autoradiograms were quantitated by comparing optical densities (Loats RAS/R1000) to tritium standards. Compared to +/+, $GABA_B$ binding in *lh/lh* was significantly greater in neocortex (49%, $p < .005$) and in 2 thalamic nuclei: ventroanterolateral [VAL] (27%, $p < .025$) and reuniens [RE] (30%, $p < .05$).

Synchronous seizures were recorded by bipolar electrodes in frontal neocortex and VAL or RE (n = 7/7 *lh/lh*); but not in lateral amygdaloid nucleus or dentate hilus (0/7). Compared to vehicle, bilateral microinjections of baclofen (1-300 ng/side) into VAL but not RE increased seizures to status epilepticus.

Together, these data demonstrate that neocortex and VAL are critical to the neural network of absence seizures in *lh/lh* mice. Other experiments investigating the mechanisms of absence seizures will focus on these neuronal structures.

598.6

THE $GABA_B$ RECEPTOR IN EXPERIMENTAL MODELS OF GENERALIZED ABSENCE SEIZURES IN RATS.
C. C. Snead III*, P.K. Banerjee, and C.C. Liu, Dept. Neurology, Univ. Southern Calif. Div. Neurology, Childrens Hospital Los Angeles, Los Angeles, CA 90027

Petit mal or generalized absence seizures are characterized by the fact that they are uniformly exacerbated by enhancement of GABAergic activity. Although $GABA_A$ agonists exacerbate experimental absence seizures, $GABA_A$ antagonists do not block this phenomenon. However, experimental absence seizures in rats are both exacerbated by $GABA_B$ agonists and blocked by specific $GABA_B$ antagonists (Snead: Eur J Pharmacol 1992;213:343). Therefore, the object of these experiments was to study the binding of [^{3}H]GABA to $GABA_B$ receptors in brain in two pharmacologic models of absence seizures in rats.

Binding of [^{3}H]GABA to $GABA_B$ receptors was determined by autoradiographic techniques before, at the onset of, and at various intervals during and after absence seizures induced by pentylenetetrazole (PTZ) or γ-hydroxybutrate (GHB). No significant difference was observed in binding between control, PTZ- and GHB-treated animal at any time point examined. These data do not support the hypothesis that the $GABA_B$ receptor is up- or downregulated either immediatedly before or during absence seizures in the models tested.

598.7

NEURONAL AND GLIAL CELL ACTIVITY ACCOMPANYING HYPER- AND HYPOMETABOLISM INDUCED BY FOCAL EPILEPSY

C. Brühl[1], O.W. Witte[1]*, K.A. Hossmann[2], Neurologische Klinik, Heinrich Heine Universität, Düsseldorf, and Max Planck Institut für Neurologische Forschung, Köln, Germany

The mechanisms underlying changes of brain metabolism during epileptic activity are not well understood. In this study metabolic changes caused by penicillin-induced interictal epileptic activity in the rat motor cortex were measured with [^{14}C]-autoradiography. In addition membrane potential of neurons and glial cells was measured in several brain areas.

The metabolism in the focal area was increased up to 3fold. Contralaterally a metabolic "mirror focus" with an increase of metabolism by about 50% was found. The focus on the ipsilateral side was surrounded by a large area with a decraese of metabolism by 10 - 20%.

Within the focus the neurons displayed typical paroxysmal depolarization shifts. In the "mirror focus" pronounced inhibitions often followed by rebound excitations were found. In the hypometabolic areas also inhibitions were found though they were less often followed by rebound excitations. Average membrane potential of neurons within the hypometabolic areas was 10 mV more negative than that of neurons in the normometabolic areas. Glial cells within the focus showed large depolarizations up to 30 mV or more accompanying the interictal EEG discharges. In the "mirror focus" these depolarizations had amplitudes of 5 to 10 mV while they were absent or very small in the hypometabolic brain areas.

The experiments show that both inhibitions and PDS can be hypermetabolic. For each metabolic area typical changes of neuronal and glial cell activity can be observed. Hypometabolism was associated wit a tonic disfascilitation of the neurons. *Supported by SFB 194 B2*

598.8

TYROSINE KINASES AND SEIZURES: A NEW PATHWAY FOR SEIZURE-INDUCED NEURAL REMODELING?

CD Applegate* and AM Moss. University of Rochester School of Medicine, Rochester, NY, 14642.

Tyrosine kinases (TK) are a potent family of enzymes known to promote permanent cellular changes during ontogeny, cellular differentiation and transformation. Post mitotic neurons are rich in TK's and hippocampal TK activity has been shown to be rapidly altered by MES-induced seizures. In this study we examined the effects of pentylenetetrazol (PTZ)-induced seizures and amygdala kindled seizures on phosphotyrosine (PY) production using immunocytochemical, western blot and autophosphorylation assays. Both seizure models resulted in increased PY immunostaining in hippocampal pyramidal neurons immediately following the ictal event and PTZ seizures consistently resulted in increased PY proteins in the 39-42 Kd range using western blot analyses at this time point. Together with previous reports, these results suggest that a common feature of generalized ictal events is an increase in hippocampal tyrosine phosphorylation. Preliminary studies indicate that amygdala kindling results in increased hippocampal PY protein production by western blot and increased autophosphorylation of a 39 Kd PY protein when assayed 2 weeks, but not at 2 days or 4 weeks after the last kindled seizure. These effects were variable however (2 of 4 rats), and may reflect variability in the involvement of hippocampal circutry in amygdala kindling. In summary, data indicate that TK's are induced by seizures and further suggest, that TK's may contribute to long-term processes involved in kindling-induced neural reorganization.

598.9

ENDURING INCREASE IN MEMBRANE-ASSOCIATED PROTEIN KINASE C ACTIVITY IN THE HIPPOCAMPUS OF THE AMYGDALA-KINDLED RAT.

K. Akiyama[1]*, M. Ono[2], I. Kohira[2] and S. Kuroda[1] [1]Dept. of Neuropsychiatry and [2]Dept. of Neurology, Okayama University Medical School, Okayama 700, JAPAN.

In our previous study (Kohira I. et al. Brain Research 593:82-88, 1992), we demonstrated that membrane-associated protein kinase C (PKC) activity in the amygdala/pyriform cortex (AM/PC) and both the right and left hippocampus (HIPP) of rats kindled from the left HIPP increased significantly 16 weeks after the occurrence of the last seizure compared with control rats. The present study examined the effect of kindling from the left AM on brain PKC activity. AM-kindled rats were decapitated either 1 week, 4 weeks or 16 weeks after the last full kindled seizure with matched controls. The AM/PC and HIPP were dissected and separated into the right (contralateral) and left (ipsilateral) sites. At 1, 4 and 16 weeks after the last seizure, the membrane-associated PKC activity increased significantly only in the left HIPP, but not in the other brain regions, compared with control rats. The cytosolic PKC activity did not differ in any brain region examined. In consistent with our previous finding in the HIPP-kindling, these results showed that AM-kindling induces enduring increase in the membrane-associated PKC activity in the HIPP, but only of left (ipsilateral to the stimulation) site, and further suggest that activation of membrane-associated PKC may be involved in the enduring seizure susceptibility induced by kindling.

598.10

CERULOPLASMIN DEFECT IN EPILEPTIC (EL) MICE.

C.E. Garey*, A.L. Schwarzman, and T.N. Seyfried. Department of Biology, Boston College, Chestnut Hill, MA 02167.

Epilepsy is an episodic disorder of the nervous system arising from the uncontrolled electrical discharge of neurons. The epileptic (EL) mouse has tonic clonic seizures with secondary generalization and serves as a model for human complex partial seizures. The epilepsy in EL mice is inherited as a dominant, multifactorial trait. We previously mapped the major gene responsible for the seizures, *El-1*, to distal chromosome 9. This region of chromosome 9 is highly conserved with a region on human chromosome 3q containing the gene for ceruloplasmin (*Cp*), a copper-binding protein. We recently reported a highly significant association between EL seizure susceptibility and an inherited DNA polymorphism at the *Cp* locus. Evidence is now presented for a partial duplication in the 5' end of the *Cp* gene in EL mice. The *Cp* duplication is: a) unique to EL mice and is not present in either normal mouse strains or in the non-epileptic EL parental strain, DDY; b) associated with enhanced expression of *Cp* mRNA in EL mice; c) coinherited with seizures in backcross generations. Moreover, the duplication is associated with localized negative interference, a classical genetic phenomenon involving an enhanced frequency of double recombinants. These findings are relevant to the basic mechanisms of epilepsy as well as to theories of genetic recombination and gene mapping. (Supported by NIH 23355 and Boston College REG.).

598.11

EFFECT OF SEIZURE EXPERIENCE ON TRANSMITTER AMINO ACID ONTOGENY IN THE GENETICALLY EPILEPSY-PRONE RAT (GEPR).

S.M. Lasley* and M.C. Green. Dept. Basic Sciences, U. Illinois Coll. of Medicine, Peoria, IL 61656.

Past work has shown that the ontogeny of sound-induced seizure activity in GEPRs develops primarily within the 3rd and 4th postnatal weeks when audiogenic seizure (AGS) stimulation is applied to seizure-naive (SN) animals. The current study was conducted to determine the effect of repetitive AGS induction on the ontogeny of transmitter amino acids in moderately severe (clonic) seizure GEPRs (GEPR-3s). GEPR-3s were divided into groups receiving AGS stimulation: 1) daily from day 15-22 or day 15-30; 2) daily from day 19-22 or day 27-30; or 3) not at all (SN). Animals were then sacrificed at day 22 or 30 along with a group at day 13 (prior to the emergence of seizure susceptibility), and brain regions analyzed for aspartic (ASP) and glutamic (GLU) acid, taurine (TAU) and GABA concentrations. Groups of seizure-resistant (SR) control animals were sacrificed at the same ages. GABA content was decreased in GEPR-3s relative to SR controls regardless of seizure history and independent of postnatal age. Conversely, TAU levels were elevated in GEPR-3s compared to SR controls in all seizure experience groups and at all postnatal ages. Furthermore, both GABA and TAU content decreased with age in all GEPR-3 and SR groups. In contrast, ASP concentrations were decreased by day 22 in frontal cortex and hippocampus of SN GEPR-3s relative to SR controls. However, increases in ASP in seizure-experienced GEPR-3s relative to SN animals abolished this distinction. These findings agree with previous observations in adult GEPR-3s (Lasley, *Brain Research*, 1991) that decreased GABA is a critical component of seizure predisposition while increased TAU is important in determining seizure severity. It is also apparent that increases in ASP in young GEPR-3s can occur as a result of seizure experience as in adults.

598.12

WIDESPREAD ABNORMALITIES IN NOREPINEPHRINE-STIMULATED INOSITOL PHOSPHATE ACCUMULATION IN BRAIN REGIONS OF GENETICALLY EPILEPSY-PRONE RATS.

D.L. Yourick*, M.R. Salter and J.L. Meyerhoff. Department of Medical Neurosciences, Walter Reed Army Institute of Research, Washington, DC 20307-5100.

Genetically epilepsy-prone rats (GEPR-9) have impaired function of brain noradrenergic systems including fewer α_1-adrenergic receptors in amygdala, hippocampus and cerebral cortex. Our previous studies in this seizure-prone rat strain showed reduced inositol phosphate accumulation in cerebral cortex but not in hippocampus and amygdala/pyriform cortex (Yourick et al., Brain Res. *551*:315-318, 1991). The purpose of the present study was to expand the regional analysis of norepinephrine (NE)-stimulated inositol phosphate accumulation based on our understanding of the involvement of NE in inhibitory processes in brain. NE-stimulated inositol phosphate accumulation was reduced significantly in parietal/occipital, frontal, entorhinal, cingulate and pyriform cortex of GEPR-9 when compared to controls. The same trend existed in hippocampus and amygdala. Inferior colliculus had numerically greater NE-stimulated inositol phosphate accumulation in GEPR-9, while no effect was found in superior colliculus, midbrain, brainstem, thalamus and cerebellum. The data support the hypothesis that in cortical subregions and possibly in amygdala and hippocampus, NE-coupled phosphatidylinositol metabolism is impaired in epilepsy. These results are consistent with the suggested inhibitory role of noradrenergic systems in seizure spread and intensity.

598.13

IDENTIFICATION OF REGIONS SUSCEPTIBLE TO SEIZURE-INDUCED DEGENERATION IN THE RAT BRAIN. M. Khurgel[1*], R.C. Switzer III[2] and G.O. Ivy[1]. [1]Dept. Anatomy & Cell Biology, Univ. of Toronto, Scarborough, ON M1C 1A4, [2]Neuroscience Associates, Knoxville, TN 37922.

In this study we aimed to identify, with a greater precision than previously reported, the extent of neuronal degeneration following an episode of severe seizures induced by systemic kainic acid (KA). Nine adult Long-Evans rats were given KA (10 mg/kg, s.c.) and observed behaviorally for development of seizures. Seven out of nine animals experienced 1 h. of status epilepticus (SE), while 2 rats had only several stage 2 and stage 5 seizures, respectively. The animals survived for 12, 24, 48 and 72 hrs. Every 8th brain slice was stained with a cupric silver degeneration stain, as well as Nissl and Weil stains. The results showed a correlation between the severity of seizure activity and the resulting pathology. The earliest traces of degeneration were seen in the inner molecular layer of dentate gyrus following mild seizures. More intense seizure activity resulted in neuronal degeneration in the CA1 subfield of ventral hippocampus, in basomedial amygdala and in thalamic nucleus reuniens. Following SE, additional regions such as agranular cortex, dorsal endopiriform nucleus and area tempestas as well as the deep and superficial layers of the infralimbic region displayed prominent degenerative changes. More caudally, degenerating neurons and processes were present in the 3rd and 5th layers of the parietal cortex, in deep piriform cortex, in lateral amygdala as well as in selective thalamic nuclei and substantia nigra compacta. Also, degenerating interneurons in the stratum oriens of hippocampal CA1 and in the hilus were present in addition to dying pyramidal neurons in CA1 & CA3. Together, these findings should prove valuable for interpetation of other morphological changes as well as physiological observations in this widely used model of elipepsy.

Supported by NSERC

598.14

MAGNETIC EVOKED FIELD FROM THE TRANSVERSE CA1 HIPPOCAMPAL SLICE OF THE GUINEA PIG. S. Kyuhou* and Y. C. Okada. Magnetophysiology Lab., VAMC, Albuquerque, NM 87108 and Depts. Neurol. & Physiol., Univ. New Mexico Sch. Med., Albuquerque, NM 87131.

Magnetic evoked fields (MEFs) of the guinea pig hippocampal slice were characterized to help understand the genesis of magnetoencephalographic signals from this structure in situ. The animal (Hartley, 200-400g) was decapitated under pentobarbital anesthesia (60 mg/kg i.p.) and transverse slices containing only CA1 were prepared using a standard procedure. The longitudinal axis of pyramidal cells is arranged parallel to each other in this reduced slice; thus the cellular currents responsible for the MEFs could be readily inferred. The slice was placed on a nylon net resting on a plastic pedestal and immersed in oxygenated Ringer solution (concentrations in mM were: NaCl, 115; KCl, 5; $NaHCO_3$, 35; NaH_2PO_4, 1.2; $MgCl_2$, 1.3; $CaCl_2$, 2.5; glucose, 10) containing 0.05 mM picrotoxin. Evoked responses were elicited with a bipolar Ag-AgCl electrode placed in the stratum radiatum (50 μs, 1-3 mA). The component of the MEF normal to the bath surface was measured on a plane 3 mm above the slice with a 4-channel magnetometer. Strong MEFs (1-6 pT) could recorded with a latency of 2.5-3.5 ms. The spatial pattern of the MEF showed that it was due to the longitudinal currents of the pyramidal cells. The laminar field potential profile showed an MEF of as much as 6 pT arising from a 1 mm^3 of active tissue (0.4 mm x 1 mm x 2.5 mm) 3 mm away from the detectors. Bath application of kynurenic acid extinguished most of the signals, indicating the dominant contribution of post-synaptic currents to the MEFs. The kynurenic acid-insensitive component was abolished by tetrodotoxin, indicating the remaining response was also neuronal in origin. Supported by NIH grant NS21149, NSF grant DIR-8820556 and Dept. Veterans Affairs.

598.15

MAGNETIC EVOKED FIELDS PRODUCED BY THE LONGITUDINAL CA1 AND CA3 HIPPOCAMPAL SLICES OF THE GUINEA PIG. Y. C. Okada*, S. Kyuhou and C. Xu. Magnetophysiology Lab., V A Medical Center, Albuquerque, NM 87108 and Depts. Neurology & Physiol., Univ. New Mexico Sch. Med., Albuquerque, NM 87131

Magnetic evoked fields (MEFs) associated with synchronized population activities in the longitudinal hippocampal slices of the guinea pig were studied in order to help understand the genesis of MEG signals from this structure in vivo. The animal (Hartley, 250-400 g) was decapitated under pentobarbital anesthesia (60 mg/kg i.p.) and rectangular slices containing CA1, CA3 or both were harvested.The slice was placed on a nylon net and immersed in Ringer solution (concentrations in mM: NaCl, 117; KCl, 3; $NaHCO_3$, 35; NaH_2PO_4, 1.2; $MgCl_2$, 1.3; $CaCl_2$, 2.5; and glucose, 10; 36°C) containing 0.1 mM picrotoxin. Four pairs of bipolar Ag-AgCl electrodes stimulated the stratum radiatum or lucidum (50 μs, 1.0-1.2 mA, 2 s/stim.). The MEF component normal to bath surface was recorded on a plane 3 mm away from the tissue with a magnetometer. The field potential was recorded simultaneously with the MEFs. Strong MEFs of as much as 25 pT peak-to-peak were recorded. They were in fact strong enough to be seen on single trials. The MEF from CA1 consisted of a series of 1-2 ms long spikes (7 pT peak-to-peak), presumably due to sodium and potassium currents, lasting about 10 ms, superimposed on a slow wave. The field potential in CA1 soma layer consisted of a series of spikes, having same latencies as those in the MEF, superimposed on a slow biphasic potential. The MEF from CA3 consisted typically of a triphasic wave of as much as 25 pT peak-to-peak. The train of spikes seen in CA1 could not be seen in the MEF from CA3, indicating that the population spikes may be less synchrnonized in CA3 than in CA1. Supported by NIH grant NS21149, NSF grant DIR8820556 and Dept. Veterans Affairs.

598.16

PEPTIDE mRNA CHANGES CORRELATE WITH CONTINGENT TOLERANCE TO CARBAMAZEPINE'S ANTICONVULSANT EFFECTS. J.B. Rosen*, M.A. Smith, S.R.B. Weiss, G.S. Massenburg, and R.M. Post. Biol. Psychiat. Br., NIMH, Bethesda, MD 20892.

Previous studies have shown that tolerance develops to carbamazepine's (CBZ) anticovulsant effects on amygdala kindling. This tolerance is contingent upon when CBZ is given; tolerance develops only when rats are given CBZ before, and not after the kindling stimulus is delivered. Although contingent tolerance is robust and well documented, little is known about the biological substrates which may underlie this phenomenon. The present study demonstrates that contingent tolerance correlates with changes in the expression of various peptide mRNAs.

Rats were matched for both drug exposure (either before or after stimulation) and number of seizures. Once tolerance was evident, rats were sacrificed either 4 or 24 hours after the last seizure. A profound reduction in the normally seizure-induced expression in TRH mRNA was found in several limbic structures in the CBZ tolerant group but not in the CBZ non-tolerant group. However, TRH receptor binding was diminished in both the contingent and non-contingent rats. CRH mRNA expression in hilar cells was also reduced in the CBZ tolerant rats compared to the non-tolerant rats. Expression of enkephalin and neuropeptide Y mRNAs in limbic areas tended to be diminished in the CBZ tolerant but not the non-tolerant rats. These changes in gene expression may partially underlie contingent tolerance to CBZ.

598.17

TIME-OFF FROM SEIZURES ATTENUATES ANTICONVULSANT DRUG EFFECTS S.R.B. Weiss*, J.V. Ferrer, P.E. Anthony, R.M. Post, Biological Psychiatry Branch, NIMH, Bethesda, Md. 20892

Rats were electrically kindled in the amygdala until they were reliably experiencing stage 5 seizures. They were tested for their anticonvulsant response to carbamazepine (15mg/kg) and demonstrated a marked decrease in afterdischarge duration, seizure duration, and seizure stage. Half the animals were then given daily kindled seizures for two weeks and the other half received sham stimulation (no current) every day. No drugs were administered during this period. At the end of the interval, the rats were again tested for their anticonvulsant response to carbamazepine. Carbamazepine's anticonvulsant effects were diminished only in the animals that had not received seizures for two weeks. A time course study determined that this loss of efficacy was nearly complete by 4 days without intervening seizures. A similar set of experiments was conducted using diazepam (1-1.5 mg/kg), which also demonstrated this time-off-seizure loss of efficacy. However, in the case of diazepam, 10 days of sham stimulation was required before diazepam became ineffective. In a separate group of kindled animals, 5 days of sham stimulation was associated with a decrease in the seizure threshold compared to rats kindled 24 hours previously. Thus, seizures appear to produce endogenous anticonvulsant alterations, which can potentiate the anticonvulsant effects of drugs on amygdala kindled seizures. Elucidating the mechanisms of this phenomena may suggest endogenous neurotransmitter and peptide systems that could be exploited as novel anticonvulsant treatments.

598.18

CONTINGENT TOLERANCE TO CARBAMAZEPINE: ALTERATIONS IN $GABA_A$ RECEPTOR BINDING AND SUBUNIT mRNA. M. Clark*, G.S. Massenburg, S.R.B. Weiss and R.M. Post. Biological Psychiatry Branch, NIMH, Bethesda, MD 20892.

We have been studying tolerance to the anticonvulsant carbamazepine (CBZ) on amygdala kindled seizures. Tolerance in this model is a contingent process, since it only develops in rats treated with CBZ before the kindling stimulation and not in those animals treated after the stimulation. The present study was designed to investigate the $GABA_A$ receptor system in CBZ contingent tolerance. Receptor autoradiography utilizing various radioligands that bind to different components of the $GABA_A$ receptor system and in situ hybridization with oligonucleotides that recognize different subunits of the $GABA_A$ receptor were performed.

Kindling increased binding to benzodiazepine, picrotoxin, and GABA recognition sites in the dentate gyrus. Rats tolerant to CBZ showed decreased [^{3}H]muscimol and diazepam-insensitive [^{3}H]Ro 15-4513 binding compared to non-tolerant rats, whereas [^{3}H]flunitrazepam and [^{35}S]TBPS binding remained elevated. Kindling also increased mRNA for the α1, α4, β1, and β3 subunits (but no change in α2). There was a selective decrease in the α4 subunit in CBZ tolerant rats compared to non-tolerant rats.

The data suggest an indirect interaction of CBZ with the $GABA_A$ receptor system, since CBZ does not bind to these receptors. However, CBZ does bind to mitochondrial benzodiazepine receptors (MBR), which were suggested to be involved in steroidogenesis. The changes observed in CBZ contingent tolerance could possibly result from CBZ binding at MBR with subsequent regulation of neurosteroids that bind to the $GABA_A$ receptor system.

598.19

EXPRESSION OF GABA AND GLUTAMATE RECEPTORS IN A RAT MODEL FOR EPILEPSY. I. M. Germano, MD, L. K. Friedman, PhD, E. F. Sperber, PhD, R. S. Zukin, PhD, E. S. Goldensohn, MD*, S. L. Moshé, MD. Department of Neurology and Neuroscience, Albert Einstein College of Medicine, New York, NY, 10461

Neuronal migrational disorders in humans are often associated with medically refractory epilepsy. The present study was undertaken in an attempt to develop an animal model of neuronal migrational disorder. We treated pregnant rats with the alkylating neurotoxin methylazoxymethanol (MAM; 25mg/kg in saline, i.p.) to induce migrational disorders in the offspring.

Rat pups were sacrificed at 15 days of age. Histological examination revealed disruption of cortical layers II trough IV and loss of striatal neurons in all MAM-treated rats. Neuronal ectopias in the CA1 area of the hippocampus were seen in 58% of MAM-treated rats. *In situ* hybridization was used to measure $GABA_A$ α1 and GluR2 (AMPA/kainate-type glutamate) receptor gene expression. In areas of neuronal ectopias in the hippocampus, $GABA_A$ α1 and GluR2 mRNA expression was reduced relative to that in control (vehicle-injected) rats. In the frontal cortex, $GABA_A$ α1 mRNA expression was markedly reduced in layers II through IV but near control levels in other cortical areas. Expression of GluR2 mRNA throughout the cortex was unchanged.

These results suggest that MAM-treated animals represent a useful experimental model to study changes in seizure susceptibility secondary to migrational disorders.

598.20

NEUROTRANSMITTER IMBALANCE IN SPONTANEOUS CANINE PRIMARY GENERALIZED AND PARTIAL EPILEPSY. M. Podell*, M. Hadjiconstantinou, W.R. Fenner. Depts. of Veterinary Clinical Sciences and Psychiatry and Pharmacology, The Ohio State Univ., Columbus, OH 43210.

Comparison of neurotransmitters in spontaneous canine epilepsy was performed to determine significant imbalances as a prognostic factor in response to antiepileptic therapy. Cerebrospinal fluid from 13 primary generalized epileptic, 6 primary partial epileptic, and 13 control dogs was analyzed for excitatory (ASP, GLU, TAU) and inhibitory (DA, 5-HT, GABA, ALA) neurotransmitters or their metabolites using high-pressure liquid chromatography with electrochemical detection. Initial general linear regression analysis yielded differences in GLU ($p=0.044$) and GABA ($p=0.002$) between epileptics and controls with respect to weight and gender. Stepwise regression analysis for seizure type, age of onset and duration demonstrated significantly lower values of ALA ($p=0.045$) and GABA ($p=0.040$) with generalized cluster seizures compared to all other seizure types. No differences in age of onset or seizure duration were detected. Significantly low levels of GABA ($p=0.023$) and high levels of GLU ($p=0.030$) were present in all male epileptics compared to control male dogs. Prospective evaluation of the response to antiepileptic therapy will be correlated with this neurotransmitter imbalance.

EPILEPSY: BASIC MECHANISMS IV

599.1

ANTIEPILRPTOGENIC AND ANTICONVULSANT EFFECTS OF NBQX, A SELECTIVE AMPA RECEPTOR ANTAGONIST, IN THE RAT KINDLING MODEL OF EPILEPSY. K.Morimoto* and T.Namba. Dep. Neuro-psychiatry, Okayama Univ.Med.Sch., Okayama, 700 Japan.

To investigate the role of non-NMDA receptors in epilepsy, we examined antiepileptogenic and anti-convulsant effects of 2,3-dihydroxy-nitro-7-sulfamoyl-benzo(F)quinoxaline (NBQX), a potent and selective antagonist of AMPA receptors, in the rat kindling model of epilepsy. Systemic administration of NBQX (10-40 mg/kg, i.p.) significantly suppressed the seizure stage (control: 5.0 vs NBQX 40 mg/kg: 0.8, $p<0.05$) and afterdischarge (AD) duration (84.5±9.7 sec vs 12.7±2.9 sec, $p<0.01$) of the previously kindled seizures from the amygdala (AM) in a dose-dependent manner. The maximal effects were observed 0.5-1 h after drug injection. When the stimulus intensity was increased to twice of the AD threshold, the effects were not reversed, suggesting that the effects are not due to non-specific elevation of the focal threshold. In contrast to AM kindlied seizures, in hippocampal kindled seizures, NBQX (20-40 mg/kg) significantly suppressed only the kindled seizure stage without reducing the AD duration. Daily pretreatment with NBQX (15 or 30 mg/kg) prior to each electrical stimulation for 14 days potently suppressed AM kindling development, in which the average of the number of stimulation required to produce the first stage 5 seizure was 9.9 (7-16) in control, 21.9 (14-28, $p<0.01$) in 15 mg/kg ($p<0.01$) and 26.1 (22-30, $p<0.01$) in 30 mg/kg ($p<0.01$) NBQX groups. These results indicate that AMPA receptors have the important role in seizure induction and propagation, at least, in AM kindling.

599.2

PERIRHINAL CONNECTIONS TO THE ORBITAL AND FRONTAL CORTEX: ANATOMY AND KINDLING. M.E. Kelly*, D.C. McIntyre and W. A. Staines§. Dept. of Psychology, Carleton Univ.; §Dept. of Anatomy, Ottawa Univ., Ottawa, Ontario, Canada, K1S 5B6.

Previously we have shown that the perirhinal cortex kindles faster and with shorter latencies to clonus onset than all other limbic sites tested (McIntyre et al., *Brain Res.*, in press). These data suggest an intimacy between the perirhinal area and areas controlling the motor convulsion. In experiment 1, using both anterograde and retrograde tracing techniques, we determined the efferents of the perirhinal cortex adjacent to the amygdala. In addition to its many subcortical projections of interest to epilepsy, including the basolateral amygdala and posterior thalamic nucleus, the PRh area also was shown to project heavily to the insular-orbital and frontal motor cortices. The insular-orbital cortex has been implicated previously in kindled seizure expression (Corcoran et al., *Kindling*, 1975; Holmes et al., *Brain Res.*, 1992).

In experiment 2, we assessed the speed, latency and form of kindling from the insular-orbital and frontal cortices compared to the perirhinal cortex. Only the frontal cortex kindled as rapidly as the perirhinal area (3 trials) and with similar latencies (1-2 sec), while the insular-orbital cortex kindled more slowly (6 trials) and with much longer latencies (15 sec). On the other hand, the form of the convulsive seizures triggered from the insular-orbital cortex, like the perirhinal cortex, were purely clonic, while the form of the convulsions triggered from the frontal cortex were clonic-tonic-clonic. A synthesis and interpretation of these results will be presented.

599.3

ANXIETY AND EPILEPSY IN SENSORY KINDLED, HABITUATED, AND NAIVE GERBILS. J.W. Collins*, and W.B. Iturrian. Department of Pharmacology and Toxicology, University of Georgia, Athens, GA 30602-2356.

The link between epilepsy and anxiety is largely empirical and not well researched. Previous studies found increased anxiety in chemically or electrically kindled rats and increased emotionality after stress-induced seizures. No attempt has been made to show a decreased level of anxiety with habituated animal subjects. We examined the relationship of emotionality to seizure expression and tested for endogenous BDZ and opioid modulation in gerbils whose genetic seizure susceptibility (SS) is under behavioral control. We control SS in this model by altering the inter-test interval to produce groups with 100% (kindled), 40% (naive), or 0% (habituated) seizure expression. "Handling kindled" subjects tested in an open field showed an increased seizure frequency (76%), left behind more fecal boli, and displayed increased locomotor activity when compared to naive gerbils (28% SS). In contrast, habituated animals exhibited no seizures, had lower fecal defecation scores, and less response to novel stimuli during open field testing. In this battery of open field tests, both flumazenil, and naltrexone, or their combination (each at 1 mg/kg, ip) had subtle effects on SS. This contrasts with the marked effects of CGS8216 and naloxone on handling-elicited SS (Neurosci. Abs. 13:52, 1987). This data shows a definite link between the seizure susceptibility of this genetic epileptic gerbil and several measures of emotionality.

599.4

HIPPOCAMPAL DAMAGE INDUCED BY PERFORANT PATH STIMULATION FACILITATES KINDLING. Y.Shirasaka,R.A.Baldwin, C.G.Wasterlain*.Dept. of Neurology,BRI,UCLA Sch. of Med.,Los Angeles, CA90024 and Epi.Res.Lab.VAMC,Sepulveda,CA91343.

24hrs perforant path(pp) stimulation causes selective damage to hilar neurons, but its potential epileptogenecity has never been investigated. In this experiment, we examined kindling susceptibility in pp stimulated rats.

Adult male Wistar rats were anesthetized with continuous i.v. Urethane and stimulating and recording electrodes cemented in pp and dentate hilus,respectively. Experimental rats(n=5) were stimulated at 2Hz(paired-pulses,40msec apart) continuously with intermittent 10-sec trains of single stimuli(20V,0.1msec,20Hz) once/min. for 24hrs. Before and after stimulation, frequency dependent paired-pulse inhibition was examined at 0.1,1,2,and 4Hz. Control rats(n=4) were anesthetized and examined in the same way as stimulated rats. After 1-3months, rats were given kindling stimulation(3times daily,1msec,60Hz,2sec total duration, 100μA above after discharge threshold(ADT)) and examined for ADT and number of ADs necessary to induce 3 generalized motor seizures.

Loss of inhibition peaked at the end of stimulation. The inhibition score ((1st-2nd spike amplitude)/(1st spike amplitude)x100) decreased significantly over the 24hrs stimulation (at 2Hz, stimulated rats,-20.9±13.0; controls,92.1±9.7; $P<0.001$). There was a non significant decrease in ADT(stimulated rats,280±84μA; controls,475±171μA). The number of AD necessary to induce 3 generalized seizures was significantly smaller in stimulated rats(6.4±3.4) compared with controls(19.0±1.4, $P<0.001$).

These results show that hippocampal damage by perforant path stimulation is epileptogenic. Supported by EFA fellowship(YS),by the VA research service and by research grant NS13515.

599.5

BEHAVIORAL EVALUATION OF AUDIOGENIC KINDLING AND AMYGDALA KINDLING IN WISTAR AUDIOGENIC RATS. N.Garcia-Cairasco,* H.Wakamatsu., S.T.B.Bueno., O.Y.Galvis-Alonso. and J.A.C.Oliveira. Department of Physiology, Ribeirão Preto School of Medicine, University of São Paulo, 14049-900, Ribeirão Preto-SP-Brazil.

Acute audiogenic seizures (AS) and audiogenic kindling (ASK) are models of midbrain tonic-clonic seizures and acoustic-limbic interactions, respectively, while amygdala kindling (AMK) is a model of limbic seizures. However, it is unknown how limbic seizures are installed and interact with AS development during ASK. Also, it is not clear if the AMK induction is different in AS sensitive (S) or resistant (R) rats. For ASK induction S(n=18) and R(n=18) rats were acoustically stimulated (120 dB; 30 days). AMK was induced by 30 amygdala subthreshold electrical stimulations (four/day; 2 ms, 60Hz, 2 s) in S(n=4) and R(n=3) rats. AS, ASK and AMK were evaluated by cluster analysis (Garcia-Cairasco et al, Behav. Brain Res. 48:49-56, 1992), and by severity indexes (Racine, 1973; Pinel, 1978). S rats displayed wild running with tonic-clonic seizures at day 1. ASK decreased tonic-clonic seizures coincidently with the appearance of limbic seizures (ANOVA; $p<0.0001$). Class 1-2 limbic seizures appeared at days 3-5 of ASK initiation; class 3 and isolated galloping at days 4-6; galloping mixed with wild running and tonic seizures at day 7; class 4 at days 6-10 and class 5 at days 13-19. AMK in S rats appeared with higher scores (6-7), typical of midbrain recruiting. ASK and AMK in S rats gradatively recruited new areas and interfered with the original epileptogenic site, phenomena which may be a temporal and spacial substrate for acoustic-limbic interactions.

N.G.C., H.W., S.T.B.B. and J.A.C.O were supported by CNPq-Brazil.

599.6

CHANGES IN RECURRENT HIPPOCAMPAL INHIBITION FOLLOWING EXTENDED KINDLING OF THE PERFORANT PATH OR AMYGDALA.

N.W. Milgram*, E. Head, M. Michael , J. Ferbinteanu, S. Cammisuli, C. Reid, & R. Racine, Life Science Division, Scarborough Campus, University of Toronto, Ontario M1C 1A4.

Kindling is a procedure in which repeated electrical stimulation leads to a progressive increase in the severity of the evoked epileptiform responses and is typically terminated after animals have experienced two severe motor seizures. Several studies have reported that perforant path kindling produces an increase in recurrent inhibition in the dentate gyrus. In order to determine whether recurrent inhibition is further modified with increases in the severity of evoked seizures, we have been monitoring transmission in the dentate gyrus in animals undergoing an extended kindling procedure. As has previously been reported, progressive increases in the severity of elicited seizures were seen when kindling was extended. In animals kindled through the perforant path, inhibition increased over the early phase of kindling, and then stabilized. There was little change from the amygdala kindled rats. At this time, there is no evidence that extended kindling can lead to an inhibitory breakdown.

599.7

RECURRENT AMYGDALA PENICILLIN FOCUS IN THE RAT. POWER SPECTRA INCREASE OF KINDLING-LIKE CORTICAL AND AMYGDALINE ELECTRICAL ACTIVITY PARALLELS BRAIN LEU- AND MET-ENKEPHALIN CONTENT AND RELEASE ENHANCEMENT. **M. Asai, A. Martínez, R. Fernández-Mas and A. Fernández-Guardiola*. Inst. Mexicano de Psiquiat. and Fac. Psychol. UNAM. Mexico City, 14370.**

Electrical amygdaline kindling induces Leu- and Met-Enkephalin increase in the rat brain (Vindrola et al., Neurosci. Lett. 21:39-43, 1981). Interictal spiking is a prominent feature of Penicillin recurrent topical application in temporal lobe amygdala. In this work we tested the hypothesis of interictal spiking being the cause of endogenous opioid augmentation.

17 non anesthetized free moving rats with indwelling cortical (bi-frontal Cx) and both amygdalae electrodes plus a canula in left amygdala, 10 sham operated rats and 17 control rats were used. During 5 days Penicillin-G-Na was daily delivered (50 IU in 1 μL). With a short latency (from 1 to 60 sec) penicillin spikes appeared and propagated first to the ipsilateral and then to the contralateral frontal Cx in amygdala. Interictal activity lasted for about 6 h in waking stage reappearing occasionally during sleep. 20 min after the last Penicillin topical dose the animals were sacrificed. Enkephalin tissue content was measured in amygdala, striatum, hippocampus, hypothalamus, and cortex by RIA procedure. Enkephalin release in amygdala was evoked with 30 mM K^+. The results were expressed in pmol/g. Content of Leu-enkephalin increased significantly ($P<0.02$ or less, using Student'-"t" test) in all measured structures except in cortex. Met-Enkephalin only increase significantly in amygdala. *In vitro* release showed an important increase of both peptides in amygdala as a result of repetitive interictal spiking. CONACT (Mex.) 0778-N9110; 0694-N9109.

599.8

PERSISTENCE OF KINDLING ANTAGONISM IN RATS. *R.D. Kirkby*, T.H. Gilbert, & M.E. Corcoran. Dept. of Psychology, U. of Victoria, POB 3050, Victoria, BC, Canada, V8W 3P5.*

During concurrent alternating stimulation of two forebrain sites, one of the sites (dominant) supports typical kindling, culminating in generalized seizures. The other site (suppressed) supports only focal or partial seizures for as long as dominant site stimulation continues. Burchfiel and Applegate (1989) proposed that this phenomenon (kindling antagonism) reflects arrested kindling from the suppressed site; however, it may merely depend upon transient seizure inhibition. We have attempted to test this hypothesis.

We stimulated rats in the right septal area and the left amygdala (1 stimulation per day) until the latter site (always dominant) supported stage 5 seizures on 6 consecutive trials (Initial Phase). Control rats were stimulated only in the amygdala. During the Final Phase, which began either immediately, after 30 stimulation-free days, or after 30 additional days of Initial Phase treatments, we intermittently applied stimulation to the septal area only. We assessed the number of septal after-lischarges (ADs) required to elicit a stage 5 seizure.

We found that the interposition of a prolonged stimulation-free period did not significantly reduce the number of septal ADs necessary to provoke a generalized seizure during the Final Phase. Also, fewer septal ADs were required by rats previously exposed to alternating stimulation as compared to control rats previously stimulated only in the amygdala. This disparity may depend upon the substantial growth in septal AD duration observed in alternately stimulated rats during the Initial Phase. The data are thus consistent with the view that kindling from the suppressed site is arrested at an intermediate stage during the induction of kindling antagonism.

599.9

EFFECT OF INTRANIGRAL FLUOXETINE ON AUDIOGENIC SEIZURES IN GENETICALLY EPILEPSY-PRONE RATS (GEPRS). R.A. Browning[1]*, M. A. Statnick[1], J. W. Dailey[2], and P.C. Jobe[2]. [1]Dept. Phyiol. Southern Illiniois University, Sch. of Med. Carbondale, IL 62901 and [2]Dept. Basic Sci. Univ. Ill. Coll. of Med. Peoria IL 61656

Previous studies have shown that depletion of brain 5-hydroxytryptamine (5-HT) increases the severity of audiogenic seizures (AGS) in GEPRs, while elevations in 5-HT attenuate these seizures. Other studies indicate that an innate deficit in brain 5-HT contributes to the seizure-prone state in GEPRs. However, the site at which 5-HT exerts its anticonvulsant action on AGS is unknown. The substantia nigra (SN) has been shown to exert a modulatory role over both brainstem and forebrain driven seizures in non-epileptic rats and it receives a rich serotonergic innervation. Moreover, the systemic administration of fluoxetine, a 5-HT reuptake inhibitor, has been shown to exert anticonvulsant effects in brainstem seizure models and recent evidence indicates that these effects are mediated via an increase in extracellular 5-HT in the GEPR (Dailey et al. JPET 260: 533, 1992). In addition, bilateral infusion of fluoxetine into the SN inhibits forebrain evoked-seizures in rats (Pasini et al. Brain Res. 593: 287, 1992). The present studies were designed to determine if intranigral infusions of fluoxetine inhibit audiogenic seizures (brainstem seizures). Male severe seizure GEPRs (GEPR-9s) were pretreated with DL-5-hydroxytryptophan (5-HTP, 75 mg/kg, i.p.) 30 min before seizure testing. Fluoxetine (7.2 nmoles/side, in 0.5 ml) was infused into the SN 15 min before AGS. Rats treated with 5-HTP and intranigral fluoxetine displayed an increase in the latency to the run and to the convulsion when compared to 5-HTP treated saline infused controls ($P< 0.05$), but no change in seizure severity score was observed. The present findings suggest that fluoxetine exerts some of its anticonvulsant action on AGS through an increase in extracellular 5-HT in the SN.

599.10

INTRATHALAMIC INFUSIONS OF BOTH NMDA AND ITS ANTAGONIST, MK-801 SUPPRESS g-HYDROXYBUTYRATE INDUCED GENERALIZED ABSENCE-LIKE SEIZURES IN RATS. P. K. Banerjee* and Q. C. Snead III Div. of Neurology, Childrens Hospital of Los Angeles, Department of Neurology, University of Southern California School of Medicine, Los Angeles, CA.

Absence-like seizures arise due to low frequency rhythmic oscillations of certain thalamocortical neurons. We have reported earlier that in two different rat models of absence-like seizures, electrographic seizures appear in various dorsal thalamic nuclei synchronously with that in the cortex (Banerjee and Snead, *Soc. Neurosci. Abstr.* 18: 909,1992). Since most of the thalamocortical and corticothalamic neurons have been reported to utilize glutamate or aspartate as their neurotransmitter, the present study was undertaken to evaluate the effects of microinfusions of NMDA and its antagonist, MK-801 in different dorsal thalamic nuclei on the generation of absence seizures induced by g-hydroxybutyric acid (GHB). Bilateral infusions of NMDA (30-100ng/side) in mediodorsal (MD), central lateral (CL), paracentral (PC), ventroposterolateral (VPL) and reticular nucleus (RT) of thalamus dose dependently suppressed GHB-induced absence seizures from both thalamus and cortex. No behavioral or electrographic seizures were obseved during or after NMDA infusion. Following GHB administration to NMDA treated animals, the appearance of burst suppression activity in the EEG served as marker for the inhibition of absence seizures. The anti-absence effects of NMDA were more prominent in MD, CL and PC because doses as low as 50-70 ng/side induced immediate burst suppression with no seizures following GHB administration. In VPL and RT, a higher dose of NMDA (100ng/side) was required for complete abolition of absence seizures with immediate appearance of burst suppression. Similar dose dependent inhibition of GHB-induced absence seizures after bilateral infusions of MK-801 (20-60 ug/side) in the above thalamic sites was also observed with EEG progressing to burst suppression.

These findings suggest that excitatory amino acid mechanisms in the thalamus, in general, and mediodorsal and intralaminar nuclei, in particular, are involved in the generation of experimental absence-like seizures.

599.11

NITRIC OXIDE POTENTIATES GENERALIZED CONVULSIVE SEIZURES ELICITED BY CHOLINERGIC STIMULATION OF THE VENTROPOSTERIOR THALAMUS. S. Mraovitch*, J. Seylaz, Y. Calando. Laboratoire de Recherches Cérébrovasculaires, CNRS UA 641, Université Paris VII, Paris (France).

It has been recently suggested that nitric oxide (NO) synthesized from the amino acid L-arginine (L-Arg) by nitric oxide synthase (NOS) may contribute to the genesis of seizure activity. In the present study we sought to determine whether NO may modify convulsive seizures elicited by cholinergic stimulation of the ventroposterior thalamus (VP).

Wistar rats (300-350 g) were used in the experiment. Carbachol (CCh, 10 μg in 100 nl) was stereotaxically microinjected in the VP via a glass micropipette. Prior to CCh microinjection the animal received an i.p. injection of either 300 mg/kg of L-Arg (Sigma), 3 mg/kg of the NOS inhibitor, N^G-nitro L-arginine methyl ester (L-NAME) or L-NAME + L-Arg. After sterotaxic injection of CCh the animals were placed in an observation chamber for 2 hours.

As previously reported, microinjection of CCh alone resulted in the appearance of paroxysmal behavior known as wet-dog shakes (WDS) followed by several convulsive components (facial clonus, jaw clonus, and rearing) associated with limbic motor seizures (LMS) and generalized convulsive seizures (GCS). While pretreatment with L-NAME (n=4) abolished GCS ($p<0.05$), the occurrence of WDS, and LMS behavioral components remained unchanged. In contrast, L-Arg (n=4) significantly increased the number of episodes of WDS ($p<0.05$) and elicited generalized and continuous bilateral convulsive movements leading to death. Injection of L-NAME with L-Arg (n=3) prior to CCh, prevented the effect of L-Arg on generalized seizure activities. Neither L-NAME (n=3) nor L-Arg (n=3) alone produced changes in the rat behavior.

We conclude that NO has potentiating effects on generalized but not on limbic motor seizure activity elicited by activation of cholinergic receptors within the VP.

599.12

LOCALIZATION OF AN ANATOMICAL SUBSTRATE FOR THE ANTICONVULSANT ACTIVITY INDUCED BY D-CYCLOSERINE. S.L.Peterson*. Department of Medical Pharmacology and Toxicology, Texas A&M University Health Science Center, College Station, Texas 77843.

Previous studies in this laboratory have demonstrated that both central and systemic administration of D-cycloserine (DCS) induces significant anticonvulsant activity in maximal electroshock seizures (MES). The purpose of the present study was to determine if local application of DCS to the nucleus reticularis pontis oralis (RPO) inhibits the tonic hindlimb extension (THE) component of MES.

Bilateral 50 nmol DCS microinfusion into a discreet region of the RPO 5.4 to 5.6 mm posterior to bregma inhibited the occurrence of THE in 14 of 17 rats. Microinfusion of 25 nmol/side DCS protected 1 of 11 rats from THE. Unilateral microinfusion of 50 nmol DCS (n=8) or bilateral microinfusion of saline (n=8) had no effect on the incidence of THE.

Browning has shown that the THE component of MES is dependent on the RPO (Fed. Proc. 44:2425, 1985). The present results would indicate that the anticonvulsant activity induced by DCS may be mediated specifically by the RPO.

(Supported by NIH grant 24566)

599.13

THE ANTICONVULSANT ACTION OF FLUOXETINE IN SUBSTANTIA NIGRA IS DEPENDENT UPON ENDOGENOUS SEROTONIN. A. Pasini*, A. Tortorella, K. Gale. Dept. of Pharmacol., Georgetown University Med. Centr., Washington D. C. 20007.

Previously we reported that intranigral application of fluoxetine was anticonvulsant in rats (Brain Res. 593:287-90; 1992). In the present study, we investigated the role of 5HT in the anticonvulsant action produced by the focal application of fluoxetine into substantia nigra (SN). In order to inhibit tryptophan hydroxylase and deplete presynaptic stores of 5HT, rats were pretreated with p-chlorophenyl-alanine methyl ester HCL (PCPA). PCPA was given in 2 successive doses: 375 mg/kg i.p. and 100 mg/kg i.p., 2 days and 1 day, respectively, prior to the intranigral injection of fluoxetine. Fluoxetine (3.5 nmol), was injected into SN bilaterally. Seizures were evoked by the focal application of bicuculline into the area tempestas (AT), an epileptogenic site in the deep prepiriform cortex. In rats not treated with PCPA, fluoxetine, (3.5 nmol) bilaterally in SN, protected the rats from AT-evoked seizures. Pretreatment with PCPA, which by itself did not significantly alter seizure severity, blocked the anticonvulsant effect of intranigral fluoxetine. The directly acting 5HT receptor agonist 1-[3-(trifluoromethyl)phenyl]piperazine (TFMPP) when injected bilaterally into SN in a dose of 10 nmol also protected against AT-evoked seizures. However, pretreatment with PCPA did not interfere with the anticonvulsant action of TFMPP. Thus, the PCPA-induced depletion of endogenous 5HT selectively abolished the anticonvulsant action of intranigral fluoxetine but not of nigral 5HT receptor stimulation. This is consistent with a mechanism of action of fluoxetine as a 5HT uptake inhibitor which thereby enhances the synaptic action of endogenous 5HT. Our results support the proposal that endogenous serotonergic transmission in SN can limit the development and propagation of seizure activity generated in limbic circuits.

599.14

LOCAL INTRACEREBRAL ADMINISTRATION OF L-LACTATE IS ANTICONVULSANT. F. Fornai, A. Tortorella, D. Dybdal and K. Gale*. Dept. Pharmacology, Georgetown Univ. Med. Ctr., Washington, DC 20007.

Intense stimulation of neural activity in brain by sensory input or seizure activity has been shown to produce prolonged increases in regional lactic acid concentrations, reaching very high extracellular levels (10 mM) with seizures (Kuhr et al. J.Cereb.Blood Flow Met. 8: 848, 1988). To determine whether local neuronal excitability could be influenced by lactate, we evaluated its effect in modulating focally-evoked seizures. L-lactate, or equimolar acetate in controls, (adjusted to pH 5.5 with NaOH) was infused 1.5mm dorsal to AT at a rate of 500nmol/100nl/min for a period of 10 min. Pyruvate (50nmol/100nl/min) was included in the lactate solution to slow the metabolism of lactate by lactate dehydrogenase. Ten min after the end of the lactate or acetate infusion, rats received a microinjection of bicuculline methiodide (236pmol) in AT. This treatment evoked severe and prolonged limbic motor seizures in controls (facial and forelimb clonus with rearing and falling); in lactate pretreated animals, seizure activity was blocked. The lactate-induced seizure suppression was reversible by 3hr following lactate infusion. When the duration of lactate infusion was increased to 30 min, the duration of seizure suppression was prolonged to 5 hr, wearing off after 7-8 hr. These observations indicate that local elevation of lactate in brain can dampen neural responsiveness to excitatory influences. Thus, endogenous lactate may provide a signal linking metabolic state to neuronal excitability. In the case of seizures, increases in endogenous lactic acid may limit the duration of seizure activity, thereby avoiding the generation of cytotoxic levels of lactic acid.

599.15

ROLE OF MUSCARINIC AND NON-NMDA RECEPTORS IN AREA TEMPESTAS FOR FOCALLY-EVOKED STATUS EPILEPTICUS A. Tortorella*, F. Fornai, R. Cassidy and K. Gale. Dept. Pharmacology, Georgetown Univ Med. Ctr., Washington, DC 20007

Blockade of GABA transmission, or enhancement of glutamate or muscarinic transmission, in area tempestas (AT) evokes episodic seizure activity which recurs at 3-7 min intervals over a 40-50 min period. In the course of examining interactions between these transmitters in AT, we found that combined treatment with a GABA antagonist (bicuculline, 118 pmol) and a muscarinic agonist (carbachol, 328 pmol) in AT, produced a severe and prolonged continuous seizure state (status epilepticus), frequently lasting for more than two hours. This response was not obtained with bicuculline (118 to 240 pmol) or carbachol (328 to 656 pmol) alone, nor with combinations of bicuculline and glutamate agonists (kainate, AMPA, or NMDA) in AT. In the presence of atropine (114 pmol) in AT, the status epilepticus produced by bicuculline and carbachol was converted to episodic seizure activity, characteristic of bicuculline alone. Whereas the seizure triggering effect of individual convulsant agents in AT was prevented by local blockade of NMDA receptors (by AP-7, 100 pmol), the effect of the combination of bicuculline and carbachol in AT was not sensitive to AP-7. In contrast, the non-NMDA antagonist, NBQX (500 pmol), when applied to AT, suppressed all seizure activity evoked by bicuculline and carbachol in AT. It therefore appears that, in the presence of a GABA antagonist in AT, focal muscarinic stimulation 1) prevents the operation of endogenous, seizure-suppressing processes, and 2) renders the seizures insensitive to blockade of NMDA receptors. The fact that status epilepticus evoked by carbachol + bicuculline was blocked by a non-NMDA glutamate antagonist suggests that glutamatergic neurotransmission is required for this phenomenon.

599.16

HIPPOCAMPAL MICRODIALYSIS OF SOMATOSTATIN EVOKES A DISSOCIATED RESPONSE OF THE EXCITATORY AND INHIBITORY AMINO ACID NEUROTRANSMITTERS; EVIDENCE FOR A MODULATORY ROLE IN EPILEPTOGENESIS. A. Legido[1,3,4], J.G. McElligott[2], T. J. Parry[2], T. J. Lynch[2], J.J. O'Neill[2*] I. I. Greenstein[3], and W. D. Grover[1,3,4]. Temple University School of Medicine, Depts. of Pediatrics[1], Pharmacology[2] Neurology[3], Philadelphia PA 19140 and St Christopher's Hospital for Children[4], Philadelphia PA 19134.

The objective of this work was to study if somatostatin (SS) functions as a modulator of brain epileptogenesis in the hippocampus and to investigate if this modulatory role is mediated through excitatory (glutamate, aspartate) or inhibitory (GABA, glycine, taurine) amino acids. Hippocampal microdialysis was performed in head restrained awake rats in which the hippocampal and cortical EEG were recorded. Baseline EEG and microdialysis sampling (1μL/min) at 20 minute intervals were performed for two hours prior to the administration of two doses of SS (20 μg and 40 μg). Each dose was dialyzed into the hippocampus over a period of 20 minutes after which drug free dialysis fluid was perfused through the probe; samples were taken at the end of each SS perfusion as well as 20 and 40 minutes later. Amino acids were analyzed with HPLC. After SS administration, the amplitude and frequency of the cortical EEG increased significantly ($p<0.05$) when compared to baseline, whereas the changes of the hippocampal EEG were minimal. The hippocampal extracellular levels of excitatory amino acids glutamate and aspartate increased significantly ($p<0.05$) at 20, 40, and/or 60 minutes after the administration of 20 μg and 40 μg of SS. In contrast, there was only a slight increase in GABA ($p<0.05$) only at 40 minutes after the first dose of SS, and a small increase in glycine ($p<0.05$) only at 40 minutes after the second dose of SS. These results indicate that SS increases cortical neuronal excitability when applied to the hippocampus, and that this effect is probably due to the dissociated response of the excitatory and inhibitory amino acids. Thus, we hypothesize that neuropeptides, in particular SS, play a role in epileptogenesis by modulating amino acid neurotransmission. *(Funded by Laboratorios AUSONIA-Spain, and St Christopher's Hospital for Children-Academic Development Fund).*

599.17

DIFFERENTIAL NEONATAL ONTOGENY OF HEIGHTENED SEIZURE SENSITIVITY IN TWO STRAINS OF GENETICALLY EPILEPSY-PRONE RATS (GEPRs). C.E. Reigel* and B-K Lin. Dept. of Pharmacol., Texas Tech Univ. Health Sci. Ctr., Lubbock, TX 79430

The GEPR was derived by selective breeding of Sprague-Dawley (SD) rats for audiogenic seizure (AGS) susceptibility and currently exists as two strains. GEPR-9s exhibit full tonic extensor convulsions whereas GEPR-3s exhibit generalized clonus, each in response to sound. GEPRs also exhibit heightened sensitivity to exogenous seizure provoking stimuli, even at ages prior to AGS susceptibility. This study was conducted to determine the ontogeny of heightened seizure sensitivity in GEPRs. Flurothyl seizure thresholds were determined for paddling and tonic seizures at postnatal day (PD) 1, 3, 5, 7 and 10 in GEPR-9, GEPR-3 and SD control pups. Paddling seizures occurred at lower doses of flurothyl than tonic seizures at any age. Flurothyl elicited a high incidence of tonic seizures in SD pups at PD 1 and 3. Tonic seizures disappeared in SD pups by PD 7. Tonic seizures were elicited in GEPRs from PD 1-10. GEPR-9s exhibited lower paddling and tonic seizure thresholds than GEPR-3s and SDs from PD 1-10. Seizure severity was greater in GEPR-9s than SDs from PD 1-10. Seizure severity was greater in GEPR-9s than GEPR-3s from PD 1-5 and thereafter similar. Paddling seizure thresholds were actually higher in GEPR-3s than SDs at PD 1. Thereafter (PD 3-10), paddling thresholds were equivalent in GEPR-3s and SDs. Tonic seizure thresholds were equal in GEPR-3s and SDs from PD 1-5. Tonic seizures disappeared in SDs by PD 7. Seizure severity was equal in GEPR-3s and SDs at PD 1-3 and greater in GEPR-3s than SDs at PD 5-10. Thus, heightened seizure sensitivity developed postnatally in the GEPR-3, appearing at PD 5 or 7. Heightened seizure sensitivity was fully present in GEPR-9s by PD 1. (Supported by NS 28118.)

599.18

EFFECT OF NEONATAL CASTRATION AND ANDROGEN TREATMENT ON ELECTRICALLY-INDUCED SEIZURES IN THE ADULT RAT. M.L. Maring*, R.W. Clough[1] and R.A. Browning, Depts. of Physiology and Anatomy[1], Southern Illinois University, Carbondale, IL 62901.

Gonadal hormones exert marked effects on seizure susceptibility and severity in both humans and experimental animals. Specifically, estrogen has seizure-promoting actions, while progesterone exerts anticonvulsant effects. Testosterone is proconvulsant in some seizure models and anticonvulsant in others. In addition, there are gender-specific differences in seizure susceptibility and severity in adult rats, with females displaying greater seizure susceptibility and severity than males. It is unclear whether gender differences in seizures are due to the activational effects of steroids in the adult brain or to organizational effects of gonadal steroids in the neonatal developing brain. To investigate the role of gonadal hormones during development in gender-specific seizure display of adults, the effects of neonatal castration of male rats and androgen treatment of female rats on minimal electroshock seizure threshold (EST) and maximal electroshock seizure (MES) pattern were determined. Male Sprague-Dawley rat pups were orchidectomized and female pups were given testosterone propionate (TP, 1.25 mg, subcutaneously), 4 days after birth. EST and MES seizure responses were evaluated at >100 days of age. Neonatal castration of males significantly reduced EST from 21.2 mA in intact males to 19.8 mA in castrated males ($p<.01$). Castration was also found to increase the incidence of tonic hindlimb extension (HLE) in the MES test (100% HLE in castrates vs 50% HLE in intacts; $p<.05$) In addition, castration decreased the latency to HLE ($p<.05$), providing further evidence of increased seizure susceptibility. With the TP treatment strategy and dose employed, no differences were observed between neonatally androgenized and vehicle-treated females. Thus, it is hypothesized that testicular secretions during the critical period of neonatal development may be necessary for the expression of male-typical seizure patterns in the adult. The lack of an anticonvulsant effect in adult females following neonatal androgen exposure may be due to sexual divergence in brain development occurring prior to 4 days postpartum. Further study is needed to characterize the nature of the development of sexual divergence with respect to seizure behavior.

599.19

EFFECTS OF ANTERIOR PERIRHINAL CORTEX MICROINFUSIONS OF NMDA ANTAGONISTS AND PROCAINE ON AMYGDALA-KINDLED SEIZURES. D.K. Bilkey* and K.H. Holmes. Department of Psychology and the Neuroscience Centre, University of Otago, Box 56, Dunedin, New Zealand.

Holmes et al (*Brain Res.* 587:285, 1992) have recently demonstrated that microinfusion of the N-methyl-D-Aspartate (NMDA) antagonist 2-amino-5-phosphono-valerate (APV, 1 ul, 70 mM) into the anterior region of perirhinal (insular) cortex (PRC) produces a powerful block of an amygdala-kindled seizure elicited 5-10 minutes after the microinfusion. The most parsimonious explanation of these results is that a recurrent excitatory link between the amygdala and PRC is activated during kindling, thus allowing regenerative, NMDA-dependent excitatory activity to develop. One prediction of this model is that a lesion of the PRC should damage the recurrent circuit and suppress the kindled seizure. In order to test this hypothesis we monitored the effects of creating a functional lesion of perirhinal cortex by infusing procaine (1ul, 20%) into the PRC of five fully-kindled Sprague-Dawley rats in which NMDA antagonists had previously produced a seizure-modulating effect. Whereas 1ul infusions of both carboxypiperazine-phosphonate (CPP, 1mM) and APV had produced a large and statistically significant reduction in seizure stage and afterdischarge, procaine had only a small and non-significant effect. In contrast, procaine microinfusion into either the deep pyriform cortex (n=6) or the piriform cortex (n=5) of separate groups of fully kindled animals produced significant reductions in both seizure stage and AD duration. The above findings suggest that the relationship between amygdala and PRC is more complex than initially hypothesised. Alternative models of this interaction are currently being developed. *Supported by grants from the New Zealand Neurological Foundation.*

599.20

NMDA & NON-NMDA RECEPTOR INVOLVEMENT IN LOW MG2+-INDUCED BURSTING IN HIPPOCAMPAL AREAS CA1 AND CA3. R.F. Berman[1]* and C.A. Janusz[2]. Depts. of [1]Psychology and [1,2]OB/GYN, Wayne State Univ., Detroit, MI 48201.

Hippocampal brain slices from rats were used to characterize the effects of magnesium, MK-801, a non-competitive NMDA antagonist, and CNQX, a non-NMDA glutamate receptor antagonist under conditions of low Mg^{2+}-induced epileptiform bursting. Removal of Mg^{2+} from the superfusion medium of slices produced spontaneous and triggered bursts in both the CA1 and CA3 areas of the hippocampus, although the overall development and characteristics of bursting were different for the 2 areas. The CA1 area was more sensitive to the re-addition of Mg^{2+} to the bathing medium, as bursting could be inhibited with very low concentrations of Mg^{2+}. The amplitude of the population spike (PS) remained elevated even after return to normal Mg^{2+} conditions in both areas-indicating the development of LTP. MK-801, at a concentration that did not affect baseline synaptic activity, blocked bursting and LTP, but did not prevent an initial increase in the amplitude of the PS. CNQX blocked bursting as well as the initial increase in the PS. We conclude that an NMDA component, as well as a non-NMDA component, are involved in the enhanced synaptic activity under Mg^{2+}-free conditions.

DEGENERATIVE DISEASE: ALZHEIMER'S- β-AMYLOID XI

600.1

A HEPARIN-BINDING DOMAIN IN AMYLOID PROTEIN PRECURSOR (APP) IS INVOLVED IN THE REGULATION OF NEURITE OUTGROWTH. D.H. Small[1], V. Nurcombe[2], G. Reed[1], H. Clarris[1], N.S. Cheung[3], B.G. Livett[3], K. Beyreuther[4] & C.L. Masters[1]. Depts. of [1]Pathology, [2]Anatomy and Cell Biology and [3]Biochemistry, University of Melbourne, Parkville, Vic. 3052, Australia and [4]Centre for Molecular Biology, Heidelberg, Germany.

While the identification of rare mutations in the APP gene has demonstrated the importance of APP in the pathogenesis of Alzheimer's disease (AD), the precise role of APP has yet to be established. One possibility is that certain forms of the amyloid protein (βA4) are neurotoxic. βA4 and homologous peptides reportedly enhance glutamate-mediated neurotoxicity, whereas intact APP may be neuroprotective. A defect in the normal function or cleavage of APP might therefore contribute to the pathogenesis of AD.

In the present study, substratum-bound APP dramatically increased the rate of neurite outgrowth from sympathetic and hippocampal neurons in culture. This effect was dependent upon the presence of heparan sulfate proteoglycans (HSPGs) purified from postnatal brain. A heparin-binding site was identified close the the N-terminus of APP (residues 96-110), using molecular modeling and site-directed mutagenesis. A peptide homologous to this domain was found to bind strongly to heparin and to block the neurite outgrowth-promoting effect of APP in culture. The results suggest that the binding of secreted APP to HSPGs in the extracellular matrix may stimulate the neurite outgrowth-promoting function of APP. A defect in the ability of APP to bind to HSPGs, perhaps due to defects in proteolytic processing, may contribute to the neurodegenerative process which occurs in AD.

600.2

CHONDROITIN SULFATE PROTEOGLYCAN FORM OF THE ALZHEIMER'S B-AMYLOID PRECURSOR IN BRAIN. D. Vassilacopoulou[1], J. Ripellino[1], J. Shioi[1], M. Pangalos[1], S. Efthimiopoulos[1], R. Margolis[2], C. Mytilineou[3]*, N. Robakis[1]. Dept. of [1]Psychiatry and Fishberg Ctr. Neurobiol., and [3]Neurol., Mount Sinai Med. Ctr., [2]Dept. of Pharmacology, NYU, Med. Ctr. NY, NY.

Chondroitin sulfate proteoglycans (CSPG) are molecules with diverse biological functions including cell adhesion and cell-cell communication. Recently we showed that in a glial cell line Amyloid Precursor Protein (APP) exists as the core protein of a CSPG of about 140-250 kDa (Shioi et al., 1992, J. Biol. Chem. 267: 13819). Here we report the existence of CSPG-APP in rat and human brains. Brain tissue was homogenized, centrifuged and the resulting soluble fraction was applied to a DEAE Sepharose column and eluted with NaCl. Samples were digested with chondroitinase ABC and analyzed by SDS-PAGE/western blot using anti-APP antisera. Soluble CSPG-APP was detected and following chondroitinase treatment the high molecular weight PG was completely eliminated with a concomitant increase in the APP protein. To further investigate the cellular origin of CSPG-APP, rat neuronal and glial primary cultures were labelled with ^{35}S-sulfate. Conditioned media were immunoprecipitated with anti-APP antisera and analyzed by SDS-PAGE/fluorography. The CSPG-APP form was detected in glial but not in neuronal cultures. However, since we detected the CSPG-APP in neuroblastoma cell line N2a, we cannot exclude the possibility of neuronal expression for this molecule. Our findings suggest that the PG nature of APP may be important for the implementation of its biological function and may play a role in the development of Alzheimer's disease.

600.3

CHONDROITIN SULFATE PROTEOGLYCANS ARE A COMMON COMPONENT OF INCLUSIONS IN NEURODEGENERATIVE DISEASES

D.A. DeWitt, P. Richey, J. Silver, and G. Perry*. Case Western Reserve University, Cleveland, OH 44106

In order to determine whether chondroitin sulfate proteoglycans (CSPG) were present in lesions of Alzheimer's disease (AD), three monoclonal antibodies each to different forms of the chondroitin glycosaminoglycan were used. Sections from methacarn-fixed, paraffin-embedded tissue were treated with chondroitinase ABC to remove glycans and expose the antigenic site. Senile plaques (SP) were identified by Congo red or with antibodies to Aβ; neurofibrillary tangles (NFTs) and dystrophic neurites with antisera to τ. Unsulfated chondroitin (1B5) was found in NFTs and dystrophic neurites arround SPs. Chondroitin 4-sulfate (2B6), and chondroitin 6 sulfate (3B3) were localized to the extracellular matrix surrounding senile plaques, to dystrophic neurites and to NFTs. No immunostaining was observed when chondroitinase was absent or replaced with heparinase or heparitinase. The enzyme specificity indicates that the antibody binding is specific for chondroitin. We wanted to further describe CSPG immunoreactivity to determine whether it was associated with non-Alzheimer inclusions. Parkinson Lewy bodies and NFTs of progressive supranuclear palsy contained only chondroitin 4-sulfate and chondroitin 6-sulfate, however, diffuse Lewy bodies and Pick bodies contained all three forms of chondroitin. In order to determine whether neuronal CSPGs were associated with dying neurons lacking inclusions, the caudate of a Huntington's patient was examined. Neuons did not show chondroitin immunoreactivity suggesting that neuronal CSPGs were usually associated with inclusions but not always neuronal death. The presence of CSPG and other proteoglycans in many kinds of inclusions and the extracellular matrix of AD suggests a wider role for this family of molecules in the pathogenesis of neurodegenerative diseases. Supported by NIH grants AG 07552 and AG 09287.

600.4

ß-AMYLOID PRECURSOR PROTEIN IS A HEPARAN AND CHONDROITIN SULFATE PROTEOGLYCAN IN BRAIN.

Olga Salinero, Maria Araujo, Juan José Garrido, Laura M. Lopez-Mascaraque* and Francisco Wandosell.
Neurodegeneration Group.Inst. Cajal. Avd. Dr. Arce 37 CSIC
Madrid 28002 Spain

Extracellular deposition of ß4A amyloid peptide is the most important feature of Alzheimer disease, and could be the base of neuronal degeneration. The ß4A is derived from a family of membrane glycoproteins, termed amyloid precursor proteins (APP). After glycosylation APP's are cleaved at the extracellular portion and secreted. We wish to analyze glycosylation of APP's in normal brain, and how after brain injury, these modifications can change. First, we have analyzed the glycosylation state of the APP in normal adults rat brains. We have selected APP using affinity chromatography from soluble and membrane fractions, from hippocampi and total brain. These fractions were treated with several glycosidases and analyzed by Western-blots with some antibodies against different regions of APP. Our data show clearly the presence of two populations of APP, chondroitin and heparan sulfate proteoglycans. Second, we have studied the expression of APP after controled hippocampal damage, by intraventricular injection of glutamate analogs. We have used different antibodies against APP, in order to follow the time-course of the damage-induced APP expression.The number, intensity and the type of the labelled cells were different depending on the glutamate analogs used. The glycosylation pattern obtained from these damaged tissue show some differences from control brains.Supported by grants from *CAM*192/90 and *DGYCT* PB90-0160. *O.S.* is supported by a fellowship from *M.E.C.*

600.5

POTENTIAL ROLE OF PROTEOGLYCANS IN THE ACCUMULATION AND PERSISTANCE OF ß-AMYLOID (Aß) IN ALZHEIMERS DISEASE (AD) SENILE PLAQUES.

R. Gupta-Bansal, W. Ziehler, J. R. Wujek* and K. R. Brunden, Gliatech, Inc., Cleveland, Ohio 44122.

The presence of senile plaques is a characteristic pathological feature of AD brain. In addition to large quantities of Aß, senile plaques contain other components including chondroitin, heparan and dermatan sulfate proteoglycans. Proteases may exist within the brain whose normal function is to degrade Aß before it can form deposits. These proteases may be inhibited by the specific association of plaque components with Aß. We have examined the ability of several proteases to degrade Aß in the presence of proteoglycans, and find that both chondroitin and heparan sulfate proteoglycans protect Aß from proteolytic action. Aß and chondroitin/heparan sulfate proteoglycans were allowed to interact at pH 6.5 or 7.4 in 100 mM Tris at room temperature. The mixture was digested for 18 hours at 37°C with either papain or pronase at an enzyme-to-protein ratio of 1:10 (w/w) and analyzed by 16.5% Tris-Tricine SDS-PAGE. Subsequently, the amount of proteolyzed Aß was quantitated by densitometry. The data reveal that fibrillar Aß is more resistant to proteolytic attack than non-fibrillar peptide. Furthermore, proteoglycans protect Aß from degradation to a greater extent when the amyloid peptide is in a fibrillar structure compared to a non-fibrillar form.

Since the source of proteases that might degrade amyloid in the brain has not been identified, it is important to determine which cells secrete proteases that may play a role in Aß degradation. We have investigated the ability of astrocyte and microglia conditioned medium to proteolyze Aß in vitro. Our results suggest that microglia secrete proteases that are capable of degrading Aß. Further studies will determine whether proteoglycans protect Aß from microglial protease(s).

600.6

INFLAMMATORY FACTORS WHICH ALTER THE METABOLISM OF PROTEOGLYCANS THAT BIND A4 PEPTIDE MAY PROMOTE AMYLOIDOGENESIS.

B. Leveugle, L. Buee, W. Ding and H. Fillit*. Department of Geriatrics, Mt Sinai Medical Center, New York, NY 10029

High binding affinity occurs between APP/A4 and heparan sulfate proteoglycans (PGs). Although these interactions may have a physiologic function in regulating cell adhesion or neurite outgrowth, the pathophysiologic events which lead to codeposition of A4 and PGs into amyloid plaques are not known. We hypothesized that PG metabolism may be altered in Alzheimer's disease (AD) as a result of the acute phase response in AD brains. Thus, we investigated wether IL-1 and NGF alter the metabolism of neuroblastoma PGs wich bind A4. PGs were purified by anion exchange chromatography on a DEAE column. The chromatograms showed that the net charge of PGs secreted into the culture medium or associated with the cells is increased after stimulation of the cells with IL-1 or NGF. In addition, the amount of PGs secreted into the culture medium increased whereas there is no such change in the cell associated PGs. Change in PG sulfation may alter PG binding affinity to A4 and enhance alternative APP proteolysis and amyloidogenesis. Increased secretion of PG induced by NGF/IL-1 may promote extracellular accumulation of amyloid.

600.7

Interleukin-1 beta alters the degree of sulfation of heparan sulfate proteoglycan in endothelial cells and may contribute to the process of amyloidosis in blood vessels.

Jesse G. Eisler*[1,2], Beatrice Leuveugle[2], Wan Hong Ding[2], Howard Fillit[1,2], Fishberg Center for Neurobiology[1], and the Department of Geriatrics[2], Mount Sinai School of Medicine, One Gustave Levy Place, New York, NY 10029

The glycosaminoglycan components of proteoglycans contain variable numbers of sulfate groups which confer some of the special binding properties of proteoglycans. Recently, it has been shown that the binding of the A4 peptide to heparan sulfate proteoglycan (HSPG) may be mediated by the sulfate groups on HSPG [Fraser et al., J. Neurochem. 59:1531. 1992, Buee et al., Brain Res. 601:154, 1993]. We examined the role of interleukin-1ß (IL-1ß) on the level of sulfation of proteoglycans secreted from endothelial cells grown in culture. Mouse aortic endothelial cells were grown to confluence in DMEM medium with 10% fetal calf serum. The medium was then replaced by serum free medium and the cells labelled with ^{35}S Na_2SO_4 (10µCi/ml) and IL-1ß (10 ng/ml) for 48 hours. The cell culture medium was removed dialyzed against 7M urea, 0.15 M NaCl, 0.05 M NaOAc, 0.5% CHAPS, pH 6.0. The samples were then analyzed by ion-exchange chromatography with DEAE Sepharose CL-6B using a linear salt gradient from 0.15 M NaCl to 2.0 M NaCl. IL-1ß added to endothelial cells significantly altered the DEAE elution profile of proteoglycans secreted into the supernatant. In controls, two peaks were obtained at 0.33 M (peak A) and 0.45 M NaCl (peak B). IL-1ß caused a shift in the elution of peak A to 0.28 M NaCl and a shift in the elution of peak B to 0.5 M NaCl. The difference in the level of salt elution can be explained by an alteration in the level of sulfation of these molecules. Specific changes in sites of sulfation may alter the affinity of the heparan sulfate proteoglycans from capillary endothelial cells for the A4 peptide and promote vascular amyloidogenesis.

600.8

ß-AMYLOID PEPTIDE INTERACTION WITH GLYCOS-AMINOGLYCANS.

K.R. Brunden, N.J. Richter-Cook, N. Chaturvedi, and R.C.A. Frederickson* Gliatech Inc., Cleveland, Ohio 44122

The senile plaques found within Alzheimer's disease brain contain proteoglycans in addition to ß-amyloid peptide (Aß). Here we have examined the ability of synthetic Aß peptides to bind to the glycosaminoglycan (GAG) moieties of proteoglycans. Both Aß(1-28) and Aß(1-40) associate with heparan sulfate and chondroitin sulfate, and the interaction of GAGs with these peptides results in the formation of large aggregates that sediment upon centrifugation. The precipitation of the amyloid peptides with GAGs is pH-dependent, with increasing interaction as pH values fall below neutrality. In addition, Aß(1-28) interacts with a heparin affinity column at pH 4.0 but not at pH 8.0. The pH profile of Aß-GAG association suggests that a consensus heparin-binding domain that contains histidine residues at positions 13 and 14 of Aß may be the site of binding. The binding of GAGs to Aß appears to be ionic in nature, as dextran sulfate associates with Aß(1-28) whereas dextran does not. The interaction of GAGs with a defined motif of Aß may be relevant to the etiology and pathology of Alzheimer's disease.

600.9

HEPARAN SULFATE PROTEOGLYCAN (HSPG) CORE PROTEIN IN SENILE PLAQUES AND CEREBROVASCULAR AMYLOID OF AGED MONKEYS.

L.C. Walker[1] and H.M. Fillit[2]. [1]The Johns Hopkins Univ. Sch. of Med., Balto., MD 21205 and [2]Mt. Sinai Medical Center, New York, NY 10029.

HSPG, a component of the vascular basal lamina, is associated with some amyloid deposits in Alzheimer's disease (AD) and Down's syndrome (Snow et al., *Am. J. Pathol.* **137**:1253, 1990). HSPG binds with high affinity to ß-amyloid (Aß) and to some isoforms of the Aß precursor protein (Buee et al., *Brain Res.* **601**:154, 1993; Narindrasorasak et al., *J. Biol Chem.* **266**:12878, 1991) and, thus, may be involved in the deposition of Aß in the cores of senile plaques and in cerebral blood vessels. To determine whether HSPG is associated with Aß in nonhuman primates, we used a monoclonal antibody (7E12) to the core protein of HSPG and a polyclonal antibody that recognizes both the core protein and HS glycosaminoglycans to stain tissues from three aged rhesus monkeys (*Macaca mulatta*) and one aged squirrel monkey (*Saimiri sciureus*). In rhesus monkeys, HSPG immunoreactivity was associated with a subset (~25%) of senile plaques, cerebrovascular Aß, and diffuse Aß. Preabsorption of 7E12 with purified basal lamina HSPG eliminated immunoreactivity associated with Aß and with the vascular basal lamina. In the squirrel monkey, Aß-associated HSPG was stained only by the polyclonal antibody. Because, in monkeys as in humans with AD, HSPG is in mature and diffuse plaques, these data indicate that HSPG deposition occurs in the early stages of amyloidogenesis and is persistent. The absence of staining for this specific basal lamina HSPG core protein in many lesions suggests that this molecule may characterize a subset of Aß deposits with a unique cellular origin. Aged monkeys may be a useful model to study these issues as well as the possible role of HSPG in the formation of senile plaques and cerebrovascular amyloid.

600.10

FATE OF MEMBRANE-ASSOCIATED FORMS OF THE AMYLOID PROTEIN PRECURSOR (APP) OF ALZHEIMER'S DISEASE IN HUMAN PLATELETS DURING ACTIVATION.

O.-X. Li, M. Berndt, A. Friedhuber, K. Beyreuther and C. L. Masters*. Departments of Pathology, The University of Melbourne, and the Mental Health Research Institute of Victoria, Australia; The Baker Research Institute, Melbourne; Center for Molecular Biology, University of Heidelberg, Germany

Alzheimer's disease (AD) is characterized pathologically by the deposition of βA4 protein which is proteolytically derived from part of the transmembrane domain of APP by an unknown mechanism. Study of the structure and function of the membrane-associated forms of APP may help to elucidate this process. We have shown that human platelets contain full-length APP in addition to abundant soluble APP. Full-length APP containing the βA4 sequence is of interest as it may contribute to βA4 amyloidogenesis. Immuno-electron microscopy showed APP to be localized in α-granules as well as on the cell surface. Surface labeling with iodine showed both the full-length APP and the C-terminal truncated APP were present on platelet surface. Labelled [^{125}I]-mAb 22C11 was used to demonstrate a 2.5-fold increase surface expression of APP during thrombin activation of platelets. Activation by thrombin also resulted in the loss of the cytoplasmic domain of the full-length APP. The precise cleavage site is yet to be determined. Studying the metabolism of the amyloid containing APP species in platelets may give insight into the metabolism of APP in neurons.

600.11

CYTOSOLIC APPS ARE DECREASED FOLLOWING EXCITOTOXIC INSULT.

L. Kent, C. Zobre, M. Lewis, M. Kelley, M. Miller, D. DeHaven-Hudkins, L. Fleissner, K. Rogers*. Sterling Winthrop Pharmaceuticals Research Division, Collegeville, PA 19426.

A hallmark of Alzheimer's Disease (AD) is neuronal degeneration in association and temporal cortex. These regions contain glutaminergic neurons which appear to degenerate in AD, possibly as a result of excitotoxic insult. We hypothesized that excitotoxic damage might alter APP processing, leading to βA4 deposition. This hypothesis was tested by treating mouse cortical neuronal cultures with 500 μM NMDA for 0.5 hr and quantifying APPs by Western blot analyses using APP C-terminal directed antisera. A rapid 2-fold decrease in cytosolic APPs was observed at times which preceded markers of neuronal injury (ie. LDH release). MK801 prevented both the decrease in cytosolic APPs and the subsequent neuronal injury. Decreases in APPs were concentration and time-dependent. Similar decreases in membrane associated APPs were observed. Future studies will determine whether the decreases in APPs following injury are associated with concomitant increases in βA4 or other APP fragments.

600.12

AN HYPOTHESIS CONCERNING DISTURBED AXOPLASMIC FLOW AS A COMMON PATHOGENIC MECHANISM LINKING AMYLOIDOGENESIS AND TANGLE FORMATION IN ALZHEIMER'S DISEASE.

K. Shigematsu*,[1], E.G. McGeer[2], H. Sugiyama[1], Y. Kamioka[1], and P.L. McGeer[2]. [1]Dept. Neurol., Natl. Minami Kyoto Hospital, Kyoto, Japan, and [2]Kinsmen Laboratory of Neurological Research, Univ. of B.C., Vancouver, B.C., Canada.

The relationship between the two pathological hallmarks of Alzheimer's disease, intracellular neurofibrillary tangles and extracellular amyloid deposits is still a mystery. Tangles are characterized by argentophilicity and Alz-50 positivity, while amyloid deposits are characterized by accumulations of β-amyloid protein, derived from the amyloid precursor protein (APP). We hypothesized that a disturbance of axoplasmic flow may be the common pathogenic mechanism linking these main two pathological entities. To test this hypothesis, we injected kainic acid, colchicine or aluminum salts into rat brain as independent methods of damaging neurons and interfering with axoplasmic flow. We then examined brain sections by silver staining and by immunohistochemical staining for APP and Alz-50. In each case, affected neurons accumulated APP within hours and often showed Alz-50 immuno-positivity as well as an argentophilic reaction. After several days, APP immunoreactivity was seen extraneuronally, some within phagocytosing reactive microglia. These results support the hypothesis that an interference with axoplasmic flow could link cytoskeletal abnormalities and APP accumulation. Microglia may also play a role in disposing of excess neuronally produced APP. However, in these animal models, no production of β-amyloid protein from APP was noted.

600.13

MICROTUBULE-ASSOCIATED PROTEIN 2-LIKE IMMUNOREACTIVITY IN SENILE PLAQUES OF ALZHEIMER DISEASE BRAIN.

O.Yasuhara, I. Tooyama[1], T. Kawamata, K. Hanai[1], H. Kimura[1], P.L. McGeer, and E.G. McGeer*. Kinsmen Laboratory of Neurological Research, University of British Columbia, Vancouver, Canada, V6T1Z3; [1]Institute of Molecular Neurobiology, Shiga University of Medical Science, Otsu, Shiga, Japan

Immunohistochemical localization of microtubule-associated protein 2 (MAP2) was examined in Alzheimer disease and control brain, using two monoclonal antibodies to MAP2. Staining of neuronal cell bodies and dendrites was observed in both Alzheimer disease and control brain, which was abolished by pretreatment of sections with trypsin. Senile plaques in Alzheimer brain tissue were labeled by only one of the antibodies to MAP2. The MAP2 staining of plaques, but not somato-dendrites, was dramatically enhanced by formic acid pre-treatment of sections. However, plaque staining was relatively resistant to trypsin digestion, indicating reduced accessibility of the epitope incorporated into amyloid deposits. By a combination of pretreatment with formic acid and a low concentration of trypsin, all plaques, as well as diffuse amyloid deposits, became immunoreactive for an epitope of MAP2. Neurofibrillary tangles, dystrophic neurites and neuropil threads were not labeled with either antibody. The present study indicates that a MAP2 fragment is incorporated into amyloid deposits at an early stage of their formation. The data provide supporting evidence in favor of a neuronal origin of plaque amyloid.

Supported by grants from the Japan Foundation for Aging and Mental Health and the Alzheimer Society of B.C.

600.14

IDENTIFICATION OF PROTEINS THAT BIND THE CYTOPLASMIC DOMAIN OF AMYLOID PRECURSOR PROTEIN.

L. A. Flanagan* and T. Saitoh. Dept. of Neuroscience, UCSD, La Jolla, CA 92093.

Much remains to be learned about the function, processing, and intracellular targeting of amyloid precursor protein (APP). Since APP is a transmembrane protein, intracellular proteins that bind its cytoplasmic domain will be critical for these biological processes. For example, APP has been suggested to interact with the cytoskeleton and has been shown to undergo endocytosis; both of which require binding to intracellular proteins. For this reason we have developed a system to identify proteins from a variety of sources, including human brain, that specifically bind the cytoplasmic domain of APP in biochemical assays. Utilizing a bacterial expression system we have produced the cytoplasmic domain of APP as a fusion protein with protein A, which enables a rapid purification of the protein for binding experiments. Purified, radiolabelled fusion protein has been used as a probe in blot overlay experiments. In addition, unlabelled fusion protein has been immobilized on columns for affinity chromatography. Preliminary evidence utilizing protein A as a negative control indicates that these methods will allow us to identify proteins that specifically bind to the cytoplasmic domain of APP. The analysis of these proteins will enable us to further reveal the biochemical mechanisms underlying the biological activity of APP.

601.1

CHANGES IN THE Na^+/Ca^{2+} EXCHANGER GENE EXPRESSION IN AGING RAT BRAIN AND IN HUMAN BRAINS WITH ALZHEIMER'S PATHOLOGY. V.Janapati*, L.Yu and R.A.Colvin. Department of Biological Sciences, Ohio University College of Osteopathic Medicine, Athens, OH-45701.

Na^+/Ca^{2+} exchanger plays critical role in regulating the Ca^{2+} homeostasis in excitable cells like neurons and myocytes. Alterations in the Na^+/Ca^{2+} exchange activity can be responsible for the functional deterioration of the cells in aging and disease. We have initiated studies on the expression of the exchanger in aging rat brain and in human brains with Alzheimer's pathology. Based on the published cDNA sequence for the human heart Na^+/Ca^{2+} exchanger (Komuro I. et al., PNAS 89, 4769-4773) we have generated a probe by PCR. RNA and DNA probes were used to perform the analysis of the RNA preparations made from rat brains of different ages from 6 months to 24 months of age and also in normal human brains and brains with Alzheimer's pathology. Northern blot analysis of the total RNA extracted from whole rat brain (Sprague-Dawley) reveals the presence of two transcripts for the exchanger corresponding to sizes 15kb and 7kb. In cerebral cortex the two transcripts are almost in equal intensity at all the ages studied (6,12, 18 and 24 months) and no significant age dependent change in the intensity of the bands is observed. In cerebellum the 7kb transcript is predominant at all the ages and it appears to decrease with age. The 15kb transcript is clearly seen only at 12 months of age. No transcripts were identified on norther blots with RNA from human brain tissues. We suspect that the exchanger levels may be either lower than detectable or that the RNA prepared from postmortem tissue was degraded. We performed RNase protection assay with human brain RNA preparations with cRNA probes for the exchanger and beta actin. We observed that the ratio of exchanger/actin is lower in Alzheimer's brain compared to that of age matched normal brain. These preliminary studies have shown evidence of changes in gene expression for the Na^+/Ca^{2+} exchanger in normal aging and Alzheimer's disease.

601.2

GAP-43 MESSAGE IS DECREASED IN TANGLE-BEARING NEURONS OF ALZHEIMER'S DISEASE (AD) PARAHIPPOCAMPAL GYRUS (Callahan, L.M.*, Cheetham, J.E., Selski, D., Martzen, M.R. and Coleman, P.D. University of Rochester Medical Center, Neurobiology and Anatomy, Rochester, NY 14642).

Our lab recently reported a decrease in Gap-43 message, based on slot blot analysis, in superior frontal gyrus of AD patients containing high neurofibrillary tangle (NFT) densities (Coleman et al., 1992). This suggested tangle-bearing neurons contained a decreased level of Gap-43 message.

The study reported here combined immunocytochemistry for NFTs with in situ hybridization for Gap-43 message on the same tissue section. NFT-bearing neurons were identified and their grain density (for Gap-43 message) compared to that of non-tangle bearing neurons within the same microscopic field. Gap-43 message appeared dramatically decreased in a majority of tangle-bearing neurons compared to non-tangle bearing neighbors in parahippocampal regions of AD patients. Preliminary semiquantitive analysis of tangle-bearing neurons indicated 80% showed a decrease in Gap-43 message level.

Study of neurodegenerative diseases is challenged by the need to identify the neurons actually affected by the disease. In AD, neurons containing tangles often appear next to neurons which morphologically appear healthy. Decreased Gap-43 in tangle-bearing neurons may indicate a subpopulation of neurons exhibiting decreased plasticity in AD. (Supported by NIH AG00107,NIH AG01121, NIH LEAD AWARD AG09016, Alzheimer's Disease Center Grant NIH AG08665, and the American Health Assistance Foundation)

601.3

LEVELS OF GAP-43 MESSAGE IN ANTERIOR CEREBELLUM APPEAR UNCHANGED IN ALZHEIMER'S DISEASE COMPARED TO CONTROLS. J. E. Cheetham, M. R. Martzen*, L. Callahan and P. D. Coleman. University of Rochester School of Medicine and Dentistry, Dept. of Neurobiology and Anatomy, Rochester, NY 14642.

We have found that decreased GAP-43 message in frontal association cortex-area 9 is associated with increased density of neurofibrillary tangles (NFTs), (Coleman *et al*., 1992). This finding leads to the hypothesis that decreased GAP-43 message in AD is related to NFTs, rather than to some other aspect of AD pathology. Therefore, we predicted that in areas of brain unaffected by NFTs in AD the GAP-43 message levels should be similar to those of controls. The cerebellum is known to have a number of pathologies of AD, including diffuse plaques, microglial activation and reactive astrocytes, however, NFTs are not found in cerebellum. mRNA was extracted from anterior cerebellum and hybridized with a GAP-43 probe which was obtained by polymerase chain reaction from a human brain cDNA library. Normalization probes of poly dT and G3PDH were obtained from Pharmacia and Clontech respectively. To quantify message levels, aliquots of mRNA were slot blotted and hybridized with the appropriate probe. Autoradiograms were quantified by scanning laser densitometry. In cases examined to date, GAP-43 message levels in the anterior cerebellum of Alzheimer's cases appear to be not statistically different relative to age matched controls.

These data are consistent with NFTs playing a role in modulating the expression of GAP-43. Preliminary data from combined IHC for identification of tangle bearing neurons and ISH for localization of GAP-43 message (see abstract by Callahan *et al*.) confirms and supports this conclusion.

This work was supported by grants from the following: LEAD award (AG09016), R01 (AG01121), Alzheimer's Disease Centre (AG08665), American Health Assistance Foundation and a Neurobiology of Aging Training Grant (AG00107).

References. Coleman P. D., *et al.* (1992) Neurobiology of Aging, 13, 631-639.

601.4

ALTERATIONS IN GROWTH-ASSOCIATED PROTEINS OCCUR EARLY IN ALZHEIMER'S DISEASE. E.Masliah*, M. Mallory, M.Alford, T.Saitoh. Dept. Neurosciences, University of California, San Diego, La Jolla, CA 92093-0624

Although significant information is now available as to the possible progression of the neuropathological alterations in Alzheimer's disease (AD), the molecular events that occur in early stages of AD are not well known. We hypothesize that alterations in the patterns of expression of growth-related proteins occur early in the development of AD, and that they are related to the synaptic pathology in the hippocampus in early stages of AD. In order to test these hypotheses, we compared the relative levels and distribution of growth-related proteins and their mRNA in cases with incipient, mild and advanced AD. Cases with incipient AD presented increased amyloid precursor protein (APP), growth-associated protein 43 and platelet-derived growth factor (PDGF) immunoreactivity in the entorhinal cortex and hippocampus. These cases also displayed minimal cognitive alterations, variable numbers of diffuse amyloid deposits, tangles in the entorhinal region, and mild synaptic loss in the outer molecular layer of the hippocampus. Cases with mild and advanced AD presented moderate to severe synaptic loss in the hippocampus and neocortex. Furthermore, the antibody against PDGF-BB and p105 (a nuclear protein associated with proliferation and plasticity) immunolabeled the neurofibrillary tangles and neuritic plaques in the different stages of AD. *In situ* hybridization studies with digoxigenin-labeled oligo probes (40 mer) also showed increased message for APP and PDGF in cases with incipient AD. The involvement of growth-associated proteins in AD favors the possibility that in the early stages of the disease and abnormal sprouting response is mounted in response to the ongoing synaptic and cytoskeletal alterations.

601.5

CYTOKINE INDICES IN ALZHEIMER'S DISEASE BRAIN: INCREASES IN IL-6 AND THE ASSOCIATED ACUTE PHASE PROTEINS; ALPHA 2-MACROGLOBULIN AND C-REACTIVE PROTEIN. Julie A. Wood*, Paul L. Wood, Neill Graff-Radford, Randall Ryan, Carmencita Pilapil, Remi Quirion, and Yves Robitaille
Dept. Neurology, Mayo Clinic Jacksonville, FL; Douglas Hospital Research Centre, Montreal.

Recent immunocytochemical data have demonstrated increases in interleukin-1β (IL-1β), interleukin-6 (IL-6), and the acute phase protein, α2-macroglobulin (α2-M), in Alzheimer's disease (AD) brains. Therefore, we investigated the levels of these proteins and determined if increases in IL-1β were compensated for by a parallel increase in the endogenous interleukin-1 receptor antagonist (IL-1RA). Mature IL-1β, IL-1RA, IL-6, α2-M and C-reactive protein (CRP) levels were compared in control vs AD brains via ELISA. There were no differences in the levels of mature IL-1β or IL-1RA. However, IL-6 levels were detected in 14 of 16 Alzheimer samples but only 2 of 14 control samples. Significant increases were also seen in α2-M and CRP levels in the AD group. These data further support the hypothesis of a possible up-regulation of neuroimmune function in AD, however, it is not clear if this reaction is initiated by IL-1β.

601.6

BRAIN S-ADENOSYLMETHIONINE DECARBOXYLASE ACTIVITY IS INCREASED IN ALZHEIMER'S DISEASE. Lesley D. Morrison*, Catherine Bergeron and Stephen J. Kish. Human Neurochemical Pathology Lab, Clarke Institute of Psychiatry and Center for Research in Neurodegenerative Diseases, Toronto, Ontario, Canada.

We measured the activity of S-adenosylmethionine decarboxylase (SAMDC), a key regulatory enzyme of polyamine biosynthesis, in autopsied brain from 13 patients with Alzheimer's Disease (AD). As compared with controls, mean enzyme activity was increased by 37 to 96% in all seven examined brain regions with statistically significant increases in temporal cortex (+96%), frontal cortex (+69%) and hippocampus (+90%). The elevation in SAMDC activity may have occurred as part of a generalized polyamine response to brain injury, which has been previously described in experimental animal conditions. Above-normal SAMDC activity implies increased levels/metabolism of spermidine and spermine, two polyamines which are involved in neuronal regeneration, growth factor production, and activation of excitatory N-methyl-D-aspartate-preferring glutamate receptors. Our data suggest the involvement of the polyamine system in the brain reparative and/or pathogenetic mechanisms of AD.

601.7

MEASUREMENT OF PHENYLETHANOLAMINE-N-METHYLTRANSFERASE mRNA, PROTEINS AND METABOLITES IN SAME SAMPLE. W.J. Burke*, C.A.Schmitt, K.N. Gillespie, R. Strong, V. Reddy, T.H. Joh, D.H. Park. Dept. Neurol., St. Louis U. Med. Sch., St. Louis, MO 63110; Cornell U. Med. Col, White Plains, NY 10605.

Measurement of specific mRNA, proteins and metabolites in scarce samples obtained from brain banks poses a problem for Alzheimer's disease investigators. Homogenizing buffer optimal for one compound may prevent measurement of others. A simple way to overcome this obstacle is to pulverize frozen tissue to a fine powder and use aliquots for different assays. However, are aliquots comparable for compounds unevenly distributed in the tissue? The adrenal provides a model to test this since all phenylethanolamine-N-methyltransferase (PNMT) protein, mRNA and metabolites are in the medulla. Adrenals, mortar and pestle were frozen overnight at -80C. Tissue was pulverized to a fine powder and replicate assays of 3-5 aliquots performed for PNMT mRNA by PCR, PNMT protein by Western blot and metabolites by hplc-ec. Differences between measurements in each aliquot were analyzed using a one way ANOVA. There was no difference between aliquots in the quantitation of PNMT mRNA, PNMT protein or epinephrine (Epi). For norepi one aliquote differed from one of the other and for dopamine two aliquots differed from two of the other samples using a Tukey HSD test. Pulverized frozen tissue can be used to measure mRNA, protein and major metabolites in the same sample. More efficient use of scarce tissue samples will result.

601.8

CORRELATION OF NAA AND NAAG LEVELS WITH SEVERITY OF ATROPHY IN ALZHEIMER'S AND HUNTINGTON'S DISEASE BRAIN. L. A. Passani*, R. E. Carter and J. T. Coyle, Dept. of Psychiatry, Harvard School of Medicine / MGH, Boston, MA 02129.

Excitotoxicity caused by elevated glutamate (GLU) levels may account in part for the neurodegeneration observed in Huntington's (HD) and Alzheimer's disease (AD). Previous studies have shown increased GLU levels in affected regions of AD brain (Mc Clure, et al., 1991) and in HD cerebrospinal fluid (Perry et al., 1992). It has been suggested that N-Acetyl-Aspartyl-Glutamate (NAAG), a putative neuromodulator in glutamatergic pathways, may also act as a GLU precursor, liberating GLU from NAAG via N-Acetylated Alpha-Linked Acidic Dipeptidase (NAALADase) at certain synapses. We therefore predicted excitotoxic GLU may originate in part from NAAG, hence, decreased NAAG levels, representing increased catabolism, would directly correlate to the severity of atrophy in AD and HD brain. In our present study, levels of N-Acetyl-Aspartate (NAA) and NAAG were determined by HPLC in three different regions of HD, AD and control brains (brains obtained from Mc Lean and MGH Alzheimer's Brain Bank). The degree of atrophy in individual samples was assessed by microscopic examination and by postmortem NAA levels. In AD, preliminary results indicate correlations between severity of atrophy and decreases in NAAG levels in hippocampus, amygdala and occipital cortex whereas in HD significant decreases were observed only in the putamen. Similar patterns of reduction found for NAA suggest that the NAAG and NAA decreases may be in part due to neuronal loss. To further investigate the mechanisms underlying NAAG reductions, we will determine GLU levels and NAALADase activity.

601.9

DECREASED ACTIVITIES OF THIAMINE MONOPHOSPHATASE (TMPase) AND THIAMINE DIPHOSPHATASE (TDPase) IN TEMPORAL CORTEX OF PATIENTS WITH ALZHEIMER DISEASE (AD) V.L. Raghavendra Rao and R.F. Butterworth*. Neuroscience Research Unit, Hop. St. Luc (University of Montreal), Montreal H2X 3J4, Canada.

Thiamine in the form of its diphosphate ester (TDP) is an essential cofactor for α-ketoglutarate dehydrogenase, pyruvate dehydrogenase in the TCA cycle and transketolase in the pentose phosphate pathway. Decreased activities of these three enzyme systems (Butterworth RF and Besnard, AM, Metab. Brain Dis., 5, 179, 1990) and a characteristic deficit of glucose utilization (Friedland et al., Neurosci. Lett., 53, 235, 1985) were reported in autopsied temporal cortex samples from patients with AD. These changes may be due to decreased availability and/or metabolism of thiamine and its phosphate esters. We now report decreased activities of TMPase and TDPase in temporal cortex samples obtained at post-mortem from seven patients with confirmed AD compared to age, sex and post-mortem time matched control subjects.

	Control (7)	Alzheimer (7)
TMPase	1.07 ± 0.10	0.37 ± 0.05*
TDPase	1.00 ± 0.12	0.47 ± 0.04*

Units: μmoles Pi liberated/mg protein/hr. * $p<0.05$.

Decreased activities of these enzymes and the consequently decreased synthesis of thiamine esters could explain the previous finding of decreased activities of thiamine-dependent enzymes in the brains of AD patients. (Funded by the Medical Research Council of Canada).

601.10

AGE DEPENDENT ALTERATIONS OF PHOSPHOMONOESTER LEVELS UNDER CHRONIC AND ACUTE HYPOXIA IN FISCHER 344 RATS. K. Panchalingam*, J.W. Pettegrew. Lab. of Neurophysics, Univ. of Pittsburgh, Pittsburgh, Pa 15261

Recent biochemical and clinical observations suggest that repeated energetic stress, could in some individuals, trigger molecular and metabolic mechanisms that result in the biochemical findings in the brains of Alzheimer's disease (AD) patients. In this study we report findings of age related changes of phosphomonoester (PME) levels in rats subjected to mild chronic and acute hypoxia. The chronic hypoxia animals were subjected to 30 seconds of 100% N_2 environment every day for two weeks. The acute hypoxia animals were subjected to a single episode of 30 seconds hypoxia. Among the PME's measured, a significant elevation of phosphoserine (PS) was observed in 1 month old animals subjected to chronic and acute hypoxia. However, there was no alteration of PS in 24 month old animals. Phosphoethanolamine (PE) and phosphosphocholine (PC) were unaltered in either age group. The chronic and acute hypoxia animals differ mainly in the change in ATP level. In animals subjected to chronic hypoxia, the ATP level was not significantly affected in younger group but decreased in older group subjected to chronic hypoxia. In animals subjected to acute hypoxia, ATP level was not altered in either age group. These results indicate that there is possible link between brief, mild hypoxia and altered membrane metabolism without significantly altering energy metabolism in young rats. The decrease in ATP levels in 24 month old animals under chronic hypoxia indicates that aged animals are more susceptible to energetic stress than young animals.

601.11

ANALYSIS OF THE cAMP SIGNAL TRANSDUCTION PATHWAY IN OLFACTORY NEUROBLASTS FROM ALZHEIMER'S DISEASE AND CONTROL DONORS. I. Little*, I. Basaric-Keys, R. S. Lebovics, M. Cantillon, T. Sunderland, and B. L. Wolozin. Section on Geriatric Psychiatry, Laboratory of Clinical Science, NIMH, Bethesda, MD 20892

Recent studies (e.g. Cowburn, R. et al, J Neurochem 58:409) have demonstrated G-protein abnormalities in signal transduction in Alzheimer's disease (AD) brain. Our investigations of amyloid precursor protein (APP) processing in olfactory neuroblasts (ON) obtained from AD patients show large increases in the amount of APP C-terminal derivative detectable on immunoblot. These changes can be reversed by treatment of the cells with cAMP agonists, which raises the possibility that the cAMP signal transduction pathway is altered in AD. In order to examine this issue we have begun analyzing this pathway in ON. Initial analysis indicates that basal cAMP levels are reduced by 67% in AD, which is consistent with comparisons of cAMP levels in postmortem AD and control brain tissue (Ohm, T. et al, Brain Res 540:229). This suggests that the abnormalities in APP metabolism may stem from changes in second messenger pathways. We are currently extending these studies by using GTPγS and forskolin to probe Gs and adenylate cyclase activity respectively.

601.12

IMPAIRED ENERGY METABOLISM IN AGING AND ALZHEIMER'S DISEASE. E. M. Mutisya, A. C. Bowling, L. C. Walker, D. L. Price, L. C. Cork, L. Vecsei*, and M. F. Beal. Neurochemistry Lab., Neurology Service, Massachusetts General Hospital and Harvard Medical School, Boston, MA 02114 and Neuropathology Lab., Departments of Pathology, Neurology, and Neuroscience, and Division of Comparative Medicine, Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205.

Energy defects may play a role in aging and age-associated neurodegenerative diseases. Several studies revealed age-related impairments in the activities of some electron transport chain enzymes in rat brain and human myocytes. Studies have also shown reductions in several metabolic enzymes in patients with Alzheimer's disease (AD). To date, however, no one had shown age-related changes in energy metabolism in primate brain or systematically examined electron transport chain activity in AD patients.

In this study, we examined activities of all the oxidative phosphorylation enzyme complexes (I, II-III, IV, and V) in the frontoparietal cortices of 20 rhesus monkeys. We found significant age-dependent decreases in complex I and complex IV activities. Complexes II-III and V activities showed no significant changes. Complexes I and IV therefore appear vulnerable to changes with age.

We also examined activities of the aforementioned enzymes in postmortem frontotemporal cortical tissue from approximately 20 AD and 20 age-matched control patients. Although significant impairment of complex IV activity in AD patients was observed, similar decreases in the activities of complexes I, II-III, and V were not seen. A specific defect may therefore be associated with AD.

601.13

Up-regulated transcription of carboxyl methyltransferase gene in Alzheimer's disease brain. H. Mori*, T. Shirasawa*, H. Takahashi*, R. Endoh, K. Sakamoto*, Y.-X. Zeng** and K. Hirokawa* Department of Molecular Biology, Tokyo Institute of Psychiatry, 2-1-8 Kamikitazawa, Setagayaku, Tokyo 156, Japan; **Tokyo Metropolitan Institute of Gerontology, Tokyo 173, Japan.

In order to isolate genes specifically or characteristically expressed in AD brain, we undertook the differential screening of an embryonic brain cDNA library with ^{32}P-cDNA prepared from AD brain and control brain. We isolated 17 genes and identified them as embryonic α-tubulin, embryonic β-tubulin, hn RNP, carboxyl methyltransferase (CMT), ferritin heavy chain, type IV collagen, cofilin, profilin and 9 novel sequences. First of 17 genes, we extensively investigated CMT gene because of its constant high up-regulation of mRNA in AD, Down syndrome and aged brains. CMT gene was expressed in an age-dependent manner in the brain tissue. A confocal imaging study conducted with both anti-CMT and anti-PHF antibodies unexpectedly showed the close association of CMT with NFTs, somata of degenerative neurons and neuropil threads. This suggested that factor(s) of the repair/degeneration system accumulated on racemized protein deposits in neurofibrillary tangles. The present observation was in good agreement with the previous finding that ubiquitin was a component involved in a collection system in neurofibrillary tangles. We discuss the possible function of CMT in AD brains in related to the well-known function wherein CMT catalyzes the reformation of D-amino acid in abnormally modified proteins. Taken together with the localization of CMT in degeneration neurons and their neuropil threads other than neurofibrillary tangles, it is likely that CMT plays a significant role in the pathogenesis of AD and ageing.

601.14

PROGRESSION OF ENTORHINAL NEUROFIBRILLARY CHANGES DURING INITIAL STAGES OF ALZHEIMER'S DISEASE AS SEEN IN FLATTENED WHOLE MOUNT PREPARATIONS. H. Braak* and E. Braak. Department of Anatomy, J.W.Goethe University, D-6000 Frankfurt/Main 70.

Early stages in the development of Alzheimer-related neurofibrillary changes reveal involvement of specific pyramidal cells in the transentorhinal region. From here the pathological process spreads into the entorhinal region gradually destroying the superficial cellular layer (Pre-*a*). The severity of the change decreases as the layer is followed from the transentorhinal to the parasubicular boundary. We studied the gradual progress of entorhinal involvement corresponding to stage I to III in the development of the neurofibrillary changes [1] in flattened whole mount preparations. This technique reveals the characteristic pattern of cellular islands of the superficial entorhinal layer (Pre-*a*) as seen from the free surface and allows one to draw the boundaries of major subdivisions of the entorhinal region. Furthermore, it enables one to recognize at a glance even subtle pathological changes throughout the entire extent of both the transentorhinal and the entorhinal region.

Supported by the Deutsche Forschungsgemeinschaft.

[1] Braak and Braak, Acta Neuropathol. 82, 239-259, 1991

601.15

EFFECT OF ETHANOLAMINE ADMINISTRATION ON PLASMA AND BRAIN EXTRACELLULAR FLUID (ECF) CHOLINE LEVELS IN AWAKE RATS. D.L. Marshall, E. De Micheli* and R. J. Wurtman. Dept. Brain & Cognitive Sciences, M.I.T., Cambridge, MA 02139, U.S.A..

Ethanolamine (ETH) levels have been shown (Nitsch *et al.*, Proc. Natl. Acad. Sci. 89, 1671-1675, 1992) to be reduced in brains of patients with Alzheimer's disease. The sources of brain ETH and the results of its administration are poorly known. We thus studied the effects of ETH administration on plasma and brain ECF ETH and choline (Ch) levels using microdialysis of awake rats. ETH was administered (i.p.) at doses of 1, 5 or $10x10^{-3}$ m/kg, and plasma and microdialysate samples were analysed for ETH and Ch using HPLC. Plasma ETH peaked 5-10 min after administration, at 260 times the basal value [72.7 ± 4.1 pm/100ml plasma] after the $10x10^{-3}$ m/kg dose (n=3), 170 times basal for the $5x10^{-3}$ m/kg dose (n=4), and 30 times basal for 10^{-3} m/kg dose (n=3). Peak plasma Ch levels, attained after 3-5 min, were 300%, 260% and 150% of basal values [159.58 ± 12.81 pm/100ml plasma] respectively. ETH levels in ECF dialysates peaked 10-20 min post injection and were 1080% of the basal value [36.52 ± 7.43 pm/5min] after $10x10^{-3}$ m/kg (n=10); Ch levels were 210% of the basal value [8.03 ± 1.06 pm/5min]. $5x10^{-3}$ m/kg ETH increased dialysate ethanolamine levels to 630% of basal, and Ch levels to 150% of basal (n=8); 10^{-3} m/kg ETH increased dialysate levels only to 150% of basal (n=3) and failed to increase dialysate Ch levels significantly (n=12). These data suggest that circulating ETH may be a source of both brain ETH and Ch.

601.16

NERVE GROWTH FACTOR RECEPTOR-TARGETED PSEUDOMONAS EXOTOXIN A FUSION PROTEINS: Tools for Studies of Development and Neurodegenerative Disease. Cartwright, M., #Moehring, J., #Moehring, T., and Heinrich G*. University Hospital, Boston University Medical School, Boston, MA 02118 and #Microbiology and Molecular Genetics, University of Vermont, Burlington, VT 05405

To examine the role of nerve growth factor (NGF) in the development of the nervous system and to construct animal models of neurodegenerative disease we genetically linked functionally active NGF to domains 2 and 3 of Pseudomonas exotoxin A (PEA). The NGF portion of the fusion gene contains exon 4 and therefore a precursor region necessary for the folding and production of biologically active NGF. Domains 2 and 3 of PEA encode the translocating and enzymatic domains. DHFR minus CHO cells were mutagenized and toxin-resistent cells were selected. The fusion gene was expressed in these CHO cells and purified from medium by immunoaffinity chromatography using an anti-NGF MAb provided by Alkermes. Western blot analyses of the secreted NGF/PEA fusion proteins revealed bands of the expected as well as larger sizes. Enzyme immunoassay using anti-PEA as the first and anti-NGF as the second antibody demonstrated the presence of both epitopes. Gel permeation chromatography indicated that NGF/PEA forms stable dimers. Competion assay with ^{125} I-labelled NGF showed that NGF/PEA is targeted to the NGF receptor. We are using NGF/PEA to knock out cells carrying the high affinity NGF receptor in developing and adult animals.

THURSDAY PM SYMPOSIA

602

SYMPOSIUM: NEUROTRANSMITTER TRANSPORTERS. M.J. Kuhar, NIH-NIDA (Chairperson); S. Paul, Lilly Research Labs; G.R. Uhl, NIH-NIDA; R. Edwards, UCLA Sch. of Med.; D. Wong, Johns Hopkins Univ. Sch. of Med.

Neurotransmitter transport is an important process in maintaining synaptic transmission. The synaptic plasma membrane transporters terminate synaptic transmission by removing the neurotransmitter from the synaptic gap; this also allows some neurotransmitter to be recycled. The synaptic vesicle transporters accumulate the neurotransmitter into vesicles for subsequent release. The plasma membrane transporter and the vesicular transporter differ in their mechanism of transport. These transporters are also important targets for various drugs. In the last couple of years, a large family of sodium, chloride and temperature dependent synaptic membrane transporters have been cloned; the cloned cDNAs encode proteins very similar in overall structure. The proteins have been shown to be glycoproteins with slightly heterogenous forms. The vesicular transporters have also been cloned and show some similarity to the synaptic membrane transporters. Neurotransmitter transporters appear to be involved in some diseases and toxicities. Imaging the transporters in the living brain of humans and nonhuman primates by PET and SPECT has proven feasible.

603

SYMPOSIUM. PREFRONTAL MECHANISMS OF DISORDERED COGNITION: RELEVANCE TO SCHIZOPHRENIA. P.S. Goldman-Rakic, Yale Univ. Sch. of Med. (Chairperson); C.D. Frith, Hammersmith Hosp., London; R.T. Knight, Univ. Calif., Davis; D.R. Weinberger, NIMH - St. Elizabeth's Hosp.; J.W. Pettegrew, Univ. of Pittsburgh.

The cerebral cortex has come to the forefront of research in schizophrenia. This symposium focuses on the prefrontal cortex and the relevance of its working memory functions to thought disorder. Advanced methods of brain imaging, electroencephalography, magnetic resonance spectroscopy and behavioral analysis in patients, and experimental research on nonhuman primates are providing neurobiological insights into this challenging psychiatric disease.

606.1

RETROGRADE TRANSPORT OF LEUKEMIA INHIBITORY FACTOR (LIF) BY CULTURED RAT SYMPATHETIC NEURONS. D.R. Ure* and R.B. Campenot. Dept. of Anatomy and Cell Biology, University of Alberta, Edmonton, Alberta, T6G 2H7.

We have previously demonstrated that the LIF-induced switch in cultured rat sympathetic neurons from an adrenergic to cholinergic phenotype can occur if LIF is applied only to neurites in compartmented cultures. This observation implies the existence of a signal which travels retrogradely from neurites to cell bodies where the transcriptional changes associated with the switch are elicited. Since it has traditionally been believed in the case of nerve growth factor (NGF) that the transport of intact ligand may be important for retrograde signaling events, we have investigated the transport of ^{125}I- LIF in rat sympathetic neurons in compartmented cultures. The retrograde transport of ^{125}I-LIF is observed in this system but to a level of only approximately 15% of the retrograde transport of ^{125}I-NGF. ^{125}I- LIF transport can be blocked by treatment with colchicine (0.5 μM), dinitrophenol (5 mM), or a 100-fold excess of unlabeled LIF. ^{125}I- LIF and ^{125}I-NGF transport is accompanied by the intracellular accumulation of intact and partially processed ligand, followed by the release of very low molecular weight products into the medium bathing the neurons. These results suggest that the transport of LIF may be important for retrograde signaling events.

606.2

POTENTIAL INVOLVEMENT OF p75^{LNGFR} IN RETROGRADE TRANSPORT OF NEUROTROPHIN-4 (NT-4). R. Curtis*, K.-F. Lee[1], R. Jaenisch[1], J. Huber[2], M.V. Chao[2], R.M. Lindsay and P.S. DiStefano. Regeneron Pharmaceuticals, Inc., Tarrytown, NY 10591; [1]Whitehead Institute, MIT, Cambridge, MA 02142; [2]Cornell University Medical College, New York, NY 10021.

Retrograde transport of the neurotrophins NGF, BDNF and neurotrophin-3 (NT-3) by sensory neurons is mediated through receptor-dependent mechanisms, consistent with utilization of TrkA, B and C, respectively. NT-4, like BDNF, binds with high specificity to TrkB. However, retrograde transport of NT-4 differs from that of BDNF, in that all four neurotrophins effectively compete ^{125}-I-NT-4 transport to DRG sensory neurons. Conversely, excess unlabeled NT-4 inhibits transport of ^{125}-I-NGF, whereas BDNF does not. ^{125}-I-NT-4 transport is additive with ^{125}-I-labeled NGF, BDNF or NT-3. ^{125}-I-NT-4 is preferentially transported to p75^{LNGFR} immunopositive sensory neurons. Of the neurotrophins, NT-4 transport is most sensitive to inhibition by co-injected soluble extracellular domain of p75^{LNGFR} and is most severely reduced in mice with targeted mutation of the p75^{LNGFR} gene. NT-4 is only transported to spinal cord motor neurons after axotomy, coincident with increased expression of p75^{LNGFR} in these cells. In contrast, BDNF is readily transported to motor neurons of normal animals. These results support a role for p75^{LNGFR} in binding, uptake and retrograde transport of NT-4 by adult neurons.

606.3

REGULATION OF p75 BUT NOT trkB GENES BY DEPOLARIZING STIMULI OR BDNF IN BASAL FOREBRAIN NEURON CULTURES. R.C. Elliott*, I.B. Black, and C.F. Dreyfus. UMDNJ/Robert Wood Johnson Med. Sch., Piscataway, NJ 08854

We have previously described a rapid elevation of BDNF message levels after depolarization of hippocampal neurons. We now define potential complementary effects of depolarization on the putative BDNF receptor component genes p75 and trkB in afferent basal forebrain (bf) neurons. Potential regulation of afferent receptors by impulse activity may be mediated directly by depolarization or indirectly by increased BDNF released by the target hippocampus. Therefore, we assayed p75 and trkB mRNA in bf neuronal cultures treated with depolarizing concentrations of KCl or with BDNF.

E18 rat bf cultures were plated in fully-defined, serum-free medium and grown for seven days. Cultures were treated with either BDNF (100 ng/ml) or KCl (50 mM), added either at the time of plating (prolonged regimen) or one day prior to harvesting (acute regimen). To measure subtle changes in p75 and trkB mRNA, a highly sensitive and efficient solution hybridization technique was utilized.

Under control conditions, basal levels of p75 mRNA were an order of magnitude lower than trkB. In response to acute, 24 hour treatment with KCl or BDNF, p75 message levels rose 15-30% while trkB mRNA was unchanged. After a prolonged, 7 day exposure to depolarizing stimuli or BDNF, p75 message levels increased 2-fold; trkB mRNA levels, in contrast, remained unchanged. These results indicate that p75 expression may be responsive to environmental signals while trkB remains static, suggesting a potential role for p75 in the synaptic response to impulse activity.

(Support: NINDS, NICHD, McKnight Fdn., and the UMDNJ Fdn.)

606.4

TRKA INTERACTS WITH LOW-AFFINITY NGF RECEPTOR AND REGULATES NEURAL DIFFERENTIATION. A.H. Ross*, M.-C. Daou, D.R. Kaplan, M.B. Lachyankar, C.A. McKinnon, D.K. Poluha, W. Poluha, R. Stephens and D.E. Wolf. Worcester Foundation for Experimental Biology, 222 Maple Ave., Shrewsbury, MA 01545.

Nerve growth factor receptors (NGFR) were expressed in the baculovirus-insect cell system, and the diffusion of the low-affinity NGFR gp75 was analyzed by fluorescence recovery after photobleaching (FRAP). Coexpression of gp75 with TrkA, but not with TrkB, resulted in immobilization of gp75. Addition of NGF to gp75+TrkA cells resulted in a further immobilization, but this NGF-induced immobilization did not occur with cells expressing gp75 and a mutant TrkA with a defective protein kinase. These results demonstrate an interaction between gp75 and TrkA which is modulated by activation of the TrkA kinase domain.

The functional role of TrkA was assessed, using neuroblastoma line SHSY5Y. This line undergoes terminal differentiation upon treatment with NGF and the DNA polymerase inhibitor aphidicolin. Starting about the time of commitment to differentiation, there is up-regulation of both gp75 and TrkA mRNA's. SHSY5Y cells were transfected with a TrkA expression vector. Although our characterization of the TrkA transformants is preliminary, it is clear that these cells are much more responsive to NGF than the parent line.

606.5

NGF-INDUCED DIFFERENTIATION THROUGH TRKA IN HUMAN NEUROBLASTOMA CELLS LACKING p75^{NGFR}. D.S. Hartman*, R. Schubenel, and C. Hertel. Pharma Research, Preclinical Neurosciences, F. Hoffmann-La Roche AG, CH-4002 Basel, Switzerland.

Nerve growth factor (NGF)-induced activation of the trkA NGF receptor (NGFR) supports the survival and differentiation of neurons. Human trkA, however, has been studied extensively by expression of a hematopoetic cell-derived cDNA clone in heterologous mouse fibroblast cells, where NGF induces cell proliferation. We have now identified a human neuroblastoma cell line, IMR-32, which expresses trkA but not the low affinity NGFR, p75. Northern blot analysis of poly A+ mRNA revealed a primary trkA transcript of 2.3 kb, and a minor 4 kb transcript. Immunoblotting of crude membrane protein from IMR-32 cells with anti-trk 43.4 antiserum labeled two trkA bands with apparent molecular weights of 116 and 105 kD. Chemical crosslinking of ^{125}I-NGF affinity-labeled IMR-32 cells identified two bands of approximately 130 kD and 300 kD which were immunoprecipitated by anti-trk antiserum. Recombinant human NGF (rhNGF) was able to induce both early and late responses in IMR-32 cells. Neurite outgrowth was observed after 9 days in the presence of 5 ng/ml rhNGF. Increased autophosphorylation of trkA on tyrosine residues was detectable at 10 pM rhNGF, and maximal at 0.2 nM rhNGF; maximal levels were reached after 5 min rhNGF stimulation, and remained elevated for longer than two hours. rhNGF also induced tyrosine phosphorylation of phospholipase Cγ and phosphotidylinositol kinase-3, but not of MAP kinase. These results indicate that expression of p75 is not required for NGF-induced differentiation of human neuroblastoma cells, and suggest that tyrosine phosphorylation of MAP kinase may not play an essential role in this process.

606.6

AN EXTENDED SURFACE OF BINDING TO TRK TYROSINE KINASE RECEPTORS IN NGF AND BDNF ALLOWS THE ENGINEERING OF A MULTIFUNCTIONAL PAN-NEUROTROPHIN C. F. Ibáñez*, L. L. Ilag and H. Persson. Department of Medical Chemistry, Laboratory of Molecular Neurobiology, Karolinska Institutet, S-10401 Stockholm, Sweden.

Neurotrophin-mediated cell survival and differentiation of vertebrate neurons is caused by ligand-specific binding to the Trk family of tyrosine kinase receptors. However, sites in the neurotrophins responsible for the binding to Trk receptors and the mechanisms whereby this interaction results in receptor activation and biological activity are unknown. We show that in NGF and BDNF, discontinuous stretches of amino acid residues group together on one side of the neurotrophin dimer forming a continuous surface responsible for binding to and activation of TrkA and TrkB receptors. Two symmetrical surfaces are formed along the two-fold axis of the neurotrophin dimer providing a model for ligand-mediated receptor dimerization. Mutated neurotrophins inducing similar levels of receptor phosphorylation showed different biological activities, suggesting that structural differences in a ligand may result in dissimilar responses in a given tyrosine kinase receptor. Our results allowed us to combine structural elements from NGF, BDNF and NT-3 to engineer a pan-neurotrophin that efficiently activates all Trk receptors and displays multiple neurotrophic specificities.

606.7

COLOCALIZATION STUDIES OF TRKA, TRKB & TRKC RECEPTOR mRNAs EXPRESSION IN PRIMARY SENSORY NEURONS AND RESPONSES TO INJURY V.M.K. Verge[1]* C. Wetmore[2] & T.Hökfelt[3]
[1]Dept. of Anatomy, Univ. of Saskatchewan, Canada S7N 0W0; [2]Dept. of Cell Biology & Neuroanatomy Univ. of Minnesota, 55455; [3]Dept. of Histol. & Neurobiol., Karolinska Institute, S104 01 Stockholm, Sweden.

In vitro studies indicate that in addition to NGF, at least two other members of the NGF family of neurotrophins (NTs) namely, BDNF and NT-3 act on primary sensory neurons. To gain insights to their roles in adult rat sensory neurons *in vivo*, *in situ* hybridization studies to detect their respective putative trk receptor mRNAs (trkA-NGF, trkB-BDNF; trkC-NT-3) were performed on intact and 14 day injured (sciatic nerve transected) L5 DRG to determine whether 1- the populations of intact neurons presumably responsive to these NTs were distinct or overlapping; and 2- whether trkB and trkC downregulate their expression following injury as has been shown for trkA. In intact ganglia all three trk mRNAs are heterogenously expressed in neurons, with the truncated form of trkB also expressed in the satellite cells surrounding the neurons. Preliminary colocalization analysis reveals detectable levels of trkA and trkB mRNAs to be expressed over what appear to be mutually exclusive populations of neurons while both trkA and trkB colocalize to varying degrees with trkC mRNA expression. Collectively, most DRG neurons appear to express one of the three trk receptors. Finally, data from paired ganglia injured unilaterally show neuronal trkA, trkB and trkC expression and truncated trkB expression in satellite cells to be reduced in ganglia ipsilateral to lesion. If BDNF and NT-3 regulate expression of their receptors as NGF does, then these results suggest that peripherally injured DRG neurons, are in a NT-deprived state, at least in regard to the NGF family of NTs. This work was supported by the Canadian and Swedish MRCs.

606.8

ALTERNATE SPLICING OF THE *trk*C TYROSINE KINASE DISSOCIATES PROCESS OUTGROWTH FROM SURVIVAL. A.S. Garner * and T.H. Large. Department of Neurosciences, Case Western Reserve University, Cleveland, OH 44106-4975.

The *trk*C receptor transduces NT3 binding into cellular responses as diverse as proliferation, differentiation and survival. These heterologous effects may be regulated at the level of receptor structure and the differential activation of signaling pathways. The cloning of avian *trk*C cDNAs revealed two tyrosine kinase splice variants that are likely to alter signal transduction. A kinase insert variant adds 25 amino acids immediately after the double tyrosine residues (711 and 712) that have been shown to be important regulators of *trk* kinase activity. A kinase truncation variant utilizes the same splice acceptor site as the kinase insert to remove the middle third of the tyrosine kinase domain. This deletion switches the reading frame and replaces the last two thirds of the kinase domain with 33 novel amino acids. In order to investigate the ability of these kinase splice variants to mediate process outgrowth and survival in serum-free medium, *trk*C receptors containing either the full length kinase, kinase insert, or kinase truncation were stably expressed in PC-12 cells. NT3 stimulated process outgrowth and survival in clones expressing the full length receptor, but not the kinase truncation receptor. Although the response appeared to be attenuated, NT3 also stimulated process outgrowth from clones expressing the kinase insert receptor, but failed to rescue the cells in serum-free medium. Thus, the 25 amino acid kinase insert appears to dissociate receptor-stimulated process outgrowth from survival. Our data indicate that alternate splicing may be an important mechanism for regulating the structure and function of *trk*C receptors.

606.9

IMMEDIATE EARLY GENE RESPONSE IN PC12 CELLS EXPRESSING *TRK* FAMILY MEMBERS. Dan Soppet*, Pantelis Tsoulfas, Luis F. Parada. Molecular Embryology Group, Laboratory of Mammalian Genetics, NCI-FCRDC, Frederick, Md. 21702

To directly compare the signal transduction pathways for neurotrophin receptors we have established PC12 cell lines that express *trk*B, *trk*C and *trk*C isoforms. We have exposed these cell lines to specific neurotrophins and analyzed the neurite outgrowth response and mRNA levels of several immediate early genes including c-FOS, c-JUN, NGFIA, and NGFIB. We find that in PC12 cells expressing *trk*B both neurite outgrowth and rapid induction of immediate early genes are produced by exposure to NGF, BDNF, and NT-5 but not NT-3. In *trk*C expressing cells NGF and NT-3 treatment induce neurites and lead to a rapid increase in the levels of immediate early genes. However *TrkC* isoforms *trkC*14 and *trkC*25 do not significantly stimulate the levels of immediate early genes in PC12 cells upon exposure to NT-3 nor do they induce neurite outgrowth. These results will be discussed in terms of the mechanisms for regulating neurotrophin responsiveness of specific types of cells. Research sponsored by the National Cancer Institute, DHHS, under contract no. NO1-CO-74101 with ABL.

606.10

EXPRESSION OF P140*trk* CONFERS NGF-RESPONSIVENESS IN A HYPOTHALAMIC NEURONAL CELL LINE. J. Zhou, D. Holtzman, R.J. Weiner, and W.C. Mobley*, Dept. of Neurology, Pediatrics and the Neuroscience Program, and Reproductive Endocrinology Center, UCSF, San Francisco, CA 94143.

An immortalized hypothalamic neuronal cell line was recently developed by genetically targeted tumorigenesis using the promoter of the gonadotropin-releasing hormone (GnRH) gene to express the SV40 T-antigen. The cloned cell line GT1-1 shows several neuronal properties. These cells express neuronal, but not glial, markers. They contain a small amount $p75^{NGFR}$ mRNA, but they do not contain $p140^{trk}$ mRNA and do not respond to NGF. We asked whether expression of $p140^{trk}$ would confer NGF responsiveness. Cells transfected with the $p140^{trk}$ cDNA made $p140^{trk}$ and they differentiated and extended neurites in response to NGF. Also, NGF delayed the death of cells that results from serum deprivation. As it is true in other responsive cells, NGF treatment induced tyrosine phosphorylation of $p140^{trk}$. Our findings provide further evidence that signal transduction is mediated through the $p140^{trk}$ receptor. They suggest that a functional NGF signaling apparatus can enhance differentiation in neural tumors.

606.11

DIFFERENTIAL DISTRIBUTION OF trkC-LIKE IMMUNOREACTIVITY IN AXON TERMINALS IN THE SPINAL CORD MOTOR NUCLEI IN MONKEY AND CAT S. Cullheim*, U. Arvidsson, J. Frisén, F. Piehl, K. Fried, T. Hökfelt and M. Risling. Department of Neuroscience and Anatomy, Karolinska Institutet, Stockholm, Sweden.

The *trk*, *trk*B and *trk*C genes encode for high affinity receptors for the nerve growth factor family. Of these receptors, the *trk*C gene product seems to be selective for neurotrophin (NT)-3. As part of a larger project aiming at identifying substances of potential interest for the plasticity of spinal motoneurons, we have included an immunohistochemical study of the distribution of trk proteins in the normal spinal cord motor nuclei. Thus, after transcardial perfusion with 4% paraformaldehyde and 0.2% picric acid, spinal cord sections of adult monkeys (Macaca fascicularis) and cats were incubated with a rabbit polyclonal antibody raised against a peptide corresponding to residues 798-812 mapping within the carboxy terminal domain of the predicted mouse *trk*C encoded protein (Santa Cruz Biotech). In order to reveal a possible coexistence of trkC and serotonin (5-HT) in bulbospinal nerve terminals, which in the monkey harbour the growth-associated protein GAP-43, the tissue sections were also incubated with a guinea-pig 5-HT antiserum for double labeling. In both monkey and cat, trkC immunoreactive (IR) nerve fibers were found at all spinal cord levels. The densest innervation was found in the autonomic intermediolateral and Onuf's nuclei, but also somatic motoneuron pools received a significant contribution of trkC-IR fibers. The coexistence patterns differed between species, however, in that the majority of trkC-IR fibers were also 5-HT-IR in the monkey, while in the cat no certain such coexistence was at hand. The results suggest a role for neurotrophins, in particular NT-3, in the regulation of motoneuron activity at the spinal cord level.

606.12

IDENTIFICATION OF *trk*B RECEPTOR mRNA IN ASTROCYTES BOTH IN VITRO AND AFTER CORTICAL INJURY. R.J. McKeon*, J. Silver and T. Large. Department of Neurosciences, Case Western Reserve Univ., Cleveland, OH 44106.

After cortical injury, factors that support neuronal survival have been shown to be present in the area of damage. It is also known that, *in vitro*, astrocytes express mRNA for the neurotrophins NGF and NT-3, but not BDNF. This expression is consistent with the observation that glial scars can support neurite outgrowth from DRG's in the absence of exogenous NGF. *In vitro*, astrocytes also express high affinity *trk*B receptors, although the significance of this expression is not yet clear. In fact, a number of different *trk*B transcripts have been isolated from whole brain, some of which lack the tyrosine kinase domain necessary for signal transduction. In order to determine the types of *trk*B receptors expressed by astrocytes, both *in vitro* and *in vivo* after cortical injury, we have probed Northern blots with a 1.7 kb *trk*B probe. Our results indicate that, *in vitro*, astrocytes express at least 5 different *trk* B transcripts ranging in size from 7.5 to 1.8 kb, including one (4.8 kb) previously reported to be a full length transcript. In contrast, after cortical injury, astrocytes express at least three *trk*B transcripts, one of which (9.0 kb) has been reported to be a full length *trk* B, while the other two (7.5 and 7.0 kb) are likely to represent C-terminally truncated transcripts. These data indicate that astrocytes express full-length and truncated *trk*B receptors and the pattern of expression differs in astrocytes obtained *in vitro* or *in vivo*. *In situ* hybridization studies are being performed to confirm the expression of full length *trk*B by astrocytes *in vivo*. The potential for autocrine stimulation of astrocytes via *trk*B receptors will be determined by examining the expression of appropriate neurotrophin ligands for this receptor, including NT-4. Supported by the Spinal Cord Research Foundation of the Paralysed Veterans of America.

607.1

BLOCK OF CALCIUM CHANNELS IN CENTRAL AND PERIPHERAL RAT NEURONS BY ω-CONOTOXIN-MVIIC.

K. J. Swartz*, I. M. Mintz, L. M. Boland, and B. P. Bean. Dept. of Neurobiology, Harvard Medical School, Boston MA 02115.

The synthetic peptide ω-conotoxin-MVIIC (kindly furnished by Dr. George Miljanich) was tested against Ca channel currents in a variety of rat neurons. With 2-3 mM Ba as charge carrier, ω-CgTx-MVIIC reversibly blocked N-type channels in sympathetic neurons with an IC_{50} of ~4 nM. Both onset and reversal of block were rapid (τ_{on} ~ 6 sec with 10 nM toxin, τ_{off} ~ 15 sec). Block of N-type channels was weaker with higher Ba concentrations (IC_{50} ~ 25 nM with 5 mM Ba, ~ 600 nM with 25 Ba). The toxin also blocked P-type Ca channels in cerebellar Purkinje neurons, but both the development and reversal of block were far slower. At 80 nM toxin applied in 2 mM Ba, block developed with a τ of ~15 minutes; there was essentially no reversal with washing for 5-10 minutes. In hippocampal CA3 neurons, substantial current remains with 3 μM nimodipine to block L-type channels, 3 μM ω-CgTx-GVIA to block N-type channels, and 100 nM ω-Aga-IVA to block P-type channels. This current was reduced by ~60% by 10 μM ω-CgTx-MVIIC.

607.2

VOLTAGE-DEPENDENCE OF P-TYPE CALCIUM CHANNEL BLOCK BY SYNTHETIC ω-AGA-IVA.

I. M. Mintz* and B. P. Bean. Dept. of Neurobiology, Harvard Medical School, Boston MA 02115.

The effects of the synthetic peptide ω-Aga-IVA (Peptides International, Inc.) were found to be nearly identical to those of the purified spider toxin ω-Aga-IVA (Dr. M.E. Adams). The synthetic peptide blocked the predominant P-type Ca channel current in freshly isolated rat Purkinje neurons potently and completely (Kd ~3 nM with 5 mM Ba, Kd ~ 20 nM with 115 mM Ba). The peptide had no effect on T-type current (200 nM) or BAY K 8644-enhanced L-type current (200 nM) in rat sensory neurons or on N-type current in rat sympathetic neurons (200-800 nM). In rat spinal cord neurons, synthetic and purified peptides blocked the same component of high-threshold Ca channel current in a mutually occlusive manner. In Purkinje neurons, the on rate constant for toxin block varied linearly with toxin concentration, consistent with one-to-one binding. The off rate constant (koff) was very slow at -80 mV but was dramatically increased by depolarizations to >+70 mV. The relief of block by big depolarizations was identical in cells recorded with permeant (Cs) or impermeant (TEA or NMDG) internal ions. In 5 mM external Ba, koff increased with voltage according to a Boltzmann relationship (midpoint +120 mV and slope factor 24 mV). This relationship was shifted to more positive potentials in recordings with 110 mM Ba.

607.3

FIVE PHARMACOLOGICALLY DISTINCT HIGH VOLTAGE-ACTIVATED Ca^{2+} CHANNELS IN CEREBELLAR GRANULE CELLS.

A.D. Randall*, B. Wendland, F. Schweizer, G. Miljanich, M.E. Adams, & R.W. Tsien. Dept. Molecular & Cellular. Physiology, Stanford University, Depts. Entomology and Neuroscience, UC Riverside & Neurex Corp. Menlo Park, CA 94025.

Whole-cell recordings from cultured rat cerebellar granule neurons (480 cells) revealed five components of Ca^{2+} channel current. In 5 mM Ba^{2+} the current was entirely high voltage-activated (HVA), with maximal current at 0 mV. L-type currents were inhibited by 10 μM nimodipine and potentiated by FPL 64176. N-type currents were blocked by ω-CTx-GVIA (1 μM). In the combined presence of nimodipine and ω-CTx-GVIA, 61 ± 3% of HVA current remained,. and could be blocked by 20 μM Cd^{2+} or reversibly modulated by agonists of $GABA_B$, MGluR and adenosine receptors.

Analysis of the nimodipine/ω-CTx-GVIA-insensitive current demonstrated that only 9 ± 1% of it was non-inactivating P-type current, inhibited by 30 nM ω-Aga-IVA. Most (71 ± 3%) of it, however, was blocked by 5 μM ω-CTx-MVIIC. Blockade by this agent was slow (τ = 55 s), like that afforded Class A Ca^{2+} channel clones expressed in *Xenopus* oocytes. A 2 h pre-incubation with 500 nM ω-CTx-MVIIC, completely occluded blockade by 5 μM ω-CTx-MVIIC, but not the actions of ω-CTx-GVIA or nimodipine. The residual current, defined by its resistance to nimodipine (10 μM), ω-CTx-GVIA (3 μM), ω-Aga-IVA (100 nM) and ω-CTx-MVIIC (5 μM), was HVA and rapidly inactivating (τ_D = 22 ms). This current was rather Ni^{2+} sensitive (IC_{50}=66 μM), like the doe-1 Ca^{2+} channel (Zhang et al., Soc. Neurosci. Abs. 1993).

We refer to the ω-CTx-MVIIC-sensitive, nimodipine/ω-CTx-GVIA/ω-AgaIVA-insensitive current as "Q-type", to distinguish it from L, N, and P-type currents. Q-type current was >2-fold larger than any another component (43% of the total current). Q-type current strongly resembles α_{1A} Ca^{2+} channels expressed in *Xenopus* oocytes.in its pharmacology, voltage- and time-dependence (Sather et al. Soc Neurosci. Abs. 18: 10, 1992). Q-type current is partially blocked (up to 50%) by high doses (100/200 nM) of ω-Aga-IVA. This suggests caution in using high doses (>30 nM) of ω-Aga-IVA to determine the presence or function of P-type channels.

607.4

ω-CONOTOXINS WITH NOVEL CA^{++} CHANNEL SUBTYPE SPECIFICITY.

D.R. Hillyard, J. Imperial, R. Schoenfeld, V. Monje and L.J. Cruz*. Depts. of Pathology and Biology, University of Utah, Salt Lake City, Utah, 84132 and College of Science, Univ. Philippines, Diliman, Quezon City, Philippines.

The dihydropyridines and ω-conotoxin GVIA (ω-CgTx) have become the standard pharmacological reagents for identifying L- and N-type Ca channels, respectively. Fish-hunting cone snails have evolved a large set of peptides of divergent sequence which inhibit neuronal calcium channels; we are identifying new ω-conotoxins which preferentially target ω-CgTx-resistant (i.e., non-N) Ca channels. Nine ω-conotoxins have been characterized from four different *Conus* venoms. The existence of non-N-type-targeting ω-conotoxins in the venoms of cone snails was revealed by a molecular cloning approach. Two new peptides, ω-conotoxins MVIIC and MVIID inhibit at least two classes of calcium channels which are both ω-CgTx- and dihydropyridine- resistant. MVIID in particular has a high index of discrimination against N-type binding sites. Sequence analysis of cDNAs encoding ω-conotoxins has revealed consistently conserved and variable domains in the toxin precursor sequences. The organization of ω-conotoxin coding sequences is ideal for amplification cloning strategies to rapidly access members of the ω-conotoxin family with novel specificity for Ca channel subtypes. (Supported by GM48677 and NS27219).

607.5

OMEGA-CONOPEPTIDE SNX-230 (MVIIC) BLOCKS CALCIUM CHANNELS IN MOUSE NEUROMUSCULAR JUNCTION NERVE TERMINALS.

S. Bowersox, C-P. Ko, Y. Sugiura, C. Z. Li, J. Fox*, B. B. Hoffman, G. Miljanich. NEUREX Corp., Menlo Park, CA 94025. Dept. of Biol. Sci., Univ. of Southern Calif., Los Angeles, CA 90089.

At least two binding sites for Ca^{++} channel blocking ω-conopeptides exist in mammalian CNS. Site 1 correlates with N-type voltage-sensitive Ca^{++} channels (VSCC) defined by sensitivity to ω-conopeptide SNX-111 (MVIIA). The identity of Site 2, defined by SNX-230 (MVIIC) sensitivity, is uncertain; being neither N, L, nor T, but possibly including P and/or P-like components. We used the mouse phrenic-nerve-diaphragm and ω-conopeptides to classify the VSCC mediating synaptic transmission at the mammalian neuromuscular junction (NMJ). SNX-111 did not block nerve-stimulated muscle contractions at concentrations up to 100μM. In contrast, SNX-230 produced complete blockade at 0.8μM. SNX-230-treated preparations continued to respond to exogenous acetylcholine. Potencies of twenty ω-conopeptides and analogues revealed a strong correlation between site 2 binding and NMJ blockade. Evoked endplate potentials (epps) and spontaneous miniature endplate potentials (mepps) were recorded in low Ca^{++} and high Mg^{++} saline. SNX-111 had negligible effects on epps or mepps up to 100μM. SNX-230 at 10μM inhibited epps completely with little effect on mepp amplitude and frequency. At 1μM, SNX-230 reduced quantal content to about 15% of control; 0.1μM showed no effect. Reduction in quantal content in 1μM SNX-230 was abolished by elevating extracellular Ca^{++}. SNX-230 also blocked the increase in mepp frequency induced by K^+-elevated saline. Further, perineural focal recordings of presynaptic currents showed that SNX-230 (10μM) did not block the conducted action potential. Taken together these findings indicate that SNX-230 blocks transmitter release at the mammalian NMJ by blocking presynaptic VSCC's that correspond to the Site 2 non-L, N, T subtype. (Supported in part by NIH grant NS 30051 to CPK.)

607.6

MULTIPLE CALCIUM CHANNEL TYPES COEXIST TO REGULATE STRIATAL DOPAMINE RELEASE AND HIPPOCAMPAL GLUTAMATE RELEASE.

T.J. Turner* & K. Dunlap Dept of Physiology and Neuroscience, Tufts University School of Medicine, Boston, MA 02111

We have used peptide toxins that block distinct Ca^{2+} channel types in order to identify which types regulate excitation-secretion coupling in mammalian nerve terminals. A superfusion system with subsecond temporal resolution was used to directly measure release of radiolabeled neurotransmitter from rat brain synaptosomes. We have shown previously that K^+-evoked release of [^{3}H]glutamate from cortex is mediated in part by P-type channels, based on partial block of release by ω-Aga-IVA (a specific P channel blocker) and resistance to ω-CgTx (a specific N channel blocker). In order to determine whether secretion of other transmitters is regulated in the same way, we tested the two toxins on release in two other systems--dopamine release from striatum and glutamate release from hippocampus. ω-Aga-IVA blocked release in both of these systems, and as before the extent of block was greatest at low levels of depolarization and diminished as the KCl concentration was increased. Unlike glutamate release from cortex, ω-CgTx blocked a small but significant portion of release in these systems. When release was evoked with 60 mM KCl neither toxin had much effect on release rates, but a combination of the two toxins produced a synergisitic block of up to 60% of control values. Our conclusion is that multiple Ca^{2+} types coexist in individual nerve terminals, and that each type can contribute to neurosecretion under physiologically relevant conditions. This arrangement could lend a high degree of flexibility to the regulation of transmitter release under diverse conditions of stimulation and modulation.

607.7

FUNCTIONAL DIVERSITY OF DIHYDROPYRIDINE- AND W-CONOTOXIN-GVIA-INSENSITIVE CALCIUM CHANNELS IN RAT CEREBELLAR GRANULES. D. Pietrobon*, L. Forti, A. Tottene and A. Moretti. C.N.R. Mitoc. Physol. Center, Dept. of Biomedical Sciences, Univ. of Padova, 35121 Padova, Italy.

After incubation of 6-11 days-old primary cultures of rat cerebellar granules with 3 uM w-conotoxin GVIA (w-CgTx) in divalent-free solution, single cell-attached calcium channel recordings were performed with 90 mM Ba^{2+} and 3 uM w-CgTx in the pipette. Using neuroblastoma IMR32 cells, whose whole-cell calcium current was -80% irreversibly inhibited by w-CgTx, we had previously ascertained that this inhibition protocol was effective in completely blocking w-CgTx-sensitive single calcium channels. In cerebellar granule cells we observed two functionally different classes of calcium channels that were not inhibited by w-CgTx and were also dihydropyridine-insensitive, as judged by the lack of effect of (+)-S-202-791. The two classes, G_1 and G_2, differed in single channel conductance (21 and 16 pS, respectively), elementary current (1.35 and 0.6 pA at 0 mV), threshold for activation (-10 and -40 mV) and mean open time (0.5 and 1.2 msec). Both G_1- and G_2-type calcium channels showed little inactivation during 700 msec long test pulses, but the majority of channels in both classes completely inactivated when the potential between successive depolarizations was held at -40 mV for 4 seconds. They were observed with similar frequency, in ~30% (lower limit) of patches. In both G_1 and G_2 classes we observed further subtle functional diversity. In agreement with the single channel recordings, the whole cell calcium current showed slow inactivation during test pulses, but was largely inactivated at holding potentials of -50 mV. Only 10-15% of the current was inhibited by w-CgTx, and most of this inhibition was reversible. We are currently testing the effect of w-Aga-IVA and w-conotoxin-MVIIC on G_1- and G_2-type calcium channels.

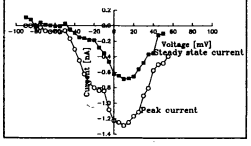
607.8

CHARACTERISTICS OF CALCIUM CURRENTS IN *HERMISSENDA* PHOTORECEPTORS. E. Yamoah* and T. Crow. Dept. Neurobiology & Anatomy, Univ. of Texas Medical School, Houston, TX 77030.

It has been proposed that the sequence of events important in plasticity of *Hermissenda* photoreceptors is mediated, in part, by Ca^{2+}. However, little is known regarding the properties of Ca^{2+} channels in these cells. Ca^{2+} currents were recorded from isolated photoreceptors using the whole cell variant of the patch-clamp technique. The composition of the external and pipette solutions in mM was; external- 300 Choline, 50 Mg^{2+}, 10 Ca^{2+}, 100 TEA, 5 4-AP, 10 Dextrose, 15 HEPES buffer, pH 7.7; pipette- 20 Na^+, 2 Mg^{2+}, 45 HEPES buffer, 10 EGTA, 20 TEA, 250 Cs^+, 300 N-methylglutamine, 5 MgATP, 1 GTP, 10 Glutathione, pH 7.4 with TEAOH).

Ca^{2+} currents appeared at potentials positive to -70 mV from a holding potential of -90 mV. The current voltage relationship showed three characteristic peaks around -60 mV, -20 mV and +10 mV; see Fig 1. The low & intermediate-voltage-activated currents (LVA,IVA) have a fast activation profile but different inactivation kinetics. The LVA has a fast and the IVA has a slow time course of inactivation. The high-voltage activated current has a slow turn-on with no inactivation. These three sub-types of Ca^{2+} channels have different sensitivity to Co^{2+},Ni^{2+},Cd^{2+}, La^{3+}, Gd^{3+}, ω-conotoxin GVIA and nifedipine. However, pharmacological separation of the currents was inadequate since the three channels can be blocked, over time, by all the blockers. Individual currents were studied with varying holding potentials or in photoreceptors that expressed one particular type of current. The kinetics, pharmacology and functions of these subtypes of Ca^{2+} currents are under investigation.

Fig.1



607.9

PROTEIN KINASE C BLOCKS SOMATOSTATIN-INDUCED MODULATION OF CALCIUM CURRENT IN CHICK SYMPATHETIC NEURONS. A.Golard, L.W.Role and S.A.Siegelbaum. H.Hughes Med.Inst. and Ctr.Neurobiology & Behavior, Columbia Univ., New York, NY 10032

Protein kinase C (PKC) has been suggested either to mediate Ca channel inhibition by neurotransmitters or, conversely, to block the inhibition of Ca currents (I_{Ca}). Here we study the role of PKC in the inhibition of I_{Ca} by somatostatin (SS) in chick sympathetic ganglion neurons. PKC activation (1μM PMA) had no effect on I_{Ca}, nor on the interaction between G-proteins and Ca channels. Pre-treatments of cells with PMA reduces the subsequent inhibition of I_{Ca} by SS. Modulation of I_{Ca} by SS normally desensitizes, with a time for half desensitization of about 3 minutes. PKC activation mimics the normal desensitization process in that high affinity responses are lost preferentially. However, while the specific PKC inhibitor Calphostin C largely reverses the effects of phorbol esters, it does not slow the normal rate of desensitization of SS responses. This indicates that PKC is not involved in the homologous desensitization of the SS receptor.

607.10

A TYROSINE PHOSPHATASE DETERMINES THE RESPONSE OF A CALCIUM-PERMEABLE CHANNEL TO PROTEIN KINASE A. G.F. Wilson*, and L.K. Kaczmarek. Dept.of Pharmacology, Yale Univ. Sch. of Med., New Haven, CT 06510.

In bag cell neurons of *Aplysia*, elevations in intracellular cAMP trigger a ~30 min discharge of action potentials. We have studied protein kinase A (PKA) modulation of a large conductance calcium-permeable cation channel which evidence suggests may provide the depolarizing drive underlying the discharge. The effect of 100 nM of the catalytic subunit of PKA depended on the channel's starting mode of activity. If the channel was continuously active, then PKA decreased the open probability (n=14); conversely, if the channel was bursting, then PKA increased the open probability by removing the channel's long-lived closed state (n=7). Bursting channels could be converted to the continuously active mode using the T-cell protein tyrosine phosphatase (PTPase; n=6), suggesting that the phosphorylation state of tyrosine residues determines both mode of activity and PKA response.

Additional experiments indicated that the PKA-induced increase in burster activity proceeds indirectly by increasing the activity of a PTPase endogenous to excised patches. If the endogenous PTPase is inhibited using 1 mM vanadate, PKA still appears to phosphorylate bursting channels, however this phosphorylation results in a decrease in activity (n=4) similar to that observed for continuously active channels. 2 nM microcystin produced no change in the normal burster response to PKA (n=4). These observations suggest that PKA-regulation of the tyrosine phosphatase may play a crucial role in triggering the discharge and illustrate that tyrosine kinases and phosphatases can directly and dynamically regulate neuronal signaling.

607.11

LSD ACTIVATES 5-HT_{1A} RECEPTORS ON DORSAL RAPHE NEURONS: EFFECTS ON Ca^{2+} AND K^+ CURRENT. N.J.Penington* and A.P. Fox. Univ. Chicago, Dept.Pharm/Phys., Chicago Il, 60637.& Dept. Pharmacol. SUNY (Health Science Center Brooklyn), New York.

Previously, we reported that 5-HT inhibited whole-cell Ca^{2+} current (I_{Ca}) and potentiated a K^+ current (I_K) in dorsal raphe (DR) neurons. Now we find that LSD mimics the actions of 5-HT by dramatically suppressing I_{Ca} while activating an inwardly rectifying K^+ conductance. LSD was previously reported to activate I_K in DR neurons. We tested the hypothesis that LSD activates 5-HT_{1A} receptors thereby potentiating I_K while inhibiting Ca^{2+} influx into the cell. In 18 cells 5-HT (1μM) inhibited I_{Ca} by 51.2 ± 1.9% and LSD (1μM) inhibited by 46.2 ± 2% (n=14). The inhibition produced by LSD showed a voltage dependence identical to that of 5-HT. Spiperone (1μM), a 5-HT_{1A} antagonist, blocked the actions of LSD (LSD inhibited I_{Ca} by only 6.9% in its presence). 2-Bol, a close structural analog of LSD that does not produce hallucinations, had no effect on I_{Ca}. However, 2-Bol (10μM n=7) did block the effect of LSD (1μM) by 59.2 ± 8%. LSD also activated an inwardly rectifying I_K that measured -177 ± 52 pA at -90mV (1μM, n=6) and reversed close to the potential expected of I_K in 20mM $[K^+]_o$ (-51mV). Incubation of the cell with 2-Bol (10μM) did not activate I_K but it blocked the effect of LSD (LSD elicited only 38.2 ± 12 pA of I_K in 2-Bol). Inhibition of 5-HT release resulting from 5-HT_{1A} receptor activation, may play an integral role in LSD's hallucinogenic actions by reducing competition between 5-HT and LSD for other postsynaptic 5-HT receptors.

607.12

ANGIOTENSIN II INHIBITS Ca^{2+} CURRENTS IN RAT SYMPATHETIC NEURONS VIA A G-PROTEIN. M. S. Shapiro, J. Herrington, L.P. Wollmuth* and B. Hille Physiology and Biophysics, Univ. Washington, Seattle, WA 98195.

We studied inhibition of N-type Ca^{2+} channels in adult rat superior cervical ganglion (SCG) neurons by angiotensin II (ATII) using the patch clamp. In whole-cell configuration with external 5 mM Ca^{2+}, 68 of 88 acutely dissociated SCG cells showed inhibition by 500 nM ATII. Peak currents elicited by 10-ms voltage steps from -80 mV to 5 mV were reduced by 32±2% (mean±SE), with an IC_{50} near 12 nM. Dialysis with 2 mM GDP-β-S for >7 min reduced ATII inhibition (to 8±2%, n=9 *vs.* 30±5%, n=10), implicating G-proteins. For cells treated overnight with 500 ng/ml pertussis toxin or heat-inactivated controls, about half responded to ATII in either case (9/15 *vs.* 7/14). AT_1 receptors seem involved since 0.2-2 μM of the AT_1-specific antagonist losartan abolished the action of 100 nM ATII (n=13). $[Ca^{2+}]_i$ measured by 100 μM Indo-1 fluorescence was little increased by 500 nM ATII (14±7 nM, n=5); nevertheless, inhibition of I_{Ca} by ATII was mostly blocked by 20 mM pipette BAPTA (to 10±3%, n=12 *vs.* 30±5%, n=12). This inhibition was not restored by pipettes with 20 mM BAPTA+10 mM Ca^{2+} to raise but still strongly buffer Ca^{2+} (8±2%, n=9). We conclude that the ATII signaling pathway is BAPTA-sensitive and uses a G-protein in the inhibition of Ca^{2+} channels in SCG neurons. Supported by NS08174, NS07332, NS07097 and the McKnight and W.M. Keck Foundations.

607.13

ONTOGENY OF P-TYPE CALCIUM CHANNEL IN THE RAT CEREBELLAR CORTEX. B.D. Cherksey*, M. Sugimori, D. Hillman and R. Llinás. Dept. of Physiology/Biophysics, NYU Medical Center, NY, NY 10016.

The time course of the development of P-channels in Purkinje cells was studied morphologically and functionally in rats of different ages: neonate (1 to 3 days), immature (6-8 days) and young (10-14 days).Morphologically, immunohistochemistry using anti P-channel polyclonal antibodies indicated a somatic location of reaction product in the neonate cells, becoming progressively more dendritic in immature cells and finally, mostly dendritic as the Purkinje cell develops over the next two weeks. Electrophysiologically whole cell patch clamp showed the presence of a low threshold calcium current at day 3, which was not FTX sensitive. A calcium current of the high threshold type appeared as the cell matured. This current was blocked by FTX and was indistinguishable from those in young or adult animals. Lipid bilayer study of neuronal membrane vesicles from the cerebellum confirmed the absence of P channel and the presence of a large (>50 pS) calcium channel, presumably of non-Purkinje cell origin, not blocked by FTX. P-type channels, blocked by FTX, were present in membranes obtained from the young animals. These results indicate that in early development, there is a transition from T-type to P-type channels, in the mammalian Purkinje cell and which correlates with the dendritic development in these cells. Support by NS13742 and AG09480.

AXON GUIDANCE MECHANISMS AND PATHWAYS VII

608.1

MOLECULAR GENETIC ANALYSIS OF TWO MEMBERS OF THE DROSOPHILA FASCICLIN IV GENE FAMILY. D. Matthes, A. Kolodkin*, and C. S. Goodman. HHMI, Dept. MCB, Univ. of California, Berkeley, CA

We previously reported on the cloning and characterization of the gene encoding a novel integral membrane protein, fasciclin IV (G-fasIV), that functions in growth cone guidance in the PNS of the grasshopper embryo (Kolodkin et al., Neuron, 1992,). Fas IV is expressed on a subset of fasciculating axons in the CNS of the grasshopper embryo; it is also expressed in peripheral tissues. In the limb bud, a pair of growth cones that pioneer a sensory pathway make an abrupt circumferential turn at a stereotyped location. Fas IV is expressed on the epidermal cells where this turn is made. Antibody blocking experiments show that fas IV functions as a key guidance molecule for this turn. Consistent with its peripheral expression on epidermal cells but not growth cones, fas IV does not function as a homophilic adhesion molecule in an in vitro assay.

In order to further characterize the function of fas IV using genetic analysis, we used a PCR-based approach to clone the Drosophila fas IV homologue. Here we report on the cloning of two members of the fas IV gene family in Drosophila. Sequence analysis suggests that one gene is the true Drosophila homologue (D-fasIV), whereas the second gene (D-fasIV-related) is more divergent. Both genes, however, encode cysteine-rich integral membrane proteins that share structural features and sequence similarity. Together they define a novel gene family. Whole-mount in situ hybridization experiments show that both Drosophila genes are expressed in the embryonic CNS and in other tissues. A P-element located within the D-fasIV-related gene provides us with the starting point for a genetic analysis of the function of this gene. Given the previous evidence that at least one member of this new gene family (G-fasIV) plays an important role in growth cone guidance, it will be interesting to determine if other members of the gene family have related functions.

608.2

ALIGNMENT OF PIONEER GROWTH CONE MIGRATION WITH AN ORTHOGONAL ARRAY OF MOLECULARLY-DEFINED EPITHELIAL DOMAINS. M. A. Singer*, T. P. O'Connor, and D. Bentley. Department of Molecular and Cell Biology, University of California, Berkeley, CA 94720.

In embryonic grasshopper limb buds, guidance information for migration of pioneer growth cones along a stereotyped route to the CNS can be provided by the epithelium and by epithelium-derived cells. This epithelium is partitioned into sharply-defined domains characterized by molecular expression patterns. The *annulin* (Singer et al., 1992, Dev. Biol. **154**, 143) and *fasciclin IV* (Kolodkin et al., 1992, Neuron **9**, 831) proteins, and endogenous alkaline phosphatase activity (Chang et al., Development, in press) each are expressed in circumferential bands of epithelial cells at specific segmentally-iterated locations along the proximodistal axis of the limb. The borders of expression of a fourth gene product, the *engrailed* (Kornberg et al., 1985, Cell **40**, 45) protein, lie orthogonal to the circumferential bands of the other three gene products. During circumferential growth, a major segment of the pioneer pathway, the growth cones migrate precisely between specific alkaline phosphatase and annulin bands, and in register with a fasciclin IV band. Subsequently, the growth cones make a 90° turn and migrate parallel to the ventral engrailed boundary. These four expression patterns thus demarcate an orthogonal grid of epithelial domains with which much of the pioneer pathway is aligned. Growth cone migration across the limb epithelium appears likely to be guided by at least three sources of information: (1) molecular gradients on the sc `the whole limb (Norbeck et al., 1992, Development **116**, 467), (2) . nal orthogonal molecular expression domains, and (3) local guidepost cells.

608.3

THE GLYPICAN FAMILY OF GLYCOSYLPHOSPHATIDYLINOSITOL-ANCHORED HEPARAN SULFATE PROTEOGLYCANS IN THE DEVELOPING RAT BRAIN. A.D. Lander*[1,2], A. Kumbasar[1], E.D. Litwack[1], and C.S. Stipp[1]. [1]Dept. of Biology and [2]Dept. of Brain & Cognitive Sciences, M.I.T., Cambridge, MA 02139.

Cell-surface proteoglycans (PGs) are thought to act as receptors or co-receptors for many of the extracellular molecules that control the differentiation, migration, and axonal projection of neurons. Of the ~25 major PGs of the developing rat brain, two of the most abundant, PGs M12 and M13, bear heparan sulfate (HS) glycosaminoglycan (GAG) chains, and are glycosylphosphatidylinositol (GPI)-anchored integral membrane proteins (Herndon and Lander, 1990, Neuron *4*:949-961). To identify these molecules, they were purified from neonatal rat brain, and protein sequences obtained from tryptic fragments of their core proteins. These and other data indicate that PG M12 is the rat form of *glypican*, a GPI-anchored HSPG first identified in human fibroblasts. PG M13, in contrast, is distinct from glypican. Using PCR, a cDNA for this PG was obtained, and western blots using anti-fusion protein antibodies confirm that the cDNA encodes M13. The full sequence of this molecule, which we have named *cerebroglycan*, indicates that it is related to glypican, exhibiting strong conservation of cysteine motifs and consensus GAG attachment sites, and 38% overall identity. *In situ* hybridization studies indicate that glypican and cerebroglycan exhibit distinct patterns of expression: Glypican mRNA is found in some populations of projection neurons of the adult brain, especially parts of the hippocampus, the thalamus, and in 1° motoneurons. Glypican is also expressed in the brain during development, as well as in non-neural tissues. Cerebroglycan mRNA, in contrast, is found only in the nervous system; its mRNA is expressed at least as early as E12 in the rat, is found throughout the nervous system at E16, but later becomes restricted to a few brain structures, before eventually disappearing entirely. The relationship of these expression patterns to those of HS-binding proteins will be discussed. Supported by NIH NS26862.

608.4

AN EXTRACELLULAR MATRIX MOLECULE PRESENT IN THE DEVELOPING VISUAL PATHWAY. S. Henke-Fahle*. Dept. of Ophthalmology, University of Tübingen, Germany.

Growth cone morphology of early growing axons varies in a position dependent manner while they navigate along their pathway, being more complex at choice points. In the optic pathway, growth cones entering the optic nerve head are the largest and most complex, suggesting that they are encountering an environment molecularly different from the retinal one they are leaving (Holt, J. Neurosc. 9, 3123-3145, 1989). A search for monoclonal antibodies that bind to cells situated along the route retinal ganglion cell axons normally follow led to the identification of a molecule present on neuroepithelial cells of the optic stalk. The monoclonal antibody (Te38) stains the cells of the optic fissure and the presumptive optic nerve in early chick embryos (E4), whereas the retina is devoid of label. At this stage, staining in the optic tectum is confined to radial cells and is displayed in a graded fashion (a>p). Immunofluorescence of living cells locates the antigen on the surface and in the immediate vicinity of cells. The solubilization properties of the antigen are consistent with those of extracellular matrix proteins. Western blot analysis of 4M urea extracts from 7d embryonic tecta revealed 3 bands of app. molecular weight 370, 400 and 440 kD. Whether this molecule provides guidance cues to retinal ganglion cell growth cones remains to be investigated. Supported by DFG He 1514/2-1.

608.5

CHONDROITIN SULFATE IS LOCALIZED AT THE MIDLINE DURING RETINAL AXON DIVERGENCE IN THE MOUSE OPTIC CHIASM. J. H. Lustgarten, R.C. Marcus, L.-C. Wang, and C. A. Mason*, Dept. Pathology, Coll. Phys. Surg., Columbia Univ., NY, NY. 10032

A growing body of evidence suggests that the divergence of retinal ganglion cell axons occurs by interactions of growth cones with components of the developing optic chiasm. Previous studies using DiI-labeled fixed tissue or time-lapse video microscopy suggest that cues localized at or near the chiasm midline may selectively repulse fibers arising from the temporal retina that project to the ipsilateral side of the brain. The molecular identity of these cues remain unknown. Certain extracellular matrix proteoglycans have been implicated as inhibitors of axon growth in the nervous system, subserving a "boundary" function at midline regions in the developing spinal cord and optic tectum. We have localized one of these molecules, chondroitin sulfate, to the midline of the diencephalon and optic chiasm in E13 - E15 mouse embryos, using the monoclonal anti-chondroitin sulfate antibody CS-56 (Sigma # C-8035) and immunofluorescence. At E13 labeling is concentrated at the midline in the ventral diencephalon. At E15 there is dense staining anteriorly in the region of the "glial knot". More posteriorly the staining localizes in the midline above the nerve fiber layer. In all cases staining is most intense at the interface of the fiber layer and the diencephalon.

To determine whether chondroitin sulfate plays a role in selective axonal guidance in the developing chiasm, we are studying the expression of the molecule by dissociated chiasm cells in culture. Under certain conditions dissociated cells from the developing chiasm aggregate to form "islands" that selectively repulse fibers growing from co-cultured explants of temporal-inferior ("TI") retina, the region giving rise to the ipsilateral projection [L.-C. Wang et al., Soc Nsci Abstracts, 1992]. Explants that give rise to crossing fibers generally grow over these islands. In many instances, chiasm cell islands express immunoreactivity for CS-56. Whether expression of this molecule correlates with selective repulsion of "TI" fibers is currently being studied. These analyses should help to elucidate whether this molecule plays a role in axon pathfinding in the optic chiasm.

608.6

EARLY RETINAL AXON GROWTH IN THE MOUSE VENTRAL DIENCEPHALON R.C. Marcus* and C.A. Mason, Dept. Pathology, Coll. Phys. Surg., Columbia Univ., NY, NY 10032.

Previous studies of retinal axon guidance suggest that both fiber-fiber interactions and the local cellular environment provide guidance cues for the growth of the permanent crossed and uncrossed projection in the mouse optic chiasm. Here we examine the pattern of growth of the earliest optic axons with reference to growth of fibers from each eye and to resident cells in the ventral diencephalon.

DiI and DiO labeling of the entire optic projection was combined with immunocytochemistry in wholemounts of the ventral diencephalon of E12.5-13.5 embryos. Several antigen markers were used based on our studies of later periods. Preparations were viewed first as wholemounts, then sectioned frontally. This method provided a 3-D analysis of axon trajectory in relation to resident cells that can only be appreciated in wholemounts.

MAb RC2 labels a midline palisade of radial glia which is penetrated by both crossed and uncrossed axons. However, all optic axons, regardless of destination, initially grow posteriorly in lateral, RC2-negative regions. Crossed axons do not grow directly into the contralateral optic tract but curve anteriorly as they approach the midline. This is in contrast to the pattern seen at E15-17, when crossed axons traverse the midline on the diagonal. Early axons course lateral to and under a population of SSEA-1-positive cells (Mason et al., Soc Neurosci Abstr, 1991) present in an inverted "V" (similar to CD44 - Sretavan et al., Soc Neurosci Abstr, 1992) suggesting these cells form an inhibitory boundary. Fiber-fiber interactions are not important in establishing the visual pathway since axons grow into the ipsilateral optic tract independent of interactions with crossed axons from the other eye. We are currently examining the relation of early axons to both F4/80-positive microglia and chondroitin sulfate (Lustgarten et al., this volume).

These analyses suggest that multiple cues are involved in the establishment of the visual pathway and that the relative contribution of cues to the divergence of retinal axons in the mouse chiasm may differ during initial vs. subsequent development.

608.7

***IN VITRO* RESPONSES OF NASAL RETINAL FIBERS TO MEMBRANE FRAGMENTS FROM SUPERIOR COLLICULUS IN MICE** B. Llirbat and P. Godement*. Institut Alfred Fessard, CNRS, 91198 Gif-sur-Yvette, France.

Studies done *in vitro* have shown that growth cones of fibers from temporal retina are inhibited by components which are present as a concentration gradient increasing along the anterior-posterior axis of the superior colliculus in mouse, chick, and fish. Such a gradient could influence the growth of fibers from temporal retina *in vivo*, leading them to avoid caudal aspects of the superior colliculus. Using the collapse assay in which membrane vesicles are added to cultures in which the growth cones of retinal fibers from nasal or temporal retina are viewed in real-time, we find that membrane fragments taken from the anterior aspects of the superior colliculus of embryonic mice (E15-17) lead to a collapse of the growth cones of nasal retinal fibers (E14), whereas posterior membranes have no such effect. Vice-versa, posterior membranes lead to a collapse of temporal fibers, but anterior membranes have no effect. Temporal growth cones react by withdrawing their lamellipodia, and the body of the growth cones retracts. Nasal growth cones react in a different manner. The lamellipodia are withdrawn, and the growth cone becomes filiform but within the next hour, some extension of the fiber tip is often observed. This response of nasal fibers is observed in cultures that are grown in serum-free conditions; in the presence of fetal calf serum, no effect is observed. These experiments suggest that complementary gradients of molecules in the retina and in the superior colliculus could be involved in setting up the polarity of retinal projections along the nasal-temporal axis of the retina. The observation of the different behaviors of nasal and temporal growth cones in response to anterior and posterior membranes suggests that different mechanisms are involved in each type of response and could be related to the fact that in vivo, nasal fibers first have to extend through anterior aspects of the superior colliculus before reaching its posterior aspects Supported by C.N.R.S., I.N.S.E.R.M., and M.R.T.

608.8

DISTRIBUTION AND FUNCTION OF T-CADHERIN DURING THE PROJECTION OF MOTOR AXONS TO THE HINDLIMB B. Fredette* and B. Ranscht, La Jolla Cancer Research Foundation, La Jolla CA 92037

The lipid-linked member of the cadherin family, T-cadherin, is expressed in the developing spinal cord and surrounding mesenchyme in regions not traversed by growing motoneurons, such as the floor plate and posterior sclerotome. Also, T-cadherin is expressed on motoneurons themselves, suggesting that T-cadherin's homophilic binding functions as a deterent to growing motor axons. Immunofluorescence in the developing chick hindlimb region revealed a distribution that is consistant with this proposal. All lumbosacral motoneurons expressed T-cadherin as they projected segmentally through the T-cadherin-negative anterior sclerotome to the plexus region at the base of the limbud (st 20-25). T-cadherin then decreased on motor axons as they refasciculated to form nerve trunks projecting to individual muscles, and remained negative until after their growth cones had invaded the T-cadherin-negative muscle masses at st 27. At st 28, T-cadherin was reexpressed heterogeneously among motor neuron pools. This may reflect differences in axonal branching patterns within muscles, since the heterogeneity arose while axons branched and formed synapses in the muscles. In muscle, T-cadherin slowly increased to extremely high levels on maturing myotube surfaces and persisted after hatching, but was excluded from synaptic junctions. In vitro experiments also indicated an inhibitory role for T-cadherin: neurite outgrowth from sympathetic and DRG neurons as well as from ventral spinal cord explants was inhibited by T-cadherin substrates. A comparison of neurite growth from T-cadherin-positive and -negative populations of motoneurons on T-cadherin substrates is presently underway to determine if the inhibition is due to homophilic T-cadherin adhesion. Thus T-cadherin in the floor plate of the neural tube and in the posterior sclerotome may act to prevent motor axon advance through these regions, and to arrest axonal growth in the maturing muscle.

608.9

CHEMOTAXIS OF GROWTH CONE OF *XENOPUS* SPINAL NEURON IN A GRADIENT OF ACETYLCHOLINE. J.Q. Zheng, M. Felder, J.A. Connor+, and M-m. Poo*, Dept. of Biol. Sci., Columbia Univ., New York, NY 10027; +Roche Inst. of Mol. Biology, Nutley, NJ 07110.

With repetitive pressure ejection of picoliters of concentrated acetylcholine (ACh) solution from a micropipette, a microscopic ACh gradient of about 10% in micromolar range was produced across single growth cones of embryonic *Xenopus* spinal neurons in cell culture. Facing such an extracellular gradient of ACh, the growth cones of cultured *Xenopus* spinal neurons exhibited a turning response toward the source of ACh. Control experiments with normal culture medium or NaCl solution did not produce such a chemotactic response. The chemotactic response of the growth cones to the gradient of ACh appeared to be mediated by neuronal nicotinic ACh receptors, since it was inhibited by d-tubocurarine. Furthermore, the chemotaxis of growth cones in ACh gradients was found to be blocked either by withdrawal of Ca^{2+} from the bath medium or addition of KN62, an specific inhibitor of calmodulin-dependent protein kinase II (CaM Kinase II). Preliminary experiments with Fura-2 ratio imaging demonstrated a small but detectable elevation of Ca^{2+} at growth cone after onset of ACh gradient but before the actual turning. Taken together, our results indicate that the chemotaxis induced by ACh gradient involved the activation of neuronal nicotinic ACh receptors and Ca^{2+}-CaM KinaseII signaling pathway. Our finding also indicates that neurotransmitters, besides serving as messengers at the chemical synapse, are potential neurotropic agents during development of the nervous system.

608.10

THE NEUROTROPIC EFFECTS OF NERVE GROWTH FACTOR IN THE REGENERATING PERIPHERAL NERVE

W. Geoff Williams, M.D.* J.R. Perez-Polo, PhD. Linda G. Phillips, M.D. Martin C. Robson, M.D. UTMB, Galveston, TX.

Nerve growth factor (NGF) exerts neurotropic effects in vitro. Little is known about the neurotropicity of NGF in vivo. Here a rat sciatic nerve model was employed wherein the nerve was transected (distal nerve was removed) and the proximal stump placed into the singlet end of a Y-shaped silicon nerve regeneration chamber. In four groups of rats, NGF at concentrations of 714, 178 and .71 μg/ml and sheep antisera to NGF was infused over 2 weeks via subcutaneously-placed Alzet minipump and catheter into one arm of the Y implant. Corresponding concentrations of cytochrome-C solution were infused into the other implant arm via a separate pump and catheter. At four weeks, tissue from the two arms of the implant was evaluated histologically and myelinated axons counted in order to compare the effects of NGF and anti-NGF versus their respective controls. In the NGF groups, there was no statistical difference among the number of myelinated axons in NGF regenerates when compared to adjacent controls. In the anti-NGF group however, the antibody-treated arm of each regenerate contained significantly less myelinated and unmyelinated axons compared to its control counterpart. There was an increase in cellular inflammation and fibrosis in the anti-NGF-treated regenerates. We conclude that endogenous NGF exerts a neurotropic effect on regenerating axons, an effect that is not affected by exogenous NGF.

609.1

UPTAKE INHIBITORS SELECTIVELY ATTENUATE DEPLETIONS IN CORTICAL AND HIPPOCAMPAL SEROTONIN AND NOREPINEPHRINE CAUSED BY 2'-NH_2-MPTP IN TWO STRAINS OF MICE. A. M. Andrews* and D. L. Murphy. Lab. of Clinical Science, NIMH, Bethesda, MD 20892.

Recently, we reported that the novel MPTP analog 1-methyl-4-(2'-aminophenyl)-1,2,3,6-tetrahydropyridine(2'-NH_2-MPTP) administered to C57BL/6 mice produces substantial decreases in forebrain serotonin (5-HT), and norepinephrine (NE) with negligible effects on brain dopamine (DA). We now report that compared to C57BL/6 mice, 2'-NH_2-MPTP causes significantly greater decreases in hippocampal 5-HT and NE (80-90% vs. 60%), and similar magnitude changes in cortical 5-HT and NE (60-75%) in Swiss Webster mice. In C57BL/6 mice receiving either fluoxetine or desipramine (10 mg/kg) prior to 2'-NH_2-MPTP, decreases in 5-HT and NE, respectively, were moderately attenuated by ~30-40% 1 week post-treatment ($p<0.05$). In Swiss Webster mice however, pretreatment with 10 mg/kg fluoxetine or paroxetine, or 10 mg/kg desipramine, completely prevented decreases in cortical and hippocampal 5-HT and NE, respectively, 3 weeks after 2'-NH_2-MPTP ($p<0.001$). Therefore, not only are Swiss Webster mice more sensitive to the effects of 2'-NH_2-MPTP in hippocampus, but they also seem to be more fully protected from the toxic effects of 2'-NH_2-MPTP by pretreatment with uptake inhibitors. Finally, these results suggest that the differential effects of fluoxetine and paroxetine versus desipramine are most likely due to the inhibition of active transport of 2'-NH_2-MPTP, or possibly a neurotoxic metabolite of 2'-NH_2-MPTP, into serotonergic or noradrenergic nerve terminals via their respective transport systems.

609.2

PEROXYNITRITE (POX) SCAVENGERS: POSSIBLE NEUROPROTECTANTS AGAINST NITRIC OXIDE (NO)-DEPENDENT TOXICITY. J.S.Althaus*, T.T.Oien, G.J.Fici, H.M.Scherch, A.F.Strautman, V.H.Sethy and P.F.VonVoigtlander. CNS Research, The Upjohn Company, Kalamazoo, MI 49001.

The toxic intermediate, POX, may form *in situ* when high concentrations of NO and $^1O_2$, generated during ischemia, react with each other. Once formed POX can then nitrate and/or hydroxylate proteins and lipids resulting in cell injury and death. The present study was undertaken to investigate the cysteine genus for POX scavengers.

POX scavengers were assayed by Attoflo®, an automated radioimmunoassay. Briefly, POX, in a dose dependent manner (0.1 to 30 mM) destroyed antibody activity by inhibiting the binding of I^{125} cAMP to a polyclonal antibody used in the assay of cAMP. At 10 mM, POX caused a 90% loss of antibody activity. Drugs were tested as protectants of this loss in antibody activity. Actives were then tested as inhibitors of POX toxicity in cerebellar granular cells treated with buthionine sulfoximine (BSO), a drug which depleted cellular glutathione and potentiated toxicity.

At 10 mM, POX caused a 90% loss of antibody activity. Cysteine protected the antibody from this lost activity in a dose dependent manner (EC_{50} = 10 mM). By comparison, the loss of activity caused by POX (10 mM) with cysteine, cysteine methyl ester or cysteine ethyl ester (at 3 mM) was only 80.7%, 55.4% or 45.3% respectively. BSO potentiated the toxicity of POX in cerebellar granular cells. Micromolar concentrations of cysteine and related compounds completely blocked this toxicity.

As research continues regarding the toxic role of NO, it is clearer that POX may be an important toxic intermediate. POX scavengers represent a new class of compounds that may be useful as neuroprotectants.

609.3

2,5-HEXANEDIONE (2,5HD) AND ACRYLAMIDE (ACR) PRODUCE DIFFERENT PATTERNS OF ELEMENTAL ALTERATIONS IN PERIPHERAL NERVE. R.M. LoPachin*, E.J. Lehning and A.J. Saubermann. SUNY Stony Brook, Stony Brook, NY 11794-8480.

The structural and functional consequences of 2,5HD-induced distal axonopathy might be mediated by subaxonal disruption of elements (e.g., Na, K, Ca) and water. To examine this possibility, we used electron probe X-ray microanalysis to measure elemental concentrations (mmol element/kg dry or wet weight) and percent water in peripheral nerve myelinated axons and Schwann cells from control and 2,5HD (oral, 0.4% w/v) intoxicated rats. Regardless of the length of neurotoxicant exposure (78, 85 and 104 days), tibial nerve internodal axon regions exhibited moderate decreases in axoplasmic Na, K and water content. In proximal sciatic nerve regions, similar patterns of elemental change were noted, although the magnitude of change was not as pronounced. We interpret these elemental alterations as a manifestation of previously reported 2,5HD-induced axonal atrophy. Giant axonal swellings were observed in both tibial and proximal sciatic nerve regions. Independent of nerve area, neither elemental composition nor water content was altered in swollen axons. These changes are unlike the complete elemental deregulation associated with swollen peripheral nerve axon areas of ACR-intoxicated rats (LoPachin et al., Tox. Appl. Pharm. 115:21, 1992). In both proximal and distal nerve regions, Schwann cells exhibited substantial derangement of Na, P, Cl, K, Ca and water. These glial alterations are injury-typic and are different from those associated with ACR neurotoxicity (LoPachin et al., Tox. Appl. Pharm. 115:35, 1992). The dissimilar patterns of elemental perturbation which characterize 2,5HD and ACR imply that the respective mechanisms of nerve injury are distinct. (Supported by NIH grant ES03830)

609.4

POWER SPECTRAL ANALYSIS (PSA): OVERNIGHT ELECTROENCEPHALOGRAPHIC (EEG) SLEEP STUDIES OF CHILDREN WITH ACUTE LYMPHOBLASTIC LEUKEMIA (ALL)

L. C. Parsons*, M. Perlis, P. Jones, K. Applegate, S. Humble, T. Skinner, R. Temes, V. Townsend, L. Crosby, I. Moore, and J. Hutter, CoN UofA Tucson 85721

Triple Intrathecal Chemotherapy (TIT/CTX) on the sleep awake cycles of children with ALL has not been studied. Purpose: to measure the PSA of the first three overnight EEG sleep cycles in four children, between the ages of 10 and 18 years, who were within 46 wks of diagnosis of ALL. Children, receiving TIT/CTX, were matched with four healthy control subjects according to age, gender, cultural ethnicity and socio-economic status. The eight children spent a conditioning night and a study night within the sleep lab. The methodology included downloading directly into a computer, from a polygraph, four channels of EEG data (F3-C3, F4-C4: frontal; C3-P3, C4-P4: central). Data were converted from an analog to digital format. EEGs were sampled at 128 samples/second for each of the overnight recordings. Rhythm Software V.7.10 (Stellate Corp.) carried out digitization in the following EEG bandwidths: δ (.75-2.5), θ (2.75-7.25), α-1 (7.5-9.75), α-2 (10.0-11.75), Σ (12.0-14.0) and B (18-30). EEG data were submitted for PSA in the form of NREM and REM cycles. Prior to PSA analysis; artifacts > 2 sec. were removed. Data were collected for power in 4 sec. epochs. Multiple epochs were averaged to yield the average 4 sec. power spectral distribution for each NREM/REM cycle. Data analysis: Analysis of variance (ANOVA) was used to assess significant main effects for patient grouping (ALL/control), cycle (1-3), or their interaction. 24 ANOVAs were calculated for frontal and central montages, averaged over lt and rt hemispheres, for both REM and NREM cycles for each frequency. Results: Significant group effects for θ ($p = .0411$) and α-1 ($p = .0452$) in the non-REM cycle and θ ($p = .0261$) and B (.0441) in the REM cycle. A marginally significant effect for REM was also obtained in REM for α-1 ($p = .0590$). The pair by group interaction was highly significant for all frontal and central analyses with p-values ranging from .0001 to .0286. Means of the frontal and central data show that each leukemic pt had higher values when compared to his/her control except in the α-2 data. One pair displayed a consistent reversal in central data. Significantly elevated power density in frontal leads in the frequencies of θ and α-2 may suggest early side effects of TIT/CTX.

609.5

EFFECT OF 1-METHYL-4-PHENYL-PYRIDINIUM (MPP^+) ON DNA SYNTHESIS IN THE MOUSE BRAIN CELL NUCLEUS. Nguyen T. Buu* IRCM, University of Montreal, Montreal (Quebec) H2W 1R7 Canada

MPP^+ is the main product responsible for the pathogenesis of parkinsonism induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) administration in humans, monkeys and mice, but not in rats. However the mechanisms by which MPP^+ causes dopaminergic neurons to degenerate and inflicts more damage in some animal species than in others is not yet understood. Recently, we found that cell nuclei from C57 mouse brain, in contrast to cell nuclei from Sprague-Dawley rat brain, accumulate and contain uptake system for MPP^+ (Buu, 1993). In this study, we determine what effect MPP^+ may have on DNA synthesis in the isolated cell nuclei.

Cell nuclei were prepared from C57 mouse brains by standard methods using detergent Triton X-100 and sucrose gradient. They were incubated for 60 min at 37^0C with ^3H methyl thymidine in the presence and absence of 1 mM MPP^+. DNA were precipitated in 15% TCA.

The results showed that MPP^+ inhibits thymidine incorporation by almost 70 percent, indicating that MPP^+ can affect a nuclear function. Whether the inhibitory effect of MPP^+ on DNA synthesis may contribute to its degenerative effect on dopaminergic neurons is being investigated.

609.6

DISSOCIATION OF CIS-PLATINUM INDUCED NEUROTOXICITY AND CHEMOTHERAPEUTIC EFFICACY. N.Y. Lorenzo, M.M. Ames, A.J. Windebank*. Mayo Clinic and Foundation, Rochester, MN 55905 USA.

The chemotherapeutic efficacy of cis-platinum depends upon production of covalent inter- and intrastrand DNA crosslinks. The mechanism of neurotoxicity is unknown. In rat dorsal root ganglion neurons (DRG) in tissue culture, we have previously demonstrated that the melanocortin analog, $ACTH_{4-9}$ may prevent cis-platinum induced axonal damage *in vitro*. In the present studies, DNA crosslinking was studied using alkaline elution. L1210 mouse leukemia cells were incubated with ^{14}C thymidine or ^3H Thymidine x-irradiated, lysed, and passed through PVC filters using alkaline conditions (pH 10.1). As previously reported by others, exposure of cells to cis-platinum significantly retarded elution in a dose dependent manner. L1210 cells were then incubated with cis-platinum (5 and 10 μg/ml) and $ACTH_{4-9}$ (1 μg/ml). This did not alter the cis-platinum effect.

Two cancer cell lines (L1210 and Hey, human ovarian epithelial cell cancer) were used. Viability was assessed by Coulter cell counting or neutral red assay. Cells were exposed to cis-platinum (1,3,5,7,10,20 μg/ml) for 3 hours followed by a 72 hour post-incubation in control medium. For both lines, cis-platinum produced dose dependent cell death (52.6% at 1 μg/ml; 96.6% at 20 μg/ml). Preincubation and coincubation with $ACTH_{4-9}$ did not alter this cytotoxicity.

Conclusion: Cis-platinum produces predictable cytotoxicity in murine and human cancer cells which is associated with DNA covalent crosslinking. This effect is not altered by concentrations of $ACTH_{4-9}$ which have been demonstrated to ameliorate neurotoxicity. This strongly suggests that the neurotoxic and chemotherapeutic effects may be dissociated.

609.7

CONVULSANT EFFECTS OF INTRAVENTRICULAR FOLATES. S.R. Snodgrass*, Z.H. Zhang, AND P.B. Bradshaw, Neurology Division, Dept. of Pediatrics, Childrens Hospital Los Angeles, Los Angeles, CA 90054 and University of Mississippi Medical Center, Jackson, MS 39216.

Past authors (Hommes, Olney) reported folate neurotoxicity in experimental animals. However, intraventricular folate administration has been used for human methotrexate (MTX) neurotoxicity. We compared intraventricular and intracisternal folate injections in rats with ether anesthesia, chloral hydrate anesthesia and no anesthesia. All folates tested produced seizures. Greater doses were needed to produce seizures when injected into the cisterna magna, or when chloral hydrate was used as anesthetic. Oxidized folates were more potent convulsants than 5-methyltetrahydrofolate, tetrahydrofolate or methotrexate. Folic acid and methotrexate convulsant effects were additive when both were given together. Folate convulsant effects were reduced by sodium ascorbate pretreatment, by simultaneous injection of the NMDA receptor blocker MK-801, and by benzodiazepines. Folate convulsant effects were seen with or without antioxidant. Folates, like penicillin, should never be injected into the ventricles or cisterna magna of humans (Snodgrass, Molec. Neurobiol. 6:41, 1992). Rats convulsing after intraventricular injection

Folate dose	10 ug	30 ug	100ug
5-methyltetrahydrofolate		1/5	2/5
folic acid	1/5	2/5	6/7
methotrexate	0/5	0/5	2/5
dihydrofolate		1/4	4/5

FA (10) + MTX (30) 3/5 FA(30) + MTX (50) 5/5

609.8

TRYPTOPHAN (TRP) PRETREATMENT ATTENUATES THE NEUROTOXIC EFFECTS OF PARA-CHLOROAMPHETAMINE (PCA). C.W. Bradberry*, R.N. Iyer, J.S. Sprouse, G.K. Aghajanian, R.H. Roth Yale Univ. Sch. Med., Dept. of Psychiatry and the West Haven VA Medical Center, West Haven, CT 06516.

The mechanism of toxicity of amphetamine analogs such as PCA and (+)-3,4-methylenedioxymethamphetamine (MDMA) on 5-HT neurons remains unclear, perhaps involving the release of dopamine (DA) and/or 5-HT (possibly peripheral 5-HT). We have previously demonstrated that TRP potentiates the ability of MDMA to release 5-HT from slices of dorsal raphe in vitro, suggesting it might also have this effect in vivo. We examined the impact of TRP pretreatment upon PCA (2 mg/kg i.p.) neurotoxicity, assessed by whole tissue levels of 5-HT one week following treatment. This is a low dose producing partial loss of 5-HT levels (30-50% depletion). TRP pretreatment (400 mg/kg of the methyl ester, i.p. 20 min prior to PCA) completely reversed the decrease in tissue 5-HT in striatum, partially reversed it in prefrontal cortex, and had no effect in the hippocampus. In order to determine what impact TRP had on PCA-induced neurotransmitter release, a separate group of animals had microdialysis probes implanted in striatum 24 hours prior to PCA/TRP administration. TRP pretreatment signifcantly increased PCA-induced 5-HT release from 7- to 24-fold, and increased PCA-induced DA release from 2.5- to 4-fold, indicating that enhanced central 5-HT release does not increase PCA neurotoxicity despite an accompanying increase in DA release. Supported in part by MH 14092, DA 08073, the State of Connecticut, and a NARSAD Young Investigator Award to C.W.B.

609.9

AUTOANTIBODIES TO NERVOUS SYSTEM PROTEINS AS MARKERS OF LEAD NEUROTOXICITY. HAN El-Fawal, AS Little, ZL Gong and HL Evans*, Nelson Institute of Environmental Medicine, NYU Medical Center, Tuxedo, NY, 10987.

Environmental toxicants may induce changes in the nervous system that are not easily detectable in humans. We have developed an ELISA to measure serum autoantibodies to neuronal and astrocyte structural proteins [neurofilament triplet proteins (NF68; NF160; NF200), myelin sheath (myelin basic protein: MBP) and glial fibrillary acidic protein(GFAP)] as early markers of neurotoxicity. Since the nervous system is "immunopriviliged", these proteins may act as autoantigens following neuronal degeneration and blood-brain barrier compromise. F344 rats (>42 d) were exposed to 0, 50 or 450 ppm lead acetate (Pb) in the drinking water. At 3, 7, 14, 21 and 42 d of exposure rats were euthanized and blood collected for assays and Pb determination. There were no overt signs of toxicity as indicated by body weight and home-cage behavior. Only serum from exposed rats had detectable autoantibody titers. Immunoglobulin (Ig) M and G to neural proteins, particularly IgM, were detected as early as 3 d of Pb exposure. There was a dose-dependent response in autoantibody levels between 50 and 450 ppm. The elevation of IgM vs IgG titers was consistent with a primary antigen (autoantigen) challenge. An early elevation in anti-NF, particularly NF68, and anti-MBP titers was consistent with Pb's targeting of axons and its ability to produce demyelination. Detection of anti-GFAP titers was consistent with decreased GFAP in brains of the same rats, suggesting damage to astrocytes. This study indicates that the assay of autoantibodies to nervous system proteins may provide a sensitive and accessible biomarker of neurotoxic effects. A pilot study of exposed humans showed the feasibility of this technique for occupational and environmental health surveillance. (Supported by API and grants ES00260 and ES04895)

CARDIOVASCULAR REGULATION: SPINAL AND PERIPHERAL CONTROL

610.1

EXERCISE INHIBITS BARORECEPTOR-SENSITIVE NEURONES IN THE NUCLEUS TRACTUS SOLITARIUS (NTS) OF THE ANAESTHETISED CAT. P.N.McWilliam*, S.E.McMahon, J.C.Kaye
Cardiovascular Studies, Univ. of Leeds, Leeds, LS2 9JT, UK.

Afferent signals from receptors in skeletal muscle contribute to the increase in heart rate and blood pressure during exercise. This study investigates whether hindlimb contraction elicited by L7 ventral root stimulation inhibits evoked activity in NTS baroreceptor-sensitive neurones. Extracellular recordings were made from interneurones in the NTS activated by electrical stimulation of the carotid sinus nerve. The effect of a 2 second hindlimb contraction on the evoked activity of the neurones was studied. Hindlimb contraction significantly reduced the incidence of evoked firing in a large number of neurones. The majority of these were baroreceptor-sensitive, the remainder were either chemoreceptor-sensitive or unclassified. Ionophoresis of the γ-aminobutyric acid (GABA) antagonist, bicuculline methiodide, antagonised the inhibition produced by hindlimb contraction. Intravenous administration of the neuromuscular blocker vecuronium bromide abolished the inhibition of the evoked activity. Therefore evoked activity in baroreceptor-sensitive neurones in the NTS is inhibited by hindlimb contraction and this inhibition is GABA mediated. This inhibitory mechanism may contribute to the cardiovascular changes in exercise.

610.2

MICRODIALYSIS OF AN OPIOID AGONIST INTO THE L_7 DORSAL HORN OF ANESTHETIZED CATS ATTENUATES THE EXERCISE PRESSOR REFLEX. A.F Meintjes, A. Ally and L.B. Wilson*. Harry S. Moss Heart Center, UT Southwestern Med. Center, Dallas, TX 75235-9034.

We tested the effects of microdialyzing [D-Ala^2]-Methionine-Enkephalinamide (MET-ENK), an opioid agonist, into the L_7 dorsal horn of 5 anesthetized cats on the mean arterial pressure (MAP) and heart rate (HR) responses to static muscle contraction. A dialysis probe was inserted into the L_7 dorsal horn, ipsilateral to the contracting triceps surae muscles. Contractions were induced by stimulating the distal cut ends of the L_7 and S_1 ventral roots for 1 minute. Tension, MAP and HR were recorded continuously. Control contractions (9.5±0.9kg) increased MAP and HR by 62±9mmHg and 20±6b/min, respectively. After 40 minutes of microdialyzing MET-ENK (20μM), contraction (8.3±0.6kg) increased MAP and HR by 30±8mmHg and 10±2b/min, respectively. The MAP response after MET-ENK was significantly less than control ($p<0.05$). Thus, microdialysis of MET-ENK into the L_7 dorsal horn attenuates the pressor and HR responses to static muscle contraction, thereby implicating an opioid mechanism in the integration of muscle afferent nerve activity.

610.3

GESTATIONAL EFFECTS ON CENTRAL AND EFFERENT NEURAL COMPONENTS OF CARDIAC RECEPTOR REFLEXES IN THE RAT. T. Hines and S. W. Mifflin*. Univ. Texas Health Sci. Center, San Antonio, TX 78284.

To determine if gestational effects on central and efferent neural components of the cardiac receptor reflex contribute to the vasorelaxation of pregnancy, activity of cells in the nucleus tractus solitarius (NTS) receiving cardiopulmonary afferent input, and in renal sympathetic nerves (RSNA), as well as arterial and right atrial pressures (MRAP) were measured in response to intra-atrial boluses of saline (50-300μl) or phenylditguanide (PDG, 1-16ug/kg) in anesthetized, sino-aortic denervated pregnant (P, n=9) and virgin (V, n=13) rats. Effects of acute volume expansion are as follows:

		50μl	100μl	200μl	300μl
%Δ MRAP	P	82.3±30.4*	105.4±46.4*	102.6±45.8*	192.4±84.8*
(*p<0.05vs virgins)	V	11.81±2.36	11.36±1.84	13.77±1.89	19.62±2.85
% RSNA	P	95.25±1.18	92.88±1.7	92.36±1.84	91.17±1.9
	V	93.83±1.18	90.9±1.18	90.02±1.17	90.4±0.99
ΔCELL FIRING	P	0.5	3.2±1.9	3.9±0.4	2.9±1.5
(spikes/sec)	V	1.0±0.2	5.3±2.0	3.6±1.6	2.2±1.5

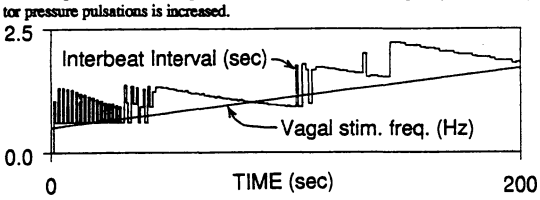
Graded doses of PDG produced dose-dependent hypotension and sympathoinhibition in virgin (n=8) but not pregnant (n=4) rats. Excitatory or inhibitory responses in NTS units were unrelated to dose of PDG in either group.

We conclude that despite markedly larger increases in MRAP in gravid rats during acute volume expansion, cardiac receptors mediate comparable NTS neuronal activity and renal sympathoinhibition in P vs V rats. Thus, gestational effects on reflex NTS and renal nerve activity do not contribute importantly to greater vasorelaxation in the pregnant rat. (Supported by HL36080 and HL08454)

610.4

MATHEMATICAL MODEL OF BARORECEPTOR-VAGAL CONTROL OF HEART RATE SHOWS ENTRAINMENT OF HEART BEAT TO VAGAL STIMULATION. W. C. Rose and J. S. Schwaber*, *Neural Computation Group, E.I. DuPont de Nemours & Co., Wilmington, DE 19880-0323.*

We have developed a mathematical model of vagal control of heart rate as part of our efforts to understand the neural computational aspects of baroreflex control of the heart. It has been observed experimentally that over some frequency ranges the vagus can entrain the heart, i.e. as vagal impulse frequency *increases*, the interbeat interval *decreases*, contradicting conventional expectation (Levy et al., *Circ. Res.* 30:286, 1972). Our model reproduces this counterintuitive behavior, as shown below, by incorporating phase-dependent resetting of an SA nodal oscillator. Although the overall trend shows interbeat interval lengthening as vagal shocks are delivered more frequently, there are ranges over which the opposite occurs. A vagal impulse arriving late in the cardiac cycle is more effective in delaying the onset of the next beat than an impulse arriving early; this property can lead to entrainment and may be due to the dynamics of $I_{K(ACh)}$ (Dexter et al., *Circ. Res.* 65:1330, 1989). When combined with a model of baroreceptor control of vagal activity this leads to interesting behavior including increases in heart rate when frequency of baroreceptor pressure pulsations is increased.



610.5

ELECTRON MICROSCOPIC LOCALIZATION OF POSTSYNAPTIC GLYCINE RECEPTOR (93 KD) IN SYMPATHETIC PREGANGLIONIC NEURONS: RELATIONSHIPS TO GABA- AND NON-GABA-CONTAINING SYNAPTIC TERMINALS. J.B. Cabot*, A. Bushnell and V. Alessi. Dept. of Neurobiology and Behavior, SUNY at Stony Brook, Stony Brook, NY 11794-5230.

Recent anatomical data suggest that rat sympathetic preganglionic neurons (SPNs) receive both glycinergic and GABAergic synaptic inputs. In vitro physiological studies indicate that SPNs respond with Cl^- conductance increases after extracellular application of either GABA or glycine. Interestingly, the GABA responses are partially blocked by the glycine receptor antagonist strychnine at concentrations that completely block responses to glycine (Clendening and Hume, **J.Neurosci.** 10:3977). The present study sought to determine if the intracellular component of the glycine receptor complex (GlyR, 93 kd) was associated with GABAergic synaptic inputs to SPNs.

SPNs in 4 rats were retrogradely labeled with cholera β-subunit. Vibratome sections of T1-T3 were immunoreacted for GlyR and embedded. Serial thick (1μm) and thin sections were collected; most thin sections were immunogold labeled for GABA-like immunoreactivity. All but 1% of terminals on identified (somas, proximal dendrites) and unidentified processes in the SPN neuropil which were opposite GlyR (n=320) contained pleomorphic(58%), pleomorphic and dense core(17%), or pleomorphic and round(24%) vesicles. In tissue immunogold labeled for GABA: **(a)** 303 of 1112 terminals sampled were $GABA^{(+)}$; 17% of $GABA^{(+)}$ terminals were on identified SPN processes, and of these 49% were opposite GlyR; **(b)** 207 of 1112 terminals were opposite GlyR; 45% of these were $GABA^{(+)}$ and 27% of that population were on identified SPN processes; **(c)** 60% of GlyR in unidentified(n=167) and 38% in identified(n=40) processes were opposite $GABA^{(+)}$ terminals. GlyR in SPNs is not uniquely associated with non-GABAergic synaptic input. Supported by HL24103.

610.6

GLYCINE MODULATION OF CARDIOVASCULAR RESPONSES EVOKED BY SPINAL NMDA RECEPTOR ACTIVATION IN CONSCIOUS RABBITS. Huang W. and West M.*, Dept Med, Uni Qld, Prince Charles Hospital, Brisbane, Qld 4032, Australia.

Recent in vitro studies indicate glycine enhances the effects of N-methyl-D-aspartate (NMDA) receptor activation. We have investigated the effects of intrathecal glycine on cardiovascular responses produced by intrathecal stimulation of spinal cord NMDA receptors in conscious rabbits. We also assessed plasma norepinephrine responses induced by hypotension. Following glycine, responses to NMDA and L-glutamate, but not α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA, 0.001-1 μg), were greatly enhanced. Baroreflex induced elevation in plasma norepinephrine was greater following glycine (194 + 31 vs 439 + 38 pg/ml, n=4, $p<0.05$). In the presence of glycine antagonist cardiovascular responses to NMDA were abolished. The results show that (i) glycine amplifies cardiovascular tonic and baroreflex responses (ii) activation of spinal NMDA receptors depends upon the presence of glycine. We conclude that the glycine receptor site within the NMDA receptor complex has an important role in central regulation of the circulation.

610.7

DETERMINISTIC MODEL OF EFFERENT SYMPATHETIC (SYM) ACTIVITY IN NEONATAL SWINE. H.-P. Koepchen, A. W. Przybyszewski L. P. Eberle, B. W. Hundley, P. M. Gootman. (SPON: ENA) Depts. Physiol., SUNY - Hlth. Sci. Ctr., Bklyn, NY 11203 and Freie Universität Berlin, Germany.

Simultaneous recordings of efferent cervical SYMP (CS), splanchnic (SPL) sympathetic and phrenic activity were obtained in Saffan-anaesthetized, paralyzed and artificially ventilated piglets (1 - 38 days of age) along with aortic pressure, EKG, end-tidal CO2. We have previously reported (Neurosci. Abst., 1992,18:1182) that peaks in power spectra (ps) of CS and SPL were correlated in piglets > 19 days old. The ps of longer (10 sec) periods 10 times averaged has shown that the respiratory (RESP) rhythm dominated in CS and cardiac rhythm in SPL. Nitroprusside (NP) injection caused suppression of the cardiac rhythm in SPL. Observation of the CS and SPL activity showed that there were short periods in both nerves when cardiac or RESP rhythm dominated. In piglets > 19days SPL and CS activity can be described as coupled nonlinear oscillators. The difference between both systems was only in their nonlinear properties, SPL was more nonlinear than CS. Both oscillators were forced with sinusoidal signals modulated with a cardiac rhythm at 4 times greater strength than RESP. This model elicited changes in the signals similar to those observed experimentally in CS and SPL. Characteristic broadening, obtained experimentally in ps peaks, were observed in our model only for quasiperiodic or chaotic oscillations. These properties were shown by plotting the relationship between CS and SPL in phase-space. In 10 sec time periods the attractor (att) changed its position in time, which appeared very similar to the Rössler hyperchaos. The structure of the att was very sensitive and disappeared after NP injection. (Supported by NIH grant HL-20864.)

610.8

REACTION OF ADRENAL SYMPATHETIC PREGANGLIONIC NEURONS (SPNs) TO DEAFFERENTATION CAUSED BY SPINAL CORD INJURY. A.V. Krassioukov* and L.C. Weaver. John P. Robarts Research. Inst. & Dept. of Physiol. Univ. of Western Ontario., London, Ont., N6A 5K8, Canada.

We hypothesized that arterial pressure instability due to exaggerated sympathetic reflexes after spinal cord injury results from changes in the SPNs in response to loss of their supraspinal afferent input. We examined morphological changes in SPNs retrogradely labelled by cholera toxin after spinal cord injury in rats. We also, we assessed changes in immunoreactivity to the growth associated protein GAP-43, to the synaptic vesicular protein synaptophysin and to the indicator of reactive astrogliosis, glial fibrillary acidic protein. After midthoracic cord hemisection all rats had paralysis of one hindlimb, indicating chronic spinal injury. A comparison of SPNs above and below the lesion revealed significant loss of dendrites and decreased cell size in response to loss of supraspinal input. Astrogliosis surrounded SPNs as far as 7 segments below the injury only on the side of the lesion but were found above the injury only at the lesion itself. Because expression of synaptophysin is normally high, differences above and below the injury were difficult to detect. GAP43 was increased in the gray matter below the side of the transection. These findings suggest that SPNs undergo significant structural change after deafferentation, associated with or dependent on astrogliosis and that exaggerated reactions may be due to new synapse formation. We also compared the morphology of SPNs below an injury to that of SPNs in cultured explants of the spinal cord taken from 7 day old rat pups. SPN morphology above an injury was compared to that of the normal 7 day pups. In culture, SPNs were smaller and had fewer processes, like SPNs below an injury *in vivo*. Numbers of SPN dendrites in 7 day rats were similar to numbers in SPNs above an injury. This shows that the living explant of the spinal cord *in vitro* can be a good model of deafferentation of the SPNs such as that occurring after spinal cord injury.

610.9

GROWTH OF EMBRYONIC CHICK HEART CELLS IN MEDIA SUPPLEMENTED WITH LIPOPROTEIN DEPLETED SERUM (LPDS) REGULATES EXPRESSION OF GENES CODING FOR G-PROTEINS AND MUSCARINIC RECEPTORS. A.P. Gadbut*, J.B. Galper. Cardiovascular division, Brigham and Women's Hospital and Harvard Medical School, Boston, MA 02115.

Growth of chick atrial cells in media supplemented with LPDS has previously been shown to increase intracellular cholesterol and parasympathetic responsiveness. These changes were associated with increased levels of muscarinic receptors (MR) as measured by [^{3}H] QNB binding and G-protein α-subunits (Gα) as measured by ADP ribosylation with pertussis toxin (Haigh et.al. JBC 263:15608). To determine whether these changes reflect increases in gene expression we used RNAse protection to compare levels of mRNA coding for MR and Gα in cells grown in medium supplemented with fetal calf serum or LPDS. In atrial cells from chicks 14 days in vivo growth in the presence of LPDS resulted in a 3 fold increase in mRNA coding for M2 and M3 MR subtypes while mRNA coding for αi2 and αi3 increased 2 and 3 fold respectively. These changes were reversed in cells grown in LPDS plus 30 μM Mevacor (an HMG-Co A reductase inhibitor). However, incubation of cells in medium suplemented with LPDS and a squalene oxido-cyclase inhibitor decreased total cell cholesterol but, did not reverse the increase in mRNA levels. Hence some factor in the cholesterol biosynthetic pathway proximal to squalene may play a role in regulating the expression of genes coding for MR and G-proteins.

TRAUMA

611.1

OMEGA-CONOPEPTIDE REDUCES THE EXTENT OF CALCIUM ACCUMULATION FOLLOWING TRAUMATIC BRAIN INJURY. H. Badie, M.L. Smith, D.A. Hoyda*, K. Fu, P. Pinanong, A. Samii and D.P. Becker. Division of Neurosurgry, UCLA School of Medicine, Los Angeles, Ca 90024.

The Omega-conopeptide SNX-111 (a N channel calcium (Ca^{++}) blocker) (NEUREX Corp.) was administered (3 or 5 mg/ kg, i.v.) to rats 1 h following a lateral fluid percussion (2.4-2.6 atm) brain injury. Animals were subsequently assessed for Ca^{++} accumulation at 6, 12, 24, 48 or 96 hrs after injury utilizing $^{45}Ca^{++}$ autoradiography. A total of 79 animals (223-367 g, male) were used with 28 serving as sham operated controls. All surgical procedures were conducted under general anesthesia (1.5-2.0 % enflurane, 33% O_2, 66% NO_2). $^{45}Ca^{++}$ was quantified using optical densitometry with relative assymetry measurments (Left-Right/Total) conducted for 20 structures. The results indicated that following fluid percussion brain injury, saline treated animals exhibited a marked accumulation of $^{45}Ca^{++}$ primarily within the ipsilateral cerebral cortex and hippocampus. This injury-induced accumulation of $^{45}Ca^{++}$ lasted for as long as 48 hrs. However, in animals who received SNX-111, the Ca^{++} accumulation was attenuated both in terms of extent and duration. Within the parietal cortex, animals receiving SNX-111 exhibited a reduction of $^{45}Ca^{++}$ accumulation at 24 hrs (Saline=0.312, 3 mg=0.048, 5 mg=0.061). This effect continued out to 48 h particularly in the higher dose (Saline=0.238, 3 mg=0.139, 5 mg=0.052). Within the dorsal hippocampus, $^{45}Ca^{++}$ accumulation was reduced by administration of SNX-111, starting at 12 hrs post-injury (saline=0.0601, 3 mg=0.048, 5 mg=0.056). This drug-induced effect was even more pronounced when measured 48 hrs after the insult (saline=0.033, 3 mg=0.017, 5 mg=-0.005). These results demonstrate that SNX-111 can reduce the injury-inducedaccumulation of Ca^{++} even when administered 1 h after surgery. (NEUREX Corp. & NS 30 308)

611.2

MRI STUDY OF RAT BRAIN EDEMA AFTER HEAD TRAUMA: EFFECTS OF HU-211, A NONPSYCHOTROPIC CANNABINOID A. Biegon*,L. Belayev, P. Bendel, M. Novikov, R. Bass and E. Shohami Phrarmos Corp., Rehovot; Weizmann Inst. Sci., Rehovot and Hebrew University, Jerusalem, Israel

HU-211 is a nonpsychotropic cannabinoid which improves the neurological outcome following closed head injury in the rat. We have used magnetic resonance imaging (MRI) to assess the spread of brain edema following head trauma and the possible effect of HU-211 on this parameter. Head trauma was induced by dropping a calibrated weight from a specific height onto the exposed skull (left side, anterior to bregma) of ether-anaesthetised rats. Animals were given chloral hydrate (350mg/kg i.p) and placed in a 4.7 Tesla magnet 30 minutes after the trauma for a 10 min T2 weighted scan (TR=2.5sec, TE=55msec, Slice thickness=1mm, center to center slice separation=1.2mm, 128x256 matrix, FOV=5cm). One hour after trauma, rats received an i.v injection of HU-211 5mg/kg in emulsion (N=9), or the appropriate vehicle (N=11). The scan was repeated 24 hours posttrauma. The extent (volume) of initial damage to the brain was calculated from the volume difference between the right and left hemispheres. The volume of edema 24 hours later was calculated from the area of hyperintense regions (besides ventricles) on all the slices where such regions were observed after thresholding, multiplied by 1.2mm. The ratio between the volume of initial damage and edema volume was calculated for each individual animal to control for variations in initial damage. This ratio was significantly lower in the treated group (mean±sem vehicle, 5.95±1.6; HU-211, 1.66±0.55; $p<0.033$, Student's t-test, two tailed), indicating that HU-211 reduced the spread of edema after head trauma.

611.3

MACROPHAGE RESPONSE DURING AXONAL DEGENERATION ALONG THE PNS/CNS DORSAL ROOT PATHWAY MAY BE IMMUNE MEDIATED. **D.B. Ellegala, A.M. Avellino, K.C. Andrus, and M. Kliot*. Dept. of Neurosurgery, Univ. of WA and Seattle VAMC, Seattle, WA 98108.**

Wallerian degeneration of axons differs dramatically in the adult mammalian PNS and CNS. For example, macrophage invasion with clearance of myelin and axonal debris occurs more rapidly in the PNS than the CNS (Perry et al, 1987; Stoll et al, 1989). The mechanisms underlying this difference are unknown. One possibility is that macrophages respond to molecules preferentially expressed in the PNS (Avellino et al, 1992; Griffin, 1993). Alternatively, macrophages may be part of an immunologic response directed at antigens exposed at the site of injury. To test this latter hypothesis, we studied macrophage invasion along the PNS/CNS dorsal root pathway in adult Lewis rats following various types of axonal injury.

Initial experiments involved cutting lumbar dorsal roots in the PNS followed by immunostaining for macrophages (ED1 monoclonal antibody) at 3, 7, and 14 days. Macrophages invaded the PNS portion of cut dorsal roots but stopped abruptly at the interface with the CNS (i.e. dorsal root entry zone=DREZ). Following a crush injury to the DREZ, involving both PNS and CNS tissue, macrophages were no longer confined to the PNS but now appeared across the DREZ and in the white matter of the CNS at each time point. In order to distinguish the local effects of surgical trauma from the possibility that a DREZ lesion might be sensitizing macrophages to both PNS and CNS antigens, a crush injury to the DREZ of a thoracic dorsal root was combined with transection of lower lumbar dosal roots on the contralateral side. Macrophages were now observed throughout the PNS and CNS portions of the cut lumbar dorsal roots at all time points. These results demonstrate that macrophage invasion during Wallerian degeneration along the PNS/CNS dorsal root pathway is influenced by the site of axonal injury. They suggest that this cellular response is immune mediated and directed at antigens exposed at the injury site.

Supported by a NIH Neurosurgical Training Grant (MK), VA funds, and a Magnuson Scholar Award (DBE).

611.4

REACTIVE AXONAL CHANGE IN A MICROPIG MODEL OF BRAIN INJURY: EVIDENCE FOR A HUMAN-LIKE PROGRESSION OF AXONAL CHANGE. Valadka, A.B., J.T. Povlishock*, J. Shah, and S. Walker. Dept. of Anatomy and Division of Neurological Surgery, Med. Col. of Va., Va. Commonwealth Univer., Richmond, VA 23298.

In an effort to develop an experimental animal model which better replicates the pathobiology and the temporal course of the reactive axonal change seen in human traumatic brain injury (TBI), we have evaluated a micropig model of fluid-percussion brain injury. Under anesthesia, 15 micropigs were subjected to mild to moderate TBI. Four hours to 5 days postinjury they were prepared for the LM and TEM visualization of antibodies targeted to the neurofilament (NF) subunits, tau, and ubiquitin. TBI elicited a temporal progression of reactive axonal change, comparable of that seen in humans. Within 4-6h of TBI, scattered axons showed a focal intraaxonal increase in the NF subunits which lost their normal alignment. Over 12-24h, this process of NF misalignment continued. Typically, these events were associated with impaired axoplasmic transport, causing focal axonal swelling and detachment. While these swollen reactive axonal segments predominated at 24h postinjury, scattered reactive profiles reminiscent of those seen at the 4-6h were also identified. At 2-5 days, these reactive changes continued with further expansion of the reactive axonal swellings. Again, swollen yet non-disconnected axons were also identified. Some showed complex multi-lobulated profiles with dilated NF and organelle-containing regions. Collectively, the evolution, the temporal course, and the heterogeneity of these reactive axonal changes are comparable to those seen in human TBI. This suggests that the micropig is an excellent model for better understanding the pathobiology of this injurious process. NS-20193

611.5

DISTRIBUTION OF DIFFUSE AXONAL INJURY FOLLOWING INERTIAL CLOSED HEAD INJURY IN MINIATURE SWINE

D.T. Ross,* D.F. Meaney, D. H. Smith, J. Brasko, L.E. Thibault, and TA. Gennarelli Head Injury Center, Div. of Neurosurgery, Univ. of Pennsylvania, Philadelphia, PA 19104

Diffuse axonal injury (DAI) is one of the most frequently encountered types of brain damage resulting from closed head injury. The development of a non-primate model of DAI would greatly facilitate studies of the pathophysiological mechanisms of DAI and the testing of potential neuroprotective therapies in a final pre-clinical screening paradigm. The present study was designed to histologically verify whether inertial injury in the coronal plane could produce DAI in miniature swine.

Animals were anesthetized with 3% isoflurane, secured to the inertial injury device by a snout clamp, and their heads accelerated rapidly (msec) once through a 120 degree arc in the coronal plane. These injuries produced transient (<15 minute) posttraumatic unconsciousness. All animals made a good recovery and were sacrificed between 6 hours and 10 days after injury. Neurofilament immunohistochemistry with antisera to non-phosphorylated (SMI-32) and phosphorylated epitopes common to heavy and medium neurofilament proteins was used to identify axonal injury.

Axonal injury was present in 9 of 12 animals subjected to inertial acceleration. These lesions, characterized by SMI-31 positive axonal retraction balls, were present at the white matter/ gray matter junction at the crests of gyri in the dorsolateral regions of the frontal, parietal and temporal cortices and along margins of the lateral ventricles. .A high density of pyramidal neuron perikarya in layers III and V within cortical gyri associated with subcortical DAI were intensely positive for phosphorylated neurofilament (SMI-31) immunohistochemistry and a high density of moderately labeled pyramidal cell perikaya were present in gyri not associated with DAI. These results validate the use of miniature swine in studies of axonal injury and demonstrate that axonal injury analogous to that seen in the mildest form of DAI (grade l) can be produced in these animals without producing prolonged coma. (supported by NIH Center Grant NS-08803-22)

611.6

REDUCED EVOKED *IN VIVO* HIPPOCAMPAL RELEASE OF ACETYLCHOLINE AT 2 WEEKS AFTER TRAUMATIC BRAIN INJURY (TBI).

[1]C. E. Dixon*, [1]J. Bao, [2]K. Johnson, and [1]R. L. Hayes. [1]Department of Neurosurgery, University of Texas-Houston Health Science Center, Houston, TX 77030 and the [2]Department of Pharmacology, University of Texas Medical Branch at Galveston, Galveston, Texas.

Recent findings suggest that chronic spatial memory deficits following experimental traumatic brain injury may, in part, be attributable to deficits in central cholinergic neurotransmission. Cholinergic deficits induced by TBI may be manifested by a decrease in the levels of evoked ACh released *in vivo*. This study employed microdialysis to measure scopolamine evoked release of ACh at 2 weeks post-injury, a time associated with significant spatial memory deficits. Two weeks prior to measurment, 10 rats were injured by controlled cortical impact (6 m/sec impact velocity, 1.7mm deformation). Sham rats (n = 10) were identically prepared but not injured. All rats were anesthetized with an isoflurane mixture and a microdialysis probe (3 mm tip) was placed into their dorsal hippocampus. Samples (30 μl) were collected every 20 minutes. Two samples were collected to establish basal levels of ACh. Scopolamine (1 mg/kg) was then injected i.p. and 4 more samples were collected and analyzed by HPLC. The data show that at 2 weeks post injury, the injured animals release less ACh in response to scopolamine than sham animals. Impaired release may be a result of either deinnervation, an impairment of the presynaptic negative feedback system, or a deficit in the cholinergic neuron's ability to synthesize ACh. Supported by CDC-R49 CCR606659.

611.7

MAGNETIC RESONANCE NEUROGRAPHY REVEALS SPIN-SPIN RELAXATION RATE (T_2) CHANGES CORRELATED WITH ONSET AND RECOVERY FROM SYMPTOMS IN TRAUMATIC & COMPRESSIVE NEUROPATHY.

A. G. Filler*, J. S. Tsuruda, C. E. Hayes, and M. Kliot. Dept. of Neurological Surgery and Dept. of Radiology, University of Washington, Seattle, WA 98195.

A series of advances over the past year have made it possible to non-invasively generate detailed 3D images of nerves in living human patients (Filler et al, *Lancet*, 341:659; Howe et al, *Mag. Res. Med.* 28:328). A striking characteristic of these magnetic resonance images is prolongation of the T_2 relaxation rate in the nerve proximal to sites of traumatic injury or compression. Upon resolution of weakness and numbness, we have observed reversion of the T_2 relaxation rate toward a normal range. The proximal extent of the T_2 changes extend over many centimeters and in several cases include changes in the dorsal root ganglion.

It is not yet clear whether these changes are due to alterations in the flow of axoplasm, blocked flow of endoneurial fluid, or shifts in some other nerve water compartment. However, this is the first image demonstration of altered physiology in nerve extending well proximal to sites of altered action potential conduction in clinical situations in humans.

These phased array MR images demonstrate the internal fascicular pattern of nerves both because of the fat content of the interfascicular epineurium and because this tissue retains very short T_2 under these conditions, appearing relatively unaffected by the pathology downstream. Thus, the perineurial portion of the blood/nerve barrier, which should be permeable to general edema fluid, does constrain movement of the altered fluid within the nerve.

With these new clinical tools, the evaluation of alterations in the various nerve water pools, (intra-organelle, Schwann cell cytoplasm, axoplasmic, etc.) will become an increasingly important focus for understanding the neurobiology of human pain and peripheral nerve dysfunction.

611.8

A STRESS-INDUCIBLE HEAT SHOCK PROTEIN (HSP68) IS EXPRESSED IN RAT DRG FOLLOWING AXOTOMY.

B. Tedeschi, R.P. Ciavarra, and C.W. Morgan*. Depts. of Anatomy & Neurobiology and Microbiology & Immunology, East. Virg. Med. Sch., Norfolk, VA 23501.

Direct exposure of most organisms or cells to potentially lethal metabolic stressors induces the expression of HSP68, a member of the 70kD heat shock protein family. In order to assess whether axotomy elicits a neuronal Stress Protein response, rat sciatic nerves (SN) were crushed at ~35mm from the L5 Dorsal Root Ganglia (DRG). Twenty-four hours following axotomy, DRG proteins were briefly radiolabelled (1 hr. pulse) in vivo and the radiolabelled proteins subsequently analyzed by two-dimensional polyacrylamide gel electrophoresis and fluorography. Results showed that prior SN axotomy, but not sham axotomy, resulted in the expression of the stress-inducible HSP68 protein in DRG. Moreover, such stress-inducible HSP68 expression was probably limited to the DRG neurons since no detectable HSP68 was expressed by the initial segment of SN following axotomy. These results suggest that the induction of Stress Proteins is one neuronal response to axotomy.

611.9

INCREASE IN TISSUE QUINOLINIC ACID AND INDUCTION OF INDOLEAMINE-2,3 DIOXYGENASE AFTER SPINAL CORD INJURY.

A.R. Blight*[1], M.P. Heyes[2] and K. Saito[2], Div. Neurosurgery[1], Univ. North Carolina, Chapel Hill, NC 27599, and Section on Analytical Biochemistry[2], NIMH, Bethesda, MD 20892.

Numerous mechanisms have been suggested to contribute to secondary cell damage and long-term neurological deficits following spinal cord trauma. Among these, is "bystander damage" from secretory activity of the large number of inflammatory phagocytes that accumulate in the tissue within the first few days. We therefore examined one of the neurotoxic products of activated macrophages, the NMDA receptor agonist, quinolinic acid (QUIN), which has been implicated in a wide range of neuropathologic states (Brain 115:1249, 1992). The lower thoracic spinal cord of 5 anesthetized, adult guinea pigs was injured by brief compression (J.Neurol.Sci. 103:156, 1991). Five days post-injury, animals were re-anesthetized and tissue samples (c.30mg each) were taken from the site of injury, upper cervical spinal cord, and somatosensory cortex and analyzed by mass spectrometry. A 10-fold elevation of QUIN concentration occurred at the injury site, with no significant changes in uninjured cervical spinal cord or cortex, compared with 5 normal and 5 sham-operated control animals. Concentrations of the kynurenine pathway enzyme, indoleamine-2,3 dioxygenase (IDO), and the pathway intermediate L-kynurenine (KYN) were increased approximately 3-fold at the site of injury, in proportion to QUIN changes. These measurements support roles for IDO and other KYN pathway enzymes in increased QUIN production in injured cord. The pathologic significance of such changes remains to be determined. (Partly supported by NINDS grant NS 21122)

611.10

PHARMACOLOGICAL ACTIVITY OF THE GINKGO BILOBA EXTRACT (EGb 761) ON THE VESTIBULAR COMPENSATION PROCESS IN THE CAT.

M. Lacour* and B. Tighilet. Lab. Neurobiologie des Restaurations Fonctionnelles, URA CNRS 372, Univ. Provence, Ct St Jérôme, 13397 Marseille Cedex 20, France.

The effects of postoperative treatment with a Ginkgo biloba extract (EGb 761) has been studied in unilateral vestibular neurectomized cats in a previous work. It has been shown that systemic administration of EGb 761 (50mg / kg / d, i.p.) induced a more rapid recovery of the postural and locomotor functions as compared to control untreated groups, indicating that EGb 761 improved the plasticity mechanisms involved in vestibular compensation. The aim of the present work was to determine which of the two main biochemical groups made up of the various compounds contained in the EGb 761 (terpenes versus flavonoids) was the most active in this recovery process, to precise the best route of administration of the substance (i.p. versus p. o.) and to test the dose-dependent effects. For this purpose, we have compared the time course of the equilibrium function recovery in 7 groups of cats: one untreated group (n = 5 : controls), two groups treated with the total extract administrated p. o. at 40 mg / kg / d (n = 4) or 80 mg / kg / d (n = 4), two groups treated with the total extract injected i. p. at 50 mg / kg / d (n = 5) or 25 mg / kg / d (n = 4), and two groups treated with a special extract without the terpene fraction, i. p., at 25 mg / kg / d (n = 4) or 10 mg / kg / d (n = 4). Treatment was always given until complete recovery of the equilibrium function as quantified by the rotating beam test. Results showed that the efficacy of the special extract do not containing the terpenes was comparable to that of the total extract, suggesting that the non-terpenic (flavonoids) fraction was the most active chemical constituent in this experimental model of CNS plasticity. Moreover, the pharmacological activity of the total extract (EGb 761) was significantly better when given i. p. as compared to the p. o. route of administration, and daily doses of 25 mg / kg were as effective as higher doses (50 mg / kg) but much more active than lower doses (10 mg / kg). These experimental data in the cat confirm that EGb 761 treatment serves as useful therapy in supporting functional recovery in the brain and improves the knowledge on the working and activity of such a complex substance.

611.11

FLUID PERCUSSION INJURY CAUSES LOSS OF SEPTAL AND VENTRO-BASAL FOREBRAIN CHOLINERGIC NEURONS. R. H. Schmidt* and M.S. Grady. Dept. Neurosurgery, Univ. Washington, Seattle, WA 98195.

Although traumatic brain injury (TBI) disturbs consciousness, cognitive function, concentration, problem solving and behavior, the neural mechanisms of this are not known. Because these functions are mediated, in part, by various cholinergic (ACh) and catecholaminergic projections, we sought to determine how these systems were affected by experimental TBI. Fluid percussion injury (FPI), 3.2 atm, or sham injury was delivered to halothane-anesthetized rats via either midline or lateral injury cannulas (N=4-5 each group). Injury resulted in an average loss of righing reflex of 13-14 minutes without any gross damage to the brains. After 11-15 days, the brains were stained in serial section for choline acetyltransferase, tyrosine hydroxylase, dopamine beta-hydroxylase, acetylcholinesterase and NADPH diaphorase. Midline injury resulted in a significant decrease in septal and ventrobasal forebrain ACh neurons bilaterally, averaging 36% in area Ch1, 45% in area Ch2, and 41% in area Ch4, $P<0.05$. Lateral injury resulted in ipsilateral ACh neuron loss of similar magnitude, $P<0.05$, but only an 11-28% loss contralaterally. Surviving ACh neurons in Ch4 appeared shrunken and with shorter processes. There was no quantitative loss of ponto-mesencephalic ACh neurons (areas Ch5 and Ch6). Dopaminergic neruons in the ventral mesencephalon and diencephalon, noradrenergic neurons in the pons and medulla, their axon bundles and terminal ramifications did not show any evidence of injury, however quantitative cell counts were not done. This demonstration of a loss of ACh neurons shortly after FPI may serve to explain the cognitive dysfunction these animals demonstrate in water maze testing. Whether this neuron loss is permanent, what terminal fields may be affected, the mechanism of this decrease, and whether it occurs in human victims of TBI remains to be determined. (Supported by the Brain Trauma Foundation).

611.12

SUPEROXIDE DISMUTASE IMPROVES POST-TRAUMATIC CORTICAL BLOOD FLOW IN RATS. J.K. Muir*, M. Tynan, R. Caldwell and E.F. Ellis, Department of Pharmacology and Toxicology, Medical College of Virginia, Richmond, VA 23298

Oxygen free radicals have been implicated in the cerebrovascular abnormalities that occur as a result of traumatic brain injury. These abnormalities include sustained dilation, decreased reactivity to hypocapnia, enothelial lesions and altered reactivity to vasoactive substances. The purpose of this study was to determine if pretreatment with superoxide dismutase(SOD), a superoxide radical scavenger, could alter post-traumatic cortical blood flow (CBF) in rats.

Sprague-Dawley rats (n=36) were initially anesthetized with thiopental (75 mg/kg, ip) and pentobarbital was used to maintain anesthesia. The animals were ventilated with room air and blood gases were maintained within normal limits. One craniectomy was made over each hemisphere and the dura left intact. The fluid percussion injury device was attached over the right parietal cortex and a laser-Doppler probe for measurement of CBF was positioned over the left parietal cortex.

SOD, 24,000 units/kg bolus followed by 1,600 units/kg/min continuous infusion, was administered i.v. beginning 10 minutes prior to an injury of 2.0-2.2 atmospheres. A second group of animals received a similar volume of saline throughout the protocol. The blood pressure and heart rate responses to trauma were not different between the two groups. The saline control group showed a pronounced reduction in CBF (~35%) between 10-60 minutes post-trauma. SOD significantly elevated CBF throughout the post-injury period and the improved CBF was mediated by enhancing cortical blood volume and blood velocity. One hour after injury, CBF in the SOD group was 12±8% below pre-injury baseline, while in the saline group this value was 34±6% below baseline. Supported by NS 27214 and DA 07027.

BIOLOGICAL RHYTHMS AND SLEEP IV

612.1

DIFFERENTIAL EFFECTS OF MODAFINIL AND AMPHETAMINE IN CANINE NARCOLEPSY: EVIDENCE FOR A UNIQUE MECHANISM FOR MODAFINIL. J. L. Vaught[1*], S. Nishino[2], J. Shelton[2], W.C. Dement[2] and E. Mignot[2]. [1]Cephalon, Inc. West Chester, PA.,[2] Sleep Disorders Center, Stanford University School of Medicine, Palo Alto, CA.

Modafinil [2-(diphenylmethylsulfinyl) acetamide] is a structurally unique and mechanistically novel stimulant approved in France and under development in the US for the treatment of narcolepsy. The purpose of the present study was to compare and contrast the effects of Modafinil and amphetamine in a canine model of narcolepsy. In polysomnographic studies on 4 narcoleptic canines with electrodes implanted for recording of the electroencephalogram, electroculogram and electromyogram, Modafinil dose-dependently suppressed slow wave sleep for up to 4 hours following dosages of 5 and 10 mg/kg iv. Amphetamine was also effective at dosages of 100 or 200 ug/kg iv. Preliminary results also indicate that Modafinil, unlike amphetamine, had no significant effects on cardiovascular parameters and rectal temperature at these effective dosages. In contrast, in the Food-Elicited Cataplexy Test, Modafinil in dosages up to 8 mg/kg had no effect on cataplexy. Amphetamine completely suppressed cataplexy at 120 ug/kg with an ED 50 of 50 ug/kg iv. These results are consistent with the known therapeutic effects of Modafinil in human narcolepsy. Moreover, these data indicate that Modafinil and amphetamine have clear differences in their pharmacological profile. Thus, Modafinil represents a mechanistically novel approach to the treatment of human narcolepsy.

612.2

KETAMINE BLOCKADE OF NMDA-GATED CATION CHANNEL DURING WAKING INCREASES NREM DELTA INTENSITY IN THE RAT I. Feinberg* and I. G. Campbell, VA/UCD Sleep Laboratory, UC Davis, Davis, CA 95616

The homeostatic model of NREM sleep (1) proposed that the brain recuperative processes of sleep occur during slow-wave sleep, and that the intensity of recuperative processes is reflected by the amplitude and density of delta waves. It further hypothesized that the amount of "recuperation" required is proportional to prior waking duration and the intensity of activity (reflected by metabolic rate) in plastic neuronal systems during waking.

Subanesthetic dosages of ketamine increase the metabolic rate of plastic brain systems, including the hippocampus and other limbic structures (2). This metabolic effect is thought due to blockade of the NMDA-gated cation channel. To test the intensity component of the homeostatic model, we administered ketamine during the dark (waking) period to rats. Three i.p. injections were given; each succeeding dose was injected when the behavioral effects of the previous dose had worn off. In separate experiments, doses of 15, 25 and 50 mg/Kg were administered. Saline injections given similarly served as controls. Delta (1-4 Hz) EEG was measured with PASS PLUS a commercially-available method of period/amplitude analysis whose reliability and validity have been established.

Ketamine produced significant increases in delta wave amplitude ($F=7.9$, $p<0.05$) and density ($F=22.3$, $p<0.0001$) during subsequent NREM sleep. While consistent with the homeostatic model, these results are subject to several alternative explanations: ketamine produces widespread metabolic effects and it stimulates multiple receptors. In spite of these uncertainties, these results are of interest because they represent the most powerful pharmacologic stimulation of NREM delta EEG yet observed. They gain added interest because of recent evidence that EAAs are important transmitters in the suprachiasmatic nucleus.

1. Hammer, RP and Herkenham, M. (1983) J.Comp. Neurol. 220:396-404.
2. Feinberg, I. (1974) J. Psychiat. Res. 10:283-306.

612.3

TEMPERATURE COMPENSATION OF A CIRCADIAN CLOCK FROM A HOMEOTHERM. R. Keith Barrett* and Joseph S. Takahashi. NSF Center for Biological Timing, Department of Neurobiology and Physiology, Northwestern University, Evanston, IL 60208.

Temperature compensation is a fundamental property of all circadian clocks examined to date; however, temperature compensation in a euthermic homeotherm has not been demonstrated. Dispersed primary chick pineal cells were entrained for 3 days to a light cycle at temperatures between 31°C and 40°C then transferred to constant darkness (DD) at the same temperatures for 5 days. The rhythmic secretion of melatonin in DD was used as an assay of the period of the clock. Circadian period was temperature compensated between 34°C - 40°C with a temperature coefficient, Q_{10}, of about 0.83. Melatonin synthesis, in contrast, was strongly dependent upon temperature. Temperature also affected the expression of the rhythm: the rhythm persisted with minimal damping at 40°C (bird physiological body temperature) but damped rapidly at 34°C and was abolished at 31°C. A phase response curve (PRC) was measured from dispersed pineal cells exposed to a temperature pulse during the first cycle in DD. The pulses were presented every 3 hours over a 24 hour period and phase shifts in the melatonin rhythm were measured. The PRC was remarkably similar to the PRC to 6 hour light pulses in chick pineal cells. These results suggest that the effects of light and temperature on the circadian clock are similar and that the cellular and molecular mechanisms mediating these inputs could have common features. These findings are of interest because higher vertebrate clocks might not have a requirement for temperature compensation because the internal thermal milieu is homeostatically regulated.

Supported by the Cellular and Molecular Neuroscience Training Program NIH T32 NS 07140 at Northwestern University (R.K.B.) and by NIH R37 MH 39592 (J.S.T.).

612.4

DAILY MELATONIN ADMINISTRATION SYNCHRONIZES CIRCADIAN PATTERNS OF BRAIN METABOLISM AND BEHAVIOR IN PINEALECTOMIZED HOUSE SPARROWS. JUN LU* AND VINCENT M. CASSONE. Department of Biology, Texas A&M University, College Station, TX

The circadian system of house sparrows is composed of two primary oscillators: the visual suprachiasmatic nucleus (vSCN) and the pineal gland. Considerable evidence indicates that these two oscillators are mutually coupled. We have asked whether melatonin administration can synchronize cerebral metabolism as with synchronization of behavior in this species. To test this hypothesis, all house sparrows were pinealectomized. After recovery from surgery, each individual was placed in a chamber in which perch-hopping activity was recorded. Once birds' locomotor activity were completely abolished in constant dim light, 12 birds were periodically administered a melatonin drinking solution (862 μM in 0.01% ETOH) 12 of every 24 hrs. For control, 10 birds were periodically administreted 0.01% ETOH only. The actograms showed that melatonin synchronized activity patterns while 0.01% ETOH did not. At ZT06 (6 hr after melatonin was administered to the experimental group or 6 hr after 0.01% ETOH was administered to the control group) and at ZT18 (18 hr after melatonin or 18 hr after 0.01% ETOH), birds (6/experimental group, 5/control group) were intra-muscularly injected with 2-[^{14}C]-deoxyglucose (2DG, 200 μCi/kg). After 1 hr birds were anesthetized, decapitated, and brains were quickly removed and frozen. Brains were then transversely cut at 20 μm. Slides were processed for 2DG autoradiography while adjacent slides were incubated in 50 pM 2[^{125}I]iodomelatonin (IMEL) to determine specific IMEL binding. Our results indicate that melatonin not only synchronizes locomotor activity but also synchronizes 2DG uptake and IMEL binding patterns in many cerebral structures associated with circadian and visual functions, but not in structures devoid of IMEL binding. **Support By AFOSR Grant 90-NL-0244**

612.5

PRENATAL INJECTIONS OF D_1-DOPAMINE RECEPTOR AGONIST SKF 38393 ENTRAIN CIRCADIAN RHYTHMS OF HAMSTER PUPS

N.Viswanathan* and F.C.Davis. Dept. of Biology, Northeastern Univ., Boston, MA 02115.

Melatonin is a possible maternal entraining signal for the developing circadian pacemaker in hamsters. Prenatal injections of melatonin to pregnant hamsters predictably set the phases of the offsprings' circadian rhythms. Induction of *c-fos* in the fetal rat SCN by the D_1-dopamine receptor agonist, SKF 38393 (Weaver et al., PNAS 89:9201, 1992) suggests that maternal entrainment could be mediated by the activation of a dopaminergic system in the fetus. The present study was undertaken to examine this possibility. Pregnant Syrian hamsters, maintained in constant dim light throughout the study, received SCN lesions on day 7 of gestation. Between gestation days 11-15, two groups of hamsters received two intra-peritoneal injections each day 12 hours apart. One of the injections contained the SKF 38393 (8 mg/kg) and the other contained saline only. At weaning, pups from both groups were introduced individually into running wheel cages to measure their activity/rest rhythms. The phases of activity onset on the day of weaning were calculated from the freerunning rhythms. Prenatal injections of SKF 38393 unambiguously set the phases of the pups' circadian rhythms; SKF at 0800 established an average phase of 16.80 hr while SKF at 2000 established an average phase of 4.20 hr. Interestingly the average phases established by SKF 38393 were approximately 180° different from those established by melatonin as reported in previous studies. The different phases established by the SKF 38393 and melatonin suggest that there are different maternal signals which act in opposite ways on the developing circadian pacemaker to mediate entrainment. It is possible that melatonin acts as a signal representing night while an unidentified maternal signal which activates a dopaminergic system acts as a signal representing day. Supported by NIH grant HD 18686.

612.6

COMPLETE LESIONS OF THE SUPRACHIASMATIC NUCLEI ELIMINATE CIRCADIAN RHYTHMICITY OF BODY TEMPERATURE AND LOCOMOTOR ACTIVITY IN GOLDEN HAMSTERS.

R. Refinetti*, C. M. Kaufman and M. Menaker. Department of Biology, University of Virginia, Charlottesville, VA 22903.

The effects of suprachiasmatic and control lesions on the circadian rhythms of locomotor activity and body temperature were studied in golden hamsters maintained in constant light as well as in constant darkness. Large suprachiasmatic lesions, but not control lesions, eliminated circadian rhythmicity in both variables, as determined by inspection of actograms and chi-square-periodogram analysis. These results do not support the hypothesis that the body temperature rhythm is controlled by a circadian pacemaker distinct from the main pacemaker located in the suprachiasmatic nuclei. Comparison of the "robustness" of the rhythms of activity and body temperature in unlesioned and lesioned animals suggests that previous results supporting the hypothesis of distinct pacemakers may have been artifactual, resulting from inadequate data analysis of the expressed rhythms of animals sustaining incomplete lesions.

This work was supported by the NSF Center for Biological Timing, by NIH NRSA award MH-10146 (to R.R.), and by NIH grant HD-13162 (to M.M.).

612.7

GASTRIN-RELEASING PEPTIDE (GRP_{1-27}) PHASE SHIFTS THE MAMMALIAN CIRCADIAN PACEMAKER.

Hugh D. Piggins* and Benjamin Rusak. Department of Psychology, Dalhousie University, Halifax, Nova Scotia, CANADA B3H 4J1.

In mammals, a photically entrainable circadian pacemaker is housed in the suprachiasmatic nuclei (SCN) of the hypothalamus. Cells of the rodent SCN contain a number of neuropeptides including the 27 amino acid gastrin-releasing peptide (GRP_{1-27}). We examined the effects of injecting GRP_{1-27} into the SCN on circadian activity rhythms. Male Syrian hamsters with cannulas implanted in the SCN were maintained in constant dim red illumination (<5 lux, supplied by photographic safelights). At 10-14 day intervals, animals received SCN injections of 1 µl volumes of saline vehicle or GRP_{1-27} (150 pmol) at various phases of the circadian activity cycle.

Saline injections had small or no phase-shifting effects on rhythms at any phase tested. In contrast, GRP_{1-27} caused significant phase-delays in activity rhythms when injected at circadian times (CT) 10-14 (where CT 12=activity onset). GRP_{1-27} also phase-advanced some animals when injected at CT 20-02. SCN injections of GRP_{1-27} at lower doses (5.22, 16.66, and 50 pmol) at CT 12-14 also phase-delayed activity rhythms in a dose-related manner. These data indicate that injections of GRP_{1-27} potently reset the circadian pacemaker in hamsters, and that the phase response curve for GRP_{1-27} is similar to that for light. These results raise the possibility that endogenous GRP_{1-27} participates in photic entrainment processes.

Supported by AFOSR (US) and NSERC (Canada) and a postdoctoral fellowship from the MRC (Canada).

612.8

GFAP IMMUNOREACTIVITY DISTRIBUTION IN THE ASTROCYTES OF THE SYRIAN HAMSTER SCN : CIRCADIAN AND NYCTHEMERAL FLUCTUATIONS.

M. Lavialle and J. Servière . INRA, 78352 Jouy-en-Josas, France.

Previous studies have shown that immunoreactivity (Ir) for the astrocyte-specific protein, glial fibrillary acid protein (GFAP), is particularly dense in the suprachiasmatic nuclei (SCN) of rodents. The SCN is an intrinsic pacemaker responsible for initiating, regulating and maintaining a variety of circadian rhythms. The SCN itself exhibits clear rhythms in glucose consumption, electrical activity and peptides expression which can be sustained in isolated explants. The present study was aimed to investigate whether the GFAP-Ir distribution might also oscillate according circadian time (CT) over the 24h period. Adult male hamsters raised in an LD 12:12 cycle were either maintained in LD, or transferred into darkness (DD) for 8 cycles. To assess internal clock time, locomotor activity was recorded (activity onset = CT12). Animals were perfused (4% paraformaldehyde) at different CTs (CT06, 10, 14, 18). Brains sections were processed for GFAP-Ir. The results revealed distinctive levels of Ir with the strongest staining at the end of the inactive phase (CT10) and the weakest at the beginning of the active phase (CT14). These GFAP-Ir fluctuations entrained by the LD cycle persisted in DD. Thus GFAP-Ir distribution exhibits a circadian rhythm with a profile similar to that of glucose consumption, characterized by a marked trough at CT14. The fluctuations in GFAP-Ir can be considered as another index of the rhythmic activity of the clock. The intense immmunoreactivity, the circadian variations in the GFAP-Ir distribution and the synchrony with the oxidative metabolism rhythm strengthen the hypothesis of a functional syncytium improving neuron-astrocyte communication and increasing neuronal synchrony in the clock.

612.9

THE REGULATION OF MULTIPLE CIRCADIAN OUTPUTS BY THE SUPRACHIASMATIC NUCLEUS AND SYMPATHETIC NERVOUS SYSTEM.

Wade S. Warren and Vincent M. Cassone*. Department of Biology, Texas A & M University, College Station, Texas 77845

The mammalian suprachiasmatic nucleus (SCN) is the master pacemaker controlling a wide variety of circadian behavioral and physiological processes. However, the motor pathway(s) by which these diverse processes are controlled are unknown. The only known motor output of this system is the regulation of pineal melatonin synthesis via the sympathetic nervous system. It is therefore possible that other peripheral circadian rhythms are similarly regulated. To address this issue, we have employed intraperitoneal AM transmitters and a data acquisition control system (Dataquest III, Mini-Mitter Inc., Sunriver, OR) to simultaneously record body temperature (BT), general activity (GA), wheel running activity (WR) and heart rate (HR) in the same rat. Two experiments were performed: 1) the effects of SCN lesion (SCNX; N=5) vs sham-operated (SHAM; N=5) and 2) the effects of sympathectomy with the drug guanethidine (GUAN; N=7) vs saline control (SAL; N=5). SCNX abolished circadian patterns in all motor outputs. All SHAM animals showed robust rhythms in all measures. These data indicate that all circadian rhythms measured here are generated by the SCN, including BT, and that residual rhythmicity is the result of incomplete lesions. GUAN selectively abolished HR rhythmicity. This drug decreased HR circadian power to a level similar to the decrease caused by SCN lesions. Other rhythms (BT, GA and WR) were unaffected. These results suggest that the SCN may influence some peripheral targets via circadian regulation of the sympathetic nervous system, but that other circadian outputs are regulated via other, unknown pathways. **Supported by AFOSR Grant 90-NL-0244.**

612.10

ISOLATION OF THE FIRST CIRCADIAN CLOCK MUTATION IN THE MOUSE.

M.H. Vitaterna*, P.L. Lowrey, J.D. McDonald[1], W.F. Dove[1], L.H. Pinto, F.W. Turek, and J.S. Takahashi. NSF Center for Biological Timing, Department of Neurobiology and Physiology, Northwestern University, Evanston, IL 60208, and [1]McArdle Laboratory for Cancer Research, University of Wisconsin Medical School, Madison, WI 53706.

In order to isolate mutations that define circadian clock genes in the mouse, we conducted a brute-force screen for mutants produced with N-ethyl-N-nitrosourea (ENU). Male C57BL/6J male mice were exposed to ENU, then bred with wild-type C57BL/6J females. The offspring, which would be heterozygous for induced mutations, were screened for circadian phenotype. 299 such offspring have been tested for abnormalities in circadian rhythmicity, using the rhythm of locomotor activity as a marker. Phase angle of entrainment to a 12 hours light: 12 hours dark cycle and free-running period in constant darkness were measured. For all the mice tested, the average time of activity onset coincided with the time of lights-off, and the average free-running period in constant darkness was 23.7 hours. One animal was found with a normal phase angle of entrainment but with a free-running period of 24.8 hours, a period 0.7 hours longer than any other animal. This male was bred with three females, to produce a total of 23 offspring. Upon testing the offspring, 13 were found with normal period, and 10 with long periods. Subsequent matings have supported the hypothesis that a single gene semi-dominant mutation is responsible for the lengthened period phenotype. Work is presently underway to characterize further the mutant phenotype and to map the mutation.

Supported by grants from the MacArthur Foundation, NIH, and NSF.

612.11

SEROTONIN MODULATES THE PHOTIC RESPONSE OF THE SCN CIRCADIAN OSCILLATOR IN THE SYRIAN HAMSTER.
M.A.Rea*, J.D. Glass1 and C.S.Colwell2 Circadian Neurobiol. Res. Group, Armstrong Lab. (CFTO), Brooks AFB, TX 78235. 1Department of Biol. Sci., Kent State Univ., Kent OH, 44242. 2Mental Retardation Res. Ctr., UCLA Med Ctr, Los Angeles, CA 90024.

The SCN receive a major serotonergic (5-HT) projection from the midbrain raphe which terminates predominantly in the retinorecipient region of the nucleus. This fact together with the observation that disruption of the 5-HT projection alters the PRC for photic stimulation of the SCN suggests that serotonin may modulate the response of the SCN oscillator to light. We have investigated the effects of 5-HT and the selective 5-HT_{1A} receptor agonist, 8-OH-DPAT, on (1) optic nerve-stimulation-induced field potentials in the hypothalamic slice preparation, (2) light-induced phase shifts of the free running activity rhythm, and (3) light-induced c-fos expression in the SCN. Both 5-HT (0.5 - 25 μM) and 8-OH-DPAT (0.1 - 10 μM) dose-dependently inhibited optic nerve evoked field potentials in the SCN. Systemic administration of 8-OH-DPAT (5 mg/kg i.p.) completely blocked light-induced (40 lux for 10 min) phase advances (CT19; Saline = 67 ± 10 min; DPAT = 12 ± 15 min; $p<0.05$) and delays (CT14; Saline = -48 ± 7 min; DPAT = -7 ± 9 min) of the free running activity rhythm. This effect was dose dependent between 0.05 - 5 mg/kg). In addition, DPAT (5 mg/kg) caused a regional attenuation in light-induced c-fos expression in the SCN which corresponded to the distribution of 5-HT terminals in the nucleus. These results indicate that 5-HT may serve to regulate the photic response of the SCN oscillator, possibly by acting through a 5-HT_{1A} receptor-dependent mechanism.
Supported by AFOSR 2312W6 (MAR).

RESPIRATORY REGULATION

613.1

POTENTIAL ROLE OF NITRIC OXIDE AS A TRANSMITTER IN THE CAT CAROTID BODY.
D. Chugh*, P. Grimes, M. Katayama, A. Mokashi, R. Stone and S. Lahiri. Department of Physiology and Department of Ophthalmology and Scheie Eye Institute, University of Pennsylvania School of Medicine, Philadelphia, PA 19104.

The function of the richly vascularized and innervated carotid body (CB) could be altered by endogenous nitric oxide (NO), since NO is synthesized and released by both vascular endothelial cells and some peripheral nerve fibers. We studied the CB in vitro for effects of nitric oxide synthase (NOS) inhibitor on carotid chemosensory nerve (CCN) activity and for the localization of NOS. The CB was perfused-superfused with Tyrode's solution at 37°C, and CNN activity was recorded continuously. Graded doses of nitro-L-arginine-methyl ester (L-NAME, 25-300 μM) were administered over a period of 2 to 40 min during normoxia. L-NAME stimulated CCN activity after a long latency of 2.9 ± 1.8 min, and the response reached peak (3-4 fold increase) at 9.9 ± 3.3 min. During prolonged perfusion with L-NAME, CCN activity remained high but fluctuated. Sodium nitroprusside (1-2 μM) strikingly reduced the response to L-NAME. Hypoxia (PO_2 = 60 Torr) augmented and hyperoxia (>400 Torr) attenuated the effect of L-NAME. In the CB, NOS immunoreactivity and NADPH diaphorase activity were localized in varicose nerve fibers mainly associated with blood vessels, as well as in the soma and processes of several intrinsic ganglion cells. Taken together, the results suggest an inhibitory role of NO in the CB.
(Supported in part by T32 HL-07027, HL-43413 and EY-05454.)

613.2

CAROTID BODY DENERVATION (CBD) ENHANCES HYPOVENTILATION INDUCED BY COOLING THE ROSTRAL VENTROLATERAL MEDULLARY SURFACE IN AWAKE GOATS.
P.J. Ohtake, H.V. Forster, L.G. Pan, T.F. Lowry, M.J. Korducki, E.A. Aaron, and Z.J. Bosnjak*. Physiology, Anesthesiology, Med. Col. of Wis.; Phys. Ther.; Marquette U.; Zablocki VA; Milwaukee, WI 53226.

The traditional view is that ventrolateral medullary (VLM) structures contribute to the control of breathing through intracranial chemoreception but more recent data on anesthetized and decorticate animals suggests VLM structures may also integrate all chemoreception, tonically facilitate dorsal respiratory neurons and/or be the site of respiratory rhythmogenesis. The purpose of the present study was to investigate the role of carotid chemoreceptor afferents in relation to VLM contributions to respiratory control. Accordingly, we used our recently developed model (*FASEB J* 7:A403, 1993) to transiently cause VLM surface neuron dysfunction in the awake goat. Reversible neuronal dysfunction at specific sites was induced by flowing ice water (for 30 sec) through thermodes (3x3 mm) chronically implanted bilaterally on the surface of the VLM. Under halothane anesthesia, cooling (thermode = 20°C) of the rostral VLM caused apnea within 5 sec which was sustained for 20 sec after cooling ceased. In contrast during cooling at the same site in the awake state during eupnea, hypoxia, and hypercapnia, apneas were only 5-20 sec in duration. Following study in the intact state, CBD was performed and confirmed by the lack of respiratory response to i.v. bolus injection of NaCN. Following CBD, cooling the VLM surface produced apneas which were at least twice as long as apneas observed prior to denervation. This pattern of response was observed during all awake conditions (eupnea, hypoxia, and hypercapnia) and during anesthesia. Our present interpretation is that these data are consistent with the idea that rostral VLM structures and carotid chemoreceptors provide independent tonic facilitation to more dorsal medullary neurons involved in the control of breathing.
[Supported by NIH 25739, Veterans Administration and Heart and Stroke Foundation of Canada]

613.3

FAST RHYTHMS IN DISCHARGES OF EXPIRATORY RECURRENT LARYNGEAL SINGLE FIBERS.
W.-X. Huang*, C.N. Christakos, M.I. Cohen, and Q. He. Dept. of Physiol., Albert Einstein Col. Med., Bronx, NY 10461.

In 5 unanesthetized decerebrate, paralyzed, vagotomized cats, discharges of decrementing expiratory (E) recurrent laryngeal (RL) fibers (94 units, comprising 120 unit pairs) were recorded simultaneously with bilateral RL population (multifiber nerve bundle) and phrenic nerve discharges. In 4/5 cats, spectral analysis revealed existence of uncorrelated rhythms in E activities. There were no significant coherences between bilateral populations, between 99/103 unit pairs, and between units and populations, even though 84/103 units had rhythmic discharges as indicated by autospectral peaks at 18-88 Hz. In contrast, 1 cat showed highly correlated rhythms (at 49 Hz), as indicated by: a) high coherences (> 0.9) between bilateral populations; b) for 7/17 unit pairs, high coherences (0.26-0.91); c) for 7/17 individual units, high unit-population coherences (0.43-0.94). Thus these rhythms resemble the high-frequency oscillations (HFO) found in inspiratory nerve activities (Cohen et al., Brain Res., 417:148-152, 1987). The rarity of comparable correlated rhythms in RL E activities indicates weakness of interactions in networks driving these activities; but their occurrence in some cases (1/5 cats) indicates the presence of connections in these networks that may be activated to produce coherence of rhythms.
(Supported by N.I.H. Grant HL-27300.)

613.4

CHARACTERIZATION OF VENTROLATERAL MEDULLARY NEURONS THAT DEPOLARIZE IN RESPONSE TO HYPOXIA.
P.C. Nolan* and T.G. Waldrop. Dept. of Physiology & Biophysics, Neuroscience Program and College of Medicine, Univ. of Illinois, Urbana, IL 61801.

This laboratory has shown that neurons in cardiorespiratory regions of the ventrolateral medulla respond to hypoxia by increasing their firing rate both *in vivo* and *in vitro*. This response persists in a chemical synaptic blockade (low Ca^{++} high Mg^{++}) medium *in vitro*. Recent studies employing the whole-cell patch clamp recording technique have demonstrated that this increase in excitability is due to a depolarization associated with an increase in membrane conductance. Current studies employed the use of a brain slice preparation with 400 μm brain slices in an interface chamber perfused with nutrient media equilibrated with 95% O_2/5% CO_2. Exposure to one to three minutes of severe hypoxia (5% CO_2/95% N_2) and/or moderate hypoxia (10% O_2/5% CO_2/85% N_2) elicited a rapid depolarization in eight-four percent of VLM neurons studied. The majority of these neurons responded to hypoxia with a gradual depolarization which was maintained throughout the stimulus; this response was partially TTX-sensitive. Another group of neurons proceeded rapidly into a recoverable depolarization blockade. Neurons that did not depolarize were either unresponsive or hyperpolarized during hypoxia. Neurons in this region of the medulla were demonstrated to monosynaptically project to the intermediolateral cell column by employing retrograde transport of rhodamine labeled microspheres. These results suggest neurons in this cardiorespiratory region of the medulla are inherantly sensitive to low oxygen, and this sensitivity may be responsible for the increase in cardiorespiratory activity elicited by acute hypoxic insult.
(Supported by NIH 32876, AHA-IL Affiliate and PHS HDO7333)

613.5

KAPPA OPIOID INFLUENCE ON CARDIORESPIRATORY AND STATE CONTROL DURING POSTNATAL DEVELOPMENT.
I. R. Moss*, R. E. Faltus, J. G. Inman and A. Laferrière. Developmental Respiratory Lab., Dept. Pediatrics, McGill University, Montreal, Québec H3H 1P3.

Effects of specific kappa opioid antagonism with Norbinaltorphimine (NorBNI) on sleep-wake state (SWS), mean arterial pressure (MAP) and heart rate (HR), and on posterior cricoarytenoid and diaphragmatic electromyographic activities were assessed in 3 to 13 and 23 to 33 day-old, chronically instrumented and unanesthetized piglets. Each piglet was studied twice daily, once before and once after NorBNI, 4 mg/kg i.v., for five consecutive days while lying in a sling within a plexiglass box. Each study session included 10 min trials with ambient air followed by 10% O_2-N_2. SWS distribution, MAP, HR, and most respiratory-related measures matured with age. NorBNI enhanced MAP and HR in both age groups, but did not alter any SWS or respiratory functions in either group. Thus, in counterdistinction to the specific influences of mu and delta opioid systems on SWS and cardiorespiration in young neonates, as reported by us previously, the kappa opioid system influences cardiovascular regulation throughout ontogeny, but plays no role in the regulation of state or breathing in swine. NHLBI HL36939 Support

613.6

EVIDENCE FOR CONCURRENT LOCAL AND REMOTE REGULATION OF IMPULSE SYNCHRONY IN BRAINSTEM NEURAL ASSEMBLIES.
B. G. Lindsey*, K. F. Morris, Y. M. Hernandez, and R. Shannon. Dept. Physiol. & Biophysics, Univ. South Florida Med. Ctr., Tampa, FL 33612.

Cardiorespiratory-related neuronal assemblies defined by their impulse synchrony are found in the brainstem midline. (*J. Neurophysiol.* 67:905-930; *Soc. Neurosci. Abst.* 15:1191). Their dynamic organization may reflect continuous cooperation that promotes appropriate expression of cardiorespiratory motor and autonomic activity. This work provides evidence that midline assemblies are coordinated with and influenced by neurons of the ventrolateral medulla. Neuronal spike trains were recorded simultaneously with planar microelectrode arrays. Sixteen groups of functionally linked neurons composed of both midline and ventrolateral cells were detected in 12 Dial-urethane anesthetized, vagotomized, artificially ventilated cats. Features in cross-correlograms and snowflakes provided evidence for concurrent serial and parallel regulation of impulse synchrony and suggested that elements of up to 3 "layers" of interneurons were monitored simultaneously. Gravitational representations demonstrated respiratory-phase dependent synchrony among neurons distributed in both domains. The data support two hypotheses. 1. Synchrony of some midline neurons is generated by divergent inhibitory actions of both midline and ventrolateral neurons. 2. Such putative distributed "synchrony-promoting" neurons are also functionally linked; changes in their effective connectivity contribute to temporal variations in the synchrony of midline assemblies. Supported by NS19814.

613.7

MATURATIONAL DIFFERENCES IN 660 nm LIGHT SCATTERING FROM VENTRAL MEDULLARY SURFACE OF KITTENS DURING GRADED HYPERCAPNIA AND HYPOXIA.
D.M. Rector*, D. Gozal, X.-W. Dong, R.K. Harper and R.M. Harper. Dept. of Anatomy & Cell Biology, UCLA Sch. of Med.;Childrens Hospital Los Angeles, USC Sch. of Med., Los Angeles, CA.

Developmental differences in ventilatory responses to hypoxia and hypercapnia are well described in kittens. To determine whether these maturational effects parallel neuronal responses in the ventral medullary surface (VMS), we recorded the time course of VMS light scattering changes to varying concentrations of inhaled CO_2 in O_2 and O_2 in N_2. Imaging procedures allowed for simultaneous measurements of large neural populations with high spatiotemporal resolution. The VMS was surgically exposed in 18 anesthetized kittens aged either 10, 20, or 30 days, and in 6 adult cats. A 3.2 mm coherent optical fiber bundle, attached to a charge-coupled device (CCD) camera, was positioned over the VMS. Neural tissue was illuminated with 660 nm light emitting diodes, and ECG-synchronized images of reflected light were digitized every 3 seconds throughout ventilatory challenges. Mean pixel intensities, which measure overall neural activity, were plotted over time. Both types of stimuli induced accelerated initial response slopes in 20- and 30-day kittens relative to 10-day and adult animals. We speculate that the age-dependent slower VMS neural responses coincide with maturational contributions of peripheral chemoreceptor function.

(Supported by HD-22506 & NIDR DE 07212)

613.8

DEVELOPMENT OF RESPIRATORY PATTERNING TO ANTERIOR HYPOTHALAMIC WARMING IN THE KITTEN.
J. Zhang*, H. Ni, D. Gozal and R.M. Harper. Brain Research Institute and Dept. of Anatomy and Cell Biology, UCLA School of Medicine, Los Angeles, CA 90024.

Local warming of the preoptic area/anterior hypothalamus (POAH) modifies respiratory rate and elicits panting. We hypothesized that the respiratory responses to thermal POAH manipulation in the kitten vary with age. We examined changes of respiratory components following POAH warming during quiet sleep (QS) in 10-, 20-, 30-, and 40-day old kittens and in adult cats. The POAH was warmed (0.5MHz, 85-190mW) bilaterally by two electrode pairs and POAH temperatures were recorded by a thermocouple. EEG, ECG, diaphragmatic, neck EMG and core body temperature were simultaneously recorded.

During QS, warming of the POAH by 1.2-4°C elicited an increase in respiratory rate in all animals. However, an associated decrease in tidal volume occurred, together with a decrease in minute ventilation (V_I) in 10-day kittens. Despite similar decreases in tidal volume, modest increases in V_I occurred at 20-40 days, and marked increases were observed in the adult. The relative hypoventilation observed in the 10-day kittens directly resulted from insufficient increases in respiratory drive. We conclude that POAH warming during QS preferentially increases respiratory rhythm rather than volume. In general, this relationship will result in increased V_I. However, maturational changes significantly affect the magnitude of this effect, and indicate the presence of a potentially vulnerable period in the youngest animals.

Supported by HD22506

613.9

ASSESSMENT OF VENTRAL MEDULLARY SURFACE ACTIVITY DURING GRADED HYPOXIA IN THE CAT WITH HIGH TEMPORAL RESOLUTION OPTICAL IMAGING.
D. Gozal*, D.M. Rector, R.K. Harper, X.-W. Dong and R.M. Harper. Dept. of Anatomy & Cell Biology, UCLA Sch. of Med.;Childrens Hospital Los Angeles, USC Sch. of Med., Los Angeles, CA.

The ventral medullary surface (VMS) displays divergent light scattering changes in response to varying degrees of ventilatory-induced hypoxia. We examined the temporal properties of VMS regions at high sampling rates to determine neural activity patterns associated with hypoxia. The VMS was surgically exposed in 7 adult anesthetized cats. A 3.2 mm coherent optical fiber bundle, attached to a charge-coupled device (CCD) camera, was positioned over the VMS. Neural tissue was illuminated with 660 nm light emitting diodes, and images were digitized at 60 fields/sec before and during ventilatory challenges with 6% and 12% O_2 in N_2. Spectral analysis of the overall reflectance changes revealed the existence of spectral peaks at 8-13 Hz, which increased in power and frequency during mild hypoxia (ANOVA, $p < 0.003$). However, during moderately severe hypoxia, multiple spectral peaks at frequencies within the 8-13 Hz range were consistently observed ($p < 0.02$). We conclude that significantly different activity patterns are elicited by varying degrees of hypoxia in the VMS. We speculate that mild hypoxia is associated with activation of discrete neural pools, while deeper hypoxia triggers activation of multiple neuronal populations within the structure.

(Supported by HL-22418 & NIDR DE 07212)

614.1

A NEW METHOD FOR THE STUDY OF VESTIBULAR FUNCTION, EYE MOVEMENTS AND MULTISENSORY FUSION IN HUMANS AND ANIMALS : MOBILE ROBOTS.

A. Berthoz*, S. Glasauer and I. Israël. Lab. de Physiol.de la Perception et de l'Action. Collège de France-CNRS. Paris France.

Vestibular function has been studied with stimulators which could provide either angular rotation or translation and no method is yet available for the study of the motor or perceptual responses to combined angular and linear motion except eccentric rotation. This method has already been successfully used, with a smaller version of the robot, for the study of the influence of movement on hippocampal neurons in the monkey (O'Mara et al. Neurosc. Soc. Abstr.18. 1992).

We have adapted a mobile robot (Robuter TM. ROBOSOFT Cie France) to provide complex quantified horizontal plane movements in human subjects. This four wheels robot contains a computer which can be tele-controlled by modem and has an odometry system for trajectory measurement. A multidirectional seat has been fixed on the platform.

This robot has been programmed to provide rotations of angular and linear velocity and accelerations in the range of vestibular receptors sensitivity. The robot can therefore give the traditional stimuli for independent stimulation of canals or otoliths. In addition it can move the subject along complex trajectories in the horizontal plane (squares, circles) by programming sequences of rotations and linear movements. Results concerning vestibular nystagmus, path integration and visual vestibular integration will be presented.

614.2

INPUT-OUTPUT TRANSFORMATIONS IN MEDIAL VESTIBULAR NUCLEUS NEURONS *IN VITRO*.

S. du Lac* and S.G. Lisberger, Dept. of Physiology and W.M. Keck Center for Integrative Neuroscience, UCSF, San Francisco CA 94143.

As a first step toward placing the membrane and synaptic properties of vestibular interneurons in a functional context, we have measured the transformations from intracellularly-injected current to firing rate in neurons recorded from the medial vestibular nucleus (MVN) in slices of chick brains. The intrinsic membrane properties and spike patterns of avian MVN neurons were similar to those reported for rodent MVN neurons *in vitro*. Chick MVN neurons fired spontaneously at regular intervals (firing rate ranged from 7 to 68 spikes/sec), displayed an inward rectification during hyperpolarizing current steps, and exhibited a rebound depolarization following current step offset.

Steps of depolarizing current produced increases in firing rate that depended linearly on the amplitude of the injected current. Firing rate was highest just after the onset of the step, declined slightly during the duration of the 1 sec step, and decreased transiently below baseline following step offset. Sinusoidal modulations of current produced sinusoidal modulations of firing rate with little harmonic distortion over the frequency range tested (0.1 to 10 hz). Firing rate phase led that of injected current; phase lead increased from 1-4 deg at 0.1 hz to 30-40 deg at 10 hz. Firing rate gain (peak to peak firing rate/current amplitude) increased slightly or was flat with increasing frequency. In contrast, when cells were held hyperpolarized to spike threshold with DC current, small amplitude sinusoidal modulations of current produced sinusoidal modulations of membrane potential with the properties expected of a low-pass filter: as modulation frequency increased, phase lag increased and gain decreased. We conclude that active conductances in vestibular neurons change neuronal filtering properties but preserve linearity.

614.3

LONG-TERM SYNAPTIC PLASTICITY IN THE RAT MEDIAL VESTIBULAR NUCLEUS STUDIED USING PATCH-CLAMP RECORDING IN AN *IN VITRO* BRAIN SLICE PREPARATION.

G.A. Kinney*, B.W. Peterson and N.T. Slater Dept. of Physiology, Northwestern Univ. Med. School, 303 E. Chicago Ave., Chicago, IL 60611.

The brainstem vestibular nuclei are believed to be a critical site for the long-term adaptive change in vestibulo-ocular reflex gain, but little information is available regarding potential cellular substrates of synaptic plasticity in this brain region. *In vivo* studies suggest that cerebellar projections to vestibular nuclei play an important role in this phenomenon. Neurons in the rat medial vestibular nucleus were recorded using whole-cell patch-clamp methods in 400 μm thick transverse slices cut at the level of nVIII, and stimulating electrodes were placed in the nVIII and m.l.f. to evoke monosynaptic EPSPs. EPSPs evoked by stimulation of either site were not affected by the NMDA receptor antagonist D-AP5 (20 μM), but were blocked by CNQX (10 μM). An AP5-sensitive component of both EPSPs was revealed in Mg^{2+}-free, bicuculline-containing medium. Tetanic stimulation of the nVIII or m.l.f. failed to produce consistent long-term changes in the AMPA receptor-mediated EPSP amplitude. Bath application of both the $GABA_B$ receptor agonist baclofen (1-10 μM) and the metabotropic glutamate receptor agonist 1S,3R-ACPD (100 μM) produced a blockade of both EPSPs. The effects of 1S,3R-ACPD were reversible. However, following the washout of baclofen, the amplitude of the nVIII-evoked EPSP exhibited a long-term depression. A stable reduction of 40% was observed after the washout of baclofen, whereas the m.l.f.-evoked EPSP was not significantly affected. The results provide evidence for a $GABA_B$ receptor-mediated form of long-term synaptic depression of the nVIII-evoked EPSP. This might be physiologically produced by descending cerebellar inputs to mediate long-lasting changes in the gain of the vestibulo-ocular reflex.

Supported by USPHS Grants EY 06485 and NS 17489.

614.4

RESPONSES OF RAT VESTIBULAR NUCLEI NEURONS TO NATURAL STIMULATION AND LABYRINTH POLARIZATION.

J. Kleine and O.-J. Grüsser*. Dept. Physiol. Freie Univ. 1 Berlin 33, Germany

In exploring the rat neocortex for vestibular responses we applied electrical polarization of the labyrinth by means of interaural DC or sinewave stimulation (≥ 20 μA). In order to evaluate the effects of labyrinth polarization, action potentials were recorded from the nerve cells of the brainstem vestibular nuclear complex (VNC) in pentobarbital anaesthetized pigmented rats.

(1) Neurons responding selectively to semicircular canal stimulation, otolith stimulation, or to both as well as polymodal units (labyrinth responses and neck muscle responses) all responded well to DC- or sinewave labyrinth stimulation.

(2) In many units phase-coupled responses were obtained even to low amplitudes (≤ 50 uA) in a frequency range above 10 Hz.

(3) Most units were activated either by positive or negative polarization of the labyrinth, whereby reversal of current led to inhibition.

(4) Responses to natural stimulation (sinewave rotation in pitch, roll or yaw, steady inclination) could be systematically superimposed on the responses to simultaneous labyrinth polarization (sinewave or DC).

(5) Low amplitude (0.5-1 Hz) transaural sinewave polarization of the labyrinths is a very handy method to evoke strong but selective vestibular responses while searching for higher-order vestibular neurons in the thalamus or in neocortical regions. Hereby the afferent vestibular neuron activity is highly synchronized.

614.5

VOR ADAPTATION: EFFECTS ON THE VELOCITY-TO-POSITION INTEGRATOR.

M. Shelhamer, D.S. Zee, C. Tiliket, D. Roberts and D.A. Robinson*, Johns Hopkins Hospital, Baltimore, MD 21287.

We investigated the effect of short-term vestibulo-ocular reflex (VOR) adaptation in normal humans on the velocity-to-position integrator that holds eccentric gaze. Following 1 hr of sinusoidal head oscillation (0.2 Hz), with the visual surround manipulated to produce an increase (X1.7), decrease (X0.5), or reversal (X(-2.5)) of VOR gain, we measured eccentric gaze-holding in darkness. Eccentric saccades were followed by sustained drift (1-2 deg/sec). The drift was centrifugal after X0.5 viewing, implying an unstable integrator. The drift was centripetal after X1.7 or X(-2.5) viewing, implying a leaky integrator. The gaze-holding deficits were context specific; they only appeared when the head was oriented in the same position (re gravity) as during training. We propose that 1) the changes in the neural integrator reflect an attempt to modify the phase (timing) relationships of the VOR, and 2) the relative directions of retinal slip and of eye velocity during training determine whether the integrator becomes unstable (producing more phase lag) or leaky (producing less phase lag).

We also assessed the effect of oscillation of the visual surround alone on VOR adaptation. This stimulus produced both a leaky integrator and an increase (10%) in VOR gain. Furthermore, during combined visual-vestibular stimulation, the gain decreased when the ratio of the movement of the visual scene to the movement of the head was relatively small (X(-2.5) viewing) and the gain increased when the ratio was relatively large (X(-7) viewing). These results suggest that one of the signals used for VOR adaptation is inferred head rotation based upon activity in central vestibular neurons that receive both labyrinthine and visual (optokinetic) signals.

614.6

THE ROLE OF THE NUCLEUS OF THE OPTIC TRACT (NOT) IN ADAPTIVE GAIN CONTROL OF THE VESTIBULO-OCULAR REFLEX (VOR).

H.Reisine*, S.B. Yakushin, and B. Cohen . Depts. of Neurology and Physiology and Biophysics, Mt. Sinai Sch. of Med., New York, NY 10029.

Combined visual-vestibular (conflict) stimulation was used to reduce the step gain (eye velocity/head velocity) of the VOR in monkeys during forced rotation. Animals were sinusoidally oscillated (0.2 Hz, ±60°/s) about their yaw axis, with head fixed, while upright in light in the presence of a subject-stationary surround consisting of alternating black and white stripes. VOR gain (0.95±0.05) was reduced 20 to 30% within two hours and maximally by ≈30% after 4 hrs (n=6). Further stimulation (up to 8 hrs) brought no further reduction in gain. Muscimol, a $GABA_A$ agonist, (0.8μg in 0.8μl) was injected unilaterally into NOT of animals who had had a gain reduction of ≈25% after 2 hrs of conflict stimulation. The VOR gain of contralateral slow phase velocity ($SPVel_C$), relative to the injection site, produced by ipsilateral rotation, returned to levels obtained before conflict stimulation, while the gain of ipsilateral slow phase velocity ($SPVel_I$) to contralateral rotation remained at the reduced (adapted) levels following stimulation. In the same animals following muscimol injection into the NOT, suppression of the gain of $SPVel_C$ during conflict stimulation was reduced (from 0.2 to 0.8), while suppression of the gain of $SPVel_I$ remained at pre-injection levels. The similarity of the effects muscimol injection in NOT on VOR suppression and adaptive gain control (at least for a gain decrease) is consistent with previous findings of others demonstrating a relationship between them. Moreover, it suggests that NOT plays a role in producing both suppression and adaptive gain control of the VOR.

Grants: EY00296, EY01867.

614.7

RESPONSES OF CAT PONTOMEDULLARY RETICULAR NEURONS TO HORIZONTAL ROTATION. R.H. Schor*, L.A. Cotter and B. J. Yates. Dept. Otolaryngology, Univ. Pittsburgh Sch. Med., Pittsburgh, PA 15213.

Is there a population of brainstem neurons which reflect the spatial and temporal properties of the vestibulocollic reflex (VCR)? Although few reticulospinal (RS) neurons appear to receive pure vertical semicircular canal input (Bolton et al., JNP 67:1992), previous work with electrical polarization of horizontal and anterior canal nerves (Peterson et al., JNP 43:1980) indicates that some RS neurons exhibit the low frequency phase lag characteristic of the horizontal VCR. In this study, we have examined the response dynamics of RS neurons to natural horizontal plane rotation.

Experiments were performed on decerebrate cats; recordings were made in the pontomedullary reticular formation. Responsive neurons were studied with sinusoidal horizontal rotation at 0.1 to 2 Hz. Fifteen neurons were identified as RS neurons by antidromic activation from C1 or L1.

Neurons responding to horizontal rotation were commonly encountered. 80% of the population, and all of the RS neurons, showed a Type II response. Most response dynamics resembled those of horizontal canal afferents, with gain and phase related to stimulus velocity. About a quarter of the neurons (4/15 RS neurons) had similar gain characteristics, but exhibited a progressive phase lag (re velocity) exceeding 45° at 1 Hz. The mean gain of the RS population (at 1 Hz) was 1.6±0.3 spikes/sec/° (0.25 spikes/sec per °/sec).

We saw no neurons responding to horizontal rotation that exhibited the low frequency phase lag characteristic of the VCR. These data, in combination with previous studies of vestibulospinal and RS neurons, suggest that some of the spatial and temporal properties of the VCR arise from convergence of vestibular signals within the spinal cord. (Supported by NIH NS24930 and DC00693).

614.8

VESTIBULO- RESPIRATORY AND SYMPATHETIC REFLEXES IN THE DECEREBRATE CAT. B.J. Yates*, J. Jakus and A.D. Miller. Lab. of Neurophysiology, Rockefeller Univ., New York, NY 10021.

Previous studies demonstrated that vestibular stimulation can affect respiration rate and sympathetic outflow. However, little is known about the latency, pattern, or receptors of origin (semicircular canals or otolith organs) of vestibulo-sympathetic and vestibulo-respiratory reflexes. This study sought to determine: 1) the minimal reflex latencies, 2) which vestibular receptors elicit the responses, and 3) what direction of head movement is most effective in evoking the activity.

Electrical stimulation of the vestibular nerve produced short-latency (< 15 ms) responses recordable from the phrenic (inspiratory) and abdominal (expiratory) nerves; in contrast, vestibulo-sympathetic reflexes recorded from the splanchnic nerve had long latencies (≥ 100 ms). Vestibulo-sympathetic and vestibulo-respiratory responses elicited by natural vestibular stimulation (head rotation in animals with upper cervical dorsal root rhizotomies) were in phase with table position and had relatively uniform gain across stimulus frequencies (0.1-1 Hz), suggesting that these responses were elicited by otolith receptors. The best direction of head rotation for eliciting vestibulo-sympathetic responses was nose-up tilt. In contrast, vestibular responses elicited in the abdominal and phrenic nerves had preferred directions that differed from experiment to experiment.

These data are consistent with vestibulo-sympathetic reflexes playing a role in compensation for orthostatic hypotension, which could result during nose-up tilt (since the cat has a long longitudinal axis). The variability in preferred direction of vestibulo-respiratory reflexes suggests that these responses may be multi-functional.

Supported by NIH grants DC00693 (B.J.Y.) and NS20585 (A.D.M.) and the Irma T. Hirschl Trust (B.J.Y.).

614.9

VERTICAL VESTIBULAR INPUT TO UPPER CERVICAL COMMISSURAL NEURONS. V.J. Wilson*, K. Endo, J. Kasper, and B.J. Yates. Lab of Neurophysiology, Rockefeller Univ. New York, NY 10021.

We have investigated, in decerebrate paralyzed cats, whether upper cervical commissural (CO) neurons play a role in the vertical vestibulocollic reflex. We looked for the pattern of inter-receptor convergence, and for evidence that CO neurons could be in a polysynaptic otolith pathway to contralateral neck motoneurons (Bolton et al. J. Neurophysiol. 1992, 67:1695). Antidromic stimuli were delivered with 1 or 2 tungsten-in-glass electrodes in the contralateral ventral horn. Judging from thresholds, 10 neurons were driven from the ventral grey matter, 7 from the grey or nearby white matter; 25 were not driven from the electrodes available. There were no obvious differences between responses of these three classes. Most neurons had typical canal (n=18), otolith+canal (n=8) or otolith (n=12) responses. Like vestibulo- and reticulospinal fibers, neurons with canal input had response vector orientations near canal planes, or had convergence from ipsilateral canals. 27/38 neurons, whether otolith- or canal-driven, had vectors pointing contralaterally. In conclusion, CO neurons could be part of an otolith pathway to motoneurons. More generally, their actions could modify the effectiveness of VS and RS fibers that have similar spatial properties and make synapses with neck motoneurons. Supported by NIH grants NS02619 and DC00693.

614.10

CERVICAL STRAIN IN VESTIBULAR UNITS. James H. Fuller* Dept. Oral Anatomy, Univ. of Illinois, Chicago IL 60612

Visually evoked head movements in trained cats were briefly (ca 100 msec) interrupted mid-flight by an electro-mechanical brake and single units were recorded in the caudal vestibular nuclei. A class of units (Category 3) was characterized by perturbation-linked ultrashort (1-5 msec) and short (5-100 msec) latency responses.

The single spike (<0.1 msec jitter), ultrashort latency, responses (1.4-1.8 msec) to the brake were duplicated by purely mechanical stimuli. The monosynaptic response to both electro-mechanical and mechanical perturbation is evoked by minute (ca 10 micra), high frequency (>100Hz) vibration of the brake shaft by solenoid activation.

The short latency responses (idiosyncratic burst-pause intervals) were associated in time and intensity with 1) brake onset, 2) voluntary head torque undulations (the animal's attempts to overcome the brake), and 3) head release. Six other methods evoked the short latency responses by passive/active maneuvers: the unit firing was most consistently associated with cervical strain. This suggests the paciniform receptors identified in the perivertebral area transduce a new modality in neck sensory information over a secure, direct, fast-conducting pathway from neck to the medial-inferior vestibular nuclei.

614.11

VESTIBULAR AND VISCERAL INPUTS TO NUCLEUS SOLITARIUS AND ADJACENT STRUCTURES IN THE CAT BRAINSTEM. L. Grélot, J. Jakus, A.D. Miller* and B. J. Yates. Lab. Neurophysiology, Rockefeller Univ., New York, NY 10021.

Nucleus solitarius and adjacent structures receive inputs from a variety of visceral receptors; inputs from different sources are partially segregated in this region. There is also some evidence to suggest that the caudal dorsomedial brainstem receives vestibular inputs, but this possibility has not yet been investigated thoroughly. Vestibular connections with this region could elicit cardiovascular and respiratory changes that compensate for stresses to homeostasis which occur during movement and changes in posture, and could also have a role in motion sickness. In this study we sought to determine the locations of neurons in nucleus solitarius and adjacent structures that receive vestibular input, and to ascertain whether these neurons receive convergent visceral input.

Experiments were conducted on adult cats that were decerebrated or anesthetized using urethane + chloralose. Vestibular inputs were elicited by electrical stimulation of the vestibular nerve. In initial experiments we recorded from the medial portion of the solitary nucleus and nucleus intercalatus. Many of these neurons responded to vestibular nerve stimulation, some at latencies ≤ 4 ms suggesting that they received direct input from the vestibular nuclei. Many adjacent neurons responded to stimulation of the abdominal vagus nerves, but only 2/26 neurons received convergent vagal and vestibular input. Thus, the role of vestibular inputs to the medial solitary complex is currently unclear. Future experiments will explore the dorsolateral solitary nucleus, and determine whether cardiovascular and vestibular inputs converge in this region.

Supported by NIH grants NS20585 (ADM) and DC00693 (BJY), Irma T. Hirschl Trust (BJY), and Ministere des Affaires Etrangeres, France (LG).

614.12

ULTRASTRUCTURAL EVIDENCE OF VESTIBULAR EFFERENT SYNAPSES OF INTRINSIC, AFFERENT ORIGIN. Muriel D. Ross.* Biocomputation Center, NASA-Ames Research Center, Moffett Field, CA 94035.

Ultrastructural and computer-aided three-dimensional reconstruction studies of rat vestibular gravity sensors have shown that maculas are organized for weighted, distributed, parallel processing of sensory input. There are two main integrated circuits: highly channeled and distributed modifying. Type I cells provide highly channeled output to calyces but type II cells are part of the distributed modifying circuitry. Type II cells, calyces and afferents are under the modulation of efferent synapses that, historically, have been attributed entirely to terminals of nerve fibers of extrinsic origin. Work with serial sections has demonstrated that a specific subset of efferent endings has an intrinsic origin from processes of vestibular afferents. They are part of the intrinsic modifying circuit. This report deals primarily with those short processes that can be traced to a synaptic ending in a single electron micrograph, since these are the most convincing examples. Other efferent processes arise from afferents as small-diameter nerve fibers and travel for several micrometers before ending. The terminals of these short and long processes are filled with clear vesicles ~40-60 nm in diameter, but some vesicles are ~70-90 nm in diameter and have an electron-opaque core. Other vesiculated terminals have a more electron-opaque appearance and contain mostly the smaller diameter vesicles. Further research is necessary to distinguish better between the two kinds of efferent terminals since the latter subset is the group possibly of extrinsic origin. The present findings are conclusive anatomical evidence that a mechanism is in place for intrinsic modulation of macular output. This modulation would be driven by ongoing activity of the afferents and rapidly expressed through vesiculated terminals of processes contributing to the intrinsic distributed modifying circuit.

This research is supported by NASA and by NIMH Grant # MH47305-02.

615.1

GENDER DIFFERENCES IN BRAIN VOLUME AND CEREBRAL BLOOD FLOW (CBF): A POSITRON EMISSION TOMOGRAPHY (PET) AND MAGNETIC RESONANCE IMAGING (MRI) STUDY OF NORMAL CONTROLS. N.C. Andreasen*, L.A. Flashman, D.S. O'Leary, D.M. Mosnik, V.W. Swayze, G.L. Watkins, L.L. Boles Ponto, and R.D. Hichwa. Dept. of Psychiatry, and PET Imaging Center, Univ. of Iowa, Iowa City, IA, 52242.

Studies using both PET and SPECT have found women to have a higher rate of blood flow/metabolic activity than men, although there have also been reports of no gender differences. Gender differences in brain size have been independently observed in both postmortem and MR studies, but the effect of this variable on CBF is unclear. The present study investigated gender differences in brain size using MR and in CBF using PET. Forty-one male and 31 female healthy normal subjects were examined using both MR and PET. Male and female groups did not differ in age, years of education or socioeconomic status. The [^{15}O] water radiographic method was used with a GE 4096 Plus Scanner to obtain quantitative measurements of perfusion (ml/min/100g) of tissue during a baseline condition. Females (mean=54.1 ml/min/100 g, sd=7.5) had a significantly higher rate of whole brain CBF (t=2.1, p<.04) than males (mean=50.1, sd=8.5). MR data were collected on a 1.5 Tesla GE Signa Scanner with an SPGR sequence generating contiguous 1.5 mm coronal slices. Males (mean=1322.2cc, sd=119.0) had significantly larger brain volumes (t=4.5, p<.001) than females (mean=1195.1, sd=108.4). The gender effect for CBF remained significant when brain volume was statistically controlled by analysis of covariance. In addition, a positive correlation between brain volume and CBF was found for males (r =.37 P<.03), but not females (r = 0.0). These results indicate that women as a group have higher whole brain CBF than men, that this effect is independent of brain size, and that there is a gender difference in the relationship between brain size and blood flow.

615.2

CENTRAL CORRELATES OF SPEECH AND LANGUAGE PRODUCTION IN HUMANS STUDIED WITH $H_2{}^{15}O$ POSITRON EMISSION TOMOGRAPHY. A.R. Braun*, C.L. Ludlow M. Varga, S. Stager and G. Schulz. VSS, NIDCD, NIH, Bethesda, MD 20892

Eight right-handed normal volunteers underwent a series of rCBF PET scans following i.v. bolus infusions of 30mCi of $H_2{}^{15}O$. Scans were performed on a Scanditronix PC2048-15B tomograph. Blood curves were generated automatically for derivation of arterial input functions and transmission scans were utilized for attenuation correction. Individual scans were stereotaxically normalized to a common coordinate system and task conditions were compared by generating statistical parametric maps (SPM, MRC Cyclotron Unit, Hammersmith Hospital). When compared to rest, simple movements of the larynx, tongue, lips and mandible were associated with *bilateral* activation of anterior cerebellar cortices, inferior primary motor cortices, supplementary motor area, putamen, and ventral thalamus as well as superior temporal gyri in the region of the primary auditory cortices (in each instance p <.01, corrected for multiple comparisons). When compared to these simple movements, more complex production of phonemes *without* linguistic content was associated with activation - again *bilateral* - of the insular, anterior cingulate and supplementary motor cortices, superior temporal (anterior auditory association) cortices, as well as left lateral premotor cortex. Compared to both simple and complex motor tasks, language production - either spontaneous conversational speech or formal sentence construction - was associated with markedly *lateralized* effects: activation of the left lateral premotor cortex, left dorsolateral prefrontal cortices (Brodman 8, 9, 10), and head of the left caudate nucleus as well as activation of the posterior temporal cortices (in the region of Wernicke's area), which was greater in the left than the right hemisphere. Both language tasks were also associated with bilateral activation of medial prefrontal (area 10), and posterior cingulate cortices, and spontaneous conversational speech was additionally linked to bilateral activation of the orbitofrontal cortices (area 11). In contrast *singing*, when compared with *speaking* the words to a familiar song, was associated with predominantly *right* cerebral hemispheral activation - in globus pallidus, insula, superior temporal, inferior prefrontal (area 47) and precentral gyri - as well activation of the *left* anterior cerebellar hemisphere. These results demonstrate that different cortical and subcortical brain regions subserve the motor and linguistic elements of speech production in humans and can be used as a baseline in future studies designed to evaluate the central correlates of disorders of speech and language.

615.3

POSITRON EMISSION TOMOGRAPHY AND EYE MOVEMENTS DURING REM SLEEP. C. C.-H. Hong, J. C. Gillin, B. M. Dow, J. Wu* and M. S. Buchsbaum**. Dept. of Psychiatry, UCSD and VAMC, La Jolla, CA 92093-9116; *Dept. of Psychiatry and Behavioral Medicine, UC Irvine, CA 92717; **Neuroscience PET Lab., Mt. Sinai School of Medicine, New York, NW 10029-6574

In order to study the neural substrate for eye movements (EMs) during REM sleep, we reanalyzed the ^{18}FDG PET scan data obtained from normal subjects in a previous study (Buchsbaum et al. Life Sciences 45:1349-1356,1989). Nine subjects were studied during REM sleep, and six control subjects were studied during waking as they periodically moved their eyes. The number of EMs during REM sleep were positively correlated with local cerebral glucose metabolism (LCGM) in the areas corresponding to (1) the saccadic-EM system (frontal eye field & dorsolateral prefrontal cortex, only on the right side), (2) the midline attentional system (cingulate & medial frontal cortex, precuneus), and (3) the parietal visual spatial attentional system (bilateral superior parietal lobules, right inferior parietal lobule); and negatively correlated with LCGM in the left inferior parietal lobule. Positive correlation between waking EMs and LCGM were observed in the same areas except inferior parietal lobule. These findings suggest that EMs saccadically scan targets in the dream scene. Our data have implications for dream theories, cerebral lateralization, and schizophrenia research.

615.4

POSITRON EMISSION TOMOGRAPHY (PET) STUDY OF REGIONAL CEREBRAL GLUCOSE METABOLISM (rCMRglc) IN DEMENTED PATIENTS WITH FRONTAL LOBE FEATURES. U. Freo*, P. Pietrini, A. Dani, C.L. Grady, J. Salerno, M.B. Schapiro. Laboratory of Neurosciences, National Institute on Aging, Bethesda MD 20892.

To examine the pattern of cerebral glucose metabolism associated to the cognitive decline seen in dementia with frontal lobe features (DFL), we studied 3 subjects (2 F, 1 M; 45, 56 and 58 year/old, respectively) with a slowly progressive dementia, characterized by early personality changes, behavioral disinhibition and later perseveration and apathy. High-resolution PET scan exams (Scanditronix PC1024-7B, Uppsala, Sweden; FWHM 6mm) with [18-F]2-fluoro-2-deoxy-D-glucose were performed in the "resting state" (ears plugged/eyes patched, minimal room noise). Attenuation correction was measured by transmission scan. Results were compared with two groups of 18 sex- and age-matched healthy volunteers by Z-score analysis. Absolute rCMRglc values were expressed in mg/100 g tissue/min; in addition, to minimize inter-subject variability, rCMRglc values were "normalized" to the mean global grey matter CMRglc. An average rCMRglc reduction greater than 50% ($p < 0.01$) was found in frontal, prefrontal and cingulate regions in all the 3 DFL patients as compared to the matched healthy controls. In addition, a significant, although smaller, rCMRglc reduction in association parietal and temporal areas (usually early affected in Alzheimer's disease) was seen in the two female patients, who also had a more severe and wider cognitive impairment. Primary sensory and motor neocortical areas, hippocampus, amygdala, cerebellum and basal ganglia were relatively spared in all patients. These results indicate that a specific pattern of rCMRglc reductions consistent with behavioral and cognitive deterioration is present in patient with DFL and further suggest that DFL should be considered a specific clinical entity.

615.5

CORRELATIONS BETWEEN GLUCOSE UTILIZATION VALUES FROM PET AND PEAK AMPLITUDES OF ERP COMPONENTS IN A NORMAL STANDARDIZATION STUDY. C.E. Naylor*, F.B. Wood and D.L. Flowers. Section of Neuropsychology, Bowman Gray School of Medicine, Winston-Salem, NC 27157.

For N=40 normal males and females, local maxima and regional averages of glucose consumption using [18]-F-2-deoxyglucose (FDG) uptake were compared to peak amplitude measures of averaged evoked potential components collected simultaneously with the uptake, during a visual single stimulus paradigm requiring letter discrimination. Subject paced stimuli occurred at an average rate of 1 per 2.5 seconds throughout the glucose uptake. Task accuracy was high, but with measurable variance, across all subjects.

By far the most robust correlations involved the late negative component (peaking at approximately 700 msec post stimulus), relating most scalp locations to most local glucose measures. More specific correlations were found in the ventral extrastriate visual processing pathways, involving early and mid-latency components such as P1 and N2, but these explained only relatively small fractions of the local glucose metabolic variance, compared to the large effects involving late negativity.

The results are interpreted as suggesting caution in the interpretation of underlying sources of task-related glucose activation.

615.6

UTILITY OF LOCAL GLUCOSE MAXIMA AS MEASURES OF TASK RELATED CORTICAL ACTIVATION WITH PET. D.L. Flowers*, F.B.Wood, J.W. Keyes, and F.H. Fahey. Section of Neuropsychology and Dept. of Radiology, Bowman Gray School of Medicine, Winston-Salem, NC 27157-1043.

N=40 normals performed a letter discrimination task, using subject-paced stimuli at an average rate of 1 per 2.5 seconds during uptake of [18]-F fluorodeoxyglucose (FDG). In this PET validation, local maxima outperformed local averages as dependent measures because of:

(1) Compatibility with the more veridical representation of actual activity in spherical and irregular phantoms;

(2) Sharper correlational demarcation of a visual extrastriate cortical pathway in posterior cortex;

(3) Demonstration of the expected dorsolateral prefrontal cortex couplings both to angular gyrus and to caudate; and

(4) Demonstration of an inverse correlation between task accuracy and extrastriate visual cortical areas.

According to the phantom studies, local glucose utilization maxima combine information about size and activation intensity of small (1 cm.) cortical regions.

According to the correlational studies, local maxima measure cortical activation in terms that are compatible with known or expected models of task-related cortical connectivities.

615.7

NEW SINGLE-SUBJECT METHOD FOR PARAMETRIC ANALYSIS OF REGIONAL CEREBRAL BLOOD FLOW (rCBF) ACTIVATION EXPERIMENTS. J. Ma. Maisog[1], T. A. Zeffiro[2], K.D. Pettigrew[3], M. Hallett[2], S.I. Rapoport[1], P. Herscovitch*[4]. LNS, NIA[1]; MNB, NINDS[2]; DESR, NIMH[3]; and PETD, CC[4]; all from NIH, Bethesda, MD 20892.

In a new single-subject method to analyze functional images, the rCBF response to an experimentally controlled parameter was characterized with a linear regression model on a voxel-by-voxel basis. $H_2{}^{15}O$ positron emission tomography scans were obtained on 8 normal subjects at rest and during an alternating wrist movement at 0.25 Hz, 0.5 Hz, and 1 Hz. Each scan state was repeated, for a total of 8 scans per subject, and all scans were stereotactically normalized (Friston et al., 1991). For each subject, the global mean CBF of each activation scan was normalized to that of the resting runs, and then, again for each subject, linear regression was performed on every voxel, with frequency of movement as the independent variable and rCBF as the dependent variable; this generated a slope image. A t-statistic test for slopes significantly different from zero was performed on every voxel. Regional maxima in the slope image were localized with a stereotactic atlas. Results showed significant linear regressions between rCBF and frequency in contralateral motor cortex and ipsilateral cerebellum during the motor task, but the exact location and slopes varied from subject to subject. This parametric imaging method can be extended to experimental designs using other external parameters, and other mathematical models besides a straight line may be used.

615.8

ASSESSING TEST-RETEST RELIABILITY OF FUNCTIONAL MRI DATA
N. J. Cohen, M. T. Banich, A. F. Kramer, H. D. Morris, P. C. Lauterbur*, C. S. Potter, Y. Cao and D. N. Levin, Dept. of Psychology, Beckman Institute, Biomedical Magnetic Resonance Lab, NCSA, University of Illinois at Urbana-Champaign and Dept. of Radiology, University of Chicago

The use of magnetic resonance imaging for functional brain mapping studies allows the same subjects to participate in multiple studies on multiple occasions and holds out the promise of being able to identify brain regions active in various processing tasks *within individual brains*. For this potential to be realized, it must first be demonstrated that *within individual brains* the same areas are activated reliably by the same cognitive challenges on multiple occasions, requiring an assessment of test-retest reliability. This report presents preliminary data on our efforts to provide such an assessment.

Eight subjects were tested on 2-3 occasions separated by 1-4 weeks. On each occasion, subjects were presented with photic stimulation in full field, left hemi-field, and right hemi-field displays, each at a 7.5 Hz reversal rate and a 3.75 Hz reversal rate; the photic stimulation conditions were interspersed with an equal number of baseline (or rest) conditions. Data were acquired on a GE Signa 1.5T MRI scanner (General Electric Corp., Milwaukee, WI) using a standard 1H head coil and a modified spoiled GRASS pulse sequence. A series of T2* weighted 3-4 5mm contiguous oblique slices was acquired with a 128x64 matrix over a 24cm FOV. Slice positions were chosen to maximally cover the visual cortex as judged by a series of T1 weighted saggital scout images. Each set of slices was obtained in 11-16 s, followed by an 8 s delay. A typical experiment consisted of 36 such multislice acquisitions.

For the 16 data sets analyzed (8 subjects x 2 testing occasions), photic stimulation reliably produced local changes in signal intensity of 2-10%, with left hemi-field stimulation typically producing greater changes on the right and right hemi-field stimulation on the left. Of the 8 test-retest data sets, 6 were sufficiently free of artifacts to assess test-retest reliability. Qualitative analysis of the functional maps, as well as of the spatial extent and numerical magnitude of the statistically derived signal intensity changes in the different stimulus conditions demonstrated substantial intra-subject correspondence between testing occasions. All 6 showed the appropriate hemispheric lateralization of greatest signal intensity changes following hemi-field stimulation on both testing occasions, with similar magnitudes of effects from test to retest. The mean ratio of change in left versus right hemisphere regions for the various stimulation conditions was 1.1 (L/R) for full-field stimulation, 2.1 (L/R) for right hemi-field stimulation, and 2.2 (R/L) for left hemi-field stimulation.

615.9

On the Nature of the Signal Change in MR Functional Brain Mapping using Gradient Echo Pulse Sequences

M. Hutchinson and V. Nenov*. UCLA.

Several techniques are now available for MR functional brain mapping. Most prominent are Echoplanar imaging (EPI) and the so-called Gradient Echo imaging (GE). The GE image, although currently more time-consuming than EPI, holds the promise of greater spatial resolution than EPI. By aquiring a number of functional images at different values of the repeat time (TR), it is shown that the signal change in activated cortex is significantly more sensitive to T_1 than to T_2^*, when a GE sequence is used. Moreover this sensitivity increases as TR is shortened. Thus it is not due to a blood flow phenomenon. This dependence on T_1 is in contrast to EPI where the change depends almost exclusively on T_2^* and is presumably a susceptibility effect. Thus GE images are sensitive to a process fundamentally distinct from that seen in EPI. It is suggested that this effect is related to fluid shifts occurring at the neuronal membrane. Since such shifts probably occur in hundreds of milliseconds after neuronal activation, then GE imaging offers the possibility of demonstrating direct neuronal events on such a timescale. A pulse sequence (spoiled GRASE)capable of making a T_1-weighted image in under a second, is outlined.

615.10

SPURIOUS EFFECTS OF DRAINING VEINS IN MAGNETIC RESONANCE FUNCTIONAL NEUROIMAGING (MRFN). R. Turner*, P.H. Jezzard, D. Le Bihan, A. Prinster. Lab. of Cardiac Energetics and Diagnostic Radiology Department, NIH, Bethesda, MD20892.

The technique of Blood Oxygenation Level Dependent (BOLD) contrast MRI has been applied to imaging human brain function with excellent spatial and temporal resolution. Doubt has been raised recently regarding the location of apparently activated cortical regions. In many gradient-echo MRI studies, large signal changes correlated with the stimulation paradigm can clearly be seen in large veins draining relevant cortical areas. These correspond to increases in oxygenation of venous blood, mixing relatively slowly with blood draining from nonactivated tissue regions, and do not indicate regions of increased neural activity. The effect, analogous to artifacts in PET blood volume studies, complicates interpretation of MRFN data and may result in erroneous conclusions.

Another MRI sequence, spin-echo MRI with long echo-time, gives very little signal from blood in veins. Theory suggests that the effect of oxygenation changes in large draining veins is also suppressed, while that of microvessels remains, due to the relative motions of paramagnetic deoxygenated red cells and tissue water molecules which irreversibly dephase the water proton spin magnetization. We used spin-echo echo-planar imaging in photic stimulation studies at magnetic fields of 4.0 T and 1.5 T. The fractional changes in signal related to brain tasks are smaller than with gradient-echo studies, but the regions of apparent activation are correspondingly more reliable. The effect increases with magnetic field, as expected.

615.11

TIME COURSE of CEREBROVASCULAR RESPONSE to NEURONAL ACTIVITY DEMONSTRATED with FUNCTIONAL MR IMAGING

MS Cohen*, JR Baker, JW Belliveau, TL Davis, RC Tootell, KK Kwong, BR Rosen MGH-NMR Center, Charlestown, MA

Neuronal activity, evoked with visual stimuli, is associated with increased signal in magnetic resonance images. Using ultra-fast magnetic resonance imaging methods sensitive to these functional changes ("fMRI methods") we examined regions of interest within primary visual cortex during exposure to flashing light stimuli. As has been shown previously, the fMRI signal change varies a function of the flash frequency. When the averaged image signal during a dark period is subtracted from the average obtained during visual stimulation, the largest signal change is observed with flash frequencies of about 8 Hz. These data are in good correspondence with cerebral blood flow changes observed by positron emission tomography (PET).

In repeated studies on a single subject the peak fMRI signal change in visual cortex at each flash frequency was similar. However, the response to 8 Hz flash was more sustained than for stimulation at either lower or higher frequencies. These data suggest that the graded response magnitudes observed in PET imaging to differing stimulus conditions may inadequately describe the cerebrovascular response to neuronal activity. By examining the signal response in single MRI voxels to repeated stimulation, we noted that the time course tended to be characteristic of particular brain locations and of particular stimulus properties. By analyzing both the locations and temporal response properties of fMRI-visible brain activity we believe that the method will lead to enhanced understanding of the neuronal response to complex stimuli.

615.12

SPATIAL RELATIONSHIP BETWEEN PHYSIOLOGICALLY-DEFINED CINGULATE HAND REPRESENTATION AND MRI-DEFINED COURSE OF THE CINGULATE SULCUS.

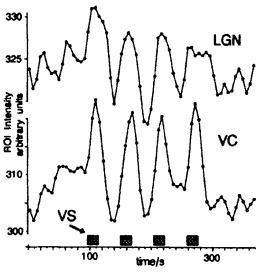
T. Paus*, F. Tomaiuolo, M. Petrides and A.C. Evans. Montreal Neurological Institute, McGill University, Montreal, Canada and Institute of Human Physiology, University of Verona, Italy.

Approximately at the level of the anterior commissure, the human cingulate sulcus (CS) gives off a vertical branch in the majority of cases (see accompanying MRI study). Since changes in rCBF related to manual responses were observed previously in this region (Paus et al. J. Neurophysiol.70(2),1993), we have now examined whether there is a relationship between the location of the activation focus (PET) and the location of the change in the CS course (MRI) in a single subject. Coordinates of the PET- and MRI-defined landmarks were obtained in 13 subjects (15 hemispheres). A close spatial relationship was observed between the averaged location of the MRI-derived point ($Y=-2.9\pm7.2$; $Z=44.6\pm3.8$) and the center of the PET-determined activation focus ($Y=-1.9\pm6.4$; $Z=47.0\pm4.8$). A significant correlation was found between Y coordinates of the PET- and MRI-determined landmarks ($r=0.71, p=0.003$). These results suggest that the location of physiologically-defined hand representation in the CS of a given subject follows the individual pattern of CS morphology.

615.13

FUNCTIONAL MRI OF LATERAL GENICULATE NUCLEUS ACTIVATION DURING VISUAL STIMULATION. A. Kleinschmidt[1], H. Steinmetz[1]*, K.D. Merboldt[2], W. Hänicke[2], J. Frahm[2]. [1]Neurolog. Klinik, H.-Heine-Universität, D-40225 Düsseldorf, [2]Biomed. NMR, MPI biophysikal. Chemie, D-37018 Göttingen

High resolution neuroimaging techniques with MRI are promising in the attempt to analyze functional connectivity in living human subjects. In this study we addressed the issue of thalamocortical transmission within the primary visual pathway. MRI was performed at 2.0 T (Siemens Magnetom) using the standard headcoil and acquiring T1-weighted FLASH images (TR/TE/a=47ms/30ms/40deg, RF-spoiled, 128x256 matrix, FOV 200mm, 4mm slice thickness, temporal resolution 6s). Within horizontal sections parallel to the bicommissural plane signal intensity changes with time were measured both in regions of visual cortex (VC) and lateral geniculate nucleus (LGN) of 8 healthy subjects. Visual stimulation (VS) was achieved by a binocularly presented pattern of diodes flashing at 10 Hz. Both in VC and LGN (as opposed to control regions) signal intensity changed with a temporal pattern reflecting VS cycles (see figure). To our knowledge, this is the first noninvasive demonstration of coupled activation of the thalamic and cortical component of the primary visual system. Functional MRI renders studies of thalamocortical transmission and of activation in small subcortical nuclei feasible; given the strategic importance of such structures within neurocognitive networks this is relevant in the healthy and the diseased human brain.



MOTOR CORTEX

616.1

FUNCTIONAL DIFFERENCES BETWEEN APICAL AND BASAL DENDRITES REVEALED FROM IMAGING CALCIUM DYNAMICS IN LAYER 5 NEOCORTICAL PYRAMIDAL CELLS. D. W. Tank* and R. Yuste. Biological Computation Research Dept., AT&T Bell Laboratories, Murray Hill, NJ 07974.

A general question in cortical physiology is if neurons contain functionally distinct compartments such as those suggested by differences in dendritic morphology. We have used optical imaging methods to explore this issue in basal and apical dendrites of layer 5 pyramidal neurons in slices of rat somatosensory cortex. Neurons were microinjected with the fluorescent calcium indicator fura-2 and the spatial pattern of changes in intracellular free calcium ion concentration ($[Ca^{2+}]_i$) produced by intracellular current injections and antidromic stimulation were imaged with a CCD camera.

In both intrinsically bursting (IB) and regular spiking (RS) cells, intrasomatic injection of depolarizing current pulses elicited action potential trains that were always correlated with $[Ca^{2+}]_i$ increases throughout the basal dendrites, cell body and the proximal 300 µm of apical dendrite. Calcium levels in most of the apical dendrite (the distal regions up to 1000 µm from the soma) were not significantly changed. Antidromic stimulation at frequencies of 20 or 100 Hz in the presence of the synaptic blockers AP5, CNQX and BMI also produced accumulations restricted to the basal dendrites and proximal apical dendrite. Since voltage-sensitive calcium channels exist in more distal regions of the apical dendrite (see next abstract), our results suggest that brief, msec duration, voltage changes are strongly attenuated with distance along the apical dendrite with an "effective space constant" of ~300 µm. Thus, distal apical dendrites do not sense changes in the action potential output of the neuron.

This strong voltage attenuation has implications for synaptic learning rules in apical versus basal dendrites. The rule on basal dendrites may depend on the cell's action potential output, while on apical dendrites it may only reflect correlated activity of local inputs. This may underlie the specificity with which presynaptic cells choose postsynaptic apical vs. basal dendritic targets.

616.2

SPATIALLY LOCALIZED CALCIUM ACCUMULATIONS IN APICAL DENDRITES OF LAYER 5 NEOCORTICAL NEURONS. R. Yuste* K. R. Delaney , M. J. Gutnick and D. W. Tank. Biological Computation Research Dept., AT&T Bell Laboratories, Murray Hill, NJ 07974.

Given the length (up to 1mm) of neocortical apical dendrites and the strong spatial attenuation of short duration electrical events shown in the previous abstract, regenerative currents in apical dendrites may play an important role in relaying information from distal synaptic inputs to the cell body (Spencer and Kandel, 1961). We have examined the role of voltage-sensitive calcium channels (VSCCs) in apical dendrites by imaging fura-2 microinjected layer 5 neurons in rat neocortical slices during synaptic, pharmacological and intracellular stimulations.

Extracellular stimulation of layers 1, 2/3 or 4 always resulted in calcium increases in the apical dendrite that were most prominent in its middle third, 400-600 µm from the soma. Lower calcium accumulations were observed in the soma and basal dendrites. All responses were blocked by AP5, suggesting that they were triggered by synaptic activation of NMDA receptors. When slices were perfused with NMDA in the presence of TTX, a rapid, local calcium accumulation was observed in the middle of the apical dendrite, which extended subsequently to the rest of the cell. To test whether these calcium accumulations were due to calcium entry through NMDA receptors or through VSCCs opened by the NMDA-induced depolarization, we impaled apical dendrites with microelectrodes and imaged their responses during intradendritic depolarizations. In all cells, calcium accumulations were triggered in the apical dendrite, indicating the existence of VSCCs. As an additional test, we injected depolarizing currents in the soma of cells bathed in TTX and TEA to increase their electrotonic length, and AP5 and CNQX to prevent stimulation by ambient glutamate. A localized calcium accumulation in the apical dendrite could be elicited by brief depolarizing currents. Coincident with the accumulations, long lasting calcium spikes were electrophysiologically observed.

Taken together, these experiments demonstrate the existence of an area in the middle of the apical dendrite with abundant VSCCs. This area can be activated synaptically, can lead in some conditions to regenerative calcium spikes and may serve as a "trigger zone" that amplifies distal synaptic inputs.

616.3

PRIMATE PREMOTOR CORTEX: EFFECTS OF GAZE ON THE NEURONAL CODING OF LIMB MOVEMENT DIRECTION. **D. Boussaoud,* Vision et Motricité, INSERM-U94, 16 Ave. Doyen Lépine, Bron 69500, France.**

Previous studies suggested that the neuronal activity of dorsal premotor cortex (PMd, the dorsal part of Brodmann's area 6) encodes limb movement direction. We studied the effects of eye position in the orbit on the properties of PMd neurons.

Single neurons' activity was recorded in a rhesus monkey seated in a primate chair, with its head fixed, while performing a previously learned conditional task. The monkey had to contact a central touch pad with its forelimb, direct gaze to a 0.3°x0.3° visual stimulus (the fixation point, FP) and maintain fixation throughout each trial. During fixation, a 2°x2° white square (the spatial attentional cue, SAC) appeared at one of nine possible screen locations and lasted for 0.5 s. Then, following a variable delay (0.4-0.8 s.), a red or green square (the motor instruction cue, MIC) of equal size was presented at the same location for 0.5 to 3.0 s. A red stimulus instructed the monkey to move its limb from the central pad to one located to the left, a green instructed a movement to a right pad. The offset of this stimulus served as the go signal, and the monkey had to execute the appropriate movement within 0.6 s in order to receive a liquid reward. The FP could appear at five different screen locations, but the stimulus remained at identical retinal co-ordinates.

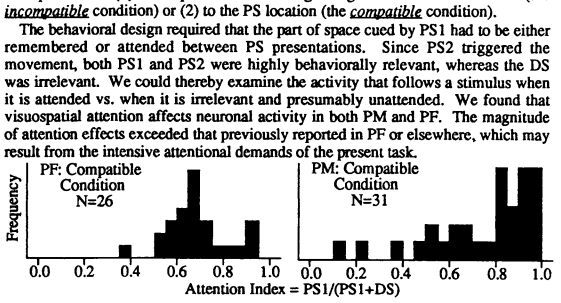
The results from a sample of 120 PMd neurons show that, for a majority of them (63%), the activity following the MIC presentation was dramatically affected by eye position. In many cases, the activity during the instructed delay period (set) for the preferred limb movement direction was highest when the retinal position of the MIC and gaze angle were in that direction. For others, the discharge was highest when gaze was deviated in the direction opposite that of the instructed limb movement. In some neurons, gaze effect was such that it reversed the direction preference. We suggest that PMd neurons code limb movement direction using, among other parameters, retinal and eye position information.

616.4

EFFECTS OF ATTENTION ON VISUOMOTOR ACTIVITY IN THE PREMOTOR AND PREFRONTAL CORTEX OF A PRIMATE S.P. Wise* and G. di Pellegrino. Laboratory of Neurophysiology, NIMH, Poolesville, MD 20837

We examined neuronal activity in the premotor (PM) and prefrontal (PF) cortex while a rhesus monkey performed a demanding spatial matching task. The monkey fixated and held a manipulandum beneath a central light emitting diode (LED), which had 8 LEDs arranged in a circle around it. On each trial, after 1 of the 8 LEDs illuminated for 0.5 s (the first prime stimulus, PS1), 0 to 4 of the other LEDs illuminated sequentially for 0.1 s at delays of 0.55 or 0.75 s each as distractor stimuli (DS). At the end of this delay period, the PS illuminated again (PS2) for 0.1 s whereupon the monkey had to execute a forelimb movement within 650 ms (and could break fixation). There were 2 response conditions: the monkey moved the manipulandum (1) to a predetermined target regardless of PS location (the *incompatible* condition) or (2) to the PS location (the *compatible* condition).

The behavioral design required that the part of space cued by PS1 had to be either remembered or attended between PS presentations. Since PS2 triggered the movement, both PS1 and PS2 were highly behaviorally relevant, whereas the DS was irrelevant. We could thereby examine the activity that follows a stimulus when it is attended vs. when it is irrelevant and presumably unattended. We found that visuospatial attention affects neuronal activity in both PM and PF. The magnitude of attention effects exceeded that previously reported in PF or elsewhere, which may result from the intensive attentional demands of the present task.



616.5

DORSOMEDIAL FRONTAL CORTEX OF THE MACACA MONKEY: EYE-EAR RELATED NEURONS.

L Bon * and C. Lucchetti Istituto di Fisiologia, University of Trieste, Trieste 34127 Italy.

The dorsomedial frontal cortex is involved in eye motor control. We recorded the activity of 139 cells in the rostral part of dorsomedial frontal cortex of one monkey, trained for fixation and saccade tasks. We followed the rules of the European Community for animal care before, during and after the experiments. We recorded the eye and ear movements by a search coil device in two dimensions. This device allowed to define only the timing between unit activity and ear movement and not parametric characteristics of movements. In addition we recorded the ear movements by an infrared TV system in order to define the movement direction. The firing discharge of 116 cells was related to ear (73%), eye (8 %) and eye-ear (19 %) movements. These neurons showed three different patterns of activity: burst, tonic and burst-tonic. When the eye and ear movements started at the same time the cell showed an increase of activity. These preliminary results suggest that the rostral part of dorsomedial frontal cortex (area 8b) of the monkey is involved in ear movements and eye-ear coordination, probably in orienting processes.

Supported by CNR, MURST 40% and 60%.

616.6

MULTI-SLICE FUNCTIONAL MRI OF HUMAN MOTOR AND SOMATOSENSORY CORTEX

CE Stern*, JR Baker, KK Kwong, JW Belliveau, BR Rosen Harvard Medical School and MGH-NMR Center, Charlestown, MA 02129.

Recent developments in functional magnetic resonance imaging (fMRI) allow for the non-invasive examination of changes in blood flow and blood oxygenation (Kwong et al., *PNAS*, 1992). Early fMRI reports examining motor cortex employed single slice, surface coil techniques, which limited the extent of brain that could be studied. Advances in multislice, high speed echo-planar imaging (EPI) techniques allowed us to obtain simultaneous, bilateral measurements of MR signal changes in motor, somatosensory and premotor cortical areas during baseline, motor, and somatosensory paradigms. Gradient echo (GE) and asymmetric spin echo (ASE) imaging sequences were used to track regional changes in signal intensity. Between six and 22 slices of 7mm thickness were positioned through the primary motor and somatosensory areas. Additional flow sensitive scans were used to locate blood vessels, and were subsequently used to dissociate vessel sensitive vs. gray matter signal changes. Each acquisition series started with the collection of resting baseline images, during which the subject remained still and relaxed. Subsequently, subjects performed a motor task, repetitively touching the thumb to each finger sequentially, alternating between movement of the right and left hand. For somatosensory stimulation, the experimenter sequentially stimulated the subject's finger tips in a passive version of the motor task. During active hand movement, MR signal intensity rose significantly in both the primary motor and somatosensory cortex. The passive somatosensory task was successful in dissociating regions selectively associated with motor vs. somatosensory processing. These recent advances in multislice, high speed EPI techniques, in combination with multiple behavioral tasks, allow for the examination of dynamic interactions at multiple cortical sites.

616.7

EVIDENCE FOR INTRACORTICAL PLASTICITY IN HUMAN MOTOR CORTEX FOLLOWING AMPUTATIONS.

LG Cohen*, J Brasil-Neto, M Daum, T Findley, J Macedo, A Pascual-Leone and M Hallett, Human Cortical Physiology Unit, Human Motor control Section, NINDS, NIH, Bethesda, U.S.A.)

Following amputation, the human motor system reorganizes (Brain 114:615-627). To determine the site along the human neuroaxis where motor plasticity takes place, we recorded from muscles immediately proximal to the stump and the homonymous contralateral muscles in eight patients with lower limb amputations. Stimulation was delivered to motor cortex with a 9 cm magnetic coil (TMS) and electrically with surface electrodes (TES, n=5) optimally positioned for activation of each muscle. Long descending tracts were stimulated electrically (SES, n=5) at C7(cathode). Intensity of stimulation was ~120% of threshold. Supramaximal muscle responses were elicited by peripheral nerve stimulation.

TMS recruited larger responses and thresholds for activation were lower from muscles ipsilateral to the stump than from homonymous muscles in the normal side. These differences were larger in patients who had the amputation more than 1 year before testing. TES and SES evoked responses with amplitudes that were not significantly different on the two sides. These results show that a substantial percentage of motor reorganization occurs intracortically. Since this phenomenon is time dependent, it might involve establishment of new synaptic contacts in neuronal nets targeting corticospinal tract neurons.

616.8

ECHOPLANAR IMAGING (EPI) OF FUNCTIONAL REORGANIZATION IN HUMAN SENSORIMOTOR CORTEX AFTER TRANSIENT ISCHAEMIC DEAFFERENTATION OF THE FOREARM.

J. B. Fieldman[1], L. G. Cohen[1], P. Jezzard[2], T. Pons[3], N. Sadato[1], D. LeBihan[4], R. Turner[2], M. Hallett[1]*, HMCS/NINDS[1], LCE/NHLBI[2], LN/NIMH[3], and DRD/CC[4], NIH, Bethesda, MD 20892

Somatosensory cortical reorganization has been demonstrated following amputation. EPI permits noninvasive image acquisition in fractions of a second and, in conjunction with a long echo-time (TE), also allows visualization of changes in blood oxygenation secondary to neural activity. Using EPI, we examined the pattern of activation in cortical areas representating muscles proximal to a transiently deafferented limb in order to study the temporal changes in *size* of the areas activated as well as their *degree* of activation.

A pneumatic cuff was placed just distal to the elbow and inflated to 30% greater than the systolic blood pressure. Images were obtained prior to inflation, every 10 minutes during ischaemia, and 10 minutes following cuff deflation. At these times, subjects performed 3 30-second sets of self-paced elbow flexions separated by 30-second rest periods. Images were acquired using T2*-weighted gradient-echo EPI sequences with a whole-body 1.5T MRI system and small z-axis head gradient coil. High resolution whole brain images were taken using a conventional gradient-echo sequence for anatomical reference. Z-score images [(activation data)-(baseline data)/(standard deviation during rest phase)] were compared over time for any changes in areas activated by the motor task.

Regions of interest (ROIs) showing good temporal correlation with the paradigm were defined from the z-score images. These ROIs increased in area proportional to the length of ischaemia. Increases in area were apparent 10 min. after cuff inflation, being largest at 40 min., just prior to cuff deflation. Ten minutes after deflation, size of activated areas had not yet returned to baseline. For a given ROI, percentage increase in signal intensity relative to baseline increased with length of ischaemia, from an initial 1.5% with baseline elbow flexion to 6% at maximal ischaemia.

616.9

REORGANIZED CORTICAL BLOOD FLOW AND MAGNETIC STIMULATION MAPS FOLLOWING UPPER LIMB AMPUTATION IN MAN.

JJM Kew, MC Ridding, PN Leigh*, JC Rothwell, RSJ Frackowiak, DJ Brooks, MRC Cyclotron Unit, Hammersmith Hospital, London, UK; Department of Neurology, Institute of Psychiatry, London, UK; and MRC Human Movement and Balance Unit, Institute of Neurology, London, UK.

We used positron emission tomography (PET) and transcranial magnetic stimulation (TMS) mapping to determine whether loss of peripheral afferent input induces cortical reorganization in human upper limb amputees. Regional cerebral blood flow (rCBF) was measured in traumatic and congenital amputees at rest and during repetitive, paced, flexion-extension movements of the shoulder ipsi- and contralateral to the amputation. TMS was used to map the cortical representation of the deltoid muscle ipsi- and contralateral to the amputation at rest and during tonic voluntary contraction of the deltoid.

Comparison of the rCBF changes during shoulder movements showed more extensive activation of the contralateral sensorimotor cortex (SMC) during movement of the amputated limb in both groups of subjects. Abnormal activation of the contralateral superior parietal cortex was present in traumatic, but not congenital, amputees. In traumatic amputees, the stimulation map for the deltoid ipsilateral to the amputation was abnormally expanded at rest, but relatively normal in size during voluntary activation.

The results suggest that a more extensive population of SMC neurons are recruited during arm movement ipsilateral to an amputation. Abnormal activation of the superior parietal cortex seems only to occur following traumatic amputation and may be related to the 'phantom limb' phenomena experienced by these patients.

(Supported by the Medical Research Council)

616.10

SUBDIVISIONS OF THE ANTERIOR CINGULATE CORTEX IN MAN: RESPONSE SELECTION AND MOTOR OUTPUT.

R. Kawashima, T. Klingberg, B.T. O'Sullivan and P.E. Roland*. Lab. of Brain Research, Karolinska Institute, S10401 Stockholm, Sweden.

Part of the anterior cingulate cortex has been coined as an anterior attentional area. Under the hypothesis that this area is functionally heterogeneous, we measured the regional cerebral blood flow with ^{15}O-butanol and PET in subjects doing the following tasks. In three **go/no-go** tasks, stimuli in three sensory modalities were present simultaneously, but in one modality the stimulus changed. The subjects pressed a button each time the frequency decreased/light dimmed. The control was simultaneous presentation of constant stimuli. In **length discrimination** the subjects tactually discriminated the length of cylinders. The control was similar surface exploring movements (without cylinders). In the two **reaching** tasks the subjects, eyes closed, pointed to targets they had seen previously. The control was rest. We subtracted the brain images of control from the respective test images, and by a consistency analysis of these images it was apparent that all go/no-go tasks and length discrimination, but not reaching, activated a field located just below the left cingulate sulcus (Talairach 8, 37, 42). Since there were no fields related to movements in the length discrimination image, and because reaching did not activate, the common denominator for activation was response selection. In the cingulate sulcus (4, 22, 46) there was another field activated in reaching and go/no-go tasks, but not by length discrimination (motor components subtracted). The common denominator for activation of this field was hand and arm movements, and it may mark the location of a supplementary motor area.

616.11

REGIONAL CEREBRAL BLOOD FLOW CHANGES DURING UNIMANUAL AND SIMULTANEOUS BIMANUAL MOVEMENT. E.A.Franz*, T.A.Zeffiro and S.Y.Bookheimer Psychology Dept, UC Berkeley; MNB-NINDS, NIH, Bethesda, MD 20892.

It has been suggested that the motor system exhibits regional specialization with regard to bilateral movement control, with the supplementary motor area (SMA) and putamen playing central roles. We investigated the neuroanatomical localization of these processes with positron emission tomography.

rCBF changes were recorded using bolus injection of $H_2{}^{15}O$ in 8 subjects performing a metronome-paced sequential finger opposition task with left, right, or both hands. Scans were spatially standardized to a common coordinate system and statistical parametric maps were generated by making planned comparisons of task and control conditions.

rCBF increases were seen in primary motor cortex, cingulate motor area, ventral premotor area, SMA, cerebellar nuclei and cortex, and putamen in relation to unilateral movement of either hand. The left-right asymmetry of the increase with respect to the hand used varied among regions, with the most asymmetry seen in primary motor cortex. Although no regions were active exclusively in relation to bimanual movement, regions more active during the bimanual than either unimanual task included the SMA, the cerebellar nuclei and the putamen, suggesting a special role for these areas in the planning or execution of simultaneous bilateral movement.

616.12

BILATERAL ACTIVATION OF THE HUMAN SOMATOMOTOR CORTEX BY DISTAL HAND MOVEMENTS. R. Hari*, R. Salmelin, N. Forss, J. Knuutila. Low Temp. Lab., Helsinki Univ. of Technology, 02150 Espoo, Finland.

We used a 122-channel whole-head neuromagnetometer to study cortical areas activated in relation to distal hand movements. Six normal human subjects performed self-paced index finger flexions, wrist flexions, and a sequence of complex digit movements in different sessions. The magnetic signals were averaged from a button press. High-amplitude signals were observed over the somatomotor hand areas within 100 ms from the button press. The sources were modelled with current dipoles, one in each somatomotor hand area. Index finger movements were associated with purely contralateral activation. However, wrist flexions and skilled finger movements evoked additional intense ipsilateral activity. The movements were also associated with dampening of spontaneous activity starting 1 s before the button press and a rebound within 1 s afterwards. The reaction was much stronger over the contralateral hemisphere but was also seen over the ipsilateral side. Some distal hand movements thus seem to be associated with bilateral activation of the human somatomotor cortex.

DRUGS OF ABUSE: COCAINE

617.1

INTERACTIVE EFFECTS OF PRENATAL COCAINE AND NICOTINE EXPOSURE ON POSTNATAL DEVELOPMENT AND BEHAVIOR IN THE OFFSPRING. S.K. Sobrian,[1*], K. Hodge[1], P. Webb[2], F. Racey[2], S. Ali[2], W. Slikker, Jr.[2], and R.R. Holson[2]. [1]Department of Pharmacology, Howard University College of Medicine, Washington, DC 20059 and [2]Divisions of Reproductive and Developmental Toxicology and Neurotoxicology, National Center for Toxicological Research, Jefferson, AR 72079.

As prenatal exposure to either cocaine (C) or nicotine (N) has been shown to alter maternal variables and induce a variety of subtle changes in the offspring, the present study investigated the possibility of synergistic effects of co-administration on offspring development. On gestation days 8-21, Sprague Dawley rats were exposed daily to either 5.0 mg/kg of N (NS), 20 mg/kg of C (CS), or both N (5.0 mg/kg) and C (20 mg/kg) (NC). N was administered by osmotic minipump and C by sc injection. Saline injected dams fitted with saline-filled pumps (SS), and untreated dams pair-fed (PF) to NC females served as controls. Maternal toxicity was not observed in any of the groups. Offsprings' body weight during the first four postnatal weeks were unaltered by prenatal drug exposure; however the development of surface righting was delayed in CS pups. Moreover, only CS offspring exhibited alterations in non-drugged and drug-challenged behaviors. CS offspring were under-responsive to the stimulatory effects of apomorphine; both activity and stereotypy were reduced. Behavioral responses to C and N challenge were similar in all groups. In contrast, a significantly larger number of CS offspring responded on both trials of an alternation task. Two measures of non-drugged activity, tested at 3 and 8 weeks of age revealed no prenatal treatment effect. Treatment effects on dopamine D_1 and D_2 binding in the caudate were not observed. Prenatal exposure to N alone did not alter any of the behaviors tested; moreover, the combination of N and C did not exacerbate any of the behavioral changes seen in CS offspring. These results support the hypothesis that C is a behavioral teratogen in rats.

617.2

THE EFFECT OF IN UTERO COCAINE AND AMPHETAMINE ON STRIATAL NEUROTROPHIC ACTIVITY. C.M. Buhrfiend*, B.W. Santi, D.K. Sierens, and P.M. Carvey. Dept. Neurology, Rush-Presbyterian-St.Luke's Med. Ctr., Chicago,IL 60612.

We have previously shown that in utero cocaine (COC; 30 mg/kg/day) exposure during E8-15 in rabbits decreased striatal DA and striatal-derived neurotrophic activity. In order to determine if these results were the result of the DA agonist properties of cocaine or generalized vasoconstriction, we treated gravid females with COC (30 mg/kg b.i.d., s.c.), amphetamine (AMPH; 5 mg/kg/day), haloperidol (HAL; 1.5 mg/kg/day), HAL+AMPH, or vehicle (VEH) during E8-21. The rat pups were sacrificed on P14, P28, and P56, perfused, and the brains removed. Striatal tissue punches were evaluated for DA using HPLC and the remainder of the striatum was dissected, homogenized in Hanks Balanced Salt Solution (HBSS), centrifuged, and the supernatant extracts added to freshly plated primary, dissociated, rostral mesencephalic tegmentum (RMT) tissue cultures at equivalent protein concentrations. The number of cells with processes were assessed 40 hours later as an index of striatal trophic activity.

Striatal DA content in COC pups was reduced at P14 by 51% but was elevated c. 50% at both 1 and 2 months. In contrast, in utero AMPH exposure did not significantly affect striatal DA. Striatal trophic activity at 2 weeks was very low in all animals and similar to that observed in the cerebellum such that trophic assessments were not valid. By 1 and 2 months however, striatal trophic activity in control animals was elevated by 43 and 39% respectively, relative to COC exposed animals ($p<0.05$;p=N.S.). Trophic activity in AMPH exposed animals at 1 and 2 months was reduced 39 and 63% respectively ($p<0.05$ for both). Of perhaps greater importance was the observation that the DA antagonist HAL, did not prevent the amphetamine induced decreases in striatal trophic activity. These preliminary data suggest that reductions in striatal trophic activity accompany in utero cocaine and amphetamine exposure and are not necessarily related to alterations in DA. Since HAL did not prevent the AMPH-induced trophic reductions, it is likely that the trophic alterations are the result of the vasoconstrictive effects of AMPH and not its DA agonist properties.

617.3

PRENATAL EXPOSURE TO COCAINE DECREASES SENSITIZATION TO COCAINE IN ADULT MALE RATS. R.F. McGivern* & M. Hutcheson. Dept. Psychology, San Diego State Univ., San Diego CA 92182.

Multiparous time-pregnant Sprague-Dawley dams (Charles River, Portage MI) were injected daily with cocaine HCL (3.3, 10.0 or 30.0 mg/kg, sc) at 0800 and 1630 hrs from days 14-21 of gestation; control dams were injected with saline or left undisturbed (7-8 dams/treatment group). Offspring were weaned at 24 days of age and group housed by sex and treatment. At approximately 120 days of age, male litter representatives were injected with cocaine (10 mg/kg, ip) or saline for 8 consecutive days 5 minutes prior to being placed in a 12" diameter open field for 60 min. On day 14, 6 days after the last daily injection, both saline and cocaine injected animals were injected with cocaine (10 mg/kg, ip) prior to being placed into the same arenas. Behavior was recorded on videotape on days 1,8 and 14, and scored by blinded experimenters. Results revealed that males exposed to 3.3 or 30 mk/kg of cocaine prenatally were insensitive to the stimulatory effect of the drug on locomotor activity on day 1, while locomotor activity of animals exposed to 10 mg/kg increased similar to controls. 30 mk/kg animals remained insensitive on day 8 and were also insensitive to the stimulatory effect of cocaine on rearing behavior on days 1 and 8. 10 & 30 mg/kg saline injected males exhibited significantly less habituation from days 1-8 than controls. Sensitization was observed on day 14 in controls with respect to stereotypy when cocaine injected animals were compared with animals previously injected with saline. No sensitization was observed in animals exposed to cocaine prenatally. Prenatal cocaine exposure has been reported to increase striatal D2 receptor binding in the rat (Scalzo et al., 1990), results which may be implicated in the response to cocaine in our males exposed prenatally to 30 mg/kg. However, our overall behavioral results indicate that cocaine has differential actions on the developing male brain which are non-linear and dose dependent, perhaps reflecting the drug's anesthetic vs reuptake blockade properties. (Supported by NIDA, DA-04490).

617.4

COCAINE-INDUCED HYPOTENSION AND BRADYCARDIA ARE MEDIATED BY CNS BETA-ADRENERGIC RECEPTORS. R.A. Gillis, H.K. Erzouki, and Y.M. Hernandez* Georgetown Univ. Med. Ctr., Washington, DC 20007.

We have shown that cocaine (COC) administered into the hindbrain via the vertebral artery (VA) decreases cardiac sympathetic nerve activity, mean arterial pressure (MAP) and heart rate (HR) and that these effects are not due to a local anesthetic action of the drug (JPET 257: 511, 1991). Presently, we investigated the possibility that COC's inhibitory effects are due to blockade of catecholamine reuptake. The effects of the beta-adrenoceptor blocker, propranolol (PR), on cocaine-induced hypotension and bradycardia were examined in 7 alpha-chloralose anesthetized cats. COC (1 mg) was injected into the VA 60 min before and 5 min after VA administration of PR (0.05 mg). PR blocked the decrease in MAP produced by COC (MAP decreased -44±8 mm Hg, $p<0.05$, before PR and -9±5 mm Hg after PR) and attenuated COC's decrease in HR (-48±22 beats/min before and -13±2 beats/min after PR). Injection of COC 90 min after PR administration produced decreases in MAP (-43±11 mm Hg, $p<0.05$) and HR (-52±20 beats/min, $p<0.05$) that were almost identical to those seen prior to PR. PR alone produced a small decrease in HR (-11±3 beats/min, $p<0.05$) with no change in MAP. I.v. administration of the same dose of PR (0.05 mg) did not affect the hypotension elicited by COC in the VA. To determine whether the COC-induced decrease in HR was due to excitation of vagal outflow, 5 cats were pretreated with the peripheral muscarinic blocker, propantheline (PTH). PTH, 0.5 mg/kg, iv, completely antagonized the decrease in HR elicited by COC (1 mg) in the VA. These data suggest that COC blocks the reuptake of catecholamines in the hindbrain and that the excess catecholamines act on beta-adrenoceptors to decrease sympathetic outflow and increase parasympathetic outflow.

617.5

REDUCED VTA DOPAMINE TRANSPORTER mRNA OCCURS AFTER CESSATION OF CHRONIC COCAINE ADMINISTRATION. C. Cerruti*, N.S. Pilotte, G.Ubl, and M.J. Kuhar. Neuroscience Branch NIH-NIDA, Balto., MD 21224.

Administration of cocaine to Lewis rats for two weeks using a schedule of intermittent delivery that mimics self-administration patterns results in a decrease in dopamine transporter (DAT) binding 10 days but not 1 or 3 days after cessation of treatment. The decrease in binding occurs in the nucleus accumbens (NAC) but not in the striatum, and is long lasting. We carried out in situ hybridization studies with a probe for DAT utilizing tissue prepared in the same manner as described for DAT binding studies. Animals were treated with cocaine or saline and then withdrawn from treatment for 10 days. In the ventral midbrains of saline-treated animals, highest mRNA hybridization grain densities were found in cells in the substantia nigra pars compacta, low grain densities were found in the interfascicular (IF) and caudal linear (CL) nuclei, and intermediate levels were found over cells in the paranigral, rostrolinear and parabrachial nuclei. In cocaine-treated animals DAT mRNA hybridization grain densities were significantly lower only in IF and CL, nuclei that project to NAC rather than striatum. This reduction in DAT gene expression corresponded to the reduction in DAT binding in NAC. The number of cells labeled per section did not change in these nuclei. The data indicate that gene expression can be altered after cessation of cocaine treatment. Such changes might conceivably contribute to craving and relapse to cocaine use during periods of abstinence from cocaine.

617.6

HIGH AFFINITY PROBES FOR COCAINE RECOGNITION SITES ON THE DOPAMINE TRANSPORTER. L. M. Gracz*, B.K. Madras, R. Hanson, D. Elmaleh, P. Meltzer. Harvard Medical School, New England Reg. Primate Res. Ctr., Southborough, MA, 01722, Massachusetts General Hospital, Boston, Organix, Inc., Woburn, MA.

Accumulating evidence suggests that cocaine recognition sites on the dopamine transporter are important mediators of the behavioral effects cocaine. In order to improve the sensitivity of probes for these sites, three high affinity ligands for the dopamine transporter were radiolabeled and characterized in caudate-putamen of cynomolgus monkeys. [^{3}H]Lu 19-005, a phenylindanamine, was approximately > 300 times more potent than [^{3}H]cocaine for labeling sites on the dopamine transporter. The novel cocaine congener [^{3}H]CDCT, one of the most potent drugs yet developed, also bound to these sites in the picomolar range. [^{125}I]IACFT, A novel high affinity iodinated probe distributed primarily to dopamine-rich brain regions following i.v. administration. Of these ligands, IACFT is the most selective for the dopamine over the serotonin transporter. Low densities of the dopamine transporter may be detected with these compounds. DA06303, MH14275, DA00499, RR00168.

617.7

CHRONIC COCAINE ADMINISTRATION IS ASSOCIATED WITH BEHAVIORAL SENSITIZATION AND DOPAMINE TRANSPORTER UPREGULATION. L.G. Miller*, J.M. Koff, L. Shuster. Dept. of Pharmacology and Exp. Therapeutics, Tufts Univ. School of Medicine, Boston, MA

Chronic cocaine administration has been associated with sensitization, in increase in drug effect, in contrast to the tolerance observed with many psychotropic compounds. Since cocaine acts at the presynaptic dopamine transporter, we evaluated sensitization and dopamine transporter binding in vivo in several mouse strains. All strains of mice evaluated showed increased activity after cocaine compared to saline injections. BALB/cByJ, DBA/2J and C57BL/6J mice exhibited sensitization after 5 daily injections of cocaine at 20 and 40 mg/kg/d, whereas B6AF1/J mice showed sensitization at 20 but not 40 mg/kg/d. CD1 mice did not exhibit sensitization at either dose. Dopamine transporter binding in vivo was increased in DBA/2J and B6AF1/J mice after 5 injections of cocaine, 40 mg/kg/d but not in the other strains evaluated. In contrast, continuous infusion of cocaine at the same daily dose and duration odid not produce sensitization or binding chances in DBA/2J mice. The time course of transporter binding alterations after intermittent cocaine exposure indicated no change at day 1, increased binding at 3 days, a return to control levels at 7 days, and decreased binding at 14 days. These data indicate that both sensitization and alterations in dopamine transporter binding occur after chronic cocaine injections, but these thances are unlikely to be directly related.

617.8

SELECTIVE LABELING OF THE STRIATAL AND CEREBRAL CORTICAL DOPAMINE TRANSPORTER IN RAT AND HUMAN BRAIN BY THE COCAINE ANALOG [^{125}I]RTI-121. J. W. Boja*, T. Kopajtic, F.I. Carroll, H.H. Seltzman, C.D. Wyrick, J. Lever, J. Staley, D.C. Mash and M.J. Kuhar. Molecular Pharmacology Section, NIH-NIDA Addiction Res Center, Baltimore, MD 21224.

The cocaine analog RTI-121 has previously been shown to be very selective for the dopamine transporter (Boja et al., Neuroreport 1992). [^{125}I]RTI-121 bound to both a high (0.2 nM) and low affinity site (1.93 nM) with a density consistent with that of the dopamine transporter. The pharmacological profile of specific [^{125}I]RTI-121 binding was also consistent with that of the dopamine transporter. In the cerebral cortex [^{125}I]RTI-121 bound to both a high and low affinity site with affinities similar to that demonstrated in the striatum, however at a much lower density. Displacement of specific [^{125}I]RTI-121 binding by various pharmacologic agents also demonstrated a profile consistent with the DAT. Autoradiographic studies in the rat brain demonstrated intense labeling of the striatum, nucleus accumbens and olfactory tubercle. The substantia nigra was also labeled, but at a lower intensity. This is in contrast to the pattern of labeling of the dopamine and serotonin rich areas that is demonstrated when [^{125}I]RTI-55 ([^{125}I]β-CIT) is used as the ligand. [^{125}I]RTI-121 bound to both a high and low affinity site in the human caudate with affinities similar to that demonstrated in the rat brain. These results demonstrate that [^{125}I]RTI-121 with its high affinity and selectivity is a superior ligand for the DAT.

617.9

REPEATED BOUTS OF CONTINUOUS COCAINE INDUCE PROGRESSIVELY CHANGING PATTERNS OF AXONAL DEGENERATION AND BEHAVIOR. G. D. ELLISON*, S. IRWIN, AND G. SULUR. Department of Psychology, UCLA, 405 Hilgard Ave., Los Angeles, CA 90024

We recently reported that rats given 3 to 5 days of either continuous amphetamine or cocaine showed a strong pattern of silver-stained degenerating axons in lateral habenula (LH) and fasciculus retroflexus (FR). Daily injections of these drugs did not induce degeneration. In order to determine whether subsequent bouts of continuous cocaine would continue to induce this effect, rats were implanted with slow-release cocaine pellets for 5 days and then sacrificed, or were then given either a 10 day or a 3 month recovery period and then a second 5 day cocaine bout and then sacrificed. Behavior was monitored every 3 hours round the clock throughout the 5 day period of drug administration.

During the first cocaine bout, the rats showed initial motor stereotypies which waned at about day 3 as other behaviors, including parasitotic grooming, emerged. With repeated bouts both of these stages were exaggerated, with striking parasitotic grooming appearing in the 3 month animals. The number of silver-stained axons in LH and FR following the second pellet was not reduced but was generally greater than following the first pellet. This suggests a similar potentiation of neurotoxicity and "late-stage" behaviors with repeated cocaine bouts.

617.10

MOLECULAR AND PHARMACOLOGIC SPECIFICITY OF STRIATAL c-FOS ACTIVATION BY COCAINE IN JUVENILE RATS. Lisa M. Genova, Barry E. Kosofsky, Kathleen Gogas*, and Steven E. Hyman. Laboratory of Molecular and Developmental Neuroscience, Massachusetts General Hospital, Harvard Medical School, Boston, MA, 02129.

Cocaine acts acutely to potentiate dopaminergic neurotransmission in the striatum. In Situ hybridization was used to characterize mRNA localization of various neuropeptides and receptors present in neurons that are activated by an acute dose of cocaine. The immediate early gene, c-fos, was used as a marker of cocaine-induced neuronal activation. We hybridized 35S-labelled c-fos cRNA and non-radioactive, digoxigenin-labelled D1, D2, Substance P, or Enkephalin cRNA on the same sections. Cocaine (30 mg/kg/ip) administration 45 min prior to sacrifice activated predominately D1 rather than D2 striatal neurons. Almost all cells containing D1 receptor mRNA expressed c-fos mRNA, whereas c-fos and D2 mRNA were infrequently colocalized. In adjacent sections, Substance P mRNA was colocalized in striatal neurons that were simultaneously expressing c-fos mRNA, whereas striatal neurons containing Enkephalin mRNA seldom demonstrated c-fos induction. Rats treated with saline (vehicle control) displayed no detectable striatal c-fos mRNA.

Cocaine's induction of striatal c-fos was mostly abolished by pretreatment (30 min before cocaine) with SCH23390 (.5 mg/kg/ip), a D1 antagonist. In these animals, there was exclusive activation of a small subset of cells confined to the dorsolateral striatum. In Situ analyses of sections labelled for c-fos and D2 mRNA or c-fos and D1 mRNA revealed that the dorsolateral striatal cells activated by SCH23390/cocaine pretreatment contained D2 and not D1 mRNA. These experiments suggest that the indirect aminergic agonist cocaine activates striatal neurons that contain predominantly Substance P and D1 mRNA and that cell activation is mediated almost entirely by a D1 mechanism.

617.11

CELLULAR AND DEVELOPMENTAL CORRELATES OF NEURONAL ACTIVATION BY COCAINE IN RAT STRIATUM. Barry E. Kosofsky*, Lisa M. Genova, and Steven E. Hyman. Laboratory of Molecular and Developmental Neuroscience, Massachusetts General Hospital, Harvard Medical School, Boston, MA 02129.

We have utilized in situ hybridization (ISH) to define the phenotype of striatal neurons activated at acute timepoints (45 minutes) after cocaine administration (30mg/kg/ip) to rat pups of different ages (P8, P15, P28, and adults). The immediate early gene c-fos serves as an acute marker of striatal cell activation by cocaine. We combined 35S-labelled c-fos cRNA probes with nonradioactive, digoxigenin labelled cRNA probes (Dopamine receptors D1 or D2, or the Neuropeptides Substance P or Enkephalin), and performed double ISH. Striatal sections of naive, saline, and cocaine exposed pups from litters of each age were analyzed to determine the topographic distribution and cellular correlates of cocaine induced c-fos expression. At each age examined, c-fos positive striatal cells were predominantly colabelled with Substance P cRNA and D1 receptor cRNA. There were occasional Enkephalin or D2 receptor positive striatal neurons in which c-fos was induced by cocaine. Conversely, not all striatal neurons which expressed either Substance P mRNA or D1 receptor mRNA demonstrated cocaine induced c-fos activation. The phenotype of activated striatal neurons was consistent across all postnatal ages examined. However, the topography of activated cells at each age varied with c-fos+/ Substance P+ and c-fos+/ D1+ cells restricted to medial predominant clusters on P8, a homogeneous lateral predominance on P15, and dorsocentral striatal predominance on P28 and in adults. These findings suggest that at all postnatal ages examined Substance P containing, D1 receptor bearing neurons comprise the majority of striatal cells induced by cocaine to express c-fos. Additional organizational features, with topographic and temporal specificity (perhaps extrinsic connections) may define which subset of Substance P+ or D1+ neurons are activated at each postnatal age.

617.12

COCAINE-INDUCED CONDITIONED TASTE AVERSIONS: COMPARISONS IN LEW/N & F344/N RATS. J. R. Glowa*, A. E. Shaw, and A. L. Riley. Laboratory of Medicinal Chemistry/NIDDK, National Institutes of Health, Bethesda, MD 20892 and Dept. of Psychology, The American University, Washington, DC 20016.

Differences in self-administration behavior between LEW/N and F344/N rats suggest a genetic predisposition toward drug abuse. The current study compared doses of cocaine required to produce a conditioned taste aversion (CTA) in these strains. Fluid, either a 0.1% saccharin solution, or water, was available during 20-min daily sessions. Saccharin sessions were always followed by a dose (0-50 mg/kg, sc.) of cocaine, and separated by three water sessions. Fluid consumption was assessed over four saccharin sessions. Vehicle had no effect on saccharin consumption. In contrast, when cocaine followed saccharin, rates of consumption decreased over successive saccharin sessions in a dose-related manner in both strains. The lowest dose (18 mg/kg) decreased consumption in LEW/N rats but not in F344/N rats. An intermediate dose (32 mg/kg) decreased consumption maximally in LEW/N rats, and only marginally in F344/N rats. The highest dose (50 mg/kg) decreased consumption completely in LEW/N rats, and almost completely in F344/N rats. The enhanced sensitivity of the LEW/N rat to the noxious effects of cocaine extends various behavioral and biochemical differences in effects of drugs of abuse in these strains. However, the increased sensitivity of the LEW/N rat to a noxious effect of a drug of abuse may suggest that this strain does not exhibit a genetic predisposition to factors related only to the abuse potential of drugs, to the extent that reinforcing and noxious effects differ.

VISUAL CORTEX: EXTRASTRIATE—FUNCTIONAL ARCHITECTURE

618.1

ANALAYSIS OF RETINOTOPY IN OWL MONKEY EXTRASTRIATE CORTEX BY VISUAL FIELDSIGN. M.I. Sereno*, C.T. McDonald, M. O'Dell, and J.M. Allman. Cognitive Science Department, University of California, San Diego, La Jolla, CA 92093 and Division of Biology 216-76, California Institute of Technology, Pasadena, CA 92115.

Cortical visual areas can be divided into those with a mirror-image transformation of the visual field (e.g., V1, VP) and those with a non-mirror-image transformation of the visual field (e.g., V2, MT) (when viewed from the cortical surface). With densely sampled retinotopy, the local visual fieldsign (mirror vs. non-mirror) can be calculated from the angle, ϕ, between the direction of the gradients in eccentricity, r, and angle, θ, of the receptive field centers (r and θ are measured relative to the center of gaze). This measure was used to subdivide owl monkey visual cortex into regions that were then correlated with myeloarchitecture.

Detailed retinotopic maps (250-600 closely spaced penetrations) were obtained from the parietal and occipitotemporal cortices of 6 anesthetized owl monkeys. At the end of each experiment, the cortex was physically flat-mounted, sectioned parallel to cortical laminae, and stained with the Gallyas technique. The penetration map digitized from the cortical surface photograph was warped to align it with the final location of a set of marker lesions in the flattened cortex using a deformable template algorithm. Eccentricity and angle data were then interpolated onto uniform grids that were used to compute a fieldsign map.

V1, V2, and MT stand out as large expanses of uniform fieldsign. The areas in between show a more complex organization. There are three strips of cortex representing the lower visual field with alternating fieldsign that generally correspond to our previously described DLp (mirror-image), DLi (non-mirror-image), and DLa (mirror-image). The topography of these areas is much less regular than that of MT, with many interdigitating fingers; and DLp contains a small upper field representation. In the region previously labeled DI, we found an upper field representation with the same fieldsign as the directly adjoining lower field V2; the lower field of V2 continues rostrally into the upper field of DI without a discontinuity. Area DM contains both lower and upper fields and it is situated medial to DI. Many of the details of the fieldsign map are correlated with subtle changes in the pattern of myelination. The fieldsign technique brings out a feature of cortical retinotopic maps that is difficult to see in raw receptive field plots; the resulting picture of retinotopy was more complex than expected.

618.2

VISUAL FIELD REPRESENTATION AND FUNCTIONAL COMPARTMENTS WITHIN SINGLE V2 STRIPES A.W. Roe* and D.Y. Ts'o. Division of Neuroscience, Baylor College of Medicine, Houston, TX 77030

The second visual cortical area, V2, of primates has been shown to contain a stripe-like organization. The canonical view is that there are three sets of functionally distinct stripes, subserving color, form and depth/motion. We have found that within single V2 stripes, however, the distribution of functional properties is far from uniform and homogeneous. Optical imaging and electrophysiological experiments show multiple distinct clusters or patches of various functional properties within individual V2 stripes. For example, within a single V2 stripe nominally identified as "thin", separate regions of color cells, luminance cells and even disparity cells may be found.

We have also examined the representation of the visual field within such single stripes, by making single tangential electrode penetrations along individual stripes. Our previous studies of the visual map in V2 have shown that across the stripes in V2, the visual field is multiply represented and discontinuous. Along a stripe, we also find such discontinuities at the boundaries between functionally distinct subcompartments. Our preliminary findings suggest that even within a single stripe, the parameters of visual field coverage, such as receptive field size and scatter are different within each functional subcompartment. These results suggest that the parcellation of visual processing, in terms of function and visual field representation is more complex than the original notion of only three distinct stripe compartments within area V2.

(Supported by grants EY06347, EY08240, ONR N00014-91-J-1865 and the McKnight Foundation).

618.3

FUNCTIONAL CONNECTIVITY WITHIN V1 AND V2: PATTERNS AND DYNAMICS D.Y. Ts'o*, A.W. Roe and J.Shey. Division of Neuroscience, Baylor College of Medicine, Houston, TX 77030

We have studied the rules of functional connectivity within V1 and V2 by using cross-correlation analysis, guided by optical imaging maps of cortical organization. One issue that we have examined is the varying degrees of divergence and convergence of cortical connectivity. In some cases, interactions were found between V1 - V1 pairs and V1 - V2 pairs which had nonoverlapping receptive fields, separated by several receptive field diameters. However, V1 - V2 cross-correlogram peaks tended to be much broader than V1 - V1 peaks, suggesting a temporal dispersion perhaps due to a much greater degree of divergence and convergence in the connections of V2 cells. Particular pairings varied greatly in the dependence of the interactions on receptive field overlap, suggesting that different functional compartments have differing degrees of divergence and convergence.

Correlations also were observed to change with state. One factor that can govern such changes is the nature of visual stimulus. For example, among color cells, the type of color stimuli influenced the shape and strength of the correlogram. An analysis of the change in correlation over time also demonstrated substantial changes in the form and strength of correlograms. One such behavior was a gradual strengthening of the correlogram over time during repeated visual stimulation. Since in general the average firing rates of the cells did not alter substantially, these changes may reflect a modulation of synaptic efficacy or coupling. While each functional compartment exhibits a characteristic pattern of functional connectivity, this connectivity may be subject to state-dependent influences.

(Supported by grants EY06347, EY08240, ONR N00014-91-J-1865 and the McKnight Foundation).

618.4

PATCHY INTRINSIC CONNECTIONS FOLLOW ISO-ORIENTATION SITES IN CAT VISUAL CORTICAL AREA 18

Z.F.Kisvárday[1*], D.-S.Kim[2], U.T.Eysel[1], T.Bonhoeffer[2#] [1]*Dept.Neurophysiol. Ruhr-Univ.Bochum, Bochum PO Box 102148, F.R.G.* [2]*MPI Brain Res.,Frankfurt PO Box 710662,F.R.G.* #*Present address: MPI Psych. 8033 München-Martinsried, F.R.G.*

A general concept in the functional organization of the cerebral cortex is that lateral patchy connections link groups of neurons possessing similar functional properties(i). Interestingly, experimental data obtained in area 18 violated this concept in that neuronal groups with dissimilar orientation preferences were connected(ii). In order to know whether area 18 indeed represents a radically different organization scheme to that of area 17 we revisited the area 18 connections using a combination of optical imaging and iontophoretic injection of biocytin. Optical imaging provided orientation maps over 12-23 mm^2 regions. Biocytin labelling was analysed in series of horizontal sections. In two cats, axonal terminals of anterogradely and of retrogradely labelled fibres were drawn from the entire depth of the cortex. Then a mesh-grid containing the same pixel-size as the optical image was overlaid the entire boutonal map of each section in precise register. For aligning between the optical and the anatomical maps 5-8 identified electrode penetration marks were used. The number of labelled terminals was counted in each pixel and a frequency distribution according to different orientation values was calculated. The results show that all of the remote patches coexisted with sites showing statistically similar orientation preferences to that of the injection site. Thus according to our results the long-range excitatory system of area 18 follows the organization principle of area 17.

Z.F.K. and U.T.E are supported by the Deutsche Forschungsgemeinschaft.

(i) Gilbert CD and Wiesel TN (1989) J.Neurosci. 9:2432-2442.

(ii) Matsubara et al (1985) Proc.Natl.Acad.Sci.(USA) 82:935-939.

618.5

OPTICAL IMAGING OF ORIENTATION AND BINOCULARITY IN VISUAL AREAS 1 AND 2 OF SQUIRREL MONKEY (*SAMIRI SCIUREUS*) CORTEX. G. Blasdel, M. Livingstone, and D. Hubel*, Department of Neurobiology, Harvard Medical School, Boston, MA 02115.

We used optical imaging combined with single unit recording to study the organization of orientation preferences and binocularity in visual areas 1 and 2 (V-1 and V-2) in squirrel monkeys whose lissencephalic cortex offers an obvious advantage. Single unit recordings were used to verify orientation maps obtained with optical imaging. In V-1 of these animals we found that orientation preferences repeated with a period of approximately 460 μm, compared with periods of 640 and 760 μm that are observed parallel and perpendicular to the ocular dominance columns in macaques. In V-2 orientation preferences repeated every 1.26 mm in directions parallel to the stripes of intense cytochrome oxidase staining that run perpendicular to the border between V-1 and V-2. Differential images of orientation, generated by subtracting images of responses to orthogonal orientations, were maximal along the edges of the thick cytochrome oxidase stripes, but were less pronounced along their centers as well as in the pale and thin stripes. Differential images of binocularity, obtained by subtracting responses to monocular stimulation from responses to binocular stimulation, showed single, narrow (370μm), dark bands running down the centers of the thick stripes, adjacent to one or two paler bands. These results thus indicate a substructure of the thick stripes that is not apparent from CO staining. In single unit recordings from the centers of the thick stripes we found cells that were selective for binocular disparity, and that were often unresponsive or poorly responsive to monocular stimulation. These cells were clustered in groups that preferred near, far, and zero disparity. (Supported by EY05403 and the Office of Naval Research)

618.6

COMPARING MAPS OF FUNCTIONAL ARCHITECTURE OBTAINED BY OPTICAL IMAGING OF INTRINSIC SIGNALS TO MAPS AND DYNAMIC PATTERNS OF CORTICAL ACTIVITY RECORDED WITH VOLTAGE-SENSITIVE DYES. D. Shoham*, Z. Gottesfeld and A. Grinvald. Dept. of Neurobiology, Weizmann Institute of Science, Rehovot 76100, ISRAEL.

In previous years our group has used real-time optical imaging utilizing voltage-sensitive dyes to study the spatio-temporal organization of the activity of large neuronal populations in the visual cortex. In these earlier studies we used an array of 12x12 photodiodes to image the cortical activity in real-time at a high sampling rate (up to 1.5kHz). An additional imaging technique based on activity-dependent intrinsic changes of cortical reflectance was used for complementary measurements – to image the functional architecture of the cortex at a high spatial resolution. These two methods were never applied in the same experiment.

Recently we have developed a new imaging system that allows both high spatial resolution (64x64 detectors) and high temporal resolution (up to 2kHz), with a signal to noise ratio of about 3000:1. We are currently using this system to combine for the first time the two imaging techniques in a single experiment, on the same patch of cortex. In the first set of experiments using this combined approach we produced orientation maps of cat areas 17 and 18. The spatial maps obtained with the two methods were basically the same. However, when voltage-sensitive dyes were applied we could see not only the static architecture but also the temporal structure of the response, including for example its on and off components. These preliminary results demonstrate the power of using a fast, high-resolution camera and combining the two optical imaging techniques to get detailed measurements of both the spatial and the temporal aspects of cortical activity.

618.7

RELATIONSHIPS BETWEEN FUNCTIONAL ORGANIZATION FOR DIRECTION OF MOTION AND FOR ORIENTATION SELECTIVITY IN CAT AREA 18. A. Shmuel, A. Arieli, D. Malonek and A. Grinvald*, Dept. of Neurobiology, Weizmann Inst., Rehovot, Israel 76100.

Previous electrophysiological studies in cat area 18 indicated that neurons preferring similar directions of motion are clustered together (Payne et. al, *Brain Res.* 1980; Tolhurst et. al., *Exp. Brain Res.* 1981; Swindale et. al., *J. Neurosci.* 1987). Optical imaging based on intrinsic signals suggested that such clustering is much weaker than that observed for orientation selectivity in area 18, if it exists at all (Bonhoeffer et. al., *J. Neurosci.* 1993). We improved the sensitivity of optical imaging, and here report the functional organization, parallel to the cortical surface, of direction of motion, and its relationship to that of orientation selectivity.

Achromatic rectangular wave gratings or random dot patterns, moving coherently in different directions, were presented binocularly. Single-condition functional maps for iso-orientation and iso-direction domains were generated using the standard procedure for obtaining optical maps from cortical images. In cat area 18 the iso-orientation domains activated by gratings moving in opposite directions exhibited a considerable overlap. However, closer analysis revealed a patchy distribution of cortical regions exhibiting preference for one direction of motion over the opposite direction. Thus, neurons exhibiting preference for a particular direction of motion are clustered, forming a mosaic of direction preference domains. We confirmed that orientation and direction selectivity are closely related. Each iso-orientation patch is divided into regions with opposite direction of motion preference. From the amplitude of the functional maps, we found that the clustering index (density) for orientation preference was 2-3 times larger than that observed for direction of motion preference. The organization for direction of motion preference revealed by random dot stimuli was nearly identical with that obtained from gratings stimuli.

618.8

OPTICAL IMAGING OF ORIENTATION, DIRECTION AND RETINOTOPIC ORGANIZATION IN AREA MT OF THE OWL MONKEY - *D. Malonek*, R.B.H. Tootell and A. Grinvald* Dept. of Neurobio., Weizmann Inst., Rehovot 76100, ISRAEL and Dept. of Neurobio., Harvard Med. School, Boston, Mass. 02115

Area MT (V5) is unique among cortical areas in that a large proportion of its neurons are directional selective. We have previously shown (Malonek et al. Neuroscience Abstracts 1992), using optical imaging, that in owl monkey area MT, populations of cells that have similar direction-of-motion preference are clustered together. In addition we showed that populations of cells that have similar *orientation* preference are also clustered, and that these orientation preference maps are 3-5 times stronger than the direction of motion preference maps .This pronounced organization for orientation, rather than axis-of-motion, preference led us to compare functional maps obtained with moving and stationary gratings.

Here we show that counterphase gratings activated the same regions as moving gratings but these stimuli were 4 times less effective than gratings moving at optimal velocity. We investigated further the relationship between functional organization for orientation and direction-of-motion. Orientation preference maps contain two types of pinwheels: 180° and 360°. These maps can be further subdivided in terms of their preference for direction-of-motion. These directionality maps showed that direction-of-motion preference changes smoothly across the cortical surface. However, several lines of discontinuity which separate opposite directions of motion preference were observed. We tested whether velocity was mapped across the cortical surface and found that maximal responses were obtained at velocities of about 10 °/sec but the overall organization for orientation and direction of motion preference was velocity independent. In addition we determined the retinotopic organization of area MT using stimuli that activated only parts of the visual field, and showed that a stimulus which subtends an elevation of ±5° around area centralis activated more than 70% area MT.

618.9

FUNCTIONAL MRI (fMRI) EVIDENCE FOR MT/V5 AND ASSOCIATED VISUAL CORTICAL AREAS IN MAN. R.B.H. Tootell*, K.K. Kwong, J.W. Belliveau, J.R. Baker, C.E. Stern, R.L. Savoy, H. Breiter, R. Born, R. Benson, T.J. Brady, B.R. Rosen. Harv. Med. Sch., Boston, MA 02115; MGH-NMR Ctr. 149 13th St., Charlestown, MA 02129; Rowland Inst., Cambridge, MA 02142

Presumptive area MT homologues (aka V5 in Old World primates) have been demonstrated in prosimians and New and Old World monkeys. PET data (e.g. Zeki et al., 1991) and preliminary anatomical data suggest that it exists in man as well. Here MT and other motion-responsive areas of human visual cortex were functionally charcterized using fMRI, and compared to macaque areas. Twelve subjects were scanned using gradient echo and control sequences in a 1.5 T EPI machine with surface coils. Five cortical areas were activated by moving stimuli (dot arrays or gratings): V1, V2, presumtive (p) pMT/V5, pMSTd, and pV3/V3a. Only pMT and pMST were highly selective for moving stimuli, as opposed to stationary stimuli during fixation. pMT is larger than pMSTd and both are located within the general region indicated previously in PET studies. Using moving square wave gratings of systematically varied contrast, contrast gain curves were obtained from each of the five areas within a given subject. Area V1 has a threshold of ~ 4%, and fMRI signal amplitudes increase monotonically at higher contrasts. Areas pMT, pMST and pV3 are much more sensitive to contrast than V1 (threshold < 1.5%, saturated above 5%): this is consistent with the magnocellular-stream dominance of their macaque namesakes, and with single unit evidence from macaque MT and V1 (Sclar et al., 1990). Supported by EY07890 to R.B.H.T. and MH50054 to J.W.B.

618.10

SEARCHING FOR STEREOPSIS IN HUMANS USING ULTRAFAST FUNCTIONAL MRI: STIMULI, ANALYSIS TECHNIQUES, AND PRELIMINARY DATA. **R.L.Savoy*, K.K.Kwong, M.S.Cohen. The Rowland Institute for Science, 100 Edwin H. Land Blvd, Cambridge, MA 02142; MGH-NMR Center, 149 13-th St.,Charlestown, MA 02129**

A variety of visual stimuli were designed that shared the common feature of using changing fields of random dots presented to the two eyes during both "control" and "experimental" periods. During control periods, the dots were the same for each eye; during the experimental periods the dots were different for the two eyes, so as to elicit images in depth (random dot stereograms) via free-fusion. Thus, V1 activation was expected and found during both periods. Differences between the two types of periods would necessarily be due to the images seen stereoscopically. Furthermore, any motion signals perceived monocularly would be comparable during both periods.

Data analysis programs were developed to automatically highlight the pixels within a given slice that showed significant stimulus locking. Also, by examining the relative variance at each pixel, the data analysis programs could detect heartbeat artefacts, even though the sampling frequency was below that which would permit straightforward frequency filtering.

Preliminary data (one subject, two experimental runs in the same experimental session, each run consisting of 3 pairs of control versus motion-in-depth, slices parallel to the calcarine fissure) revealed an area that showed a highly significant stimulus locked response variation. This area was in the same slice and position in both runs, and, based on its location, was clearly not the putative human V1 nor MT homologue.

618.11

STIMULUS DEPENDENT MRI SIGNALS EVOKED BY ORIENTED LINE-ELEMENT TEXTURES IN HUMAN VISUAL CORTEX A. Karni*[1], L.G. Ungerleider[1], J. Haxby[2], P. Jezzard[3], L. Pannier[4], C.A. Cuenod[4], R. Turner[3] and D. LeBihan[4] NIMH[1], NIA[2], NHLBI[3] & DRD-CC[4], NIH, Bethesda MD 20892.

A considerable number of neurons is devoted to the parallel extraction of the orientation of line elements. Here we use a set of simple textures - arrays composed of identical oriented line elements (0.5 deg. each) - to evoke event related changes in the local MRI signal (activation).

An echo planar imaging (EPI; TR = 3000ms; TE = 40ms) sequence and/or a conventional gradient echo (SPGR; TR = 60ms; TE = 40ms; flip angle 40) sequence were run on a 1.5T GE scanner. The stimuli were presented at a rate of 3Hz with element orientation alternating between horizontal and vertical. A consecutive baseline-test-baseline-test design was employed, with each interval 30 - 40 sec long, sampled (per slice) 6 - 12 times; 4 slices (2 - 4mm thick) were sampled in parallel. The observer's task was to fixate and discriminate small target letters at the center of display, ensuring consistent retinotopic mapping.

Results: I) For hemifield and quadrantic stimuli, a consistent, large activation (3 - 16% change, rise-time 6 - 9 sec) was measured by both sequences along the banks of the calcarine fissure (striate cortex) and on the convexity of the occipital lobe (extrastriate areas 18,19). II) A small texture target (6 line elements, subtending 1.5 x 2.0 deg. - presented at 5 - 7 deg. from fixation) induced a consistent, localized signal (2.4 - 6%) in both striate and extrastriate areas up to about 16 mm from the occipital pole. III) Preliminary data for a similar texture target embedded in a background of orthogonal line elements (compared to a homogeneous texture) showed consistent signals (2.4 - 6%, rise time 12 - 15 sec) evoked in the same locations as the target in (II).

Our results indicate that early stages of human visual cortical processing are strongly driven by oriented contours, including contours defined by local orientation discontinuities (gradients).

618.12

VISUAL FORM DETECTION IN MAN USING COLOUR AND MOTION CUES: FUNCTIONAL ANATOMY BY POSITRON EMISSION TOMOGRAPHY (PET). B. Gulyás[1]*, C. A. Heywood[2], D. Popplewell[2], P. E. Roland[1], and A. Cowey[2]. [1] Laboratory for Brain Research and Positron Emission Tomography, Nobel Institute of Neurophysiology, Karolinska Institute, Box 60400, S-104 01 Stockholm, Sweden, and [2] Department of Experimental Psychology, University of Oxford, Oxford OX1 3UD, South Parks Road, UK

With the purpose of mapping anatomical structures in the human brain participating in the processing and analysis of form information mediated by motion or colour cues, we measured regional cerebral blood flow (rCBF) changes in ten young male volunteers with PET, using 15O-butanol as tracer. During the measurements, the subjects performed two pairs of discri-mination tasks, each related to a specific and a reference task (form from motion, motion discrimination; form from colour, colour discrimination). The individual rCBF images were standardized in shape and size with the help of the Karolinska Institute's computerized brain atlas system. 'Specific task - reference task' subtraction (ΔrCBF) images were determined, which were then averaged across the subjects. The resulting images were analysed for statistically significant changes between specific and reference tasks.

The discrimination of form by means of motion cues activated regions bilaterally in the inferior and lateral occipital gyri, in the lingual, anterior cingulate, medial frontal and orbitofrontal gyri, and in the left fusiform and right inferior temporal gyrus. Form discrimination by colour cues resulted in activation bilaterally in the inferior temporal, lateral occipital, and orbito-frontal gyri, in the left precuneus and intraparietal sulcus, and in the right precentral gyrus. The regions activated in the two kinds of form discrimination did not overlap. The present findings suggest that the human brain can use different functional pathways in processing and analysing information resulting in the very same perceptual entity.

618.13

VISUAL SPATIAL COMPARISONS INVOLVE GYRUS CINGULI POSTERIOR: EVIDENCE FROM PET ACTIVATION STUDIES. P. Dupont[1], G.A. Orban, R. Vogels, A. Schoups*, G. Bormans[1] J. Nuyts[1] and L. Mortelmans[1]. Lab. Neuro- en Psychofysiologie, K.U.Leuven GHB, Med. School; [1]PET Center, Dept. of Nuclear Medicine, UZ GHB, B-3000 Leuven, Belgium

Local cerebral blood flow was measured with PET in 14 subjects performing six tasks. In a first triplet a grating was presented centrally and the subject had either to detect the occurrence (DET), identify the orientation as vertical or not (ID) or compare the orientation of two successive gratings (TSD). In the second triplet two gratings (a vertical and an oblique one) were presented simultaneously on the vertical meridian (1.9° ecc). The subjects' task was either to detect the occurrence (DDET), to identify the side of the vertical orientation (SID), or to compare the orientation of the two gratings (SSD). Despite the small orientation differences, the subjects performed the tasks well at a rate of 50 trials/min. Compared to the DET, ID and TSD activated mainly areas 18 and 19 in the right hemisphere. Compared to the DDET, SID and TSD activated areas 18, 19 and 7 bilaterally. Comparing SSD with SID yielded a significant activation of right posterior cingulate (area 31).

These data confirm the task dependency of visual processing and suggest that area 31 elaborates the message from area 7 to achieve spatial comparisons.

EXCITATORY AMINO ACIDS: EXCITOTOXICITY V

619.1

THE TIME COURSE OF GLUTAMATE-INDUCED NEURONAL DEATH. Janet M. Dubinsky* Dept. Physiology, Univ. Texas Health Science Center, San Antonio, TX 78238.

Glutamate excitoxicity has been viewed as a multicomponent insult involving both a rapid osmotic lysis following influx of Na^+, Ca^{2+}, and Cl^-, and a delayed calcium-mediated neuronal death. It has been widely assumed that the former mechanism operates initially, during exposure to excitatory amino acids while the later occurs over the course of the ensuing 24 hr. In order to study the mechanism of delayed toxicity, cell survival has been routinely assayed 24 hr after toxic insult. However, it is not known how quickly the neurons die or if the population declines synchronously, as might be expected from a simultaneously-triggered, calcium-mediated event. In this study, death among cultured hippocampal neurons was monitored by trypan blue cell counting at varying times in the 48 hr following a 5 min exposure to 500μM GLU. These time course measurements revealed that neuronal death occured continuously, with a high initial death rate that steadily declined with time. The rate of cell loss was proportional to the size of the surviving population or dependent upon some product released in proportion to cell number. The survival curve followed an exponential decline, suggesting that death occured in a stochastic fashion following the original insult. These experiments were supported by NIH grant AG10034.

619.2

THE QUANTITY OF Ca THAT ENTERS A NEURON TO CAUSE ITS DEATH IN GLUTAMATE TOXICITY.
M. Schramm* and S. Eimerl, Dept. Biological Chemistry, Hebrew University, Jerusalem 91904, Israel

Qualitative evidence shows that Ca mediates acute and delayed glutamate toxicity. However, the quantitative relationship, between Ca uptake and cell death has remained obscure. It has also been difficult to establish a quantitative correlation between toxicity and the rise in $[Ca^{2+}]i$ during exposure to glutamate. We have determined the total amount of ^{45}Ca entering cultured rat cerebral granule cells (9 DIV) during exposure to NMDA and glutamate, and measured resulting cell death 45 min later. The amounts of endogenous Ca have also been monitored. Plotting ^{45}Ca uptake against % cell death, the experimental points fit well on a linear regression line (r 0.85; $p < 0.001$) which hits the ^{45}Ca axis at 2 nmol/5 x 105 cells/40 µg protein (0% cell death). At 6 nmol ^{45}Ca uptake, cell death reached 30%. Results obtained with cells cultured for another week (16 DIV) fit a parallel regression line (r 0.88; $p < 0.001$), touching the ^{45}Ca axis at 0.75 nmol. Cell death reached 60% at 6 nmol ^{45}Ca uptake/5 x 10^5 cells (10 fmol ^{45}Ca /cell, about three times the endogenous Ca). The older cells thus demonstrate a higher toxicity, driven by the same amount of ^{45}Ca taken up. The calculated concentration of the total ^{45}Ca taken up, ≈15 mM, exceeds that measureable in Ca^{2+} imaging by 4 orders of magnitude. The procedures developed furnish quantitative information on the dynamic relationship between calcium and neurotoxicity.

619.3

INTRACELLULAR CALCIUM BUFFERING IS IMPAIRED IN FIBROBLASTS FROM PATIENTS WITH MITOCHONDRIAL DEFECTS. A.M. Moudy[a*], D.C. DeVivo[b] and S.M. Rothman[a]. Dept of Anatomy & Neurobiology[a], Washington Univ, St. Louis, MO 63110 & The Neurological Institute[b],New York, NY 10032.

Excitotoxic neuronal damage and cell death have been associated with toxic rises in intracellular calcium (Ca^{++}_i) levels. Recently, a number of investigators have speculated that nerve and muscle cells from patients with abnormal energy metabolism will be especially sensitive to excitotoxicity. We have now found that cultured fibroblasts obtained from patients with three mitochondrial disorders [MELAS syndrome; cytochrome oxidase deficiency (COX); and pyruvate dehydrogenase deficiency (PDH)] are unable to buffer Ca^{++}_i rises normally.

We induced Ca^{++}_i rises by abruptly depolarizing cultures with an extracellular solution containing 60 mM K^+ to trigger opening of voltage-gated Ca^{++} channels. In cultures loaded with the Ca^{++} sensitive dye fura-2, Ca^{++}_i was measured by ratioing fluorescence intensity produced by flashes of 340 and 380 nm light. In control cells exposed to high K^+, Ca^{++}_i increased transiently, then rapidly returned to baseline. The 340/380 fluorescence intensity ratio (**R**) at 20 min was not different from baseline (0.65 vs 0.64; p=.06, Wilcoxon; 99 cells of 8 patients). In MELAS fibroblasts, **R** increased from 0.89 at baseline to 1.01 at 20 min ($p<.001$; 83 cells of 2 patients). In COX fibroblasts, **R** changed from 0.94 to 1.06 ($p<.001$; 58 cells of 2 patients). In PDH cells, **R** rose from 1.02 to 1.06 ($p<.05$; 63 cells of 2 patients). Baseline and 20 min **R** values differed significantly between control and all disease groups ($p<.001$, Dunn). In toxicity studies, cells incubated for 24 hrs in high K^+ solution had >99% survival, suggesting that there is little, if any, acute cellular damage from these paradigms.

If Ca^{++} homeostasis is affected similarly in the nerves of patients with these disorders, this could explain the neuronal loss that characterizes a variety of inborn metabolic errors. Supported by NS19988.

619.4

AGE-DEPENDENT STRIATAL EXCITOTOXIC LESIONS PRODUCED BY THE ENDOGENOUS MITOCHONDRIAL INHIBITOR MALONATE. M. Flint Beal*, Emmanuel Brouillet, Bruce Jenkins, Ross Henshaw, Bruce Rosen and Bradley T. Hyman. Neurochemistry Laboratory, Neurology Service, and MGH-NMR Center, Department of Radiology, Massachusetts General Hospital and Harvard Medical School, Boston, MA 02114.

Intrastriatal injection of the reversible succinate dehydrogenase (SDH) inhibitor malonate produced age-dependent striatal lesions, which were significantly greater in 4 and 12 month old animals than in 1 month old animals. Both histologic and neurochemical studies showed that the lesions were significantly blocked by the non-competitive NMDA receptor MK-801. Neurochemical characterization showed significant decreases in GABA and substance P concentrations with relative sparing of somatostatin concentrations. Histologic assessment showed sparing of NADPH-diaphorase neurons. Chemical shift magnetic resonance imaging showed that malonate produces age-dependent increases in striatal lactate concentrations. There were transient ATP depletions, which were less severe than those seen with 3-nitropropionic acid. The results strengthen the possibility that a subtle impairment of energy metabolism may play a role in the pathogenesis of HD.

619.5

MECHANISM OF QUINOLINIC ACID FORMATION AFTER INFECTION WITH EITHER THE HUMAN OR SIMIAN IMMUNODEFICIENCY VIRUS. M.P. Heyes*, K. Saito, S.P. Markey, A.A. Lackner and E.O. Major. Lab. of Clinical Science, NIMH; Lab. of Viral and Molecular Pathogenesis, NINDS, Bethesda, MD 20892; and California Primate Center, Davis, CA 95616.

Infection with the human- or simian- immunodeficiency virus (HIV, SIV) is associated with neurologic deficits and encephalitis, in conjunction with elevated levels of the neurotoxin quinolinic acid (QUIN) in brain. In the present study, infection of macaques with various serotypes of SIV or the type-D retrovirus was associated with induction of indoleamine-2,3-dioxygenase, kynurenine hydroxylase and kynureninase in brain, in proportion to tissue and CSF QUIN levels. No changes in 3-hydroxyanthranilate-3,4-dioxygenase occurred. The largest increases were found in macaques with local inflammatory cell infiltrates. In a second study, human macrophages (THP-1 cells) and/or human fetal brain cultures (neurons, oligodendrocytes and astrocytes) were infected with HIV or stimulated by interferon-γ (IFN-γ) or endotoxin in media containing [$^{13}C_6$]-L-tryptophan. Macrophages converted [$^{13}C_6$]-L-tryptophan to L-kynurenine and [$^{13}C_6$]-QUIN when infected with either HIV-1 or stimulated by IFN-γ. However, while mixed brain cultures alone converted [$^{13}C_6$]-L-tryptophan to L-kynurenine in response to HIV-1 or IFN-γ, no [$^{13}C_6$]-QUIN was formed, unless macrophages added to the culture. The synthesis of [$^{13}C_6$]-QUIN by activated macrophages was attenuated by 4-chloro-3-hydroxyanthranilate (IC_{50}=0.1μM), 6-chlorotryptophan (IC_{50}=53μM), norharmane (IC_{50}=54 μM), indomethacin, superoxide dismutase, actinomycin-D, or interleukin-4. We conclude that macrophages are an important localization for induction of kynurenine pathway enzymes and QUIN formation in brain inflammation, and that QUIN synthesis can be modulated by either cytokines or inhibitors of the kynurenine pathway.

619.6

PLATELET-ACTIVATING FACTOR (PAF) IN THE PATHWAY OF HIV-RELATED NEURONAL INJURY. Dongxian Zhang*, Yun-Beom Choi, J. Offermann, H. E. Gendelman†, and Stuart A. Lipton. Dept. of Neurology, Children's Hospital and Program in Neuroscience, Harvard Medical School, Boston, MA 02115; †Dept. of Microbiol., Univ. of Nebraska Med. Ctr., Omaha, NE 68198.

At least one pathway to neuronal injury in AIDS appears to involve the release of toxins by macrophages that have been either infected with HIV-1 or stimulated with the HIV coat protein gp120. This form of neurotoxicity can be largely prevented by NMDA antagonists (reviewed in Lipton, TINS 1992;15:75). Recently, cytokines and arachidonic acid metabolites have been shown to be produced by macrophages activated by gp120 exposure or HIV infection (Wahl et al., PNAS 1989;86:621; Genis et al., J. Exp. Med. 1992;176:1703). Here, we demonstrate that gp120 stimulates the release of toxins into retinal cultures and that the candidate macrophage toxins, PAF and TNF-α, lead to injury of retinal ganglion cell neurons. In the first experiment, overnight exposure of rat retinal cultures to 20 pM gp120 resulted in injury to approximately half of the retinal ganglion cells. A specific gp120 antiserum prevented this toxic effect. In each case, culture medium assayed by HPLC contained ~25 μM glutamate. When gp120-containing 'conditioned medium' (CM) from this first experiment was added to a second set of cultures, a similar degree of neuronal injury was observed. However, fresh gp120 antiserum did not completely ameliorate this toxic effect, suggesting that toxic substances other than gp120 had accumulated in the CM. In a third series of experiments, the addition of carbamyl-PAF (1 μM) and TNF-α (1000 U/ml), but not TNF alone, resulted in neuronal injury that could be attenuated with the NMDA antagonist memantine (6 μM).

619.7

1-AMINOCYCLOPROPANECARBOXYLIC ACID (ACPC) PROTECTS AGAINST DYNORPHIN A (DYN)-INDUCED SPINAL CORD INJURY IN RATS. J.B. Long[1] and P. Skolnick*[2]. [1]Neuropharm. Br., Dept of Med. Neurosci., Div. of Neuropsych., Walter Reed Army Inst. of Res., Wash., D.C. 20307 and [2]Lab. of Neurosci., NIDDK, Bethesda, MD 20892.

Lumbar subarachnoid injection of DYN causes ischemia, neuronal degeneration, and persistent hindlimb paralysis in rats. The protective effects of a variety of competitive and non-competitive antagonists of the NMDA receptor complex indicate that excitatory amino acids (EAAs) mediate DYN-induced spinal cord injury. Moreover, spinal cord CSF concentrations of the EAAs glutamate and aspartate are increased 5-6 fold during the time that spinal cord blood flow is reduced by paralytic doses of DYN. ACPC is a high affinity partial agonist for the glycine binding site within the NMDA receptor complex, and acutely blocks NMDA- and cocaine-induced convulsions. Chronic treatment with this compound appears to yield a reversible desensitization of the NMDA receptor complex, suggesting that ACPC might provide an effective means of ameliorating degenerative mechanisms mediated through this ligand-gated ion channel. Therefore, we examined the effects of acute and chronic ACPC on recovery from the persistent hindlimb motor deficits elicited by L4-L5 subarachnoid injections of 20 nmoles of DYN. When administered 30 min before DYN injection, ACPC (50, 100, & 200 mg/kg, i.p.) caused significant, dose-dependent improvements in motor scores by 24 hours postinjection. When given as 6 daily injections followed by 1 injection-free day immediately preceding DYN administration, ACPC (200 mg/kg, i.p.) also significantly improved neurological recovery. These results support earlier indications that: 1) ACPC provides an effective means of antagonizing excitotoxic phenomena, and 2) chronically-administered ACPC desensitizes the NMDA receptor complex.

619.8

Use-dependent binding demonstrates that NMDA receptor channels do not open during "ischemia" *in vitro*. J. J. Vornov* and K. E. Bruce Depts. of Neurology and Neuroscience The Johns Hopkins School of Medicine, Baltimore, MD 21205

In some models of ischemia, neuronal injury is dependent upon glutamate activation of the NMDA receptor. We now present evidence in a tissue culture model that toxic NMDA receptor activation begins not during ischemia, but upon restoration of oxidative metabolism.

Dissociated cultures of rat cortical neurons were subjected to a 15 min period of simulated ischemia by blockade of oxidative metabolism and glycolysis with potassium cyanide and 2-deoxyglucose. Injury was assessed after 24 hours by measurement of LDH release. NMDA receptor antagonists (CPP, MK-801) protected against injury if added after simulated ischemia, during recovery. Protection was completely lost if the antagonist was removed before the restoration of oxidative metabolism. We then directly measured NMDA receptor activation with [^{3}H]-TCP binding. TCP binding is use-dependent because the binding site is in the open channel of the NMDA receptor. TCP binding was rapid, reversible and specifically displaced by MK-801. Binding was increased by toxic concentrations of glutamate or NMDA. However under ischemic conditions, binding was reduced from baseline by approximately 50%. Immediately after restoration of oxidative metabolism, binding had returned to near control levels. We found no evidence for massive NMDA receptor activation under ischemic conditions. During recovery, normal levels of NMDA receptor activation may injure vulnerable neurons. Alternatively, receptor activation may be transient or delayed.

619.9

POST-STROKE NEUROPROTECTION BY MEMANTINE MINIMALLY AFFECTS BEHAVIOR AND DOES NOT BLOCK LTP. PE Stieg*[1], S Sathi[1], SP Alvarado[2], PS Jackson[2], JW Pelligrini[2], H-SV Chen[2], SA Lipton[2], FE Jensen[2], Neurosurgery[1] and Neurology[2], Children's Hospital and Brigham and Women's Hospital, Harvard Medical School, Boston, MA 02115

In vitro studies demonstrate that memantine (Mte) antagonizes NMDA activity by open-channel blockade and decreases neurotoxicity (*J. Neurosci.* 1992;12:4427-4436). Mte is well-tolerated clinically, and this may be related to its faster off-rate compared to MK-801 at neuroprotective doses (*ibid*). Pretreatment with Mte reduces infarct size in a rodent stroke model (*ibid*). We evaluated infarct size and neurobehavior in rats administered Mte following stroke induction by photothrombosis (Mte 20 mg/kg i.p. 30 min after stroke, then 1 mg/kg b.i.d for 48 hours). Mte-treated rats did not exhibit sedation, alteration of withdrawal and righting reflexes, or weight loss. Mte treatment resulted in significantly smaller infarct volumes (mean = 36.7 mm^3, n=7) compared to controls (mean = 81.0 mm^3, n=6) ($p<0.001$). Because behavior was not significantly affected *in vivo*, we examined whether neuroprotective concentrations of Mte affected LTP in brain slices *in vitro*. Previous *in vitro* data revealed 6μM Mte to be neuroprotective (*ibid*). In 4/4 slices, 6μM Mte did not block LTP (145% of baseline epsp slope 1hr post tetanus). In contrast, neuroprotective doses of APV (50μM) completely blocked LTP (3/3 slices). These results suggest that Mte may be clinically useful in stroke treatment, given its selective blockade of the NMDA receptor under ischemic conditions with preservation of physiologic NMDA activity.

619.10

ADMINISTRATION OF THE SOLUBLE REDOX COFACTOR PYRROLOQUINOLINE QUINONE (PQQ) FOLLOWING HYPOXIA/ISCHEMIA REDUCES INFARCT SIZE. FE Jensen[1,2]*, G Gardner[1], A Williams[1], P Gallop[1], E. Aizenman[3], PA Rosenberg[1]. Neurol., Children's Hosp.[1], Brigham and Women's Hosp.[2], Harvard Med. Sch., Boston, MA; Neurobiol., Univ. Pittsburgh Sch. Med, Pittsburgh, PA[3]

PQQ is a putative essential nutrient that has been shown *in vitro* to diminish NMDA-evoked current and neurotoxicity by direct oxidation of the NMDA receptor redox site (*J. Neurosci.* 1992;12:2362-2369). PQQ has also been shown to have antioxidant effects and function as a free radical scavenger (*J. Pharm. Exp. Ther.* 1990;281:980-985). Administration of PQQ prior to hypoxia/ischemia in a rodent stroke model significantly reduces infarct size (*Soc. Neurosci. Abs.* 1992;18:756). The present study evaluated the efficacy of a single dose of PQQ (20 mg/kg i.p.) administered immediately following bilateral carotid ligation and hypoxia (30 min at 8% O_2) in 7 d.o. rat pups. Analysis of histologic sections from animals sacrificed at 48 hrs post hypoxia/ischemia revealed a significant neuroprotective effect of PQQ. Infarct area was 59.4 +/- 15.6% of total cortical area (n=8) in PQQ-treated animals, compared to 99.8 +/- 0.3% in matched controls ($p<0.02$). Thus, post-treatment with PQQ resulted in a 59% reduction in infarct size. In conclusion, PQQ appears to be useful in the treatment of hypoxia/ischemia when administered following the acute insult. Future experiments will determine the maximal delay for post-treatment in this model. Given the known actions of PQQ, both as a free radical scavenger and a selective pro-oxidant at the NMDA receptor redox site, there may be multiple mechanisms of neuroprotection by PQQ *in vivo*. (Supported by NS31718, NS29365; Amer. Heart Assoc; WR Hearst Fdn.)

619.11

FURTHER EVIDENCE THAT PYRROLOQUINOLINE QUINONE (PQQ) INTERACTS WITH THE NMDA RECEPTOR REDOX MODULATORY SITE E. Aizenman*, F.E. Jensen, P.A. Rosenberg, P.M. Gallop and L.-H. Tang, Dept. Neurobiology, Univ. of Pittsburgh, Pgh., PA 15261, and Depts. of Neurology and Biological Chem., Children's Hosp. and Harvard Med. Sch., Boston, MA 02115.

The putative essential nutrient PQQ has been shown to be an oxidizing agent for the NMDA receptor redox modulatory site (Aizenman et al., *J. Neurosci.* **12**, 2362; 1992). In the present study, we have performed additional experiments to establish the mode of action of this substance. As shown previously, 50 μM PQQ reverses the potentiating action of 4 mM dithiothreitol (DTT) on NMDA-induced whole-cell responses in rat cortical neurons in culture. However, following NMDA receptor alkylation with 500 μM N-ethylmaleimide (NEM; Tang & Aizenman, *J. Physiol.* **465**, 303; 1993) the effects of PQQ are largely abolished. Single channel measurements from outside-out patches revealed that 50 μM PQQ could readily reverse the increase in frequency of NMDA channel openings produced by 1 mM DTT, similar to what was previously shown for another oxidizing agent, DTNB (Tang & Aizenman, *ibid.*). Furthermore, even higher concentrations (200 μM) of PQQ did not alter NMDA single channel conductance or open time distribution. Together, these results indicate that PQQ modulates the NMDA receptor by directly oxidizing the redox site. Supported by NS29365.

619.12

CULTURED NEURONS RESCUED FROM AMINO ACID EXCITOTOXICITY BY HU-211, A NON PSYCHOTROPIC CANNABINOID. N. Eshhar, S. Strim, M. Liscovitch* and A. Biegon, Pharmos Ltd. and Weizmann Inst. Sci., Rehovot, ISRAEL

. Excessive stimulation of glutamatergic receptors is intimately involved in mechanisms of neurotoxicity and neuronal cell death. HU-211, a non-psychotropic cannabinoid-derived compound has been shown to act as a functional noncompetitive NMDA receptor antagonist in vivo and in vitro. In the present study, we examined the ability of HU-211 to prevent neuronal degeneration produced by different glutamatergic receptor agonists in culture. Primary cerebral cortical cell cultures were prepared from 18- to 20-day old rat fetuses by enzymatic dissociation. Resulting cell suspensions were plated on a confluent cortical glial-cell feeder layer (prepared 2 weeks earlier). Cells at 10 days in culture were exposed to 500μM NMDA and/or to 1000μM quisqualate, either alone or in the presence of 10μM HU-211. The percent of viable cells was assessed by ELISA measurements of oxidative metabolism 24 hours later. Exposure to the toxin was associated with 20-50% neuronal death, which was totally prevented by HU-211. The ability of HU-211 to prevent cell death mediated by quisqualate suggests that its neuroprotective activity may be mediated by more than one receptor and/or mechanism. Exploring this possibility, we found that HU-211 at 10μM totally displaced tritiated QNB binding from the cholinergic muscarinic receptor in brain homogenates, whereas it had no effect on phospholipase D activity in solubilized brain membranes. While the mechanism underlying the neuroprotective activity of HU-211 is still under investigation, the compound offers promise as a therapeutic agent for conditions such as stroke and head trauma, in which excitotoxicity has been shown to play a major role.

NEURONAL DEATH V

620.1

THE MORPHOLOGY OF CEREBELLAR PURKINJE CELLS GROWN *IN VITRO* FROM LURCHER AND WILD-TYPE MICE Keith W.T. Caddy*, and Martin L. Doughty. Department of Physiology, University College London, Gower Street, London, WC1E 6BT, U.K.

The Lurcher mutant mouse has been studied for a number of years and the development of the lesion in the animal characterized both qualitatively and quantitatively (Caddy and Biscoe, 1979, *Phil. Trans. Roy. Soc.* **287**:167). We know that the Lurcher Purkinje cells (P-cells) exhibit characteristic abnormal ultrastructure by 10 days post natal (P10) and suffer complete degenerative loss by P26. As a consequence of this 90% of its granule cells are lost through trans-synaptic degeneration. Lurcher<-->wild-type chimeric studies (Caddy and Herrup 1990, *J. comp. Neurol.* **297**:121 & 1991, *J. comp. Neurol.* **305**:421) have shown that only the wild-type P-cells survive in these mice and they are morphologically abnormal. It was with this knowledge that an *in vitro* study was begun. We have been culturing explants of cerebellar cortical slices from 2 day old Lurcher and wild-type mice to compare the growth and development of the P-cells with those found in the above studies. We have analyzed explants grown for up to 25 days *in vitro* (DIV) using immunocytochemistry and electron microscopy (EM). The results of the EM study, presented here, indicate that the P-cells grown *in vitro* from the Lurcher mutant mouse are not significantly different morphologically to those grown from the wild-type. The fine structure of the P-cells in both genotypes *in vitro* are different from the cells seen *in vivo*. In the cultures we have found a range of synaptic profiles, both typical of those seen *in vivo* and abnormal structures unique to the culture environment. Quantitative analysis of Calbindin-D immunoreactivity indicates there is no significant difference in P-cell numbers in Lurcher and wild-type cultures (10-25 DIV). We are continuing this study with a quantitative analysis of the growth of the P-cell neurites in cultures of Lurcher and wild-type cerebellar cortex. (Supported by The Wellcome Trust).

620.2

TETRAHYDROAMINOACRIDINE HAS A LONG-TERM NEUROSURVIVING EFFECT ON CULTURED CEREBELLAR GRANULE CELLS. R. Ishitani*[1], K. Sunaga[1], M. Kimura[1] and D.-M. Chuang[2]. 1)Group on Cellular Neuropharmacology, Josai Univ., Sakado, saitama 350-02, Japan. 2)Section on Molecular Neurobiology, BPB, NIMH, Bethesda, MD 20892, U.S.A.

The long-term neurosurviving effect of 9-amino-1,2,3,4-tetrahydroacridine (THA), a putative antidementia agent, was studied in cultured granule cells. Dissociated cerebellar granule cells from 8-day-old Sprague-Dawley rats were grown in BME medium, containing 10% fetal calf serum and 25 mM KCl. Without repeated glucose supplement these neurons died between 15 and 18 days later in culture. Ultrastructural changes included disruption of neurites, followed by cell body degeneration characterized by dilation of the rough endoplasmic reticulum and the condensed marginated chromatin (pyknosis). By contrast, no primary alterations of mitochondria or lysosomes were observed. The addition of THA (1 to 10 μM) to cultures clearly prolonged these neuronal survival, at least by 2 days in vitro. This long-term neurosurviving effect was reproduced by using cycloheximide or actinomycin D, but not carbachol, 4-aminopyridine, NMDA or leupeptin. Furthermore, SDS-PAGE analysis indicated that certain minor proteins were reduced by THA. These data suggest that THA maintains neuronal survival by suppressing an endogenous, active death program.

620.3

INTRACELLULAR CALCIUM AS SECOND MESSENGER IN MATURE CEREBELLAR GRANULE CELLS UNDERGOING APOPTOSIS. C. Galli, O. Meucci, A. Scorziello, S. Schinelli*, G. Schettini. Ist. Neurobiologia, CNR, Roma; Dip. Farmacol., Fac. di Medicina, Universita' di Napoli Federico II, Ist. Farmacol., Fac. Farmacia, Universita' di Pavia. ITALY.

We have investigated the role of the intracellular calcium concentration, $[Ca^{2+}]_i$, in apoptosis in mature cerebellar granule neurons. Apoptosis was induced by lowering the extracellular potassium concentration from standard 25mM to 5mM. In healty mature neurons cultured in 25 mM KCl the $[Ca^{2+}]_i$, measured by fura-2 fluorescence imaging, is 143+/-22 nM. Upon downshifting the extracellular potassium concentration to 5mM KCl the $[Ca^{2+}]_i$, immediately decreased to 68+/-11 nM. The time course of apoptosis was evaluated by intact nuclei counts and DNA fragmentation and subsequently correlated to measurements of $[Ca^{2+}]_i$. Levels of $[Ca^{2+}]_i$ remained low throughout the entire process. Depolarising potassium rescues neurons previously kept in 5mM KCl and this effect was related to the restoration of high level of $[Ca^{2+}]_i$. Agents able to inhibit the apoptotic process (forskolin and IGF-1) were investigated in order to establish a possible involvement of $[Ca^{2+}]_i$ in their neuronal survival promoting activity. 10μM forskolin was able to counteract the decrease of $[Ca^{2+}]_i$ observed downshifting to 5mM KCl. The effect of forskolin was inhibited by 1μM KT5720, a specific protein kinase A inhibitor, showing that activated-protein kinase A is able to maintain elevated $[Ca^{2+}]_i$ levels, despite non-depolarising conditions. Contrary to forskolin, IGF-1 had no effect on the $[Ca^{2+}]_i$ in KCl deprived neurons. In fact, albeit their low $[Ca^{2+}]_i$, IGF-1 treated neurons were perfectly viable. These data support the notion of sustained $[Ca^{2+}]_i$ as a key component in the survival promoting action of forskolin and depolarising KCl and, on the other hand, show that IGF-1 bypasses this step, acting probably downstream the increase of intracellular Ca^{2+}.

620.4

REGULATION OF PROTEIN KINASE ACTIVITIES BY TAURINE AND β-ALANINE DURING EXCITOTOXICITY IN CAT AND MOUSE CEREBELLAR CULTURES. E. Trenkner, J.A. Sturman* and D-J. Liu. NYS Institute for Basic Research, Staten Island, NY 10314.

Increased levels of protein kinase C (PKC) activity have been linked to excitotoxic cell death by demonstrating that PKC specific inhibitors prevent excitotoxicity. We have shown that glutamate induced excitotoxicity can be prevented by taurine in mice, while β-alanine rescues cerebellar cells in cat from taurine toxicity. This study suggests a mechanism for the preventive role of taurine and β-alanine respectively.

In mouse, which synthezises taurine throughout life, glutamate (10^{-3}M) stimulates PKC activity up to 60% as compared to untreated cultures. In the presence of 10^{-2}M taurine, the glutamate induced PKC activity is down-regulated to untreated levels. β-Alanine had no affect on PKC activity in mice. Taurine alone had no significant affect on PKC activity. In contrast to mice, cat, monkey and man acquire taurine mostly through diet. We have shown that taurine is toxic in cat cerebellar cultures, whereas β-alanine promotes survival, suggesting a different mechanism. When treated with NMDA (10^{-3}M) PKC activity was raised by 35%, similar to that induced by β-alanine (10^{-2}M) alone; taurine had no significant effect. When added together NMDA-induced PKC activity was reduced, suggesting that β-alanine mimics the role of taurine in cats. We suggest that the rescue mechanism of taurine or β-alanine is based on the down regulation of glutamate induced PKC activity. Supported by NSF grant BNS-8910218 to E.T. and NIH grant HD-16634 to J.A.S.

620.5

PROTEIN-TYROSINE PHOSPHORYLATION AS A TARGET OF SUSTAINED LEVELS OF CYTOPLASMIC CALCIUM IN DIFFERENTIATING PC12 CELLS AND CEREBELLAR GRANULE CELLS. T. Koike* and S. Tanaka. Department of Natural Science, Saga Medical School, Saga 849, Japan.

There is ample evidence suggesting that membrane depolarization with elevated K^+ promotes neuronal survival *in vitro*. We have previously shown that neuronal survival under depolarizing conditions is well correlated with sustained levels of cytoplasmic free calcium in sympathetic neurons and cerebellar granule cells (Koike and Tanaka, PNAS, 88:3892, 1991 etc). Data available so far (Tanaka and Koike, BBA, 1175:114, 1992 etc) indicate that elevated K^+ may act through the post-translational modification. In an attempt to identify a possible down-stream target of the death-preventing effect of elevated K^+, we are utilizing differentiating PC12 cells that undergo neuronal death after withdrawal of NGF (Koike, Prog.Neuro-Psychopharmacol. & Biol-Psychiat., 16:95, 1992). Immunoblotting with anti-phosphotyrosine antibody revealed that membrane depolarization stimulates tyrosine phosphorylation of p130 in a Ca^{2+}-dependent manner. The tyrosine phosphorylation of p130 was hampered when the cells were depolarized in the presence of herbimycin A, an inhibitor of tyrosine kinase activity. This drug also abolished the death-preventing effect of elevated K^+ in a dose-dependent manner (IC_{50}=2μM) suggesting a correlation between the two. We also found that membrane depolarization stimulates tyrosine phosphorylation of a distinct set of proteins in cerebellar granule cells *in vitro*. These results suggest that protein-tyrosine phosphorylation is a common down-stream target of sustained levels of cytoplasmic free calcium under depolarizing conditions.

620.6

NMDA RECEPTOR ANTAGONISTS AND ANTIBODIES AGAINST A NEURITE OUTGROWTH DOMAIN OF THE B2 CHAIN OF LAMININ ENABLE NEURITE OUTGROWTH OF THE WEAVER GRANULE NEURONS ON A LAMININ SUBSTRATUM. P. Liesi*.Department of Anatomy.University of Helsinki.Finland and Laboratory of Molecular and Cellular Neurobiology, NIAAA, NIH, Rockville, MD 20852.

Neurite outgrowth of the granule neurons from normal mouse cerebellum is supported by substrate bound laminin and synthetic peptides derived from the neurite outgrowth domain of the B2 chain of laminin. In contrast, the migration deficient weaver granule neurons fail to send out neurites or move on a laminin substratum. As glutamate exitotoxicity has been suggested as a putative mechanism for neuronal death in all neurodegenerative models, I studied the interrelationship of the glutamate system and the laminin system in vitro.The cerebel ar neurons of the weaver mutant mice or normal control litter mates were trypsinized and plated on a laminin substratum in a serum free medium. The cells were allowed to attach for one hour after which the culture medium was changed and 7 μm of MK801 or 100 μm of.APV or 20μg/ml of Fab-fragments of antibodies to neurite outgrowth domain of the B2 chain of laminin were added into the culture medium. The cells were cultivated for 24 hrs, fixed,and stained for L1 antigen to allow identification of the granule cells.Quantitative analysis of the cultures showed that the weaver neurons were rescued in terms of their neurite outgrowth potential by NMDA receptor antagonists as well as the domain specific laminin antibodies. Their immunohistochemical and biochemical properties also normalized during the short cultivation period exposed to these agents. These results indicate that the glutamate system and laminin system are interrelated, and offers new ways of thinking regarding neurodegenerative disorders.

620.7

SURVIVAL OF SUBPLATE NEURONS IN CULTURES OF DEVELOPING NEOCORTEX A. Hohn*, K.L. Allendoerfer, A. Toroian-Raymond, and C.J. Shatz. Dept. of Molecular and Cell Biology, U.C. Berkeley, CA 94720.

During mammalian cerebral cortical development, a transient population of early born neurons takes up a position underneath the forming cortical plate. These neurons, called subplate (SP) neurons, are essential for the formation of thalamocortical pathways, but by adulthood, most of these neurons have been eliminated by cell death. By studying SP neurons in culture, we examined the hypothesis that they might depend for survival on trophic support from their target cells in thalamus and superior colliculus or on incoming fibers from subcortical sources. SP neurons were labeled by injecting ^{3}H-thymidine into ferrets at embryonic day 24 (E24), and survival was analyzed in three ways: 1) Cortices were analyzed for the presence of labeled SP neurons at ages from postnatal day 1 (P1) to 4 months, in order to establish a time course of SP cell death in vivo. Whereas little variation in the number of labeled cells was seen between P1 and P24, at 4 months few labeled cells could be detected. 2) Organotypic slice cultures from cortical tissue were prepared before the onset of SP neuron cell death and maintained throughout the period when cells die in vivo. In slices prepared at P1 and maintained in culture for 3 weeks, or in slices prepared at P15 and maintained for 7 weeks, large numbers of labeled SP neurons could be detected. Furthermore, neurons in the SP layer could be labeled with antibodies against neuropeptide Y, suggesting that at least some SP neurons maintain a normal phenotype in culture and are able to extend neurites into other cortical layers. 3) Fetal cortices were dissociated and cultured at E30, when SP neurons comprise about 50% of the neuronal population. The number of SP neurons, as measured by counts of ^{3}H-thy$^+$, MAP2$^+$ cells, remained at high levels (55-70% survival) throughout the six days in culture. Taken together, these observations demonstrate that SP neurons are able to survive for extended lengths of time in culture, in the absence of their subcortical connections such as those to or from the thalamus. Thus, SP neuron survival may be regulated by local interactions within the cerebral wall. Supported by Swiss Natl. Found. (AH), EY02858 and the Alzheimer's Assn. (CJS).

620.8

DYING NEURONS IN DEVELOPING AND ADULT CEREBRAL CORTEX ARE DETECTED WITH IgG-SPECIFIC ANTIBODIES. J.A. Dunn[1], J.D. Kirsch [1], J.R. Naegele*[1,2] [1]Dept. of Biology, Wesleyan University, Middletown, CT 06459 and [2]Child Study Center, Yale University School of Medicine, New Haven, CT 06473

Subplate neurons of the mammalian cerebral cortex die perinatally. During this period, they express a variety of proteins also contained in the surviving cortical plate neurons. In contrast, a 56 kDa cytosolic polypeptide identified by a monoclonal antibody SP1 (mAb SP1) is restricted to the subplate neurons (Naegele et al. '91). Lesion studies, biochemistry, and immunocytochemistry were carried out to determine the identity of the SP1 antigen and its involvement in cell death. Lesions of adult cat cortex revealed the SP1 antigen in dying, but not healthy cortical cells. Biochemical analyses showed that the SP1 antigen is not limited to neurons but is also enriched in liver and serum. On Western blots, mAb SP1 recognized IgG purified from cat cortex and cat serum. Comparisons between mAb SP1 and a goat anti-cat IgG (Vector) demonstrated that mAb SP1 was specific for IgG heavy chains. Immunocytochemical staining of kitten cortex showed that both mAb SP1 and the IgG antiserum label only subplate neurons. Two distinct neuronal subsets were double-labeled. One, located deeper in the subplate, had small round somata. The other, located more superficially, had large, inverted pyramidal-shaped somata. Additionally, two-color immunofluorescent double-labeling studies demonstrated that cat IgGs and the SP1 antigen co-localize in the same subplate neurons, suggesting that they are the same molecules.
Supported by MH49351 and EY09749

620.9

SHUNTING OF CYST(E)INE FROM PROTEIN SYNTHESIS TO GLUTATHIONE PROTECTS CORTICAL NEURONS FROM GLUTAMATE-INDUCED OXIDATIVE STRESS. R.R. Ratan, T.H. Murphy, and J.M. Baraban*. Department of Neuroscience, Johns Hopkins University, Sch. of Med., Baltimore, MD 21205.

Immature, embryonic rat cortical neurons are susceptible to glutamate neurotoxicity through a non-receptor mediated mechanism involving cystine transport inhibition, glutathione depletion and oxidative stress. We utilized this in vitro preparation and inhibitors of macromolecular synthesis to evaluate whether oxidative stress can activate a pathway of cell degeneration similar to growth factor deprivation. We found that glutamate-induced neuronal death is prevented by inhibitors of macromolecular synthesis. Protection is associated with enhanced availability of acid-soluble cyst(e)ine and restoration of cellular glutathione levels. N-acetylcysteine, an agent which delivers exogenous cysteine intracellularly and raises glutathione, is also protective; while buthionine sulfoximine, an inhibitor of glutathione synthesis, prevents protection by inhibitors of macromolecular synthesis. These results suggest that protection provided by these agents, in this paradigm, derives from shunting of the amino acid, cysteine, from global protein synthesis into the formation of the anti-oxidant, glutathione; and raise the possibility that oxidative stress may activate a pathway of cell degeneration similar to growth factor deprivation.

620.10

AFFERENT INFLUENCES ON NEURON DEATH AND CELL BIRTH IN A FOREBRAIN MOTOR REGION THAT CONTROLS LEARNED VOCAL BEHAVIOR IN ZEBRA FINCHES. F. Johnson* and S.W. Bottjer. Dept. of Biology, University of Southern California, Los Angeles, CA 90089-2520.

We examined the ability of afferent input to influence the survival of neurons that form the descending motor pathway of forebrain song-control regions in the zebra finch brain. The robust nucleus of the archistriatum (RA) receives two afferent inputs (from the lateral magnocellular nucleus of the anterior neostriatum - lMAN, and from the higher vocal center - HVC) and RA projection neurons control the motoneurons that innervate the syrinx (vocal organ). Because the ingrowth of HVC input is delayed, lMAN provides the majority of afferent input to RA during early vocal learning. lMAN afferent input to RA is of particular interest since lMAN is necessary for vocal learning only during a restricted period of development. lMAN was electrolytically lesioned in birds of various ages to determine whether the survival of RA neurons depends on lMAN afferent input, and if so, whether such dependence varied as a function of the ingrowth of HVC afferent input (35 days of age), or the end of the period during which lMAN is necessary for vocal learning (60 days of age). Removal of lMAN afferent input induced widespread pyknosis and the loss of over 40% of RA neurons among 20 day-old birds that were in early stages of vocal learning. However, by 40 days of age, at which time HVC axons have elaborated terminal arbors within RA, lMAN lesions no longer induced neuron death in RA. These data indicate that many RA neurons require lMAN afferent input for their survival during early vocal learning, and that the ingrowth of axon terminals from HVC may provide an alternate source of afferent support in older birds. In 20 day-old birds, removal of lMAN afferent input also triggered a burst of intense mitosis in RA (1700 mitotic figures/mm^3) at 2 days post-lesion. The early, acute nature of the mitotic events suggests that cell division in RA may be a specific response to the loss of lMAN afferent input.

620.11

TOPOGRAPHICAL ORGANIZATION AND TIME COURSE OF LOSS OF RETROGRADE HRP LABELING OF CORTICOSPINAL NEURONS AFTER SPINAL CORD TRANSECTION. R.L. McBride*, P.I. D'Cruz, and E.R. Feringa. VA Medical Center and Department of Neurology, Medical College of Georgia, Augusta, GA 30910.

As indicated by the retrograde transport of HRP, axotomized rat corticospinal neurons progressively deteriorate after T-9 spinal cord transection. To understand why some neurons are affected more by axotomy than others (and thus may be less likely to regenerate if treated), we studied the organization of the temporal sequence of HRP labeling loss after cord transection. Complete T-9 cord transections were performed on 7-week-old female rats. An HRP pellet was placed at T-2 3, 6 or 20 weeks after transection. Compared to controls [808 ± 38 (n=7)], the number of labeled corticospinal neurons in transected rats progressively decreased: 3 weeks, 592 ± 79 (n=9); 6 weeks, 459± 126 (n=7); 20 weeks, 348 ± 31 (n=7). Labeling loss in the cortical area representing the lower trunk and hindlimb began caudally and medially, and progressed laterally and rostrally. We conclude that labeling loss by corticospinal neurons is related to somatomotor cortex somatotopic organization.

620.12

DOES THE FLOOR PLATE INDUCE BASAL PLATE FORMATION ? S. Hirano*[1] and H. Tanaka[2]. [1]Dept. Anatomy, Niigata Univ. Sch. Med., Niigata 951, JAPAN and [2]Dept. Neuroscience & Immunology, Kumamoto Univ., Kumamoto 862 , JAPAN

We have previously shown that motoneurons fail to develop when the neural tube is separated from the floor plate (Hirano et al.,*Science* 251: 1991). However, the mechanisms which regulated motoneuron development have not yet been established. Two factors may influence motoneuron development: the neuroepithelial layer of the basal plate, which gives rise to the neuroblasts that form the ventral horn motoneurons, may fail to develop normally or the neuroblasts themselves die shortly after their formation. In order to evaluate these possibilities, the neural tubes of chick or quail embryos (H-H stage 12-13) were incised along the lateral side of the floor plate at the level of the segmental plate. Embryos were then assayed for dorso-ventral differences in mitotic activity using BrdU and for cellular death using in situ labeling of DNA. Monoclonal antibodies DBM and VBM were used to identify the dorsal and ventral aspects of the neural tube, and SC1 and 23C10 to distinguish the motoneurons from non-motoneurons, respectively. In the floor plate-deprived half of the neural tube, the basement membrane expressed only DMB antigen, and neurons expressed only 23C10 antigen, indicating that both the ventral basement membrane and motoneurons failed to develop. BrdU analysis revealed no dorso-ventral differences in mitotic activity which might have resulted if the neuroepithelial layer failed to develop normally. Moreover, there was no localized concentration of cells containing DNA fragments indicating that neuroblasts did not die after formation. These data suggest that the development of the basal plate, and the subsequent development of motoneurons, is induced by the floor plate.

620.13

ULTRASTRUCTURAL CHANGES IN SUPRAOPTIC NEURONS OF CHRONIC ALCOHOL-FED RATS. C. Ruela, N. Sousa, M.D. Madeira and M.M. Paula-Barbosa*, Dept. of Anatomy, Porto Medical School, 4200 Porto, Portugal.

We demonstrated[1] that the supraoptic nucleus (SON) of chronic alcohol-fed (A) and chronic dehydrated (D) rats were larger than that of controls (C). The volume increase of SON was mainly due to an increase in neuronal size, greater in (A) than in (D). By applying morphometric ultrastructural techniques we attempted to discern the underlying causes for the augment of the neuronal volume. Groups of six animals twelve month age were studied. The evaluations were carried out in 10 photomontages of SON neurons per animal and the following parameters were estimated: a) volume and surface densities of R.E.R. and Golgi apparatus and b) volume density of secretory granules. Based on these parameters and on the cytoplasmic volume, which was previously calculated by employing the nucleator, the absolute values of those organelles were calculated. An increase in the volume of all parameters was found in groups (A) and (D) when compared with (C), more marked in (A). We conclude that the larger size of SON neurons is dependent on an increase in the volume of the organelles related to peptide synthesis although in (A) it might also be related to changes in the neuronal transport mechanisms as demonstrated by the marked accumulation of secretory material.

1) Madeira, M.D. et al, Neuroscience 1993

620.14

DNA FRAGMENTATION IS ASSOCIATED TO NEURONAL DEATH IN THE DEVELOPING RAT SUPERIOR COLLICULUS Grazia Galli[1], Maddalena Fratelli[1] and Lucia Galli-Resta[2]* [1]Istituto Ricerche Farmacologiche Mario Negri - Milano - Italy and [2]Istituto di Neurofisiologia CNR - Pisa - Italy.

Cell death is a peculiar aspect of the development of the nervous system. While the developmental strategies regulating death of vertebrate neurons have been well studied *in vivo*, the biochemical mechanisms of cell death have been largely analysed in various *in vitro* models. Cell death, as it has been best characterized *in vitro*, occurs through a process, named apoptosis, which involves in most instances the degradation of cellular DNA to discrete oligonucleosomal fragments. We show here that this distinctive biochemical event is also associated to death of developing mammalian neurons *in vivo*. As a model we have analysed the developing rat superior colliculus where naturally occurring cell death can be substantially increased by retinal afferent removal. After eye enucleation we dissected away the superior colliculus of postnatal rats and extracted DNA. Southern hybridization with labelled total DNA indicates the presence of a ladder of DNA fragments typical of apoptosis.

620.15

ACTIVATION OF COMPLEMENT AND UPREGULATION OF SULFATED GLYCOPROTEIN (SGP)-2 mRNA IN THE HYPOGLOSSAL NUCLEUS FOLLOWING PERIPHERAL NERVE INJURY. M. Svensson* and H. Aldskogius. Department of Anatomy, Karolinska Institutet, Box 60400 S-104 01 Stockholm, Sweden

In a previous study we observed expression of complement factors C3, C3d, C4d and C3 mRNA as well as endogenous immunoglobulin G in the rat hypoglossal nucleus following hypoglossal nerve transection, suggesting that activation of the complement cascade had taken place in the vicinity of the axotomized motorneurons. The complement factor and IgG expressions were associated with reactive microglial cells indicating that these cells have a central role in complement activation as a local source of complement factors.

In the present study we have used in situ hybridization to examine the expression of sulfated glycoprotein (SGP-2) mRNA in axotomized hypoglossal neurons. SGP-2 is the rat analogue to human clusterin, a potential complement inhibitor. Low levels of SGP-2 mRNA were expressed in the intact hypoglossal neurons. After injury, there was a marked increase in this expression. The results lend further support to the hypothesis that the complement cascade is activated in the vicinity of axotomized neurons, which in turn may be protected by an upregulation of complement inhibitor(s). The balance between activation of complement and complement inhibitors might have an impact on the degenerative components of the axon reaction, and in particular the events leading to nerve cell death.

620.16

VITAL ASSAY FOR NEURONAL CELL DEATH IN ORGANOTYPIC CULTURES Richard Adams[1*], Zoltán Molnár[2], Matthew Nesbit[2] and Colin Blakemore[1,2] MRC Research Centre in Brain and Behaviour[1] and University Laboratory of Physiology[2], Parks Road, Oxford OX1 3PT, UK.

Cell death is an important feature of the development of the nervous system. We have been investigating methods of assaying cell mortality. Our aim was to find a vital assay that can be used to map patterns of cell death and to use this to follow natural events as well as the effects of experimental manipulations. Acridine orange is a vital dye that crosses the plasma membrane and stains the nucleus with a green emission on excitation with light of 488nm. Propidium iodide also binds to chromatin but differs from acridine orange in two respects: it cannot cross the plasma membrane at normal resting membrane potentials and it has a longer Stoke's shift, giving a red emission of light when excited by the blue 488nm laser line. Thus, a short staining of tissue with a mixture of these dyes produces green fluorescence in all cell nuclei with an additional red fluorescence of the nuclei of those cells that are at a depolarized potential – largely indicative of dead or dying neurons in culture. We use confocal microscopy to image the 3-D distribution of stained nuclei within organotypic cortical and thalamic cultures and thalamo-cortical co-cultures stained for 30-60 minutes with 10μg/ml of each of these dyes. The red and green emissions were separated and collected simult-aneously by two photomultiplier channels and the two 3-D datasets were reconstructed into a two-colour projection.

In slices of P0 rat cortex kept in culture for several days we see occasional dead cells throughout the depth of the tissue but a predominance of such cells within the subplate and marginal zone - regions of transient cells known to be lost at this stage of development (Luskin and Shatz, 1985 J. Neurosci **5**:1062). Second, we have compared the survival of thalamic neurons in slices of E16 and P2 thalamus cultured with and without cortex in the same dish. Preliminary results are consistent with the idea that the cortex provides a soluble trophic component that prolongs the survival of neurons within the slice of thalamus (Cunningham *et al.* 1987 Dev. Brain Res. **37**:133).

MOTOR SYSTEMS

621.1

AUGMENTED STRIATAL ACETYLCHOLINE (ACH) RELEASE FOLLOWING PERINATAL HYPOXIC-ISCHEMIC INJURY: AN IN VIVO MICRODIALYSIS STUDY. T. Qo, R.E.Burke*, Dept of Neurology, Columbia University, NYC, NY, 10032.

We have previously shown that following hypoxic-ischemic (H-I) injury to the developing brain, there is an increase in both morphologic and biochemical pre-synaptic markers of striatal cholinergic neurons. There are increases in the density of neurons, choline acetyltransferase-positive neuropil, and ^{3}H-hemicholinium binding. In addition, there is preservation of total and M1 muscarinic receptor binding. To explore the functional significance of these changes, we have measured the striatal release of ACH under basal and muscarinic-antagonist stimulated conditions using microdialysis in vivo. 7 day rat pups underwent unilateral carotid ligation and exposure to 8% O_2 for 3-3.5 hours. At 9-12 weeks of age rats received a stereotaxic implantation of a striatal guide cannula. After >48 hour recovery, microdialysis was performed for 6 hours under awake free-roaming conditions. In the 6 hour studies of basal ACH release rats with striatal H-I injury (N=8) were not significantly different from rats subjected to H-I showing no injury (N=10), sham-operated (N=5) or normal (N=7) controls. However, following stimulation of release by infusion of atropine (3 μM) there was a significantly higher induced release of ACH in the H-I injury group (859±459 percent change;N=6) than in the non-injured H-I group (99±23;N=24) or Sham controls (152±58;N=7) ($p<.01$, ANOVA) . A similar augmentation of induced release was observed with infusion of the M1 antagonist pirenzepine (30 μM): H-I injury 405±155 (N=4); H-I no injury 74±21 (N=29) ($p<.01$). We conclude that there is a functional increase in striatal ACH release under stimulated conditions following H-I injury. NS26836, UCP, PDF.

621.2

HYPERBARIC OXYGENATION INCREASES AROUSAL AND INDUCES BREATHING MOVEMENTS DURING NREM SLEEP IN THE FETAL LAMB < 135 DAYS GESTATION. M.H. Tiktinsky, S.U. Hasan**, B.Bishop*, F.C. Morin III, Dept. of Physiol. and Ped. SUNY-Buffalo, Buffalo, N.Y. 14214. U. of Calgary**, Calgary, AB, Canada T2N 4N1.

Fetal breathing movements (FBMs) occur 40% of the total time and almost exclusively during rapid eye movement sleep (REM). Oxygenation via lung distension increases the duration of fetal arousal and induces FBMs during non rapid eye movement sleep (NREM) in fetal lambs > 135 d gestation (ga). We studied the effect of O_2 without lung distension on behavioral states and FBMs. Seven fetal lambs (129-132 d ga) were chronically instrumented to record electrocorticogram, electro-oculogram, nuchal EMG, diaphragm EMG, and carotid arterial blood pressure, pH and gas tension to assess fetal behavioral states, FBMs, and cardiovascular status. Fetal arterial O_2 (PaO_2) was raised from 25 ± 1 to 46 ± 1 torr by mechanically ventilating the ewe with 100% O_2 at 3 ATA in a hyperbaric chamber. Hyperbaric oxygenation (HBO_2) increased the duration of fetal arousal and the occurrence of FBMs during arousal and NREM. The duration of arousal tripled from 13 ± 11 to 38 ± 12% of the total time. The mean longest period of arousal increased from 3 ± 5 to 18 ± 6 mins. The mean time from the start of HBO_2 to the onset of arousal was 8 ± 4 mins. The occurrence of BMs increased from 37 ± 13 to 63 ± 30% of the total time. During arousal, the occurrence of FBMs tripled from 24 ± 23 to 72 ± 34% of the time. During NREM, FBMs occurred in 5 of 7 fetuses from 15 and 100% of NREM time. The mean longest episode of BMs increased from 16 ± 6 to 49 ± 29 mins. We conclude that oxygenation without lung distension increases fetal arousal and FBMs, especially during arousal and NREM, in fetuses 129-132 d ga. The increase in PaO_2 at birth may contribute to the transition from placental to lung gas exchange.

Funded by HL # 41387

621.3

FUNCTIONAL ASYMMETRY OF THE SPINAL CORD IN NORMAL CHILDREN AND AFTER BRAIN DAMAGE. M.S.Sinyaya*,A.M.Shelyakin,D.U.Pinchuk and O.V.Bogdanov. Pavlov Institute of Physiology,Russian Academy of Sciences, Institute of Experimental Medicine,St.Petersburg,199034,Russia.

The goal of this study was to compare the excitability of the motoneurons. We have recorded the H- and M-responses of the muscle gastronemius and calculated the asymmetry coefficient in the three following groups: 1) in normal children (from 8 to 13 years of age, right-handed persons; 2) in children with spastic type cerebral palcy (CP) (right hemiplegia) before and after rehabilitation of motor functions; 3) in the adult. It was observed that the normal children have a functional asymmetry of the spinal cord. A decrease of excitability of the right muscle gastronemius as compared with the left one was found in this group of children. The asymmetry was found to disappear in the adult and was absent in children with CP. The application of the functional biofeedback method has led to the normalization of electrophysiological parameters (EEG, EMG) and has created the asymmetry of the spinal cord and brain. We suppose that the functional asymmetry of the spinal cord is one of the mechanism of the formation and development of the interhemisphere asymmetry.

621.4

DIFFERENTIATION OF MOTONEURON ELECTRICAL PROPERTIES IN ORGANOTYPIC CULTURE OF RAT SPINAL CORD. H. Xie, and L. Ziskind-Conhaim*. Dept. of Physiology and Ctr. for Neuroscience, Univ. of Wisconsin, Madison WI, 53706.

To compare the pattern and time course of motoneuron differentiation in vitro to that in vivo, thin slices (300 μm) of embryonic spinal cords (E15-E16), with or without dorsal root ganglia, were placed on porous, collagen-coated membranes (Costar Co.) and incubated at 30°C for 3-8 weeks. The frequency of spontaneous synaptic activity significantly increased in organotypic culture, but the increase was not correlated with the presence of dorsal root ganglia. Furthermore, dorsal horn stimulation produced synaptic potentials in most motoneurons.

Intracellular recordings were used to determine the changes in motoneuron resting potential, input resistance, action potential threshold, action potential maximum rate of rise, and motoneuron repetitive firing. The electrical properties of E15-E16 motoneurons cultured for 3-4 weeks were similar to the properties of E18-E21 motoneurons in hemisected spinal cord. Motoneuron electrical properties change significantly at birth, but similar differentiation did not occur in culture.

Prolonged depolarization (300 ms) generates a train of action potentials in neonatal but not embryonic motoneurons. However, such depolarization generated a train of action potentials in 31% of cultured motoneurons. The development of repetitive firing was not correlated with the level of synaptic activity or the presence of dorsal root ganglia.

Our findings suggested that differentiation of motoneuron electrical properties was halted in culture, despite the significant increase in the level of spontaneous activity. Supported by RCDA (NS01314) and NS23808 to L. Z-C.

621.5

CORTICORUBRAL AND CORTICOTHALAMIC PROJECTIONS FOLLOWING UNILATERAL FRONTAL CORTEX LESIONS IN FETAL CATS. P. Carlson-Kuhta*, L.D. Loopuijt and J.R. Villablanca. Mental Retardation Research Center, UCLA, Los Angeles, CA 90024.

We have shown (e.g., *Behav. Brain Res.* 19: 205-226, 1986) that behavioral recovery is greater in the cat when a cerebral hemispherectomy is sustained neonatally (N-HEMI) as compared to the same lesion sustained in adulthood (A-HEMI). Coexisting with the better behavioral outcome is a greater "reinnervation" of the red nucleus (n.) and thalamus in the N-HEMI than in A-HEMI cats (e.g., *Brain Res.* 453: 17-31, 1988). In the present study we sought to determine if a robust "reinnervation" would also be present in cats when a unilateral resection of the frontal neocortex occurred fetally. An unilateral frontal cortex lesion, including the cruciate sulcus and surrounding areas, was performed in 4 cats prenatally (E43-E55). Cats grew to adulthood and then received an injection of [^{3}H]leucine-proline into the remaining sensorimotor cortex. Terminal field densities were estimated in the red n. and thalamus by computer, using video analysis software (Jandel Scientific). Bilateral corticorubral projections were found in the red n. of fetally lesioned cats. The average labeling amount for the red n. ipsilateral to the injection was 543 particles/mm^2 and the contralateral red n. was 66 particles/mm^2. Thus the contralateral red n. only had 12% as much label compared to the ipsilateral red n. By comparison, in control cats the red n. contralateral to the injection had particle counts of only 2% of the amount in the ipsilateral red n. (N = 4 cats). For the thalamus three areas were examined: 1) intralaminar nuclei, 2) ventrobasal complex, and 3) ventrolateral n. Initial analysis of the thalamic nuclei also suggests only a small amount of contralateral corticothalamic projections. Our preliminary results suggest that the "reinnervation" of the red nucleus and thalamus following a prenatally sustained, frontal-cortex lesion is less than has been reported for similar lesions in neonatal cats (*Dev. Brain Res.* 32: 15-30, 1987; *Neurosci. Res.* 12:122-139, 1991). Behaviorally, our fetal-lesioned cats showed less recovery (sparing) of function than neonatal cats (P8-P14) with the same lesion (*Soc. Neurosci. Abstr.* 17: 894, 1991) Grants USPHS HD-05958 and 04612.

621.6

DEVELOPMENT OF CORTICOSPINAL PROJECTIONS TO THE CERVICAL AND LUMBAR SPINAL CORDS OF THE RAT. D.L. O'Donoghue*, R.E. Small, D. McFeeters, S. Fuller, C.R.D. Poff and (+) D.R. Humphrey. Depart. of Anatomical Sci., Univ. of Oklahoma Health Sci. Center, Oklahoma City, OK, 73190; + Lab. of Neurophysiology, Emory Univ. Sch. of Med., Atlanta, GA, 30322.

The precise projection pattern in the corticospinal system serves as a substrate for the control of fine motor functions in most mammalian species. The current series of experiments were designed to test the specificity of the growing corticospinal projections at a time of apparent imprecision in the numbers of corticospinal cells. The collateralization of corticospinal axons were analyzed using multiple retrogradely transported dyes. These studies were intended to quantify branching of corticospinal axons early in development of the rodent tract. The number, distribution, and overlap of cells labeled from the cervical spinal cord (labeled with one dye) were compared with those projecting into the lumbar cord (labeled with a second dye). Roughly only 3.5% of the labeled corticospinal cells showed both dyes when the dyes were placed in a six day old animal. Thus, few cells, even at this early age, showed widely divergent axons. This early pattern is further refined into adulthood. These data indicate the early growth of the corticospinal tract shows a high degree of specificity even though the number of labeled cells is greatly increased. Grant support by the Presbyterian Health Foundation (DOD) & NIH-NS20146 (DRH).

621.7

NORADRENERGIC AND SEROTONERGIC PATHWAYS MAKE SPECIFIC CONTRIBUTIONS TO RECOVERY OF MOTOR FUNCTION AFTER NEONATAL SPINAL CORD LESIONS AND TRANSPLANTS B.S. Bregman,* H.N. Dai, D. Gao, E. Kunkel-Bagden. Dept. of Anatomy and Cell Biology, Georgetown University, Washington, D.C. 20007.

Transplants of fetal spinal cord tissue mediate recovery of locomotor function after spinal cord injury in newborn and adult rats. This recovery of function is dependent upon the anatomical integration of the transplant with the host spinal cord. In order to determine the mechanisms underlying transplant mediated recovery we have begun to examine the contribution of particular pathways to recovery. Rats received spinal cord lesions and transplants at birth, and recovery of locomotor function was assessed quantitatively. After development and recovery were complete rats received intracisternal 6-OHDA injections to remove the noradrenergic input to the transplants or 5,7 DHT injections to remove the serotonergic input to the transplants and the effects on locomotor function were again assessed. Quantitative analysis of locomotion indicates that 6-OHDA lesions results in loss of transplant mediated recovery in some, but not all, aspects of locomotor function. There were significant deficits in base of support, stride length and limb rotation. Individual limb responses (hopping) and accurate locomotion over grid runways were not altered. The consequences of removing 5-HT input differed from that observed after removing noradrenergic input. For example, there were no alterations in hindlimb rotation following loss of serotonergic input. These results suggest TP mediated recovery is not simply a generalized influence on the host spinal cord, rather, particular pathways make specific contributions to recovery of function. Supported by NIH NS 27054 and American Paralysis Association to BSB.

621.8

PATTERNS OF EXPRESSION OF MYOSIN HEAVY CHAIN AND SARCOPLASMIC RETICULUM CA^{2+}-ATPase ISOFORMS IN DEVELOPING CAT HINDLIMB MUSCLES G.A. Unguez*, R.J. Talmadge, D.J. Pierotti, R.R. Roy and V.R. Edgerton. Dept. Physiological Science and Brain Research Inst., UCLA, LA, CA 90024.

Although the contractile and histochemical properties of muscle fiber types have been extensively investigated in the adult cat, there has been minimal characterization of the immunohistochemical properties of muscle fiber proteins to match the physiological properties during development. The present study provides an immunohistochemical analyses of soleus (Sol), medial gastrocnemius (MG) and tibialis anterior (TA) of fetal (E55 in utero), newborn (P1), 10- (P10), 15- (P15), 20- (P20), 30- (P30), 40-day (P40), and adult cats. Muscles were frozen in isopentane cooled in liquid nitrogen. Immunolabelling was performed on serial cryostat sections using monoclonal antibodies (mAbs) specific for fast and slow muscle isoforms for the Ca^{2+}-ATPase of the sarcoplasmic reticulum (SR) and for embryonic/neonatal (emb/neo), slow, and subtypes of fast myosin heavy chains (MHCs). The mAbs against slow and fast SR labelled mutually exclusive populations of fibers in TA, MG, and Sol at all stages investigated. In contrast, the MHC mAbs demonstrated co-labelling of different isoforms in some fibers in each muscle at all, except adult, stages. The slow mAb labelled fibers exclusive to one population in the TA, Sol, and MG in the adult. The emb/neo mAb labelled all fibers until P10 when the label was lost only from those fibers labelled with the slow MHC and SR mAbs, but not labelled with fast MHC mAbs. A population of slow fibers appeared to reach an adult phenotype by P10 in Sol. Further, these early differentiated adult slow fibers had a larger cross-sectional area than those fibers co-labelled with the emb/neo mAb in each muscle from E55 to P30. In contrast, fibers labelled with fast MHC mAbs also labelled with emb/neo mAbs until P40. Thus, it appears that SR and MHC protein systems within individual fibers reached a differentiated state asynchronously and the adult MHC pattern of expression was established after P40 in the cat. Supported by NIH Grants NS-16333 and NIDR NRSA DEO7212.

621.9

A COMPARISON OF THE TENSION TRANSDUCTION DEVICE AND GAIT ANALYSIS MODEL FOR NERVE INJURY AND REGENERATION. P.M. Santos, S.L. Williams, S.E. Seinko Thomas, B. Spyropoulous, H.R. Konrad*, Division of Otolaryngology, Southern Illinois University, Springfield, IL 62794

We have developed two animal models to study the function of the peroneal nerve and anterior tibialis muscle group. Presently the study of nerve regeneration is mostly limited to electrophysiological and histological techniques which lack direct measurement of nerve-muscle force and function. The models described herein utilize the anterior branch of the sciatic nerve, i.e. the peroneal nerve which primarily innervates the anterior tibialis muscle to dorsiflex the foot through the ankle.

The tension transduction device allows accurate force measurements of muscular contraction of dorsiflexion upon peroneal nerve surgical exposure and electrical stimulation. Within animal and between animal testing has revealed very low variability of testing (<2% difference for left/right force development for eight animals). The gait analysis model is designed after human gait studies relating to "drop foot" secondary to decreased function of the peroneal nerve-anterior tibialis muscle group. High speed frame videotaping of rat gait captures the increased ankle angle and other parameters. Data analysis demonstrated no significant left/right differences of gait parameters within non-injured nerves. Nerve crush injury resulted in obvious decreased function with both models. The ankle angle using gait analysis returned to normal by week five; however, force development using the tension transduction device demonstrated a persistent decrement of function two months after injury.

The tension transduction device is more sensitive than the gait analysis model but requires a surgical procedure thereby incurring a slight but reproducible decrement (7%) in nerve muscle force compared to the contralateral control with repeat testing.

621.10

POSTNATAL DEVELOPMENT OF RAT CORTICOSPINAL MOTOR NEURONS: DETAILED QUANTITATIVE EVIDENCE FOR MASSIVE COLLATERAL ELIMINATION AND MINIMAL CELL DEATH

S. Dale*, M. Qudega, S. Varon and T. Hagg

Dept of Biology, University of California San Diego, La Jolla, CA 92093.

The postnatal development of rat corticospinal motor neurons (CSMN) was studied using the sensitive neuronal tracer cholera toxin B subunit (CTB), injected into the upper cervical dorsal spinal cord on the first postnatal day (P0), P3, P10, P20 and at adulthood. CTB labeled neurons were visualized using immunocytochemistry and counted throughout the whole cortex. At P0, CSMN were found to an extent similar as that previously reported in P3 animals with other neuronal tracers. Between P0 and P3, the total number of labeled neurons increased by 24% to a maximum. At P10, the number of labeled CSMN had decreased to 60% of the number at P3. At P20, the number of CSMN had decreased to 52% and remained essentially constant throughout further development and adulthood. The number of labeled neurons in rats injected at P0 and analyzed at P20 was approximately 10% lower than the number in similarly injected littermates which were analyzed at P3, suggesting that only a small portion of the 'disappearing' CSMN die. Thus, the remaining 38% apparently undergo elimination of their spinal projection between P3 and P20. Detailed quantitative analysis of the CSMN distribution demonstrates that neuronal death occurs predominantly in the lateral cortex (perirhinal and entorhinal cortex). The neurons whose spinal projection is "pruned" are localized throughout the cortex, but especially in the parietal and lateral cortex. Support: NINCDS grants NS-16349 and 27047.

621.11

DEVELOPMENT OF CYCLIC MOTILITY IN POSTNATAL RATS. M. Dyer* and C. R. Almli. Lab. of Develop. Neuropsychobiol., Wash. Univ. Sch. Med., St. Louis, MO 93110

Newborn and fetal rats display cyclic spontaneous movements (CSM) at 1 min periodicity. This study assessed the development of the CSM pattern from PN 1 to PN 40.

Rat pups were individually videotaped for 20 min, and movements of individual limbs were scored to computer. Time series for summed forelimb/hindlimb movements were analyzed with FFT.

Results show that on PN 1, forelimbs and hindlimbs exhibit CSM at a 1 min periodicity. At approximately PN 10, the predominant periodicity of CSM is 4 min, with no differences between forelimbs and hindlimbs. Most of these spectra also show the 1 min periodicity. This dual periodicity pattern of CSM does not change substantially with further development.

These results suggest that the fetal/neonatal CSM pattern with a 1 min periodicity continues postnatally, but becomes integrated into a 4 min periodicity that may represent a basic rest-activity cycle.

621.12

MOTOR CORTICAL PLASTICITY IN AGED RATS THAT SUSTAINED UNILATERAL CORTICAL LESIONS AT BIRTH. G.L. Tillotson*, T.P. Hogan, E.J. Neafsey and A.J. Castro. Dept. of Neurology, Hines VA/Loyola and Dept. of Cell Biology, Neurobiology and Anatomy, Loyola University School of Medicine, Maywood, IL 60153.

This study was undertaken to examine the long term effects of neonatal cortical lesions on motor cortical organization . On postnatal day 1, animals sustained large right cortical lesions by aspiration under hypothermic anesthesia. Littermate controls received only hypothermic anesthesia. At 20-24 months of age, animals were anesthetized with ketamine hydrochloride (100mg/kg i.p. with supplemental doses given as needed) and the hair over the forelimbs and hindlimbs was clipped to permit better observations of evoked movements. Animals were placed on a heating pad in a stereotaxic frame, and the cisterna magna was opened to prevent cortical swelling. A craniotomy was made over the left frontal/parietal cortex, the dura removed and the exposed brain covered with saline. Using the method of intracortical microstimulation (ICMS), electrode penetrations were made in a systematic fashion throughout the motor cortex. Observed evoked movements were recorded with threshold currents (the lowest current reliably evoking the response). Following mapping, animals were overdosed with sodium pentobarbital and perfused with saline and buffered formalin. Brains were removed and cut on a cryostat for inspection of the cortical lesions.

Control animals showed no change in the motor map, indicating that normal aging does not affect the motor cortical efferent system when studied with ICMS. However, animals that sustained large neonatal cortical lesions showed reorganization with shifting of the motor map 2.5-3mm anteriorly, indicating cortical remodeling that is persistent into senescence.

(Supported by NIH Grant NS 13230).

621.13

ALTERATIONS IN MOTONEURONAL αCGRP, GAP-43 AND ChAT mRNA LEVELS FOLLOWING MONONEUROPATHY PRODUCED BY FIXED DIAMETER NERVE "CONSTRICTION" IN THE RAT. C.E. Blanco*, T. Mosconi, L. Kruger and P. E Micevych. Dept. of Anatomy & Cell Biology, Brain Research Institute, UCLA School of Medicine, Los Angeles, CA 90024-1763.

Mononeuropathy induced by cuffing the sciatic nerve with polyethylene tubing of fixed diameter results in degeneration of a large proportion of heavily myelinated axons distal to the sleeve. This suggests that the motor component of the sciatic nerve is damaged, and the procedure may result in muscle denervation (Mosconi and Kruger, Soc. Neurosci. Abst., 18:289, '92). Behavioral observations suggest that motor function is maximally impaired within two weeks after nerve "constriction" and is noticeably recovered after four weeks. The purpose of this study was to determine whether the motoneuronal expression of specific mRNAs associated with axonal regeneration (growth associated protein 43; GAP-43), synaptic remodelling (α-calcitonin gene-related peptide; αCGRP) and neuromuscular transmission (choline acetyltransferase; ChAT) were altered by acutely-induced mononeuropathy. Two weeks after slit polyethylene rings were placed on one sciatic nerve, animals were transcardially perfused under pentobarbital anesthesia. The lumbosacral spinal cords were removed and subsequently processed for GAP-43, αCGRP or ChAT *in situ* hybridization histochemistry using ^{35}S-labelled ribonucleic acid probes. GAP-43 and αCGRP mRNA levels were significantly greater in the retrodorsal lateral nucleus (RDLN; sciatic nerve motoneuron pool) of the lumbosacral spinal cord ipsilateral to the site of nerve reaction as compared to the contralateral RDLN. In contrast, ChAT mRNA levels were lower in the ipsilateral RDLN. These observations suggest that the molecular response of motoneurons to "constriction" mononeuropathy is similar to the alterations in mRNA expression during denervation-reinnervation of skeletal muscles. Supported by HD 7228, NS 9176 and NS 5685.

621.14

EARLY POSTNATAL ANTAGONISM WITH MK-801 EXTENDS THE SPARING OF FUNCTION EFFECT AFTER MIDTHORACIC SPINAL TRANSECTION. D.L. Maier* and D.J. Stelzner. Dept of Anatomy & Cell Biology, SUNY Health Science Center, Syracuse, NY 13210.

Weber and Stelzner ('77) found that many hindlimb responses recover after complete midthoracic spinal transection (MTST) in neonatal rats (birth-12 days of age) that do not recover in 15 day-adult operates. Developmental events in the second postnatal (PN) week of life serve to limit plastic processes in the spinal cord. This critical period may be related to N-methyl-D-aspartate (NMDA) receptor-mediated remodeling and stabilization of synapses as descending inputs mature. We hypothesized that chronic blockade of the NMDA receptor during development would extend the sparing of function effect to older operates. Treatment with MK801 from PN day 7-17 significantly facilitated hindlimb recovery after MTST on PN day 18, compared to saline treated operates. The recovery response was characterized by postural and locomotor abilities, reflexivity and tonus, contact placing and coordinated stepping abilities. To determine if the MK801-mediated effect on recovery was due to a reduction in injury-induced ischemic damage to the spinal cord, animals received MK801 30 min prior to MTST on PN day 18. To determine if the MK801-mediated effect was due to manipulation of plasticity only during spinal cord maturation, older animals received MK801 from PN day 19-29 and received a MTST on PN day 30. In each case, recovery of function did not occur. Thus, sparing of function in MK801 treated MTST rats depends on chronic disruption of events during early PN development of the spinal cord that limit the adaptive capabilities of the spinal motor system. Supported by grant NS 14096 (DJS) and NRSA 1F32NS09235-01 (DLM).

621.15

CORRELATED REORGANIZATION OF CERVICAL CORTICOSPINAL TERMINATIONS AND MOTONEURON DENDRITIC FIELDS DURING POSTNATAL DEVELOPMENT IN THE RAT. M.H.J.M. Curfs, A.A.M. Gribnau and P.J.W.C. Dederen (SPON: European Neuroscience Association). Dept. Anatomy and Embryology, University of Nijmegen, P.O. Box 9101, 6500 HB Nijmegen, The Netherlands.

In order to study the development of the corticospinal tract (CST) in relation to that of two motoneuron (MN) pools supplying extensor and flexor muscles in the distal forearm as described previously (Curfs et al., Development 117 (1993) 535-541), HRP gels were implanted in the sensorimotor cortex of Wistar rats varying in age from postnatal day 0 (P0) to P60. After 48 hr 30 μm transverse sections were made of the spinal cord and reacted for HRP using an intensifying incubation method. Drawings of the labelling in the seventh and eighth cervical spinal segments were made being the segments in which the above mentioned MN pools are located.

A delay of 2 days was found between the arrival of the first labelled CST axons in the dorsal funiculus (P2) and their outgrowth into the adjacent gray matter (P4). More fibres are gradually added in an almost radially proceeding way until maximal number and extension is reached by P10. By then the entire gray and large parts of the white matter are covered by labelled CST axons. From P10 onwards the number of labelled fibres decreased and they even disappeared from particular areas such as the lateral part of the ventral and dorsal horn and large parts of the ventral and lateral white matter.

The main conclusion is that the postnatal development of the CST and the MNs in the rat is correlated: both show an overshoot in the first postnatal week, and from the second week onwards, when progressively more mature coordinated movements appear, axons and dendrites respectively are selectively eliminated.

621.16

DISCHARGE PATTERNS IN DEVELOPING HYPOGLOSSAL MOTONEURONS OF THE RAT. P. A. Núñez-Abades*, G. Barrionuevo and W. E. Cameron. Departments of Behavioral Neuroscience and Psychiatry, University of Pittsburgh, PA 15260.

We have investigated time dependent changes in the discharge pattern of a population of brainstem motoneurons *in vitro* (n=74) during the first month of postnatal life. At birth, all ventromedial hypoglossal (genioglossal) motoneurons displayed an adaptation in their repetitive discharge; the number of adapting cells decreased during the first week of postnatal life to 27% giving rise to an almost exclusive (90%) non adapting cell type at ages older than 13 days. These changes in the discharge pattern were accompanied by changes in the shape of the action potential. Specifically, in the first postnatal week, there were significant decreases in the spike half-width and in the duration of the medium AHP ($mAHP_{dur}$), and a more rapid repolarization of the action potential. The spike half-width was positively correlated with the $mAHP_{dur}$ ($r=0.61$, $P<0.001$) implying that broaden spikes permitted a greater influx of Ca^{2+} which, in turn, produced the prolonged $mAHP_{dur}$ recorded at 1-2 days. Genioglossal motoneurons also showed a trend to increase their maximal frequency (highest discharge rate achieved prior to inactivation of the firing mechanism) from 25.5±10.1 Hz at 1-2 days to 42.2±19.7 at 19-30 days. This ability to discharge more rapidly was positively correlated ($r=0.54$, $P<0.001$) with a more rapid repolarization of the action potential. We propose that new channels shaping the action potential and the $mAHP_{dur}$ and ultimately shaping the pattern of discharge are developed during the first week.

This work was supported by NIH grant HD 22703.

622.1

TARGET SPECIFIC GROWTH OF DOPAMINERGIC NEURONS IS ENHANCED BY STRIATAL-NIGRAL CO-GRAFTS IN AFRICAN GREEN MONKEYS.

J.R. Sladek, Jr.*[1], S.K. Schilling[1], B.C. Blanchard[1], T.J. Collier[2], J.D. Elsworth[3], J.R. Taylor[3], R.H. Roth[3], and D.E. Redmond, Jr.[3]. [1]Dept. of Neurosci., Chicago Med School, North Chicago, IL 60064, [2]Dept. of Neurobiology and Anatomy, Univ. of Rochester , Rochester, NY 14642, [3]Depts. of Psychiat. and Pharm., Yale Univ. Sch of Med., New Haven, CT 06510.

We examined the ability of embryonic striatum to enhance survival and growth of dopaminergic (DA) neurons co-grafted into the striatum of adult monkeys. Earlier experiments in rats resulted in enhanced fiber outgrowth from grafted DA neurons and more rapid improvement in agonist-induced rotational behavior (Yurek et al., Exp. Neur. 109:191, 1990). Grafts of embryonic striatum and ventral mesencephalon were implanted simultaneously into the left caudate nucleus, either juxtaposed (in caudal caudate), or separated by at least 2mm (in rostral caudate); single grafts of only ventral mesencephalon were placed into two sites in the contralateral caudate as controls for DA cell survival and fiber outgrowth. Grafts were placed into seven adult, male, monkeys that previously received MPTP, were DA-depleted, but were asymptomatic. Brains were examined for tyrosine hydroxylase (TH) immunohistochemistry and DA and HVA levels six months after grafting. Grafts contained large numbers of well developed DA neurons that ranged from 3000 to 22,000 per host (mean of 11,000). Fibers from grafted DA neurons grew preferentially toward and into the striatal grafts. Striatal grafts contained dense patterns of TH fibers that either filled the striatal graft neuropil or formed "patches." DA levels were markedly increased in the vicinity of the grafts. Striatal grafts that were placed at a distance to nigral grafts appeared to receive TH fibers from the host brain; nigral grafts also appeared to be the source of TH fibers that coursed toward the striatal grafts from contralateral nigral grafts. This study suggests that maximal DA cell survival and target-specific neurite outgrowth occur when mesencephalic grafts are placed in a position to benefit from putative target-derived trophic factor(s) and that striatal grafts can stimulate a regenerative response by the partially depleted nigrostriatal system. Supported by PO1-NS24032, RSA MH00643 to DER and the Axion Research Foundation.

622.2

SPROUTING OF HOST DOPAMINE FIBERS IN RESPONSE TO GRAFTS OF EMBRYONIC STRIATUM IN ADULT AFRICAN GREEN MONKEYS.

R.T. Wechsler*[1], J.R. Sladek, Jr.[1], B.C. Blanchard[1], T.J. Collier[2], J.D. Elsworth[3], J.R. Taylor[3], R.H. Roth[3], and D.E. Redmond, Jr.[3]. [1]Dept. of Neurosci., Chicago Med Sch., N. Chicago, IL 60064, [2]Dept. of Neurobio. & Anat., Univ. of Rochester, Rochester, NY 14642, [3]Depts. of Psychiat. and Pharm., Yale Univ. Sch of Med., New Haven, CT. 06510

We are examining the ability of embryonic striatum to influence growth from host DA systems by intrastriatal implantation into partially DA-depleted animals in an effort to understand the potential for this approach to repair and protect against neurotoxin degeneration. Ten male monkeys received multiple implants of embryonic striatum from one of several embryonic stages ranging from E41 to E59. Brains were analyzed immunochemically for TH-positive neurons and fibers six months after transplantation. Concurrent analysis of DA and HVA was performed. Striatal origin of grafts was verified by dual staining for choline acetyltransferase (ChAT) and TH. Eight animals received grafts within the caudate nucleus; two received grafts within the putamen. Dense DA fiber staining was seen within these grafts. In some instances, it appeared in the form of patches; in others it appeared to completely fill the striatal graft. The source of this DA pattern for caudate grafts is most likely from nigrostriatal fibers. Additionally, a putamen graft received fibers from the basal forebrain. ChAT-positive neurons occurred within the grafts in a density comparable to normal striatum and gave origin to patterns of neurites that overlapped with DA-rich areas. This study suggests that the embryonic striatum may possess a strong trophic influence on DA neurons that can result in regenerative sprouting either from partially damaged DA neurons or collateral sprouting from unaffected DA neurons. The partially DA-depleted animal model used here, represents a useful system for comparison to the early stages of Parkinson's disease at which time repair and/or protection of the DA systems may be possible through the use of a specific trophic factor delivered through an intrastriatal graft of embryonic striatum. Supported by PO1-NS24032, RSA MH00643 to DER and the Axion Research Foundation.

622.3

EFFECTS OF FRONTAL CORTICAL AND SUBSTANTIA NIGRA LESIONS ON GLIOSIS IN THE RODENT STRIATUM.

H.E. Cannon-Spoor*, R. Heim, A.S. Herranz, M. Poltorak and W.J. Freed.
Neuropsychiatry Branch, NIMH Neuroscience Center at St. Elizabeths, Washington, DC 20032 .

We have previously shown that lesions of motor and somatosensory cortex induce astrogliosis in almost the entire striatum. The areas of increased gliosis do not correlate well with damage of the corticostriatal projections, since there is an additional intense periventricular gliosis ipsilateral to the lesions. In order to examine the effect of lesion location, we studied GFAP immunoreactivity in the striatum of the mouse brain after lesions of the medial (cingulate) cortex and lateral (motor and somato-sensory) cortex, after 3 and 12 weeks of survival, and compared it to the immunoreactivity in non-lesioned controls. Numbers of GFAP+ astrocytes were significantly increased in the entire striatum after both medial and lateral lesions at 3 weeks, but remained increased in only the dorso-lateral striatum after 12 weeks. Periventricular gliosis was also found after both medial and lateral lesions, but disappeared by 3 months. To determine the influence of the nigrostriatal pathway on gliosis after cortical lesions we also studied rats with unilateral damage to the substantia nigra (SN). Animals received either small or very large lesions of the frontal cortex. In both groups, dorso-medial striatal gliosis was evident and the striatal localization of increased GFAP+ immunoreactivity did not differ from mice with cortical lesions alone. The results indicate that the periventricular dorso-medial striatum tends to preferentially develop gliosis after cortical lesions, and may be relatively sensitive to disturbances in corticostriatonigral circuits.

622.4

A transgenic model of dopaminergic synaptogenesis using the thymidine kinase obliteration technique to ablate melanotropes of the mouse pituitary.

Richard G. Allen*, Charles Carey, Joel D. Parker, Marty T. Mortrud, and Malcolm J. Low. The Oregon Health Sciences University, Portland, OR 97201

We produced 3 founder lines of transgenic mice harboring a fusion gene composed of the pituitary-specific promoter of the pre-pro-opiomelanocortin gene, driving the herpes simplex viral-1 thymidine kinase. Treatment of these mice with 70 mg ganciclovir/kg body weight (i.p., twice daily for 10-12 days) results in >99% ablation of the pituitary intermediate lobe (IL), a major target for dopaminergic fibers emanating from the hypothalamus. The extent of ablation was determined by immunocytochemistry (ICC) and RIA and was >85% effective between days 3-6 of treatment. Initial ICC indicated that corticotropes are also substantially reduced. ICC staining for tyrosine hydroxylase shows the presence of dense, bundled catecholamine nerve fibers in the vicinity of the ablated IL, compared to diffuse, punctate staining seen in the normal IL. The ablation of the dopamine target cell group, the IL melanotropes, suggests to us the usefulness of this model for the study of neuroendocrine plasticity and synaptogenesis. It has been demonstrated that dopaminergic nerve endings regenerate in the IL within 4 weeks of 6-OH-dopamine destruction, L.C. Saland et.al. 1991. By ablating the IL during postnatal catecholamine innervation of the IL, and by ablating the IL in adult mice, we can determine the requirements for growth and/or regeneration of the terminals for their target IL cells. In summary, we have developed three lines of transgenic mice which can serve as models of neuroendocrine plasticity and dopamine synaptogenesis. This work was supported by NSF grant BNS9108426 to RGA.

622.5

NEURONS IN THE MEDIAL SEPTAL NUCLEUS (MS) ARE CAPABLE OF RESTORING THEIR TRANSMITTER SYNTHESIS AFTER FIMBRIA-FORNIX TRANSECTION

T. Naumann*, P. Kermer and M. Frotscher. Institute of Anatomy, University of Freiburg, D-7800 Freiburg, Germany.

Most MS neurons lose their capability to synthesize transmitter-related enzymes after fimbria-fornix transection (ff-t). We recently showed that identified septohippocampal neurons survived axotomy for up to 10 weeks and displayed fine-structural characteristics of controls. However, the fate and functional role of these neurons remained unclear.

A total of 35 adult Sprague-Dawley rats received a bilateral ff-t. In 10 animals MS projection neurons were prelabeled with Fluoro-Gold (FG) 1 week prior to axotomy by intrahippocampal injections. After various survival times these animals as well as age-matched control rats were processed for choline acetyltransferase (ChAT) immunocytochemistry. The completeness of the bilateral lesions was controlled by acetylcholinesterase staining of both hippocampi.

Three weeks post lesion only 18.8 % of ChAT-positive neurons in controls were counted. Six months after axotomy this value increased nearly 3-fold to 51 %. A fine-structural analysis of backlabeled, axotomized ChAT-positive neurons showed that most of them contained FG resulting from retrograde transport before ff-t. We conclude that many neurons in the MS have the capacity to restore their transmitter synthesis after long survival times. This long-lasting process could not be detected at 10 weeks post lesion where ChAT-positive neurons were still reduced to 22 % of controls.
(Supported by the DFG: Leibniz Program and Fr 620/4-1)

622.6

CELL ADHESION AND EXTRACELLULAR MATRIX MOLECULES ASSOCIATED WITH REGENERATING CNS AXONS AND SCHWANN CELLS OF PERIPHERAL NERVE GRAFTS IMPLANTED IN ADULT RAT THALAMUS.

Yi Zhang, G. Campbell*, P. N. Anderson, A. R. Lieberman, R. Martini¶ and M Schachner¶. Dept of Anatomy, Univ Coll London, ¶Dept of Neurobiol., Swiss Fed Inst Tech Zürich.

In order to gain insight into the possible molecular mechanisms underlying axonal regeneration of CNS neurons, we have investigated the localization of the neurite outgrowth-promoting cell surface molecules L1, N-CAM, its highly sialylated form E-N-CAM and of the neurite outgrowth-promoting extracellular matrix component tenascin in peripheral nerve grafts implanted into the thalamus of adult SD rats. After 1-13 wk, sections were processed for EM immunocytochemistry using a monoclonal antibody against E-N-CAM and polyclonal antibodies against N-CAM, L1 and tenascin. In normal adult rat thalamus, some nonmyelinated axons were N-CAM+ and L1+ but E-N-CAM- and tenascin-. Between 1 and 10 wk after graft insertion, sprout-like regenerating CNS axons in both the brain adjacent to the graft and in the graft were strongly labelled for N-CAM, E-N-CAM and L1. L1 and N-CAM were also found on Schwann cell (SC) surfaces where they were apposed to other SCs, but not on the surface abutting the basal lamina. Tenascin was found at the surface of SCs abutting the basal lamina but not deep within the columns in the absence of axons. In contrast to regenerating PNS axons which frequently lie between SC basal laminae and SC surfaces and are often tenascin-, CNS axons in the grafts were predominantly clustered in the L1+ and N-CAM+ regions at the centre of SC columns and were tenascin+. A few tenascin+ sprouts were identified in the thalamus adjacent to grafts at 2 wk post operation and their numbers increased at 8-13 wk. These results show that regenerating CNS axons express both L1 and N-CAM, which are known to promote neurite extension on SC surfaces *in vitro*. Furthermore, these axons were also immunoreactive for E-N-CAM and tenascin, suggesting that these molecules may play a role in interactions with neighbouring cells.

622.7

MOLECULAR CHANGES IN PURKINJE CELLS (PC) AND DEEP CEREBELLAR NUCLEI (DCN) NEURONS AFTER LESION OR INSERTION OF A PERIPHERAL NERVE GRAFT INTO THE ADULT RAT CEREBELLUM. E.Vaudano[1, 2, *], C.Woolhead[1], P.N.Anderson [1], A.R.Lieberman[1] and S.P.Hunt[2]. [1]Dept. of Anatomy, University College London, London, WC1E 6BT, and [2]Div. Neurobiology MRC LMB, CB2 2QH, Cambridge, UK.

Adult rat DCN neurons can regenerate their axons along a peripheral nerve (PN) graft implanted into the cerebellum, while the PC of cerebellar cortex appear not to be able to do so. To find out if such dichotomy might be correlated with differences in the molecular response of DCN neurons and PC to injury or to the presence of a PN graft, a segment of autologous sciatic nerve was implanted in the cerebellum of adult rats deeply anaesthetized with halothane, and after 7-28 days survival the immunohistochemically detectable levels of the product of the immediate early gene c-jun, of the growth associated protein GAP-43, of phosphorylated neurofilaments (NF) and of the low affinity NGF receptor (NGFr) as well as the expression of GAP-43 mRNA as shown by in situ hybridization, were compared in these two types of neuron. In another group of animals a simple stab wound was made in the cerebellum and the animals were examined at 7 days survival. Neurons immunopositive for c-jun, and expressing GAP-43 mRNA, were found in the DCN ipsilateral to a lesion/graft in all cases examined, while neurons immunopositive for NF and GAP-43 were found in the DCN after simple lesion but not after PN graft. In all cases the DCN neurons were immunonegative for NGFr. PCs adjacent to a lesion/graft had increased immunoreactivity for the NGFr, but were always found negative for the other molecules investigated. Thus injured DCN neurons, which have the established capacity to regenerate their axons along a PN graft, show a prolonged expression of c-jun and have high levels of GAP-43, as has been described for regenerating peripheral neurons. In contrast cerebellar PCs seem not to be able to mount such a response and this might be an important factor in their failure to regenerate axons into PN grafts.

622.8

BANDS IN THE TRANSCOMMISSURAL OLIVOCEREBELLAR PROJECTION FOLLOWING UNILATERAL PEDUNCULOTOMY IN THE NEWBORN RAT ARE ALIGNED WITH ZEBRIN II-DEFINED PURKINJE CELL COMPARTMENTS. M. Zagrebelsky[1], F. Rossi[1], R.C. Hawkes[2] and P. Strata*[1]. 1: Dept. of Human Anat. & Physiol., Univ. of Turin, Italy; 2: Dept. of Anatomy and Neuroscience Research Group, Univ. of Calgary, Canada.

Following the unilateral transection of the inferior cerebellar peduncle in neonatal rats, a process of transcommissural sprouting of the olivocerebellar projection is observed. Anterograde tracing of the surviving olivary axons, two months after the pedunculotomy, shows that the compensatory projection follows a parasagittal band pattern highly symmetrical with that seen in the not-denervated hemisphere. The anti-Zebrin II monoclonal antibody reveals a subset of Purkinje cells that are arranged into parasagittal compartments separated by similar bands of unlabelled cells. These compartments are related to the normal distribution of the olivocerebellar projection. In the present study we have combined PHA-L tracing and anti-Zebrin-II immunocytochemistry to determine if the alignment between climbing fibre stripes and Purkinje cell bands is maintained by the compensatory projection. Numerous PHA-L labelled olivocerebellar axons crossed the midline in the cerebellar white matter to terminate in the denervated hemicerebellum in distinct parasagittal climbing fibre stripes, symmetrical with those in the intact side. In both hemicerebella there was a strict correlation, up to the single cell level, between climbing fibre stripes and Purkinje cell compartments. These observations indicate that target specificity is maintained during the process of transcommissural sprouting. Therefore we conclude that transcommissural reinnervation in the neonate is not random, but rather is directed by specific cues.

622.9

INGROWTH OF SEROTONERGIC AXONS INTO INAPPROPRIATE TARGETS ARISES FROM AXON COLLATERALS. H. Bernstein-Goral*, P. Diener-Ostfield, M. M. McAtee, and B. S. Bregman. Georgetown University School of Medicine, Washington, DC 20007.

Axotomized raphe-spinal neurons are capable of permanent regenerative axonal growth into and through fetal spinal cord (SC) transplants placed into the site of either a spinal cord hemisection or transection in the neonatal rat. If an inappropriate target such as fetal hippocampus (HC) is introduced into a neonatal hemisection, serotonergic (5HT) axons initially innervate the transplant, but are later withdrawn during development. After a neonatal transection, however, 5HT axons do not regenerate into an inappropriate target. The current study was designed to determine whether the transient ingrowth of 5HT axons into an inappropriate transplant arises from axonal sprouting of neurons which maintain collaterals to uninjured spinal cord targets. A retrogradely transported fluorescent tracer (FB or DiO) was injected into the transplant and a second tracer (DY or DiI) was injected in spinal cord caudal to the lesion site in 10 day old rat pups with a neonatal hemisection, plus either a fetal SC or HC transplant. Double labeled neurons were present in brain within the caudal raphe nuclei, red nucleus and sensorimotor cortex with a HC transplant at the site of the hemisection. Long distance growth of regenerating axons through a SC transplant was reflected by a large proportion of single labeled (DY or DiI) neurons in the axotomized raphe and RN. We suggest that 1) the transient serotonergic innervation of inappropriate targets arises from axon collaterals and 2) the requirements for axonal growth after injury differ for regenerating axons and sprouting axons. Supported by NS 19259 and RCDA NS 01356 to BSB.

622.10

FATE OF PROTEINS INJECTED INTO AXONS OF APLYSIA NEURONS: RETROGRADE TRANSPORT VERSUS UPTAKE INTO MULTIVESICULAR BODIES. R. Schmied*, X-P Zhang, and R.T. Ambron. Anatomy and Cell Biology, Columbia Univ., New York, NY 10032.

During axonal growth and remodeling, newly synthesized proteins arrive from the cell body while those that are obsolete must be removed. To identify factors that regulate the fate of proteins in axoplasm, we injected a variety of proteins into axons of neurons regenerating *in vitro*. The proteins either: 1) remained diffusely distributed at the injection site; 2) were retrogradely transported to the cell body; or 3), were taken up into organelles. Transported proteins included human serum albumin (HSA) coupled to a 7 amino acid signal peptide (sp). The sp is important, since HSA without sp was taken up into organelles, as was HSA coupled to another peptide similar to sp.

To identify organelles that take up the HSA, we injected 15 nm colloidal gold particles (Au), coated with either HSA or HSA-sp, into the axon. 1 hour after injection, the cells were fixed and examined by EM. Almost all of the HSA-Au (n=120) were inside organelles. 24% were inside multivesicular bodies (MVBs); organelles with similar morphology contained acid phosphatase. Other HSA-Au were in vesicles and large vacuoles. In contrast, 62% sp-HSA-Au (n=671) were associated with an amorphous, electron-dense material in axoplasm. Of the rest, 16% were inside vacuoles or vesicles, 10% were in MVBs, and 9% were on the surface of vesicles. These findings indicate that soluble proteins can be removed from axoplasm by lysosomal structures and that sp protects HSA from uptake, either by enabling it to gain access to the retrograde transport system, or by blocking a feature of HSA that makes it susceptible to uptake.

622.11

EVIDENCE THAT THE RETROGRADE TRANSPORT - NUCLEAR IMPORT PATHWAY CONVEYS AN INJURY SIGNAL AFTER AXOTOMY OF APLYSIA NEURONS. R. T. Ambron*, R. Schmied, and A. Osipov. Anatomy and Cell Biology, Columbia University, New York, NY 10032

A pathway in Aplysia neurons conveys proteins from the distal axon to the cell body and then into the nucleus. We believe this pathway communicates the needs of the axon periphery to the biosynthetic centers in the cell body. Transport through the axon is rapid and exclusively in the retrograde direction and access to the transport and import processes requires the same 7 amino acid signal peptide (sp). Using an affinity-purified antibody to the sp, we identified several endogenous sp-containing proteins, including 75, 83, and 110 kDa species that were present in both axoplasm and the nucleus, indicating that they use the pathway (Schmied et al. *J. Neurosci.* 1993).

One function of the pathway might be to convey a signal back to the nucleus after axonal injury. To test this idea, we crushed Aplysia peripheral nerves *in situ* and placed a ligation between the crush site and the cell soma. Other nerves were ligated only, as controls. 1-2 days later, axoplasm was extruded from the retrograde side of each ligation and equal amounts were subjected to SDS-PAGE. In 3 experiments, employing nerves from a total of 11 animals, Western blots showed that sp83 and sp97 were enriched in the axoplasm behind the ligation of crushed relative to non-crushed nerves; presumably both constituents would have been transported to the cell body and into the nucleus had the ligation not been present. In addition, the amount of sp97 was greatly diminished on the cell body side of the crush site. These results are consistent with a role for the transport/import pathway in conveying these proteins during regeneration. This can be tested by injecting these proteins into neurons in order to mimic the electrophysiological properties elicited by axotomy.

623.1

A GOLGI STUDY OF OLFACTORY BULB TRANSPLANTS IN THE RAT. M. D. Gooden[1], J. N. Kott[1], B. R. Fink[3]*, L. E. Westrum[1,2]. Depts. of Neurosurgery[1], Biol. Struct.[2], and Anesthesiol.[3]. Univ. of Washington, Seattle, WA 98195.

Studies in this laboratory have shown that olfactory bulb transplants (OBTXs) differ from normal OBs in regard to cellular architecture. In contrast to the distinct laminar organization found in normal OBs, OBTXs manifest a more disorganized composition. Cellular types tend to "cluster" together; glomeruli likewise seem to fuse, losing their distinct ovoid shape. In addition, the various cell types and glomeruli are found in aberrant places throughout the OB. Our study was undertaken to examine the atypical features of OBTXs, using variations of the rapid Golgi method. This procedure has the advantage of delineating whole-cell configurations in detail, thereby permitting a more definitive look at bulbar structure in terms of specific cell types, neuronal orientation, and glomerular composition. Golgi processing also allows comparisons between the overall architectures of OBTXs and normal OBs. Tritiated thymidine autoradiography is used in conjunction with Golgi processing in order to distinguish donor from host tissue. Donor OBs are taken from 14 day old rat fetuses and TX into 1 day old (unilaterally bulbectomized) rats of the same strain. At 8-12 weeks, the rats are anesthetized, sacrificed, and the brains processed by variations of the rapid Golgi method. Results demonstrate that all cell types [mitral, tufted, juxtaglomerular (JG), granule and fusiform] are present in the TX. There is, however, a preponderance of fusiform cells as compared to normal OBs. Apical and basal dendritic processes of mitral cells are sometimes atypically oriented. Olfactory nerve axons frequently penetrate clusters of glomeruli after traveling in long, cable-like fascicles. Several cell types participate in the clustering, and their dendrites seem to contribute to glomerular structures. Results therefore suggest that, although all cell types are present in the TX, there are significant architectural differences between normal and TX OBs. (Supported by NIH Grant NS09678. LEW is a research affiliate of the CDMRC).

623.2

DIFFERENCES BETWEEN NERVE GROWTH FACTOR RECEPTOR (NGFR) AND PLATELET DERIVED GROWTH FACTOR B-CHAIN (PDGFB) EXPRESSION IN THE ENSHEATHING GLIA OF THE DEVELOPING AND TRANSPLANTED RAT OLFACTORY BULB. J. N. Kott[1*], B. L. Goheen[1], M. E. Lee[2], M. A. Bothwell[3], L. E. Westrum[1,2]. Depts. of Neurosurgery[1], Biol. Struct.[2], and Physiol. and Biophys.[3], Univ. of Washington, Seattle, WA 98195.

Ensheathing cells (EC) are a glial subtype that is interposed between the peripheral and central nervous system (CNS) along the pathway of incoming axons of the olfactory receptor neurons. These neurons are replaced throughout life, and of necessity, the axons of newly generated ONs must pass from the periphery into the CNS. It has been hypothesized that it is some unique characteristic(s) of the ECs that enable(s) this repenetration into the CNS. At least two growth associated molecules have been attributed to the layer of ECs surrounding the olfactory bulbs (OBs): 1) p75NGFR and 2) PDGFB*.

Using immunolocalization in adjacent paraffin sections, we are examining the distribution of these two molecules in developing and transplanted (TX) OBs. Such examinations suggest nearly separate sublayers within the overall EC layer. In the normal immature OB, PDGFB reactivity is common to most if not all components of the internal sublayer of ECs, while NGFR reactivity is more sparse, and restricted to the external sublayer. In the TX OB, NGFR reactivity appears to be limited to a small subpopulation of PDGFB reactive cells. These studies clearly demonstrate that the EC layer is not made up of a homogenous population of cells, but rather is separable on the basis of the two growth associated molecules. (Supported by NIH Grants NS09687, NS29582, and NS07144. LEW is an affiliate at the CDMRC.)
* Anti-PDGFB kindly provided by Dr. R. Ross and Mochida Pharmaceutical Company, Tokyo, Japan.

623.3

SYNAPTIC RECONNECTIVITY OF TRANSPLANTED OLFACTORY BULBS IN RATS. [1]B.L. Goheen,* [1]I.N. Kott, [1]N.L. Anderson and [1,2]L.E. Westrum. [1]Depts. of Neurosurgery, [2]Biol. Struct., Univ. of Washington, Seattle, WA 98195.

Previous studies suggest that transplanted olfactory bulbs (TX OBs) display reconnectivity between host olfactory nerve (ON) and donor TX OB at the behavioral and light microscopic levels. In this study TX OBs are examined with the electron microscope (EM) using immunolocalization with an antibody against olfactory marker protein (OMP)* enabling us to differentiate between host ON axon terminals and donor TX OB processes. Subjects are anesthetized and perfusion fixed with an aldehyde mixture, and the TX OB is dissected out and embedded directly in medcast/araldite without osmium fixation. Ultrathin sections of the tissue for EM are reacted for OMP which is subsequently conjugated to colloidal gold labeled secondary antibody for visualization. The sections are stained using osmium vapors, followed by uranyl acetate and lead citrate. ON fibers are OMP reactive, allowing us to visualize mature neurons from host innervating the TX OB. OMP positive axonal terminals establish synaptic contact with unlabeled donor processes, which correlates with the observed reinnervation in host animals. These findings suggest that host ON axons are able to innervate the TX OB and clearly demonstrates that synaptic connectivity is established between host ON and donor OB. (Supported by NIH Grants NS09678 and NS07144. LEW is a research affiliate of the CDMRC. *Anti-OMP kindly provided by Dr. Frank Margolis).

623.4

CROSS-STRAIN TRANSPLANTATION OF OLFACTORY BULBS IN RAT. [1]M. E. Lee*, [1]J. N. Kott, [1,2]L. E. Westrum, [3]R. D. Lund. [1]Depts. of Neurosurgery and [2]Biol. Struct., Univ. of Washington, Seattle, WA 98195 USA; [3]Dept. of Anatomy, Univ. of Cambridge, Cambridge, England CB2 3DY.

We have previously shown that olfactory bulb (OB) transplants (TXs) into rat hosts of the same strain survive, are reinnervated and become connected with host brain, whereas mouse OB TXs placed in rats are rejected. In an attempt to evaluate the usefulness of cross-strain TXs we are transplanting OBs from Long-Evans (LE) rat fetuses into Sprague-Dawley (SD) rat hosts of similar ages to our previous studies. Time-mated LE dams received injections of tritiated thymidine on embryonic days (E) 12-14. OBs from LE fetuses (E14-15) are immediately transplanted into newborn SD rats in the site from which the host OB was removed. At 2 weeks and later sagittal frozen sections of forebrain were stained for cells and fibers or immunoreacted for olfactory marker protein (OMP antibody kindly provided by Dr. Frank Margolis), the latter a label for mature primary olfactory nerve fibers. Autoradiography was carried out on alternate series of sections to identify and characterize the donor OB TX. The LE OB TXs occur in a high proportion of SD hosts, are attached to the olfactory peduncle and are robust, often near the size of the normal OB. The cytoarchitecture is somewhat disorganized as in SD only TXs, but autoradiography demonstrates large numbers of heavily labeled donor cells of various sizes. OMP material shows primary olfactory nerve fibers innervating the LE OB TX whereas the cell-fiber stains show continuity between the TX and the ventral forebrain. These results indicate that cross-strain transplantation in this system is successful and suggests that this model might hold promise for immunological manipulations, similar to but less problematic than cross-species TXs (xenografts). (Supported by NIH Grant NS09678. LEW is a research affiliate of the CDMRC).

623.5

EFFECTS OF AMPA LESIONS OF NBM AND SUBSEQUENT EMBRYONIC TRANSPLANTS ON CORTICAL SEROTONERGIC MECHANISMS IN RATS. F. A. Abdulla, M.-R. Calaminici, M. A. Simmonds*, J. D. Sinden and J.D. Stephenson. Institute of Psychiatry, Departments of Psychology and Neuroscience, Denmark Hill, London SE5 8AF and School of Pharmacy, Department of Pharmacology, Brunswick Sq, London WC1N 1AX, U.K.

The effects of destroying the cholinergic innervation to the cerebral cortex by unilateral S-AMPA (2 injections of 0.5μl of 10 mM) lesions of nbm and of subsequent transplants to the cortex on the sensitivity of frontal cortex neurones to raphé nuclei stimulation (a 300 μA square wave for 5ms) and to 5HT applied iontophoretically (30 nA ejection current for 20s) were studied in anaesthetized rats. Responses were sought within 2s of raphé nuclei stimulation or during or within 20s of drug application and compared to baseline firing rates.

The percentage of frontal cortex neurones responding to raphé stimulation 8-10 weeks post-lesion, when AChE-positive fibres were almost completely absent from the area, was significantly reduced, 31/83 compared to 46/79 neurones ($\chi^2=7.07$, $P<0.01$, 5 rats in each case) while the percentage of neurones responding to 5-HT increased, 70/83 compared to 49/79 neurones ($\chi^2=10.33$, $P<0.005$). Six-8 weeks after cholinergic-rich transplants of basal forebrain (E-15) to the lesioned frontal cortex, when transplant-derived fibres were distributed to the recording area, sensitivity of frontal cortex neurones to raphé nuclei stimulation and to iontophoresied 5-HT was partly restored. Non-cholinergic transplants of hippocampus (E-17) to frontal cortex were ineffective. Sensitivity to glutamamte was not affected by the procedures.

The observed changes in frontal cortex neurones sensitivity to raphé nuclei stimulation and to 5-HT applied iontophoretically could be due to functional changes in the serotonergic cortical input following nbm lesion and may account for dysfunction of the serotonergic system in aging and in Alzheimer's disease.

623.6

CORTICAL GRAFTED C6 RAT GLIOMA AND XENOGRAFTED FRESH HUMAN GLIOBLASTOMA CELLS EXPRESS GUANIDINOBENZOATASE. W.J. Goldberg* and J.J. Bernstein. Stroke & Trauma Lab. and Lab. of CNS Injury and Regeneration, Dept. Veterans Affairs Medical Center, Washington, DC 20422.

C6 rat glioma cells and fresh human glioblastoma cells grafted to rat cortex migrate throughout the host brain. C6 cells form large tumor masses in immunocompetent rats whereas fresh human glioblastoma cells do not. In the present experiments 10^6 C6 cells were grafted as suspensions into freshly made implantation pockets in host rat cerebral cortex. In a second series of animals, suspensions of human malignant glioma cells were xenografted into athymic host cortex to test tumor mass formation. Animals were sacrificed at 1,3,5,7,14,21 and 30 days post-implantation. Using the fluorescent active-site probe 9-aminoacridine, paraffin sections were examined for the presence of the serine protease guanidinobenzoatase (GBZ), which is associated with many metastasizing malignant tumors. Paraffin sections of the original human gliomas were also examined. A class of GBZ+ cells was observed in the original human gliomas and cultures of C6 rat glioma cells. Grafted cells retain the ability to express GBZ. Individual GBZ+ rat and human cells migrated on the glia limitans and parallel and intersecting nerve fiber fascicles of the corpus callosum. Large tumor masses containing GBZ+ cells formed in the implantation pocket. Secondary tumor foci containing GBZ+ cells formed around blood vessels. Supported by DVA and NIH, NCI, CA48956.

623.7

IMMUNOCYTOCHEMICAL EXPRESSION OF SOME METABOLIC MARKERS IN NEOCORTICAL TRANSPLANTS. J. Rosenstein and R. Walsh*. Depts. of Anatomy and Neurosurgery, The George Washington University Medical Center, Washington, D.C. 20037.

By its nature transplantation defines a different developmental status for fetal tissue when it is divorced from its normal environmental cues. It is not yet understood if connectivity levels are a reflection of the biochemical nature of grafted neurons which may discourage appropriate contacts or conversely if the connectivity levels are indicative of neuronal metabolism. In order to determine potential metabolic maturity in neocortical grafts, a series ranging between 10 days and 15 months postoperative was examined for the immunocytochemical expression of neuron specific enolase (NSE) and the glucose transporter (GT). Anesthetized rats were perfused with 4% paraformaldehyde. NSE is a reliable marker for neuronal developmental and synaptic maturity and GT is a marker for barrier competent cerebral vessels. When compared to a normal developmental series where all neurons express NSE, at early times NSE in the grafted neurons was practically non-existent and at later times remained weak; at 15 months only a minority of grafted neurons which appeared normal by Nissl staining standards, had normal NSE expression. GT which is expressed on embryonic vessels, was repressed on graft vessels for up to 4 weeks following surgery but reached near normal levels at later times. These results demonstrate a reduced expression in two metabolic markers, NSE and GT, following neocortical grafting. The presumed metabolic reduction when compared to normal activity may be due to incomplete migration patterns, reduced connectivity or potential ischemia causing lowered protein synthesis. (NS-17468).

623.8

RELATIONSHIP BETWEEN β-ADRENERGIC RECEPTORS AND ANTIDEPRESSANT ACTIVITY OF MONOAMINERGIC NEURAL TRANSPLANTS IN THE RAT NEOCORTEX. D. Dougherty*, J.R. Unnerstall, C.E. Sortwell and J. Sagen. Dept. Anat. and Cell Biol., University of Illinois at Chicago, Chicago, IL 60612.

We have shown that the transplantation of monoaminergic tissue into the frontal neocortex of rats can reduce behavioral deficits in rodent depression models. Further, both α- and β-adrenergic antagonists not only reverse the effects of the transplants on the behavior but in fact exacerbate the deficit, suggesting changes in receptor sensitivity. Although our previous α_1- and β-receptor binding studies showed no significant changes in the number of monoaminergic sites between transplant and control in the small area around the transplant, we decided to look for more widespread receptor changes. Therefore, this study investigates changes in β-receptor density in coronal sections of brain associated with the transplants using receptor autoradiography. Monoaminergic tissue (adrenal medulla, pineal gland or a combination of both) was transplanted into the frontal cortices of separate groups of rats. Striated muscle was transplanted for control in other groups. Another group was treated with imipramine daily for six weeks. All animals were tested for behavioral deficits using the forced swimming test six weeks post-transplantation. Significant reductions in immobility scores were obtained in imipramine treated animals and all three monoaminergic transplant groups, but not in control transplanted animals. Following testing, animals were decapitated and 10 μm coronal sections were taken through the transplant region. [^{125}Iodo]pindolol (600 pM) was used as the ligand to investigate β binding sites. As previous receptor binding studies have shown, significant decreases in β-receptor binding following imipramine treatment were seen. In contrast, no such decreases were seen in any of the transplant groups. These results suggest that previously seen behavioral and pharmacological changes in the transplanted animals are not associated with large changes in β-receptor density. Supported by NIMH MH47491.

623.9

DO EMBRYONIC NEURAL GRAFTS INDUCE REPAIR BY THE INJURED JUVENILE NEOCORTEX?
J. Ourednik, W. Ourednik, H. Van der Loos & B. M. Riederer*. Institute of Anatomy, University of Lausanne, 1005 Lausanne, Switzerland.

Pieces (0.02mm^3) of presumptive neocortex from 14 day old mouse embryos were placed into freshly prepared cavities (2mm^3) in the parietal cortex of 20 day old mice (n=21; one graft per cavity). The survival times of the hosts were 3 days (n=10) or 2 months (n=11), and for each group, sham-operated animals (cavities without graft, n=10 and 7, respectively) were prepared in parallel. The identification of graft and host tissue in mice with long-term survival was made possible by the choice of two mouse substrains (C57BL/J6) which express two different allelic forms of the Thy-1 antigen and therefore are distinct in their immunohistochemical staining pattern. Moreover, ^{3}H-thymidine (^{3}H-T) was used to monitor cell division by injecting either host or donor.

At 3 days after operation, the graft, labeled with ^{3}H-T prior to transfer, could be well distinguished from the host tissue as a compact mass of undifferentiated radioactively labeled (DNA synthesizing) cells, while ^{3}H-T labeling of the host showed a significant number of radioactive cells in the tissue surrounding the grafted cavity. Haematoxylin staining revealed cells with mitotic figures in graft and host. When immuno-stained for cell type-specific markers, dividing graft and host cells were either positive for the glial marker GFAP or for the neuronal marker MAP2. At 2 months after operation, in 8 out of 11 grafted mice new and organized cortical tissue had taken the place of the cavity. In 6 of these animals, the grafted region contained, however, no cells positive for the donor-specific Thy-1 antigen, but the area was positive for the Thy-1 antigen of the host.

Our data suggest that the graft may induce some restorative mechanism within the injured cortical area and that cellular proliferation in the host may contribute to the final restoration of the grafted region in an unexpectedly important way. Support: Swiss NSF 31-30932.

623.10

MORPHOMETRIC STUDY OF FETAL BRAIN TRANSPLANTS IN THE INSULAR CORTEX AND NGF EFFECTS ON NEURONAL DEVELOPMENT. S. Díaz-Cintra*, P. Rivas-Manzano, E. Pérez-Torrero, M. Escobar, L. Parra, Bermúdez-Rattoni F. and L. Cintra. Investigaciones Biomédicas, Fisiología Celular, y Facultad de Ciencias, UNAM., México D.F.

Twelve rats previously insular bilaterally cortex lesioned, received one of the three similar neural grafts, one with only insular fetal cortex (IC) , other with IC, and NGF plus their DMEM, and third one, with IC and DMEM. The IC tissue was from fetuses of E16 and applied into the lesioned area. After 15 days of recovery, all animals underwent to silver techniques. The histological analysis showed well impregnated neurons, glial cells and mitotic figures in all grafts. Morphometric study was made, in a total of 175 cells arising from these groups, and analyzed using an imaging (BIOCOM) system. Results showed significant increase in the minor axis of neurons in the DMEM group. Astrocytes, had significant reductions in their minor axis in the DMEM and NGF groups and mitotic figures presented significant increases in number and mayor and minor axes in the NGF group. (Support DGAPA IN204689 and IN204892).

623.11

NEGATIVE LONG-TERM EFFECTS OF INSULAR CORTEX GRAFTS ON LEARNING. M. L. Escobar*, C. E. Ormsby, N. Jiménez and F. Bermúdez-Rattoni. Instituto de Fisiología Celular, UNAM, México, D.F. 04510.

It has been shown that insular cortex (IC) grafts induce recovery on the ability to acquire learning tasks on previously IC lesioned animals at 60 days after graft. Moreover, we have demonstrated that cortical grafts with nerve growth factor accelerates recovery of the acquisition of learning abilities at 15 days post-implantation.

In the present work, we evaluated the long-term effects of IC-grafts, alone or combined with neurotrophic factors, on the ability to learn. Four groups of male Wistar rats received bilateral lesions in the IC and one group remained as an unoperated control. Ten days later, three of the lesioned groups received homotopic fetal IC grafts combined with either nerve growth factor (20μg/ml), epidermal growth factor (20μg/ml), or vehicle. The fourth group remained as a lesioned control. All groups were trained 130 days later in an inhibitory avoidance and Morris water maze tasks. We found that at 130 days after grafting the animals that received IC grafts with or without neurotrophic factors did not show recovery in the ability to learn both tasks, compared with control and lesioned animals. These results showed that rats with homotopic IC grafts, despite the presence of neurotrophic factors, failed to show behavioral recovery, after 130 days post-transplantation.
Supported by DGAPA IN204689 & CONACyT 0178-N0107.

623.12

FATE SHIFTING OF CEREBELLAR PRECURSOR CELLS UPON TRANSPLANTATION INTO THE DEVELOPING DENTATE GYRUS. *C. Vicario, M.G. Cunningham and R.D.G. McKay*, NIH, NINDS, Laboratory of Molecular Biology, Building 36, Room 3D02, Bethesda, MD 20892, USA.*

The aim of this work was to ask if environmental factors might determinate the final fate of cerebellar precursor cells. Cell suspensions prepared from the cerebella of postnatal-day-2 (P-2) rat and mouse were stereotaxically transplanted into the developing dentate gyrus of rat (P-2) or mouse (P-4). The implanted cells were distinguished by thymidine labelling or by the expression of a transgene, the *lacZ* gene under the control of a neuron-specific enolase promoter. Cryostat sections from grafted animals were stained for cell-type specific antigens, reacted with 5-Br-4-Cl-3-indolyl-β-galactoside (X-Gal) and processed for autoradiography. Cerebellar cells grafted into the dentate gyrus integrated into the granule cell layer expressing calbindin (CBP) and, during kainic acid-induced seizure activity, upregulated c-FOS proteins. Transplanted cells expressed glial fibrillary acidic protein (GFAP) when found in the hilar region and in the molecular layer of the dentate gyrus. These results indicate that there are cells in the developing cerebellum of rat and mouse which can give rise to hippocampal neurons and astrocytes in response to local signals. These observations suggest that there is an equivalent multipotential stem cell in different regions of the developing mammalian CNS.

623.13

TRANSPLANTATION OF NORMAL EMBRYONIC CEREBELLAR CELL SUSPENSIONS INTO THE CEREBELLUM OF LURCHER MUTANT MICE.
J.A.Heckroth* and M. H. Foster. Indiana University School of Medicine, Biomedical Research Institute, Terre Haute, IN, 47809.

Cerebellar cell suspensions of normal embryonic day 12 embryos were injected into the cerebellar parenchyma of *lurcher* mutant hosts of 10-12 days (n=8), 17-20 days (n=12), or 1-3 months (n=10) of age. Following a 1-2 month survival period the graft hosts were sacrificed, transcardially perfused, and their brains processed for immunohistochemistry using anti-CaBP (28 kilodalton vitamin D dependent calcium binding protein) serum provided by Dr. Anthony Norman (University of California, Riverside). Adult *lurcher* cerebella without grafts exhibit only a few isolated Purkinje cells. Successful grafts were visible as clusters of immunoreactive cells surrounding the transplantation site. Grafted Purkinje cells infiltrated all layers of the cerebellum, but exhibited a predilection for occupying the host molecular layer. Dendrites of grafted Purkinje cells which ramified within the molecular layer did not appear to adopt their characteristic planar morphology. In some cases immunoreactive axons were observed to connect the region of the graft with the host cerebellar nuclei. Grafts placed into the 17-20 day old hosts were occasionally very large with extensive innervation of the host nuclei by grafted neurons. Grafts in 10-12 day old hosts exhibited less extensive spread in the host cortex, but still displayed significant infiltration of the host molecular layer, and in some cases innervation of host cerebellar nuclei. Successful grafts in juvenile *lurcher* hosts may permit the trophic rescue of host neurons which would undergo retrograde transneuronal degeneration in the absence of transplanted target neurons.
Supported by the National Ataxia Foundation, and by grant 5 S07 RR5371 awarded by the BRSG Program, Division of Research Resources, NIH.

623.14

SPINAL CORD GRAFTS IN OCULO: FUNCTIONAL CONNECTIONS WITH COGRAFTS OF SPINAL CORD OR SKELETAL MUSCLE, EFFECTS OF CNTF.
K. Trok, M. Eriksdotter-Nilsson*, M. Palmer[1], B. Hoffer[1], L. Olson. Dept. of Histology & Neurobiology, Karolinska Institutet, Stockholm, Sweden, and [1]Dept. of Pharmacology, Univ. of Colorado Health Sciences Center, Denver, CO 80262, USA.

Fetal spinal cord tissue survived grafting to the anterior chamber of the eye of adult rat hosts. We have previously demonstrated that such grafts can receive inputs from co-grafts of serotonin as well as noradrenaline neurons and also become functionally connected to cortex cerebri cografts. In this study we wished to examine how treatment with ciliary neurotrophic factor (CNTF), cografting with another fetal spinal cord and cografting with fetal skeletal muscle can influence development and maturation of spinal cord tissue. (1) E18 fetal spinal cord grafts treated with CNTF at grafting and, by intraocular injections 5, 10, 15, and 20 days after grafting did not differ in volume increase compared to controls as measured through the cornea. Animals were sacrificed after 6 weeks and the grafts examined histologically. We observed a larger number of large neurons in the CNTF-treated grafts. (2) Fetal rat E15 spinal cord was grafted together with E20 skeletal muscle to the anterior chamber. Controls received single grafts of either spinal cord or skeletal muscle. Spinal cord grafted together with skeletal muscle grew to larger volumes than single spinal cord grafts. Contractions in the skeletal muscle cografts after stimulation of the spinal cord demonstrated specific functional contacts between the grafts. After maturation in oculo the double grafts were examined histologically and immunohistologically using antisera to neurofilament (NF), glial fibrillary acidic protein (GFAP), acetylcholinesterase (AChE) and calcitonin gene-related peptide (CGRP). The immunohistochemical results also demonstrate that spinal cord can innervate skeletal muscle. (3) The pieces of fetal E15 spinal cord were sequentially cografted to the anterior chamber. The double grafts were allowed to mature in oculo. Preliminary electrophysiological examinations indicate functional contacts between these spinal cord double grafts. We conclude that spinal cord grafts can establish functional contacts with both adjacent spinal cord grafts and skeletal muscle and that CNTF appears to stimulate the grafted motoneurons.

623.15

SUCCESSFUL DEVELOPMENT OF MAMMALIAN EMBRYONIC EYE TRANSPLANTS WITHIN THE ANTERIOR CHAMBER.
H. Klassen*, M. K. Klassen, N. Chaudhry, P. Liggett, C. Barnstable, Dept. of Ophthalmology, Yale Univ. Sch. of Med., New Haven, CT 06510

Retinal grafts can respond to photic stimuli in a phasic, intensity dependent manner and transmit this information to the host neural circuitry, resulting in a motor response. A major limitation of retinal grafts is the failure to maintain topography when innervating host visual centers. It is not known why retinotopy is lost in this model. Transplantation of isolated retina disrupts the normal relationship between adjacent tissues, as well as the normal semi-spherical retinal shape. Conversely, by preserving these relationships, grafted whole eyes might achieve a more accurate axonal projection pattern. The survival of such grafts is, however, less assured given the greater immunogenic load presented to the host. Furthermore, it has not been established that a complex organ, such as the eye, can develop from an early embyonic graft in mammals. Eyes were removed from Long Evans rats at embryonic day (E) 15-17 and transplanted to the anterior chamber of adult albino or pigmented rats. Similarly, embryonic eyes from both rat and rabbit (E14-18) were grafted to the anterior chamber of adult rabbits. After 14 days host eyes were fixed for histology. Grafted eyes survived without immunosuppression and were extensively vascularized by host vessels. The eye grafts exhibited a substantial degree of tissue differentiation, with distinct retinal laminae and a high degree of spherical geometry. Of particular interest was the intimate apposition of photoreceptors and RPE. Taken together, these results show that early embryonic eye can differentiate into a normal-appearing organ following transplantation. This finding has important functional implications and suggests that such grafts may be capable of extending topographic projections.

623.16

HISTAMINERGIC INNERVATION OF CENTRAL AND PERIPHERAL TARGET AREAS IN INTRAOCULAR DOUBLE GRAFTS.
H. Bergman*, C. Bäckman[1] and A-C Granholm.[1] Dept. Cell Biology, Univ. of Linköping, Sweden, and Dept. Basic Science, University of Colorado Health Science Center, Denver, CO[1].

Histaminergic neurons of the tuberomamillary nucleus (TM) in the posterior hypothalamus send projections to virtually all regions of the rat brain. In order to investigate innervation pattern and plasticity of histaminergic neurons into different central and peripheral target areas, fetal hypothalamic tissue containing histaminergic neurons was grafted into the anterior chamber of the eye of adult Fisher 344 albino rats. Four weeks later, a second graft was placed in contact with the TM graft, and the double grafts were left to mature for 4-5 weeks *in oculo*. The co-grafts were fetal medial forebrain, hippocampal, cerebellar or lung tissue. Growth curves revealed that all central target tissues survived transplantation well and grew *in oculo*. The hippocampal target tissue seemed to benefit from a double transplant of TM tissue, and grew at least 3 times larger than single hippocampal control grafts *in oculo*. The lung tissue did not survive well, and some grafts showed signs of rejection 2-3 weeks after grafting. Immunohistochemical evaluation with antibodies against histamine, synapsin, glial proteins and choline acetyltransferase was performed. A dense pattern of synapsin-positive profiles was found in all transplants. Histamine-immunoreactive neurons in TM grafts innervated central target tissues to a variable degree. Highest densities of histamine-positive fibers were found in medial forebrain and hippocampal tissues, while cerebellar grafts contained very sparse fibers. The lung grafts did not appear to be innervated by histamine-positive fibers. These results indicate that the TM area of the hypothalamus may influence brain tissue growth, and that histaminergic innervation can be target specific.

623.17

REINNERVATION OF TRANSPLANTED LANGERHANS' ISLETS IN LIVER AND UNDER THE KIDNEY CAPSULE.
H. Houwing, R. van Asperen, P.T.R. van Suylichem[1], J.H. Strubbe, S.F. de Boer* and A.B. Steffens. Dept. of Animal Physiology and [1]Dept. of Surgery, University of Groningen, The Netherlands.

In islets of Langerhans, cholinergic stimulation leads to an enhanced insulin release in the early phase of a meal. Noradrenergic stimulation inhibits insulin release during exercise. Former physiological studies indicated that a low meal-induced early insulin respons and a clear exercise-induced reduction in insulin concentration was returned after islet transplantation in diabetic rats. We aimed to study whether islets, transplanted in the liver or under the kidney capsule, become reinnervated with both cholinergic and noradrenergic nerve fibers. Diabetic rats were provided with isogenic islet grafts of 50 % of the normal pancreatic endocrine volume via the portal vein in the liver or under the kidney capsule. After 2 weeks immunocytochemical stainings were performed on the noradrenergic enzymes tyrosine hydroxylase (TH) and dopamine-ß-hydroxylase (DBH), on the cholinergic enzyme choline acetyltransferase (ChAT) and on neuron specific enolase (NSE). In the grafts under the kidney capsule, ChAT staining was more pronounced at the capsule site and seemed to originate from the capsule, while TH staining was more pronounced at the parenchyma site. The TH and DBH staining was mainly visible around blood vessels in the islets in the liver and under the kidney capsule. In both transplantation sites NSE was present in the islets. These results give evidence for a reinnervation of islets grafts, which might be responsible for increasing and decreasing insulin levels.

623.18

GUIDANCE CHANNELS CONTAINING ADULT SCHWANN CELLS INDUCE CHOLINERGIC NERVE REGENERATION IN THE SEPTO-HIPPOCAMPAL SYSTEM.
D. Hoffman*[1] and P. Aebischer[1,2]. [1]Division of Biology and Medicine, Brown University, Providence, R.I. 02912; [2]Division of Surgical Research, CHUV, University of Lausanne, Switzerland.

The limitation of CNS axonal regeneration after injury may be due in part to an inhibitory post-lesion environment. While endogenous CNS components do not support axonal elongation, regeneration can occur with the provision of exogenous tissue from the peripheral nervous system, particularly Schwann cells. The means by which Schwann cells mediate CNS regeneration and the nature of the Schwann cell-axonal relationship have not yet been determined. The present study examines the ability of Schwann cells to induce septo-hippocampal regeneration when pre-seeded within a polymer nerve guidance channel, and the subsequent Schwann cell-axonal association at the transmission electron microscope (TEM) level. Schwann cells isolated from adult Fisher rat sciatic nerves were suspended in an extracellular matrix-containing gel, and surrounded by a hollow permselective poly(acrylonitrile/vinyl chloride) polymer tube. Following a unilateral aspirative lesion of the fimbria-fornix, adult Fisher rats received cell-containing tubes in the lesion cavity, placed so as to abut the septum and the hippocampus. Cholinergic axons regenerated through the channel lumen of tubes containing Schwann cells. A complete absence of cholinergic ingrowth was noted in control channels which contained extracellular matrix alone. Examination under TEM demonstrated the regeneration of numerous unmyelinated axons, groups of which were engulfed by Schwann cells. Few unmyelinated axons grew into control tubes. These studies show that when transplanted into the lesioned brain, Schwann cell-seeded nerve guidance channels provide directionally oriented biosynthetic bridges which induce CNS regeneration via a direct association between Schwann cells and groups of regenerating axons.

623.19

NGF-PRODUCING GRAFTS IMPLANTED INTO UNILATERALLY LESIONED RAT FIMBRIA-FORNIX ELICIT HIPPOCAMPAL REINNERVATION AND FUNCTIONAL RECOVERY. K.L. Eagle*, G.R. Chalmers, M.H. Tuzsynski, and F.H. Gage. Dept. of Neurosciences, UCSD, La Jolla, Ca. 92093

Nerve growth factor (NGF) has been shown to induce regeneration of axotomized NGF receptor-positive (NGFr-IR) cholinergic neurons. We have demonstrated that primary fibroblasts genetically modified to produce NGF, when suspended in a collagen matrix and implanted into a rat fimbria-fornix (FF) lesion cavity, promote growth of axons across the graft and into the hippocampus (HPC). In the present experiment, rats were given unilateral aspirative FF lesions and then either grafts of NGF-producing (NGF) or ß-galactosidase-producing (ßGal) fibroblasts, or no graft (FFx). After 6 months, NGF-producing grafts exhibited growth of neurofilament-positive processes in close association with astrocytic glial fibrillary acidic protein immunoreactive (GFAP-IR) processes. Similarly, NGFr-IR fibers often paralleled GFAP fibers, passing through the thick layer of astrocytic processes both to enter the graft from the host and to enter the HPC from the graft. The density of HPC NGFr-IR fibers was measured in four different hippocampal sites: the most caudal point of the dentate gyrus (DG1), just rostral to DG1 on the dorsal blade of the dentate gyrus (DG2), CA2, and the most caudal region of CA1. NGF-grafted animals show no significant NGFr-IR difference from normal control rats in the four sites measured, while ßGal-grafted rats have significantly less NGFr-IR density than control or NGF rats in DG2 and CA1. In a locomotor habituation task, all rats displayed the same baseline level of activity during initial exposures, but after a 24 hour delay both NGF-grafted and normal rats showed significantly greater habituation than ßGal-grafted or lesion-only rats. These results suggest that rats grafted with NGF-producing fibroblasts exhibit regrowth of neurites, that they receive enhanced reinnervation of the graft and HPC, and that this morphological effect is reflected in attenuated deficits in locomotor habituation.

SYNAPTIC STRUCTURE AND FUNCTION IV

624.1

ACTIVITY DEPENDENT PLASTICITY OF ELECTROTONIC SYNAPSES ON THE MAUTHNER (M) CELL. Alberto Pereda* and Donald S. Faber *Dept. of Anatomy & Neurobiology, Medical College of Pennsylvania, Philadelphia, PA 19129.*

Single eighth nerve afferents establish both gap junctions and chemical synapses with the M-cell's lateral dendrite, such that low threshold extracellular stimulation of the posterior eighth nerve produces a mixed electrotonic and chemical excitatory post-synaptic potential (VIIIth EPSP). Different tetanizing paradigms evoke long term potentiation or depression of both components of this response, depending in part on the initial level of synaptic efficacy. We are further investigating the determinants of activity-dependent plasticity of the gap junctions. Short or long duration tetanic stimulations of the eighth nerve produced transient (3-6 min.) and pronounced enhancements of coupling (m=509.6 %, s.e.m.=173.2 %, n=11), accompanied in all but one case by an increase in the chemical EPSP. Plasticity of electrical synapses was also illustrated in three experiments in which spontaneous decoupling occurred. Under those conditions stimulating tetani were able to re-establish the electrotonic potential. Paired pre- and postsynaptic recordings have been used to correlate changes observed at single connections with modifications of the population VIIIth EPSP. Unitary electrotonic potentials recorded at the input site (lateral dendrite) had a wide range of amplitudes (0.3-1.2 mV), suggesting the junctional conductance may vary appreciably among individual afferents (dendritic filtering may also contribute to this variability). Tetanic nerve stimulation can produce persistent (>5 min) changes in the amplitude of the unitary coupling potential. However, in 7 of 10 trials where the population response was modified, the size of the unitary potential either was unaffected or was altered in the opposite direction. These findings suggest that the electrical synapses of this group of afferents have different levels of efficacy and that activity dependent modifications of the VIIIth EPSP represent the net effect on this population.

624.2

EVIDENCE FOR ACTIVE CONDUCTANCES IN THE DENDRITES OF MOTONEURONS. M.E. Larkum, M.G. Rioult, H-R. Lüscher*. Physiologisches Institut, University of Bern. CH-3012 Bern, Switzerland.

We recorded electrical activity in the dendrites of presumed motoneurons using organotypic slice cultures from embryonic rat spinal cord stained with a voltage-sensitive dye (di-8-ANEPPS). Fluorescence changes were measured with an array of 124 photodiodes (each 16 µm × 16 µm using a 40X oil immersion objective) and the signal sampled and digitized at 3000 Hz per diode for up to 150 ms. We recorded evoked action potentials (AP) using an intracellular microelectrode by injection of current (0.1 to 1.0 nA, 50 msec) at the soma. By comparing the decay in amplitude of the AP's along the dendrites with the electrotonic decay of hyperpolarizing pulses (-0.4 to -0.6 nA, 50 msec) we were able to compensate for the decrease in fluorescence signal in the dendrites due to the decrease in membrane surface area and also for the inhomgeneities due to dendrites not in the focal plane. The decay of the AP amplitude along the dendrites up to 130 µm from the soma was less than the decay of the hyperpolarizing pulse. To examine the possiblity that calcium ions were involved in the active propagation of the AP we measured accompanying changes in calcium concentration using the photodiode array and the calcium indicator Fluo-3. Fast increases in calcium concentration could be observed that were restricted to the soma. Calcium increases were not seen in the dendrites.

These observations lead us to suspect by exclusion that sodium ions are probably responsible for AP propagation in the dendrites of motoneurons. (SNF 31-37553.89 to H.-R. L.)

624.3

THE PASSIVE ELECTROTONIC STRUCTURE OF MOTONEURONS IN SLICE CULTURES ESTIMATED BY COMBINING PATCH CLAMP AND MORPHOLOGICAL DATA. **D.Ulrich* and H.-R.Lüscher, Dept. of Physiology, University of Bern, 3012 Bern, Switzerland**

Motoneurons in slice cultures were filled with biocytin during whole cell recording. Na^+ and K^+ conductances were blocked with TTX in the bath and Cs^+ in the pipettes, respectively. The cells were stained with HRP and reconstructed using a semi-automatic reconstruction system (Eutectic 3D NTS). Voltage and current transients were generated by short pulses of current or voltage, respectively and could be fitted by 3-4 exponential functions. The motoneurons consisted of a polygonal soma with 3-6 dendritic trees of different length. The slowest time constants of the voltage and current decay were used to calculate the electrotonic length (L_{pas}) of an equivalent cylinder. The mean electrotonic lenght of the different trees (L_{neu}) was estimated from the dendrograms assuming a cytosolic resistivity of 250Ωcm and a capacity of 1µFcm^{-2}. The resulting L_{pas} of 0.76±0.18 (n=6) was close to the L_{neu} of 0.68±0.16. However, estimates of L_{pas} by the use of higher order time constants overestimated L_{neu} by a factor of 2-4. We conclude that the cable parameters describe an average electrotonic structure of the motoneurons although the conditions by which dendritic trees can be reduced to an equivalent cylinder are not exactly met in the cells examined. (SNF 31-27553.89)

624.4

Electrophysiological and Ultrastructural Properties of Identified Mesopontine 5-HT Neurons in Culture. **M.D Johnson* and A.G. Yee. Dept. of Neurobiology, Harvard Medical School, Boston, MA 02115.**

Studies indicate that mesopontine 5-HT neurons are electrophysiologically and morphologically heterogeneous *in vivo*. To examine the relationship between the electrophysiological and ultrastructural properties of 5-HT neurons, neonatal Long-Evans rats (P0 - P3) were decapitated, the mesopontine raphe nuclei were removed and enzymatically dissociated, and the cells were maintained in microcultures for several weeks. The small area of the microcultures confined growing processes, increased the probability that neurites would form synapses with target cells, and facilitated the ultrastructural study of identified neurons. Intracellular recordings from 5-HT-immunoreactive neurons revealed the presence of short latency glutamatergic, GABAergic, and serotonergic potentials in these cells. 5-HT neurons also formed serotonergic and glutamatergic synapses on target cells (Johnson, Soc Neurosci Abstr 18:1530). For electron microscopy, 5-HT neurons in 8 microcultures were identified by peroxidase-linked 5-HT immunocytochemistry or by a 15-30 minute exposure to the autofluorescent 5-HT analogue, 5,7-dihydroxytryptamine, prior to embedding in Epon. 5-HT and non-5-HT axon terminals formed synaptic contacts on both labeled and unlabeled target neurons. Most 5-HT axons contained round or pleiomorphic, as well as large dense-cored vesicles. In 3 microcultures containing solitary 5-HT neurons, intracellular recordings and subsequent EM analysis suggested that differences in synaptic physiology may be reflected in the number and type of synapses present. Thus, this system is useful for studying correlations between synaptic electrophysiology and ultrastructural morphology in 5-HT neurons. Supported by NS02253 and the Freudenberger Fund. MJ is a recipient of a Harvard Ryan Fellowship and an American Psychological Association Minority Fellowship.

624.5

FAST DEACTIVATION OF NON-NMDA RECEPTORS IN OUTSIDE-OUT PATCHES. B. Edmonds*, R. A. Silver, D. Colquhoun and S. G. Cull-Candy. Dept. of Pharmacology, University College London, London WC1E6BT, England.

The response of non-NMDA receptors to rapid application of glutamate was investigated in outside-out patches from granule cells cultured from rat cerebellum (4 days in culture, age 7-8 days). A near saturating concentration (1-5 mM) of glutamate was applied for varying durations to investigate the time course of deactivation upon removal of glutamate, and desensitization in response to a long application. The deactivation time courses resulting from 0.2 & 1.0 ms applications were not found to be different, and single exponential fits to the data gave an overall mean time constant of 0.64 ms. Desensitization was slower; the mean time constant for an exponential fit was near 3 ms. Since the non-NMDA component of the synaptic current decays with a time constant of about 1 ms in these cells (Silver *et al.*, 1992), our results suggest that desensitization alone is too slow to account for the decay, supporting the idea that transmitter is present only briefly in the synaptic cleft. However, deactivation appears to be faster than the synaptic decay rate, so the latter may well be influenced by the time course of glutamate concentration in the cleft.

624.6

MODEL OF THE QUANTAL ACTIVATION OF NMDA RECEPTORS AT A HIPPOCAMPAL SYNAPTIC SPINE. T.M. Bartol Jr., T.J. Sejnowski*. Computational Neurobiology Laboratory, Salk Institute, 10010 N. Torrey Pines Rd., La Jolla, CA 92037.

A Monte Carlo simulation of a hippocampal area CA1 synaptic spine was undertaken. The simulation included the three-dimensional geometry of a synaptic spine, the release and random-walk diffusion of each transmitter molecule in a quantal packet of glutamate, and the activation by glutamate of NMDA receptors present on the postsynaptic membrane. The kinetic rate constants used to model the NMDA receptor were held fixed at the values reported by Lester & Jahr (The Journal of Neuroscience, 1992, 12:635-643). Thus, the free parameters in the model were the number of transmitter molecules contained in a quantal packet (N), and the NMDA channel density on the postsynaptic membrane (σ_{nmda}). N and σ_{nmda} were adjusted so that ~10 NMDA channels would be in the open state at the peak of receptor activation (Bekkers & Stevens, Nature, 1989, 341:230-233). The time course of glutamate concentration in the cleft following quantal release had a rapid component decreasing from ~2 mM to 0.5 mM in <100 μs and a slower component which kept glutamate concentration at ~0.1 mM for over 1 ms. In agreement with experimental results (Lester & Jahr) the ensemble average time course of the modeled NMDA receptor activation was unaffected by the value of N or σ_{nmda}. It was also found that over a limited range of values for σ_{nmda}, a best fit to the peak activation criteria could be acheived by keeping the product of $N*\sigma_{nmda}$ constant and that outside this range a fit could only be obtained by increasing N at a given value of σ_{nmda}. This result suggests that $N*\sigma_{nmda}$ may be operating at a minimum value in vivo and that the actual value of N could be determined if one could measure σ_{nmda}.

Research supported by the Howard Hughes Medical Institute.

624.7

ENGINEERING CHARACTERIZATION OF SYNAPTIC JUNCTIONS IN THE HIPPOCAMPUS. F. G. Ascarrunz, K. Flach, L. E. Adler* and R. J. MacGregor Dept. of Aerospace Engineering Sciences, Univ. of Colorado, Boulder CO 80309

In this study we compile the quantitative data available in the literature, on the anatomy and electrophysiology of the hippocampal system. The objective is to characterize the synaptic junctions to establish a means of comparing the dynamics of these junctions. The quantitative data are used to derive a set of parameters that express the connectivity and dynamic characteristics of the junctions. We tentatively organize synaptic junctions into 4 broad categories, diffuse excitatory junctions, discrete excitatory junctions, selective inhibitory junctions, and discrete inhibitory junctions. Diffuse excitatory junctions are characterized by post-synaptic potentials (PSP) which are small compared to the threshold of the receiving neuron. The synaptic junctions are generally on distal dendrites and the convergence ~ divergence. At discrete excitatory junctions, the PSP sizes are comparable to threshold and the divergence is much greater than the convergence. Selective inhibitory junctions control the inputs to the neuron and are characterized by small PSP's of long duration. The synapses are on distal dendrites and seem to be activated in a feed forward sense. The discrete inhibitory connections control the outputs of a neuronal network through recurrent inhibition. These junctions are characterized by large PSP's of short duration, and a high redundancy factor. These synapses are on proximal dendrites, cell bodies and axon initial segments. We compare the afferents to different neuronal populations in the hippocampus in terms of these parameters. We show that by identifying and characterizing the operational constraints of synaptic junctions, one can provide a basis for choosing a modeling approach that can best represent the anatomy and electrophysiology of the neuronal system investigated. Ref; R. J. MacGregor, Theoretical Mechanics of Biological Networks, Academic Press 1993. This study was supported by NIMH: 5P50 MH 44212-06

624.8

Diversity in EPSC size and release probability of synapses on individual CA1 cells in the rat hippocampus.

Morten Raastad* and Rupert Lipowski
Institute of Neurophysiology, University of Oslo.

If the excitatory synapses on CA1 pyramidal cells can undergo modifications of efficacy in vivo, different axons synaptically connected to the same cell would be expected to present different release properties and/or postsynaptic amplitude. We have studied the question of diversity of synaptic properties by using a technique for reliable activation of a single synaptically connected axon.

Tight-seal voltage clamp recordings from CA1 cells in hippocampal slices were used to record excitatory postsynaptic currents (EPSCs). Inhibitory currents were blocked by 10 uM bicuculline. Schaffer collaterals were activated with a limited range of stimulus intensities around the threshold for the occurrence of EPSCs. Activation of a single fibre was inferred when EPSCs appeared at a certain stimulation intensity, and a stable distribution of EPSC amplitudes was observed over a range just above this stimulation intensity.

EPSC recordings with stable electrode series resistance, membrane resistance, Ca^{++} concentration, stimulation frequency and comparable rise times showed clearly different EPSC amplitude distributions and different proportion of release failures when different single fibre inputs to the same cell were activated. There was no apparent correlation between probability for failures and mean EPSC size, speaking for a diversity in postsynaptic response between different inputs.

624.9

HOW THE SIZE DISTRIBUTION OF THE POSTSYNAPTIC AREAS ON HIPPOCAMPAL (CA1) DENDRITIC SPINES MIGHT INFLUENCE THE AMPLITUDE HISTOGRAMS OF MINIATURE SYNAPTIC CURRENTS. K.M. Harris* Dept. Neurology, Children's Hospital and Harvard Medical School, Boston, MA 02115

Amplitude histograms of excitatory postsynaptic currents (epcs) in area CA1 of the hippocampus can show broad, positively skewed distributions ranging from 2 to 30 pA.[1,2] There is however, considerable heterogeneity in the shapes of the histograms. Several explanations could account for this heterogeneity and for the occurrence of peaks in their distributions, including variation in the number of vesicles released, in the amount of neurotransmitter per vesicle, and in the size of the postsynaptic response. Here we have examined the possibly that the surface area of the dendritic spines occupied by the postsynaptic density (PSD), i.e. the receptive region, may be an anatomical marker of the postsynaptic contribution to these amplitude histograms. In previous studies[3,4] we have reconstructed through serial electron microscopy the entire area of PSDs occurring on 572 dendritic spines in the middle of s. radiatum in hippocampal area CA1. The frequency histogram of these PSD areas is positively skewed ranging in size from 0.01 to 0.76 μm^2; and PSDs spanning most of this range in size can occur along a 5-8 μm segment of a single CA1 dendrite. A Chi-square analysis revealed that this distribution is not likely to be unimodal ($X^2 = 89$, df = 34, $p < 0.01$, for bin widths of 0.02 μm^2, excluding the two end bins) suggesting the possibility that postsynaptic size may contribute to variation in the size of epcs thereby influencing the shape of the amplitude histograms. Supported by NS21184, The Alfred P. Sloan Foundation, and the MR center grant P30-HD18655.

1) Manabe T, Renner P, and Nicoll RA (1992) Nature 355:50-5.
2) Liao D, Jones A, Malinow R. (1992) Neuron 9:1089-97.
3) Harris KM, and Stevens JK (1989) J. Neuroscience 9:2982-97.
4) Harris KM, Jensen FE and Tsao B (1992) J. Neuroscience 12:2685-2705.

624.10

HIGH-SPATIAL RESOLUTION IMAGING OF Ca^{2+} TRANSIENTS IN LIVING HIPPOCAMPAL NEURONS USING CONFOCAL MICROSCOPY D.B. Jaffe[1*], and T.H. Brown[1,2], Depts. of Psychology[1] and Cellular & Molecular Physiology[2], Yale Univ., New Haven, CT 06520.

Long-lasting forms of synaptic plasticity, such as LTP or LTD, are believed to be triggered by synaptically-mediated changes in $[Ca]_i$. The high-spatial resolution of confocal microscopy in combination with recently developed Ca-sensitive dyes can now be used to visualize changes in $[Ca]_i$ at the synaptic level.

Living CA1 and CA3 pyramidal neurons in thick rat hippocampal slices (300 μm) were filled with long-wavelength Ca-sensitive dyes (Fluo-3 or Ca-green) using standard sharp microelectrode techniques. Cells near the surface of the slice (within 50 μm) were chosen to facilitate high-spatial resolution imaging of dendrites and dendritic spines. Fluorescence imaging was performed using laser scanning confocal microscopy (Bio-Rad MRC-600) either with a 63x oil, 1.2 NA objective (inverted system) or a 40X, 0.75 NA water immersion objective (upright system).

We have examined the spatial distribution of dendritic Ca^{2+} accumulation produced by two different sets of afferent synapses, demonstrating specific patterns of $[Ca]_i$ increases for separate inputs. We have also begun studying increases in $[Ca]_i$ in the large proximal dendritic spines of CA3 pyramidal neurons, the thorny excrescenses or thorns. We are currently applying DIC optics and video techniques to patch clamp in the whole-cell configuration identified neurons close to the surface to the slice. This will further facilitate high-spatial resolution imaging of changes in $[Ca]_i$ within the dendrites and dendritic spines of hippocampal neurons. Supported by ONR and NIMH.

624.11

THE 3-D LOCATIONS OF THORNY EXCRESCENCES ON HIPPOCAMPAL CA3 PYRAMIDAL NEURONS. R.B. Gonzales*, Y.M. Rangel, and B. J. Claiborne, Division of Life Sciences, The University of Texas at San Antonio, San Antonio, TX 78249.

Hippocampal CA3 pyramidal neurons demonstrate prominent spines, or thorny excrescences, at the site of mossy fiber innervation. These postsynaptic structures are found on both the apical and basal dendrites of those pyramidal neurons located near the hilar region. Because synaptic currents decay as they spread toward the cell body, the location of thorny excrescences is an important variable in predicting the effect of mossy fiber inputs on CA3 pyramidal neurons (Siegel et al., Soc. Neurosci. Abst., 18:566). Here we compare the locations of thorny excrescences on the apical and basal portions of the dendritic tree.

CA3 neurons (n=15) in hippocampal slices from adult rats were intracellularly labeled with HRP and reconstructed in three-dimensions using a computer-microscope system (Claiborne, B.J., in Methods in Neuroscience, p. 315, Academic Press, 1992). Results showed that the excrescences on apical dendrites were located an average of 64 $\pm$ 2 um from the cell body (mean $\pm$ SEM; n=181), whereas excrescences on basal dendrites were located only 30 $\pm$ 1 um from the cell body (n=146). These values were significantly different (Mann-Whitney; P<0.001). The lengths of the excrescences were also measured. The mean aggregate length of apical excrescences was 7 $\pm$ 0.6 um, while the average length of basal excrescences was 6 $\pm$ 0.4 um. These results show that although excrescences on basal dendrites are similar in length to those on apical dendrites, they are located much closer to the cell body. We are presently using computer simulations to investigate the possible functional significance of this anatomical variation. (Supported by NIH/NIGMS GM08194 and the Texas Higher Education Coordinating Board)

624.12

COMPONENTS OF EPSCS IN RAT CA1 PYRAMIDAL CELLS: SHAPE, SPACING AND PAIRED-PULSE POTENTIATION. J.Isaac, Y. Chen, D.A.Turner and H.V.Wheal*, Dept.Physiol. & Pharm., Univ. Southampton, Southampton, U.K. SO9 3TU and Neurosurg. & Neurobiol., Duke Univ. and Durham VAMC, Durham, NC 27710.

Components of evoked EPSCs recorded in CA1 neurons are often assumed to be equally spaced and "quantal" in the sense of uniform dendritic termination and amplitude. We have recorded "minimal" EPSCs and analyzed the data for components, using the waveform shape, amplitude and also an unconstrained Bayesian statistical approach, using paired-pulse manipulations.

Stationary EPSCs (n=16) showed variable rise time (1.3-7.9 msec), half-width (18.2-51.7 msec) and could often be separated by subgroup analysis into components with different shape and amplitude. The mean amplitude was 6.89$\pm$4.1 pA, consisting of an average of 5 components, with a background mean nsd = 1.61$\pm$0.5 pA. The average component spacing was 3.9 $\pm$0.9 pA, with a ratio of components to nsd = 2.54$\pm$0.72. The components were often irregularly spaced (n = 12); combined with the variable shape this likely indicates multiple dendritic sites of origin. The paired-pulse paradigm resulted in an average of 30% increase in amplitude; however, this altered primarily component probability rather than spacing.

These data suggests that electrotonically separated synaptic events exhibit different site-specific amplitudes and shapes. Thus, differences between sites should be explicitly considered in EPSC analysis rather than simply assuming uniformity of dendritic synaptic events. **Supported by grants from the Wellcome Trust, NINDS and VAMC.**

624.13

Hippocampal microisland cultures as a model of CNS synaptic transmission. S.J. Mennerick*, J. Que, A. Benz, D.B. Clifford, and C.F. Zorumski. Depts. of Psychiatry and Neurology, and Program in Neuroscience, Washington Univ. Med. Sch., St. Louis, MO 63110.

There are many advantages of models of vertebrate CNS synaptic transmission that are not influenced by complex network properties. We have begun to characterize one such model by comparing properties of postnatal rat hippocampal neurons grown on microislands (MIs), where synaptic connections are restricted to one or two cells, with control neurons from the same animals grown in high density cultures (HDCs).

Using whole cell patch clamp techniques, differences were found in passive membrane properties of MI neurons versus HDC neurons matched for age and somal diameter. MI neurons displayed significantly less contribution of a slow-charging component of the membrane capacitance toward total membrane capacitance. This result may reflect less extensive neuritic arborizations in neurons grown under the physical constraints of MI cultures.

There was a much higher probability of encountering monosynaptic responses in MIs versus HDCs. Single-cell MIs more frequently displayed excitatory autaptic responses (6/8 neurons) than HDC cells (1/8 neurons). Two-cell MIs also displayed a higher incidence of monosynaptic connections than pairs in HDCs (81% vs. 38%, p<.01; n=42). Despite the higher incidence of evoked synaptic responses and a trend toward larger responses on MIs, no differences were detected in the timecourse, amplitude, or frequency of miniature excitatory postsynaptic currents.

These studies demonstrate two advantages of the MI preparation for the study of CNS transmission: simpler passive membrane properties and a higher probability of encountering monosynaptic connections than in typical culture systems. The results also suggest that MIs could be exploited to study the convergence and divergence of synaptic connections during development.

624.14

DENDRITIC SPINES IN CULTURED HIPPOCAMPAL NEURONS: DEVELOPMENT AND MORPHOLOGY. M. Papa, M. Bundman*, V. Greenberger and M. Segal Dept. Neurobiol., The Weizmann Institute, Rehovot, 76100, ISRAEL.

Dendritic spines are the sites of excitatory synaptic communication among central neurons. The small size of the spines and the complexity of synaptic connectivity among neurons prohibits their systematic analysis in-vivo. We have, therefore, developed conditions for monitoring dendritic spines in cultured neurons. Embryonic rat hippocampal neurons were dissociated, plated on polylysine-coated glass cover slips and grown in culture for 1-3 weeks,. Cultures were fixed with 10% formalin and individual neurons were stained with DiI and visualized in a confocal laser scanning microscope. Some cultures were immunostained for synaptophysin and others prepared for EM analysis.

About 99% of the neurons contained spiny dendrites. The mean length of the spines decreased from 1.77 μm to 1.16 μm between 1 and 3 weeks in culture. There were clearly-defined spine heads at 3 weeks in culture. The density of spines increased 2.7 fold between 1 and 3 weeks, reaching a mean density of 4.3 spines per 10 μm of dendrite length. At 3 weeks, spines were associated with synaptophysin-immunoreative labeling, resembling synaptic terminals. At the EM level, dendritic spines were similar to those in-vivo. They contained no microtubules or polyribosomes, but were filled with a fluffy, flocculent material, and made asymmetric synapses with terminals containing spherical vesicles. These studies provide the basis for further analysis of the rules governing the formation, development and plasticity of dendritic spines under controlled, in-vitro conditions.

624.15

BLOCK OF ENDOGENOUS ADENOSINE DEAMINASE (ADA) HAS LITTLE EFFECT ON SYNAPTIC TRANSMISSION IN RAT HIPPOCAMPAL SLICES. P.J. Zhu* and K.Krnjević, Anaesthesia Res. Dept. McGill Univ. Montréal, PQ, H3G 1Y6, Can.

Adenosine is a potent inhibitory neuromodulator in the CNS. ADA, which breaks down adenosine, might have an important role in the control of adenosine levels. In field recordings from CA1, the role of endogenous ADA in synaptic transmission was studied by applying the specific ADA inhibitor **pentostatin** (1-30 μM). There was no consistent effect on responses elicited by Schaeffer collateral stimulation (n=20). Exogenous ADA (0.5 U/ml) enhanced the field EPSP and population spike (n=9), but not the afferent volley (n=6); stimulus strength for 50% maximum of EPSP and population spike was decreased by 8.7% and 10.7%, respectively. These actions of exogenous ADA were blocked by pentostatin (n=6). Similar effects were seen in intracellular recordings (n=2). The present results confirm previous evidence that ongoing adenosine release depresses synaptic transmission hippocampus (Haas and Greene, 1988 Naunyn-Schmiedeberg's Arch Pharmacol. 337:561); but they suggest that endogenous ADA plays little, if any, role in modulating this effect - in keeping with the reported low ADA content of the hippocampus (Geiger and Nagy 1986, J. Neurosci. 6:2707). *Supported by Medical Research Council of Canada.*

624.16

HIPPOCAMPAL PYRAMIDAL NEURONS EXCITE NONPYRAMIDAL CELLS VIA SINGLE RELEASE SITES. A.I.Gulyás[1], R.Miles[2], K.Tóth[1,2] and T.F.Freund[1]*
[1]Inst. Exp. Medicine, Hungarian Acad.Sci., Budapest, P.O.B. 67, Hungary; [2]Lab. Neurobiologie Cellulaire, INSERM U261, Inst. Pasteur, 75264 Paris, France.

Combined physiological and anatomical experiments were carried out to study excitatory synaptic transmission between pyramidal cells and nonpyramidal cells in the guinea-pig hippocampus in vitro. Simultaneous intracellular recordings were made from CA3 pyramidal cells and inhibitory cells located close to stratum pyramidale. Both neurons were filled with biocytin, and were subsequently visualized with the ABC method. Excitatory interactions were detected in about 10% of the recorded pairs. EPSPs evoked by pyramidal cell action potentials in the postsynaptic interneuron had a mean amplitude of 0.5-1.5 mV and a rise time of 1.2-2.8 ms (n=18). The transmission failed with a probability of 0.05 to 0.45. At the end of the electrophysiological session Three cell pairs were successfully recovered with complete axonal and dendritic trees. A basket cell and two dye-coupled pyramidal cells were labelled in the first slice (EPSP amplitude 0.75±0.3 mV), a basket cell and a single pyramidal cell in the second (0.65±0.25 mV), and a pyramidal cell together with an interneuron arborizing in strata lacunosum moleculare and oriens in the third (1.15±0.25 mV). Reconstruction in the light microscope showed that pyramidal cell axons in each slice established a single contact on the dendrites of the nonpyramidal cells. All three contacts were shown to be asymmetrical synapses in the electron microscope.

We conclude that pyramidal cells excite CA3 inhibitory cells by liberating transmitter from a single morphologically identified site. This arrangement permits direct measurements of the probability of transmitter release, and variation in the effects of transmitter released from a single site. The present findings suggest that there is a high degree of divergence and convergence in the pyramidal cell –> interneuron connection, and place an important constraint on different mechanisms of synaptic plasticity.

624.17

PHYSIOLOGY AND ANATOMY OF LOCAL CIRCUIT CONNECTIONS BETWEEN PYRAMIDAL CELLS IN RAT NEOCORTEX. A.M. Thomson*, J. Deuchars & D.C. West Department of Physiology, Royal Free Hospital School of Medicine, London NW3 2PF, UK

Pairs of synaptically connected pyramidal neurones were recorded intracellularly in slices of rat motor cortex. After recording the excitatory postsynaptic potentials (EPSPs) evoked in one neurone by action potentials in the other, the two cells were filled with biocytin. Slices were fixed in 2% glutaraldehyde/2% paraformaldehyde, sectioned at 60μm and incubated in Avidin-HRP, which was localized using DAB as chromogen. Sections were subsequently processed for light and electron microscopy. Connections between deep layer pyramidal neurones could involve either basal or apical postsynaptic dendrites, but the input from one presynaptic neurone was relatively restricted in its distribution. Preliminary correlations between fluctuation analysis and morphology indicate that i) EPSP time course is determined by electrotonic distance from the recording site and ii) coefficient of variation may correlate with the number of contributory presynaptic terminals. In one example, the postsynaptic recording site was in the apical dendrite, 140μm from the soma and the EPSP evoked by apparent terminals onto branches of this dendrite evoked slow spikes and fast spike bursts.

624.18

PHYSIOLOGY AND ANATOMY OF LOCAL CIRCUIT CONNECTIONS BETWEEN PYRAMIDAL NEURONES AND INTERNEURONES IN RAT NEOCORTEX. J. Deuchars, A.M. Thomson & D.C. West, SPON: Brain Research Association. Department of Physiology, Royal Free Hospital School of Medicine, London NW3 2PF, UK.

In slices of rat motor cortex, simultaneous intracellular recordings were obtained from an interneurone and a pyramidal cell. Synaptic connections were demonstrated electrophysiologically and connected cells filled with biocytin. Both inhibitory postsynaptic potentials (IPSPs) recorded in the postsynaptic pyramid and excitatory postsynaptic potentials (EPSPs) recorded in the postsynaptic interneurone were studied. However, in no case was a reciprocal connection observed. Fast spiking interneurones that evoked fast IPSPs in pyramidal neurones had aspiny dendrites, axons that ramified extensively in the vicinity of the soma and appeared to contact basal dendrites and somata of postsynaptic pyramids. Single axon IPSPs could be several mV in amplitude, and followed high presynaptic firing rates with a decline in IPSP amplitude, but only rare complete failures of transmission. These IPSPs fluctuated in amplitude, but CV (coefficients of variation) were small. Pyramidal neurones evoked fast EPSPs in postsynaptic interneurones which typically displayed profound paired pulse facilitation and large CVs.

624.19

NEW PREPARATORY METHODS DO NOT AFFECT FIELD POTENTIALS IN HIPPOCAMPAL SLICES. D. L. Tauck*, J. E. Tullis, and R. L. Sasich, Laboratory of Neurophysiology, Department of Biology, Santa Clara University, Santa Clara, CA, 95053.

Accumulated evidence suggests that hypothermia protects against anoxia in a variety of neuronal preparations. Hippocampal slices initially incubated for 45 minutes at 21°C have many more normal CA1 pyramidal cells than slices incubated at 37°C (Newman, et al., 1992, *Brain Res.* 575:159-163). Similarly, preventing the neuronal swelling caused by anoxia allows preparation of brain slices from adult rats with electrophysiologically viable motoneurons (Aghajanian & Rasmussen, 1989, *Synapse* 3:331-338). This is accomplished by substituting sucrose for NaCl in the artificial cerebrospinal fluid (ACSF) used during the dissection and subsequent recovery period.

The present study shows that neither hypothermia nor the use of sucrose-ACSF affects the maximum amplitude of the population spike recorded in hippocampal area CA1 at 33°C. Slices initially incubated at 20°C in normal ACSF generated an average maximal population spike of 17.4 ± 4.2 mV (mean ± S.D.; n=4); incubation at 33°C resulted in a maximal response of 16.7 ± 2.0 mV (n=4). In slices initially perfused with sucrose-ACSF, the average maximal response was 20.7 ± 3.1 mV (n=4) in slices incubated at 20°C and 18.8 ± 5.7 mV (n=4) in slices incubated at 33°C.

PRESYNAPTIC MECHANISMS IV

625.1

4-AMINOPYRIDINE (4-AP) DOES NOT AFFECT PRESYNAPTIC INHBITION BY MUSCARINE IN RAT HIPPOCAMPAL CA1 *IN VITRO*. B.J. Mack, P.A. Smith & W.F. Colmers* Dept. Pharmacology, Univ. of Alberta, Edmonton, Canada

Multiple receptors mediate presynaptic inhibition of excitatory transmission at the Schaffer collateral-CA1 pyramidal cell synapse. Neuropeptide Y (NPY) and baclofen appear to share a common mechanism, with a common response to application of 4-AP and 4-AP, low Ca^{2+}, while adenosine's actions differ in their response to these conditions. Here we test the hypothesis that muscarinic inhibition at this synapse would respond to 4-AP and low Ca^{2+}, 4-AP in the same manner as NPY.

The slope of the population EPSP evoked by stratum radiatum stimulation and recorded in stratum radiatum of CA1 was adjusted to between 70% and 90% of the maximum response and maintained under all conditions. Concentration-response curves to muscarine (0.1-30 μM) were constructed in control saline (1.5 mM Ca^{2+}), in 30 μM 4-AP, in 4-AP with low (0.75 mM) Ca^{2+}, and in saline with low (1.0 mM) Ca^{2+} alone. 4-AP had no effect on the concentration-response curve to muscarine, in contrast to previous findings with NPY, baclofen or adenosine. Low Ca^{2+}, or low Ca^{2+}, 4-AP both increased the inhibition caused by muscarine.

The results suggest that presynaptic muscarinic receptors in hippocampus mediate inhibition of transmitter release by a different mechanism than do NPY, baclofen or adenosine. While results from similar experiments suggest that NPY and baclofen may inhibit Ca^{2+} influx into presynaptic terminals, it is still unclear how muscarinic receptors act there. It is possible that a change in excitability, e.g., activation of a large potassium or chloride current, may underlie muscarine's action. It is certain, however, that the mechanism of muscarinic presynaptic inhibition differs from those of the other three receptors studied.

Supported by MRC (Canada). BJM is supported by the Rick Hansen Foundation.

625.2

NEUROPEPTIDE Y PRESYNAPTICALLY INHIBITS EXCITATORY SYNAPTIC TRANSMISSION ONTO CA3 PYRAMIDAL CELLS BY A Ca^{2+}-DEPENDENT MECHANISM. A. Rory McQuiston* and William F. Colmers. Department of Pharmacology, University of Alberta, Edmonton, Alberta, Canada.

Although neuropeptide Y (NPY) inhibits all feedforward and recurrent excitatory synaptic inputs to CA3 pyramidal cells in hippocampus *in vitro*, the mechanism of NPY's action remains to be determined. We investigated the mechanism of presynaptic inhibition of NPY on CA3 pyramidal cells by recording spontaneous excitatory postsynaptic currents (sepsc's) in acutely prepared rat hippocampal slices utilizing the whole cell patch clamp method.

Slices were perfused with aCSF containing 100 μM picrotoxin to eliminate spontaneous inhibitory synaptic currents and cells were acquired with electrodes (3 to 7 MΩ) made from 1.5 mm borosilicate glass. Bath application of NPY (1 μM) reversibly decreased the sepsc frequency distribution and also decreased the amplitude distribution, as determined by a Kolmogorov-Smirnov test. However, further evaluation of the sepsc amplitude distribution (by plotting the cumulative mean sepsc amplitude against the sepsc amplitude) demonstrated that there was a selective reduction in the number of large amplitude sepsc's without a subsequent change in the distribution of the smaller amplitude sepsc's (in the amplitude range of the unitary event). These results are consistent with an exclusively presynaptic site of NPY action, reducing the likelihood that multiple, summating sepscs are observed. Furthermore, in the presence of tetrodotoxin to eliminate action potential-dependent sepsc's (and thus presumably calcium-dependent sepsc's), neither the frequency nor amplitude distribution of sepsc's were affected by 1μM NPY.

These data are consistent with a presynaptic site of NPY action in CA3, and suggest that NPY inhibits Ca^{2+}-dependent transmitter release in hippocampus.

Supported by MRC (Canada).

625.3

ADRENERGIC EXCITATION OF HIPPOCAMPAL INTERNEURONS.
V. A. Doze*, D. E. Bergles, S. J Smith and D. V. Madison.
Department of Molecular & Cellular Physiology, Beckman Center, Stanford University School of Medicine, Stanford, CA 94305-5426.

Whole-cell recordings were made from pyramidal cells and from identified interneurons in the CA1 region of adult rat hippocampal slices. Epinephrine-10μM (EPI) increased both the frequency and the amplitude of spontaneous action potential-dependent IPSCs recorded in CA1 pyramidal neurons. This action was mediated by an alpha adrenoceptor. In contrast, EPI did not affect either the frequency or the amplitude of spontaneous action potential-independent (miniature) IPSCs recorded in CA1 pyramidal cells.

EPI depolarized interneurons causing them to fire action potentials more frequently. This action was also mediated by an alpha adrenoceptor. The EPI-induced depolarization of the interneuron membrane was accompanied by an decrease in input resistance (with a reversal potential of ~-40mV) consistent with an increase in a non-selective cation conductance. These results suggest that alpha adrenoceptors are selectively localized on inhibitory interneurons and are coupled to a non-selective cation channel. Activation of these adrenoceptors by EPI causes excitation of the inhibitory interneurons. These results further confirm that the adrenergic system modulates interneuron activity and thus may have an important role in regulating tonic inhibition.

625.4

CHOLINERGIC INHIBITION OF MINIATURE EPSCS IN THE RAT HIPPOCAMPUS.
G.A. Cohen,* D.E. Bergles, S.J Smith, and D.V. Madison. *Dept. of Molecular and Cellular Physiology, Stanford University School of Medicine. Beckman B-115, Stanford, CA 94305-5426.*

Muscarinic receptor activation is thought to presynaptically inhibit the release of glutamate in the hippocampus. To test this hypothesis directly, the effect of the cholinergic agonist carbachol was studied on miniature excitatory post-synaptic currents (mEPSCs) in the rat hippocampus. Recordings were obtained using whole-cell electrodes filled with cesium gluconate or potassium gluconate based solutions. In pyramidal cells, the average tonic frequency of observed mEPSCs was less than 0.1 Hz, with a range of 0 to 1.5 Hz. Events had slow rise times (2-10 msec) and average amplitudes of 2-3 pA, only slightly larger than the 1 to 1.5 pA background noise. Carbachol (10uM) was applied to < 5% of cells which exhibited mEPSCs with sufficiently large frequency and amplitude. In these cells, carbachol induced a significant decrease in frequency.

Whole cell recordings from lacunosum-moleculare and stratum radiatum interneurons revealed mEPSCs with an average frequency of 5 Hz (range 2-15 Hz), amplitude of 8 pA (>100 pA maximum), and rise times of 0.5 to 2 msec. Carbachol (10uM) caused > 50% reduction in the frequency of these events. These results provide further evidence for a presynaptic inhibitory action of cholinergic receptors on glutaminergic terminals.

625.5

ACTIVATION OF METABOTROPIC GLUTAMATE RECEPTORS MODULATES FEEDFORWARD EXCITATION OF HIPPOCAMPAL INTERNEURONS.
D. E. Bergles* and S. J Smith. *Department of Molecular and Cellular Physiology, Stanford University, Stanford, CA 94305-5426.*

The metabotropic class of glutamate receptors has been recently shown to have diverse modulatory actions on hippocampal neurons, including supression of synaptic inhibition in area CA1 and modulation of Ca^{2+} channels. To examine the modulatory effects of this receptor class on the excitation of interneurons in the hippocampus, we have developed a combined imaging and electrophysiology setup which allows us to visualize and record from interneurons 50 - 200μm deep in adult rat hippocampal slices (400-500μm) through a combination of infrared imaging with a CCD camera and Nomarski optics.

The selective agonist for these receptors, (1S,3R)-1-aminocyclopentane-1,3-dicarboxylic acid (1S,3R-ACPD)(50-100μM) reversibly increases the frequency of spontaneous excitatory postsynaptic currents (EPSCs) recorded from interneurons located in stratum radiatum and lacunosum-moleculare, presumably reflecting the known excitatory action of 1S,3R-ACPD on CA3 pyramidal neurons. However, the amplitude of the evoked EPSC into these interneurons, obtained through stimulation of the Shaffer collaterals, is depressed in the presence of 1S,3R-ACPD, suggesting that this agonist has an additional action at the CA3 pyramidal neuron - interneuron synaptic terminals. Consistant with this hypothesis, 1S,3R-ACPD reduced the frequency of spontaneous miniature EPSCs (recorded in 1μM TTX). These results suggest that activation of metabotropic receptors can modulate the release of excitatory transmitter through an action at the presynaptic terminal, and may play an important role in the feedforward excitation of stratum radiatum and lacunosum-moleculare interneurons and thereby inhibition of CA1 pyramidal cells.

625.6

INTERACTIONS BETWEEN PRESYNAPTIC INHIBITION AND FREQUENCY FACILITATION AT HIPPOCAMPAL SYNAPSES.
K. P. Scholz* and R J. Miller, Dept. of Pharm. and Physiol. Univ. of Chicago, 60637.

Conditions that reduce the probability of transmitter release are known to increase the degree of facilitation at the neuromuscular junction (c.f. Rahamimoff, 1968). This may be a general property of synaptic transmission. Presynaptic inhibition induced by adenosine or GABAB receptors has been shown to increase paired-pulse facilitation in the hippocampal slice. However, at hippocampal synapses, the properties of facilitation during paired-pulses or during longer trains of stimuli are poorly characterized. We have begun to examine the properties of two-pulse and four-pulse facilitation and the interactions of facilitation with inhibition of release induced by elevated extracellular Mg^{2+}, activation of A1 adenosine receptors and block of presynaptic Ca^{2+} channels by ω-CgTX GVIA. These experiments were performed on hippocampal pyramidal neurons cultured on a photolithographically defined substrate that limited the number of presynaptic neurons to one. Upon switching the extracellular solution from 1 mM Ca^{2+}/2 mM Mg^{2+} to 2 mM Ca^{2+}/1 mM Mg^{2+}, the amplitude of the excitatory postsynaptic current (EPSC) increased by 319± 43%(n=8). In addition, the facilitation ratio (amplitude of second/amplitude of first) for 2 EPSCs evoked at a 60 msec inter-stimulus interval (ISI) shifted from 1.5 to 0.93. Addition of the adenosine receptor agonist cyclopentyladenosine (CPA;100 nM) inhibited the EPSC by 65±7% and shifted the facilitation ratio to 1.5. This effect was readily reversible. Application of ω-CgTX GVIA (250 nM) until the block of the EPSC resembled the inhibition produced by CPA resulted in an increase in the facilitation ratio to 1.3, which was not significantly different than in CPA. During four-pulse facilitation (ISI=25 msec) CPA inhibited the first EPSC by 57±8% (n=5) whereas the fourth EPSC of a train was inhibited by only 26±7. Similar results were obtained in low Ca^{2+} solution. The results do not support a role for voltage-dependent recovery of Ca^{2+} channel inhibition in the increase in facilitation that accompanies presynaptic inhibition.

625.7

HIPPOCAMPAL EXCITATORY SYNAPSES: EVIDENCE FOR PREFERRED SUB-LEVELS IN THE AMPLITUDE DISTRIBUTION OF MINIMAL ALL-OR-NONE SYNAPTIC CURRENTS (EPSCs).
J. F. Storm,* *Inst. of Neurophysiology., University of Oslo, Norway.*

Single-fiber EPSCs in CA1 neurons may be due to a single quantum each: the amplitude distribution of minimal evoked EPSCs resembles that of minis (Raastad&al.,1992,Eur.J.Neurosci.4:113); and facilitation gives no clear increase in the mean non-zero EPSC amplitude (Storm & Raastad,Soc Neurosci.Abstr.17:1486). Still, the size of minis and minimal evoked EPSCs fluctuate much (Bekkers&al.PNAS 87:5359; Manabe&al.Nature 355:50). Here I report evidence that minimal evoked EPSCs exhibit preferred amplitudes.

EPSCs were recorded (whole cell) from CA1 pyramidal cells in slices from young rats. Afferent fibers were stimulated via a patch pipette, with pairs of stimuli (40-60ms interval) of minimal intensity (>50% failures). The facilitation (mean EPSC1 > EPSC2) was usually entirely due to a reduced failure rate, with no detectable increase in the mean amplitude of non-zero EPSCs. This suggests that each EPSC is not due to multiple independent units (e.g. quanta) but either a single unit (quantum), or multiple coupled units. The EPSC amplitude varied 2-10-fold from trial to trial, but often clustered at 2-5 "preferred" levels for periods of 50-500 trials. The "preferred" amplitudes were 2-20 times more frequent than expected from the overall distribution. When the non-zero EPSC2 was reduced (<EPSC1), indicating depression coexisting with facilitation (Storm,Soc.Neurosci.Abstr.18:1340), EPSC2 sometimes showed a stepwise reduction, as if composed of subunits, although the mean of EPSC1 and EPSC2 preceded by failures were equal, suggesting all-or-none EPSCs.

These observations suggest that single-fibre EPSCs are determined at two steps: one for failure rate, another for amplitude. And: single fibre EPSCs show (periods of) preferred amplitudes. Possible mechanisms include concerted release of multiple quanta or subquanta, or alternation between synaptic sites.

625.8

ADENOSINE INHIBITS SYNAPTIC TRANSMISSION BY REDUCING THE PRESYNAPTIC CALCIUM INFLUX AT CA3-CA1 SYNAPSES OF GUINEA PIG HIPPOCAMPUS.
L.G. Wu* and P. Saggau. Division of Neuroscience, Baylor College of Medicine, Houston, TX 77030.

Adenosine is an important regulator of excitatory transmitter release in the central nervous system and at the neuromuscular junction. Recent evidence suggests that adenosine presynaptically inhibits synaptic transmission. Such a presynaptic inhibition could result from either a reduction of voltage-dependent Ca^{2+} current or an inhibition of the transmitter releasing machinery downstream from Ca^{2+} influx. Using a technique to selectively load presynaptic terminals of CA3-CA1 synapses in hippocampal slices with the Ca^{2+} indicator fura-2, we investigated the effects of adenosine on both the presynaptic Ca^{2+} influx and the corresponding field EPSP (fEPSP) induced by a single electrical stimulation of the Schaffer collateral/commissural pathway. Application of 50 mM adenosine reduced the amplitude of the Ca^{2+} influx by 24±2% and the initial slope of the fEPSP by 73±5%. These effects were dose-dependent, partially occluded by the N-type Ca^{2+} channel blocker ω-conotoxin, mimicked by the adenosine A1 receptor agonist cyclopentyladenosine, and blocked by the A1 receptor antagonist 8-cyclopentyl-1,3-dipropylxanthine (DPCPX). DPCPX by itself enhanced both presynaptic Ca^{2+} influx and fEPSP, suggesting a tonic action of adenosine on synaptic transmission. We have demonstrated previously by applying the nonspecific Ca^{2+} channel blocker cadmium, that there is an approximately fourth power relationship between fEPSP and Ca^{2+} influx. Taken together, these results suggest that activation of presynaptic adenosine A1 receptors inhibits evoked transmitter release primarily by reducing the presynaptic Ca^{2+} influx.

625.9

BACLOFEN INHIBITIS SYNAPTIC TRANSMISSION PRIMARILY BY REDUCING THE PRESYNAPTIC CALCIUM INFLUX AT CA3-CA1 SYNAPSES OF GUINEA PIG HIPPOCAMPUS. P. Saggau*, L.G Wu and R.H. Thalmann+. Division of Neuroscience & +Department of Cell Biology, Baylor College of Medicine, Houston, TX 77030.

The predominant inhibitory neurotransmitter in the hippocampus is γ-aminobutyric acid (GABA). Activation of presynaptic $GABA_B$ receptors has been found to cause an inhibition of transmitter release. Two possible mechanisms may couple the activation of $GABA_B$ receptors to the inhibition of transmitter release: 1) decrease of the presynaptic Ca^{2+} influx induced by the action potential, 2) inhibition of the transmitter release machinery downstream from the presynaptic Ca^{2+} influx. Following a selective loading of presynaptic terminals of CA3-CA1 synapses in hippocampal slices with the Ca^{2+} indicator fura-2, we investigated the effects of baclofen, a $GABA_B$ agonist, on both the presynaptic Ca^{2+} influx and the corresponding field EPSP (fEPSP) induced by a single electrical stimulation of the Schaffer collateral/commissural pathway. Application of 50 μM baclofen reduced the amplitude of the Ca^{2+} influx by 31±4% and the initial slope of fEPSP by 83±4%. These effects were: 1) dose-dependent, 2) partially occluded by the N-type Ca^{2+} channel blocker ω-conotoxin (0.5 μM), and 3) blocked by the $GABA_B$ antagonist CGP35348 (500 μM). The relationship between the decrease of fEPSP and Ca^{2+} influx caused by baclofen followed about the same fourth power function as found by applying the voltage-dependent Ca^{2+} channel blocker cadmium. As it is unlikely that interneurons significantly contribute to the detected Ca^{2+} signals, these findings suggest that activation of presynaptic $GABA_B$ receptors causes the inhibition of evoked synaptic transmission primarily by reducing the presynaptic Ca^{2+} influx.

625.10

TIME COURSE OF POTENTIATION OF SYNAPTIC INPUTS IN RAT MOSSY CELLS. B.W. Strowbridge* and P.A. Schwartzkroin. Dept. of Neurological Surgery, University of Washington, Seattle, WA.

Mossy cells, the major cell type in the rat dentate hilus, receive strong excitatory input from collaterals of granule cell axons enroute to the CA3 region of hippocampus. We and others have demonstrated that spontaneously-active granule cell axons generate the spontaneous, large-amplitude EPSPs that are observed frequently in mossy cells. We also observed that strong depolarization of a single mossy cell can potentiate these EPSPs for periods lasting several minutes.

We now extend these findings by demonstrating that this form of potentiation, as well as potentiation evoked by repetitive perforant path stimulation, have a common, stereotyped time course. We quantified spontaneous EPSPs by measuring membrane potential variance in 1 sec. windows of a continuously-sampled record, filtered to remove action potentials. We found that 2-3 minute periods of depolarizing current pulses (approximately 1 nA, 300 ms on, 400 ms off) often triggered a transient period of potentiation, which built up over a period of 30-60 seconds. The peak variance reached was consistent between trials and was similar to the maximum variance attained during "spontaneously-occurring" potentiation epoches. The period of maximal potentiation ranged from a few seconds to minutes, then decayed at a rate usually slower than during the onset. A similar time course was observed after a 30 second period of 2 Hz perforant path stimulation. Short periods of potentiation (lasting a few seconds) also were observed following interictal discharges in CA3. These findings suggest that potentiated EPSPs may reflect a common mode of circuit activity in the dentate that can be triggered by a variety of excitatory inputs.

Supported by NIH grants NS15317, NS18895, and NS09188.

625.11

MACROSCOPIC CURRENTS FROM HIPPOCAMPAL MOSSY FIBER PRESYNAPTIC TERMINALS. *R. Gray**, Division of Neuroscience, Baylor College of Medicine, Houston, TX 77030

Little is known about the types of voltage-gated ion channels present in presynaptic terminals. We have approached this problem by studying the mossy fiber presynaptic terminal in the hippocampal formation. Their large size (3–8 μm diameter) and location adjacent to the CA3 cell body region make them excellent candidates for biophysical analysis. We have used enzyme treatment, microdissection, and trituration to dissociate the CA3 area of the hippocampus from adult guinea pigs and isolate CA3 pyramidal neurons with attached mossy fiber terminals as well as single isolated mossy fiber terminals.

Totally isolated mossy fiber presynaptic terminals were identified with the carbocyanine fluorescent dye 3,3'-diethyloxadicarbocyanine iodide and those still attached to CA3 pyramidal neurons with the dye and by their position on the proximal apical dendrite of the neuron. Whole-terminal currents were measured using perforated-patch recording. We used high-potassium internal saline and HEPES-buffered artificial cerebral spinal fluid (aCSF) bath saline in these experiments to study the activity of potassium and sodium channels. Currents observed varied from single channels to small macroscopic currents.

A putative "A" current was recorded from 3 presynaptic terminals. This outward current was transient (half inactivation in 50 ms) and displayed steady-state voltage-dependent inactivaton (50% inactivation near −60 mV). Inward sodium currents were recorded from 4 presynaptic terminals. Activation occurred at potentials positive to −60 mV from a holding potential of −80 mV, and peak inward currents occurred at −20 mV. Activation was half maximal at −32 mV with a steepness of 7.6 mV and half inactivation was at −80 mV with a steepness of −6 mV. (Supported by NIH NS29871 and MH44754.)

625.12

IS THE LONG LASTING INFLUENCE ON REFLEX EXCITABILITY RELATIVE TO PRESYNAPTIC ACTIVATION HISTORY PRODUCED BY GABAb-MEDIATED PRESYNAPTIC INHIBITION? F.J. Thompson*[1], R. Parmer[1], R.G.Fessler[2]. Depts. of Neuroscience[1], Neurosurgery[2], Univ. of Florida Brain Institute, Gainesville, Florida 32610-0244

We recently reported a decrease in rate-sensitive depression of lumbar monosynaptic reflexes (MSRs) subsequent to midthoracic spinal injury (Thompson et al., 1992; 1993). The reflex magnitudes produced by repetitive sensory inputs were 250% increased over those in normal animals. These changes emphasize basic questions regarding fundamental mechanisms which regulate reflex excitability. To identify a more specific neurosubstrate essential for the expression of rate-depression we analyzed tibial MSR excitability before and following pharmacologic stimulation or blockade of specific receptors in ketamine anesthetized adult rats. Intrathecal application of a specific GABAb agonist (-L-bacolofen) to the L_5 spinal cord resulted in an increase in rate-depression of 250%. In contrast, following intrathecal application of 50 μg of a specific GABAb antagonist (CPG-35348, CIBA-Geigy) to the L_5 spinal cord, decreased rate-sensitive depression of tibial monosynaptic reflexes (MSRs) resulted in 10 Hz MSRs that were 250% larger than observed in the pretreatment controls. Intraspinal injection using micropipettes to deliver < 5μg CGP-35348 resulted in more robust changes with a faster time course of onset. These data support the conclusion that reflex excitability is modulated by a GABAb-mediated rate-depression of reflex magnitude relative to activation history. These preliminary findings indicate that focal application of a specific GABAb antoagoist produced changes which mimicked, in part, those observed subsequent to thoracic cord lesions. (Supported by NIH-NINCDS (NO1-NS-7-2300 and 5-PO1-NS-27511) and the Florida Impaired Drivers and Speeders Trust Fund.

625.13

$GABA_B$ RECEPTOR-INDEPENDENT PAIRED-PULSE DEPRESSION OF SYNAPTIC INHIBITION IN AREA CA3 OF THE RAT HIPPOCAMPUS. N.A. Lambert* and W.A. Wilson Dept. Pharmacology, Duke University Medical Center, Durham, NC 27705.

We have observed paired-pulse depression (PPD) of inhibitory postsynaptic currents (IPSCs) in CA3 pyramidal neurons that is not occluded by the $GABA_B$ receptor agonist baclofen or blocked by the antagonist CGP 35348 (*J.Neurophysiol.* 69:630). When pairs of IPSCs were evoked (in the presence of DNQX, APV and 1mM CGP 35348) at intervals of 15ms-4s the second response was depressed by ~20%. This depression was not due to a shift in E_{IPSC}, and was not blocked by naloxone (10μM) or phorbol-12,13-diacetate (10μM). It was, however, reversibly attenuated by increasing external Mg^{2+} from 1 to 5mM.

In separate experiments IPSCs were evoked by minimal stimulation. Minimal IPSCs were evoked at stimulus intensities that occasionally failed to evoke a response, had amplitudes that fluctuated and were similar to the amplitude of spontaneous IPSCs, and were completely and abruptly blocked by TTX (1μM). Minimal IPSCs, which presumably resulted from activation of single inhibitory fibers, also displayed $GABA_B$ receptor-independent PPD. Unexpectedly, minimal IPSCs were unaffected by baclofen or CGP 35348 in many of these experments, despite a coincident reversible depression of spontaneous IPSC amplitude and frequency.

These results suggest that, in addition to PPD resulting from activation of presynaptic $GABA_B$ receptors, a separate mechanism produces PPD of synaptic inhibition in the hippocampus. In addition, the release of GABA from some inhibitory synapses is apparently not regulated by $GABA_B$ receptors. Supported by the VA, NS-17771 and MH-15177-15.

625.14

DYNAMIC INTERCONVERSION BETWEEN TONIC AND TRANSIENT RELEASE OF GABA IN CULTURED RAT HIPPOCAMPAL NEURONS. J. Vautrin,* A.E. Schaffner and J.L. Barker.
Lab. of Neurophysiology, NINDS, NIH, Bethesda, MD 20892.

18 day-old embryonic rat hippocampal neurons cultured for 3-20 days were clamped with CsCl patch pipettes. Transient synaptic currents and random baseline fluctuations reversed at E_{Cl^-}, were blocked by bicuculline and exhibited the identical channel kinetics, indicating the presence of both tonic and "quantal" forms of GABA release in these cultures. Transient synaptic signals are traditionally ascribed to the release of transmitter quanta preformed in vesicles, while tonic, random activations of synaptic receptors have been interpreted as a "non-vesicular" form of neurosecretion. Remarkably, rapid conversions from tonic to transient or from transient to tonic $GABA_A$ receptor-coupled Cl^- channel activity were recorded in these neurons during experimental conditions that are known to modify internal Ca^{2+} homeostasis like the addition of Zn^{2+}, or ionomycin or tetrodotoxin (TTX) or depolarizing the presynaptic neuron. These experimental treatments also changed the amplitude and time course properties of the Cl^- transients in the presence of TTX. These results suggest that pulsatile release of GABA, rather than representing the release of preformed quanta, might be directly driven by intracellular, quantal transient increases in the local Ca^{2+} concentration supplied by extracellular and/or internally-stored Ca^{2+}.

625.15

ATP TRANSMITS SYNAPTIC RESPONSES AND STIMULATES INTERNAL Ca INCREASE IN RAT CULTURED HIPPOCAMPAL NEURONS. K. Inoue*, K.Nakazawa, S. Koizumi, T. Watano, T. Obama, K. Fujimori & A. Takanaka. Division of Pharmacology, National Institute of Health Sciences, 1-18-1 Kamiyoga, Setagaya, Tokyo 158, Japan.

We have reported that ATP directly activates small sustained currents, and indirectly transient currents, presumably by evoking glutamate release in rat cultured hippocampal neurons (Neurosci. Lett., 134, 215, 1992). Here we show the synaptic currents transmitted by ATP using whole-cell recordings and mutual connections between ATP- and glutamate-neurons using the fura-2 method. A subpopulation of cultured neurons evoked repetitive inward currents (-10 to - 380 pA at -60 mV of a holding potential). They were abolished in low-Ca (100nM) buffer, and were decreased to the level of miniatures in the presence of 3μM tetrodotoxin. These currents were blocked reversibly by ATP-receptor blockers, suramin (100μM), reactive blue 2 (RB2, 100μM) and α,β-methylene ATP (100μM). ATP (10μM) also increased internal Ca which was blocked by suramin (100μM) or RB2 (100μM). These data suggest that ATP has a role of synaptic transmission with internal Ca increase in rat cultured hippocampal neurons.

625.16

ENDOCYTIC UPTAKE OF GLUTAMATE BY CHOLINERGIC GROWTH CONES ENABLES ESTABLISHMENT OF GLUTAMATERGIC SYNAPTIC TRANSMISSION. Y. Dan*, H-j. Song, R. Wong+ and M-m. Poo. Dept. Biol. Sci., Columbia University, N.Y., N.Y. 10027. +SUNY Hlth. Sci. Ctr. at Brooklyn.

Growth cones of cholinergic spinal neurons from Xenopus embryos rapidly establish functional synapse with co-cultured myotomal myocyte after growth cone-myocyte contact, as shown by immediate appearance of spontaneous and nerve impulse-evoked synaptic currents in the contacted myocyte. In the present study, these cholinergic neurons were incubated in a medium containing 60 mM of glutamate for 30 min and their growth cones were tested for the ability to establish functional synaptic transmission using glutamate as transmitter. Acutely dissociated hippocampal pyramidal neurons were isolated from CA1 regions of adult rat hippocampi by the method of Kay and Wong (J. Neurosci. Methods, 16, 227, 1986), and whole-cell clamped before manipulated into contact with the growth cone of glutamate-incubated Xenopus cholinergic neurons. Spontaneous synaptic currents were observed in many of the neurons tested. Pharmacological evidence suggests these currents were induced by glutamate secretion from the incubated neurons. The currents exhibited a skewed amplitude distribution in the range of 10 to 200 pA, similar to that observed for the ACh secretion from same neurons and for glutamate secretion from other glutamatergic terminals. Impulse-evoked synaptic currents were also detected in some cases, but repetitive presynaptic stimulation resulted in rapid depression of the evoked release. Taken together, these results suggest (1) direct topological relationships between endocytic pathways and transmitter secretion pathway in the growth cone of developing neurons, and (2) phenotypic transformation of neuronal types using different transmitter molecules may involve little more than a supply of transmitters and transporter proteins for vesicular accumulation of the transmitter.

625.17

GTPγS BLOCKS SYNAPTIC RELEASE OF GLUTAMATE AT RAT HIPPOCAMPAL SYNAPSES IN CULTURE. P. S. Jackson*, K. M. Harris, and L. I. Benowitz., Departments of Neurosurgery and Neurology, Children's Hospital and Program in Neuroscience, Harvard Medical School, Boston, MA 02115.

Non-hydrolyzable guanine triphosphate analogs have been shown to alter regulated exocytosis in a variety of cellular systems such as mast cell degranulation[1] and to alter synaptic release at the squid giant terminal[2] through their effects on a small G-protein. The present study was designed to test the role of small G-proteins in exocytosis at mammalian central nerve terminals. Whole cell recordings were made from pairs of synaptically coupled neurons in tissue culture. Guanine nucleotide analogs were introduced into the presynaptic cell and allowed to diffuse down the axon to the presynaptic terminal. When GTPγS (1mM) was included in the presynaptic patch pipette solution, the magnitude of the postsynaptic response began to decline ~8-10 minutes following the initiation of whole cell access. Within five minutes following the beginning of the decline, there was no detectable post-synaptic response following presynaptic stimulation. This pattern of inhibition was mimicked by GppNHp (1mM) but not by GTP (0.5mM) nor by the inclusion of F^- in the patch solution (135mM). These results suggest that the cycling of a small G-protein at the presynaptic terminal is necessary for synaptic release. The failure of F^- to block release argues against the involvement of a large, heterotrimeric G-protein in this inhibition. We are currently evaluating whether there is a morphological correlate of this inhibition by using electron microscopy to examine the vesicle distribution in affected nerve terminals. In addition we are evaluating approaches to selectively manipulate specific G-proteins.

Supported by the Boston Neurosurgical Foundation, NIH EY05690, NS21184 and T32ET07110

(1) Oberhauser, A. F. *et al., Nature* **360**, 270-273 (1992).
(2) Hess, S. D. *et al., Science* **259**, 1169-1172 (1993).

625.18

MULTIPLE VESICULAR RELEASE OF TRANSMITTER FROM HIPPOCAMPAL NEURONS. G. Tong* and C. E. Jahr. Vollum Institute, Oregon Health Sciences University, Portland, OR 97201.

Transmitter is thought to be released in quantal units by vesicular exocytosis but it is unclear how many vesicles can be released simultaneously from a single bouton. If more than one vesicle is released, the peak concentration of free transmitter in the synaptic cleft and the time during which it is elevated above an effective concentration will increase. We have monitored the concentration time course of synaptically released transmitter in conditions of low and high release probability by measuring the percent inhibition of the NMDA receptor component of the epsc in cultured hippocampal neurons produced by the low affinity competitive antagonist, L-AP5 (Clements et al.,*Science* 258:1498,1992). If transmitter remains elevated for longer periods, less inhibition will result because more receptors will become available for transmitter binding as antagonist dissociates. In the presence of 3 mM extracellular Ca^{2+}, the amount of block of NMDA epscs by L-AP5 (400 μM) (46.2±3.3%) was significantly less than that in the presence of 1 mM Ca^{2+} (59.6±2.2%; n=7, p<0.0001, paired *t*-test). The percent inhibition of whole-cell NMDA responses by L-AP5 was unaffected by extracellular [Ca^{2+}] (1-3 mM; n=6). The amount of block of NMDA epscs by L-AP5 was also reduced when 4-AP (300 μM) was used to increase the release probability (57.3±3.3% in the presence of 4-AP, 70.8±1.8% in control, n=5, p=0.0033). Furthermore, when paired-pulse facilitation of NMDA epscs was induced, inhibition of the second NMDA epsc by L-AP5 (54.9±3.5%) was less than that of the first epsc (62.9±3.3%; n=6, p=0.0013). AMPA receptor component of the epsc remained the same in the presence and absence of L-AP5 (n=5) indicated that L-AP5 has no effect on presynaptic release. These data suggest that multiple vesicular release of transmitter occurs in the central synapse.
Supported by NIH grant NS21419.

625.19

PRIMING OF GLUTAMATE RELEASE PERSISTS FOLLOWING REMOVAL OF PHORBOL ESTER AND IS CALMODULIN-INDEPENDENT. D.M. Terrian*, C.M. Manring, D.K. Ways[1], and D.A. Zetts. Depts. Anatomy and [1]Medicine, East Carolina University School of Medicine, Greenville, NC 27858.

The K^+-evoked release of endogenous glutamate, but not opioid peptides, from isolated hippocampal nerve endings (synaptosomes) is significantly enhanced following the sustained activation of protein kinase C (PKC). Although a phorbol ester (phorbol 12,13-dibutyrate, PDBu) was used to sustain PKC activation, the selective enhancement of glutamate release persisted for at least 20 min after the complete removal of PDBu (Terrian et al., Hippocampus 3:205-220, 1993). In contrast to the acute effects of PDBu, sustained PKC activation lead to an enhancement of the slow phase of glutamate release that was no longer sensitive to PKC inhibitors. This effect of PDBu cannot be attributed to a reduction in membrane potential, since PDBu pretreatment did not alter the Ca^{2+}-independent component of K^+-evoked release. Moreover, this novel effect of PDBu displayed a strict requirement for localized Ca^{2+} entry, increasing glutamate release evoked by depolarization but not the Ca^{2+} ionophore, ionomycin. A plausible explanation for these results might be that PDBu-induced phosphorylation of calmodulin (CaM)-binding substrates of PKC could enhance the release of glutamate through the phosphorylation-induced liberation of free CaM and activation of CaM kinase II. However, three lines of evidence argue against the involvement of such a PKC-CaM kinase II "cross-talk" mechanism: 1) enhancement of glutamate release is not diminished under conditions which do not support CaM kinase II activity in intact synaptosomes, equimolar of $BaCl_2$ for $CaCl_2$; 2) the level of cytosolic free CaM is not altered in synaptosomes following pretreatment with PDBu and its removal; and 3) the level of autonomous CaM kinase phosphotransferase activity is not altered following an identical pretreatment with PDBu. Thus, the persistent activation of synaptosomal PKC selectively primes the glutamate release apparatus to respond to localized Ca^{2+} entry by a CaM-independent mechanism.

625.20

CALCIUM INDUCES VESICULAR GLUTAMATE RELEASE FROM PERMEATED SYNAPTOSOMES. J.J.H. Hens, W.E.J.M. Ghijsen#, W.H. Gispen and P.N.E. De Graan*. Rudolf Magnus Inst., Vondellaan 6, 3521 GD Utrecht, and #Exp. Zoology, Amsterdam, the Netherlands.

Glutamate (glu) is the most important excitatory transmitter implicated in long-term potentiation (LTP). To unravel the presynaptic mechanisms leading to enhanced glu release during LTP, fundamental knowledge about the molecular mechanisms underlying recruitment, docking and fusion of glu-containing small clear-cored vesicles is required. Therefore, we developed a permeated synaptosome preparation, which enables us to introduce macromolecules and to study their effects on Ca^{2+}-induced glu release. Highly purified synaptosomes were permeated with streptolysin-O (SLO) and glu release was measured in supernatant samples by HPLC. Ca^{2+}/EGTA buffers were used to control $[Ca^{2+}]_i$. Increasing $[Ca^{2+}]_i$ from 10^{-8} M (basal level) to $3x10^{-5}$ M induced a release of 2 nmol glu/mg of synaptosomal protein. This Ca^{2+}-induced glu release is clearly from vesicular origin, because it was completely inhibited by tetanus toxin light chain (300 nM). Ca^{2+}-induced glu release is measured on top of a basal cytosolic efflux of glu (at 10^{-8} M Ca^{2+}), which is about 55% of the total glu content. Significant stimulation of vesicular glu release was observed at $[Ca^{2+}]_i \geq 3x10^{-6}$ M, and maximal at $3x10^{-5}$ M. The maximal amount of glu released from permeated synaptosomes is about the same as Ca^{2+}-dependent K^+-induced glu release from intact synaptosomes. The Ca^{2+} sensitivity of glu release from SLO-permeated synaptosomes (EC_{50} ~10^{-5} M) does not markedly differ from that of noradrenaline and cholecystokinin-8 release from the same preparation. Ca^{2+}-induced glu release was not inhibited by protein kinase C (PKC) inhibitors PKC_{19-36} ($3x10^{-5}$ M) and H-7 (10^{-4} M), whereas polymyxin B (200 IU/ml) blocked it completely. At these concentrations all three compounds inhibited the phosphorylation of the nervous tissue-specific PKC substrate B-50/GAP-43 in SLO-permeated synaptosomes. Our data show that the SLO-permeated synaptosome system is suitable to study vesicular glu release, and indicate that PKC activation and PKC-mediated B-50 phosphorylation are not involved in steps in the mechanism of glu release after the Ca^{2+} trigger.

625.21

MEASUREMENT OF GLUTAMATE RELEASE FROM HIPPOCAMPAL SLICES WITH MICROSCOPIC PHOTON-COUNTING DETECTION SYSTEM L. Li, M. Arnold, R. Malinow*. Departments of Chemistry and Physiology & Biophysics, U. of Iowa.

Glutamate is the major excitatory neurotransmitter in the vertebrate central nervous system. The long-term goal of this project is to develop a chemical sensor capable of real-time detection and quantification of glutamate during its release from neurons. A bicatalytic/chemi-luminescence reaction sequence using glutamate oxidase and horseradish peroxidase has been developed in which light is generated in the presence of glutamate. The light is monitored by a microscopic photon-counting detection system. Millisecond temporal resolution and micromolar detection limit have been obtained based on this sensing system. A calcium-dependent release of glutamate has been successfully measured during potassium depolarization of rat hippocampal slices. This sensing approach will be used to measure glutamate transients during electrical stimulation of rat hippocampal slices. The kinetics of the signal may provide an estimate of the distance between the enzymatic sensor and the site of glutamate release and thereby allow an estimate of peak glutamate concentration at the synaptic cleft during synaptic transmission. Changes in transmitter release after different perturbations such as LTP may also be monitored.

POSTSYNAPTIC MECHANISMS II

626.1

REGULATION OF AN NMDA-MEDIATED EPSP BY A SLOW DENDRITIC $GABA_A$-MEDIATED IPSC IN PIRIFORM CORTEX. A.Kapur*, R.A.Pearce and L.B.Haberly. Neuroscience Training Program and Departments of Anatomy and Anesthesia, Univ. of Wisconsin, Madison, WI 53706.

The observation that blockade of $GABA_A$-mediated inhibition is necessary for the induction of associative LTP in piriform cortex (Kanter & Haberly, J. Neurosci., in press) has prompted study of the regulation of NMDA-mediated EPSPs by inhibitory processes. As previously reported (Kapur et al, Soc. Neuro. Abs. 18:1497), blockade of $GABA_A$ receptors by bath applied bicuculline (BIC) resulted in a large increase in the NMDA component of the response to burst stimulation of afferent fibers (4 pulses at 100 Hz). Subsequent study has revealed that local application of BIC in the distal dendritic region facilitated the NMDA component whereas BIC application at the soma did not. Dendritically applied BIC had little or no effect on the large feedback IPSP evoked by stimulation in the association fiber layer that was blocked by somatic application. This suggests the presence of a previously unrecognized $GABA_A$-mediated IPSP in the dendritic region that is mediated by a feedforward pathway. One factor that could contribute to the stronger action of the dendritic than somatic IPSP on release of the NMDA component is spatial proximity; however the recent demonstration of fast and slow Cl^- mediated IPSPs in hippocampus (Pearce, Neuron 10:189, 1993) suggests that differences in the duration of dendritic and somatic IPSCs could also contribute. To test this hypothesis, timecourses of $GABA_A$-mediated IPSCs evoked by stimulation in layer Ia (distal dendrite-afferent fiber zone) and layer II-deep Ib (proximal dendrite-cell body zone) were compared. IPSCs were isolated by addition of DNQX (20 μM) and D-APV (15 μM) to the bath (to block EPSPs) and 5 mM QX-314 to the recording pipette (to block the $GABA_B$-mediated IPSC). Whole-cell recordings were obtained under voltage clamp. Time courses of decay of IPSCs evoked at both depths were best fit with the sum of 2 exponentials with time constants of 8-16 ms and 30-80 ms (28°C). However, the slow component was significantly ($p<0.005$) larger from layer Ia than from layer Ib-II stimulation (relative amplitude of slow component: 54±6.9(SE)%, n=4, for Ia vs 21±5.5%, n=8, for Ib-II). This suggests that the slower time course of the dendritic IPSC may contribute to its ability to strongly regulate the NMDA component. Supported by NS19865 (LBH) and NS01548 (RAP).

626.2

FUNCTIONAL DIVERSITY OF GABA-ACTIVATED CURRENTS IN PURKINJE VERSUS GRANULE NEURONS IN RAT CEREBELLAR SLICES. G. Puia*, E. Costa, and S. Vicini. FGIN, Georgetown Univ., Washington DC.

In rat cerebellar slices, we compared spontaneous inhibitory postsynaptic currents (sIPSCs) to Cl^- currents produced by the fast application (< 0.2 ms) of GABA pulses to outside-out membrane patches excised from Purkinje and granule neurons. We used whole-cell recording with a symmetrical Cl^- solution and after blockade of excitatory synapses with excitatory amino acid receptor antagonists. sIPSCs had largely variable peak amplitude and were reduced in frequency and amplitude by TTX (1 μM) and abolished by bicuculline methiodide (10 μM). In 42 granule neurons, sIPSCs had a rise time of 0.6 ± 0.3 ms (mean ± SD of the average of 50 events/cell) while in 49 Purkinje cells sIPSCs had a rise time of 1.5 ± 0.7 ms. At a holding potential of -60 mV, average sIPSCs in Purkinje cells decayed with a single exponential curve with a time constant (τ) of 17 ± 6.5 ms, while in granule cells sIPSCs' decay was best described by the sum of two exponential curves with a fast τ of 7 ± 1.6 ms and a slow τ of 59 ± 16.8 ms; the contribution of the slow component to the peak current ranged from 15 to 100 %. The fast application of a brief pulse of GABA (1 mM) to 9 patches excised from granule cell elicited rapidly rising Cl^- currents with decay best described by the sum of two exponential with a fast τ of 3 ± 1.8 ms and a slow τ of 102 ± 48 ms. In 6 outside-out patches from Purkinje neurons the decay of the currents produced by fast GABA application was described by a double exponential curve with a large preponderance of a fast τ of 5.1 ± 2.3 ms and the presence (<10%) of a slow τ of 95 ± 36 ms. The fast decay of GABA-activated Cl^- currents in excised patches could be related to the presence of a fast component of desensitization produced by the sustained application of 1 mM GABA. These results demonstrate distinct functional properties of GABA receptors Cl^- channel complexes perhaps related to the large heterogeneity in the molecular assembly of subunits forming various $GABA_A$ receptor subtypes. Supported by NIH grant R01 MH49486-01A1.

626.3

DIFFERENCES BETWEEN SPONTANEOUS INHIBITORY POSTSYNAPTIC CURRENTS (sIPSCs) RECORDED IN GRANULE CELLS AND HILAR NEURONS OF THE DENTATE GYRUS. I. Soltesz* and I. Mody. Depts. of Anesthesiology & Neurology, UT Southwestern Med. Ctr., Dallas, TX.

Recordings with sharp microelectrodes have failed to demonstrate notable sIPSCs in hilar neurons (HNs). Yet, receptor binding and molecular biological studies have clearly identified $GABA_A$ receptors on HNs, and morphological investigations have revealed a specific GABAergic innervation of HNs.

We have examined characteristics of sIPSCs in HNs using whole-cell voltage-clamp recordings in standard 400 μm thick horizontal adult rat brain slices maintained at 35-36°C. All recordings were done in the presence of the glutamate receptor antagonists CNQX (10 μM) and D-AP5 (40 μM). The frequency of sIPSCs recorded in this manner in HNs in symmetrical Cl^- was between 15-50 Hz, comparable to that found in granule cells (GCs). The decay kinetics of sIPSCs in HNs were examined, and the single channel conductance together with the number of active subsynaptic $GABA_A$ receptor channels were determined by non-stationary noise analysis. Consistent with different molecular subunit assemblies of subsynaptic $GABA_A$ receptor channels, HNs differed from GCs in having a 24% longer decay time constant and a 22% smaller single channel conductance. The average number of subsynaptic $GABA_A$ receptor channels contributing to sIPSCs were similar in GCs and HNs. Cell attached recordings of K^+ channel activity were performed to ascertain the relationship between the resting membrane potential (MP) and E_{Cl} in HNs and GCs. A change in the MP following bath application of GABA or muscimol should be reflected in the altered size of K^+ channel openings. Invariably, GCs were depolarized (15-25 mV) by $GABA_A$ receptor agonists, whereas HNs responded with either a small change in the MP (<5 mV) or a GC-like depolarization. No significant GABA-induced hyperpolarizations were noted in HNs.

Supported by NINDS grant NS-30549.

626.4

RECEPTOR SATURATION, CHANNEL KINETICS, AND THE APPLICABILITY OF NOISE ANALYSIS DETERMINED THROUGH MODELING OF QUANTAL SYNAPTIC CURRENTS. Y. De Koninck*[1] and I. Mody[2]. [1]Dept. Neurology & Neurological Sci., Stanford Univ., Stanford, CA, and [2]Depts. of Anesthesiology & Neurology, UT Southwestern Med. Ctr., Dallas, TX.

The method of non-stationary noise analysis (Sigworth, *J.Physiol.* **307**:97, 1980; *Biophys.J.* **35**:289, 1981) can be used to extract information about the properties of single channels underlying synaptic currents (Robinson *et al. Biophys.J.* **59**:295, 1991). However, the technique relies heavily on the appropriate scaling of ensemble averages to individual synaptic currents. The large intrinsic variance that occurs at different phases of a given synaptic current (Faber *et al. Science* **258**:1494, 1992) may preclude proper scaling of the ensemble averages and lead to systematic errors.

We used stochastic simulation of synaptic currents to determine the relationship between the kinetic properties of subsynaptic channels and the resulting intrinsic variance during the time-course of unitary synaptic currents. We estimated the accuracy of the noise analysis in evaluating the properties of channels underlying synaptic currents that were generated with different kinetic properties and receptor saturation.

The accuracy of the non-stationary noise analysis was mainly dependent on the relationship between the onset kinetics of the current and the open times of subsynaptic receptor channels but not on receptor saturation. [1]For currents with rapid onset, accurate estimates of the properties of subsynaptic channels could be obtained even at low levels of saturation. Moreover, the level of saturation of the underlying receptor channels could be resolved by spectral analysis. Thus, such analysis appears more appropriate for the study of synaptic currents with rapid kinetics of onset (*e.g.*, AMPA, $GABA_A$),than for slower onset synaptic currents (*e.g.*, mediated by NMDA receptor channels; see Edmonds & Colquhoun, *Proc.Roy.Soc.Lond.B* **250**:279, 1992).

Supported by NINDS grants NS-27528 and NS-30549. Y.D.K. is a Fellow of Medical Research Council of Canada.

626.5

EVOKED SHIFTS IN EXTRACELLULAR pH, BICARBONATE AND CO2 IN RAT HIPPOCAMPAL SLICES. *M. Chesler* & JCT Chen. Dept. Physiol.. & Biophys./Dept. Neurosurg., NYU Med. Ctr., 550 First Ave., NY, NY 10016.*

To elucidate the nature of synaptically evoked extracellular pH (pH_o) shifts (1), we determined the dynamics of extracellular bicarbonate ($[HCO_3^-]_o$) and CO_2 in the CA1 region of rat hippocampal slices, using pH and $CO_3^=$ microelectrodes (2). Baseline & evoked acidosis (stim. of Schaffer collaterals, 20 Hz, 5 s) were due entirely to elevation of PCO_2. Baseline pH_o was 7.14 ± 0.078 (mean ± SD, n=90 trials in 17 slices) with a PCO_2 of 78 ± 18 torr. Evoked acid shifts & associated increases in PCO_2 averaged 0.062 ± 0.01 pH units/12 ± 6.0 torr (n=17). The early alkaline shift (0.059 ± .025 pH units, n=76) was associated with a $[HCO_3^-]_o$ rise of 0.98 ± 1.32 mM & PCO_2 fall of 7.0 ± 6.1 torr. Picrotoxin (PTX) had no consistent effect on $[HCO_3^-]_o$ & PCO_2 transients. In the presence of the carbonic anhydrase (CA) inhibitor benzolamide, the alkaline shifts were amplified 5-fold (1), however, the calculated $[HCO_3^-]_o$ paradoxically decreased by 4.99 ± 4.53 mM, suggesting that in the absence of catalyzed HCO_3^- formation, a HCO_3^- uptake mechanism was unmasked. In a smaller number of untreated slices, stimulus-evoked decreases in $[HCO_3^-]_o$ were also sometimes noted, suggesting a transient CO_2/HCO_3^- disequilibrium under "normal" conditions. Thus, the amount of extracellular CA may be insufficient to insure maximum buffering of rapid base loads. Indeed, recordings with $pH/CO_3^=$ and CO_2 electrodes indicated that the equilibrium-based Henderson Hasselbach equation does not hold in many instances of repetitive stimulation. Extracellular buffering power is therefore a time-dependent variable in the brain extracellular space, which may depend on the degree of synchronous synaptic activity. (1) Chesler & Kaila TINS 15:396 (2) Wietasch & Kraig Am. J. Physiol. 261:R260.

Supported by NIH grant NS27011.

626.6

SYNAPTIC MECHANISM OF STIMULATION-INDUCED ALKALINE pH SHIFTS IN THE RAT HIPPOCAMPAL SLICE. K. Kaila*, P. Paalasmaa, T. Taira and J. Voipio, Departments of Zoology and Physiology, P.O. Box 17, SF-00014 Univ. Helsinki, Helsinki, Finland.

The synaptic basis of stimulation-evoked alkaline transients was studied in rat hippocampal slices (300-400 um) kept in an interface chamber. The interstitial pH was recorded in the pyramidal layer of area CA1 using double-barrel H^+-selective microelectrodes. Stimulus trains (10-100 Hz) with a constant number (100) of pulses or with a constant duration (5-10 s) were applied to the Schaffer collaterals. Inhibition of extracellular carbonic anhydrase (CA_e) activity by benzolamide (BA) or prontosil-dextran 5000 (PD 5000) was used to distinguish between HCO_3^-/CO_2- dependent ($GABA_A$ receptor-mediated) and H^+-dependent (glutamatergic) alkaline shifts: the former are known to be inhibited, and the latter enhanced, upon Ca_e inhibition. Regardless of the number of stimuli applied, alkaline shifts evoked by stimulation at a relatively low frequency (5-10 Hz) were enhanced by BA and by PD 5000, and they were not blocked by picrotoxin (PiTX). In sharp contrast to this, the alkaline shifts induced by trains of 100 pulses applied at 50-100 Hz were attenuated by BA, PD 5000 and by PiTX. We conclude that the relative contributions of glutamate and $GABA_A$ receptor-mediated effects to activity-induced alkaline shifts depend on the pattern of stimulation. In particular, the $GABA_A$-mediated component dominates initially in trains given at a high frequency. The results also suggest that, even when elicited using low-frequency stimulation, the alkaline shift is not solely attributable to excitatory synaptic activity. This view gains support from the finding that, in the presence of 100 uM pentobarbital, the alkaloses evoked by 5-10Hz trains were attenuated following CA_e inhibition. Taken together, our observations fit well with the excitatory and inhibitory synaptic circuitry of the CA1 region and with the fatigue properties of GABAergic transmission.

626.7

MORPHOELECTROTONIC TRANSFORMS IN THREE CLASSES OF RAT HIPPOCAMPAL NEURONS. K.Y.Tsai[1,3], N.T.Carnevale*[1,3], B. J. Claiborne[4], and T.H.Brown[1-3]. Depts. of Psych.[1] and Cell. & Mol. Physiol.[2], the Center for Theoretical and Applied Neuroscience[3], Yale Univ., New Haven, CT 06520 and Div. of Life Sci.[4], Univ. of Texas at San Antonio, San Antonio, TX 78285.

We compared the electrotonic structure of three classes of rat hippocampal neurons by applying morphoelectrotonic transforms (METs) [Brown et al, 1992, In: *Single Neuron Computation*] to realistic compartmental models [Claiborne et al, *op. cit.*]. This generates an intuitive graphical display of voltage or current/charge attenuation [Carnevale & Johnston, *J. Neurophysiol.* 1982] by recasting the branched architecture of the cell using branch lengths proportional to the logarithm of the attenuation for each segment.

The METs disclosed several facts about these cells. First, voltage attenuation is asymmetric (worse for signal transfer toward the soma (Vin) than away from it (Vout)). Second, significant voltage attenuation for Vin occurs along major processes, even at DC and low frequencies (~5 Hz). Third, voltage attenuation in both directions worsens rapidly as frequency increases. Fourth, the Vin and Vout METs of dentate granule cells are comparable to METs of the basilar dendrites of CA1 and CA3 pyramidal neurons, although granule cells have a simpler branching pattern. Finally, Vout METs of the apical dendritic trees of CA1 and CA3 neurons are qualitatively different. CA1 neurons appeared to have one or two long main trunks with many short side branches; whereas in CA3 neurons the ramifications were more equal in length. The CA1 neurons had a greater overall electrotonic length.

We conclude that the MET is a useful approach to understanding comparative electrotonic structure. *Supported by ONR , ARPA & CTAN.*

626.8

ESTIMATING VOLTAGE-DEPENDENT CONDUCTANCE PARAMETER VALUES FOR DENTATE GRANULE CELL MODELS. W.R. Holmes*. Neurobiology Program, Dept. of Biological Sci., Ohio U., Athens, OH 45701.

Models that include voltage-dependent ionic conductances need values for parameters used in the equations that describe the conductances. These values are usually determined by "trial and error" or an exhaustive stochastic search scheme. However, selecting values (i.e., gbars) for conductances in dendrites is difficult. Spruston and Johnston (1992) and Staley et al. (1992) report that input resistance (R_N) and τ_0 are larger at depolarized potentials in dentate granule cells. These data and voltage-dependent conductance descriptions modified from Yuen and Durand (1991) were used in a model of a dentate granule cell to constrain estimates of dendritic conductance values.

When voltage-dependent conductances were restricted to the soma, R_N and τ_0 were only slightly larger at depolarized potentials. To match the experimental data, soma conductance would have had to increase 10-fold for a 10 mV subthreshold change in potential. No acceptable soma conductance gbar values could be found to do this.

When voltage-dependent conductances were included on the dendrites, much larger changes in R_N and τ_0 were obtained at depolarized potentials. When soma gbar values were fixed and dendritic conductance distributions were uniform, dendritic conductance gbar values could be found to produce the same magnitude of R_N and τ_0 changes at depolarized potentials as observed experimentally.

Different conductance kinetics or adding other conductances could affect these results. However, if the model conductance descriptions have a solid experimental basis, data for R_N and τ_0 at different potentials allow one to constrain estimates of gbar values in the dendrites. Of course such estimates will be non-unique, especially if conductance distributions are non-uniform.

626.9

CABLE PROPERTIES AND MORPHOLOGY OF LATERODORSAL TEGMENTAL (LDT) NEURONS *IN VITRO*. A. Surkis*, B. Taylor, C. Peskin and C.S. Leonard, Center for Neural Science, New York University, 6 Wash. Pl., NY, NY 10003.

Considerable evidence supports a role for brainstem cholinergic neurons of LDT in controlling the alternation between rapid eye movement and slow wave sleep. Since an understanding of the integrative properties of these cells is necessary to further elucidate this role, we have investigated the passive membrane properties of guinea pig LDT neurons with intracellular recordings in a brain slice preparation. Electrophysiological data were obtained by direct current injection using long (300msec,~0.5nA) and short (1msec,~1nA) pulses. Morphological data were obtained by computer reconstruction of the same neurons after biocytin injection and DAB visualization. The studied cells (n=5) were presumed to be cholinergic due to the correspondence of their physiology with that of LDT type II cells of which 93% (n=60) were identified as cholinergic based on NADPH-diaphorase histochemistry. Analysis of dendritic shape revealed the cells to be poor candidates for an equivalent cylinder approximation. Specifically, electrotonic lengths of the branching paths of a given dendrite were highly variable, dendrite diameter was non-uniform, and branching deviated from the Rall 3/2 power law, particularly for higher order branches. As a result, a numerical solution of the cable equation for cells of arbitrary geometry was implemented and solved for each of the filled cells. This solution does not assume a square pulse stimulus, so a more varied data set can be used. Cable parameters were determined by choosing values which produced the best fit to the recorded voltage traces. Synaptic inputs will be included in the model to investigate the integrative properties of the cells. Supported by NS27881.

626.10

DOSE ACTIVATION OF CALCIUM-DEPENDENT CHLORIDE CHANNEL CAUSE POST-TETANIC DEPOLARIZATION IN RABBIT PARASYMPATHETIC NEURONS? M. Ishimatsu, T. Nishimura, T. Tokimasa* and T. Akasu. Dept. of Physiol., Kurume Univ. Sch of Med., Kurume 830, Japan

Intracellular recordings were made from neurons in rabbit parasympathetic ganglia on surface of the urinary bladder *in vitro* at 36 ± 1 °C. Direct action potentials were evoked by brief (4-20 ms) cathodal current injection (0.1-1 nA) through a recording electrode. Cells were classified into two groups regarding the duration of afterhyperpolarization (AHP) of spikes. In a majority of neurons tested (n=250), a single action potential was followed by fast and slow AHPs (J. Auton. Nerv. Syst., 1988). In contrast 21 cells consisted of a distinct sub-population having a short duration of AHP (<50 ms) following a single spike. A train of direct action potentials at frequency of 5-20 Hz for 1-30 s was followed by a post-tetanic depolarization (PTD) instead of a post-tetanic hyperpolarization (PTH) in neurons of the latter sub-population (PTD-neuron). The amplitude and duration of the PTD were a function of the number of action potentials in the train. The PTD was associated with a decrease in input resistance. The amplitude of the PTD was increased by membrane hyperpolarization. The estimated reversal potential of the PTD was -25 mV. Low chloride solutions augmented the amplitude and duration of the PTD. Low sodium, high potassium or low potassium solutions did not significantly affect the PTD. Nominally free calcium solutions and ω-conotoxin (500 nM) abolished the PTD. These data suggest that activation of the calcium-dependent chloride channels is responsible for the PTD. In a particular PTD-neuron, the PTD was converted to the PTH during experiments without changing a resting potential. Contrary, the PTH was converted to the PTD by apamin (5 nM). These data imply that the rabbit parasympathetic neurons are endowed with a mechanism switching from the PTD to the PTH and *vice versa*. Supported in part by The Ishibashi Research Fund.

626.11

DO DEPOLARIZING MEMBRANE OSCILLATIONS REGULATE SPONTANEOUS DISCHARGE OF ACTION POTENTIALS IN FELINE PARASYMPATHETIC NEURONS? T. Nishimura[*1], T. Akasu[1] and J. Krier. [1]Dept. of Physiol., Kurume Univ. Sch. of Med, Kurume 830, Japan and Dept. of Physiol., Michigan State Univ., East Lansing, MI 48824, U.S.A.

The properties of depolarizing membrane oscillations (DMOs) of neurons in colonic parasympathetic ganglia (CPG) were studied *in vitro* using intracellular recording techniques. Neurons were classified into two types, spontaneously discharging or quiescent neurons. For quiescent neurons, action potentials (APs) and DMOs were evoked by injection of cathodal currents of long duration (3 s-5 min) and at varying intensities (0.01-1.5 nA). Each action potential was preceded by a prepotential with rates of rise ranging between 0.12-0.25 V/s. DMOs had rates of rise ranging between 0.05 and 0.23 V/s and occurred for the duration of the cathodal depolarizing pulses. Their amplitude and frequency increased with larger membrane depolarization, suggesting that DMOs are voltage-dependent. Hexamethonium (100 μM), d-tubocurarine (3 μM), atropine (1 μM), phenoxybenzamine (1 μM) and propranolol (1 μM) did not affect DMOs, suggesting that they are an intrinsic property of CPG neurons. DMOs were blocked by tetrodotoxin (0.3-3 μM) and by low sodium solutions whereas high and low potassium solutions and low chloride solutions had no effect. These data suggest that a non-inactivating sodium current is involved in the generation of DMOs. For spontaneous discharging neurons, prepotentials associated with APs and DMOs also occurred. Muscarine (100 nM) initiated DMOs in quiescent neurons and converted to spontaneous discharging neurons. In contrast, UK14304 (100 nM), an α_2-adrenoceptor agonist, abolished DMOs and converted spontaneous discharging neurons to quiescent neurons. These data suggest that DMOs regulate the excitability of feline CPG neurons. Supported in part by The Ishibashi Research Fund and NIH-DK29920.

626.12

OPIOID MODULATION OF K AND Ca CURRENTS IN MAMMALIAN NERVE TERMINALS. M.I. Banks*, K. Bielefeldt and M.B. Jackson Department of Physiology, University of Wisconsin-Madison, WI 53706

Opioid peptides modulate transmitter release from nerve endings in the central and peripheral nervous system. In the posterior pituitary, dynorphin A (DYN) reduces secretion of oxytocin by an unknown mechanism. We characterized the effects of DYN on K and Ca currents using patch clamp recordings from nerve terminals in thin slices of the posterior pituitary. DYN (2 μM) increased the peak and late K current, elicited by steps from -100 to 0 mV, by 29 ± 21% and 48 ± 29%, respectively (n=5), an effect that was blocked by the opioid antagonist naloxone (10 μM, n=5). This process involved a G protein-stimulated phosphorylation, as it was blocked by intracellular application of GDP-β-S (0.1 mM, n=5) and the protein kinase inhibitor staurosporine (50 nM, n=5). In 4 of 6 single channel recordings from cell attached patches, DYN increased the activity of large conductance K channels by ~70%, while channel activity decreased by ~15% when DYN was given in the presence of naloxone (n=4). In recordings of whole-terminal Ca currents, DYN reduced the peak and late Ca current, elicited by steps from -60 to 0 mV, by 13 ± 3% and 23 ± 6%, respectively (n=4), while Ca currents increased slightly when DYN was applied in the presence of naloxone (n = 4). Thus, DYN may reduce neurosecretion in the posterior pituitary by two mechanisms: a decrease in terminal excitability due to activation of K currents, and a decrease in the Ca^{2+} trigger for secretion via a reduction in Ca currents.

626.13

ELECTROPHYSIOLOGICAL CHARACTERISTICS OF STELLATE GANGLION NEURONS OF THE GUINEA-PIG. R.Hendriks, M.M.Gad-El Karim, J.B.Angevine* and D.L.Kreulen, University of Arizona, Departments of Pharmacology and Medicine, Tucson, AZ 85724.

The stellate ganglia form part of the upper paravertebral chain and lie below the mid - cervical ganglia. They variously control activity of the heart, lungs, trachea and gastrointestinal tract. We report here, for the first time, electrophysiological characteristics of stellate ganglion neurons of the guinea-pig. Intracellular recordings were made from neurons in both right and left stellate ganglia *in vitro* via glass microelectrodes (tip resistances: 45-100 MΩ). The average resting membrane potential of the neurons was -42.1±1.2mV (n=8). Input resistances for these cells ranged from 33-138 MΩ. Tonically firing cells accounted for 87% of the total sample. Firing rates in response to intracellular current pulses attained instantaneous peak values of between 50 & 80Hz. Stimulation of surrounding interganglionic nerve trunks, elicited action potentials (1/2 width = 1.8ms, peak amplitude = 42mV) in all the neurons. Antidromic action potentials were also observed. Synaptic input onto the recorded cells were of three types: slow EPSPs, fast EPSPs and intermediate IPSPs. Supported by DK36289, HL 27781.

626.14

MUSCARINIC EXCITATION OF RAT DORSOLATERAL SEPTAL NUCLEUS (DLSN) NEURONS *IN VITRO*. H. Hasuo* and J. P. Gallagher. Dept. of Pharmacol. and Toxicol., Univ. Texas Med. Br., Galveston, Texas 77555.

Whole-cell patch-clamp recordings were made from rat DLSN neurons *in vitro*. Under current clamp mode, muscarine (5-20 μM) caused bursting activity and markedly enhanced a depolarizing afterpotential (ADP). These facilitatory actions are similar to those reported previously using sharp electrodes (Hasuo & Gallagher, 1990). This ADP was dependent on extracellular calcium and sodium ions. Directly evoked calcium spikes were either enhanced , depressed or not affected by muscarine(10 μM). The amplitude of ADPs was increased even when the preceeding calcium spike was slightly surpressed by muscarine. When cesium ions were used intracellularly, muscarine(10 μM) caused an inward current in neurons voltage-clamped at -80 mV(HP=-80mV). In the majority of neurons, the current induced by muscarine was independent of potential over the range -60 to -120 mV and was associated with an increase in conductance. Extrapolation of the I-V relationship during the inward current yielded a reversal potential of -8mV (n=6). This inward current was reduced by lowering extracellular sodium ions. In some neurons at HP of -40 mV, muscarine produced an inward current associated with a decrease in conductance whose reversal potential was -80mV. These results suggest that muscarine increases the excitability of DLSN neurons by multiple conductance systems and the augmentation of the ADP is not simply due to an increase of calcium influx through voltage dependent calcium channels. Supported by MH-39163.

626.15

EXCITATORY INPUTS FROM DISTAL COLON/RECTUM TO NEURONS IN PELVIC PLEXUS GANGLIA OF MALE GUINEA-PIG. J. Lin and J. Krier*. Dept. Physiol., Mich. State Univ., East Lansing, Michigan 48824.

Intracellular recording techniques were used *in vitro* to study the electrophysiological properties and excitatory synaptic inputs to neurons in pelvic plexus ganglia. The preparation consisted of pelvic ganglia (PG), pelvic nerves (PN), hypogastric nerves (HGN), rectal nerves (RN), colonic nerves (CN), urethra/urinary bladder nerves (UUN) with or without an attached segment of the distal colon/rectum which extended 5-7 cm orad from the external anal sphincter. PG neurons had a mean resting transmembrane potential of -55.1 ± 0.6 mV (n=124), mean action potential amplitude of 75.0 ± 1.3 mV (n=124) and mean cell input resistance of 44.1 ± 3.0 MΩ (n=44). Forty-eight % of neurons exhibited a repetitive discharge of APs during cathodal current injection of long duration (5-10 sec) into cells. Electrical stimulation of all nerve trunks attached to PG elicited fast excitatory post-synaptic potentials (f-EPSPs) and action potentials (APs) (76 % of neurons tested) that were reversibly blocked by hexamethonium (C_6, 1-10 μM). Electrical stimulation of nerve trunks also evoked antidromic APs (37 % CN/RN; 27 % UUN; 8 % HGN; 16 % PN). Antidromic APs were resistant to hexamethonium (1-3 μM) and were abolished at stimulus frequencies between 20 and 50 Hz. In preparations where a fluid-filled segment of distal colon/rectum was attached to the pelvic ganglia via CN and RN, and intraluminal pressures recorded under non-isovolummetric conditions, 30% of neurons exhibited spontaneous f-EPSPs and APs. The spontaneous electrical activity was reversibly abolished by C_6 (1-10 μM) or gamma-aminobutyric acid (1-10 μM) and irreversibly abolished by sectioning CN and RN nerves. When punctate mechanoreceptive stimuli were applied to the serosal/longitudinal muscle layer, the amplitude of spontaneous f-EPSPs and the frequency of spontaneous f-EPSPs and spontaneous APs increased. Under isovolummetric recording conditions, colonic distension increased spontaneous electrical activity that rapidly adapted. The data indicate that some pelvic plexus neurons receive sensory inputs either directly or indirectly from rapid adapting mechanoreceptors in distal colon/rectum. These sensory inputs involve nicotinic cholinergic synapses.(NIH-DK-29920)

626.16

ELECTROPHYSIOLOGICAL RECORDINGS FROM NEURONS IN RAT MEDIAN PREOPTIC NUCLEUS (MnPO) IN VITRO. D. SPANSWICK, R. NISSEN, M. HERMES, C.R. YANG, L.P. RENAUD*. Neuroscience, Loeb Research Institute, Ottawa Civic Hospital, Ottawa, Canada, K1Y 4E9.

The MnPO, which plays a critical role in body fluid homeostasis, receives a prominent input from the subfornical organ (SFO). To evaluate their intrinsic and synaptic properties, whole-cell patch-clamp recordings were obtained from MnPO neurons in rat brain slices that retained afferents from the SFO. MnPO neurons had mean resting potential and input resistance of -60 ± 8 mV and 961 ± 84 MΩ, respectively. These cells exhibited two prominent intrinsic membrane features that were evoked by hyperpolarizing current pulses: 1) an inward rectification; 2) a low-threshold spike (LTS) evoked at the break of a hyperpolarizing pulse. Some neurons possessed a transient outward rectification typically indicated by a delay in the return to rest of a hyperpolarizing square pulse. Other MnPO neurons responded to a brief depolarizing pulse (10 ms) with a prolonged (1-5 s) discharge of action potentials. MnPO neurons also displayed spontaneous reversed IPSPs which summated to give rise to bursts of spikes and were reversibly reduced by $GABA_A$ antagonist bicuculline (15 μM). Electrical stimulation of the SFO evoked fast EPSPs that were reversibly blocked by a glutamate non-NMDA antagonist CNQX (10 μM). These results indicate that MnPO neurons possess diverse electrophysiological properties and receive both GABAergic and glutamatergic inputs. (Funded by the Canadian Heart and Stroke Foundation).

626.17

WHOLE-CELL RECORDINGS FROM VISUALLY IDENTIFIED AMYGDALA NEURONS IN RAT BRAIN SLICES. S. Chattarji*, D.B. Jaffe and T.H. Brown, Department of Psychology, Yale University, New Haven, CT 06520.

Interest in the amygdala stems from its critical role in certain forms of rapid learning. Until recently, very little was known about the basic cellular neurophysiology of amygdala neurons. Previous efforts to bridge this information gap combined three complementary experimental methods – the amygdala brain slice preparation, the single-microelectrode clamp, and video-enhanced contrast, differential interference contrast microscopy (VEC-DICM) (Chapman et al., *Synapse* 6:271-278, 1990; Keenan et al., *Brain Res. Bull.* 21:373-383, 1988). The present study extends this strategy by using whole-cell recording in conjunction with VEC-DICM adapted to an upright microscope (Stuart et al., *Pflügers Archiv*, in press, 1993).

Male Sprague Dawley rats (14-28 days) were used to cut amygdala slices (300 μm) in the horizontal plane. Slices were placed in a submerged-type recording chamber at room temperature mounted on a fixed-stage, upright microscope (Zeiss Axioskop) with a water immersion lens (40X, 0.75 NA) and DIC optics. Standard whole-cell patch-clamp techniques were used to record from basolateral and central amygdaloid neurons observed on a video monitor.

Cells showed considerable diversity with respect to passive and active membrane properties (n=15). The overall mean (±SEM) values of the three passive membrane properties that were measured were as follows: resting potential, V_m = -68.7 ± 1.2 mV; input resistance, R_N = 293.6 ± 34.7 MΩ; time constant, τ_m = 54.0 ± 5.2 msec. There was also considerable variability in the firing patterns produced by depolarizing current injections. Physiologically identified neurons are also being characterized morphologically using intracellular labeling with fluorescent dyes in conjunction with confocal microscopy. (Supported by NIH grant 5-R01-N527130-04)

626.18

ACTIVITY OF BULLFROG SYMPATHETIC NEURONES *IN VIVO*. A-Y Ivanoff and P.A. Smith*, Dept. Pharmacol., Univ. Alberta, Edmonton, Canada, T6G 2H7 and Bogomoletz Inst., Kiev, Ukraine, 252024.

Although the biophysical properties of B- and C-neurones in bullfrog sympathetic ganglia (BFSG) have been carefully analysed (Adams *et al., J. Expl. Biol.* 124, 259, 1986) little is known about their electrical activity *in vivo*. For example, it is not known whether the muscarinic and peptidergic 'slow' responses play any role in ganglionic transmission *in vivo*. Bullfrogs were anaesthetised with urethane (2mg/g) and their IXth paravertebral sympathetic ganglion exposed by a ventral incision. Ganglia were supported on a small plastic ring attatched to a micropositioner and the ongoing activity in ganglionic neurones monitored with microelectrodes (3M KCl) and with suction electrodes placed on the postganglionic nerves. 37 out of 87 cells were silent and the remainder produced action potentials (a.p.) and/or subthreshold e.p.s.p's. The average frequency of activity in B-cells was 0.11±0.01Hz (n=60). C-cells exhibited bursts of 3-8 spikes or e.p.s.p's (at 15-40Hz) once every 2-4s. Mild stimulation of the skin evoked a slow depolarization in B-cells which was accompanied by discharge of a.p.'s. A spike-triggered averaging technique (Skok & Ivanoff, *J. Auton. Nerv. Sys.* 7, 263, 1983) showed that a pre-ganglionic volley evoked a suprathreshold synchronous discharge in about 21 cells (range=13-36, n=14). Tetanic stimulation of C-fibres evoked a slow depolarization in B-cells which was associated with a.p. generation. This reflected the late-slow e.p.s.p. and 'neuropeptide action at a distance' *in vivo* (Jan *et al., Proc. Natl. Acad. Sci.* USA 76, 1501, 1979). Thus, non-nicotinic 'slow' responses may play a role in *in vivo* transmission in BFSG. Since the B-cells innervate mucous glands in the skin (Horn *et al., J. Comp. Neurol.*, 278, 570, 1988), non-nicotinic transmission may mediate a 'sympathetic reflex' activated by stimulation of the skin. Supported by the MRC of Canada.

PHARMACOLOGY OF SYNAPTIC TRANSMISSION II

627.1

INHIBITION OF HIPPOCAMPAL ACETYLCHOLINE RELEASE BY MUSCARINIC AUTORECEPTORS EXHIBITS NO RECEPTOR RESERVE. T.W. Vickroy*, W.L. Malphurs and N.M. DeFiebre, Depts. Physiological Sciences and Neuroscience, Univ. Florida, Gainesville, FL 32610.

The extent of reserve among muscarinic autoreceptors on hippocampal cholinergic nerve terminals was examined in superfused calcium–naive synaptosomes. Autoreceptors were occluded with the irreversible muscarinic cholinergic receptor (mAChR) antagonist propylbenzilylcholine mustard (PrBCM) and then tested for acetylcholine (ACh)–induced inhibition of calcium–evoked [^{3}H]ACh release. PrBCM reduced the density of [^{3}H]quinuclidinyl benzilate binding sites (46, 72 and 90% reductions after 3, 6 or 10 nM PrBCM, respectively), while having no apparent influence on the binding affinities or relative proportions of high– and low–affinity binding sites for the M_1–selective antagonist pirenzepine nor the agonist ACh. In control tissues, ACh was a potent (EC_{50} = 240 nM) and efficacious (maximum inhibition [E_{MAX}] of stimulated [^{3}H]ACh release = 65%) autoreceptor agonist. However, following PrBCM treatment, E_{MAX} for ACh was greatly attenuated (35% and 17% for 3 and 6 nM PrBCM, respectively) with no concurrent changes in the EC_{50} or slope factor. Comparisons of equieffective agonist concentrations before and after receptor occlusion revealed a direct linear relationship between autoreceptor occupancy and inhibition of [^{3}H]ACh release with close agreement between the calculated agonist dissociation constant (K_A = 220 nM) and the EC_{50} for ACh. Pretreatment with 100 nM atropine completely prevented PrBCM–induced reductions in mAChR binding and autoreceptor function. These results indicate an absence of spareness among inhibitory muscarinic autoreceptors on hippocampal nerve endings (supported by NS–28568).

627.2

MUSCARINIC RECEPTOR ACTIVATION MODULATES NEURONAL ACTIVITY IN THE CENTRAL SUBNUCLEUS OF THE NUCLEUS TRACTUS SOLITARII (NTSc). W.Y. Lu, R.S. Neuman, J. Reynolds, D. Bieger*. Faculty of Medicine, Memorial Univ., St. John's, Canada A1B 3V6

Muscarinic acetylcholine receptor (mAChR)-mediated neuronal activity in the NTSc plays a pivotal role in generating premotor programs for esophageal peristalsis in the rat (Bieger, Dysphagia 6:147-164, 1991). Esophageal distension induces rhythmic unit discharges in the NTSc, as well as rhythmic esophagomotor output. After application of methscopolamine (MSCP) to the NTS surface, distension-induced NTSc neuronal activity persists but its rhythmicity along with peristalsis is abolished. To examine the cholinergic input to the NTSc in more detail, whole cell recordings were made from rat NTSc neurons in horizontal brainstem slices. Electrodes contained (in mM): K-gluconate 145; $MgCl_2$ 2; Hepes 5; EGTA 1.1; $CaCl_2$ 0.1; K_2ATP 5. Electrical stimulation (0.1 ms, 1-5 V) of solitary tract afferent fibres in the slice evoked EPSPs that were resistant to 100 μM MSCP but dramatically reduced by 1 mM kynurenate or γ-D-glutamyl-glycine. High intensity (6-10 V) stimulation elicited EPSPs with spikes riding on top. The bursting pattern of spikes was altered by 5-10 μM (+)-cis-dioxolane, a mAChR agonist. Pressure-ejection of L-glutamate (0.1 nmol) in the NTSc region produced a short term (8-12 s) membrane depolarization (15-25 mV). Pulse-application of muscarine (10-20 pmol) produced a low amplitude (3-5 mV), long lasting (> 10 min) depolarization, and enhanced the glutamate response. These data suggest: (1) the esophageal afferent input is not cholinergic, but instead excitatory amino acidergic; (2) the pattern of NTSc neuronal activity can be modulated by activation of mAChR. (Supported by the Medical Research Council of Canada.)

627.3

Prejunctional M2-Muscarinic Inhibition of Sympathetic Neurotransmission in the Guinea-Pig Inferior Mesenteric Artery. T.L. Anthony, S.R. Knoper* and D.L. Kreulen. University of Arizona, Department of Pharmacology, Tucson, AZ 85724.

Activation of muscarinic receptors inhibits adrenergic neurotransmission to the vascular system. Our aim was to characterize the subtype of pre-junctional muscarinic receptor on sympathetic nerves in the mesentery. Intracellular recordings were made in arterial muscle cells through glass micropipettes (tip resistance: 60-100 MΩ). The average resting membrane potential of the mesenteric arteries was -60 ± 4 mV (n = 17). Excitatory junction potentials (e.j.p.s) were evoked via stimulation of post-ganglionic sympathetic nerve fibers. The average amplitude of the stimulation-evoked e.j.p.s. was 5.3 ± 0.3 mV (n = 5). Superfusion of muscarine (1 μM; n = 5) resulted in a 18 % reduction in e.j.p. amplitude. Superfusion of carbachol (1 μM; n = 5) reversibly blocked the stimulation-evoked e.j.p.s. In either of the muscarinic agonists there was no effect on the resting membrane potential. Pretreatment with muscarinic antagonists reversed the inhibitory effects of carbachol on e.j.p.s with the following rank order of potency: atropine > AF-DX 116 > pirenzepine (IC_{50}'s (μM) = 0.1, 0.2, 0.8).These results demonstrate the presence of inhibitory M2-muscarinic receptors on the sympathetic nerve terminals that innervate mesenteric arteries in the guinea pig. Supported by DK36289, HL 27781.

627.4

ADENOSINE RECEPTORS MODULATE INSPIRATORY DRIVE TO PHRENIC MOTONEURONS. X-W. Dong*, G. Liu, & J. L. Feldman, Systems Neurobiology Lab., Dept. of Physiological Science, UCLA, Los Angeles, CA 90024-1527.

Activation of brain adenosine receptors depresses ventilation. We examined the effects of adenosine on transmission of endogenous excitatory amino acid mediated inspiratory activity to phrenic motor neurons (PMNs) in the isolated brainstem-spinal cord preparation from neonatal rats. PMN activity was recorded under whole cell patch-clamp conditions. Drugs were applied via pressure ejection from micropipettes positioned over the PMN pool. The adenosine analogue N^6-cyclopentyladenosine (CPA; 25 μM) reversibly reduced peak inspiratory-modulated synaptic current (I_{insp}) to 74±6% (n=6) of control. To examine the role of endogenous adenosine, we applied the antagonist 3-isobutyl-1-methylxanthine (IBMX, 0.5-1 mM) which increased I_{insp} by ≈15% above control; similar effects were produced by the selective A_1 receptor antagonist 8-cyclopentyltheophylline (CPT; 50 μM). This indicates a tonic endogenous release of adenosine. To elucidate underlying mechanisms, we performed analysis during the expiatory period. None of these drugs produced significant changes in steady-state membrane current, but the frequency and peak amplitude of spontaneous excitatory postsynaptic currents (EPSCs) were reduced by CPA and increased by IBMX and CPT. Moreover, following 1μM TTX perfusion, bath application of CPA reduced the frequency of miniature EPSCs to below half of their pre-CPA value. This reduction was reversed with bath application of IBMX. We suggest that adenosine receptors are involved in the modulation of the transmission of endogenous inspiratory drive to PMNs, possibly by a presynaptic mechanism. Supported by NIH Grant NS 24742.

627.5

BLOCKADE OF GABAERGIC INHIBITION REVEALS A NMDA RECEPTOR MEDIATED COMPONENT IN EPSPS ELICITED BY STIMULATION OF THE THALAMIC AFFERENTS TO CAT PRIMARY AUDITORY CORTEX. T. Krucker, C.L. Meier, P.L. Herrling*. Sandoz Research Institute, P.O.Box, CH-3001 Bern, SWITZERLAND.

Processing of sensory information within the auditory system has been well characterized using histological and electrophysiological techniques. However, relatively little is known about the neurotransmitters involved. The present study was performed to elucidate the possible role of excitatory (EAA) and inhibitory (i.e., GABA) amino acids in neurotransmission within the primary auditory cortex (AI). The main afferent to the AI, the ipsilateral medial geniculate body (MGB), was electrically stimulated and evoked responses were recorded intracellularly in the AI region in halothane anesthetized cats. A seven-barrelled iontophoresis pipette glued alongside the recording electrode allowed localized application of EAAergic and GABAergic compounds onto the recorded neuron. In most AI neurons, MGB stimulation evoked a short latency, short duration EPSP followed by a long-lasting IPSP. Pattern, duration, and amplitude of synaptic potentials were highly variable and strongly dependent on stimulation intensity. Reduction of $GABA_A$ receptor-mediated inhibition by iontophoretic application of either bicuculline or SR95531 markedly enhanced MGB stimulation-evoked EPSPs. Only the late component of this enhanced EPSP was reversibly blocked by the competitive NMDA receptor antagonists AP7 at currents which selectively blocked neuronal excitation induced by iontophoretic application of NMDA. Our results demonstrate that in the cat 1) NMDA receptors in AI are activated following MGB stimulation and that 2) a dominant $GABA_A$ receptor mediated inhibition usually masks this NMDA receptor activation under our experimental conditions.

627.6

BRAIN-DERIVED PEPTIDES DEPRESS SYNAPTIC TRANSMISSION VIA $GABA_B$ RECEPTORS IN RAT HIPPOCAMPUS *IN VITRO*. H. Xiong*, J.M. Wojtowicz and A. Baskys. Depts. of Physiology and Psychiatry, Univ. of Toronto and Clarke Inst. of Psychiatry, Toronto, Ont. M5S 1A8, Canada

Cerebrolysin (CB) is a brain-derived peptide preparation which consists of free amino acids (AAs) and biologically active peptides (MW < 10kD), and has been found to have therapeutic effects on neurodegenerative, ischaemic and traumatic brain disorders. To understand the actions of CB on central neurons, we studied its effects on evoked field potentials (FPs) in hippocampal slices taken from young (14-30 d) Wistar rats of either sex. In agreement with a preliminary study (Wojtowicz et al., Soc. Neurosci. Abstr. 18:987, 1992), bath applied CB (10μl/ml) was found to strongly and consistently depress the FPs evoked in CA1 area by 65.9±4.1% (mean±S.E., n=24) in a concentration dependent manner. Interestingly, CB-induced depression of FPs was consistently and reversibly antagonized by a $GABA_B$ receptor antagonist CGP35348 (50-100μM, n=15). In contrast, $GABA_A$ receptor antagonist bicuculline (20-50μM) had no apparent effect (n=14), indicating that CB selectively acts on $GABA_B$ receptors. Bath applied baclofen (2μM), a $GABA_B$ receptor agonist had similar depressant effects (84.9±3.2%) on FPs, and this action was blocked by CGP35348 (n=3). Application of CB peptide fraction (PF, 10μl/ml, n=4) or AAs (10μl/ml, n=3) caused 43.1±13.8% and 37.9±4.6% depression of FPs respectively. CGP35348 was found to have much stronger effects in antagonizing the actions induced by CB (46.9±3.7%, n=14) and PF (45.2±9.3%, n=4) than AAs (21.0±12.5%, n=3), suggesting that the major active component of CB is a peptide or peptides, and that the mechanism mediating AAs-induced depression is different from that caused by CB. These results indicate that CB inhibits synaptic transmission via acting on $GABA_B$ receptors. Supported by EBEWE Initiative, Austria and MRC of Canada.

627.7

EFFECTS OF TERRITREM-B ON MOTOR NERVE TERMINALS OF MOUSE SKELETAL MUSCLES AND CHOLINERGIC RESPONSES OF SNAIL NEURON. M.C.TSAI*, W.H.HSIEH, H.C.CHOU, V.L.ARVANOV, F.T.PENG and K.H. LING. Institute of Pharmacology and Toxicology, College of Medicine, National Taiwan University, Taipei, Taiwan, R.O.C.

Effects of territrem-B(TRB), a mtcotoxin, on (1)twitch tension of mouse diaphragm, (2)motor nerve terminal activity of mouse *triangularis sterni* nerve-muscle preparation and (3)cholinergic responses of snail central neuron were studied. TRB increased the indirectly elicited twitch tension and it also induced spontaneous muscle twitching of mouse diaphragm. The spontaneous muscle twitching induced by TRB was blocked if d-tubocurarine was further added. TRB potentiated the ACh induced current of snail neuron, while it had no effect on GABA or glutamate elicited currents. The perineural waveforms were recorded with extracellular electrodes placed in the perineural sheaths of motor nerves. At 10 μg/ml, TRB decreased the component of waveform associated with potassium current while it had no effect on the sodium, calcium as well as calcium activated potassium currents of the nerve terminal. It is concluded that TRB affected potassium current in the nerve terminal. The effect may contribute to its actions on synaptic transmission. (This study was supported by a grant, NSC-81-0412-B-002-536, from National Science Council, Taipei, Taiwan, R.O.C.)

627.8

AMINOPYRIDINES REVERSE SAXITOXIN (STX)- AND TETRODOTOXIN (TTX)-INDUCED CARDIORESPIRATORY DEPRESSION. R.M. Bauer*, B.J. Benton and F.-C.T. Chang, Pathophysiology Div., USAMRICD, APG, MD.

Aminopyridines have been shown to reverse TTX blockade of synaptic transmission *in vitro*. In the present study, we examined the effects of 4-aminopyridine (4-AP) and 3,4 diaminopyridine (3,4-DAP) on STX- and TTX-induced cardiorespiratory depression. Anesthetized guinea pigs were instrumented to record respiratory-related medullary units, arterial pressure, diaphragm electromyogram (DEMG), electrocorticogram, electrocardiogram, end-tidal CO_2 and arterial pH, CO_2 and O_2. STX or TTX infusion (0.3 μg/kg/min, IV) elicited vascular hypotension, bradycardia and bradypnea leading to DEMG inhibition. Artificial ventilation was provided when DEMG was reduced by 50%. Toxin infusion was stopped upon DEMG inhibition and subsequently, 4-AP (2 mg/kg, IV) or 3,4-DAP (8 mg/kg, IV) was given. Arterial pressure and heart rate returned to pre-toxin levels and DEMG reappeared rapidly after 4-AP or 3,4-DAP. Spontaneous breathing resumed within minutes and was sustained for several hours without additional aminopyridine therapy. These findings should aid in designing new strategies for therapy against STX or TTX intoxication.

627.9

[^{3}H]BIMU-1 selectively labels sigma$_2$ binding sites in guinea-pig hippocampus. Douglas W. Bonhaus*, Dana N. Loury, Lyn B. Jakeman, Zung To, Andrea DeSouza, Richard M. Eglen and Erik H.F. Wong. Dept. of Neurosciences, Institute of Pharmacology, Syntex Discovery Research, 3401 Hillview Avenue, Palo Alto, California, 94304 USA

The binding of [^{3}H]BIMU-1 ([^{3}H]endo-N-(8-methyl-8-azabicyclo [3.2.1]oct-3-yl) -2,3-dihydro-3-ethyl-2-oxo-1H- benzimidazole-1-carboxamide hydrochloride), a benzimidazolone with high affinity for 5-HT$_3$ and 5-HT$_4$ receptors, was characterized in guinea pig hippocampal membranes. [^{3}H]BIMU-1 binding was insensitive to serotonin but was displaced by haloperidol, and 1,3-di-o-tolyl-guanidine (DTG) with affinities appropriate for the labelling of a sigma site (Ki of 6.3 and 31 nM respectively). The affinity profile of ligands displacing [^{3}H]BIMU-1 binding in guinea pig hippocampus was consistent with the selective labelling of a sigma$_2$ site since the sigma$_1$ selective benzomorphans (+) pentazocine and (+)N-allylnormetazocine only weakly displaced the binding (Ki greater than 1 uM). The affinity of BIMU-1 for sigma$_2$ binding sites (Ki = 32 nM) was more than 200 fold greater than that for sigma$_1$ binding sites (Ki = 6.3 uM), dopamine (D$_1$ and D$_2$), other serotonin (5-HT$_{1A}$, 5-HT$_{2A}$, 5-HT$_{2C}$) and muscarinic (M$_1$, M$_2$, M$_3$ and M$_4$) receptors (Ki > 10 uM).

These data suggest that [^{3}H]BIMU-1 selectively labels sigma$_2$ sites in guinea-pig hippocampus. [^{3}H]BIMU-1, under appropriate experimental conditions, is thus the first sigma$_2$ binding site radioligand to be characterized.

627.10

EFFECT OF 2,4-DITHIOBIURET ON SMOOTH ENDOPLASMIC RETICULUM OF PC12 CELLS. D.M. Autio, M.B. Rheuben and W.D. Atchison*. Depts. of Anatomy, Pharmacology and Toxicology, and the Neuroscience Program, Michigan State University, East Lansing, MI 48824.

2,4-Dithiobiuret (DTB) causes a delayed onset neuromuscular weakness in rats which is associated with decreased quantal content, alterations in postsynaptic ion channel properties, and abnormalities in the ultrastructure of the nerve terminal. The latter include features typical of degenerating or diseased nerve terminals as well as a proliferation of smooth endoplasmic reticulum (SER), swelling of mitochondria and evidence for a decrease in intraterminal calcium concentrations at early stages of neurointoxication (Jones, 1989, Acta Neuropathol. 78: 72). To evaluate the possibility that DTB affects neurotransmission and initiates its toxic effects by interfering with the ability of the SER to modulate cytoplasmic calcium concentrations, we examined DTB treated PC12 cells. The toxicity of DTB to cultured cells was assessed with a fluorescent viability assay. An increase in the proportion of dead cells over the 8-9% seen in control cultures first occurred at exposures of 10μM for 24 hours and reached 30% at 100μM. In moderately differentiated cells having processes up to 10 cell diameters and several varicosities, the SER did not seem to be specifically targeted by 10-40μM DTB, 4-72 hrs; gross abnormalities in the structure of the smooth ER were not seen with TEM, and large changes in the quantity were not detected at the light microscope level with fluorescent stains $DiOC_6$ or Rhodamine B. Other signs of neuronal degeneration (blebbing of the plasmalemma, large intracellular droplets, mitochondrial abnormalities) preceded or accompanied any evidence for abnormalities in the SER. The effect of DTB on the SER *in vivo* may occur secondary to a more general toxic action, or may require a greater degree of differentiation of the neuronal cells than examined in this study. Supported by NIH Grant NS20683.

627.11

EFFECTS OF DIISOPROPYLFLUOROPHOSPHATE (DFP), PARAOXON AND ALDICARB (ALD) ON PRESYNAPTIC NERVE TERMINALS AND POSTSYNAPTIC RECEPTORS. W.M. Cintra[1,2*], E.S. Rocha[1], L.E.F. Almeida[1], Y. Aracava[1,2] and E.X. Albuquerque[1,2]. [1]Lab. Mol. Pharmacol., IBCCF/UFRJ, RJ, Brazil 21944; [2]Dept. Pharmacol., Univ. MD Sch. Med., Baltimore, MD 21201.

The non-anticholinesterase effects of organophosphorus compounds and carbamates have been well demonstrated (*Fundam. Appl. Toxicol.* 5:S182, 1985). Here we have obtained evidence of diverse effects of DFP, paraoxon, and ALD on synaptic transmission in various preparations. The effects of ALD on directly and indirectly elicited muscle twitch were studied in the frog (*Leptodactylus ocellatus*) sciatic nerve-sartorius muscle preparation. At concentrations that completely inhibit ChE, ALD (0.1-10 μM) increased nerve-elicited twitch tension to 125% of control. At 100 μM, ALD initially increased twitch tension to 200% of control, followed by a return to control levels within 30 min. At 800 μM, ALD increased twitch tension to only 150%, which decreased to 85% of control after 30-min exposure and then returned to 140% after a 30-min wash. With the nicotinic acetylcholine receptor (nAChR) blocked by d-tubocurarine (100 μM), ALD (100 μM) only caused a 40% decrease of direct-elicited twitch at 30 min, indicating that the above mentioned potentiation was not due to an effect of ALD on the muscle contractile properties. The patch-clamp technique was used to study miniature excitatory postsynaptic currents (mEPSCs) in cultured fetal rat hippocampal neurons (15-30 days) and nicotinic whole-cell currents in cultured rat myoballs (15 days). In the neurons, paraoxon (100 μM), but not DFP or ALD (each up to 10 μM), enhanced the frequency of mEPSCs, an effect that was insensitive to tetrodotoxin. Paraoxon and DFP (100 μM-1 mM) also shortened the decay phase of the mEPSCs. In myoballs, paraoxon (> 100 μM) reduced the peak amplitude of ACh (50-500 μM)-induced currents. Thus, it is likely that a blockade of postsynaptic neuronal nAChRs may at least in part underlie the shortening of mEPSCs. These results indicate that modulation of transmitter release and direct interactions with post-synaptic nAChRs may be involved in the effects of these anti-ChEs in synaptic transmission. Supp.: US Army Med. Res. Devel. Comm. Cont. DAMD17-88C-8119; CNPq & Mol. Pharmacol. Train. Prog.-FINEP/Brazil.

627.12

EFFECTS OF ANAESTHETIC AGENTS ON REGIONAL DOPAMINE RELEASE IN THE RAT BRAIN: A 3-METHOXYTYRAMINE STUDY. S. J. Chrapusta, M. F. Egan, F. Karoum and R. J. Wyatt*. Neuropsychiatry Branch, NIMH Neuroscience Center at St. Elizabeths, Washington, D.C. 20032.

According to a number of microdialysis and voltammetry reports, anaesthetics may differentially affect dopamine (DA) release. For example, halothane was shown to elevate extracellular DA in the rat striatum, while most other anaesthetics were found to depress it. We investigated the effects of a variety of anaesthetics using tissue 3-methoxytyramine (3MT) levels as an index of DA release. 3MT was measured using combined gas chromatography-mass spectrometry with negative chemical ionization.

Male Sprague-Dawley rats weighing 340-420 g were anaesthetized with either a mixture of halothane (about 2%) and oxygen, or 400 mg/kg ip chloral hydrate, 50 mg/kg ip pentobarbital sodium, 150 mg/kg ip ketamine or 1.25 g/kg ip urethane. An injection of the MAO inhibitor pargyline (75 mg/kg, ip) was given at 50 min, and the animals were killed 10 min later by microwave brain irradiation. No effect was found in the hypothalamus or substantia nigra. Ketamine elevated 3MT (155% of control, $p<0.01$) in the frontal cortex, indicating an enhancement of DA release, while no change was observed in the nucleus accumbens and striatum. Halothane, chloral hydrate, pentobarbital sodium and urethane decreased 3MT levels in the frontal cortex (by 40%, 47%, 54% and 53%, respectively; $p<0.05$), nucleus accumbens (by 25%, 24%, 48% and 40%, respectively; $p<0.05$) and striatum (by 31%, 35%, 38% and 46%, respectively; $p<0.01$), indicating marked decreases in DA release. 3MT formation normalized 24 hours after halothane, pentobarbital sodium, and chloral hydrate treatment (other drugs were not tested). The results indicate that anaesthesia significantly affects DA release from neuron terminals but not cell bodies. The reason for the discrepancy between the halothane-induced changes in 3MT formation found here and extracellular DA reported by others is not clear.

627.13

MODULATION OF EXCITATORY SYNAPTIC RESPONSES IN RAT SPINAL DORSAL HORN NEURONS BY (1S,3R)-1-AMINOCYCLOPENTANE-1,3-DICARBOXYLIC ACID. LJ. Kolic* and M. Randic. Dept. of Vet. Physiol. and Pharmacol., Iowa State University, Ames, Iowa 50011

We examined the effects of the activation of metabotropic glutamate receptor (mGluR) by (1S,3R)-1-aminocyclopentane-1,3-dicarboxylic acid (1S,3R-ACPD), a selective agonist, on the excitatory synaptic transmission in the rat spinal dorsal horn (DH). The excitatory synapse between small primary afferent fibers (0.5-8.5 m/s) and neurons in the laminae I-IV was examined by intracellular recording from DH neurons in the longitudinal spinal slice preparation of rats (20-26 days). The strength of primary afferent transmission was assayed by recording the size of the presumed monosynaptic (n=10) and/or polysynaptic (n=7) EPSPs that result from stimulation of L4 and L5 spinal nerves with electrical shocks of low (5-10V, 10-50μs) and/or high (20-35V, 0.5ms) intensity. Bath application of 1S,3R-ACPD (10-100 μM for 4-18 min) caused a prolonged depolarization (5.4 ± 0.6mV, n=14) of the membrane potential accompanied by an increase in membrane noise and the burst firing. During the superfusion of 1S,3R-ACPD (25-100μM) the peak amplitude of the presumed monosynaptic and polysynaptic EPSPs was reversibly depressed (to 49 ± 8% of control; m ± SEM, n=13). With smaller doses of the drug ($\leq$50μM) a long-lasting potentiation of EPSPs, especially those evoked by high intensity stimulation, was observed (178 ± 16%,n=7). Following removal of 1S,3R-ACPD a potentiation (up to 30 min) of synaptic transmission (by 200 ± 30%, n=9) was observed in 11 of 17 cells. However, 4 of 8 monosynaptic EPSPs exhibited a long-lasting depression (to 38 ± 10%). The effects of 1S,3R-ACPD on the burst firing and EPSPs were reduced by (RS)-4-carboxy-3-hydroxyphenylglycine (500μM). The results demonstrate that distinct and long-lasting modulation in synaptic efficiency can be induced by metabotropic receptor activation which may have important implications for synaptic transmission and plasticity (LTP/LTD) in the spinal DH (supported by NS-26352 and IBN-9209462).

627.14

MEDIATORS OF THE S-EPSP IN CAT PANCREATIC GANGLIA. L. Sha, L. L. Ou and J. H. Szurszewski*. Dept. of Physiology, Mayo Clinic, Rochester, MN 55905.

Repetitive stimulation of intrapancreatic nerves evokes a S-EPSP in the majority of pancreatic neurons. Exogenous acetylcholine (ACh) or 5-HT evokes a slow depolarization through muscarinic or 5-HT_{1P} receptors. The purpose of this study was to determine the percentage of neurons which have a S-EPSP and respond to either ACh or 5-HT. Intracellular recordings were made from 176 neurons from 90 cats. A S-EPSP was evoked in 138 neurons. ACh (20 mM, 5-300 mS, 40 lb/in^2) evoked a slow depolarization in 61 of 81 neurons tested. Of the responding neurons, 77% also had a S-EPSP while 50% of the non-responding neurons had a S-EPSP. These data suggest that a cholinergic muscarinic mechanism accounted for the S-EPSP in approximately 30% of the neurons which had a S-EPSP. 5-HT (20 mM, 500 mS, 40 lb/in^2) evoked a slow depolarization in 61 of 137 neurons tested. Of the responding neurons, 85% also had a S-EPSP. A S-EPSP was recorded in 71% of the non-responding neurons. These data suggest that a serotonergic mechanism accounted for the S-EPSP in approximately 8% of the neurons which had a S-EPSP. These data suggest that S-EPSPs are mediated by ACh, 5-HT and other neurotransmitters which have yet to be identified. (Supported by DK 17632.)

627.15

A FUNCTIONAL ROLE FOR NITRIC OXIDE IN LOCUS COERULEUS: IMMUNOHISTOCHEMICAL AND ELECTROPHYSIOLOGICAL STUDIES Z.-Q. Xu, V.A. Pieribone, X. Zhang, S. Grillner*[1] andT. Hökfelt. Dept. of Histology and Neurobiology and [1]Nobel Institute of Neurophysiology, Karolinska Institute, P.O. Box 60400, 10401 Stockholm, Sweden

The present study was undertaken to examine a possible role of nitric oxide (NO) in synaptic transmission in the locus coeruleus (LC). Using immunohistochemical methods, the localization of nitric oxide synthase (NOS)-like immunoreactivity was examined at both the light- and electron microscope levels. After incubation with antiserum to NOS, immunoreactive cell bodies were seen close to the LC, extending dendritric processes into the LC. Also a few large and small NOS-positive cell were seen intermingled with the numerous tyrosine hydroxylase (TH)-positive cells of the LC. There NOS-positive cells were always TH-negative. Single NOS-positive processes, often with a varicose appearance, could be seen in the LC. Some processes, mainly of dendritic nature, closely approached blood vessels in the LC. At the ultrastructural level NOS-positive cell bodies, axonal terminals and dendrites were seen in the LC. Synaptic contacts were observed between unlabelled boutons and labelled dendrites, often with spines but also between labelled boutons and unlabelled dendrites, and between labelled and unlabelled dendrites. Intracellular recordings were made from LC neurons in rat brain slices. Focal electrical stimulation of the area around the LC evoked an EPSP which could be blocked by CNQX (10 μM, n=12). Bath application of the NOS inhibitors, nitro-L-arginine methyl ester (L-NAME, 15 μM) or N^G-monomethyl-L-arginine (L-NMMA, 80μM), potently enhanced the EPSP up to 127.71% ± 4.12% ($p<0.001$, n=16) and 121.7% ± 3.78% ($p<0.0015$, n=7) of the control, respectively. The enhancement caused by L-NAME was reversed by co-administration of L-arginine (n=6), a precursor of neuronal NO. Hemoglobin, which binds extracellular NO, also enhanced the EPSP up to 118.84% ± 4.86% ($p<0.025$, n=6) of the control. Neither NOS inhibitor, L-arginine nor hemoglobin had effects on resting membrane potential or impedance. The results suggest a role for NO in excitatory synaptic transmission in the LC.

627.16

P-TYPE CALCIUM CHANNELS ARE RESPONSIVE FOR TRANSMISSION AT THE PARALLEL AND CLIMBING FIBER SYNAPSES IN PURKINJE CELLS. M. Sugimori*, B. Cherksey and R. Llinás. Dept. Physiology/Biophysics, New York University Medical Ctr., NY, NY 10016.

Patch current and voltage clamp studies in adult guinea pig cerebellar slices were implemented to test the effect of different calcium channel blockers on synaptic transmission at the Purkinje cell level. Both climbing fiber and parallel fiber postsynaptic responses were evoked by electrical stimulation at the granular layer level. In agreement with previous pharmacological results indicating that inferior olivary cells express P-type calcium channels (Manfridi et al., *Soc. Neurosci. Abst.* 18:974, 1992), it was found that the climbing fiber transmission was blocked by the addition of purified FTX at a concentration of 1:1000. Quite unexpectedly, given that the somata of granule cells demonstrate mostly L channels (Slesinger & Lansman, *J. Physiol.* 435:101-121, 1991), parallel fiber activity in Purkinje cells was also blocked at similar FTX doses. By contrast, Ω conotoxin at 1 to 5 µM concentration and dihydropyridine at 10 µM concentration, had no effect on this transmission. Finally, the effect of local application of glutamate on Purkinje cells was unaffected by any of the three calcium blockers. We conclude that synaptic transmission by both climbing and parallel fibers are mediated by the activation of P channels. Support by NS13742 and AF9620-92.

627.17

17β-ESTRADIOL INHIBITS ω-CONOTOXIN SENSITIVE (N-TYPE) CALCIUM CHANNELS IN RAT STRIATAL NEURONS. P.G. Mermelstein*, J.B. Becker and D. J. Surmeier. Neuroscience Program, Reproductive Sciences Program and Psychology Dept., Univ. of Michigan, Ann Arbor, MI 48104 and Dept. of Anatomy and Neurobiology, Univ. of Tennessee, Memphis, Memphis, TN 38163.

Until recently, steroid hormones had been believed to act only upon cells containing genome activating receptors. However, recent evidence suggest that steroids can also have specific and rapid effects at the cellular membrane. Neurons in the female rat striatum are candidates for immediate actions of 17β-estradiol. 17β-Estradiol has previously been reported to rapidly influence striatal dopamine release, dopamine receptor concentrations and behaviors mediated by the striatum. Yet, traditional estrogen receptors are not found within the striatum, suggesting estradiol may be acting via a non-established mechanism.

Using whole-cell patch clamp, we have found 17β-estradiol able to reversibly block calcium currents in dissociated striatal neurons from 3-5 week and 10 week old female rats. Inhibition of calcium current by 17β-estradiol was not seen after application of ω-conotoxin, an N-type calcium channel blocker, suggesting 17β-estradiol's effects are specific to N-type calcium channels. 17β-Estradiol's inhibition of N-type calcium channels was reliably seen within seconds of administration and at picomolar concentrations. Stereospecificity of this response was demonstrated with 17α-estradiol, which required at least 100x more concentrated doses than 17β-estradiol to produce a similar effect. These results suggest that at physiological concentrations, 17β-estradiol can have immediate actions upon neuronal membrane activity.

sponsored by NIMH 5T32MH14279, BNS9021966, & NS2889

627.18

PROPERTIES OF MEMBERS OF THE α-BUNGAROTOXIN SENSITIVE ACETYLCHOLINE RECEPTOR FAMILY. K. Maury[1], C. Gotti[2], F. Clementi[2]*, M. Ballivet[1] and D. Bertrand[1]. [1]Dpt of Physiology, CMU, CH-1211 Geneva 4 and [2]CNR Center of Cytopharmacology, Milan, Italy.

Using polyclonal antibodies raised against synthetic peptides unique to the α7 and α8 subunits, two α-Bungarotoxin receptors subtypes (α7 and α7-α8) have been purified from chick optic lobe. Both have the same affinity for nicotinic antagonists but the α7-α8 subtype has higher affinity for agonists. Intranuclear injection of α8 cDNA in *Xenopus* oocytes led to the expression of functional receptors whose EC_{50} to acetylcholine was about 100 fold lower than for α7. However co-injection of α7 and α8 in equimolar concentrations gave receptors indistinguishable from those obtained with α7 alone. These results suggest either that α7 masked the properties of α8 or that these two subunits could not co-assemble in vitro. However, the difference in agonist sensitivity between α7 and α8, found both in vitro and in vivo, indicates that assembly of these two subunits in different ratios may be one mechanism involved in the generation of functional receptor diversity.

SODIUM CHANNELS II

628.1

ATX-MODIFIED Na^+ CHANNELS FROM LOBSTER WALKING LEG NERVES IN PLANAR LIPID BILAYERS. **C. Castillo, C. Piernavieja, E. Recio-Pinto* Instituto Internacional Estudios Avanzados (IDEA), Caracas 1015A, Venezuela. Cornell University Medical College, Depts. of Anesthesiology and Physiology, New York 10021.**

ATX-modified, voltage-dependent Na^+ channels from lobster walking leg nerves were studied in symmetrical 0.5M NaCl. ATX-activated channels underwent long and short openings and closings (msec to min range) at all potentials. Channel openings occurred in a burst-like manner. Two open states were observed, a short-lived (msec-sec) large conductance (60pS) state and a long-lived (sec-min) low conductance (10pS) state. 98% of the channels displayed the low conductance state, and 86% of the channels displayed both conductance states. The probability of the channel displaying the large conductance state was highest between +40 and -40mV. At positive potentials, the channel slowly inactivated (entered an irreversible non-conductive state). The channel's fractional open time (f_o) increased with depolarization, with a midpoint potential ($V_{\frac{1}{2}}$) value of -13mV and an apparent gating charge of 0.9. At -60mV, the mean burst life-time was 22sec, and the channel's mean f_o was 0.05. At +60mV, there were two bursts types, with mean-life times of 3 and 24 min, and the channel's mean f_o was 0.33. At +60mV, the mean open time was 4.1min and the mean closed time was 6.8min. The permeability ratio of Na^+ over K^+ was 1.93 for the low conductance state, and 4.01 for the large conductance state. Supported by grants S1-2179 CONICIT-Venezuela, TWAS and Vollmer Research Fund, CEC-Caracas.

628.2

CESIUM INHIBITS BOTH THE TTX-INSENSITIVE SLOW SODIUM CURRENT (I_{NaS}) AND DEPOLARIZING AFTER-POTENTIALS IN HIPPOCAMPAL CA1 PYRAMIDAL NEURONS K. Hoehn* and B.A. MacVicar, Neuroscience Research Group, University of Calgary, Alberta, Canada, T2N 4N1

We have reported a novel voltage-dependent, TTX-insensitive, slow Na^+ current (I_{NaS}) which is present in striatal and hippocampal neurons (Neuron, 10:543-552, 1993). This current appears to underlie the Na^+-dependent depolarizing after-potentials (DAPs) recorded from hippocampal CA1 pyramidal neurons under conditions in which Ca^{2+}- and K^+-dependent processes are blocked. It may contribute to alterations in neuronal firing and epileptiform bursting. To investigate I_{NaS} further, whole-cell voltage clamp techniques were used on hippocampal CA1 pyramidal neurons acutely isolated from 21-28 day postnatal rats using trypsin and hyaluronidase. I_{NaS} was observed when the pipette solution contained either Tris phosphate, Tris chloride, N-methyl-D-glucamine phosphate, or N-methyl-D-glucamine fluoride. I_{NaS} was not observed with pipette solutions of cesium with either chloride, fluoride or phosphate. However, I_{NaS} was still observed when up to 30 mM cesium was added to the Tris phosphate intracellular solution. These observations suggest that high concentrations of intracellular Cs^+ block I_{NaS}. Extracellular Cs^+ also blocked I_{NaS} and this block was reversible. In the presence of 2mM extracellular Cs^+, I_{NaS} was decreased by about 60% while 16 mM Cs^+ blocked about 90% of I_{NaS}. In another set of experiments, current-clamp recordings were obtained from CA1 hippocampal pyramidal neurons in the *in vitro* slice preparation. Under conditions in which Ca^{2+}-dependent processes were blocked using medium containing 3mM $MnCl_2$, 5 mM $MgCl_2$ and 0.05 mM $CaCl_2$, we have shown previously that the DAPs are Na^+-dependent. While DAPs were still observed in the presence of 2 mM Cs^+ in the extracellular solution, they were blocked reversibly in 16 mM extracellular Cs^+. These data support the hypothesis that I_{NaS} underlies the DAPs observed in hippocampal neurons following spike activation and suggest a means whereby the involvement of I_{NaS} in epileptiform activity (e.g. epileptiform bursting observed in hippocampus in 0 mM extracellular Ca^{2+}) can be investigated. (Supported by the M.R.C of Canada and AHFMR).

628.3

TTX SENSITIVITY OF SODIUM CHANNELS IN ELECTROCYTES FROM *STERNOPYGUS* ELECTRIC ORGAN. L. McAnelly*, M. B. Ferrari, and H. Zakon. Dept. of Zoology, Univ. of Texas, Austin, TX 78712

Action potential (AP) duration of the myogenically derived electrocytes of the weakly electric gymnotid fish, *Sternopygus*, covaries with the electric organ discharge (EOD) of the individual fish: electrocytes from fish with low frequency EOD's produce longer duration APs. Variation in the electrocyte AP duration is in turn correlated with variation in Na channel inactivation kinetics (Ferrari, 1993): sodium channels in electrocytes from fish with low frequency EODs display slower inactivation kinetics than those from fish with high frequency EODs.

In some cell types (mammalian muscle, dorsal root ganglion) different Na channel kinetics may be associated with TTX sensitivity: quickly inactivating TTX-sensitive Na channels and more slowly inactivating TTX-insensitive channels. We examined the TTX sensitivity of Na channels in *Sternopygus* electrocytes over a range of EOD frequencies using a two electrode voltage clamp. Na current in all electrocytes is totally blocked by 250-600 nM TTX. Thus, there is no evidence for a TTX-insensitive Na channel in this preparation. Preliminary dose response curves of TTX block of Na current show no systematic variation in TTX sensitivity across EOD frequency. Average Kd was 22 nM (range 12-35 nM, n=4).

(Supported by ONR, NSF and NIH)

628.4

ANDROGEN MODULATION OF A VOLTAGE-SENSITIVE SODIUM CURRENT. M. B. Ferrari* and H. H. Zakon Zoology Dept., Univ. of Texas, Austin, TX 78712

Sternopygus macrurus is a weakly electric gymnotiform fish which serves as a model system to study the effects of sex steroids on cell excitability. The *Sternopygus* electrocyte action potential (AP) displays a large natural variation in duration (3-14 msec) among individual fish. This natural variation in the AP is a sexually dimorphic character, with longer duration APs found in males. Chronic androgen treatment in fish of either sex increases AP duration.

In electrocytes, the variations in AP duration are correlated to variations in the macroscopic sodium current. Long duration APs are correlated with slow sodium current kinetics. Therefore, we examined if chronic androgen treatment would alter the sodium current kinetics in these cells. Voltage-clamp recordings of the sodium current were made before and 3-4 weeks after implanting fish with either 5α-dihydrotestosterone or empty silastic capsules. When compared to baseline recordings, the androgen implants resulted in significantly slower sodium current inactivation kinetics compared to controls ($p < 0.008$, Mann-Whitney U, $n = 6$ fish per group). This provides the first direct evidence for chronic androgen modulation of a voltage-sensitive ionic current. Electrocytes are ideal model cells for studying the biophysical and molecular mechanisms underlying androgen modulation of cell excitability, and are particularly useful to study modulations of sodium current kinetics. Supported by ONR, NSF, and NIH.

628.5

DISTRIBUTION OF CYCLIC GMP-GATED SODIUM CHANNELS AMONG TISSUES: IN SITU HYBRIDIZATION AND RNASE-PROTECTION ASSAY STUDIES IN RAT. E.D. Potter*, C. Ding, M.A. Levine, and S.E. Guggino. The Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205.

What is the distribution of cyclic GMP (cGMP)-gated sodium channels, similar to those found in retinal rod neurons, throughout the body? Are these channels (nearly) identical to the retinal rod sodium channel? To answer the first question, we performed in situ hybridization upon various tissues in rat. Silver grain autoradiographs developed following hybridization of ^{35}S-labeled synthetic oligomer probes (whose sequences were based upon rat and human retinal cGMP sodium channel sequence), indicate presence of mRNA coding for a cGMP-gated channel in: rat retina, pineal, lung, epithelial layer of trachea and jejunum, and possibly in kidney. Negative controls include RNAse-pretreatment of tissues, "sense" probes, and excess "cold" competing probe. Probes based upon different areas of channel sequence, showed similar tissue distribution.

To answer the second question, we are performing RNAse-protection assay (RPA) studies upon total RNA from various rat tissues. If RPA studies indicate that cGMP-gated channels in certain tissues are related, but not identical to the retinal rod channel, partial sequence of such channels will help resolve the question of channel identity. However, in situ studies indicate that similar cGMP-gated channels exist in various rat tissues.

628.6

DISTRIBUTION OF SODIUM CHANNELS ON DENDRITES OF NEURONS IN HIPPOCAMPAL, CORTICAL AND CEREBELLAR RAT BRAIN SLICES

T. A. Benke* and K. J. Angelides, Departments of Neuroscience and Cell Biology, Baylor College of Medicine, Houston, TX 77030

Neuronal dendrites have long been though to be largely passive, but recent information suggests otherwise and that the presence of excitatory voltage-dependent channels can have a great effect on neuronal excitability and even plasticity. The presence of sodium channels on neuronal dendrites has been inferred by electrophysiological and optical methods using sodium-sensitive fluorescent dyes. However, immunocytochemistry using type-specific antibodies has failed to demonstrate their presence anywhere but neuronal somata and axons, though the techniques to demonstrate their presence were not as sensitive as current methods employing scanning confocal fluorescent laser microscopy (SCFLM). Using SCFLM and previously characterized polyclonal antibodies prepared against purified sodium channels and anti-peptide antibodies to type II sodium channels, we have found that sodium channels are distributed on dendrites in the hippocampus, cortex and cerebellum of rat brain. Structures which co-stained with monoclonal antibodies to MAP-2 dendritic protein were classified as dendrites and could be followed for several hundred micrometers through 50 mm slices of fixed rat brain. Dendrites of pyramidal and apyramidal neurons in the CA1-CA3 regions and cortex contained sodium channels from their soma to nearly the tips of their dendrites. Dendrites of cerebellar purkinje neurons also contained sodium channels. We have found no evidence to indicate that sodium channels are present on dendritic spines. The presence of sodium channels on dendrites conveys the dendrites with greater information processing and storage capabilities. Compared to passive dendrites, as few as two neighboring sites of synaptic input to a dendrite can become potentiated without depolarizing the entire dendrite, mimimizing the necessary length of the dendrite over which the inputs take place as well as the number of inputs or associations. Thus, local regions of the dendrite can serve as individual information processing and storage units. We have confirmed this theoretical possibility with computer models that incorporate the latest information on channel distribution and kinetics.

628.7

HETEROGENEITY OF VOLTAGE-DEPENDENT Na^+CONDUCTANCE IN DORSAL ROOT GANGLION NEURONS OF ADULT RAT. M.A. Rizzo, S.G. Waxman, and J.D. Kocsis*. Dept. of Neurology,Yale School of Medicine, New Haven, CT and Neuroscience Research Center, VAMC, West Haven, CT 06516.

Electrical diversity of dorsal root ganglion neurons (DRGs) is conferred in part by differences in the properties of their voltage-dependent Na^+channels. Using the whole-cell patch-clamp method, Na^+currents were recorded from adult rat DRGs maintained in culture from 1 to 3 days. *Small* (≅20 μm diameter) neurons had a predominantly slow Na^+conductance whose properties varied among different neurons tested. In most neurons, activation began at -50 to -30 mV (conditioning potential -120 mV). The Na^+conductance in one population of *small* neurons was saturated at test potentials between 0 and +20 mV, while in others, saturation could not be achieved at potentials up to +50 mV. Steady-state inactivation was weakly voltage-dependent and complete removal of inactivation often could not be achieved at very negative (-160 mV) conditioning potentials. Activation half-times were voltage-dependent and varied among cells from 0.8 to 2.5 ms at +40 mV (19 °C) and included a variable delay. Inactivation half-times were voltage-dependent and a variable fraction of the conductance did not inactivate during >20 ms depolarization. Although inactivation half-times varied over a greater than three-fold domain among different neurons, they remained uniform and stable within a given neuron. Slow conductances were TTX insensitive (100 nM). Most *medium-sized* (≅40 μm diameter) neurons had only a fast, TTX-sensitive Na^+conductance which also showed neuron to neuron variations. Fast conductances were consistently saturable. Some *medium-sized* neurons had both fast and slow conductances. In conclusion, DRG neurons appear to be individually tuned. The expression of Na^+channels is constrained within each neuron to one or two kinetic species despite the potential for broad diversity. In addition to a fast Na^+conductance necessary for action potential generation, DRG neurons contain slow, weakly voltage-dependent and nonsaturable Na^+conductances which may have a role in subthreshold excitatory activity.

628.8

A PHARMACOLOGICALLY AND KINETICALLY DISTINCT Na^+-DEPENDENT AFTERPOTENTIAL IS PRESENT ON RAT CUTANEOUS BUT NOT MUSCLE AFFERENT FIBERS. O. Honmou*[1], D. A. Utzschneider[1], C. M. Bowe[2], S. G. Waxman[1], and J. D. Kocsis[1]. [1]Dept. of Neurology, Yale Med. Sch., New Haven, CT. 06510; and VAMC, West Haven, CT. 06516, [2] Dept. of Neurology, Univ. California, Davis, CA. 95616.

The effects of the potassium channel blocker, 4-aminopyridine (4-AP) and tetraethylammonium (TEA) on myelinated cutaneous (sural nerves) and muscle afferent fibers (deefferented muscle branches in tibial nerves) were investigated using intra-axonal recording techniques in vitro. The nerves were removed from 5 week old Wistar rats and placed in an in vitro recording chamber. Application of 4-AP (1 mM) on muscle afferents but not cutaneous afferents led to a slight broadening of the action potential; cutaneous afferents developed a pronounced delayed depolarization with burst firing which was followed by an afterhyperpolarization (AHP). TEA (10 mM) alone had little effect on both fiber types, but in combination with 4-AP the AHP was eliminated and a single stimulus induced repetitive firing on cutaneous fibers. The delayed depolarization was Na^+-dependent and Ca^{2+}-independent. Hyperpolarization increased and depolarization decreased the amplitude of the delayed depolarization. In cutaneous afferents tetrodotoxin blocked both the action potential and the delayed depolarization. However, the delayed depolarization but not the action potential was blocked by low concentrations (100 μM) of lidocaine; higher concentrations of lidocaine effected the action potential as well. These results indicate that cutaneous afferents can be distinguished from muscle afferents based on their sensitivities to 4-AP. Moreover, evidence is presented to suggest that the delayed depolarization of cutaneous afferents is generated from a pharmacologically and kinetically distinct Na^+ conductance not present on muscle afferents.

628.9

RT-PCR AMPLIFICATION OF VOLTAGE-SENSITIVE NA^+ CHANNEL mRNAs IN CULTURED RAT SPINAL CORD ASTROCYTES. Y. Oh, J. A. Black, S. Yokoyama, and S. G. Waxman*. Dept. of Neurology, Yale Univ. Sch. of Med., New Haven, CT 06510 and Neuroscience Research Center, West Haven, CT 06516

Astrocytes have been shown to express voltage-sensitive Na^+ channels with regional and developmental variation. So far, three major subtypes (Form I, II and III) of the Na^+ channel from rat brain and recently a partial cDNA of a putative glial-specific Na^+ channel from rat cerebral astrocytes have been cloned. In the present study, we have applied a combined technique of reverse transcription and polymerase chain reaction (RT-PCR) to identify the type(s) of voltage-sensitive Na^+ channel expressed in cultured rat spinal cord astrocytes. Spinal cord astrocytes were isolated from neonatal Sprague-Dawley rats and cultured for 7 days before RNA extraction and RT-PCR. We have designed three different primer sets to amplify coding regions and three different primer sets to amplify 3' non-coding regions of these cloned Na^+ channels. Interestingly, we were able to amplify all three major subtypes of rat brain Na^+ channels as well as a putative glial-specific Na^+ channel in spinal cord astrocytes. Adult rat brain, used as a positive control, was also shown to express all these Na^+ channels. However, in adult rat liver we were unable to amplify any of these Na^+ channels. We have also found that rat skeletal muscle could express form I and III but no form II of rat brain Na^+ channels. The present study provides the first direct evidence that astrocytes can express rat brain voltage-sensitive Na^+ channel mRNAs, which have been considered as mainly neuronal type Na^+ channel messages, in addition to a putative glial-specific Na^+ channel. [Supported in part by the VA and NMSS]

628.10

SODIUM CHANNEL mRNAs IN SPINAL CORD ASTROCYTES *IN VITRO*. J.A. Black*, S. Yokoyama, Y. Oh, K.B. Zur, H. Higashida, B.R. Ransom and S.G. Waxman. Dept. Neurology, Yale Sch. Med., New Haven CT and VAMC, West Haven CT, and Dept. Biophysics, Kanazawa Univ. Sch. Med., Japan.

Voltage-sensitive sodium channels are most often responsible for generating action potentials in neurons; these ion channels have also been described in astrocytes *in vitro* and *in situ*, though their function is less clear. Three distinct sodium channel mRNAs (R_I, R_{II}, and R_{III}) have been cloned from rat brain; a fourth mRNA (R_{IIA}) may represent alternative splicing. Recently, a partial clone of a putative glial cell-specific mRNA (NaG) has also been described. Since morphologically and antigenically distinct cultured astrocytes possess different electrophysiological properties, which may reflect specific sodium channel isotypes, we examined the expression of sodium channel mRNA in these glial cells utilizing non-isotope *in situ* hybridization methods.

Digoxigenin-substituted riboprobes were constructed for specific coding and 3'-noncoding regions of R_I, R_{II}, and R_{III}, and for a coding region in NaG. In addition, 40-mer probes specific for R_I, R_{II}, and R_{III} were synthesized and tailed with digoxigenin-dUTP. Astrocyte cultures were established from P0 rat spinal cords and maintained for up to 21 days *in vitro* (div).

The results demonstrate a developmental progression of mRNA expression. At 4 div, flat, fibroblast-like and stellate astrocytes expressed R_{III} mRNA, and to a lesser extent R_{II} mRNA. By 7-14 div, R_{II} mRNA expression was predominant and R_{III} mRNA expression was attenuated; within this timespan, NaG mRNA became weakly expressed. Stellate astrocytes typically were more intensely labeled than flat astrocytes. At all times in culture and for all astrocytes, R_I was not detectable. These observations suggest that differences in electrophysiolgical properties of cultured astrocytes do not reflect expression of a single sodium channel isotype. [Supported in part by DVA, NMSS and NIH]

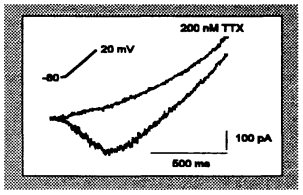
628.11

A SURVEY OF PERSISTENT Na^+-CURRENTS IN RAT AND HUMAN NEURONS. A. Alonso, J.A. White†, A. Olivier & A. R. Kay†*, Dept. Neurology & Neurosurgery, McGill Univ., MNI, Montreal, Canada H3A 2B4; and †Dept. of Biol. Sci., Univ. of Iowa, Iowa City, IA 52242

A persistent sodium current (I_{Na}^P) plays a fundamental role in determining the nonlinear properties of some neurons. I_{Na}^P activates and deactivates rapidly (<1ms) and inactivates only very slowly (< 20 % over 1 sec) and is completely blocked by 100 nM TTX.

Neurons were acutely dissociated from rat and human brain. I_{Na}^P was observed in whole-cell voltage clamp recordings in neurons derived from rat neocortex, entorhinal cortex, basal forebrain & cerebellum. When I_{Na}^P was present, it displayed the stereotyped behavior described above. The ratio of I_{Na}^P to rapidly inactivating Na current was not fixed, suggesting that if I_{Na}^P does arise through "mode-switching" the switching may be modulated, or alternatively the channels might be distinct.

I_{Na}^P was observed in acutely dissociated neurons from human entorhinal cortex (below) with kinetic properties similar to those found in the rat.



Whole-cell recording from acutely dissociated neurons from human entorhinal cortex. Voltage-clamp response to slow ramp command. Intracellular solution (mM): 120 CsF, 1 $CaCl_2$, 10 Hepes, 10 EGTA, pH 7.25. Extracellular: 120 NaCl, 2 $CaCl_2$,2 $MgCl_2$, 15 TEA Cl, 5 4 AP, 0.5 $CdCl_2$, 10 Hepes, 25 Glucose, pH 7.4

Supported by grants from MRC (to AA) and ONR and NIH (to ARK)

628.12

A FAST, ZINC-SENSITIVE, TTX-RESISTANT Na^+-CURRENT IN THE MEDIAL ENTORHINAL CORTEX. J.A. White*, A. Alonso† and A.R. Kay, Dept. of Biol. Sci., Univ. of Iowa, Iowa City, IA 52242; and †Dept. Neurology & Neurosurgery, McGill Univ., MNI, Montreal, Canada H3A 2B4

Acutely dissociated neurons from the superficial layers of the medial entorhinal cortex (mEC) of the mature rat were studied under voltage-clamp using the whole-cell patch-clamp configuration. In addition to the normal TTX-sensitive Na^+ current (I_{TTX-S}) traditionally found in neurons from the adult mammalian CNS, neurons from the mEC exhibit a tetrodotoxin-resistant Na^+ current (I_{TTX-R}). I_{TTX-R} was found in both putative stellate and pyramidal neurons from the mEC. I_{TTX-R} is blocked by high levels of TTX (IC_{50} ~ 500 nM) and is abolished by replacement of NaCl with TrisCl in the recording medium. The TTX-resistant current is kinetically indistinguishable from I_{TTX-S}, with a half-activation voltage of -36 mV, a half-inactivation voltage of -64 mV, very fast activation kinetics (τ_m < 1 ms) and fast inactivation kinetics (τ_h = 3 ms at V = -20 mV). However, I_{TTX-R} can be distinguished from I_{TTX-S} based on its enhanced sensitivity to block by cadmium (IC_{50} ~ 1 mM), lanthanum (IC_{50} ~ 10 μM), and zinc (IC_{50} = 9 μM). I_{TTX-R} shares many characteristics with the TTX-resistant Na^+ current found in cardiac muscle, including high sensitivity to block by divalent cations. The sensitivity of I_{TTX-R} to block by Zn^{2+} and the presence of Zn^{2+} in the neuropil surrounding neurons in layers II and III of the mEC suggest the possibility that synaptically-released zinc may reduce the excitability of neurons in the mEC by blocking I_{TTX-R}. Modeling results support this hypothesis.

Supported by grants from NIH and ONR (to ARK) and MRC (to AA).

628.13

ANTIBODIES TO RAT BRAIN SODIUM CHANNEL SUBTYPE III PURIFIED FROM THE EGGS OF IMMUNIZED HENS. M. Jarnott*, F.J. Alvarez‡, D.A. Harrington‡, R.E.W. Fyffe‡ and A.M. Corbett†, Depts. of Anatomy‡ and Physiology and Biophysics†, Wright State Univ., Dayton, OH 45435.

Antibodies to synthetic peptides corresponding to specific regions of the voltage-gated sodium channel from rat brain have been produced by several groups to study the distribution of channel subtypes within the brain. However, these antibodies, commonly produced in rabbits, are not usually present in high titers and must be used at very low dilutions in immunochemistry. Egg yolks from immunized hens have been shown to be a rich alternative source of antibodies against both synthetic peptides and purified proteins, with higher titers than are normally found in serum. In this study, laying hens were immunized with a synthetic peptide corresponding to a unique region of the carboxyl terminus of sodium channel subtype III from rat brain (amino acids 1980-1995) coupled to keyhole limpet hemocyanin. IgY (50-60 mg/yolk) was extracted from 5 days' pooled egg yolks using a PEG-chlorofom isolation procedure (Polson, 1990). Antibodies were purified by passage over an antigen affinity column containing the peptide coupled to bovine serum albumin immobilized on Sepharose, followed by elution at low pH. Analysis of the resulting fractions by ELISA and with immunoblots of crude brain membranes demonstrated specific labeling with postimmune but not preimmune extracts, which could be blocked by preincubation of the antibody with peptide. No cross-reactivity was observed with peptides corresponding to similar regions of sodium channel subtypes I and II. Specific staining of cell bodies of adult rat brain and spinal cord was observed in tissue sections using antibody dilutions up to 1:200. This work supported by NIH Grants NS28377 and NS25547.

ION CHANNEL MODULATION AND REGULATION III

629.1

REGULATION OF THE NUMBER OF VOLTAGE-DEPENDENT Na AND K CHANNELS IN CULTURED NEUROBLASTOMA CELLS. J. K. Hirsh* and F. N. Quandt, Dept. Physiology, Rush Univ., Chicago, IL 60612.

The expression of proteins in neurons may be regulated by various factors such as electrical activity, however factors which control the expression of Na and K channels have not been extensively investigated. The number and type of voltage-dependent channels expressed in N1E-115 mouse neuroblastoma cells were found to be dependent on growth conditions. Expression of voltage dependent currents was quantified by measuring whole-cell currents recorded from neurons 25 to 35 μm in diameter in normal saline solution. Differentiated neurons express two types of voltage-dependent K channels. One type (K_f), rapidly activates and inactivates following a step depolarization while a second type (K_s) exhibits much slower gating. The ratio of K_f to (K_s + K_f) increased when cells were inhibited from dividing by growing them in solution having low serum and dimethylsulphoxide. These non-dividing cells, grown in media also containing 1 μM A23187 for three days had a low ratio of I_{Na} to total I_K. However cells grown in 50 mM external K, had a factor of 2 higher ratio of K_f to total K current. In order to determine whether regulation occurs at the level of transcription, levels of mRNA for voltage-dependent channels are being assayed for cells grown under various conditions. Genes for the expressed Na and K channels in N1E-115 cells are being identified using polymerase chain reaction-specific amplification of cDNA from isolated mRNA. Amplified products indicate that these cells express channels from both K_{V1} and K_{V3} families. Supported by the National Multiple Sclerosis Society.

629.2

MODULATION OF SINGLE ATP-GATED CHANNEL ACTIVITY BY EXTRACELLULAR ZINC IONS IN RAT SYMPATHETIC NEURONS. R. Clowes* and D.A. Brown. Department of Pharmacology, University College London, London WC1E 6BT, U.K.

Adenosine 5'-triphosphate (ATP) is a neurotransmitter in both the central and peripheral nervous systems. We examined ATP-activated currents and their modulation by extracellular zinc (Zn^{2+}) in cultured rat superior cervical ganglion cells. In whole-cell recordings, ATP activated a non-specific cation current with a null potential near 0 mV. The current was carried in part by Ca^{2+} and was blocked by the P_2-purinoceptor antagonist, suramin. Low concentrations of Zn^{2+} (0.5-10μM) strongly potentiated the current amplitude. The effect of Zn^{2+} was dependent on the concentration of agonist: Zn^{2+} lowered the threshold of activation without increasing the maximum response. The ATP-induced rise in intracellular Ca^{2+}, measured using Indo-1 fluorescence, was also enhanced by Zn^{2+}. Single channels activated by ATP were recorded using outside-out, nystatin-perforated vesicles. The channels were small (1.2 pA at -80 mV) and showed considerable flicker in the open state. Zn^{2+} significantly increased the ATP-evoked channel open probability without affecting the unitary current amplitude. Thus, changes in the concentration of extracellular Zn^{2+} may modulate activation of the receptor by ATP, thereby affecting ATP-mediated Ca^{2+} entry into the cell. Supported by the Wellcome Trust.

629.3

A NOVEL SUBTYPE OF SULFONYLUREA-SENSITIVE K^+ CHANNEL MODULATED BY DOPAMINE IN RAT STRIATUM. Yong-Jian Lin*, Gabriela J. Greif and Jonathan E. Freedman. Dept. Pharmaceutical Sciences, Northeastern Univ., Boston, MA 02115.

We studied the ability of the sulfonylurea drugs tolbutamide and glibenclamide (glyburide) to block the 85 pS K^+ channel activated by $D_{2/3/4}$ dopamine receptor agonists in dissociated rat corpus striatum (caudate-putamen) neurons. In cell-attached patch-clamp recordings in the presence of 10 μM quinpirole, tolbutamide (IC_{50}~60 nM) was 10-100 times more potent than glibenclamide (IC_{50}~2 μM), a rank-order potency opposite to that typically observed at ATP-sensitive K^+ channels. Furthermore, the K_{ATP} channel opener diazoxide (50 μM) was relatively ineffective at opening the 85 pS channel. However, these channel openings were elicited in the absence of dopamine receptor agonist when the cells were treated with the metabolic inhibitor rotenone (5 μM), demonstrating activation of these channels under energy-depleting conditions. These results suggest that the 85 pS K^+ channel modulated by dopamine receptors in rat striatum belongs to a new class of sulfonylurea- and metabolism-sensitive K^+ channels. Thus, there may be a link between dopaminergic transmission and mechanisms protecting against excitotoxicity. (Supported by NIH FIRST award MH-48545.)

629.4

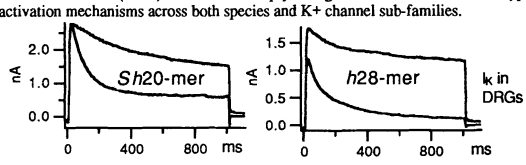
DIRECT G-PROTEIN COUPLING OF D_2 DOPAMINE RECEPTORS TO K^+ CHANNELS IN RAT STRIATAL NEURONS. Gabriela J. Greif*, Yong-Jian Lin and Jonathan E. Freedman. Dept. Pharmaceutical Sciences, Northeastern Univ., Boston, MA 02115.

We studied the coupling of $D_{2/3/4}$ dopamine receptors to an 85 pS K^+ channel in cell-attached patch-clamp recordings from freshly dissociated rat corpus striatum (caudate and putamen) neurons. Multipolar cells of ≥10 μm diameter were most likely to display this channel activity. Openings of the 85 pS channel were observed on these cells when 10 μM dopamine or quinpirole was present in the patch pipette (26% of 172 cells tested), but were never observed in the absence of agonist (n=130 cells). Application of dopamine via a macropipette to the cell membrane outside the patch did not elicit openings (n=41), suggesting spatial proximity between the receptor and the channel. Substituting the GTP-binding protein activator mastoparan (100 μM) for agonist in the pipette also elicited 85 pS channel openings. The membrane-permeable cyclic AMP analogs Rp-cAMPS and Sp-cAMPS (100 μM via macropipette) did not have gross effects on channel opening, arguing against a major, direct role for cAMP. This suggests that receptor-channel coupling may be through a mastoparan-sensitive G-protein, independently of a diffusible second messenger. (Supported by NIH FIRST award MH-48545.)

629.5

ACTIONS OF INACTIVATION PEPTIDES ON VOLTAGE-ACTIVATED K^+ CURRENTS IN RAT DRGs AND A CLONED BRAIN K^+ CHANNEL, MK-1 (mKv1.1). F. Heblich, B. Robertson, A. Opalko & D.G. Owen* Wyeth Research (U.K.) Ltd., Taplow, Berkshire SL6 0PH, U.K.

It has been shown that in the *Shaker* B potassium channel, an N-terminal sequence mediates fast inactivation (Zagotta et al. Science **250** 568, 1990). Using standard patch clamp techniques, we have examined the effects of this peptide (MAAVAGLYGLGEDRQHRKKQ) (*Sh*20-mer) on a cloned potassium channel from mouse brain (MK-1) expressed in CHO cells, and on native voltage-activated K+ currents in rat DRG neurones, which are known to express the rat homologue of MK-1, RCK-1 (rKv1.1). The *Sh*B peptide transformed MK-1 from a non-inactivating current into a rapidly decaying current (τ_d = 12±1.2ms at +60mV,n=12). Similarly, the decay of K+ currents (principally the transient outward current) in DRGs were accelerated when exposed to the *Sh*B peptide internally. We have extended these observations to include another inactivation particle, a 28 amino acid peptide (*h*28-mer) based on the N-terminal sequence of a human K+ channel (MISSVCVSSYRGRKSGNKPPSKTCLKEE), hKShIIIc (hKv3.4) (Rudy et al., J. Neurosci. Res. **29** 401, 1991). This peptide also enhanced inactivation of DRG outward currents and produced rapid inactivation in MK-1 channels, with a τ_d of 66±9ms at +60mV (n=24). The results imply a degree of conservation in N-type inactivation mechanisms across both species and K+ channel sub-families.



We would like to thank Bruce Tempel for the stable expression of MK-1 in CHOs, and Janette Scott for tissue culture.

629.6

STUDIES ON THE SITE OF ACTION OF 4-AMINOPYRIDINE ON THE CLONED POTASSIUM CHANNEL MK-1 (mKv1.1). B. Robertson*, J. C. Garratt, D. G. Owen & G. J. Stephens Wyeth Research (U.K.) Ltd, Taplow, Berkshire SL6 0PH, U.K.

The interaction between 4-AP and the first 20 amino acids from the N-terminus sequence of the *Shaker B* channel ('inactivation peptide') was studied using the whole-cell patch clamp technique in Chinese hamster ovary (CHO) cells transfected with the vector containing the DNA encoding MK-1 potassium channels. Transfected cells expressed an outward current that activated from -40 mV, and reversed at the potassium equilibrium potential. The half maximal conductance of MK-1 was at -10 mV and had a slope factor of 11 when fitted with a Boltzmann function. There was only a very slight (<10%) inactivation of MK-1 even at very large positive voltages. 4-AP caused dose- and time-dependent block of the outward current when applied extracellularly or intracellularly with IC_{50}'s of 147 and 117 μM respectively. Inclusion of the inactivation peptide in the patch pipette solution (at 2 mg/ml) transformed MK-1 into a rapidly inactivating current with a time constant of decay of 12 ± 1.2 ms at +60 mV (n=12). In the presence of the inactivation peptide, extracellular 4-AP was less potent with an IC_{50} of 537 μM. Since the inactivation peptide is known to have a receptor on the cytoplasmic side of MK-1 these findings would support the hypothesis that 4-AP acts intracellularly; possibly at the same site as the inactivation peptide.

We would like to thank Bruce Tempel for MK-1 and expressing CHOs, and Janette Scott for tissue culture.

629.7

SINGLE DELAYED RECTIFIER K^+ CURRENTS OF FROG SKELETAL MUSCLE ARE MODULATED BY G PROTEINS. M. Vazquez and J. A. Sanchez*. Department of Pharmacology CINVESTAV-IPN, A. Postal 14-740, Mexico D.F. 07000 and Department of Physiology UNAM, Mexico, City D.F. Mexico.

Modulation of delayed rectifier K^+ channels (I_K) of frog muscle was investigated in isolated vesicles formed from the sarcolemma of twitch skeletal muscle (Rana montezumae). Methods: Patch clamp recordings in the inside-out patch configuration in vesicles formed as described by Standen et al. (J. Physiol. (1985) 364:339-358). Solutions (mM): Pipette contained KCl=120, $CaCl_2$=2, HEPES=5. Bath contained KCl=120, $MgCl_2$=2, EGTA=5, HEPES=5, ATP=2. pH=7.2 and T=22-25°C. Results: I_K were identified by their sensitivity to TEA and by reversal potential measurements in asymmetrical solutions. The single channel conductance (γ) was ca. 60 pS. Continuous recordings for 50 s revealed the presence of bursts followed by silent periods of ca. 5 s. Substitution of Cl^- by F^- greatly increases the duration of the silent periods in a concentration dependent manner. In $[F^-]_i \geq 30$ mM I_K virtually disappears. The effects are reversible. No significant changes in γ were observed. GTPγS (100 μM) had similar effects and GDPβS and Pertussis toxin prevented the effects of both F^- and GTPγS. Modulation did not involve changes in open time.

Supported by CONACyT grant #0287N.

629.8

MODULATION OF DELAYED RECTIFIER POTASSIUM CHANNEL FROM ANTERIOR PITUITARY CELL LINE BY PROTEIN PHOSPHORYLATION.

S. Chung*, L.K. Kaczmarek Dept. of Pharmacology, Yale Medical School, New Haven, CT 06510

The perforated-patch configuration was used to record the delayed rectifier currents in metabolically intact GH_4C_1 cells. The current was isolated by removal of bath Ca^{2+}. The inactivation of the current was well fitted by a sum of exponentials (500-900 ms, and 1.2-3 s). Bath application of a cAMP analog, dibutyryl cAMP, decreased the current at all voltages. The peak currents decreased by 12%, while the end currents of the 500 ms step from -80 to +40 mV decreased by 28%. Both of the inactivation times were reduced (300-500 ms, and 0.8-1.3 s). Cell-attached patch recordings were made of the delayed rectifier channel activity at the single channel level. The most frequent channel in the patch was 5 pS channel which inactivated with time constant of 3-7 s. Dibutyryl cAMP reduced the inactivation time to 2-4 s. Ensemble currents of single channel activities showed that the effect of dibutyryl cAMP was to decrease the current. The neuropeptide somatostatin, which is known to activate a protein phosphatase in this cell [White et al. *Nature* **351**, 570 (1991)], reversed the effect of dibutyryl cAMP. These studies strongly suggest that the delayed rectifier channel of this cell is modulated by protein phosphorylation.

629.9

ACTIVATION OF PKC BLOCKS THE METABOTROPIC GLUTAMATE RECEPTOR MEDIATED INHIBITION OF CALCIUM CURRENTS IN DENTATE GRANULE NEURONS. T. A. Valiante, M. A. Abdul-Ghani, P. S. Pennefather, P. L. Carlen*. MRC Nerve Cell and Synapse Group, Playfair Neuroscience Unit, University of Toronto, Canada.

Intracellular calcium has been implicated in several neuronal processes including modulation of transmitter release, enzymatic activity like protein kinases and phosphatases, neuronal plasticity, and neurotoxicity. Since calcium influx through the cell membrane alters intracellular calcium levels, modulation of calcium currents plays a fundamental role in altering neuronal function. We have studied the modulation of calcium currents recorded from dentate granule neurons in the hippocampal slice preparation by the metabotropic glutamate receptor (mGluR) and protein kinase C (PKC) using the whole cell configuration of the patch clamp technique. Activation of mGluRs by trans-ACPD (50 μM) produced a reversible reduction in calcium currents, that was blocked by the inclusion of 1.2 mM GDP-β-S in the patch pipette. Activation of PKC by the phorbol ester PMA (5 μM) reduced calcium currents, and blocked the inhibition of calcium currents by trans-ACPD. Our results show that activation of mGluR on dentate granule cells, as on CA3 neurons (Schwartz et al., Nature 361:165-168 1993), inhibits calcium currents by a process that is modulated by PKC.

Acknowledgement: T.A.V. is an MRC student and M.A.G. is an MRC fellow.

629.10

NMDA CURRENT IN SUPRACHIASMATIC NEURONS AND ITS MODULATION BY CALCIUM IONS. M. Raggenbass, S. Alberi and J.J. Dreifuss*. Department of Physiology, University Medical Center, CH-1211 Geneva 4, Switzerland.

Due to their small size, suprachiasmatic neurons have been difficult to study with standard intracellular recording techniques. We have used whole cell patch clamp recordings and hypothalamic slices of the rat in order to characterize the effect of N-methyl-D-aspartate (NMDA). Cell input resistances ranged from 500 to 1000 MΩ. In current clamp conditions, NMDA at 50-100 μM induced a 10-30 mV depolarization in most neurons. In the voltage clamp mode, NMDA generated an inward current of 10-60 pA, which was insentitive to 1 μM TTX and which reversed at about 0 mV. The current/voltage relation contained a region of negative slope conductance. The NMDA current was reversibly reduced or suppressed by D(-)-2-amino-5-phosphonopentanoic acid (D-AP5) at 20-40 μM. It could be enhanced by lowering the extracellular magnesium concentration from 1 to 0.01 mM, or by adding 10 μM glycine to the perfusion solution. In 7 out of 8 neurones, lowering the extracellular calcium concentration from 2 to 0.01 mM caused a 1.5- to 2.5-fold potentiation of the NMDA current. Increasing the calcium concentration from 1 to 5 mM did not affect significantly the amplitude of this current. These results indicate that NMDA receptors are present on suprachiasmatic neurons and suggest that these receptors may in part mediate glutaminergic synaptic transmission in the suprachiasmatic nucleus. They also suggest that, in addition to magnesium ions, calcium ions may also exert a modulatory action on the NMDA-induced current.

629.11

RUN-UP OF NMDA-ACTIVATED CURRENT IN *XENOPUS* OOCYTES EXPRESSING NMDA RECEPTOR SUBUNITS IS SENSITIVE TO CYTOSOLIC Ca^{2+}. U. Brauneis, K. Masood, C. Yi and F. F. Weight*. Lab. Molecular & Cellular Neurobiology, NIAAA, NIH Rockville, MD 20852

The recent cloning of the NMDA receptor has presented the opportunity to investigate functional differences between subunits and subunit combinations. In this study different subunit combinations were expressed in *Xenopus* oocytes and currents elicited by NMDA were measured using two-electrode voltage-clamp. In some combinations, repeated application of NMDA for 20-50sec at 5-7min intervals resulted in an increased current amplitude with each subsequent application, until a level was attained that was stable for several hours. This "run-up" in Ca^{2+}-containing bath solution, expressed as %$\pm$SE of the first measured current, was: $\epsilon 2/\zeta 1 = 144 \pm 13\%$, $\epsilon 1/\zeta 1 = 115 \pm 13\%$. No "run-up" was observed using the $\epsilon 3/\zeta 1$ subunit combination ($98 \pm 7\%$). With the $\epsilon 2/\zeta 1$ combination, the injection of 10nl EGTA (50mM) into oocytes 15min-3h prior to recording abolished current "run-up" ($106 \pm 4\%$). "Run-up" also occurred in bath solutions containing Ba^{2+} instead of Ca^{2+} ($126 \pm 7\%$). This makes unlikely the possibility that the increased current is merely due to a Cl^- conductance activated by the influx of Ca^{2+} through NMDA channels. As $\epsilon 1/\zeta 1$ and $\epsilon 2/\zeta 1$ but not $\epsilon 3/\zeta 1$ show sensitivity to protein kinase C (PKC) activators, we looked at the role of PKC in "run-up" and found that pretreatment of the oocytes with the PKC inhibitor staurosporine (5μM for 1h) had no apparent effect on "run-up". These observations suggest that "run-up" of NMDA-activated current with repeated receptor stimulation is dependent upon cytosolic Ca^{2+}, but may not involve PKC.

629.12

TIME COURSE OF NMDA RECEPTOR CALCIUM-DEPENDENT INACTIVATION WITH INTACT INTRACELLULAR ENVIRONMENT. A.Kyrozis* and A.B.MacDermott. Dept.Physiology & Cellular Biophysics and Center for Neurobiology & Behavior, Columbia Univ., New York, NY 10032.

The kinetics and amplitude of the response to NMDA receptor (NMDA-R) activation varies with intracellular [Ca^{2+}] ([Ca^{2+}]$_i$). Using perforated whole-cell recording to avoid exogenous Ca^{2+} buffering and wash-out of critical intracellular molecules, we have observed that the peak but not the steady state response to exogenous NMDA is invariably suppressed when preceded by a voltage step that elicits Ca^{2+} entry. This indicates that [Ca^{2+}]$_i$ can regulate NMDA-R under normal cellular buffering conditions. To determine the kinetics of Ca^{2+}-dependent modulation of NMDA-R, we briefly applied a saturating dose of glutamate (Glu, 30 μM) to embryonic dorsal horn neurons grown in culture under 2 different experimental conditions. NMDA-R component was isolated by including (in μM) CNQX(30), Gly(100) & strychnine(5) in all drug solutions. First, fast application of Glu in 20μM Ca^{2+} was followed by Glu in 2mM Ca^{2+}. The switch to 2 mM Ca^{2+} caused current decay to a steady state amplitude of 26$\pm$2% of the amplitude in low Ca^{2+}, with time to 2/3 of total relaxation $t_{2/3}$=90$\pm$10 msec (mean$\pm$SEM, n=4). Second, following application of Glu in 20 μM Ca^{2+}, the solution was rapidly switched to APV (25μM) to avoid Glu rebinding to NMDA-Rs, a condition designed to simulate synaptic transmission. When APV was in 2 mM Ca^{2+}, the current decay in 4 of 5 cells was significantly faster ($t_{2/3}$=139$\pm$17 msec) than the decay with APV in 20μM Ca^{2+} (230$\pm$6 msec for the same cells). These results indicate that Ca^{2+}-dependent NMDA-R inactivation has a faster onset than previously described and that it may enhance the decay of NMDA-R-mediated synaptic events. (supported by GM32099)

629.13

HISTAMINE MODULATES NMDA-CURRENTS IN HIPPOCAMPUS. H.L. Haas*, I.N. Sharonova, D.R. Stevens, V.Uteshev, V.S. Vorobjev and I.B. Walsh Institute of Physiology II, Heinrich-Heine-University, DW-4000 Düsseldorf, Germany.

N-methyl-D-aspartate (NMDA) currents were recorded from isolated rat hippocampal neurones, using patch-clamp techniques and a rapid perfusion system. Histamine, at 0.5 to 100 μM, reversibly enhanced NMDA currents up to 150%. The effect cannot be ascribed to activation of the known histamine receptors (H_1, H_2, H_3) or to an action on the NMDA agonist site or the glycine co-agonist site. It is occluded by spermine and suppressed by the putative polyamine site antagonist diethylenetriamine (DET). NMDA channel mean open time and mean amplitude were minimally affected but mean burst duration was enhanced from 27 $\pm$ 3 ms to 51 $\pm$ 11 ms by 5 μM histamine.

In hippocampal slices, in the CA 1 area, AP5-sensitive multiple population spikes in Mg^{2+}-free medium were enhanced by histamine, but NMDA-currents evoked by local pressure application and epsps following stratum radiatum stimulation in the presence of DNQX and bicuculline were depressed by about 20%.

These results suggest an interaction of histamine with the polyamine binding site on the NMDA receptor complex. This new modulatory action enables the histaminergic system to determine time and loci of NMDA-receptor mediated events such as memory formation according to behavioral state.

629.14

MODULATION OF NMDA AND AMPA RESPONSES BY cAMP-DEPENDENT PROTEIN KINASE AND PROTEIN KINASE C IN SPINAL DORSAL HORN NEURONS. R. Cerne* and M. Randić, Dept. of Vet. Physiol. and Pharmacol., Iowa State University, Ames, IA 50011

Glutamate-gated ion channels mediate excitatory synaptic transmission in the central nervous system, including spinal dorsal horn, and are involved in synaptic plasticity, neuronal development and excitotoxicity. These ionotropic glutamate receptors are classified according to their preferred agonists as AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid), KA (kainate), and NMDA (N-methyl-D-aspartate) receptors. The present study of NMDA and AMPA receptors expressed in acutely isolated spinal dorsal horn (DH) neurons of young rats (7-15 days) reveals that these receptors are subject to modulation through the adenylate cyclase cascade and protein kinase C (PKC). Whole-cell voltage-clamp recording mode was used to examine the effects of adenosine 3',5'-monophosphate (cAMP)-dependent protein kinase (PKA) and protein kinase C on the responses of DH neurons to NMDA and AMPA. Whole-cell current responses to NMDA and AMPA were enhanced by 8 Br-cAMP, a membrane permeant analog of cAMP or by intracellular application of cAMP, or catalytic subunit of PKA (cPKA) in a proportion of DH neurons. In addition, the AMPA responses were potentiated by phorbol esters, the agents that activate protein kinase C and by intracellularly applied PKC. Our results indicate that NMDA and AMPA receptors are modulated by PKA and PKC and that this modulation is potentially an important mechanism to control excitability of spinal DH neurons and the efficacy of synaptic transmission (Supported by NS-26352 and IBN-9209462).

629.15

ANCHORING OF THE REGULATORY SUBUNIT (RII) IS REQUIRED FOR cAMP-DEPENDENT PROTEIN KINASE MODULATION OF AMPA/KAINATE RECEPTORS ON HIPPOCAMPAL NEURONS. C. Rosenmund*, D.W. Carr, J.D. Scott and G.L. Westbrook .Vollum Institute, Oregon Health Sciences University, Portland, OR 97201.

Neuronal regulation by cAMP is largely mediated by phosphorylation of target proteins by the cAMP-dependent protein kinase (PKA). Rapid and preferential phosphorylation of key target substrates can be facilitated by receptor-specific activation of localized pools of PKA. Compartmentalization occurs via binding of the regulatory subunit (RII) to A-Kinase-Anchoring-Proteins (AKAPs) that are themselves attached to cell membranes or cytoskeletal structures such as the postsynaptic density.

AMPA/kainate receptors that mediate fast EPSCs at central synapses have been shown to be regulated by PKA (Wang et al., 1991; Greengard et al., 1991). We examined the role of AKAPs in mediating this action of PKA in cultured hippocampal neurons. Multiple AKAPs were demonstrated in cell extracts from these neurons by overlay assays probed with RII. The binding of RII to AKAPs was competitively inhibited by a 24 amino acid peptide derived from the RII binding domain, but not by a 16 amino acid fragment from the same region. If PKA localization is necessary for modulation of AMPA receptors in intact neurons, whole-cell dialysis with the 24mer should displace bound RII, and therefore prevent phosphorylation. Currents evoked by kainate (10 μM) or (S)-AMPA (1 μM, in the presence of 100 μM cyclothiazide) were well maintained in the presence of ATP (5 mM, n=12). However, in the presence of PKA inhibitory peptide (PKI, 1 μM), currents evoked by either agonist ran down to ~60% of the initial amplitude during 30 minutes of whole-cell recording (n=16). This is consistent with the reported role of PKA in maintaining AMPA channel activity. Intracellular dialysis with the 24mer peptide mimicked the effect of PKI (n=9), but the 16mer peptide had no effect (n=7). The inhibition could be overcome by intracellular dialysis with the catalytic subunit of PKA (n=6), suggesting that the peptides did not directly interfere with catalytic activity of the kinase. Our results suggest that PKA-mediated regulation of AMPA receptors or an associated regulatory protein requires anchoring of the kinase near the receptor. Supported by USPHS grants MH46613 and GM 48231.

629.16

^{3}H-PROPOFOL BINDING AND PROPOFOL ACTION ON RECOMBINANT $GABA_A$ RECEPTORS EXPRESSED IN HEK 293 CELLS AND *XENOPUS* OOCYTES. E. Sanna*, J.E. Dildy-Mayfield, S.J. McQuilkin, R.L. Klein, P.J. Whiting, M.P. Mascia, G. Biggio and R.A. Harris. Dept. of Pharmacol., Univ. of Colorado & Denver VAMC, Denver, CO 80262, Merk, Sharp & Dohme Res. Lab., Essex, U.K., and Dept. Exper. Biology, Univ. of Cagliari, Italy.

The general anesthetic propofol (Pro) potentiates $GABA_A$ receptor function, but the precise mechanism and specificity of this action are still unclear. We measured ^{3}H-Pro binding in HEK 293 cells transiently transfected with human $GABA_A$ receptor subunit cDNAs and used *Xenopus* oocytes to assess the effect of Pro on GABA-gated Cl^- currents. Specific ^{3}H-Pro binding was not greater in HEK 293 cells transfected with either homomeric (α1, β1, or γ2S) or dimeric subunits (α1β1, α1γ2S, or β1γ2S) than in non-transfected cells. Significantly higher (4-5 fold) specific ^{3}H-Pro binding was detected in HEK 293 cells transfected with α1β1γ2S or α5β1γ2S subunit cDNAs. Stimulatory action of Pro on GABA-gated Cl^- currents recorded in oocytes was consistent with the above binding data. In oocytes expressing α1β1γ2S receptors, Pro (1-25 μM) induced a reversible and dose-dependent potentiation of GABA-gated currents (~ 800%, at 25 μM), whereas in oocytes expressing α1β1 receptors, its effect was much lower (~ 200 %, at 25 μM). Conversely, pentobarbital induced a larger potentiation of GABA responses in oocytes expressing α1β1 compared to α1β1γ2S receptors. Comparing Pro's effects on glutamate receptors, no detectable ^{3}H-Pro binding was observed in 293 cells transfected with GluR6 receptor cDNA, and Pro had no effect on kainate responses in oocytes expressing either GluR6, GluR1 or brain mRNA. NMDA responses in oocytes expressing brain mRNA were inhibited 8 and 34% by 25 and 300 μM Pro, respectively. These binding and functional results indicate that the anesthetic effects of Pro may be largely mediated through actions on $GABA_A$ receptors, and this interaction may be influenced by the $GABA_A$ subunit composition.

629.17

2,3-BUTANEDIONE MONOXIME (BDM) BLOCKS GAMA-AMINOBUTYRIC ACID (GABA) GATED CHLORIDE CURRENT OF ACUTELY ISOLATED MURINE HYPOTHALAMIC NEURONS. T. Carino, J.H. Ye, and J.J. McArdle*. Dept. Pharmacol., New Jersey Med. Sch. (UMDNJ), Newark, NJ 07103-2714.

We have investigated the effects of BDM on GABA activated chloride current (I_{GABA}) of hypothalamic neurons acutely isolated from young mice. The nystatin perforated patch technique was used to record whole cell I_{GABA} from neurons rapidly superfused with drug containing solutions. When neurons were subjected to a holding potential of -50 mV, application of 300 μM GABA produced a rapidly rising inward current which slowly decayed in the continued presence of agonist. Superfusion of neurons with a solution containing GABA and BDM concentrations ranging from 2 to 50 mM caused a progressive suppression of peak I_{GABA}. This effect of BDM was completely reversible upon washout. BDM is known to suppress voltage activated calcium and potassium currents of neurons. Our preliminary data demonstrate that BDM can also suppress a ligand-gated ion channel. Supported by NIAAA AA08025 and NIH NS31040.

629.18

2,3-BUTANEDIONE MONOXIME (BDM) BLOCKS GLYCINE GATED CHLORIDE CURRENT OF ACUTELY ISOLATED MURINE HYPOTHALAMIC NEURONS. J.H. Ye* and J.J. McArdle. Dept. Pharmacol., New Jersey Med. Sch. (UMDNJ), Newark, NJ 07103-2714.

Data from this lab, and others, demonstrate that BDM suppresses voltage gated calcium, sodium and potassium currents of various preparations. In an attempt to determine whether BDM also blocks ligand-gated currents, we evaluated its effects on glycine activated chloride current (I_{Gly}) of hypothalamic neurons acutely dissociated from young mice. The nystatin perforated patch technique was used to record I_{Gly} from neurons rapidly superfused with drug containing solutions. Pulses of 0.01 to 1 mM glycine alone rapidly induced a peak I_{Gly}. Desensitization caused a gradual decay of I_{Gly}. BDM concentrations ranging from 0.05 to 50 mM caused a dose dependent suppression of I_{Gly}. BDM appears to cause open channel block since the peak I_{Gly} remained largely unaltered while the slowly decaying portion was greatly diminished. This block occurred rapidly and was completely removed upon washout of the BDM containing solution. Other work in this laboratory demonstrates that BDM also blocks GABA gated chloride current. Thus, BDM acts non-selectively to suppress voltage and ligand gated ion currents. Supported by NIAAA AA08025 and NIH NS31040.

ACETYLCHOLINE RECEPTORS: NEURONAL NICOTINIC II

630.1

NICOTINIC ANTAGONISTS DISTINGUISH BETWEEN SYNAPSES ON SYMPATHETIC B AND C NEURONS IN THE BULLFROG W-X Shen* and JP Horn Department of Neurobiology, University of Pittsburgh, School of Medicine, Pittsburgh, PA 15261.

The block of nicotinic EPSPs in sympathetic B and C neurons by curare, hexamethonium, and mecamylamine was compared using trains of 40 preganglionic stimuli at frequencies between 0.5 and 20 Hz. Antagonism of nicotinic transmission had 2 components.

Use-independent IC_{50}s were measured from block of the first extracellular compound action potential in the train. The C cell AP was more sensitive than the B cell AP to all 3 drugs. However, the relative selectivity (B_{IC50}/C_{IC50}) varied from 1.5(curare) to 5.7(mecamylamine).

Use-dependent effects appeared as rundown or fade of the compound AP during trains. In the B cell system, fade occured at frequencies as low as 0.5 Hz and at drug doses which had little effect on the first AP in the train. By contrast, fade of C-cell responses in all 3 drugs occured only at higher frequencies (10-20 Hz). Intracellular recording was used to analyze differences in fade between B and C cells. In curarized (20 μM) B cells, EPSPs but not iontophoretic repsonses to ACH faded at 2 Hz. This suggests fade is presynaptic (eg. depression). EPSCs recorded from B cells in control Ringer under single-electrode voltage-clamp faded in a frequency-dependent manner between 2-20 Hz. By contrast, EPSCs in C cells grew during trains at 2 Hz and the apparent facilitation was larger at 10Hz. Fade appeared in C cell EPSCs at 20 Hz.

The data suggest that B and C cells may differ in terms of their nicotinic receptors and the dynamics of ACH release.

Supported by NIH grants NS21065 and NS01427 and by a Grant-in-Aid from the American Heart Association, PA Affiliate.

630.2

NICOTINIC ACETYLCHOLINE RECEPTOR FUNCTION IN NEURONAL SUBPOPULATIONS. M. E. McNerney and J. F. Margiotta* Dept. of Physiology & Biophysics, Mount Sinai School of Medicine, NY, NY 10029.

The chick ciliary ganglion contains choroid and ciliary neuron subpopulations, which both express functional nicotinic acetylcholine receptors (AChRs). Choroid and ciliary neurons can be distinguished by criteria that include differences in their preganglionic terminals, peripheral targets, ganglionic position, receptor pharmacology and soma size. We are now examining the two populations for differences in the expression and regulation of functional AChRs. Size measurements from ≈700 ciliary ganglion neurons, that were freshly-isolated at embryonic day 14 (E14), revealed a bimodal distribution of soma surface areas; calculation of 95% confidence limits for each mean demonstrated that the two populations could be segregated by size. Choroid neurons were identified as having soma areas ≤550 μm^2, and ciliary neurons as having soma areas ≥1200 μm^2, in accord with observations from other laboratories. ACh responses were obtained from E14 neurons in subsequent whole cell recordings at -70 mV following microperfusion with 10 or 500 μM ACh. In each trial, the peak conductance response was normalized to the neuron's membrane capacitance, which varies directly with membrane area. The normalized responses to 10 μM ACh were about two-fold greater for choroid than for ciliary neurons. A similar distinction in the normalized peak responses to 500 μM ACh was observed, while no differences between the two populations in rates of receptor desensitization were detected. Preliminary single channel recordings reveal that two AChR conductance classes of roughly 25 pS and 40 pS are present on both choroid and ciliary neurons. Characterization of the relative abundance and kinetic properties of the individual AChR channel classes is in progress. These results reveal distinct ACh responses in neuronal subpopulations that may be explained by differential regulation of AChR function or density. Supported by NIH # NS24417.

630.3

THREE DISTINCT NICOTINIC RECEPTOR-MEDIATED RESPONSES RECORDED INTRACELLULARLY IN DIFFERENT REGIONS OF CHICK BRAIN. V.A. Chiappinelli*, L.L. McMahon, K.M. Wolf and L. Yum, Dept. Pharm. and Phys. Sci., Saint Louis Univ. Sch. of Med., St. Louis MO 63104

We used whole-cell patch-clamp and sharp electrode recordings to examine nicotinic responses in embryonic chick brain slices. We find very distinct responses to receptor activation in three different brain nuclei.

Lateral Spiriform Nucleus (SPL). In this nucleus, we have identified postsynaptic nicotinic receptors with a high affinity for nicotine that are insensitive to blockade by alpha-bungarotoxin (ABgT) and kappa-bungarotoxin (Neuron 5:307, 1990). We now report that nicotinic agonists also produce a large increase in the frequency of spontaneous inhibitory postsynaptic currents (IPSCs), an effect which is blocked by 30 μM dihydro-β-erythroidine. The increase in IPSC frequency is totally blocked by 1 μM tetrodotoxin (TTX).

Ventral Lateral Geniculate Nucleus. One cell type in this nucleus shows little direct response to nicotinic agonists, but exhibits a marked increase in bicuculline-sensitive IPSCs in response to 30 μM carbachol. Unlike the SPL response this effect is not blocked by 1 μM TTX, indicating that presynaptic nicotinic receptors are likely to mediate the increase in IPSCs.

Edinger-Westphal Nucleus. Cells in this nucleus exhibit an excitatory response to nicotinic agonists that is very sensitive to blockade by methyllycaconitine (MLA; ED_{50}=3 nM). By contrast, the direct nicotinic response in SPL neurons is blocked only at much higher doses of MLA (ED_{50}=3 μM). Separate binding experiments indicate that MLA has high affinity (IC_{50}=4 nM) for ^{125}I-ABgT sites in chick brain, and suggest that the Edinger-Westphal nicotinic receptor is of the ABgT-sensitive type. NS17574.

630.4

ACETYLCHOLINE-BINDING SUBUNITS OF α-BUNGAROTOXIN-SENSITIVE AND α-BUNGAROTOXIN-INSENSITIVE NICOTINIC ACETYLCHOLINE RECEPTORS: PATTERNS OF EXPRESSION IN CHICK RETINAL NEURONS. Kent T. Keyser*, Harvey Karten and Jon Lindstrom. Dept. of Neurosci., University of California, San Diego, CA 92093 and Dept. of Neurosci., University of Pennsylvania, Philadelphia, PA 19104.

α subunits of α-bungarotoxin-insensitive acetylcholine receptors (nAChRs; α3, α4, α5) and α-bungarotoxin-sensitive nAChRs (αBgtAChRs; α7, α8) have been purified, their cDNAs cloned, and antibodies have been raised against them. Studies have shown that receptor subtypes containing the different α subunits differ pharmacologically. We have used antibodies against α3, α4, α5, α7, and α8 to determine the patterns of expression of these subunits in chick retinal neurons.

Populations of amacrine and ganglion cells contained α3 subunits while α4 subunit labeling was weak throughout the retina. α5 subunits were found in amacrine, ganglion and displaced ganglion cells. α8 subunits were found in amacrine, bipolar and ganglion cells while α7 subunits were found only in amacrine and ganglion cells. The distribution of α3 and α5 subunits in the IPL was very similar and closely matched that of dendrites of cholinergic amacrine cells. α8 was found in both the inner and outer plexiform layers, but α7 subunit labeling was found only in the IPL in a pattern distinct from that of α8 labeling. The distributions of α7 and α8 in the IPL overlapped, but were more extensive than, that of dendrites of cholinergic amacrine cells. In summary, the five α subunits are widely distributed throughout the retina. Since receptors containing different subunits differ pharmacologically, the effects of ACh or its agonists depends upon what receptor subtype(s) is/are expressed by the neurons in various retinal circuits. Supported by EY07845 (K.T.K.), EY06890 (H.J.K.) and CTR, CSTR, MDA and NS11323 (J.L.).

630.5

FUNCTIONAL PROPERTIES OF THE SUBTYPES OF NICOTINIC ACETYLCHOLINE RECEPTOR (nAChR) IN RAT HIPPOCAMPAL NEURONS. M. Alkondon[1]* and E.X. Albuquerque[1,2]. [1]Dept. Pharmacol. Exp. Ther., Univ. Maryland, Sch. Medicine, Baltimore, MD 21201; [2]Lab. Mol. Pharmacol, IBCCF, Fed. Univ. Rio de Janeiro, Brazil, 21944.

Recent work from our laboratory indicated the presence of functionally distinct nAChRs in rat hippocampal neurons grown in primary culture (Alkondon & Albuquerque, *JPET, in press*, 1993). In the present work, the diversity in the functional subtypes of the hippocampal nAChRs was further explored by studying the characteristics of the macroscopic currents measured using the whole-cell mode of the patch-clamp technique. Application of ACh (3 mM) evoked the following current variants: (i) A rapidly decaying current (named type IA) elicited in a majority of neurons (83%) and potently blocked by α-bungarotoxin (α-BGT), κ-bungarotoxin (κ-BGT), and methyllycaconitine (MLA); (ii) A slowly decaying current that was elicited either alone (type II) or together with the rapidly decaying current (type IB), and was potently blocked by dihydro-β-erythroidine but less so by α-BGT, κ-BGT and MLA; (iii) A slowly decaying current (type III) elicited in a small population of neurons (~2%) and relatively insensitive to blockade by α-BGT, κ-BGT and MLA, but that could be blocked by low concentrations of mecamylamine. The three current types, which most likely represent three distinct subtypes of nAChR in hippocampal neurons, showed further differences in their functional properties. Type IA currents exhibited little or no rectification, whereas type II showed a marked rectification. Another striking difference was reflected in the presence in type IA, but not in the other two current types, of a marked run-down in the current amplitude during the first 30 min of whole-cell recording. Addition of ATP-regenerating solution (consisting of 4 mM Tris ATP, 20 mM phosphocreatine, and 50 units/ml creatine phosphokinase) to the pipette solution prevented the run-down of IA currents to a great extent, suggesting that the high-energy phosphate compounds, which are dialysed away during the whole-cell recording, may be required for the maintenance of nAChR-mediated currents. Thus, the proposed subtypes of nAChR found in hippocampal neurons varied not only in their sensitivities to pharmacological agents, but also in their intrinsic properties, such as rectification and their regulation by phosphorylation-dephosphorylation mechanisms. Support: USPHS Grants NS-25296 and ES-05730.

630.6

CALCIUM ION INFLUX IN CLONAL CELL LINES EXPRESSING NICOTINIC ACETYLCHOLINE RECEPTORS. Cynthia M. Eisenhour, Elzbieta Puchacz* and Ronald J. Lukas. Div. Neurobiology, Barrow Neurological Institute, Phoenix, AZ 85013, USA.

Ca^{2+}/Na^{+} permeability ratios for muscle nicotinic acetylcholine receptor (nAChR) channels are reported to be lower than those for neuronal nAChR and for putative alpha-bungarotoxin-sensitive nAChR (Bgt-nAChR). Bgt-nAChR have been implicated in the intracellular accumulation of Ca^{2+} in neuronal cells. We studied agonist-stimulated ion flux in rat PC12 or human SH-SY5Y cells, which express both neuronal (ganglia-type) nAChR and Bgt-nAChR, and in human TE671/RD cells, which express muscle-type nAChR. Agonist dose-response profiles for $^{45}Ca^{2+}$ influx are similar to those for $^{86}Rb^{+}$ efflux for each cell type (e.g., maximum flux occurs at 100 uM nicotine for ganglia-type nAChR). $^{45}Ca^{2+}$ flux is much smaller in TE671 cells than in PC12 or SH-SY5Y cells and in every case is reduced rather than enhanced when cell membrane potentials are reduced. Antagonism of $^{45}Ca^{2+}$ influx or $^{86}Rb^{+}$ efflux by d-tubocurarine or pancuronium is identical for a given cell type. Alpha-bungarotoxin (Bgt) blocks both types of fluxes in TE671 cells but fails to block either $^{45}Ca^{2+}$ or $^{86}Rb^{+}$ fluxes in PC12 or SH-SY5Y cells. While we may have failed to detect Bgt-nAChR-mediated $^{45}Ca^{2+}$ influxes and/or to discount the possibility that Bgt-nAChR can activate release of Ca^{2+} from intracellular stores, these data indicate that toxin-insensitive nAChR can mediate Ca^{2+} influxes in neural crest-derived PC12 or SH-SY5Y cells.

630.7

A HETERO-BETA NEURONAL NICOTINIC ACETYLCHOLINE RECEPTOR EXPRESSED IN *XENOPUS* OOCYTES. L. Colquhoun*, K. Dineley & J. Patrick. Division of Neuroscience, Baylor College of Medicine, One Baylor Plaza, Houston, Tx, 77030.

Functional neuronal nicotinic receptors possessing distinct pharmacological properties can be expressed in *Xenopus* oocytes by pairwise injection of DNA encoding one alpha (α2,α3, α4) and one beta (β2, β4) subunit (1). Both alpha and beta subunits contribute to the specific pharmacological differences between pairwise combinations. There are many cases where the properties of nAChR channels measured *in situ* do not correlate with any pairwise combinations expressed in oocytes. One possible explanation is that more than 2 types of subunit are involved in receptor formation and there is evidence for involvement of 3 subunit types (α3,α5 and β2)in a nicotinic receptor out of chick ciliary ganglion (2). DNA for the subunits α3, β2 and β4 were intranuclearly injected into oocytes and voltage clamp recordings made after 3-7 days incubation. When the 3 subunits were injected together a receptor with mixed properties was obtained.The irreversible open channel blocker chlorisondamine was used to show that the major receptor population contained both β2 and β4. Cytisine was a potent agonist suggesting the presence of β4 and neuronal bungarotoxin partially blocked the response, indicating the presence of β2. We are currently using subunit specific antibodies to show the association of α3,β2 and β4 immunoreactive protein in a receptor expressed in *Xenopus* oocytes.

1. Leutje,C.W & Patrick,J. J. Neurosci. 11, 837-845.
2. Vernallis,A.B., Conroy W.G & Berg,D.K, Neuron, 10, 451-464.

630.8

PARTICIPATION OF α5 in NEURONAL NICOTINIC AChR CHANNELS. J.A. Ramirez Latorre, X. Qu and L. Role* Dept . Anat. & Cell Biol. in the Ctr. Neurobiol. & Behav., Columbia University P&S, 630 W 168th St NY, NY 10032.

In spite of a broad expression pattern of the α5 gene, this nAChR subunit has not been shown to form a functional channel either alone or in combination with known β subunits. Examination of the α5 sequence reveals substitutions in both the ligand binding and putative ion permeation domains. Previous studies of muscle AChRs predict that changes in these regions would decrease agonist affinity (Yellen et al., 1991) and increase single channel conductance (Imoto et al., 1986). To test whether α5 can participate in functional AChR complexes we examined the properties of channels expressed in *Xenopus* oocytes injected with α5 cDNA alone or α5 in combination with other α and β subunit cDNAs (α4, α5, β4 sequences gift of Ballivet). Injection with α4 and β2 produced a 22pS channel and injection of α5 alone produced no detecable current in response to applied ACh as previously reported. Co-injection of α5 cDNA with both α4 and β2, however, produced a new nAChR channel with conductance states of 45 and 50 pS. When the ratio of α5 cDNA was increased to 5-10x that of the co-injected α4 and β2, the 45pS openings were more frequent than the 22pS. Determination of the dose-dependence of ACh-evoked currents for both the α4β2 and the α4α5β2 combinations revealed a K_m of 7μM for α4β2 as reported, but a K_m of 300μM for α4α 5β2. Cysteine replacements in the M2 region of α5, and subsequent treatment with cysteine reactive reagents will be used to further test participation of the α5 subunit in the new channels. (supported by NS22061).

630.9

IMMUNOHISTOCHEMICAL LOCALIZATION OF THE α6 SUBUNIT OF THE NEURONAL NICOTINIC ACETYLCHOLINE RECEPTOR IN THE ADULT RAT BRAIN. F.M. Goldner[1], M. Quik[2] and J.W. Patrick[1]*. [1]Division of Neuroscience, Baylor College of Medicine, Houston, TX 77030 and [2]Department of Pharmacology, McGill University, Montreal, Canada

Nine different rat genes which code for putative subunits of the neuronal nicotinic acetylcholine receptor have been identified, cloned and sequenced. One of these, α6, shows the sequence characteristics of a nicotinic receptor subunit, but no contribution of this clone to a functional receptor has been demonstrated in the *Xenopus* oocyte expression system. To determine whether the receptor protein is expressed in the brain, a polyclonal antibody against recombinant protein representing the predicted extracellular domain of the receptor was raised and the serum purified over a peptide column. Western analysis with the α6 antibody identified two bands with apparent molecular weights of about 98 and 60KD. The latter band is consistent with the size for α6 as predicted from the cDNA sequence. Preincubation of the antibody with an excess of the α6 peptide used in the purification step prevented its interaction with the above two protein bands suggesting a specific interaction. The antibody was used in immunohistochemical studies to assess the anatomical distribution of the receptor protein in frozen sections of adult rat brains. α6 immunoreactivity is restricted to neurons, staining the cytoplasm and plasma membranes of cell somata and proximal processes whereas cell nuclei are devoid of immunostaining. Immunoreactivity was highest in the substantia nigra and ventral tegmental area as expected from previous *in situ* hybridization data. Other areas that were previously shown to be positive for α6 mRNA such as the reticular nucleus of the thalamus, the anterior pretectal area and the locus coer-uleus also showed immunoreactivity with the anti-α6 antibody. Overall, there was a good correspondence between *in situ* hybridization and immunohistochemistry, although immunoreactive neurons were seen in the cerebral cortex where no α6 mRNA has been detected. These data suggest that the α6 protein is expressed in rat brain.

ACETYLCHOLINE RECEPTORS: NICOTINIC ANTAGONISTS AND AGONISTS

631.1

TOXINS, BUT NOT OTHER ANTAGONISTS, STABILIZE THE RESTING STATE OF THE NICOTINIC ACETYLCHOLINE RECEPTOR. Marjorie Moore[1], Frederick Kauffman*[2], and Michael P. McCarthy[1]. [1]CABM/Pharmacology, UMDNJ-RWJMS,Piscataway, NJ, 08854. [2] College of Pharmacy, Rutgers U., Piscataway, NJ 08855.

We have used 3-(trifluoromethyl)-3-(*m*-[^{125}I]iodophenyl)diazirine ([^{125}I]TID) to characterize the effects of antagonists on the conformation of the neuromuscular, nicotinic acetylcholine receptor (AChR) from *Torpedo californica*. [^{125}I]TID labels all four subunits of the AChR, and is a sensitive probe of AChR conformation. The snake venom toxins α-bungarotoxin and *Naja naja* toxin both increase the degree of [^{125}I]TID incorporation. The small molecule antagonists tubocurarine and gallamine have no effect on [^{125}I]TID labelling at low concentrations, but at higher concentrations reduce [^{125}I]TID incorporation. *In vitro*, preparations of AChR are mixtures of both resting state and desensitized AChR. The effects of snake venom toxins are most likely due to their stabilization of the resting state form of the AChR, leading to increased [^{125}I]TID incorporation. However, the snake venom toxins will not induce the resting state in AChRs which have been desensitized by reconstitution in desensitizing lipids or by solubilization in desensitizing detergents. Tubocurarine and gallamine appear to act as partial agonists. At low concentrations they do not induce the resting state, but desensitize the AChR at higher concentrations (>0.5-1mM), leading to diminished [^{125}I]TID incorporation.

631.2

THE REDUCED FORM OF NEREISTOXIN IS RESPONSIBLE FOR REDOX EFFECTS ON NEURONAL NICOTINIC RECEPTORS (nAChRs). Y. Xie*, R.H. Loring & G.S. Jones, Jr. Dept. of Pharmaceut. Sci., Northeastern Univ., Boston, MA 02115

We have shown that nereistoxin (NTX, 4-dimethylamino-1,2-dithiolane) blocks nAChRs in chick retina and ciliary ganglion by reducing the disulfide bond found in the agonist binding site. We hypothesized that NTX is reduced intracellularly to dihydro-NTX (DHNTX; 2-dimethylamino-1,3-propanedithiol) as the reactive species that reduces nAChR's. In whole-cell patch-clamp recordings from isolated ciliary neurons, inclusion of 100 μM NTX in the pipette has no effect, but 100 μM DHNTX slowly blocks nicotinic responses, and this effect is reversed by external application of the oxidizing agent dithiobisnitrobenzoic acid (DTNB). 100 μM NTX applied externally acts only as a reversible nicotinic antagonist, but recovery from external DHNTX blockade requires reoxidation with DTNB. The quaternary derivative of NTX, (4-trimethylamino-1,2-dithiolane) acts as a weak oxidizing agent on nAChRs of chick ciliary ganglia treated with the reducing agent dithiothreitol, while the reduced quaternary version (4-trimethylamino-1,3-propanedithiol) readily reduces ciliary ganglion nAChR's. Preliminary studies with reduced charatoxin (2-methylthio-1,3-propanedithiol, another analog of DHNTX) also demonstrate reduction of nAChR's in chick retina. These data suggest that analogs of NTX that diffuse through membranes can reduce nAChR's, while the quaternary derviatives must be reduced to dithiols to affect the nAChR redox state. (Supported by NS22472 and the STRC).

631.3

THE EFFECTS OF STRYCHNINE, CHLORISONDAMINE (CHL) AND CYTISINE ON THE INHIBITORY NICOTINIC RESPONSE OF DORSOLATERAL SEPTAL NUCLEUS (DLSN) NEURONS E.M. Sorenson* and J.P. Gallagher. Dept of Pharmacol. and Toxicol., Univ. of Texas Med. Br., Galveston, TX 77555-1031.

This laboratory has previously demonstrated that 1,1-methyl-4-phenylpiperazinium (DMPP) produces a direct inhibitory response at rat DLSN neurons which is blocked by mecamylamine (50 μM) and kappabungarotoxin (0.5 μM). We have now examined the effects of the antagonists CHL and strychnine on this response. CHL is believed to have a mechanism of action similar to that of mecamylamine while strychnine blocks the nicotinic receptor consisting of α_7 subunits expressed in oocytes (Séguéla et al., J. Neurosci. 13:596, 1993). We have also determined whether the nicotinic agonist cytisine, which appears to be an antagonist at nicotinic receptors containing the β_2 neuronal nicotinic subunit(Luetje and Patrick, J. Neurosci. 11:837, 1991), blocks the inhibitory response.

A control response to DMPP was recorded intracellularly from DLSN neurons. The cells were then superfused with strychnine, CHL, or cytisine for at least 10 min and the response of the cell to DMPP was retested. Strychnine 10-100 μM did not block the effects of DMPP. CHL (50 - 500μM) reduced the magnitude of the hyperpolarization in response to DMPP but only completely blocked the DMPP response when superfused at 500 μM. This inhibition was not always reversible, even after prolonged washing. Cytisine (500 μM) was ineffective as either an agonist or an antagonist.

These results suggest that the α_7 subunit is not a constituent of the inhibitory nicotinic receptor. Since low levels of β_2 subunit are expressed in the DLSN (Wada et al., J. Comp. Neurol. 284:314 1989), the possibility exists that the inhibitory receptor contains the β_2 subunit in combination with an as yet uncloned α subunit.

Supported by DHHS 1F32 DA 05447-02 and The Council for Tobacco Research, USA, Inc.

631.4

THE NICOTINIC ANTAGONISTS, MECAMYLAMINE AND PEMPIDINE, ARE NONCOMPETITIVE INHIBITORS OF BRAIN NICOTINIC RECEPTOR FUNCTION. W. Cao*, M.J. Marks, A.C. Collins. IBG Univ. Colorado, Boulder, CO 80309

Mecamylamine (Mec) and pempidine (Pem) do not inhibit binding to brain nicotinic receptors, but several studies indicate that these two compounds block behavioral effects of nicotine (Nic). This suggests that Mec and Pem are noncompetitive inhibitors of brain nicotinic receptors, perhaps acting by binding to the ion channel associated with the receptor. This possibility was assessed by determining the inhibition profiles of Mec and Pem for three behavioral responses to Nic and for a recently-developed ion flux assay that seems to measure the activity of a brain nicotinic receptor that binds L-[^{3}H]nicotine with high affinity; this is probably a receptor that contains the α-4 variant of the receptor. Dose-response curves were constructed for inhibition of Nic responses using Nic doses of 0, 0.5, 1.0 and 1.5 mg/kg. Both Mec and Pem inhibited the behavioral actions of Nic, but the IC_{50} values did not vary depending upon Nic dose. Similar results were obtained with the in vitro test. Both Mec and Pem inhibited Nic-induced (0, 10, 100 μM) increases in $^{86}Rb^+$ efflux from synaptosomes prepared from mouse brain. The IC_{50} values for inhibition of ion flux were not affected by Nic concentration. Thus, both in vivo and in vitro tests produced data that are consistent with the argument that Mec and Pem are noncompetitive inhibitors of brain nicotinic receptor function. Supported by DA-03194 and DA-00116.

631.5

PARAOXON AND DIISOPROPYLFLUOROPHOSPHATE (DFP) INDUCE OPEN CHANNEL BLOCKADE OF NICOTINIC ACETYLCHOLINE RECEPTORS (nAChR). J.A.L. Santos[1], B.S. Nascimento[1], Y. Aracava[1,2*] and E.X. Albuquerque[1,2]. [1]Lab. Mol. Pharmacol., Fed. Univ. Rio de Janeiro, Brazil and [2]Dept. Pharmacol. Exp. Ther., Univ. Maryland Sch. Med., Baltimore, MD 21201.

It has been shown that DFP interacts with the nAChR, blocking nerve-elicited endplate currents in a manner similar to that of certain local anesthetics (*JPET* 189:499, 1974). We further investigated the actions of DFP on the muscle nAChR, comparing them with those of the pesticide paraoxon and the local anesthetic piperocaine. Single-channel currents activated by acetylcholine (ACh) were studied in frog (*Leptodactylus ocellatus*) interosseal muscle fibers under the cell-attached patch-clamp condition. DFP (0.1-1 mM) produced a voltage-dependent blockade of the open state of ACh-activated channels, increasing the frequency of closures and decreased intra-burst open times at negative potentials. The DFP-induced closures, corresponding to the blocked state, were 10 times longer than those detected in control currents. Paraoxon (1 mM) applied together with ACh (0.2 μM) also blocked the nAChR channels in their open conformation. However, paraoxon induced randomly separated brief events, with no detectable burst pattern, thus indicating a more stable blockade. Both OPs mildly reduced the frequency of openings induced by ACh. Piperocaine, on the other hand, blocked the nAChR more potently, decreasing both the frequency and the duration of the open state at 1 to 10 μM and at negative membrane potentials completely the openings at concentrations higher than 25 μM. In consonance with the voltage- and time-dependent blockade reported on macroscopic currents (*Mol. Pharmacol.* 16:909, 1979), we observed that under the conditions of single channel current recordings the blockade by piperocaine was favored. In addition, paraoxon at 1 mM exhibited agonist activity, activating currents resembling those activated by ACh and also a population with an increased level of noise in the open state. Both currents were not inhibited by α-bungarotoxin. Support: CNPq, Finep, Mol. Pharmacol. Training Program, U.S. Army Med. Res. Devel. Comm.

631.6

EFFECTS OF BUPIVACAINE ON NEURONAL NICOTINIC ACETYLCHOLINE RECEPTORS IN RAT HIPPOCAMPAL NEURONS. Y. Yu[1], R.A. Siodin[2*] and E.X. Albuquerque[1,3]. Depts. [1]Pharmacol. Exp. Ther. and [2]Biophysics, Univ. Maryland, Sch. Medicine, Baltimore, MD 21201; [3]Lab. Mol. Pharmacol., IBCCF, Fed. Univ. Rio de Janeiro, Brazil 21944.

The local anesthetic, bupivacaine, has been shown to act as an open-channel blocker at the nicotinic acetylcholine receptor (nAChR) in frog sartorius muscle (*Mol. Pharmacol.*, 26:293,1984). In the present work, we investigated the effects of bupivacaine on neuronal nAChRs, which have subunit compositions different from that of their muscle counterpart. Using the patch-clamp technique, nicotinic single-channel and whole-cell currents were studied in fetal rat hippocampal neurons cultured for 10-30 days. The agonist, acetylcholine (ACh), in the absence or presence of bupivacaine, was applied to the patches through a U-tube. In agreement with a recent report (M. Alkondon & E.X. Albuquerque, *JPET, in press*, 1993), four types of whole cell currents (IA, IB, II, and III) could be induced by ACh (100-500 μM). Bupivacaine (50-500 μM) decreased the peak amplitude of these currents, in a concentration- and voltage-dependent manner, and shortened the decay phase of slowly desensitizing currents in a concentration-dependent manner. These effects were all reversible upon washing. At the concentration of 500 μM, bupivacaine blocked nearly 50% of the peak amplitude of the ACh response, but did not affect the NMDA, quisqualate, and kainate responses in the hippocampus. In an outside-out patch excised from a neuron cultured for 16 days, the mean open and burst times of ACh (30μM)-activated single channels were decreased by bupivacaine (0.5 μM). These results suggest that bupivacaine may act as an open-channel blocker of neuronal nAChRs. This is the first report of an open-channel blocker of neuronal nAChRs in the rat hippocampus, and illustrates that in spite of the difference in subunit composition between neuronal and muscle nAChRs there might be a highly conserved region in the ion channels of these receptors at which open channel blockers can bind and exert their effects. Support: USPHS NS25296 and ES05730.

631.7

CENTRAL NICOTINIC BLOCKADE BY CHLORISONDAMINE ADMINISTERED IN VITRO OR IN VIVO. H. El-Bizri* and P.B.S. Clarke. Dept of Pharmacology and Therapeutics, McGill Univ., Montreal, Canada H3G 1Y6.

Chlorisondamine (CHL) is a nicotinic antagonist which produces a persistent central nicotinic blockade lasting for at least 12 weeks after a single in vivo administration. We used (-)-nicotine induced ^{3}H-dopamine release from rat striatal synaptosomes as a functional assay to investigate the mechanisms of nicotinic blockade produced by CHL. After *in vitro* administration of CHL, nicotinic blockade was concentration-dependent (10^{-8}-10^{-4} M), and the potency of CHL was increased 26-fold by concurrent administration of a brief pulse of nicotine prior to nicotine challenge. Block following in vitro CHL was not surmounted by nicotine (10^{-7}-10^{-4} M) challenge. After *in vivo* administration of CHL, blockade persisted in vitro and was also not surmounted by nicotine (10^{-7}-10^{-4} M) challenge. CHL (given in vitro or in vivo) also blocked nicotinic responses to ACh but did not affect responses to amphetamine or high K^+. Prolonged wash reversed nicotinic blockade resulting from in vitro, *but not in vivo*, administration of CHL. This suggests that in vitro and in vivo administration of CHL produces CNS nicotinic blockade by different mechanisms. Funded by the MRC of Canada.

631.8

ESERINE (PHYSOSTIGMINE) BLOCKS NICOTINIC RESPONSES IN THE RAT CNS. P.B.S. Clarke*, M. Reuben, and H. El-Bizri. Dept of Pharmacology and Therapeutics, McGill Univ., Montreal, Canada H3G 1Y6.

The AChE inhibitor eserine (physostigmine) exerts agonist and antagonist actions at Torpedo electroplaque nicotinic receptors (Shaw et al 1985, Mol. Pharmacol. 28:527-538). We tested for both actions in the rat CNS, by studying ^{3}H-dopamine release induced by (-)-nicotine from superfused rat striatal synaptosomes. Superfusion of eserine (0.3 - 300 μM) for 30 min resulted in a concentration-dependent blockade of a subsequent nicotine 1 μM challenge (IC50 approx. 30 μM). Neostigmine produced a similar effect, whereas DFP somewhat reduced nicotinic responses only at 300 μM. Eserine (0.3 - 300 μM), given as a brief pulse, did not mimic nicotine. Nicotinic blockade by eserine (30 μM) was noncompetitive and was selective; responses to the nicotinic agonists cytisine and DMPP were reduced, whereas responses to high K^+ were not. Eserine (300 μM) completely blocked responses to nicotine, slightly reduced responses to amphetamine, and did not alter responses to high K^+. Thus, it appears that eserine blocks CNS nicotinic responses in a pharmacologically selective manner, possibly independent of its ability to inhibit acetylcholinesterase. Funded by the MRC of Canada.

631.9

RECEPTOR BINDING AND FUNCTIONAL EFFECTS OF A SERIES OF KNOWN AND NOVEL NICOTINIC LIGANDS. P.M. Lippiello*+, K.G. Fernandes+, W.S. Caldwell+, M.J. Marks++, and A.C. Collins++. Research & Development, R.J. Reynolds Tobacco Co.+, Winston-Salem, NC 27102 and Institute for Behavioral Genetics++, University of Colorado, Boulder, CO 80309.

A number of potential nicotinic ligands were evaluated in inhibition binding assays to assess their relative potencies in displacing L-[^{3}H]-nicotine binding to rat brain membranes and [^{125}I]-α-bungarotoxin binding to mouse brain membranes. These compounds were classified into six structural series, representing both minor plant and tobacco alkaloids and synthetic compounds. Of the compounds tested in binding assays, those having the lowest K_i values for [^{3}H]-nicotine binding (<10 μM) were studied using ligand-evoked dopamine release from rat brain striatal synaptosomes. The most potent compounds were further evaluated using Rb^+ efflux from mouse brain thalamic synaptosomes, and a physiological test battery in mice (heart rate, body temperature, respiration, y-maze rears and crosses, acoustic startle). Based on dopamine release and/or Rb^+ efflux, compounds were classified as full or partial agonists. Although the K_i values for inhibition of [^{3}H]-nicotine binding by the most potent agonists differed by more than two orders of magnitude (2 nM - 900 nM), the range of EC_{50} values for dopamine release (100 nM-1.3 μM) and for Rb^+ efflux (200 nM-2.5 μM) differed only by a factor of ten. In general, [^{3}H]-nicotine binding affinity did not correlate well with the EC_{50} or maximal effect for Rb^+ efflux and dopamine release or with *in vivo* behavioral effects, which may reflect varying degrees of receptor desensitization by these ligands. However, relative potencies in the *in vitro* functional assays were reasonably good predictors of *in vivo* effects. The information gained from these studies may provide a useful basis for identifying and developing novel nicotinic ligands as research tools and potential therapeutics for neurodegenerative diseases. (A.C. Collins was supported by a Research Scientist Development Award from NIDA DA-00116).

631.10

α-CONOTOXINS: SMALL PEPTIDE LIGANDS OF THE NICOTINIC ACETYLCHOLINE RECEPTOR. M. M. Grilley and B. M. Olivera.* Department of Biology, University of Utah, Salt Lake City, UT 84103.

α-conotoxins, a family of competitive antagonistic peptides isolated from *Conus* snail venoms, have emerged as useful tools for the elucidation of nicotinic acetylcholine receptor (nAChR) structure and function. At least seven distinct α-conotoxins have been identified and sequenced, ranging in size from 13 to 19 amino acid residues. We have used a subset of these toxins, one of whose structure has been solved by 2D-NMR, to examine the subunit organization of the nAChR. Sites of photoaffinity labeling of derivatized toxins on nAChR from *Torpedo* electroplax were determined, and, considering the placement of photoreactive groups within the toxin tertiary structure, it was possible to map the portions of the receptor surrounding the ligand binding site. Ordering of subunits about the established pseudo-symmetrical rosette structure of the receptor most consistent with the results obtained is $\alpha\gamma\alpha\beta\delta$.

The α-conotoxins also show great potential for use as discriminators of phylogenetic or receptor subtype differences. Some phylogenetic and subtype specificities have been observed among the α-conotoxins that have been examined to date. In addition, no two species have yet been found to produce conotoxins of identical sequence, and only a small fraction of the members of the genus have been screened for the presence of nAChR antagonists. (Supported by NIH Fellowship DA05485-02)

631.11

OSTREOTOXIN I, A FRACTION FROM A TOXIC CARIBBEAN DINOGLAGELLATE, ALTERS THE IONIC CONDUCTANCE OF NICOTINIC ACh RECEPTOR CHANNELS. J.A. Mercado, G. Escalona de Motta*, T.R. Tosteson# and I. Gonzalez#. Inst. of Neurobiology and Dept. of Biology, UPR Rio Piedras 00931 and #Dept. of Marine Sciences, UPR Mayaguez 00968.

Ostreopsis lenticularis is one of the benthic dinoflagellates implicated in the Caribbean as producers of toxins related with human ciguatera intoxication. Methanolic extracts obtained from three different clonal cultures of O. lenticularis were toxic to mice and inhibited with approximately equal potency the acetylcholine (ACh)-induced contractions of frog sartorius muscles. In addition, these extracts reduced the average amplitudes of miniature endplate potentials (mepps). After one hour of exposure to 0.3 mg of dried extract/mL average mepps amplitude was reduced by 72% but neither the time course of individual mepps nor their frequency were altered. Reverse phase High Pressure Liquid Chromatography (HPLC) was used to fractionate an extract according to polarity. In single channel patch clamp experiments addition of the more polar of the resulting fractions to an ACh-containing pippette caused the appearance of lower conductance ACh-activated channels in cultured chick embryo myocytes. No effect on channel lifetime was observed. We conclude that a component of this toxic dinoflagellate extracts is a polar toxin (ostreotoxin I) that reduces the ionic conductance of nicotinic ACh receptor channels. (Supported by NIH grants GM08102 and NS07464 and NOAA, USDC Sea Grant College Program at UPR Mayaguez.)

631.12

ANTAGONISM OF NICOTINIC RECEPTORS BY DELPHINIUM ALKALOIDS. P. Dobelis[1], J. E. Madl[1], G. D. Manners[2], J. A. Pfister[3], J. P. Walrond[1]*. [1]Dept. of Anatomy & Neurobiol., Colo. State Univ., Ft. Collins, CO 80523 USA, [2]Western Regional Res. Ctr., USDA, Albany, CA 94710 USA, [3]Poisonous Plant Res. Lab., USDA Logan, UT 84321 USA.

Delphinium spp. contain the norditerpenoid alkaloid methyllycaconitine (MLA), a nicotinic antagonist in the central and peripheral nervous systems. We have purified several related norditerpinoid alkaloids (MLA; 14-deacetylnudicaline, 14-DN; Barbinine; and Anthranoyllycoctonine, ALC) and tested their ability to block neuromuscular transmission in an *in vitro* lizard neuromuscular preparation. Intracellular recordings of miniature end plate potentials and extracellular recordings of compound muscle action potentials showed all of these toxins to be nicotinic antagonists. Our studies indicate that 14-DN is the most potent (EC_{50} = 0.5 μM), followed by barbinine (3 μM), MLA (7 μM), and ALC (20 μM). In contrast, the order of LD_{50}'s is ALC > barbinine > > MLA = 14-DN with MLA and 14-DN being the most potent. The apparent disparities in potency suggest that the mechanism of toxicity of these alkaloids may derive, at least in part, from effects on non muscle nicotinic acetylcholine receptors. It will be of interest to determine the effectiveness of these norditerpenoid alkaloids as nicotinic antagonists in the autonomic and central nervous systems. (Supported by USDA Agricultural Research Service and the Colorado Experiment Station).

631.13

THE EFFECTS OF DIHYDRO-B-ERYTHROIDINE (DHBE) ON METHYLCARBAMYLCHOLINE (MCC) - INDUCED CHANGES IN BRAIN NICOTINIC RECEPTORS AND RELATED BEHAVIORS IN THE RAT. X-H. Yang* and J.J. Buccafusco, Department of Pharmacology and Toxicology, Medical College of Georgia, and the DVAMC, Augusta, GA 30912.

Chronic intracerebroventricular (icv) treatment with MCC, a selective nicotinic agonist produced up-regulation (increased Bmax) of cortical nicotinic receptors (nAChR). The purpose of this study was to determine whether this regulation is associated with the development of changes in sensitivity to the behavioral effects of MCC, and also whether these changes could be influenced by icv pretreatment with the competitive nicotinic antagonist DHBE. Rats were injected twice daily with 30 μg, icv of MCC over 10 days. The development of changes in Bmax for nACHR paralleled the development of tolerance and return to normal sensitivity to a characteristic nicotinic behavior, the prostration syndrome. In contrast, the MCC-induced water drinking response evoked in the same animals actually was sensitized over the same period. When icv injection of DHBE (6 or 60 μg) preceded each MCC injection, MCC-induced prostration (but not MCC-induced water drinking) was inhibited. DHBE pretreatment also significantly inhibited the MCC-induced increase in Bmax for nAChR. Thus, activation of the agonist recognition site on the nAChR by MCC was required for the expression of changes in binding parameters. Also, since DHBE pretreatment reduced MCC-induced changes in receptor binding, but did not block all behaviors induced by MCC, it appears that MCC may act through both DHBE-sensitive and DHBE-insensitive receptor subtypes. Supported by a grant from the DVAMC.

631.14

PHARMACODYNAMIC AND PHARMACOKINETIC STUDIES ON ERYSODINE: A POTENT NEURONAL NICOTINIC RECEPTOR ANTAGONIST. C.-H. Kang*, J.L. Raszkiewicz, S. Swanson, J.D. Brioni, M.W. Decker, S.P. Arneric and J.P. Sullivan, Neuroscience Research, Pharmaceutical Products Division, Abbott Laboratories, Abbott Park, Il, 60064.

The CNS pharmacology of erysodine, a natural alkaloid isolated from the Erythrina seed, is poorly understood. We have recently reported (Anderson et. al., Soc for Neurosci, 1993) that erysodine is a potent (K_i = 5 nM) neuronal nicotinic acetylcholine receptor (nAchR) antagonist. In these experiments, we sought to determine 1) Is erysodine a centrally active nAchR antagonist in rat? and 2) How do the brain and plasma pharmacokinetics correlate with the CNS pharmacodynamic responses?

Erysodine (30 µmol/kg, i.p.) had no effect on locomotor activity in rats when administered on its own. When administered 4 mins prior to (-) nicotine (1.9 µmol/kg, i.p.), erysodine attenuated the initial (10 min) (-) nicotine-induced suppression of locomotor activity and the stimulatory effect of (-) nicotine on activity at 40 min. The effect of (-) nicotine on locomotor activity is also blocked by mecamylamine (5 µmol/kg, i.p.) and chlorisondamine (10 µg i.c.v.) (Decker et al. Behav. Neural Biol., in press). Brain levels of erysodine, comparable to (-) nicotine were rapidly achieved. At 10 min, brain levels of erysodine and (-) nicotine were 280 ± 31 and 318 ± 42 ng/g brain (n=3), respectively. Brain levels of erysodine remained elevated (306 ± 45 ng/g brain; n=3) at 40 mins. The brain:plasma ratio for erysodine was 1.5 at 10 mins and 1.3 at 40 mins. In contrast, plasma levels of (-) nicotine were much lower (5 fold) than the corresponding brain levels. Following i.v. administration, erysodine (4.3 µmol/kg) was found to have an elimination half-life of 29 mins but was not bioavailable following p.o administration (38 µmol/kg).

This study demonstrates that erysodine is rapidly accumulated into brain where it blocks the behavioral effects of (-) nicotine at brain levels similar to the behaviorally effective brain levels of (-)nicotine.

631.15

IN VITRO PHARMACOLOGY OF ERYSODINE: A HIGH AFFINITY ANTAGONIST OF NEURONAL NICOTINIC ACETYLCHOLINE RECEPTORS D.J. Anderson*, S.P. Arnerić, S. Swanson, J.L. Raszkiewicz, and J.P. Sullivan, Neuroscience Research, Pharmaceutical Discovery Division, Abbott Laboratories, Abbott Park, IL 60064.

Very few high affinity, competitive antagonists for neuronal nicotinic acetylcholine receptors (nAChRs) have been identified. Dihydro-β-erythroidine (DHβE) serves as the best example. Erysodine (ERY) is a related alkaloid also isolated from the Erythina seed. The purposes of this study were: 1) to characterize the binding properties of ERY, and 2) to characterize ERY in a functional response assay.

Initial characterization of ERY by concentration-inhibition of [^{3}H]cytisine ([^{3}H]CYT) binding revealed a K_i of 5 nM as compared to 35 nM for DHβE. Both compounds at 1 µM blocked 0.1 µM (-)nicotine (NIC) induced release of [^{3}H]dopamine ([^{3}H]DA) from striatal slices. Additionally, ERY displayed 40-fold selectivity for displacing [^{3}H]CYT binding over [^{125}I]α-bungarotoxin binding.

In saturation isotherms of 0.1-5 nM [^{3}H]CYT binding to rat brain washed membranes, increasing concentrations of ERY effected increases in apparent K_Ds with no change in B_{MAX}, indicating competitive binding. A K_D of 7 nM was determined from a kinetic plot of apparent K_Ds.

ERY shifted to the right concentration-response curves of NIC facilitation of [^{3}H]DA release from preloaded rat striatal slices. The shifts were parallel with no effect on the maximal response to NIC. A pA_2 of 8.34 was determined from a Schild plot of the resulting dose ratios.

Here it is shown that ERY is a high affinity competitive antagonist at neuronal nAChRs for both receptor binding and a functional nicotinic response. It has been recently demonstrated (Kang et al., *Soc for Neurosci*, 1993) that ERY accumulates in the brain where it blocks the effects of NIC on behavior. Therefore, ERY has potential for use as a peripherally administered, centrally active neuronal nAChR antagonist.

631.16

IN VIVO CHARACTERIZATION OF ERYSODINE, AN ERYTHRINA ALKALOID THAT BINDS TO NEURONAL NICOTINIC ACETYLCHOLINE RECEPTORS (nAChRs). A.B. O'Neill, J.D. Brioni, M.J. Buckley,* C.H. Kang, J.P. Sullivan, J.L. Raszkiewicz, S. Swanson, S.P. Arneric, and M.W. Decker,. Neuroscience Research, Pharmaceutical Products Division, Abbott Laboratories, Abbott Park, IL 60064.

Erysodine is an erythrina alkaloid related to the potent competitive nAChR antagonist dihydro-ß-erythroidine (DHßE), but the effects of erysodine at nAChRs are not well-characterized. Binding studies conducted in our group suggest that erysodine binds with high affinity to nAChRs (Anderson et al., this meeting). In the current study, we explored the effects of systemically-administered erysodine in mice.

Erysodine (0, 3, 10, or 30 µmol/kg IP) was administered prior to a nicotine (6.2 µmol/kg IP) challenge in male, CD-1 mice. Nicotine significantly reduced rectal temperature in mice pretreated with saline. Erysodine, by itself, did not affect body temperature but dose dependently attenuated the hypothermic effect of nicotine. In a separate experiment, we found that DHßE (0, 1.5, 3, or 5 µmol/kg IP) also produced a dose-related blockade of nicotine-induced hypothermia. In contrast to its effects on nicotinic receptor mediated hypothermia, erysodine (10 µmol/kg) did not affect muscarinic receptor mediated hypothermia produced by oxotremorine. These doses of erysodine were found to be inactive in the elevated plus-maze model of anxiety, but pretreatment with erysodine (30 µmol/kg) effectively blocked the increase of open-arm exploration seen with nicotine (0.62 µmol/kg). Brain and plasma levels for mice administered 30 µmol/kg of erysodine were determined to be 92.2±13.6 ng/g and 30.5±5.3 ng/ml, respectively.

Thus, erysodine readily enters the brain after systemic administration and antagonizes the hypothermic and anxiolytic-like effects of nicotine in mice.

631.17

BOROCAPTATE REVERSAL OF RESPIRATORY PARALYSIS INDUCED BY BLOCKADE OF NICOTINIC CHOLINERGIC RECEPTORS. G. Daniel and T. R. LaHann*. Center for Toxicology Research, College of Pharmacy, Idaho State University, Pocatello ID 83209.

Borocaptate sodium, a boronated drug developed for boron neutron capture therapy of cancer, rapidly reverses respiratory paralysis induced by i.v. injection of the neuromuscular junction (NMJ) blockers, gallamine and pancuronium. Gallamine triethiodide (15 mg/kg) or pancuronium bromide (2 mg/kg) administered intravenously to Long-Evans rats caused prolonged skeletal muscle paralysis and respiratory failure. In the absence of artificial respiration, these doses of NMJ blockers were fatal. If artificially respirated, however, animals recovered within 45 to 60 minutes. Borocaptate sodium (75-200 mg/kg, i.v., rate: 28 mg/kg/min) was infused 5-15 minutes after full NMJ blockade was established. Rats regained their ability to spontaneously breathe within 3 minutes of the start of borocaptate infusion, demonstrating a reversal of the skeletal muscle paralysis induced by the NMJ blockers. Borocaptate infusion re-established a normal respiratory pattern, and stable cardiovascular function was maintained throughout the time period that control (NMJ antagonist without borocaptate) rats remained paralyzed. The rapid, effective and sustained reversal of respiratory paralysis by borocaptate sodium suggests that this drug might be a useful antidote for treating overdosage of NMJ blockers. The mechanism by which such reversal occurs is currently under investigation.

631.18

PHYSOSTIGMINE (PHY) ACTIVATES $\alpha4\beta2$ NEURONAL NICOTINIC ACETYLCHOLINE RECEPTORS (nAChRs) VIA A NOVEL AGONIST SITE. E.F.R. Pereira[1,2*], J. Lindstrom[3], A. Maelicke[4], S. Reinhardt-Maelicke[4] and E.X. Albuquerque[1,2]. [1]Dept. Pharmacol. Exp. Ther., Univ. MD Sch. Med., Baltimore, MD 21201, USA; [2]Lab. Mol. Pharmacol., IBCCF, UFRJ, RJ 21944, Brazil; [3]Univ. Pennsylvania, Philadelphia, PA 19104, USA; [4]Inst. Physiol. Chem., Johannes-Gutenberg Univ. Mainz, Med. Sch., Duesbergweg, D-6500, Mainz, Germany.

It has been shown that PHY activates the α-BGT-sensitive nAChRs of frog muscle, *Torpedo* electroplax, and rat hippocampal neurons, and that its agonist effect is not blocked by competitive nicotinic antagonists, but instead by the nAChR-specific monoclonal antibody FK1 (*J. Rec. Res.*, 13:393 & 413, 1993). It has been suggested that the agonist effect of PHY could be caused by its binding to a novel nAChR agonist site. The residue Lys-125 on the receptor α subunit is an essential feature of the PHY-binding site on the nAChR (*Ann. N.Y.A.S.*, 681:140, 1993) and is highly conserved in nAChRs from different sources (Pereira *et al.*, *J.P.E.T., in press*, 1993). To determine whether the novel site is present on α-BGT-insensitive neuronal nAChRs, we took advantage of a cell line of fibroblasts that stably express the α-BGT-insensitive neuronal $\alpha4\beta2$ nAChR (M10 cells; *Mol. Pharmacol.*, 40:463, 1991). In outside-out patches excised from M10 cells that were primed with dexamethasone (1 μM), single-channel currents were activated by AnTX or PHY (each at 1 μM). Dihydro-β-erythroidine (DHβE, 1-30 nM), but neither methyllycaconitine nor FK1 blocked the channel activity induced by AnTX. The frequency of channel openings induced by PHY was unaffected by DHβE, but was decreased by FK1. Indirect immunofluorescence revealed heavy labeling of all cells with FK1, while no binding of α-BGT could be detected. Neither FK1 nor α-BGT was able to bind to M10 cells in which nAChR expression had not been induced by dexamethasone. Our findings agree with previous reports that the $\alpha4\beta2$ neuronal nAChR is insensitive to α-BGT, and demonstrate that the newly described agonist site is also present in α-BGT-insensitive nAChRs. Support: USPHS Grants NS25296, ES05730 (USA); Ma 599/17-1 (Germany).

631.19

THE NOVEL AGONIST SITE ON NICOTINIC RECEPTOR (nAChR): ACTIVATION BY PYRAZOLES, AMANTADINE, AND POLYAMINES. T. Tano[1,2*], M.C. Heluy-Dantas[1], M. Marchioro[1], W.M. Cintra[1], Y. Aracava[1,2], E.X. Albuquerque[1,2], A. Schrattenholz[3] and A. Maelicke[3]. [1]Lab. Mol. Pharmacol., IBCCF, UFRJ, Brazil 21944; [2]Dept. Pharmacol. Exp. Ther., Univ. Maryland Sch. Med., Baltimore, MD 21201; [3]Inst. Physiol. Chem., Johannes-Gutenberg Univ. Mainz, Med. Sch., Duesbergweg, D-6500, Mainz, Germany.

It has been shown that pyrazole, 4-methylpyrazole (4MP), and dipyrone increase significantly the binding of perhydrohistrionicotoxin and do not affect the binding of α-bungarotoxin (α-BGT) to *Torpedo* nicotinic acetylcholine receptors (nAChRs) (*Abs. Neurosci. Soc.*, 18:1510, 1992), suggesting that these pyrazoles could activate the nAChR via a site different from that for ACh. Recently, a novel agonist site was described on *Torpedo*, muscle, and neuronal nAChRs. The nAChR activation via this site is not blocked by competitive nicotinic antagonists, can be detected even after the nAChR has been desensitized by high concentrations of ACh, and is blocked by the nAChR-specific mAB FK1 (*FEBS Lett.*, 279:216, 1991; *J. Rec. Res.*, 13:413, 1993). In the present work we investigated the actions on muscle nAChRs of these pyrazoles, as well as of some polyamines and amantadine. Nicotinic, single-channel currents were studied in frog (*Leptodactylus ocellatus*) interosseal muscle fibers under cell-attached patch-clamp configuration. All compounds (1-100 μM) activated single-channel currents. 4-MP was the most potent compound among the pyrazoles, and putrescine was more potent than spermidine. The channels activated by these drugs had higher noise levels during the open state than channels activated by ACh. The activation of the noisier channels usually began 2-3 min after formation of the GΩ seal, and was facilitated by membrane hyperpolarization. The channel activity induced by all the drugs was not blocked by α-BGT and was seen even in the presence of high, desensitizing concentrations of ACh. FK1 (1:1000) completely abolished the agonist response of those substances, and did not affect that of ACh. The results revealed that these agents activate the muscle nAChR via the newly described agonist site, and raise the questions whether the polyamines might play a physiological role as modulators of nAChR activation and whether interaction of amantadine with the new nAChR agonist site could account for the therapeutic efficacy of this drug. Supp: FINEP/UMAB Mol. Pharm. Training Program, CNPq.

631.20

TEMPERATURE DEPENDENCY OF LIGAND BINDING TO THE RAT BRAIN NICOTINIC RECEPTORS. R.J.Prince, K.G.Fernandes* and P.M.Lippiello. Duke University Medical Center, Durham, NC 27710 and Research & Development, R.J.Reynolds Tobacco Co., Winston-Salem, NC 27102

The thermodynamic properties of ligand binding to the neuronal nicotinic acetylcholine receptor were examined using a filtration assay. The affinity of L-[^{3}H]-nicotine in rat cortical membranes decreased from 1.72± 0.16nM (5) at 4°C to 2.47± 0.29nM (5) at 23°C ($P<0.1$) and 3.40± 0.18nM (4) at 37°C ($P<0.001$) (mean ± SEM (n)). A van't Hoff plot of these data yielded values of 14.79± 2.94 kJ.mol^{-1} for $\Delta H°$ and 114.7± 10.1 J.K^{-1}mol^{-1} for $\Delta S°$ indicating a large entropic component in the binding reaction. In contrast, previous studies have shown other ligand-gated ion channel ligands such as the benzodiazepines have largely enthalpy-driven binding reactions. An affinity-shift similar to that of nicotine was noted for carbamycholine (Ki 185.6 ±4.9nM (3) at 4°C; 294.2± 9.2nM (3) at 37°C). The rigid nicotinic-agonist, cytisine, however, showed a much smaller decrease in affinity between 4°C (Ki= 0.53± 0.004nM (3)) and 37°C (Ki=0.69± 0.07nM (3)). In contrast, tetramethylammonium showed an increase in affinity at 37°C (Ki=471.5± 46.4nM (4)) compared with 4°C (Ki=650.7± 38nM (4)) while decamethonium showed no significant change in its Ki (4560± 293nM (4) at 4°C; 4350± 668nM (4) at 37°C). These differences may be due to these ligands possessing only the positively charged centre of the classical nicotinic pharmacophore. Our results further suggest that receptor protein conformational changes and deviations of some ligands from the ideal pharmacophore geometry both contribute to the binding process.

GABA RECEPTORS: FUNCTION—NEUROSTEROIDS

632.1

CHRONIC PENTOBARBITAL TREATMENT PRODUCE UNCOUPLING OF THE GABA-BENZODIAZEPINE RECEPTOR COMPLEX IN MAMMALIAN CORTICAL NEURONS. R. Yu* and M.K. Ticku, University of texas Health Science Center, San Antonio, TX 78284-7764.

We have investigated the effect of chronic pentobarbital treatment on the binding of [^{3}H]flunitrazepam and its coupling to various sites associated with the $GABA_A$ receptors in cultured cortical neurons. Chronic treatment with pentobarbital sodium failed to alter the basal [^{3}H]flunitrazepam binding to the intact cortical neurons. Studies of enhancement of [^{3}H]flunitrazepam binding by various modulators showed that while the EC_{50} value of GABA, pentobarbital sodium and 5α-pregnane-3α-ol-20-one (5α3α) were unaltered, their E_{MAX} value decreased after chronic pentobarbital sodium treatment (200 μM for 5 days). The E_{MAX} value of GABA decreased from 120% to 28%; while the EC_{50} value was not altered by the chronic treatment (12 μM v/s 10μM). The E_{MAX} value of pentobarbital sodium decreased from 155% to 75%; while the EC_{50} value was not altered (210 μM v/s 200 μM). The E_{MAX} value of 5α3α decreased from 60% to 45%; while the EC_{50} value was not altered (100 nM v/s 80 nM). Using western blot analysis it was found that after chronic pentobarbital treatment (200 μM; 5 days) there was a change in the amount of the α subunit subtypes: α1 increased 122%; α2 increased 36%; and α3 decreased 39%. Taken together, these preliminary results indicate that there is a functional uncoupling of allosteric sites of GABA receptor complex after chronic pentobarbital treatment. This uncoupling appears to be heterologous in nature. The mechanism of this uncoupling appears to be a switch in the assembly of the subunits, resulting in decreased coupling. (Supported by NIH-NINDS grant # NS-15339)

632.2

CHRONIC BENZODIAZEPINE AGONIST TREATMENT PRODUCES UNCOUPLING OF THE $GABA_A$-BENZODIAZEPINE RECEPTOR IONOPHORE COMPLEX IN CORTICAL NEURONS. Xian-Jue Hu* and Maharaj K. Ticku. Dept. of Pharmacology, University of Texas Health Science Center, San Antonio, TX 78284-7764

We have investigated the effect of chronic flurazepam HCl treatment on the $GABA_A$ receptor complex in cultured mammalian cortical neurons. Chronic flurazepam (1-5 μM) treatment (1-10 days) did not produce any changes in the morphological appearance or the cell protein content of the cortical neurons. The basal [^{3}H]flunitrazepam binding was also not altered following the chronic treatment. However, chronic flurazepam treatment produced uncoupling between GABA and pentobarbital sites with the [^{3}H]flunitrazepam binding site. The EC_{50} values of GABA and pentobarbital were not significantly altered following the chronic flurazepam treatment; however, their E_{max} values were decreased by ~50%. Chronic flurazepam treatment did not alter the effect of GABA on ^{36}Cl-influx; however, it significantly attenuated the effects of diazepam and inverse agonist (DMCM) on GABA-induced ^{36}Cl-influx. Taken together, these results suggest that chronic benzodiazepine treatment produces uncoupling of GABA and pentobarbital sites with the benzodiazepine site and between benzodiazepine site and GABA receptor gated Cl^- channels. This decreased coupling may be responsible for the tolerance associated with chronic benzodiazepine treatment.

632.3

REGIONAL COMPARISON OF THE EFFECTS OF FOUR NATURALLY OCCURRING PREGNANE DIOLS ON THE $GABA_A$ RECEPTOR COMPLEX (GRC). Linda D. McCauley* and Kelvin W. Gee. Dept of Pharmacology, University of California, Irvine, CA 92717.

It is well known that the progesterone metabolite 5α-pregnan-3α,20α-diol (5α,20α) is a potent modulator of the GRC. The current studies were initiated to compare 5α,20α with the three other naturally occurring pregnane diols 5β-pregnan-3α,20β-diol (5β,20β), 5α-pregnan-3α,20β-diol (5α,20β) and 5β-pregnan-3α,20α-diol (5β,20α), all derivatives of progesterone. Each diol was evaluated for its ability to inhibit [^{35}S]*t*-butylbicyclophosphorothionate ([^{35}S]TBPS) binding and allosterically enhance [^{3}H]Flunitrazepam ([^{3}H]FLU) binding in rat brain homogenates. The rank order of efficacy for the inhibition of [^{35}S]TBPS binding in the presence of GABA in cortex, thalamus and cerebellum was 5α,20α < 5β,20β < 5α,20β ≤ 5β,20α. 5α,20α was quite potent in modulating [^{35}S]TBPS binding (IC_{50} < 100 nM) while the other three diols had IC_{50}'s in the high nanomolar range. In the absence of GABA, 5α,20α lost efficacy while the remaining diols had potencies in the micromolar range. In the cortex, 5α,20α enhanced [^{3}H]FLU binding minimally, whereas the effect of 5β,20β was similiar to 5α,20β but less than that of 5β,20α. In the thalamus and cerebellum, again 5α,20α showed little efficacy, but the other three diols had similiar efficacies. Generally, the EC_{50} for the enhancement of [^{3}H]FLU binding was highest for 5α,20β and lowest for 5α,20α. These studies underscore the importance of stereochemical configuration at positions 5 and 20 in determining neurosteroid efficacy and potency. (Supported by USPHS grants NS 24645 and NS 25986 and CoCensys)

632.4

PROGESTERONE CAN REGULATE $GABA_b$ RECEPTORS IN THE CEREBRAL CORTEX. M.I. Al-Dahan, and R.H. Thalmann*, Dept. of Cell Biology and Division of Neuroscience, Baylor College of Medicine, Houston, TX 77030.

Well-washed synaptic plasma membranes were prepared from rat neocortex for filtration assays of the binding of the $GABA_b$ agonist (^{3}H)-baclofen. We found that the binding of baclofen to these membranes varied systematically as a function of the estrous cycle, with the lowest binding occurring during the estrus stage. We then replaced ovarian steroids in animals that had been ovariectomized for 3 weeks according to a standard paradigm that approximately mimics the onset of each hormone during the estrous cycle: estrogen, followed by progesterone 48 hours later, followed by sacrifice 4 hours after progesterone. An identical protocol but without estrogen or without progesterone treatment was also included. Surprisingly, progesterone-only produced as large an increase in binding as estrogen + progesterone. The increased baclofen binding following a progesterone-only treatment could be elicited by as little as 100 µg progesterone per 100 g body weight, and was evident 4 but not 1 hour following progesterone. This effect of progesterone is unlike the classic ones in which progesterone effects are dependent upon prior treatment with estrogen. Supported by National Institutes of Health grant NS21713.

632.5

NEUROMODULATORY EFFECTS OF PROGESTERONE ON ISOLATED WHOLE TURTLE CEREBELLUM. C.Y. Chan*, J. Choe, M. Kumar. Dept. Physiol., CUNY Med. Sch., 138th St., Convent Ave., New York, 10031.

Numerous recent studies have shown the novel effects of steroids on CNS neuronal membranes. We are interested in the possible neuromodulatory actions of progesterone (P) in the integrally intact circuitry of the isolated whole turtle cerebellum, which may serve as a model for other brain regions not linked to reproduction. The tissue was continuously perfused with artificial CSF solution. Synaptic responses were evoked by stimulation of parallel fibers or the ipsilateral peduncle using bipolar electrodes. These population (field potential) responses were recorded using 2 extracellular glass micropipettes inserted in the molecular and granular layers and aligned in the same vertical axes. Data were then frame averaged. Doses of bath-applied P were typically limited to below 300 nM to avoid its anesthetic effect which could be readily discerned as suppression of the presynaptic volley. Postsynaptic excitation in both layers were dose-dependently suppressed by P by up to 30%. In 5/6 experiments, the amplitudes of EPSPs (inferred from latency) was slightly reduced or unchanged. The amplitude of IPSPs especially in the granular layer was enhanced. In other experiments, there was an apparent 20% diminution of the granular layer IPSP, which we attribute to suppression of its polysynaptic activation. In yet another 4 cases, in the presence of 100 uM picrotoxin, P showed little or no reduction of the remaining EPSP. Intracellular recording from Purkinje cells showed little evidence of direct effect of P on EPSPs though some changes in rise time and input resistance was noted. Further intracellular studies are underway to isolate P effect on EPSPs and IPSPs.

632.6

EFFECTS OF PHENOBARBITAL AND 5β-PREGNAN-3α-OL-20-ONE ON THE $GABA_A$ RECEPTOR FROM CA1 PYRAMIDAL CELLS. I.I. Celentano*, and R.K.S. Wong. Dept. of Pharmacology, SUNY Health Science Center, Brooklyn, NY 11203.

Single channel analysis of the steady state GABA response reveals prolongation of open time and burst duration by barbiturates and neurosteroids, suggesting an effect on the gating mechanism (Tymann and Macdonald, J Physiol 1989, 1992). We have begun to explore the effects of GABA receptor modulators on the initial response to rapidly applied GABA. Rapid application of 1 mM GABA to outside-out patches induces both fast and slow desensitization, while lower concentrations (≤30µM) induce only slow desensitization (Neuroscience Abstr 487.9, 1992). In the present study, we examined the effects of 500µM phenobarbital (PB) and 1µM 5β-pregnan-3α-ol-20-one (5β) on the response to 3, 30 and 1000µM GABA. Patches were excised from acutely isolated pyramidal neurons of guinea pig hippocampus (Kay and Wong, J Neurosci Meth 1986). Extracellular buffer was (in mM): NaCl 145, $CaCl_2$ 1, $MgCl_2$ 1, glucose 25, Hepes 10, pH 7.4, and intracellular buffer was: Tris HCl/base 130, NaCl 3, $MgCl_2$ 4, Na_2ATP 4, EGTA 10, HEPES 10, pH 7.3 methane sulfonic acid (final Cl^- 46). Drugs were applied by a 7-barrel flow-tube capable of displacing the solution surrounding an electrode within 1 ms. The peak response to 3µM GABA was enhanced by 106±39% (n=8) by PB and 81±7% (n=3) by 5β. The peak response to 30µM GABA was unaffected by PB, consistent with an increase in affinity for GABA. In the presence of either PB or 5β, the response to 30µM GABA desensitized with only slow kinetics. Neither drug significantly affected the peak response to 1mM GABA or the rate or extent of desensitization. This may be the first time these agents have been studied on the maximum GABA response free of limitations imposed by series resistance error or slow drug application. If these modulators act on the gating mechanism, their effects seem to be overcome by high GABA. Experiments are underway to examine the effects of other modulators.

632.7

RU5135 Antagonizes Neurosteroid and Pentobarbital Modulation of [35S]TBPS Binding to $GABA_A$ Receptors. D.W. Sapp* and R.W. Olsen. Dept. Pharmacology, Brain Research Institute, UCLA, 90024.

The amidine steroid RU5135 is an androstan derivative that lacks hormonal activity, but acts as a convulsant in the rat. RU5135 is a potent inhibitor of GABA binding and of the allosteric actions of GABA at the receptor complex. We were interested to determine whether RU5135 might also act as an antagonist at the steroid binding site on the $GABA_A$-receptor complex. We reported earlier that the steroid anesthetic alphaxalone has a biphasic effect on [35S]TBPS binding in rat cortex, enhancing at low steroid concentrations (500nM) and inhibiting TBPS binding at higher steroid concentrations (> 10µM). RU5135 has no effect on the enhancement of TBPS binding by steroids, but it does block the inhibition produced by higher concentrations. With increasing RU5135 concentrations the inhibitory effect of steroids are reversed and TBPS binding is enhanced to the same level as that observed with lower steroid concentrations. Bicuculline produces the same effect as RU5135, but with a much lower potency. RU5135 also reverses pentobarbital inhibition of TBPS binding similar to the neuroactive steroids. Pregnenolone-SO_4 and dehydroepiandrosterone-SO_4 have been reported to inhibit the neuroactive steroids, but they had no effect on alphaxalone modulation of TBPS binding. Both RU5135 and bicuculline appear to act as antagonists of the inhibitory effects of the neuroactive steroids and pentobarbital, acting at an unknown allosteric site on the $GABA_A$ receptor complex.
Supported by AA07680.

632.8

MODULATION OF $GABA_A$ RECEPTORS BY STEROIDS IN POSTERIOR PITUITARY NERVE TERMINALS. S. J. Zhang* and M. B. Jackson , Dept. of Physiology, Univ. of Wisconsin, Madison, WI 53706.

Patch clamp techniques were used to investigate the modulation of the $GABA_A$ receptor by alphaxalone (3α-hydroxy, 5α-pregnane, 11,20-dione), allopregnanolone (3α-hydroxy-5α-pregnan-20-one) and estradiol-17β (E2) in the rat posterior pituitary. Both alphaxalone and allopregnanolone strongly enhanced GABA activated Cl^- current in nerve terminals. Application of GABA (40 µM) and alphaxalone (5 µM) produces responses 2.5 times (n = 10) as large as controls. Allopregnanolone (100 nM) enhanced GABA responses by a factor of 1.9 (n = 5). Estradiol-17β (100 nM) reduced GABA responses by 10%. In single channel recordings, alphaxalone (5 µM) with GABA (40 µM) increased by a factor of 1.8 (n = 4) $GABA_A$ receptor Cl^- channel activity. Allopregnanolone (100 nM) with GABA (1 µM) produced a similar effect (2.1 times as high as the controls, n = 4). However, estradiol (100 nM) with GABA (40 µM) resulted in 74% (n = 4) of the single channel activity of the controls. The rate of desensitization of the $GABA_A$ receptor in nerve terminals was not altered by the steroids tested (n = 8). These results demonstrated modulation of the $GABA_A$ receptor of peptidergic nerve terminals by steroids at both the whole-cell and single-channel level. This finding can explain the reciprocal relation between circulating oxytocin and allopregnanolone. The identification of this previously unknown link between steroids and the neurohypophysis has broad implications for endocrine function at the system level.

632.9

SIMULATION OF SPIKE PROPAGATION BLOCK BY GABA IN POSTERIOR PITUITARY NERVE TERMINALS. Meyer B. Jackson* and Shuanglin J. Zhang, Dept. of Physiology, University of Wisconsin, Madison, WI 53706.

We have recently shown that activation of $GABA_A$ receptors in the posterior pituitary depolarizes nerve terminals and blocks action potentials evoked by local current injection (Zhang and Jackson, Science 258, 531-534, 1993). In order to establish the relevance of this result to the active spread of excitation through nerve terminal arborizations, we used the computer program NODUS to simulate action potential propagation in a realistic model of the posterior pituitary. Morphological features for this simulation were based on cable analysis and neurobiotin filling data. Sodium and potassium channel parameters were based on patch clamp experiments in both the whole-cell and outside-out patch configuration. Action potential propagation was then simulated in an axon with a single large spherical swelling in the middle. With only active sodium and potassium currents, simulations showed that action potentials initiated at one end of the axon propagate successfully through the swelling. We then introduced a chloride conductance comparable in magnitude to that activated by GABA in this preparation. The chloride conductance was added only to the swelling membrane, but this resulted in failure of action potentials to pass through the swelling. Propagation was blocked. This simulation strengthens the argument that GABA acts at nerve terminal membranes to prevent the active spread of excitation.

632.10

INHIBITION OF 5α REDUCTASE SUPPRESSES PROGESTERONE INDUCED ANESTHESIA BY BLOCKING THE ACCUMULATION OF BRAIN ALLOPREGNANOLONE. A. Korneyev*, A. Guidotti, E. Costa. Fidia-Georgetown Institute for Neurosciences, Georgetown Univ Med Sch, Washington, DC 20007.

Administration of high doses of progesterone induces sedation and anaesthesia in human and rat. Presumably this phenomenon is related to the conversion of progesterone into allopregnanolone (3α,5α tetrahydroprogesterone) a high affinity positive modulat or of GABA induced Cl^- current. We present direct evidence in favor of this hypothesis by demonstrating that inhibition of 5α reductase, the enzyme which participates in the conversion of progesterone to allopregnanolone, with 17β-(N,N-diisopropylcarbamoyl)androst-3,5-diene-3-carboxylic acid (IN) kindly provided by SmithKline Beecham, suppresses both the accumulation of allopregnanolone in the brain and the occurrence of anaesthesia following the progesterone perfusion in rat. Freely moving adrenalectomized/castrated rats were perfused at a rate of 0.3 ml/min with progesterone (6 mg/ml) solution. In the first experiment perfusion was terminated at the time of the loss of righting reflex, which was 5.9±1.1 min in control rats and 11.2±1.4 in rats treated with 50 mg/kg of IN 1 h before the experiment. In the second experiment, in order to evaluate brain steroid content rats were perfused for 7 min (independently of the loss of righting reflex) and sacrificed 20 min later. In the forebrain of control rats levels of progesterone and allopregnanolone were 150±25 μM and 7.5±2.1 μM, respectively. In the forebrain of IN treated animals, the level of progesterone was 230±49 μM and that of allopregnanolone was 0.8±0.3 μM. Interestingly, administration of IN did not change the latency of the loss of righting reflex following the perfusion with allopregnanolone nor its brain content. In conclusion IN can be used as a pharmacological tool in the study of neurosteroid functions. Supported in part by grant RO1 MH49486-01.

632.11

PROGESTIN METABOLITES, EFFECTIVE AT THE GABA RECEPTOR, ALTER PAIN SENSITIVITY IN FEMALE RATS. J. E. Duncan* and C.A. Frye. Dept. of Psychology, Bates College, Lewiston, ME 04240.

To investigate whether progestin metabolites alter pain sensitivity in a manner consistent with their efficacy at the GABA receptor complex (GBR), seven progestin metabolites were administered SC, via ICV implantation, and then ICV infusion. Listed from most to least efficacious at the GBR, administered metabolites were THP [5α-pregnan-3α-ol-20-one], THDOC [5α-pregnan-3α, 21-diol-20-one], P [4-pregnen-3,20-dione], DHP [5α-pregnan-3, 20-dione] ,17-OH-P [17α-hydroxy-progesterone], DHEAS [5-androsten-3β-ol-17-one sulfate], and PS [5-pregnen- 3β-ol-20-one sulfate]. Pain sensitivity was measured using the tailflick apparatus at 0, 5, 20, 40, 60, 80, 100, and 120 minutes post SC steroid administration of 0.0, 0.1, 0.2, 1.6, 3.2, or 6.4 mg/kg. Although peripheral administration of potent to moderate agonists of the GBR (i.e. THP, THDOC, P, and DHP) significantly elevated tailflick latencies above baseline, changes in nociception were inconsistent across dose and time. Findings from both ICV implantation and ICV infusion (0.0, 0.5, 1.0, 2.0 μg/rat) of the progestins were clearer. Potent to moderate agonists of the GBR, i.e. THP, THDOC, P, and DHP, all significantly increased tail flick latencies above baseline, whereas 17-OH-P did not. Furthermore, the GABA receptor antagonists, DHEAS and PS, did not significantly elevate tailflick latencies compared to controls. Changes in pain sensitivity occurred within 0-5 minutes post ICV administration of potent to moderate agonists of the GBR. These immediate differences were not due to stress effects given that corticosterone levels were not elevated in any of the groups. These findings indicate that progestins may alter pain sensitivity via an interaction with the GBR.

632.12

ESTRUS CYCLE AND SENSITIVITY TO CONVULSANTS AND THE ANTICONVULSANT EFFECT OF 3α-HYDROXY-5α-PREGNAN-20-ONE (3α,5α-P). D.A. Finn*, R. Ostrom and K.W. Gee. Dept. Pharmacology, Univ. of Calif., Irvine, CA 92717.

Recent work in our laboratory suggests that sensitivity of the $GABA_A$ receptor complex (GRC) to the progesterone metabolite 3α,5α-P may change to maintain homeostatic regulation of brain excitability. Therefore, the current studies were conducted to evaluate estrus cycle-related differences in sensitivity to convulsants and the anticonvulsant effect of 3α,5α-P. The threshold dose for onset to first myoclonic jerk, generalized and tonic convulsions was measured by constant infusion of (+)bicuculline, picrotoxin, pentylenetetrazol, strychnine and methyl-6,7-dimethoxy-4-ethyl-β-carboline-3-carboxylate (DMCM). Females in estrus were more sensitive than females in diestrus 1 or males to (+)bicuculline and DMCM. Administration of 3α,5α-P (15 mg/kg in β-cyclodextrin, i.p.) 15 min prior to infusion of pentylenetetrazol significantly increased the threshold dose for onset to all three convulsions and provided equal protection against tonic convulsions. The dose for onset to first myoclonic jerk was significantly higher in females in diestrus 1 than females in estrus or males. Blood levels of 3α,5α-P are currently being evaluated. These results suggest that there is a selective interaction between convulsant drugs specific for the GRC and estrus cycle-related changes in neurosteroid levels and potency. [USPHS grants NS24645 and NS25986 (KWG) and NRSA fellowship NS09264 (DAF) and CoCensys]

632.13

IMMORTALIZED GnRH-SECRETING NEURONS ARE SENSITIVE TO $GABA_A$ RECEPTOR AGONISTS. M. El-Etr, R.J. Fiddes, Y. Akwa, M. Amalric°*, P. Robel and E.E. Baulieu. Lab. Hormones, 94276 Bicêtre Cedex and °Lab. Neurobiol. Fonc., BP71, 13402 Marseille Cedex, France.

A variety of neuromodulators appears to be involved in the control of GnRH secretion: among these, GABA has been reported to stimulate GnRH release by rat arcuate nuclei-median eminence fragments through $GABA_A$ receptors ($GABA_A$-R) (Masotto et al., *Endocrinology*, 125:548-553, 1989). An immortalized hypothalamic GnRH-secreting neuronal cell line has recently been developed by genetically targeted tumorogenesis (Mellon et al., *Neuron*, 5:1-10, 1990). We detected saturable (^3H)-muscimol binding on GT1-1 cell membranes (460 ± 40 fmol/mg of protein at 50 nM) indicating the presence of $GABA_A$-R. Using Northern and Western blot analyses, we are characterizing the subunits expressed by these cells. Some steroids, such as 3α,5α-tetrahydro-progesterone (3α,5α-THP), modulate $GABA_A$-R and we are testing their effect on the binding of (^3H)-muscimol to GT1-1 cell membranes. Progesterone has been shown to facilitate GnRH release, but since no classical intracellular progesterone receptor has been detected in hypothalamic GnRH neurons, the regulatory effect of progesterone could imply nuclear receptors in connecting neurons. However, the action of progesterone might also be exerted on GnRH neurons through its 3α,5α-THP metabolite via the $GABA_A$-R and we are currently investigating this intriguing possibility. It is also worth noting that we found GT1-1 cell cultures synthesize 3α,5α-THP from progesterone.

633.1

POTENTIATION OF $GABA_A$ RECEPTOR-MEDIATED CURRENTS BY HALOGENATED BUTANES CORRELATES WITH THEIR ABILITIES TO INDUCE GENERAL ANESTHESIA. S.J. Mihic*, P.J. Whiting and R.A. Harris. Dept. Pharmacology, Univ. Colorado Sch. Med., Denver VAMC and Merck Sharp and Dohme Res. Lab., U.K.

The Meyer-Overton rule correlating a compound's anesthetic potency with its lipid:water partition coefficient is one of the cornerstones of modern anesthetic theory. Recently, a number of chlorinated/fluorinated butane compounds have been synthesized, some of which deviate from the Meyer-Overton rule. We tested three of these compounds (one anesthetic and two non-anesthetic in vivo) for their abilities to potentiate $GABA_A$ responses in Xenopus oocytes expressing $\alpha_1\beta_2$ and $\alpha_1\beta_2\gamma_{2S}$ $GABA_A$ receptor constructs. The anesthetic 1-chloro-1,2,2-trifluorocyclobutane (F3) strongly potentiated chloride currents produced by 5 μM GABA. This potentiation was greater in oocytes expressing the $\alpha_1\beta_2\gamma_{2S}$ subunits (300%) than in eggs expressing just the $\alpha_1\beta_2$ subunits (150%). In both constructs, the percent potentiation produced by F3 decreased as the GABA concentration was raised. The two non-anesthetics, 1,2-dichlorohexafluorocyclobutane and 2,3-chlorooctafluorobutane, had no effects on $GABA_A$ currents, at any concentrations tested. Even though all three of these agents are predicted to be anesthetics by the Meyer-Overton rule, only F3 behaved as an anesthetic in vivo, and only F3 potentiated $GABA_A$ responses in oocytes. These results further support the involvement of the $GABA_A$ receptor in general anesthesia. Support by VA, NIAAA and the Medical Research Council of Canada (to SJM).

633.2

GABA RECEPTOR ACTIVATION OF CUTANEOUS NOCICEPTIVE AFFERENTS B. Ault* and L. M. Hildebrand, Dept. Neurosciences, Sterling Winthrop Pharmaceuticals Research Division, Rensselaer, NY 12144.

Algogens such as capsaicin, bradykinin, acetylcholine and potassium ions applied to exposed tail skin in the rat isolated spinal cord-tail preparation evoke a nociceptive ventral root response consisting of depolarization and spiking activity. Glutamate and kainate, which depolarize C-fibers, also evoke a similar reflex. GABA has now been examined because it is known to depolarize unmyelinated afferent fibers.

GABA superfused over exposed tail skin (for 3 sec) evoked a ventral root reflex comparable to that produced by capsaicin (3 μM). The EC50 was 37±9 μM, n=12. The $GABA_A$ receptor agonists muscimol and isoguvacine had comparable effects to GABA, with EC50s of 13±7 μM and 64±20 μM respectively. The $GABA_B$ agonist baclofen (100 μM) and glycine (10 mM) had no effect. Bicuculline applied to the tail antagonized GABA (Schild slope = -1.0) with a pA2 of 5.8. Spinal application of 1 μM morphine blocked the actions of GABA and capsaicin, and was reversed by naloxone. These data indicate that $GABA_A$ receptors can activate nociceptive afferents, and thus could be involved in nociception.

633.3

THE GENERAL ANESTHETIC PROPOFOL SELECTIVELY ENHANCES $GABA_A$ RECEPTOR-MEDIATED RESPONSES FROM BOTH HIPPOCAMPAL AND CEREBRAL CORTICAL NEURONS IN RAT BRAIN SLICES. W.R. Proctor*, J.M. Brundege, E. Sanna and T.V. Dunwiddie, University of Colorado Health Sciences Center, and Veterans Admin. Medical Res. Service, Denver, CO.

We have examined the effects of the general anesthetic, propofol, on synaptically evoked responses in pyramidal cells located in the CA1 region of the hippocampus and in the frontal cerebral cortex from young adult rats. Initial studies showed very potent and irreversible inhibitory effects on EPSPs and on the resting membrane potential (RMP) of CA1 hippocampal neurons using conventional intracellular electrophysiological recordings. Further analysis showed that these effects were due to small concentrations of a breakdown product in fresh, commercially obtained propofol. Following fractional distillation, purified propofol (40 μM) added to the superfusing artificial CSF media produced no significant changes in RMP, membrane input resistance (R_{in}), EPSP amplitude, or the late IPSP ($GABA_B$) amplitude in both hippocampal CA1 neurons and cortical neurons (level IV-V). However, significant potentiation (20-100%) of the $GABA_A$ receptor-mediated chloride conductance was seen in both brain regions during application of 40 μM propofol. This result is comparable to what we have previously observed with other general anesthetics (e.g., pentobarbital and etomidate), but stands in contrast to what we have observed with ethanol, which enhances synaptically evoked $GABA_A$ mediated response in cortical neurons, but not in CA1 hippocampal neurons.

Supported by AA03527 and VA Medical Research Service.

633.4

THE VOLATILE ANESTHETICS ENFLURANE AND HALOTHANE POTENTIATE MUSCIMOL-STIMULATED $^{36}Cl^-$ EFFLUX RATE

B. Longoni *, G.C. Demontis and R.W. Olsen. Dept. of Pharmacology, School of Medicine, University of California, Los Angeles, CA 90024.

The volatile anesthetic halothane increases $^{36}Cl^-$ efflux rate in rat brain cortical slices. Halothane also increases the $GABA_A$ receptor binding affinity for ^3H-muscimol in rat brain membranes (Longoni et al. 1993 JPET in press). To assess the possibility that the $GABA_A$ receptor represents a common target for different volatile anesthetic, we studied the effect of enflurane on $^{36}Cl^-$ efflux rate in rat brain cortical slices. Like halothane, enflurane in a clinical useful range (0.46 - 4 mM) potentiates the muscimol stimulated efflux rate: moreover the effect was stronger for 2 μM muscimol than for 10 μM an almost saturating concentration. Enflurane increases monotonically the $^{36}Cl^-$ efflux rate in the absence of exogeneous $GABA_A$ agonist, at variance with the biphasic dose-effect relashionship found for halothane. Our data suggest that different volatile anesthetics share a common molecular target, namely the $GABA_A$ receptor, in their mechanism of action.

Supported by NIH Grant AA07680.

633.5

$GABA_A$ Receptor Cl^- Current Potentiation Correlates with General Anesthetic Potency. S.A. Zimmerman, M.V.Jones[1], S.M.Minaglia and N.L. Harrison*. Depts of Anesth & Crit Care and [1]Pharm & Phys Sci, University of Chicago, Chicago, Il 60637

The molecular mechanisms of general anesthetics (GAs) are unknown. We studied the effects of the GAs chloroform, enflurane, halothane, isoflurane, methohexital, pentobarbital, propofol, trichloroethanol, and urethane at known concentrations on $GABA_A$ receptor function in cultured rat hippocampal neurons. Recordings were made at 25°C using the whole-cell patch clamp technique, with an intracellular solution based on K gluconate and externally perfused with HEPES-buffered saline. GABA was applied to individual neurons by brief pressure pulses from a micropipette containing 10 μM GABA positioned near the cell body. Neurons were held at -40mV under voltage clamp. Application of GABA elicited transient outward currents that reversed in polarity around -70 mV. Anesthetic solutions were applied via the perfusion line and volatile anesthetic concentrations were determined by gas chromotography. Transient current responses to brief pulse applications of GABA were prolonged by the general anesthetic agents. Time to half decay ($T_{1/2}$) of the GABA response was measured under control and anesthetic conditions. All the GAs studied increased $T_{1/2}$ in a concentration dependent manner. A plot of the increase in $T_{1/2}$ vs log[Anesthetic] was constructed for each GAs. These plots were fit using an exponentially increasing function. The concentration of each GAs which increased $T_{1/2}$ for the GABA current by 50%, C_{50}, was interpolated from this function. C_{50} was then plotted against the published clinically effective concentration (EC_{50}) on a log-log plot for the entire range of GAs studied. The log-log plot of C_{50} against EC_{50} was fit with the linear equation: Y=1.45X+1.05, r=0.994. Concentrations of GAs which potentiate $GABA_A$ receptor current are positively and linearly correlated with clinical anesthetic concentrations. This correlation spans at least four orders of potency. This result suggests the $GABA_A$ receptor as a possible site of anesthetic action.

633.6

MODULATION OF $GABA_A$ RECEPTOR CHANNEL KINETICS BY THE VOLATILE ANESTHETIC ENFLURANE. M.V. Jones*, R.E. Twyman° and N.L. Harrison†; Depts of *Pharm & Phys Sci, †Anesth & Crit Care, Univ of Chicago, Chicago, IL 60637 and °Human Mol Biol & Gen, Univ of Utah, Salt Lake City, UT 84112

The volatile anesthetic enflurane (ENF) induces EEG seizure activity[1], neuronal spike firing[2] and modulates $GABA_A$ receptor-mediated inhibitory postsynaptic currents (IPSCs) in a complex manner.[3] We have examined ENF's effects on the kinetics of single chloride channels activated by GABA. Hippocampal neuron culture[4], single channel[5] and anesthetic measurement[4] techniques were similar to those previously described. Outside-out membrane patches were voltage-clamped at -80 mV. 1 μM GABA and 460 μM ENF (1 MAC), were bath applied. GABA activated 28 pS channel currents (97% of openings). ENF appeared to produce brief closures within openings, reducing mean open duration and causing an increase in openings and closings within bursts. The average current per second was reduced by ENF. Open durations were best fitted[5] by three exponential components (time constant (relative area)) (0.7 (0.68), 2.8 (0.22) and 6.9 (0.10) ms). ENF altered this distribution to favor fewer components and briefer durations (1.14 (0.99) and 4.39 (0.01) ms). The closed time distribution (0.50 (0.32), 5.8 (0.16), 64 (0.5) and 577 (0.02) ms) was also shifted by ENF to favor briefer events (0.31 (0.64), 2.98 (0.18), 41 (0.14) and 242 (0.04) ms). Removal of ENF reversed these effects. One possible explanation of these results is that ENF acts as an open channel blocker. Many drugs which reduce total $GABA_A$ receptor channel current also produce seizures *in vivo*. Analysis of ENF's effects on burst and cluster structure may help to reconcile these observations with the observed amplitude depression and prolongation of the IPSC[3]. 1) Prog Drug Res 26:225-228,1982; 2) Br J Anaesth 62:301-310,1989; 3) Soc Neurosci Abstr 17:102.2,1991; 4) J Physiol 449:279-293,1992; 5) J Physiol 456:215-245,1992, Supported by GM45129 (NLH) and T32GM07151 (MVJ).

634.1

Molecular characterization of cDNAs encoding an NPY-like molecule and a NPY receptor-like receptor from the CNS of the mollusc *Lymnaea stagnalis*. E. Vreugdenhil*[1], H. van Heerikhuizen[1], K. Cox[2], J. F. Burke[2], E. van Kesteren[1], R. J. Planta[1] and C. P. Tensen[1] [1]Department of Biochemistry and Mol.Biology, Vrije Universiteit, de Boelelaan 1083, 1081 HV Amsterdam The Netherlands. [2]Sussex Centre for Neuroscience, IRC, School of Biological Sciences, University of Sussex, Brighton BN1 9QG, UK.

We have cloned a cDNA encoding an NPY-like precursor from a lymnaean cDNA CNS library by a degenerate PCR strategy. The primary structure of the predicted *Lymnaea* NPY is very similar with that of *Aplysia* NPY. Although the overall homology with the human NPY precursor is low (25%) the structure of the lymnaean precursor is similar in having a signal peptide, a NPY-like peptide that is flanked by a classical Lys-Arg processing site and a carboxy terminal peptide. Interestingly, like *Aplysia* NPY, the carboxy terminal end of *Lymnaea* is having structural elements in common with the well-characterized invertebrate neuropeptide FMRFa.; this might indicate that *Lymnaea* NPY and FMRFa influence similar target cells.

To determine whether NPY receptor-like receptors are present in the CNS of *Lymnaea* we have used a similar PCR strategy to clone several cDNAs that putatively encode neuropeptide receptors. One of these cDNA clones (GRL106) exhibits a high amount of sequence identity with the human NPY receptor and also contain specific secondary structural features that are also found in its vertebrate counterpart. Therefore, this predicted protein is a serious candidate to be the cognate receptor for the lymnaean NPY or, alternatively, lymnaean FMRFa.

In situ hybridisation experiments showed that *Lymnaea* NPY is expressed in many neurons that are scattered througout the CNS of *Lymnaea*. In contrast, GRL106 is specifically expressed in the multidisciplinary studied caudo-dorsal cells (CDCs), which are giant peptidergic neurons controlling egg laying and associated behaviour and which are strongly inhibited by FMRFa. Whether GRL106 is activated by *Lymnaea* NPY and/or FMRFa. has to await the functional expression of this receptor clone.

634.2

ISOLATION OF FOUR NOVEL HUMAN PEPTIDE G-PROTEIN COUPLED RECEPTORS. T. Nguyen, A. Marchese, P.H. Wu, H. Ma, H. Heng, L-C. Tsui, X. Shi, S.R George and B.F. O'Dowd*. Addiction Research Foundation and Depts. Medicine and Pharmacology, University of Toronto, and Research Institute, Hospital for Sick Children, Toronto, Ont, CANADA.

The cloning of the first opioid receptor (OR) has recently been reported [Evans et al, Science 258:1952-1955 (1992)], and this mouse delta OR has identity with the somatostatin receptors and other peptide G protein-coupled receptors. We planned experiments to search for the human delta OR gene and other opiate receptor genes in the human genome and prepared two degenerate oligonucleotides based on the nucleotide sequence encoding the third and seventh transmembrane regions of the opioid and somatostatin receptors. The gene structure for the OR has not yet been reported, however, many of the G protein coupled receptors are encoded on single exons. Thus we used the oligonucleotides to amplify human genomic DNA and also human hippocampus cDNA library. The amplified DNA's in the size range 500 to 1000bp were subcloned into Bluescript and 150 of the resulting clones were sequenced. None of the clones encoded the orthologue of the mouse delta OR, however three clones, #11, #12 and #14 obtained from the amplification of the genomic DNA shared some identity with the delta OR and somatostatin receptor genes. We have screened a human genomic library and the full length genes are intronless in their coding regions. Clone 12 has been mapped to chromosome 10, at q11.2-21.1. Clone 33, isolated from the hippocampus cDNA library, and not found in the genomic amplification, also shared identity to the delta OR and somatostatin receptor genes. Northern blot analysis, using clone 33 as a probe, has revealed hybridization signals in rat striatum, cortex, hippocampus and cerebellum.

634.3

CLONING OF HUMAN KAPPA OPIATE RECEPTOR cDNA J. Nystrom*, H.Kong, K. Yasuda, G. Bell and T. Reisine, Dept. Pharmacol., Univ. Pennsylvania, Philadelphia, PA 19104 and HHMI, Univ. Chicago, Chicago, Ill 60637.

Dynorphin induces its biological actions by interacting with kappa opiate receptors. Kappa receptors are one of three opiate receptor subtypes. Agonists at this receptor can induce psychotomimetic effects in humans, as well as analgesia. We recently cloned a mouse kappa opiate receptor cDNA. To investigate the properties of the human kappa receptor, a human genomic library was screened with the mouse kappa receptor cDNA. The human kappa receptor gene was identified. Since the gene has introns, the mouse receptor cDNA was used to screen both human temporal lobe and hippocampal cDNA libraries to isolate human kappa receptor cDNA. Following initial screening, nine positive plaques were identified and isolated for further purification. Presently we are characterizing the inserts to identify a human kappa receptor cDNA that could be used in subsequent expression studies. These studies will be important to establish that the cDNA encodes the kappa receptor and to determine whether the human and mouse receptors have similar properties. Supported by MH45533 and MH48518.

634.4

DESENSITIZATION OF THE SOMATOSTATIN RECEPTOR SSTR2 INVOLVES BETA-ADRENERGIC RECEPTOR KINASE (BARK) J. Hines*, M. Theveniau, J. Benovic, and T. Reisine Dept. Pharmacol. Univ. Pennsylvania and Jefferson Medical Cent., Philadelphia, PA 19104.

Somatostatin (SRIF) induces it's biological actions by interacting with a family of receptors. One of the recently cloned subtypes, SSTR2 selectively binds the peptide MK 678. Continuous exposure of this receptor to agonists reduces the ability of the receptor to be labeled by ^{125}I-MK 678. This regulation of agonist binding to the receptor is dependent on the time and concentration of agonist treatment and is reversible. To investigate the molecular mechanisms involved in this regulation, we developed a peptide-directed antisera against SSTR2. The antisera detects similar levels of SSTR2 from control and agonist treated CHO cells by immunoblotting, indicating that the reduced agonist binding is not due to receptor down-regulation. For a number of receptors, desensitization involves kinase catalyzed phosphorylation of the receptor. To determine whether SSTR2 becomes phosphorylated during agonist treatment, CHO cells expressing SSTR2 were preloaded with ^{32}P-orthophosphate, stimulated with agonist, solubilized and SSTR2 immunoprecipitated with the peptide directed antisera. These studies showed that agonist pretreatment of SSTR2 induced the phosphorylation of the receptor. Agonist induced phosphorylation and regulation of SSTR2 is not likely to involve cAMP or Ca++ dependent kinases since SRIF inhibits these kinases and agents that activate these kinases, such as forskolin, PMA and calcium ionophore do not affect agonist binding to SSTR2. However, the second messenger independent kinase BARK, may be involved in SSTR2 regulation since in preliminary studies, a BARK dominant negative mutant partially blocked agonist induced regulation of SSTR2. Studies are in progress to test the role of BARK in agonist-induced regulation and phosphorylation of SSTR2. Supported by MH45533.

634.5

IMMUNOLOGICAL DETECTION OF MULTIPLE FORMS OF THE SOMATOSTATIN RECEPTOR SUBTYPE SSTR2 M. Theveniau*, K. Raynor and T. Reisine Dept. Pharmacology, Univ. of Pennsylvania, Philadelphia, PA 19104.

Five somatostatin (SRIF) receptors have recently been cloned. One subtype, SSTR2, has unique pharmacological properties and may selectively mediate SRIF inhibition of growth hormone release. To investigate it's physical properties, we developed two peptide directed antisera against SSTR2. One antisera, 2e3, specifically detects 93 kDa proteins in CHO cells stably expressing cloned SSTR2. This is a similar size as the affinity labeled SSTR2 from these cells. 2e3 also immunoprecipitates 93 kDa proteins from these CHO cells. A second antisera, 2i4, specifically immunoprecipitates high affinity SRIF binding sites from these CHO cells. These studies and others show that 2e3 and 2i4 specifically interact with SSTR2. 2e3 detects proteins of 148 kDa in rat brain and both 2e3 and 2i4 specifically immunoprecipitate high affinity SRIF binding sites solublized from rat brain, indicating that the 148 kDa protein is likely to be SSTR2. 2e3 also detects 148 kDa proteins from AR42J and 293 cells which express SSTR2. To determine whether the differences in size of the cloned SSTR2 and SSTR2 expressed in AR42J cells is due to processing, mRNA was extracted from CHO and AR42J cells, translated and the radiolabeled products immunoprecipitated with 2e3. Similar proteins of 41 kDa were immunoprecipitated. Since this is the predicted size of unprocessed SSTR2, these findings indicating that the size differences of SSTR2 detected in CHO cells versus AR42J cells are due to differential processing of SSTR2. These antisera will be useful in further investigating the nature of the differential processing of SSTR2 as well as determining other physical properties of this receptor. Supported by MH45533 and MH48518.

634.6

DEVELOPMENT OF SELECTIVE AGONISTS AT THE CLONED SOMATOSTATIN RECEPTORS SSTR2, SSTR3 AND SSTR4 T. Reisine*, W. Murphy, A-M. O'Carroll, D. Coy, J. Taylor, H. Kong, K. Yasuda and K. Raynor Dept. Pharmacol., Univ. Pennsylvania, Philadelphia, PA, 19104; LCB, NIMH, Bethesda MD, 20892; Dept. Medicine, Tulane Univ. New Orleans, LA, 70112; Biomeasures Inc. Hopkinton, MA 01748 and HHMI, Univ. Chicago, Chicago, Ill 60637

Somatostatin (SRIF) induces its biological actions by interacting with membrane associated receptors. Five SRIF receptors have recently been cloned. To develop ligands selective for the receptor subtypes that can be used to investigate their individual functions, the five cloned receptors were expressed in either CHO or COS cells and tested for their affinities for a large number of SRIF analogs. SSTR2 could be specifically labeled by the cyclic peptides BIM23027 and NC4-28B having over 1000-fold selectivity for these compounds. SSTR3 could be specifically labeled by the linear peptide BIM23056 and SSTR4 selectively interacted with the linear peptide BIM23052 and the cyclic peptide L362855. No peptides were identified that selectively interacted with SSTR1 or SSTR5. This is the first observation of very high affinity binding of linear peptides to SRIF receptors. Correlational analysis of the affinity of the receptors for these different peptides and the potencies of the compounds to inhibit growth hormone release indicates that SSTR2 selectively mediates the regulation of growth hormone secretion by SRIF. These subtype selective peptides will allow for the determination of the specific functions of the SRIF receptor subtypes. Development of these subtype selective peptides as radioligands will also be useful in detecting the expression of the individual SRIF receptors in brain, the endocrine system and in SRIF responsive tumors. Supported by MH45533 and MH48518.

634.7

Giα1 SELECTIVELY COUPLES THE SOMATOSTATIN RECEPTOR SUBTYPE SSTR3 TO ADENYLYL CYCLASE. Susan F. Law*, Silvio Zaina, Raymond Sweet, Kazuki Yasuda, Graeme I. Bell, Jeffrey Stadel and Terry Reisine. Dept. of Pharm., U. of Pennsylvania, Phila., Pa. 19104; SmithKline Beecham Pharm., King of Prussia, Pa. 19406; U. of Chicago, Chicago, Ill. 60637.

Three Somatostatin (SRIF) Receptors have recently been cloned and only one, SSTR3, appears to functionally couple to adenylyl cyclase. However, SRIF does not inhibit cAMP formation in CHO cells stably expressing SSTR3 and Giα2 or Giα3 but lacking Giα1. In contrast, SRIF does inhibit forskolin stimulated cAMP formation in CHO cells stably expressing SSTR3 and Giα1, indicating that Giα1 selectively couples SSTR3 to adenylyl cyclase. To investigate the functional domains of Giα1 necessary for interaction with SSTR3, a chimeric alpha subunit (Giα2/Giα1) was constructed consisting of the amino-terminal two thirds of Giα2 ligated to the carboxy-terminal third of Giα1. SRIF inhibited cAMP formation in cells expressing SSTR3 and the Giα2/Giα1 chimera. In contrast, a similar Giα2/Giα3 chimera did not couple SSTR3 to adenylyl cyclase further indicating that Giα3 does not contribute to SRIF inhibition of adenylyl cyclase activity. These findings demonstrate that Giα1 selectively couples SSTR3 to adenylyl cyclase and indicates that the carboxy-terminal region of this alpha subunit is involved in mediating SRIF inhibition of adenylyl cyclase activity. Supported by MH45533 and MH48518.

634.8

IDENTIFICATION OF G-PROTEIN COUPLED RECEPTORS IN A LOCUS COERULEUS-LIKE CELL LINE BY PCR. M.E. Charlton*, J.D. Alvaro, Nestler, E.J. and R.S. Duman. Laboratory of Molecular Psychiatry, Depts. of Psychiatry and Pharmacology, Yale University School of Medicine , New Haven ,CT 06508.

In recent years, studies in our laboratory have examined the molecular mechanisms underlying the actions of psychotropic drugs on locus coeruleus (LC), the major noradrenergic nucleus in brain. A unique *in vitro* model for the study of this system is a transformed murine cell line derived from a brainstem tumor which is LC-like (Suri *et al.*, J. Neuroscience 13, 1280, 1993). This cell line has been demonstrated, through regulation of adenylate cyclase activity, to express specific G-protein coupled receptors including the VIP, CRF, $GABA_B$, α_2 adrenergic, NPY, and opiate. We have used PCR and degenerate primers derived from conserved regions of three different subfamilies to isolate novel G-protein coupled receptors. Messenger RNA isolated from the cells was reverse transcribed, second strand cDNA was synthesized, and cDNA fragments which encode putative receptor amino acid sequences were PCR amplified. As visualized on agarose gels, each primer set produced PCR fragment(s) of the approximate size predicted for that receptor subfamily. One set of primers which has been extensively used (Libert, *et al.*, Science 244, 569, 1989) resulted in PCR amplification of 2 major bands which were subsequently subcloned, sequenced, and determined to encode a previously published receptor (adenosine) as well as a putative MSH receptor isolated from LC (see Alvaro et al., Soc. Neuro. Abst. 1993). Primers based on the opiate and secretin receptor subfamilies each resulted in amplification of a single major band. Further studies are in progress to identify and characterize full length clones encompassing these sequences.

634.9

ISOLATION OF MELANOCORTIN RECEPTOR cDNAs FROM LOCUS COERULEUS AND VENTRAL TEGMENTUM BY PCR. J.D. Alvaro*, E.J. Nestler, and R.S. Duman. Interdepartmental Neuroscience Program, Laboratory of Molecular Psychiatry, Depts. of Psychiatry and Pharmacology, Yale Univ. School Med.,New Haven, CT 06508.

Previously we described the use of the PCR technique to amplify G-protein coupled receptor cDNAs from bovine locus coeruleus (LC) and rat ventral tegmentum (VT) cDNA (Soc. Neurosci. 18, Abstract 193.12). Of the putatively novel receptor fragments that were subcloned and sequenced, two have since been identified. Both of these clones share significant amino acid identity with each other and are homologous to the newly discovered family of melanocortin receptors. The PCR clone isolated from the VT is identical to the recently published MSH-3 receptor. The LC PCR clone may correspond to the as yet unpublished MSH-4 receptor. Rat and human brain cDNA libraries have been screened with the LC clone, and several positive clones have been identified. Northern blot analysis of bovine poly (A)+ mRNA from various brain regions reveals that the LC clone detects two distinct messages between 4.7kb and 5.3kb in length. Levels of the smaller message are highest in the dorsal raphe and pons whereas the larger message is most abundant in pons and cortex. The sequence and pharmacological properties of this LC MSH receptor are currently being investigated.

634.10

ISOLATION OF VERTEBRATE CLONES ENCODING THE NPY/PYY Y1 RECEPTOR - IMPLICATIONS FOR MUTAGENESIS OF THE HUMAN Y1 RECEPTOR. A. G. Blomqvist*, E. Roubost, G. Martenst, I. Lundell, C. Wahlestedt# & D. Larhammar. Dept of Medical Genetics, Box 589, S-751 23 Uppsala, Sweden, †Department of Animal Physiology, Nijmegen, The Netherlands, #Cornell Univ. Med. Coll., New York, USA.

Our recent cloning of a human NPY/PYY receptor of the Y1 subtype (Larhammar et al., J. Biol. Chem. 267, 10935-10938, 1992) allows detailed studies of the receptor's structure-function relationships by site-directed mutagenesis. We have used computer modelling to identify several potentially interesting amino acid positions whose importance will be tested by functional studies after transfection of the mutated clone into mammalian cells. Another way of identifying such positions is by comparison of Y1 receptor sequences from different animal species. The sequences of human, rat and mouse Y1 receptors display only a few amino acid replacements and seem to respond identically to most or all NPY-related peptides. Therefore, we decided to isolate Y1 clones from more distantly related vertebrate species, among others the frog *Xenopus laevis* and the zebrafish *(Brachydanio rerio)*.

DNA clones from both species have been isolated and are now in the process of being subcloned and sequenced. Primary sequence information indicates about 60-70% homology between the frog and human NPY/PYY receptor Y1. The sequence will provide useful information about conserved amino acid residues that may be important in, for example, ligand interactions, second-messenger coupling, and overall tertiary structure. Amino acid replacements in the zebrafish Y1 receptor may be possible to correlate with replacements in the zebrafish NPY peptide. We have previously shown that the NPY sequence of the closely related goldfish differs at five positions from the human sequence (Blomqvist et al., PNAS 89, 2350-2354). Also zebrafish NPY clones are being characterized.

634.11

CLONING AND EXPRESSION OF NPY AND PYY IN THE RIVER LAMPREY. C. Söderberg*, V. A. Pieribone[1], J. Dahlstrand[2], L. Brodin[2], and D. Larhammar. Dept of Medical Genetics, Uppsala Univ., Box 589, S-751 23 Uppsala, Sweden, 1) Rockefeller Univ., New York, NY, USA, 2) The Karolinska Institute, Stockholm, Sweden.

The evolutionary relationships of the members of the NPY family have been unclear despite sequence information from many vertebrates. We have recently shown that NPY has remained extremely well conserved during vertebrate evolution (Blomqvist et al., PNAS 89, 2350-2354).

We present here two NPY-related cDNA clones from the river lamprey(*Lampetra fluviatilis*) isolated by low-stringency hybridizations with a shark NPY probe. The first clone corresponds to NPY as it has 83% identity to human NPY. *In situ* hybridizations detected mRNA in neurons of the dorsal spinal cord, lateral brainstem and retina. The sequence of the second clone is equally similar to NPY and PYY (61% identical to human NPY). Anatomically, it corresponds to PYY as its mRNA is found in gut cells and in a reticulospinal neuron system.

These results show that the gene duplication leading to NPY and PYY had already occurred in the common ancestor of lampreys and teleost fishes more than 450 million years ago. The anatomical similarities of these peptide systems between lamprey and rat (Pieribone et al., J. Neuroscience 12(9), 3361-3371) suggest that their functions have been strongly conserved during vertebrate evolution.

We have also isolated candidate clones for the lamprey NPY receptor Y1. They were isolated by low stringency hybridization with a rat Y1 probe to a lamprey genomic library. Partial sequencing of one clone has revealed features characteristic for 7TM receptors.

634.12

ZEBRAFISH 7TM RECEPTOR CLONES ISOLATED WITH A RAT NPY RECEPTOR PROBE. I. Lundell, Anders G. Blomqvist, and D. Larhammar*. Department of Medical Genetics, Uppsala University, Box 589, S-751 23 Uppsala, Sweden.

The G-protein-copuled receptors with seven TM (transmembrane) regions are encoded by a gene family consisting of several hundred members. The neuropeptide Y (NPY) receptors belong to this family and include at least three different subtypes called Y1, Y2, and Y3. Recently, we have isolated clones encoding the Y1 receptor subtype from rat, human, and Xenopus laevis. As we have previously isolated clones for NPY from the zebrafish, we also wished to isolate NPY receptor clones from this species to allow correlation of ligand and receptor expression in studies of tissue distribution and developmental regulation.

PCR was used to generate an NPY receptor probe corresponding to almost the entire coding region of a rat Y1 cDNA clone. The probe was labelled with P-32 and used to screen a zebrafish genomic library (kindly provided by A. Fjose, Bergen, Norway) under conditions of low stringency. Hybridization was in 6X SSC at 42°C, and final washes in 2X SSC and 42°C. Sixteen clones were isolated and their cross-hybridizing regions transferred to Bluescript II KS+ for cDNA sequencing. Analysis of the first clone revealed that it encodes a 7TM receptor with similarity to several mammalian receptors for peptide ligands. Its seventh TM region appears to be a mosaic of several other receptors why its exact identity has not yet been possible to ascertain. Sequence analyses of this as well as the other receptor candidate clones will be presented.

634.13

MOLECULAR CLONING OF A G-PROTEIN COUPLED RECEPTOR WITH HIGH HOMOLOGY TO THE DELTA-OPIATE RECEPTOR. Jean E. Lachowicz*, Yong Shen, Frederick J. Monsma, Jr. and David R. Sibley, Molecular Neuropharmacology Section, NINDS, NIH, Bethesda, MD 20892.

We have used the polymerase chain reaction technique to selectively identify G protein-coupled receptor cDNA sequences from rat brain mRNA. A partial length cDNA fragment of a novel receptor was amplified using degenerate primers derived from conserved sequences found in the third and sixth transmembrane domains of previously cloned receptors. This fragment was found to exhibit high homology to a number of peptide receptors. In order to isolate a full-length clone, a rat cerebral cortex cDNA library was screened using the randomly primed PCR fragment as a probe. A 2.4 kb cDNA clone was isolated which encodes a 367 amino acid protein containing seven putative transmembrane spanning domains. Within these regions, the closest homology was found to the δ-opiate receptor (64%). Other features which are shared with the δ-opiate receptor are two glycosylation sites in the amino terminus, a cAMP-dependent protein kinase phosphorylation site in the third cytoplasmic loop, an aspartic acid residue in the second transmembrane domain, which was previously thought to be found only in biogenic amine receptors, and a palmitoylation site in the intracellular carboxyl terminus. The receptor is also homologous with members of the somatostatin receptor family (48% with SSTR-1 in the transmembrane regions). Despite the high degree of homology to these peptide receptors, radiolabeled somatostatin-14, somatostatin-28, and opiate ligands such as naloxone, naltrindole, diprenorphine, CTOP, U-69593, ethylketocyclazocine, and bremazocine fail to bind to membranes from transfected COS-7 or CHO-K1 cells. Northern blot analysis reveals 3.2 kb and 8.2 kb transcripts in the hippocampus, hypothalamus, prefrontal and cerebral cortices. No transcript was observed in the cerebellum, medulla, striatum, olfactory bulb and spinal cord. The identity of this novel receptor subtype is currently being explored using a variety of expression strategies.

634.14

MOLECULAR CLONING OF A NOVEL G-PROTEIN COUPLED RECEPTOR FROM RAT. J.K. Harrison*, C.M. Barber, and K.R. Lynch, Dept. of Pharmacology, Univ. of Virginia Health Sci. Center, Charlottesville, VA 22908.

G-protein coupled receptors (GCRs) comprise a large gene superfamily whose encoded proteins are characterized by the presence of seven transmembrane spanning regions. Low stringency hybridization (using a radiolabelled cDNA encoding the rat angiotensin (AT1) receptor) was used to isolate a novel member of the rhodopsin family of GCRs from a rat brainstem cDNA library. This cDNA (RaBS11) was used subsequently to isolate cDNAs from other rat brainstem, pituitary, and spinal cord cDNA libraries. From these cDNAs an open reading frame (orf) was identified that encodes a protein of 354 amino acids (40 kDa). The orf does not contain sites for N-linked glycosylation (N-X-S/T). A search of the Genbank (release #73) revealed that RaBS11 was most similar to chemokine receptors for MIP-1α and IL-8 (45% and 37% identical, respectively) identified previously. Northern analysis demonstrates that RaBS11 mRNA is widely and unevenly distributed in rat tissues. RaBS11 mRNA was most prominent in the rat spinal cord, brain (where it is evenly distributed over 8 discrete regions), testes, uterus, kidney, and gut. Southern analysis of restriction-digested rat genomic DNA indicated the gene for RaBS11 is single copy in the rat. We speculate that RaBS11 encodes a receptor for a chemokine. (Supported by PO1 HL19242 and F32 HL08223)

634.15

IDENTIFICATION OF A PARTIAL cDNA CLONE OF THE INTERLEUKIN-2 RECEPTOR-β IN NORMAL MOUSE BRAIN

JM Petitto[1], Z Huang[1], K Suzuki*[2]. [1]Department of Psychiatry, University of Florida, Gainesville, FL, 32610, [2]Brain and Development Research Center, University of North Carolina, Chapel Hill, NC, 27599.

Interleukin-2 (IL-2), the prototypical cytokine, has been implicated in important CNS processes including glial cell differentiation, and more recently, as a neuromodulator. Although considerable attention has been given to the molecular biology of *lymphocyte* IL-2 receptors, little is known about the molecular pharmacology of IL-2 receptors in *brain*. Because it is essential to intracellular signal transduction, the leukocyte IL-2 receptor-β (IL-2Rβ) subunit has been the focus of much interest. In the immune system, the IL-2Rβ subunit is constituitively expressed (without the IL-2α accessory subunit), forming a high affinity receptor for IL-2 (referred to as the "intermediate" affinity IL-2 receptor). We sought to determine whether the IL-2Rβ gene is expressed in normal mouse brain, and to compare the sequence of IL-2Rβ from brain with that from immune tissue. Using PCR, we amplified a partial IL-2Rβ cDNA fragment (ca. 350 bp) from mouse brain. Brain cDNA was subcloned, and three independent clones were isolated and sequenced. Sequences for all three clones share identical nucleotide sequences with the corresponding sequence of the extracellular domain of the mouse lymphocyte IL-2Rβ. Using the same strategy, several clones obtained from two neuroblastoma cell lines are currently being sequenced. To our knowledge, these studies are the first to demonstrate IL-2Rβ gene expression in normal brain tissue. In situ hybridization studies shall be important to determine the brain regional and cell-specific localization of the IL-2Rβ receptor subtype in the CNS.

634.16

MOLECULAR CLONING OF A NOVEL TRANSMEMBRANE SERINE KINASE FROM RAT BRAIN.

K.Tsuchida and W.W.Vale*. The Clayton Foundation Laboratories For Peptide Biology, The Salk Institute, La Jolla, CA 92037.

Activin and other members of the transforming growth factor-β(TGF-β)/superfamily are involved in the regulation of many biological functions including neuronal modulation and development. This gene family includes the TGF-βs, inhibins, activins, the drosophila *dpp* protein, bone morphogenetic proteins and the Vg-related proteins. The activin and subsequently the TGF-β type II receptors were cloned and characterized and found to be putative transmembrane serine/threonine kinases. In order to identify additional members of the activin/TGF-β receptor superfamily, we designed degenerate primers based on the conserved kinase domains of type II receptors for activin and TGF-β for polymerase chain reaction (PCR). We have undertaken RT-PCR using the RNAs from rat brain and pituitary gland, and succeeded in obtaining a novel fragment which has the serine/threonine kinase motif. Subsequently, we isolated a full length clone from rat brain cDNA library. The predicted protein consists of 509 amino acids with a cysteine-rich extracellular domain, a single transmembrane domain and an intracellular kinase domain. The kinase domain shows 39% and 36% homologies to activin and TGF-β type II receptors, respectively. The single transcript of 3.7 kbs was detected in rat brain and pituitary. Functional characterization of the structurally related receptors should provide a basis for exploring the roles of these receptors in signal transduction and in the regulation of development and differentiated functions of various systems.

634.17

IDENTIFICATION OF RESIDUES OF THE HUMAN NEUROPEPTIDE Y RECEPTOR (Y1) IMPORTANT FOR LIGAND BINDING. Ph. Walker*, R. Martinez and M. Munoz . Division of Hypertension, CHUV, CH-1011 Lausanne Switzerland

Neuropeptide Y (NPY) is one of the most abundant peptide found in the mammalian brain. By activating specific G protein-coupled receptors, NPY is a mediator of diverse physiological responses. In the cardiovascular system, NPY exerts a number of important regulatory actions. NPY acts as a potent vasoconstricor on certain blood vessels. In addition, administered at non-pressor doses, NPY can strikingly potentiate the action of a number of vasopressive substances such a norepinephrine or angiotensin II. The aim of our work is to understand the molecular basis of the interactions between neuropeptide Y (NPY) and the human Y1 receptor. To work towards this goal, we first designed a transient expression system allowing a rapid testing of various mutants. In this assay, Hela cells are first infected with vaccinia virus and subsequently transfected with an expression vector in which expression of the Y1 cDNA is driven by a vaccinia gene promoter (11K late promoter). Vaccinia specific transcription factors transactivate the 11 K promoter resulting in the rapid synthesis of 1.5 to 3 x 10^6 binding sites per transfected cell with a Kd of about 2 nM. Using site-directed mutagenesis we generated a collection of single point mutants at candidate residues and tested these clones following transient expression into Hela cells. We will describe the effect of various amino acid substitutions on the ability of the mutants to bind NPY. We could identify single point mutations that abolish any ligand binding activity. We have now tagged the receptor with a foreign epitope and are currently using antibodies to assess whether the mutant receptors are expressed at the cell surface.

634.18

MUTATIONS IN THE THIRD CYTOSOLIC LOOP OF THE RAT NEUROTENSIN RECEPTOR: EFFECTS ON PEPTIDE BINDING AND SECOND MESSENGER RESPONSE. M. Watson, M. Yamada, Mi. Yamada, and E. Richelson*. Mayo Clinic and Foundation, Jacksonville, Fl 32224

The neurotensin receptor belongs to the super-family of seven transmembrane, G-protein coupled receptors. Studies of receptors from this family show that the third cytosolic (C3) loop contains sites for G-protein binding. Among these receptors, the C3 loop is not well conserved, making each receptor unique in its second messenger response. We made deletion mutations of the C3 loop of the rat neurotensin receptor in an effort to identify regions important to G-protein binding. Previous studies in our laboratory with the rat neurotensin receptor transfected into CHO-K1 cells show that it mediates cAMP formation and phosphoinositide (PI) hydrolysis. Initial investigations showed that deletion of all but the nine N-terminal and eight C-terminal amino acids of the C3 loop results in neurotensin binding with the same affinity (K_d=3 nM) as found in the transfected wild-type receptor for membrane preparations. In addition, receptor-mediated cAMP response was unchanged (EC_{50}=30 nM). Binding in whole cells and PI hydrolysis was undetectable. However, preliminary results with another mutant construct having only five additional C-terminal amino acids showed whole cell binding, but no PI hydrolysis. These results suggest that the site for cAMP response resides in the N- or C-terminal ends of the C3 loop, or another internal domain. The data also suggest that at least part of the site for the G-protein binding responsible for PI hydrolysis is found in the 26 amino acids which were deleted. Further mutational analysis is being conducted to determine specific amino acids that may comprise the actual binding sites. (Supported by Mayo Foundation and USPHS grant MH27692)

634.19

CONFORMATIONAL STUDIES OF THE SEVENTH TRANSMEMBRANE DOMAIN OF THE DOPAMINE D_2 RECEPTOR. Valerie L. Williams*, Scott H. Courtney, David I. Schuster, and Randall B. Murphy, Department of Chemistry and Center for Neural Science, New York University, New York, NY 10003.

Highly hydrophobic peptides in small unilamellar vesicles is a commonly used model for membrane-embedded receptor proteins. However, the insolubility of these hydrophobic peptides presents many obstacles in determining the critical physical parameters of this type of model system. Using a novel reverse-phase-HPLC methodology, we have a synthesized and purified the following sequence: NH_2-D-V-L-Y-S-A-F-T-W-L-G-Y-V-N-S-A-V-N-P-I-I-Y-T-T-F-N-V-COOH. This sequence represents the seventh transmembrane domain of the dopamine D_2 receptor, a membrane-embedded protein. Fluorescence spectroscopy was used as a tool in determining the microenvironment of the peptide in synthetic lipid vesicles. Studies of the peptide in its most denatured state were used as a starting point in determining the conformational changes induced by increasing the lipid/peptide ratio. The results of these studies yield information on the conformational differences of the dopamine D_2 receptor in its interaction with ligands and suggest that this peptide can be used as a biophysical model for the dopamine D_2 receptor system. (Supported by NSF and New York University).

PEPTIDES: RECEPTORS V

635.1

LOCALIZATION OF mRNAs OF ENDOTHELIN RECEPTORS IN THE RAT BRAIN. J. Zhu*, S. Fitzpatrick-McElligott, J.K.DeReil, and L.-Y. Liu-Chen. Dept. of Pharmacology and Fels Institute, Temple Univ. Sch. of Medicine, Philadelphia, PA, and E.I. DuPont & Co., Wilmington, DE.

Endothelins (ETs) are a family of vasoactive peptides. Intracerebroventricular administration of ETs causes profound vasoconstriction and hypertension. Specific binding sites for [^{125}I] ET-1 have been identified in the brain. Recently two types of ET receptors have been cloned and named ETa and ETb receptors based on differential affinities for ETs. In this study, we examined the distribution of both ETa receptor (ETaR) and ETb receptor (ETbR) at mRNA levels in the rat brain by *in situ* hybridization histochemistry. mRNAs coding for the ETaR or ETbR were detected by using antisense oligonucleotides probe (30-mer) labeled at 3'-end with ^{35}S by terminal transferase. The probes correspond to 1270-1299 of ETaR and 188-217 of ETbR sequences. Frozen rat brains were sectioned (20 μm) and sections were fixed with 4% paraformaldhyde, prehybridized for one hour and hybridized with one of the probes overnight at 37°C. The posthybridization washing was 2 x SSC at room temperature and 0.5 x SSC at 45°C, each for one hour. Sections were exposed to Hyperfilms for 2-4 days, and then undergone emulsion dipping for localization at the cellular level. High levels of both ETaR and ETbR mRNAs were found in the cerebellum, hippocampus, piriform cortex, anterior dorsal thalamic nucleus, medial habenular nucleus, supraoptic nucleus, median eminence, arcuate hypothalamic nucleus, red nuclei, pontine nuclei, oculomotor nuclei, mesencephalic trigeminal nucleus, motor trigeminal nucleus, locus coeruleus, trapezoid body, facial nuclei, nucleus of solitary tract and hypoglossal nucleus. In some regions, there were differential distribution of ETaR and ETbR mRNAs. Olfactory tubercle expressed high level of ETaR mRNA, yet low level of ETbR mRNA. In contrast, ventromedial hypothalamic nucleus had high density of ETbR mRNA, yet low density of ETaR mRNA. These results suggest that ETs may play important roles in the central nervous system, particularly in the regulation of cardiovascular and motor functions.

635.2

CHARACTERIZATION OF THE PROXIMAL PROMOTER REGION OF THE RAT SOMATOSTATIN RECEPTOR GENE, SSTR4. Yun Xu, Jinfen Song, Michael Berelowitz* and John F. Bruno. Div. Endocrinology and Metabolism, SUNY at Stony Brook, NY 11794

Primer extension and solution hybridization nuclease protection analysis were used to determine the transcriptional initiation site(s) of the rat somatostatin receptor gene, SSTR4. Four major transcriptional start sites have been identified and are located 22, 72, 123, and 125 bp upstream of the translational initiation site. Sequence analysis of the proximal promoter region of rSSTR4 gene revealed this region lacks TATA and CCAAT boxes. Moreover, the promoter region was found to be highly GC rich containing several potential SP1 binding sites. These are characteristics typically found in promoter regions of 'housekeeping' genes which are not highly regulated. In addition, the promoter region included a consensus octamer binding motif (ATGCAAAT) which binds a group of related transcription factors that are believed to regulate genes that specify organ development and cell phenotypes.

635.3

CNS EXPRESSION AND ISOLATION OF A PROMOTER FOR THE RAT VASOPRESSIN V1a RECEPTOR GENE P. Szot*, T.L. Bale, A.M. Kalliomaki, M.W. Hamblin and D.M. Dorsa. GRECC, Seattle VAMC, WA 98108 and Dept. of Pharmacology, Univ. Washington, Seattle, WA 98195.

Vasopressin binding sites with the pharmacologic properties of V1a receptors (V1aR) have previously been demonstrated in various regions of the rat brain including the septum/bed nucleus of the stria terminalis, hippocampus, amygdala and hindbrain. The location and identity of the cells responsible for the synthesis of the receptor protein are as yet undetermined. Based on the recently reported sequence of a cDNA encoding the vasopressin V1aR, in situ hybridization using an oligonucleotide "cocktail" was performed on coronal sections throughout the rat brain. The "cocktail" consisted of 3 oligonucleotides complementary to nucleotides 61-109, 573-621 and 810-858 of the vasopressin V1aR mRNA which were end-labeled with ^{33}P-dATP. Specific hybridization signal was observed in the internal granular layer of the olfactory nucleus, islands of Calleja, piriform cortex, superchiasmatic nucleus, denate gyrus, paraventricular hypothalamic nucleus, ventromedial hypothalamic nucleus, medial habenular nucleus, and the molecular and granular cell layers of the cerebellum. The cerebellum, olfactory nucleus and dentate gyrus appeared to be the most intensely labeled areas. In situ hybridization using these probes was also performed on peripheral tissues known to express vasopressin receptors, and the most intense labeling was observed in the liver. To verify the specificty of labeling observed, in situ hybridization using a ^{33}P-cRNA probe complementary to nucleotides 31-177 of the V1aR mRNA was performed on a set of alternate brain slices. The anatomical distribution of hybridization signal using this riboprobe was similar to that obtained with the oligonucleotides.

We have also isolated a genomic clone from a rat testis library which contains the 5'-upstream region of the V1aR gene. The nucleotide sequence of this clone has suggested regulation by several transacting factors, and includes at least two hormone response elements. (Support by the VA Research Service and NS 20311)

635.4

THE GENE STRUCTURE OF THE MOUSE GONADOTROPIN RELEASING HORMONE RECEPTOR W. Zhou*[1], S.C.Sealfon[1,2] [1],Fishberg Center for Reserch in Neurobiology and [2], Dept. of Neurology, Mount Sinai School of Medicine, New York, NY 10029.

We previously isolated the functional mouse gonadotropin releasing hormone receptor (GnRHR) cDNA (Tsutsumi, Zhou, et al Mol. Endocrinol. 6, P.1163, 1992) as well as variant receptor transcripts (Zhou et al, Soc. Neurosci. Abstr. V.18, Part 1, P.451, 1992). The primary structure of the receptor protein revealed that GnRHR is a member of the G protein coupled receptor (GPCR) family with putative 7 transmembrane helices (TMHs). All the variants conserved the first 3 TMHs but ended with distinct carboxyl termini. None of the variant transcripts was capable of encoding complete 7 TMHs. To elucidate the gene structure of GnRH receptor, the mouse gene was cloned. Our results showed that the coding region of the mouse GnRHR gene was composed of three short exons. ExonI covered the first 522 base pairs (bp) of the coding sequence and terminated in the middle of TM4, followed by an intron gap of >10 kilobases. ExonII was 217 bp in length and continued the coding region through TM5. ExonIII, following an intron of about 5 kilobases, contained the 3' coding region. The variant transcript cDNA sequences are generated by alternative splicing of the mouse GnRH receptor. This work is funded by NSF91-06877.

635.5

5' FLANKING DNA SEQUENCE DIRECTS TISSUE-SPECIFIC EXPRESSION OF MOUSE NPY-1 RECEPTOR. Carola Eva*, Alessandra Oberto, Rita Musso, Silvana Ricci Gamalero and Enrico Genazzani. Institute of Pharmacology, University of Torino, Italy.

Using a previously identified rat cDNA clone, we isolated the murine gene for the NPY-1 receptor subtype for NPY. The 5' flanking region of the NPY-1 receptor gene contains binding sequences for variuos transcription factors, including cAMP and glucocorticoids responsive elements. A 1,300 bp genomic fragment of the 5' flanking region of the gene drives the expression of the reporter gene luciferase in NG108-15 cells and primary cultured neurons, but not in glial and human embryonic kidney cells. Deletion of sequences 5' to nucleotide -636 did not decrease the transcriptional activity of the gene in NG108-15 cells, while greater than 70% of the activity was lost when sequences between -636 and -223 were deleted. Moreover the addition of forskolin increases luciferase activity by 2 to 4 folds. These data indicate that the information between -636 and -223,which include an AP1 site and cAMP responsive elements, is essential for full transcriptional activity of the NPY-1 receptor gene and that the gene is controlled at the transcriptional level by cAMP.

635.6

ANTISENSE INHIBITION OF BRAIN AT_1 RECEPTORS REDUCES HYPERTENSION IN SHR. Robert Gyurko, Donna Wielbo and M. Ian Phillips*. Department of Physiology, University of Florida, Gainesville, FL, 32610

Angiotensin II receptors (AT_1R) are increased in localized brain regions of spontaneously hypertensive rats (SHR). Based on antisense mediated gene expression inhibition in biological systems we hypothesized that a specific antisense oligodeoxynucleotide (ODN) to AT_1 receptor mRNA in the brain would decrease the number of AT_1 receptors, thereby decreasing hypertension in the SHR model. 12 week old male SHR's were cannulated intracerebroventricularly (ICV). After recovery, baseline blood pressure was measured by tail cuff monitor. Animals were divided into three groups: Antisense (AS) ODN, and control groups; Sense (S) ODN and vehicle (C). Each group was injected ICV with 5μl (10μg/μl) of ODN or 5μl isotonic saline. Doses were administered at 12 hour intervals. Two hours after the injections blood pressure was measured. The AS ODN treated group showed significant decrease (*, $p<0.05$) in systolic blood pressure on the second and third day of the treatment when compared to pre-treatment level, whereas neither the sense ODN, nor the saline treated control group showed significant decrease during the four-day monitoring period.

	Pre-treatment	2h	26h	50h
Control	205.3 ± 5.52	209.3 ± 4.62	184 ± 13.14	186 ± 14.13
Sense ODN	93.3 ± 10.88	180 ± 10.59	176 ± 1.7	175 ± 2.67
Antisense ODN	197.5 ± 8.27	199.5 ± 8.38	154 ± 7.54*	160.5 ± 8.02*

Preliminary autoradiographic studies showed 40% decrease in AT_1 receptor binding in the AV3V region. These data suggest that intraventricular administration of specific antisense ODN attenuates AT_1 receptor gene expression, decreasing the synthesis of the receptor protein, and significantly lower blood pressure in the SHR model.

635.7

CROSS-REACTIVITY BETWEEN AMYLIN-NH_2 AND [^{125}I]hCGRPα BINDING SITES IN RAT BRAIN AND PERIPHERAL TISSUES. D. van Rossum[1]*, D. Ménard[1], A. Fournier[2], S. St-Pierre[2] and R. Quirion[1]. [1]Douglas Hosp. Res. Centre and Dept. of Pharmacol. & Ther. and Psychiatry, McGill Univ., Montréal, QC, [2]INRS-Santé, Pointe-Claire, QC, Canada.

Amylin or Islet Amyloid Polypeptide is a 37 amino acid peptide that shows ~50% sequence homology with the Calcitonin Gene-Related Peptides (CGRP). Amylin and CGRP also share several biological actions including inhibition of glucose uptake and glycogen synthesis, hypotension and anorexia. It remains to be determined if amylin acts exclusively through a unique amylin receptor class such as reported in lung membranes and/or via CGRP receptors. We have therefore investigated the cross-reactivity between amylin-NH_2 and [^{125}I]hCGRPα binding sites by performing competition experiments (1nM-1μM) on adjacent rat brain coronal sections using *in vitro* receptor autoradiography, as well as in membrane preparations and functional bioassays on the guinea pig atria ($CGRP_1$ enriched tissue) and vas deferens ($CGRP_2$ enriched tissue). [^{125}I]hCGRPα binding in the nucleus accumbens and ventral striatum was clearly more sensitive to amylin-NH_2 (IC_{50} 50 and 20nM, respectively) than all the other areas of the rat brain examined (IC_{50} >100nM). Amylin-NH_2 was ~15 times more potent in the vas deferens (0.2 μM) than in atria (2.5μM) to produce its effects. Moreover, [^{125}I]Tyr^{37}-amylin binding sites were detected in nucleus accumbens, hypothalamus, tegmental nucleus and the locus coeruleus. In summary, although revealing much lower affinity (50-100) than CGRP for [^{125}I]hCGRPα binding sites in either brain, atria or vas deferens, amylin showed highest affinity for CGRP binding sites in the nucleus accumbens and ventral striatum followed by $CGRP_2$ and $CGRP_1$ putative receptor subtypes. Supported by the MRCC.

635.8

IN VITRO AUTORADIOGRAPHIC LOCALISATION OF AMYLIN BINDING SITES IN RAT BRAIN. P. M. Sexton[1], G. Paxinos*[2], M. A. Kenney[3], and K. Beaumont[3]. [1]St. Vincent's Institute of Medical Research, Fitzroy, Victoria 3065, Australia, [2]School of Psychology, University of NSW, Kensington, NSW 2033, Australia and [3]Amylin Pharmaceuticals Inc., San Diego, CA 92121.

Amylin is a 37 amino acid peptide hormone, structurally related to calcitonin gene-related peptide, which is co-secreted from the pancreas with insulin and acts to modulate carbohydrate metabolism. Recently high affinity binding sites for ^{125}I-rat amylin have been identified in the rat central nervous system (Beaumont et al., Br. J. Pharm. 108: 241P, 1993). Analysis of these sites, in accumbens nucleus membranes, revealed highest affinity for rat amylin but also high affinity for salmon calcitonin and somewhat lower for the β-CGRPs. In the present study we have used *in vitro* autoradiography to map the distribution of ^{125}I-rat amylin binding sites in rat brain. For this purpose 12 μm cryostat sections were incubated at 22°C for 60 min with 74 pM ^{125}I-rat amylin. The sections were subsequently washed, dried and then exposed to X-ray film for 12 days. Anatomical identifications were based on comparison of the autoradiographs with the corresponding Nissl stained sections. Moderate to high levels of binding were present in mid-caudal accumbens nucleus, fundus striati and parts of the bed nucleus of the stria terminalis and substantia innominata. This binding extended caudally into parts of the amygdalostriatal transition zone and the central and medial amygdaloid nuclei. Moderately high levels of binding also occurred in much of the hypothalamus including the medial preoptic, dorsomedial hypothalamic and medial tuberal nuclei as well as the ventrolateral subnucleus of the ventromedial hypothalamic nucleus. Other regions of high level binding included the subfornical organ, the vascular organ of the lamina terminalis, area postrema, locus coeruleus, dorsal raphe and caudal parts of the nucleus of the solitary tract.The distribution of amylin binding sites supports a potential role for these sites in regulation of fluid and electrolyte homeostasis, appetite, autonomic function and mood.

635.9

IN VITRO MODULATION OF HUMAN NEUROPEPTIDE Y-Y1-RECEPTORS BY ANTISENSE OLIGONUCLEOTIDES. F. Yee*, G. Weng, D. Erlinge, C. Bjenning and C. Wahlestedt. Div. Neurobiology, Dept. Neurology and Neuroscience, Cornell University Medical College, NY, NY, 10021.

We have recently used an antisense oligodeoxynucleotide (ODN) technique to downregulate the rat neuropeptide Y (NPY)-Y1-receptor *in vitro*, i.e. cultured cortical neurons, as well as *in vivo* (Wahlestedt et al., *Science* 259: 528, 1993). Based on the nucleotide sequence of the human NPY/PYY Y1-receptor (Larhammar et al., *JBC* 267:10935, 1992; Herzog et al., *PNAS* 89:5794, 1992), we have also applied the antisense strategy to study the regulation of this human Y1-receptor, primarily in a neuroblastoma cell line, SK-N-MC, which has previously been shown to exclusively express the Y1-receptor subtype. Several 18-mer antisense ODNs, corresponding to different regions of the receptor message, were synthesized in order to determine which site(s) would be most sensitive to downregulation by the antisense ODNs. In addition, phosphorothioate analogs, which are more stable than unmodified ODNs, have also been studied *in vitro*. Mismatched oligonucleotides, where 3-4 of the 18 bases are interchanged, were used as controls. The ODNs were tested in a dose range of 0.3-30 μM. Following treatment of SK-N-MC cells with antisense ODNs directed to a region near the amino terminus of the Y1-receptor, the density of Y1 binding sites, labeled by [125I]peptide YY, was most efficaciously reduced. Using a solution hybridization assay for quantitating Y1-receptor message, we are currently investigating whether the Y1-receptor mRNA concentrations are affected by antisense treatment. Antisense oligonucleotides may represent a novel class of therapeutic agents which have greater selectivity/specificity over traditional drug therapies and this approach is particularly useful in a case like the human NPY-Y1-receptor since specific antagonists are not yet available.

635.10

ANGIOTENSIN II (AII) STIMULATION OF GLUT-1 GENE EXPRESSION IN ASTROGLIA. C. Sumners*, W. Tang, J.M. Cummings, E.M. Richards and M.K. Raizada. Dept. of Physiology, College of Medicine, Univ. of Florida, Gainesville, FL 32610.

AII elicits trophic actions in the CNS via specific AT_1 receptors located on astroglia (Rydzewski et al. in, Cellular and Molecular Biology of the Renin-Angiotensin System; eds. Raizada et al. CRC Press, 1993, pp. 485-512). Since glucose is a major nutrient required for growth, we have investigated the effects of AII on glut-1 mRNA and deoxy D-glucose (dGlc) uptake in rat astroglia in primary culture. In these cells AII (10 nM-1 μM) caused a time-dependent stimulation of [^{3}H]-dGlc uptake which was evident as early as 3 min, reached a maximum of ~140% by 4 hr, and persisted for 24 hr. This stimulation was a result of an increase in the V_{max} and was blocked by the AT_1 receptor antagonist Losartan (1 μM), but not by the AT_2 receptor blocker PD123177 (1 μM). The levels of glut-1 mRNA were measured to determine if the AII stimulation of dGlc uptake involved transcriptional regulation. Incubation of astroglia with AII (1 nM-1 μM) resulted in time and concentration-dependent increases in glut-1 mRNA levels mediated via AT_1 receptors. Contrary to its effect on [^{3}H]-dGlc uptake, the stimulation of glut-1 mRNA by AII (100 nM) was not evident until 1 hr, reached a maximum of ~60% at 4 hr and persisted for 24 hr. These data suggest that AII causes an acute increase in dGlc uptake by activation/ translocation of glucose transporters, and a longer term increase following a stimulation of glut-1 gene expression. (Supported by PHS grants NS-19441 and HL-36610).

635.11

ISOLATION AND BIOCHEMICAL CHARACTERIZATION OF TWO DISTINCT ANGIOTENSIN AT_2 RECEPTOR POPULATIONS IN MURINE NEUROBLASTOMA N1E-115 CELLS. I.R. Siemens*, L.P. Reagan, D.K. Yee, and S.J. Fluharty. Depts. of Animal Biology and Pharmacology, and Institute of Neurological Sciences, University of Pennsylvania, Phila., PA 19104.

The murine neuroblastoma N1E-115 cell line contains a high density of angiotensin Type 2 (AT_2) receptors whose density is regulated in concert with the neuronal phenotype of these cells. We have shown that CHAPS solubilizes exclusively AT_2 receptors from N1E-115 cell membranes, as evidenced by their high affinity for CGP42112A but not Losartan. In contrast, displacement of [^{125}I]-AngII with the AT_2 nonpetide antagonist PD123319 resulted in a biphasic curve suggesting heterogeneity of the AT_2 receptor population in N1E-115 cells. Heparin-sepharose chromatography efficiently separated these two AT_2 receptor populations into two distinct protein peaks termed peak I and III. Pharmacological analysis revealed that both peaks contained exclusively AT_2 receptors insofar as they exhibited high affinity for CGP42112A and little or no affinity for Losartan. However, while PD123319 completely displaced the binding of [^{125}I]-AngII from Peak I in a monophasic fashion (IC_{50} 9.1 ± 4.1 nM; mean ± SEM; n=3), PD123319 was much less effective in displacing [^{125}I]-AngII from Peak III (IC_{50} 196 ± 27; mean ± SEM; n=3). Treatment of individual peaks with the reducing agent dithiothreitol caused a large increase in [^{125}I]-AngII specific binding in peak III, whereas a decrease in binding was observed in peak I. Moreover, GTPγS significantly reduced high affinity agonist binding in peak I but not peak III further suggesting heterogeneity in the AT_2 receptor family. Finally, immunoprecipitation studies with polyclonal antisera raised against AT_2 receptors in Peak I specifically immunoprecipitated [^{125}I]-AngII binding activity in peak I but were ineffective in peak III. Collectively, these results suggest that heparin-sepharose chromatography can efficiently isolate two distinct populations of AT_2 receptors. Supported by NS23986 and MH43787.

635.12

ANGIOTENSIN TYPE 1 ANTISENSE OLIGONUCLEOTIDES INHIBIT RECEPTOR EXPRESSION AND FUNCTION IN CULTURED CELLS AND RAT BRAIN. R.R. Sakai*, X.D.Yang, P.F.He, L.Y. Ma, Y.F.Guo, J.R. Reilly, C.N. Moga, and S.J.Fluharty. Depts. of Animal Biology and Pharmacology, and Institute of Neurological Sciences, University of Pennsylvania, Philadelphia, PA 19104.

Many of the physiological, endocrine and behavioral actions of angiotensin II (AngII) are mediated by specific AT_1 receptors located in peripheral tissues and the brain. In an effort to study the regulation and function of these receptors, we have developed short AT_1 antisense oligonucleotides. In an initial series of studies, the efficacy of the antisense oligonucleotides was examined in cultured cells. Antisense oligonucleotides were incubated with liver epithelial WB or neuroblastoma N1E-115 cells for 24 hours, and AT_1 receptor density was determined by radioligand binding assay. In both cell lines, the AT_1 antisense oligonucleotide inhibited binding by 60-70%. In a second set of experiments, the antisense oligonucleotides were used to assess AT_1 receptor expression and function in conscious rats. Three daily intracerebroventricular (ICV) injections of AT_1 antisense significantly attenuated drinking elicited by a pulse ICV injection of AngII whereas random antisense oligonucleotides were ineffective. These antidipsogenic effects were dose-dependent, reversible, and specific in that water intake elicited by carbachol was unaffected. Radioligand binding analysis of selected brain regions revealed that ICV antisense decreased AT_1 receptor density by approximately 40% in hypothalamic-thalamic-septum and brainstem membrane preparations. Collectively, these results demonstrate that both *in vitro* and *in vivo* administration of antisense oligonucleotides attenuate angiotensin receptor expression and function. Supported by NS23986, MH43787 and HD 25857.

PEPTIDES: PHYSIOLOGICAL EFFECTS III

636.1

INTRACEREBROVENTRICULAR BOMBESIN INDUCES FOS PROTEIN IN THE DOPAMINE NEURONS OF THE ARCUATE NUCLEUS IN OVARIECTOMIZED RATS C.W. Coen*, A. Gbadebo, P. Strutton, R. Persaud and I. Kalló†. Biomedical Sciences, King's College, London WC2R 2LS, UK. †Anatomy Dept, Pécs, Hungary.

We have recently demonstrated that intracerebroventricular bombesin administered to ovariectomized rats suppresses pulsatile LH release, reduces core temperature and induces FOS expression in various sites including the arcuate, periventricular and preoptic regions. Since dopaminergic activation also suppresses LH pulses and induces hypothermia, the present study was undertaken to establish whether bombesin-induced FOS-immunoreactivity occurs within dopamine cells. Ovariectomized rats were fitted with an indwelling cannula in the third cerebral ventricle and an intraperitoneal temperature-sensitive radiotransmitter (Mini-Mitter Co.) at least 10 days before the experiment. In order to minimize any effects of handling, the administration of the bombesin (1μg) or vehicle was performed from outside the cage; this occurred 3 hours after the injection cannula had been inserted into the guide cannula. The animals were perfused transcardially 90 mins after the infusion, a time at which core temperature had dropped by at least 4°C in the animals given bombesin (no change in temperature was observed after the vehicle). FOS-immunoreactivity was detected using streptavidin peroxidase together with diaminobenzidine (DAB) and ammonium nickel sulfate; the subsequent detection of tyrosine hydroxylase (TH) involved PAP and DAB. The dark blue nuclear reaction product associated with FOS was readily distinguishable from the brown cytoplasmic staining for TH. Following bombesin treatment FOS-immunoreactivity was detected in the majority of the TH-positive cells within the arcuate nucleus, but in a smaller proportion of the periventricular and in very few of the preoptic TH-positive cells. FOS was not observed in the zona incerta or substantia nigra. Further studies on the possible involvement of dopamine in bombesin-induced LH pulse suppression and hypothermia are in progress.

636.2

CHROMOGRANIN A IN RAT RETINA: INHIBITION OF DOPAMINE RELEASE. D.G. Munoz* and C.J. Gibson. Department of Pathology, The University of Western Ontario, London, Ontario, Canada N6A 5C1.

Chromogranin A (CgA) is a soluble protein of secretory granules in neuroendocrine cells, and large dense-core synaptic vesicles in neurons. Proteolytic fragments of CgA inhibit calcium influx and block release of noradrenaline from adrenal medullary cells. In spite of the widespread expression of CgA in the CNS, no central actions have been documented. Using an antibody donated by H. Winkler we demonstrated expression of CgA in both inner and outer plexiform layers of rat retina. Ganglion cells and some perikarya in the inner nuclear layer were also stained. We tested the actions of CgA in an in vitro retinal perfusion system (Gibson, J. Neurosci. Methods 32:75,1990). A ten min pulse of K^+ resulted in a five-fold increase in endogenous dopamine (DA) release (from basal levels of 26 + 3 to 130 + 17 pg) and a second K^+ pulse, 50 min later, also significantly increased DA release to 85 + 13 pg. This release was calcium-dependent. Addition of 100 nM of CgA purified from human adrenal completely abolished K^+-stimulated DA release. The inhibition was dose dependent with an IC_{50} of 3nM, similar to that previously found in adrenal medullary cells (5 nM). Thus the actions of CgA in the first CNS tissue tested resemble those documented in the periphery. Supported by separate MRC grants to CJG and DGM.

636.3

ELECTROPHYSIOLOGICAL EFFECTS OF CHOLECYSTOKININ PEPTIDES ON ACUTELY ISOLATED DOPAMINERGIC NEURONS OF THE RAT MIDBRAIN

Tony. Wu*[1] and H. L. Wang. Dept. of Physiology, Chang Gung Medical College, Kwei-San, Tao-Yuan. [1] Dept. of Neurology, Chang Gung Memorial Hospital, Taipei, Taiwan.

Autoradiographic studies indicated that cholecystokinin(CCK) receptors are expresssed in the substantia nigra (SN) and ventral tegmental area (VTA) and may be located on dopaminergic cell bodies. Previous extracellular recording studies reported that sulfated CCK-8 has an excitatory effect on midbrain DA neurons. To understand ionic and molecular mechanisms by which CCK peptides regulate membrane properties of midbrain DA neurons, we are investigating electrophysiological effects of CCK peptides on acutely isolated DA neurons using whole-cell patch-clamp recording techniques.

SN and VTA neurons were acutely isolated from 3 to 4 weeks old Sprague-Dawley rats. DA neurons were identified by following electrophysiological characteristics: (1) Spontaneous action potentials with the firing rate of 4-5 Hz. (2)A broad action potential (>2 ms). (3) Prominent afterhyperpolarizations. (4) Hyperpolarization currents induce inward rectifications. Under whole-cell current-clamp recordings, sulfated CCK-8 depolarizes midbrain DA neurons and triggers action potentials when DA neurons were hyperpolarized to near their spike thresholds. During whole-cell voltage-clamp recordings, sulfated CCK-8 induces an inward current at the holding potential of -60mV. These findings are consistent with previous studies that CCK has an excitatory effect on midbrain DA neurons. Currently, we are studying (1)Pharmacological characterization of CCK effects. (2)The identity of ionic conductances regulated by CCK peptides. (3) Molecular mechanisms by which CCK peptides modulate ion channels of DA neurons.

636.4

CHOLECYSTOKININ-B RECEPTOR AGONISTS EXCITE LOCUS COERULEUS NEURONS *IN VITRO*. L.S. Reynolds* and A.H. Ganong. Department of Neuroscience, Pfizer Inc, Central Research Division, Groton, CT 06340.

Cholecystokin (CCK) is present in many brain regions including the ventral medial hypothalamus (VMH), the hippocampus and the locus coeruleus. CCK-8 is known to excite neurons in the VMH and to increase excitability in hippocampal CA1 neurons. We have investigated the effects of CCK agonists and antagonists on spontaneously active neurons found in the locus coeruleus. Extracellular single unit recordings were made in slices of guinea pig brainstem containing the locus coeruleus. Applications of CCK-8 at 10 or 100 nM resulted in reversible increases in the firing rate of locus neurons. The selective CCK-B receptor agonists, CCK-4 and pentagastrin, also induced increases in firing of locus cells. Pentagastrin produced a dose dependent increase in locus neuron firing rates with an ED_{50} of 17 nM. A three minute application of 10 nM pentagastrin caused a rapid excitation that returned to baseline when the drug was washed out. Excitations induced by 10 nM pentagastrin could be repeated with a 15 minute interval between applications. Pentagastrin-induced excitations could be antagonized dose-dependently by the selective CCK-B antagonists CI-988 and L-365,260. L-364,718, a CCK A antagonist, did not effectively block the pentagastrin-induced excitation. These data indicate the presence of CCK-B receptors on guinea pig locus coeruleus neurons which could be related to the behavioral effects of CCK-B agonists and antagonists reported in both animal and human studies.

636.5

CCK$_B$ RECEPTORS TONICALLY MODULATE A10 DOPAMINERGIC NEURONS SELECTIVELY: NEUROCHEMICAL EVALUATION OF LY288513. D. Li, R.M. Simmons, J. J. Howbert*, K. Rasmussen, and S. Iyengar. CNS Research, Eli Lilly and Company, Indianapolis, IN 46285.

The octapeptide CCK has been postulated to modulate dopaminergic function presumably via the mesolimbic A10 dopamine pathways. Recently, it has been shown that selective CCK$_B$ receptor antagonists, including LY262691, decrease the number of spontaneously active A9 and A10 dopaminergic cells [Rasmussen et al., Eur. J. Pharmacology, 209 (1991)135-138; J.P.E.T., 264 (1993) 480-488] in anesthetized rats. The effect of LY288513, the active isomer of LY262691, was evaluated in unanesthetized rats on dopamine metabolism and release in olfactory tubercles (OLT), a mesolimbic region innervated by dopaminergic cell bodies from the VTA, by GC/MS. LY288513 was found to dose dependently decrease the levels of dopamine as well as those of its metabolites dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA) and 3-methoxytyramine (3-MT) in the OLT 60 mins. after peripheral injection (i.p.). The decrease in dopamine and its metabolites closely paralleled observed decreases in spontaneous activity of A10 cells in the VTA, both in terms of onset of action and dose response, corroborating the electrophysiological observations. In comparison, the effects of LY288513 on striatal dopamine metabolism and release were very transient and did not show evidence of a strong tonic modulation of A9 neurons, consistent with the lack of activity of this antagonist on catalepsy. This neurochemical finding is the first demonstration of the selective tonic modulation of mesolimbic A10 dopaminergic neurons by CCK$_B$ receptors in drug-naive, awake, freely moving animals. More importantly, these data further support the contention that CCK$_B$ antagonists may be useful in antipsychotic and/or anti-anxiety therapy .

636.6

INTRACISTERNALLY-ADMINISTERED CHOLECYSTOKININ PREVENTS β-ENDORPHIN FROM INHIBITING BRAIN AND LIVER DNA SYNTHESIS IN 10-DAY-OLD RATS: A MODULATORY ROLE FOR ENDOGENOUS BRAIN CHOLECYSTOKININ IN THE CONTROL OF GROWTH BY β-ENDORPHIN? J. V. Bartolome*, B. A. Lorber and M. B. Bartolome. Dept. of Pharmacology, Duke University Medical Center, Durham, N.C. 27710.

We have previously reported that intracisternal (i.c.), but not subcutaneous, administration of β-endorphin (BE) suppresses DNA synthesis (an index of cell replication) throughout the body in developing rats. The effect is restricted to the first three postnatal weeks. These findings, together with other observations, led us to postulate that endogenous CNS BE may play an important role in controlling postnatal growth.

The gut peptide cholecystokinin (CCK) is now known also to be abundant in the brain, predominantly as the sulfated octapeptide (CCK-8S) of CCK. It is believed that brain CCK may function physiologically as an opioid antagonist. We found that 0.75 ug of CCK-8S co-injected with 0.25 ug of BE i.c. prevented BE from inhibiting brain and liver DNA synthesis. CCK-8S i.c. given alone had no effect on basal DNA synthesis in the brain, but produced small but consistent increases in liver. The same exact pattern of response was obtained when unsulfated CCK-8 (also naturally-occurring in the brain) was used instead of CCK-8S, suggesting the involvement of the CCK-B receptor subtype. CCK-8S given subcutaneously did not alter liver DNA synthesis, indicating that the suppression of liver DNA synthesis by i.c. CCK-8S occurs via brain-based mechanisms, and not through direct peripheral actions after leakage from the central locus of injection into systemic blood.

These results suggest that changes in the basal levels of brain CCK during early development may alter growth by modulating tissue DNA effects of BE. Accordingly, normal development may require a fine-tuned balance of the activities of both BE and CCK brain systems. (Supported by US PHS Grant NS 25738)

636.7

EFFECT OF IONIZING RADIATION ON CORTICAL, HIPPOCAMPAL, AND HYPOTHALAMIC CHOLECYSTOKININ LEVELS IN RATS. S.B. Kandasamy*, C. Turkelson* and A.H. Harris. Behavioral Sciences Department, Armed Forces Radiobiology Research Institute, Bethesda, MD 20889, *VA Medical Center, Kansas City, MO 64128.

The effects of ionizing radiation on cortical, hippocampal, and hypothalamic CCK levels "immediately after" and "3 hr after" exposure were determined. Rats were bilaterally exposed to doses from 1 to 10 Gy of ^{60}CO γ radiation. Exposure to 1 and 3 Gy of γ rays decreased hippocampal and cortical CCK levels but had no effect on hypothalamic CCK levels. Exposure to 5 Gy decreased hippocampal CCK levels but had no effect on cortical and hypothalamic CCK levels. Exposure to 10 Gy decreased hippocampal, and hypothalamic CCK levels but had no effect on cortical CCK level. There was significantly less CCK in the cortex, hippocampus, and hypothalamus of all "immediately after" groups than in the cortex, hippocampus, and hypothalamus of the corresponding "3 hr after" groups. These results demonstrate that radiation decreases CCK levels in the cortex, hippocampus, and hypothalamus.

636.8

INDUCTION OF FOS PROTO-ONCOGENE BY INTRACEREBRO-VENTRICULAR ADMINISTRATION OF TYR-W-MIF-1 (TYR-PRO-TRP-GLY-NH$_2$). **K. GERGEN, S.L. CHANG[1], A.J. KASTIN*, AND J.E. ZADINA VA Med. Center and Dept. of Medicine and Neuroscience Training Program, Tulane University Sch. of Med., New Orleans, LA 70146, and [1]Dept. of Physiology, LSU Sch. of Dentistry, New Orleans, LA 70119.**

A peptide recently isolated from brain, Tyr-W-MIF-1, has been shown to bind to mu opiate receptors, to have opiate agonist activity in the guinea pig ileum, and to induce prolonged analgesia after intracerebroventricular (ICV) injection. In this study, we explored brain areas that may be activated by Tyr-W-MIF-1. We and others have used immunocytochemical (ICC) detection of FOS proto-oncogene protein as an anatomical marker for brain sites activated by extracellular stimuli. In this study, we provide a preliminary mapping of areas in the thalamus and hypothalamus where FOS immunoreactivity is induced by ICV injection of an analgesic dose of Tyr-W-MIF-1. Guide cannulae were implanted in the lateral ventricle of anesthetized male Sprague Dawley rats (250-350 g). Ten days later, the animals received 200 μg Tyr-W-MIF-1 or the vehicle (5 μl lactated Ringer's solution). The analgesic effect of the peptide was confirmed because all 3 animals given peptide showed the maximum (15 sec) tail-flick latency for 60 min while the mean of the 3 controls did not exceed 6 sec at any time. Three hr after the injection, the animals were perfused. Coronal brain sections were stained with anti-FOS antiserum (Ab-2, Oncogene Science) by the avidin-biotin peroxidase method. FOS was activated in the preoptic area and the paraventricular and periventricular nuclei of the hypothalamus. In the thalamus, the paraventricular nucleus showed the greatest activation. The results show that Tyr-W-MIF-1 activates FOS in several diencephalic areas, including areas associated with nociception.

636.9

INTRACEREBROVENTRICULAR ADMINISTRATION OF TYR-W-MIF-1 (TYR-PRO-TRP-GLY-NH$_2$) INDUCES PROLONGED ANALGESIA IN THE RAT. **Velga Kenigs[1], James E. Zadina, Laszlo Hackler, Richard D. Olson*[1] and Abba J. Kastin Veterans Affairs Medical Center and Dept. of Medicine and Neuroscience Program, Tulane Univ. Sch. of Med. New Orleans, LA 70146 and [1]Dept. of Psychology, University of New Orleans.**

Tyr-W-MIF-1 (Tyr-Pro-Trp-Gly-NH$_2$) is a peptide recently isolated from human and bovine brain. It was shown to have opiate agonist activity in the guinea pig ileum and to bind to mu opiate receptors. In this study, Tyr-W-MIF-1 was tested for its effects on nociception in the tail-flick test after ICV injection in the rat. Guide cannulae were implanted in the right lateral ventricle of anesthetized male Sprague Dawley rats (250-350 g). At least a week was allowed for recovery. The animals then received 200 μg Tyr-W-MIF-1 in 5 μl vehicle. Tail-flick latencies, measured every 10 min, were significantly increased with a rapid onset and remained significantly elevated for at least 50 min. Naloxone injected 11 min after the peptide (1 min after the first test) reversed the effect of the peptide, indicating opiate receptor involvement in the response. Met-enkephalin at the same dose produced only slight antinociception, and only on the first test. Some animals showed "barrel-rolling" behavior in addition to the analgesia; this behavior was not a prerequisite for the analgesia, was unusually short-lived, and had no apparent persistent effects. The results show that in addition to previously described opiate-like actions (inhibition of electrically-induced contractions of the guinea pig ileum and binding to the mu opiate receptor), Tyr-W-MIF-1 can induce significant and prolonged analgesia.

636.10

PITUITARY ADENYLATE CYCLASE-ACTIVATING PEPTIDE (PACAP) STIMULATES CYCLIC AMP (cAMP) FORMATION IN CULTURED CEREBELLAR GRANULE CELLS. A. Favit*, U. Scapagnini and P.L. Canonico. Institute of Pharmacology, University of Catania School of Medicine, Catania, Italy.

PACAP is a novel 38-residue neuropeptide, structurally similar to vasoactive intestinal peptide, which stimulates adenylate cyclase activity in rat pituitary cells as well as in other neuronal and non-neuronal tissues (Matsuo Y. et al., Brain Res., 1992, 575:113-123). In this study we have investigated whether PACAP may modify cAMP formation in cultured cerebellar granule cells, a rather pure and homogeneous population of neurons. In cultures at 9 days of maturation in vitro, a 15-min exposure to PACAP triggered a concentration-dependent increase in intracellular cAMP content. PACAP's effect started to be significant at 1-5 nM and was maximal (about a 3-fold increase) between 10-100 nM. In the presence of the phosphodiesterase inhibitor, 3-isobutyl-1-methylxantine (IBMX) (200 μM), the concentration-response curve was shifted to the left with a stimulatory effect being present already at a concentration of 0.1 nM, and the maximal effect reaching about a 6 fold-increase. To assess whether this effect was time-dependent, granule cells were exposed to 10 nM PACAP for times ranging from 30 sec to 60 min. A rapid elevation of intracellular cAMP (about 80%) was observed after a 1-min exposure. The stimulatory effect was maximal between 15 and 30 min and progressively declined at 60 min. These results provide evidence for the presence of PACAP receptors linked to the adenylate cyclase-cAMP system in cerebellar granule cells.

636.11

ELECTROPHYSIOLOGICAL ACTIONS OF SOMATOSTATIN AND PACAP ON CULTURED DORSAL ROOT GANGLION (DRG) NEURONS
D. Crute* and E. R. Perl. Dept. Physiology, CB #7545 Univ. No. Carolina, Chapel Hill, NC 27599.

To test effects of somatostatin (SRIF) and pituitary adenylate cyclase activating peptide (PACAP), two peptides identified by immunocytochemistry in subsets of the diverse DRG population, organotypic monolayer DRG cultures were prepared from 16-18 day fetal pups taken from euthanized rats. In these cultures, SRIF-like immunoreactivity (SRIF-LI) appeared in 12% of DRG neurons, all less than 30μm diameter, similar to observations on adult rat DRG. SRIF and PACAP were applied by U-tube during whole-cell and perforated patch recordings. SRIF (25nM) hyperpolarized 27%, and depolarized 14% of neurons tested under current clamp conditions (n=170). SRIF (up to 10μM) did not affect action potential duration. Synaptic blockade with Cd^{++} eliminated the SRIF depolarization but not the SRIF hyperpolarization. Neurons both larger and smaller than 30μm were hyperpolarized by SRIF. SRIF-LI was demonstrated only in neurons unaffected by SRIF. PACAP (2nM) directly depolarized (via an increased gCl^-) 15% of tested neurons (n=26), all also vigorously depolarized by GABA (1.5mM) but not hyperpolarized by SRIF. Thus, SRIF and PACAP produce different inhibitory actions on separate subpopulations of DRGs. Supported by USPHS grant NS-10321 and NRSA NS-08691 to DC.

636.12

EFFECTS OF SOMATOSTATIN ON MEMBRANE PROPERTIES OF RAT SUBICULAR NEURONS *IN VITRO*
J.R.T. GREENE and A.J.R. MASON*. University Department of Pharmacology, Oxford, OX1 3QT, UK.

The peptide somatostatin and its receptors are found at relatively high levels in the human and animal hippocampus. Changes in brain somatostatin levels occur in some mental disorders, including schizophrenia. We have investigated some effects of somatostatin on the subiculum, which provides the major output pathway of the hippocampal formation. Slices of the ventral hippocampus and parahippocampal region were taken from young adult, male Wistar rats, and maintained in an interface chamber at 34 °C. Intracellular impalements were made with conventional, sharp micropipettes and membrane potentials recorded using an intracellular amplifier in bridge mode. All drugs were applied in the bathing medium. The 39 cells studied were of the type described previously (Mason, 1993, Brain Research, 600, 174-178) that show voltage-sensitive burst-firing responses to depolarising current pulses. Application of somatostatin-14 (SOM-14) for 2 minutes (0.5 - 5 μM) caused a slow hyperpolarisation and a decrease in input resistance (Rin) in 17 of 18 cells. A sample of 13 cells had control resting potentials of 67 ± 3 mV (mean ± SD) and Rin of 24 ± 4 MΩ. Exposure to 5 μM SOM-14 caused a hyperpolarisation of 3.8 ± 1.4 mV and a decrease in Rin of 24 ± 12 %. The change in Rin was reduced but not abolished when the membrane potential was returned to control values by depolarising current injection. Repeated responses to SOM-14 were obtained from the same cell when 20 - 30 minutes elapsed between doses. The actions of SOM-14 were mimicked by [D-Trp^8] SOM-14 (5 of 6 cells), but not by somatostatin-28 [1-12] at 10 μM (8 cells). The SOM-14 induced hyperpolarisation and decrease in Rin survived exposure to tetrodotoxin (1 – 3 μM, 10 cells), indicating that these effects are mediated by a direct, post-synaptic action on the recorded cells. The voltage sensitivity of burst-firing suggests that somatostatin-induced hyperpolarisation could have important consequences for information processing by subicular neurons.

636.13

Gender Differences in Choline Acetyltransferase, Somatostatin, and Swim Maze Performance in Somatostatin Transgenic and Control Mice.
C. F. Hohmann, S. L. Kinsman, T. H. Moran, G. Wenk, W. Gibson, B. N. Ladenheim, and M. L. Oster-Granite*; The Kennedy Krieger Inst. and Dept. of Psychiatry, Johns Hopkins Univ. Sch. of Med., Balto, MD; Div. of Neural Sciences, Univ. of AZ, Tucson, AZ; Div. of Biomedical Sciences, Univ. of CA, Riverside, CA 92521-0121.

Alterations previously observed in both choline acetyltransferase (ChAT) activity and numbers of somatostatin (SOM) immunoreactive neurons in the cerebral cortex of SOM transgenic mice (TgSmst13) versus control mice resulted from pooled female and male samples that were quite variable. Therefore, we studied gender differences in TgSmst13 mice versus controls. Since both SOM and ChAT levels affect cognitive function, we correlated neurochemistry and swim maze behavior.

Neocortical ChAT activity in young adult TgSmst13 and control mice (male=12; female=14) was elevated significantly (42%; $p < 0.01$) in male controls versus female controls, but only slightly increased (not significant) in TgSmst13 males versus TgSmst13 females. In contrast, SOM peptide levels were significantly lower (52.2%, $p=0.03$) in male versus female controls, but only slightly decreased (not significant) in TgSmst13 males versus TgSmst13 females. In all groups, however, there was an inverse relationship between ChAT activity and SOM levels. Thus, SOM expression and cholinergic neuronal activity may interact developmentally. Further, sex hormones and their receptors may affect SOM expression and peptide levels, leading to the attenuated gender differences in the TgSmst13 mice versus controls.

Five TgSmst13 and control mice of each sex were subjected to a visible platform trial, an invisible platform trial, and a probe trial, respectively, in the Morris water maze. We found no statistically significant differences among any of the groups. However, control males performed worse than TgSmst13 males and than all females, particularly on the invisible platform task. These surprising results may be influenced by mouse strain (C57BL/6J). BALB/cByJ males perform significantly better than females on the invisible platform task. Furthermore, our control males had the highest ChAT activity levels, yet, the lowest SOM peptide levels in neocortex. Supported by MH46529 and HD19932.

636.14

SOMATOSTATIN TRANSGENIC MICE: BEHAVIORAL AND NEUROCHEMICAL CONSEQUENCES.
S.L. Kinsman*[1,2], C.F. Hohmann[1,3], T.H. Moran[3], B.N. Ladenheim[1] and M.L. Oster-Granite[4]; [1]The Kennedy Krieger Research Institute, Baltimore, MD; Depts. of [2]Neurology and [3]Psychiatry, The Johns Hopkins Medical Institutions, and [4]Dept of Anatomy, UC Riverside CA.

The peptide somatostatin is abundantly expressed in many brain regions in the adult mouse. Somatostatin has been implicated in the control of many nervous system functions including several behaviors and the modulation of various neurotransmitter systems. Transgenic mice containing extra copies of a mouse genomic fragment containing the preprosomatostatin gene were produced to study further the role that somatostatin plays in these functions. In a preliminary analysis of the phenotypic consequences of the insertion and function of this transgene we examined spontaneous activity and regional levels of biogenic amines in two independent lines of transgenic mice. One line of homozygous transgenic mice [Tgm Smst 13] exhibited abnormalities in locomotor behavior and regional levels of norepinephrine. Multiple measures of horizontal and stereotypic activity were significantly elevated in this line of transgenic mice as compared with controls. In this hyperactive line, levels of norepinephrine in the cortex and cerebellum were significantly higher in both males and females compared to controls Using 2x2 ANOVA, in cortex, males were 13.5% elevated and females 15.3% ($p<.05$). In cerebellum, males were 20.7% and females 12.7% elevated ($p<.01$). The other line of transgenic mice were no different from their correspondingly derived controls. These results suggest long lasting effects on brain function in one line of these somatostatin transgenic mice. Further study will be required to determine whether these changes are due to somatostatin overexpression or whether they are dependent on transgene insertional effects.

Supported by MH46529 and K08 NS01455-01S1.

PEPTIDES: PHYSIOLOGICAL EFFECTS IV

637.1

MICROINJECTION OF THYROTROPIN-RELEASING HORMONE INTO THE PARAVENTRICULAR NUCLEUS OF THE HYPOTHALAMUS STIMULATES GASTRIC CONTRACTILITY
N.S. Morrow,*[1,2] D. Novin,[2] and T. Garrick.[1,3] Center for Ulcer Research and Education,[1] Dept. of Veteran Affairs Medical Center, Los Angeles CA 90073, Depts. of Psychology[2] and Psychiatry,[3] UCLA, Los Angeles, CA 90024.

Changes in gastric contractility following microinjection of TRH into the PVN were examined in fasted, urethane-anesthetized rats. Gastric contractility was measured with extraluminal force transducers and analyzed by computer. Unilateral and bilateral PVN microinjections of TRH (.5 μg, 1 μg) significantly increased the force index of gastric contractions 0-60 m postinjection, when compared to animals microinjected with .1 μg TRH, .1 % BSA or TRH (.5 μg, 1.0 μg TRH) in sites adjacent to the PVN. The gastric force index was also significantly elevated from 61-120 m postinjection in rats receiving bilateral PVN microinjections of TRH (.5 μg, 1 μg). The excitatory action of TRH (1.0 μg) on gastric contractility was completely abolished by subdiaphragmatic vagotomies. These results suggest that TRH acts within the PVN to stimulate gastric contractility via vagal-dependent pathways (Veteran Affairs, CURE/UCLA Digestive Disease Center, NIH Grant DK 41301, UCLA University Grant SF86).

637.2

ENDOTHELIN-INDUCED C-FOS EXPRESSION IN THE RAT HYPOTHALAMUS.
K. KUROKAWA H. YAMADA, M. KAWATA* and J. OCHI. Dept. of Anatomy Shiga Univ. of Med. Sci., Otsu, 520-21, *Kyoto Pref. Univ. of Med., Kyoto, 602, JAPAN

Endothelin(ET-1, 2 and 3), a vasoconstrictive peptide, is known to exit in the brain. In order to elucidate the central action of each ET, the expression of c-Fos protein was immunohistochemically analyzed after injection of ET-1,2 and 3 into the lateral ventricle of the rat brain. During the injection experiments, animals (male Wistar rats weight 180-200g) were under anesthesia with the sodium pentobarbital (50mg/kg, intraperitoreal injection). The immuoreactivity of c-Fos was observed from 30min throughout 6hrs after intraventricular administration of each ET(0.1-1 μmol). After the injection of ET-1 or 2, the reactivity was found in the paraventricular(PVN) and supraoptic (SON) nuclei of the rat hypothalamus. It was found that these positive neurons were immunohistochemically double-stained with oxytocin or vasopression-antibodies. In the case of ET-3, the c-Fos positive neurons were also seen in the organum vasculosum laminae terminalis (OVLT) in addition to PVN and SON.

637.3

INFLUENCE OF DIMETHYLSULFOXIDE ON THE INCIDENCE OF DYNORPHIN-INDUCED PARALYSIS AND LOSS OF THE TAIL-FLICK REFLEX IN THE RAT. Z-X. Qu and L. Isaac* Department of Pharmacology, Univ. of Ill. Coll. of Med., Chicago, IL 60680

We showed that dynorphin (DYN) administered i.t. results in hindlimb reversible paralysis and loss of the tail-flick reflex (TFR) (Brain Res. 610:340, 1993). Also, we reported that DYN enhances the C-fiber evoked reflex resulting in excitotoxicity and loss of the TFR (JPET 246:508, 1988). Becker, *et al.*, (Exp. Neurol. 24:272, 1969) showed that dimethylsulfoxide (DMSO) blocks conduction in C-fibers. We hypothesized that DMSO would interrupt DYN-induced loss of the TFR but not paralysis.

We administered DMSO 5 min (0.4 - 3.0 g/kg i.p.) before DYN (30 nmol i.t.) and observed a dose-dependent protective effect on loss of the TFR. DMSO did not influence the incidence of paralysis but it improved recovery from paralysis.

These data indicate that DMSO is neuroprotective in our *in vivo* model of excitotoxicity and suggest that its mechanism may be to interrupt enhanced C-fiber activity. Additionally, these data support our hypothesis that DYN-induced loss of the TFR is neurally mediated. In conclusion, DMSO is a useful pharmacologic tool to facilitate exploration of the interaction of DYN with spinal reflexes.

Supported by NIH NS-30295

637.4

DEVELOPMENT OF TOLERANCE TO MORPHINE IN THE RAT SPINAL CORD: SELECTIVE INTERACTION WITH CALCITONIN GENE-RELATED PEPTIDE. D. Ménard[1]*, D. van Rossum[1], S. Kar[1], F. Jolicoeur[2], K. Jhamandas[3] and R. Quirion[1]. [1]Douglas Hosp. Res. Centre and Dept. of Psychiatry and Pharmacol. & Ther., McGill Univ., Qué. [2]Dept. of Psychiatry and Pharmacol., Univ. of Sherbrooke, Qué. [3]Dept. of Pharmacol., Queen's Univ., Ont., Canada.

Tolerance to the antinociceptive effects of morphine develops rapidly after its chronic administration. The mechanism involved in this phenomenon likely does not involve a direct regulation of spinal opioid receptors. A variety of neuropeptides including neurokinins and calcitonin gene-related peptide (CGRP) are found in sensory fibers that project to the substantia gelatinosa and hence may play a significant role in nociception, and the development of tolerance to morphine. We have therefore investigated the possible regulation of CGRP, neurokinins, galanin, neurotensin and neuropeptide Y receptors and immunohistochemical labelling in the dorsal horn of the spinal cord along with the development of tolerance induced by morphine. Morphine sulfate (7.5μg/μl/h) was continuously administered at lumbar level L4 using Alzet mini-osmotic pumps for 3, 5, 7 and 14 days. Tolerance was monitored by tail-flick test and was evident on day 5 of the treatment. After the respective time periods, animals were sacrificed and L4 segments processed for either immunohistochemistry or quantitative *in vitro* receptor autoradiography. No changes were detected for all peptides tested at any time periods examined except for CGRP where decreases in [^{125}I]CGRPα binding sites (30-45%) in the lamina II were observed after 5, 7 and 14 days of morphine treatment whereas CGRP-like immunoreactivity was increased at these same time points. These results suggest a selective interaction between CGRP innervation and the development of tolerance to the antinociceptive effects of morphine. Supported by the MRCC.

637.5

INTRACISTERNALLY INJECTED GALANIN (1-15) MODULATES CARDIOVASCULAR RESPONSES OF GALANIN (1-29). J.A. Narváez*, Z. Díaz, P.B. Hedlund§, J.A. Aguirre, S. González-Barón and K. Fuxe§. Dept. of Physiology. Faculty of Medicine. University of Málaga. Spain. §Dept of Histology and Neurobiology. Karolinska Institute. Stockholm. Sweden.

The presence of specific binding sites for the Galanin (1-15) fragment [GAL(1-15)] in the rat brain has recently been reported. Since they are located in cardiovascular areas of the brainstem, the aim of this work has been to study the functional significance of such binding sites. GAL(1-15) has been injected intracisternally (i.c.) in anaesthetized male rats (dose range 0.3-10 nmol/10 μl) and mean arterial pressure (MAP), heart rate (HR) and respiratory rate (RR) were recorded during 1 hour period. GAL(1-15) injection leads to a dose-dependent increase of MAP in the first minutes after the injection (peak effect) which is maintained during the whole recording period (overall effect). Also an increase of HR was obtained for the peak and overall effects, but no significant changes in RR were observed. To study the possible modulation of GAL(1-29) on the cardiovascular responses by GAL(1-15) an effective vasodepressor dose of GAL(1-29) (3 nmol) and a subthreshold dose of GAL(1-15) (0.1 nmol) were coinjected i.c. under the same experimental conditions. GAL(1-15) reversed the vasodepressor effect of GAL(1-29) an even induced an increase in MAP of about 15% from basal values. Nevertheless, the tachycardic and bradypneic responses of GAL(1-29) are not modified. Since MAP responses after the coinjection of both GAL molecules resembles the MAP increases observed with higher doses of GAL(1-15) it could be suggested that GAL(1-15) receptors might be sensitized by the parent molecule GAL(1-29) acting on another subtype of GAL receptor. This fact and the absence of modulation of GAL(1-29) induced HR responses could suggest that GAL(1-15) may be involved mainly in the central control of blood pressure while GAL(1-29) could be involved mainly in the central control of heart rate. This work was supported by Spanish CICYT (Sal91-0458) and from the Swedish MRC (04X-715).

637.6

PHARMACOLOGICAL COMPARISON OF TWO CORTICOTROPIN-RELEASING FACTOR (CRF) ANTAGONISTS: *IN VIVO* AND *IN VITRO* STUDIES. R.J. Valentino*, A.L. Curtis, D. E. Grigoradis, M.E. Page and J. Rivier. Dept. of Mental Health Sci., Hahnemann University, Philadelphia, PA 19102, Du Pont Merck Pharmaceutical Co, Wilmington, DE 19880 and The Clayton Fdtn. Labs. for Peptide Biology, Salk Institute, La Jolla, CA 92037.

The present study compared the effects of two analogues of CRF, D-PheCRF$_{12-41}$ and α helical CRF$_{9-41}$, as antagonists of CRF in *in vivo* and *in vitro* assays. In halothane-anesthetized rats, intracerebroventricular (i.c.v.) administration of both analogues inhibited the activation of locus coeruleus (LC) neuronal discharge produced by CRF (3.0 μg, i.c.v.). LC activation by hypotensive stress elicited by intravenous (i.v.) infusion of nitroprusside was antagonized by the same doses of the CRF antagonists that were effective in antagonizing CRF, suggesting that the receptors involved in LC activation by CRF and by hypotensive stress are similar. However, D-PheCRF$_{12-41}$ was approximately 100 times more potent than α helical CRF$_{9-41}$ when administered i.c.v. The IC 50's for D-PheCRF$_{12-41}$ against CRF and nitroprusside were 0.16 and 0.14 μg i.c.v., respectively. The IC 50's for α helical CRF$_{9-41}$ against CRF and nitroprusside were 18 and 27 μg, i.c.v., respectively. In contrast, D-PheCRF$_{12-41}$ was only slightly more potent than α helical CRF$_{9-41}$ in antagonizing CRF-stimulated cyclic AMP production in rat brain homogenates, with EC 50's of 50 and 200 nM for D-PheCRF$_{12-41}$ and α helical CRF$_{9-41}$, respectively. Moreover, the antagonists had similar affinities for CRF binding sites in rat brain homogenates, with Ki's of 17.6 and 9.9 nM for D-PheCRF$_{12-41}$ and α helical CRF$_{9-41}$, respectively. The results support previous studies suggesting that CRF serves as a neurotransmitter to activate the LC during hypotensive stress. The relatively lower potency of α helical CRF$_{9-41}$ *in vivo*, but not *in vitro*, may be due to a differential distribution of the antagonists after i.c.v. administration or to relative affinity differences at non-receptor CRF binding sites or proteins. Supported by PHS Grants MH 40008, MH 00840 and DK 26741.

637.7

ANXIOLYTIC AND SEDATIVE EFFECTS OF MELATONIN AND 8-OH-DPAT IN MICE. E.B. Naranjo-Rodríguez, E.B. and C. Reyes-Vázquez*. Secc. de Farmacología. Depto de Farmacia. Fac. de Química and Depto. de Farmacología. Fac. de Medicina, UNAM. México, D.F. 04510.

The sedative and anxiolytic actions of melatonin and 8-OH-DPAT, a specific 5-HT1A receptor ligand were compared in mice. Animals underwent a free exploratory test especially adapted to reveal sedation, and they were introduced to a two-box light-dark choice situation widely used for the detection and screening of anti-anxiety substances. The effects of both drugs were compared with those induced by chlorodiazepoxide and buspirone, two classical anxiolytics. Both, melatonin and 8-OH-DPAT induced sedative properties when they are applied at high doses (4-10 mg/Kg); whilst, when administered at lower doses they displayed anxiolytic-like effects, in a very similar fashion. However, the execution of such effects were different to those elicited by chlorodiazepoxide and buspirone, suggesting a different mechanism of action.

637.8

GLUTAMATE UP-REGULATES α1 AND α2 SUBUNITS OF THE SODIUM PUMP IN GLIA OF MIXED TELENCEPHALIC CULTURES BUT NOT IN PURE GLIAL CULTURES. Michael L. Brines* and Richard J. Robbins. Neuroendocrine Program, Yale School of Medicine, New Haven, CT 06510

Prior work employing an *in vitro* model of the cerebral cortex (*Brain Res.* 591 (1992) 94ff) has shown that sodium pump activity is a critical determinant for neuronal survival of glutamate stimulation. We have hypothesized that up-regulation of total brain sodium pump activity will protect against potential excitotoxins. Increased sodium pump activity could theoretically occur by changes in the reaction rate (short-term) and/or by increased levels of sodium pump protein (long-term) and is potentially complex since the three catalytic (α) subunit isoforms of the sodium pump are distributed in a highly variable, cell-specific pattern in the brain. Short-term regulation (seconds to minutes) has been well studied: brain sodium pump exhibits a large dynamic range. In contrast, the possibility of long-term modulation of sodium pump activity has not been extensively explored. We used isoform specific antibodies and ^{3}H-ouabain binding to determine whether prolonged stimulation of sodium pump activity in rodent telencephalic cultures increased total sodium pump enzyme. Exposure of mixed neuronal-glial cultures to high levels of glutamate (10mM) for 18 hours, which is highly toxic to neurons, was associated with an ~80% increase in α1 and α2 subunit expression by glia. Induction of α2 subunit immunoreactivity was also associated with comparable changes in ^{3}H-ouabain binding, suggesting that the up-regulation corresponded to functional α2 protein. Shorter (30 m) glutamate treatments, which also killed neurons, did not produce similar changes in sodium pump expression. In contrast to mixed cultures, pure astrocyte cultures had undetectable α2 and α3 and moderate levels of α1 protein, as confirmed by low levels of ^{3}H-ouabain binding. Glutamate treatment using this protocol was associated with a decrease in α1 sodium pump expression. We conclude that long-term regulation of the sodium pump can be demonstrated in glia which have developed in the presence of neurons. Both α1 and α2 isoforms of the sodium pump are involved in this response to glutamate.

637.9

GLYCYL-L-GLUTAMINE ANTAGONISM OF α-MSH: INVOLVEMENT OF PGE2. G.E. Resch* and W.R. Millington Division of Molecular Biology & Biochemistry, Univ. Missouri, Kansas City MO 64108-2792.

Glycyl-L-glutamine (Gly-Gln; β-endorphin-30-31), antagonizes certain behavioral and physiologic effects of β-endorphin and α-MSH. Preoptic area/anterior hypothalamus (POA/AH) sites were selected for a rise in colonic temperature (Tc) first with PGE2 injections (0.35 fmol in 1 μl) and second with α-MSH injections (0.06 nmol in 1 μl) vs saline vehicle. Injection of α-MSH (0.06 nmol) mixed with Gly-Gln (3 nmol) abolished the Tc response (0.0 ± 0.02°C) elicited by α-MSH alone (0.64 ± 0.17°C; P<0.01 vs control) in the same POA/AH sites. The antagonism did not occur to equimolar Gly and Gln (0.55 ± 0.05°C) nor to repeated exposure to α-MSH since serial α-MSH injections 4 h apart showed an undiminished rise in Tc. The PGE2 antagonist, SC19220, did not consistently block α-MSH, suggesting α-MSH does not act by stimulating PGE2 synthesis. Preliminary studies show that Gly-Gln also antagonized the PGE2 elicited rise in Tc, suggesting Gly-Gln does not act by blocking α-MSH receptors but may act through independent receptors to reduce membrane excitability. Supported in part by DAMD 17-90-Z-0022.

637.10

INCREASED INTRANEURONAL Ca^{2+} INDUCED BY THE DIPEPTIDE N-ACETYL-ASPARTYLGLUTAMATE. M.L. Koenig*, P.M. Rothbard, and J.L. Meyerhoff. Dept. Med. Neurosci., Div. Neuropsychiatry, Walter Reed Army Inst. Research, Wash., DC 20307-5100.

N-acetyl-aspartylglutamate (NAAG) is one of the most abundant peptides in the vertebrate brain. Since there is evidence to suggest that NAAG can act as an excitatory neurotransmitter, we have evaluated the effect of this dipeptide on neuronal Ca^{2+} homeostasis.

Primary neurons derived from fetal rat forebrain were loaded with the Ca^{2+}-sensitive dye indo-1 (2 μM; 60 min.; 37°C), and NAAG-induced changes in intraneuronal Ca^{2+} ($[Ca]_i$) were measured in single, identified neurons (5-20 per 150 μm^2 field) using an ACAS 570C interactive laser cytometer (Meridian, Okemos, MI). An HPLC analysis of the NAAG used in these studies revealed that the dipeptide represented 99.5% and free glutamate only 0.4% of what was added to the neuronal cultures. The concentration of contaminating glutamate therefore, did not contribute significantly to the observed effects.

In Ca^{2+}-containing medium, NAAG (50 μM) promoted an immediate and transient four-fold increase in $[Ca]_i$ from a basal level of 133 nM to a mean level of 520 nM. With increasing concentration of the dipeptide, a second, more sustained phase of increased $[Ca]_i$ became evident suggestive of prolonged influx. In the absence of extraneuronal Ca^{2+}, NAAG was completely without effect. Because the excitatory properties of NAAG were reduced in the presence of extraneuronal Mg^{+}, and inhibited by pretreatment of the neurons with either MK-801 (200 nM) or 2-amino-5-phosphonovalerate (500 μM), we are investigating the possibility that NAAG-induced increases in $[Ca]_i$ are mediated by the ionotropic NMDA type of glutamate receptor.

637.11

THE EFFECT OF A FMRFamide-LIKE PEPTIDE (SDPNFLRFamide) ON SYNAPTIC TRANSMISSION IN THE PARASITIC NEMATODE *Ascaris suum*. Holden-Dye, L., & Walker,R.J*. Dept of Physiol & Pharmacol., Bassett Crescent East, University of Southampton, UK. SO93TU.

Recently FMRFamide-like peptides have been identified in a number of nematodes and the widespread distribution of FMRFamide-like immunoreactivity suggests an important role for these peptides in nematode nervous system. Here we have investigated the effect of the peptide SDPNFLRFamide (PF1), first identified in *Panagrellus redivivus* (Geary et al, 1993) on excitatory neuromuscular transmission in the parasitic nematode *Ascaris suum*. Intracellular recordings were made from *Ascaris* ventral muscle cells and the ventral nerve cord was stimulated with bipolar electrodes immediately rostral to the retrovesicular ganglion. The preparation was perfused with artificial perienteric fluid (APF, composition in mM; NaCl 67, NaAcetate 67, $CaCl_2$ 3, $MgCl_2$ 15.7, KCl 3, Tris 5, pH 7.6 with glacial acetic acid with 3 mM glucose at 36°C) and drugs were added to the preparation in the perfusate. EPSPs of 5-35 mV amplitude were recorded. The EPSPs were blocked by the nicotinic receptor antagonist benzoquinonium (10μM, n=5) and by cobalt (1 mM, n=7). PF1 (0.01-1μM) decreased the amplitude of the EPSP in a dose-dependent manner with an EC_{50} of 300 nM (n=5). The block was partially reversible after a wash of 15 min or longer. The results suggest a physiological role for a FMRFamide-like peptide at the neuromuscular junction in *Ascaris*.

GEARY, T.G. et al.(1992) Peptides 13, 209-214.

We are grateful to the Wellcome Trust for financial support.

637.12

ENKEPHALINERGIC MODULATION OF THE CRAYFISH X ORGAN - SINUS GLAND SYSTEM. García, U.* and Garduño, J. Depto. de Fisiología, Biofísica y Neurociencias, CINVESTAV del IPN, Apartado Postal 14-740, 07000 México, D. F.

Immunocytochemical studies showed immunoreactive fibers to enkephaline in the medulla terminalis of lobster and shore crab eyestalks (Mancillas et al., 1981 and Jaros et al., 1985).

In the present work we show evidences of the enkephalinergic synaptic regulation of the electrical activity of the crayfish X organ - sinus gland system.

Isolated eyestalks were dark adapted during 30 minutes and inhibitory synaptic potentials were evoked by brief light pulses and recorded from XO neurons with microelectrodes filled with potassium acetate (2.6 M). These inhibitory potentials reduced the firing rate of spontaneous action potentials, both frecuency and amplitude of IPSPs were dependent the intensity and duration of the light pulses and they were blocked by naloxone (3-50 μm). Enkephaline addition (10 μM) to the bathing solution during intracellular recordings in XO cells, induced a small hyperpolarization (about 2-3 mV). However when XO cells were cultured, they showed a higher sensitivity to enkephaline micropulses of the same concentration, the hyperpolarization was dose dependent and neurons which were cultured during 48 hours showed higher sensitivity in the lamellipodium than in the cell body. Our results suggest that enkephaline increases a potassium conductance in XO cells, since TEA addition (20 mM) to the recording medium enhanced action potential duration to 10-50 ms and enkephaline reversed this prolongation. Finally, enkephaline response did not modified IK and IA currents present in these neurons.

OPIOIDS: RECEPTORS IV

638.1

BOTH DELTA-1 AND DELTA-2 OPIOID RECEPTORS REGULATE ADENYLYL CYCLASE IN NG108-15 CELLS
B. Búzás, P.S. Portoghese# and B.M. Cox*, Uniformed Services Univ., Bethesda, MD and #Univ. of Minnesota, Minneapolis, MN.

Heterogeneity of delta receptors has been suggested on the basis of *in vivo* studies: the analgesic effect of DPDPE was antagonized by [D-Ala2,Leu5,Cys6]-enkephalin (DALCE), but not by naltrindole-5'-isothiocyanate (5'NTII), while deltorphin II (DT-II)- and DSLET-mediated antinociception was antagonized by 5'NTII, but not by DALCE. This and other evidence suggests that these agonists act at different sites, which have been designated δ1 (sensitive to DPDPE and DADLE) and δ2 (activated by DT-II and DSLET). Seeking functional evidence at a biochemical level, we investigated the role of delta receptor subtypes in the regulation of adenylyl cyclase in NG108-15 cell membranes, using two reversible antagonists, naltriben (NTB) (δ2) and 7-benzylidenenaltrexone (BNTX) (δ1), which were subtype-specific in antinociceptive assays. DPDPE, DSLET and DT-II diminished the formation of cAMP in a concentration-dependent manner and with similar efficacy producing a maximal inhibition of about 50% of control. NTB significantly antagonized all the delta ligands, but not to the same extent. At 1 and 10 nM it showed significantly better antagonism of DT-II and DSLET, than of DPDPE. BNTX (100 nM) diminished the inhibition by DPDPE to a greater extent than either by DT-II or DSLET. In conclusion, on the basis of differential antagonism by NTB and BNTX these data suggest the existence of delta receptor subtypes regulating adenylyl cyclase in NG108-15 cells. We have previously shown a similar differential antagonism of δ-inhibition of adenylyl cyclase in rat brain. (Supported by grants from the National Institute of Drug Abuse.)

638.2

THE μ-OPIOID RECEPTOR AGONIST D-Ala^2-NMe-Phe^4-Gly^5ol MODULATES INHIBITORY AND EXCITATORY SYNAPTIC TRANSMISSION IN RAT NEOCORTICAL NEURONS *IN VITRO*. G. Martin, H. Pawelzik, R.A. Deisz and W. Zieglgänsberger*. *Max-Planck-Institute of Psychiatry, Clinical Institute, Clin. Neuropharmacology, Kraepelinstr. 2, 8000 München 40, Germany.*

Activation of opioid receptors has been considered to produce mainly inhibitory effects on neuronal activity. In the present study the μ-opioid receptor agonist D-Ala^2-NMe-Phe^4-Gly^5ol (DAMGO) was found to differentially affect glutamatergic transmission. It enhanced NMDA receptor-mediated excitatory postsynaptic potentials (EPSPs), and diminished non-NMDA receptor-mediated EPSPs. DAMGO also reduced inhibitory postsynaptic potentials (IPSPs).

Pyramidal neurons of the somatosensory neocortex (lamina II/III) of the adult rat were recorded intracellularly employing standard *in vitro* slice techniques. EPSPs and IPSPs were elicited by electrical stimulation in lamina V/VI and lamina III/IV, respectively. Substances were added to the perfusion medium. Pretreatment with CNQX and D-APV, respectively, was used to differentiate NMDA from non-NMDA receptor mediated components of the EPSP.

DAMGO (10^{-7} - 10^{-3} M) did not significantly affect resting membrane potential or input resistance. The amplitude of EPSPs evoked by low stimulus intensities was reduced by DAMGO, whereas EPSPs elicited by high stimulus intensities were not altered or were enhanced. After blockade of non-NMDA receptors by CNQX (2 μM), DAMGO concentration-dependently increased EPSP time integrals up to 200% compared to control (n=34). Under conditions where NMDA receptors were blocked by D-APV (100 μM), DAMGO decreased the EPSPs to 10% (n=21). The conductance during the early Cl^--mediated and late K^+-mediated GABAergic IPSPs (EPSPs blocked by 100 μM D-APV and 10 μM CNQX) was decreased by DAMGO (1 μM) to below 20% (n=6). The actions of DAMGO were antagonized by naloxone (10 μM; n=14).

The present results suggest that DAMGO has a differential effect on excitatory glutamatergic synaptic transmission depending on the receptor subtype modified by the peptide. The enhancement of NMDA receptor-mediated excitatory synaptic transmission and the decrease of inhibitory synaptic processes caused by the opioid peptide may be relevant for synaptic plasticity following chronic opioid exposure.

638.3

EVALUATION OF OPIOID ANTINOCICEPTION IN A RAT MODEL OF VISCERAL PAIN FOLLOWING LOCAL AND SYSTEMIC ADMINISTRATION. Rebecca M. Craft*, Steven R. Henley and Frank Porreca. Department of Pharmacology, University of Arizona Health Sciences Center, Tucson, AZ 85724.

The antinociceptive effects of systemically or locally administered opioid μ, κ and δ agonists were evaluated in a rat model of visceral pain involving direct administration of compounds into the urinary bladder (intravesical, *i.ves.*) via an indwelling cannula. *I.ves.* resiniferatoxin (RTX, 3 nmol), a capsaicin-like irritant, produced intense abdominally directed licking behavior, quantified by determination of time spent licking. Systemic (*s.c.* or *i.p.*) pretreatment with the μ agonists morphine (1-10 mg/kg) or [D-Ala^2, NMPhe^4, Gly-ol]enkephalin (DAMGO, 1-10 mg/kg), the κ agonists U50,488 (1-10 mg/kg) and CI-977 (0.01-0.1 mg/kg), or the non-peptidic δ agonist BW 373U86 (1-10 mg/kg) decreased time spent licking in a dose-dependent manner. The rank order of potency for inhibition of licking was CI-977 > morphine > U50,488 > BW 373U86 > DAMGO, and all agonists produced maximal suppression of abdominal licking except DAMGO (maximal effect 48% at the highest dose tested). Antinociceptive effects of systemically administered morphine, U50,488 and BW 373U86 were blocked by the receptor-selective antagonists β-funaltrexamine (μ), nor-binaltorphimine (κ) and naltrindole (δ), respectively. Local (*i.ves.*) pretreatment with DAMGO (10-1000 nmol) or BW 373U86 (1-1000 nmol) also decreased RTX-induced abdominal licking; maximal effects were 34 and 46% with DAMGO or BW 373U86, respectively (both at 100 nmol). Local U50,488 was ineffective up to 1000 nmol. Thus, systemically administered μ, κ and δ agonists all produced antinociception against a visceral chemical stimulus in the rat. Locally, only μ and δ, but not κ, agonists were effective, though less so than following systemic administration. This study suggests that opioid μ and δ receptors may be present on nociceptive afferents of the bladder and may be amenable to activation for production of peripheral analgesia.

638.4

DYNORPHIN A (1-17) ANTAGONIZES MORPHINE ENHANCEMENT OF THE DORSAL ROOT POTENTIAL. H. Ristic, P. Stewart* and L. Isaac Dept. of Pharmacology, Univ. Ill. Coll. of Medicine at Chicago, Chicago, IL 60680

Previously, we showed that dynorphin (DYN) applied to the spinal cord results in depression of the dorsal root potential (DRP) lasting 30 min which is insensitive to nor-binaltorphimine antagonism. DYN is both a κ agonist and a μ antagonist. To determine whether DYN affected the DRP through a μ action we tested its ability to influence morphine on the DRP and we challenged both DYN and morphine with β-funaltrexamine (βFNA), a μ antagonist.

We performed a laminectomy on rats under urethane anesthesia and recorded a DR-DRP from L4 after stimulation of L5. βFNA (20 nmol) or saline was administered i.t. 24 hr prior to recording whereas morphine (20 - 300 nmol) was applied topically 2 hr after DYN (9 nmol) or saline applied topically.

Morphine, dose-dependently, enhanced the DRP. Its action peaking at 30 min. This effect was antagonized by βFNA and DYN. βFNA did not affect DYN nor did it influence the DRP.

These data indicate that morphine enhances the DRP through an interaction with μ receptors whereas DYN-induced depression of the DRP is non-opioid in nature. These data suggest that DYN may act as an endogenous modulator of μ receptor mediated effects. Supported by NIH NS-30295

638.5

OPIATE RECEPTOR SUBTYPE INVOLVEMENT IN THE STIMULATION OF GROWTH HORMONE RELEASE BY β-ENDORPHIN IN FEMALE RATS. James Janik*, Rebecca Parman, Shannon Klosterman and Phyllis Callahan, Miami University, Department of Zoology, Oxford, Ohio 45056

We have reported that β-endorphin (25 ng) produced a prolactin secretory response in virgin female rats which was similar in magnitude to the suckling induced prolactin increase (Kehoe, et al., Neuroendo, 1993, IN PRESS). This secretory response seemed to involve an interaction among the μ, δ and κ opiate receptor subtypes. The purpose of this study was to determine whether or not there was interaction among these opiate receptors in the GH secretory response to β-endorphin.

Virgin female Sprague-Dawley rats in the diestrous stage of the estrous cycle were used for all experiments. Animals were surgically implanted with chronic intraventricular (ivt) cannula into the lateral ventricle and all drugs were administered ivt. On the day of the experiment, animals were variously pretreated with either vehicle, β-funaltrexamine (1 or 5 μg: a μ specific antagonist), ICI 154,129 (5, 10 or 25 μg: a δ specific antagonist), or Nor-binaltorphimine (8 μg: a κ specific antagonist) followed by β-endorphin (0.5 μg). All three antagonists were capable of abolishing the stimulatory effect of β-endorphin on GH release. As was the case for prolactin release, β-endorphin appears to increase GH secretion via actions at all three receptor subtypes (Supported by Ohio Board of Regents Research Challenge Program Grant to P. Callahan).

638.6

CALCIUM CHANNEL INHIBITION BY SOMATOSTATIN AND AN OPIOID IN HIT AND NG108-15 CELLS. E. Piros*, C.J. Evans, B. Ribalet, T.G. Hales. Depts Psychiatry, Physiol. and Anesthesiol., Ctr Hlth Sci. UCLA. Los Angeles, CA. 90024

We have compared modulation of voltage-activated Ca2+ channels by DADLE and somatostatin (SRIF) in insulinoma (HIT) and neuroblastoma x glioma (NG108-15) cells. Like SRIF, opioids inhibit insulin secretion *in vivo* (Giugliano et al. 1989, Am.J.Physiol. 257:B361). However, a direct effect of opioids on channel activity in insulin secreting cells has not been reported.

The whole-cell patch-clamp technique was used to record currents through Ca^{2+} channels carried by Ba2+ in both cell lines, with NMDG chloride or CsCl based internal solutions. Drugs were bath or pressure applied.

Ba2+ currents in NG108-15 (n = 115) and HIT (n = 18) cells, activated by depolarizing voltage steps from -80mV, had a threshold and were maximum at around -50 and 0mV, respectively. Both cell lines exhibited Ba^{2+} current run - down proportional to the frequency of depolarization. Stimulation at 0.3 Hz caused a 40% Ba^{2+} current reduction in HIT cells (n = 6), and a 20% decline in NG108-15 cells (n = 6) in 5 min. Reducing the frequency to 0.1 Hz reduced the rate of run-down in both cell types. Under these conditions DADLE (0.3-10 μM) dose-dependently inhibited Ba^{2+} currents in both cell lines. DADLE (1 μM) reduced HIT and NG108-15 cell Ba^{2+} currents by 12.8 ± 1.0% (± SEM, n = 4) and 14.9 ± 2.9% (n = 6). SRIF (1 μM) inhibited Ba^{2+} currents by 12.5 ± 0.5% (n = 4) and by 15.9 ± 2.1% (n = 5) in HIT and NG108-15 cells.

Our data suggest that like NG108-15 cells, HIT cells express SRIF and opioid receptors. We are investigating which opioid receptor and Ca2+ channel subtypes are modulated by DADLE in the insulin secreting cell line. (NIDA Center Grant #DA-05010).

638.7

OPIOID INHIBITION OF CALCIUM CHANNELS: A ROLE FOR PHOSPHORYLATION IN A LOCALIZED SIGNAL TRANSDUCTION PATHWAY. T. J. Wilding, R. E. Melfordt, M. D. Womack*, and E. W. McCleskey Dept. of Cell Biology and Physiology, Washington University School of Medicine, St. Louis, Missouri 63110, U. S. A.

Previous work from this laboratory has shown that the μ opioid agonist, DAMGO, inhibits Ca channels in rat sensory neurons (Schroeder et al., Neuron, 6: 13-20, 1991). We have been investigating the signal transduction of this inhibition using the whole-cell patch clamp method. Incubation of the cells in 500 ng/ml PTX for 16-24 hr reduced μ opioid induced inhibition from 23 ± 2 % to 4 ± 1 %. This result demonstrates the involvement of PTX-sensitive GTP-binding proteins in the pathway. Using fast solution changes, we found transduction to be complete in 4 s and to begin with a latency of 150 ms. Recording simultaneously from a perforated patch electrode and an on-cell electrode, we found that bath-applied DAMGO inhibited whole-cell Ca current but did not affect Ca channels in the small patch of membrane protected from the agonist. The rapid time course and localized transduction path suggest a direct GTP-binding protein link between the μ opioid receptor and the Ca channel. Intracellular perfusion of ATP-γ-S, which irreversibly phosphorylates proteins, rendered DAMGO inhibition irreversible. Okadaic acid (5 μM), a phosphatase inhibitor, enhanced the DAMGO-induced inhibition of Ca current from 20 ± 6 % to 40 ± 4 %. To date, phosphorylation/dephosphoryaltion have only been seen in signal transduction pathways involving second messengers; here, phosphorylation appears to play a role in a localized pathway. (†Howard Hughes Medical Institute Medical Student Research Fellow)

638.8

INSENSITIVE GUANINE NUCLEOTIDE MODULATION OF SIGMA LIGAND BINDING IN GUINEA-PIG BRAIN MEMBRANES. J.H.Connick*, G.Hanlon, C.D.Nicholson and R.J.Marshall. Organon Labs., Newhouse, Lanarkshire ML1 5SH, U.K.

We have previously investigated whether subtypes of sigma site(s) exist using [3H]-DTG and [3H]-3PPP as ligands and examining the modulation of self and cross displacement curves by Gpp(NH)p (Connick et al., Brit. J. Pharmacol. 107, 726-731 1992). However, the use of relatively nonselective radioligands may lead to an overestimation of the number of subtypes. Such observations may be further complicated where one or more site is G-protein linked since several states of a single receptor site may exist due to G-protein equilibria (Kent et al., Mol Pharmacol. 17, 14-23 1980). It is therefore of critical importance to determine whether one or more sigma site is G-protein coupled. We have examined this using a variety of guanine nucleotides to inhibit the binding of sigma ligands with differing subtype selectivity's; [3H]-pentazocine, [3H]-3PPP and [3H]-DTG. Gpp(NH)p inhibited the binding of all 3 ligands with IC50's of 953±24, 918±24 and 950±21μM respectively (n=4). The slope of all displacement curves was significantly greater than 1. Similar results were obtained with GTP, GDP, GMP and GTPγS.

These results suggest that the ability of guanine nucleotides to modulate the sigma site(s) is 1000 fold less than classical G-protein linked receptors. Furthermore, no significant differences were observed between the three ligands used, even though the sigma 1/sigma 2 and the putative agonist/antagonist properties of these ligands differ. It is possible that under the conditions used, binding was predominantly to an uncoupled state(s). If this is not the case, these data do not support the contention that sigma sites are G-protein linked.

638.9

DIFFERENTIAL DOWN- AND UP-REGULATION OF RAT BRAIN OPIOID RECEPTOR TYPES AND SUBTYPES BY BUPRENORPHINE. M.M. Belcheva+, J. Barg+, R.J. McHale+, S. Dawn+, M. T. Ho+, E. Ignatova+, F.E. Johnson*§ and C.J. Coscia+. Depts. of +Biochem. and Mol. Biol. and §Surgery, St. Louis Univ. Sch. Med., St. Louis, MO 63104.

Induction of opioid receptor adaptation by mixed agonist-antagonists such as buprenorphine (BP) has not been studied. Neonatal rats were injected with BP (0.1-2.5 mg/kg/d) and μ binding (K_d and B_{max}) to brain membranes was measured with ^{3}H-[D-Ala^2,MePhe^4,Gly-ol^5] enkephalin. At BP doses $\geq$0.5 mg/kg, μ sites were reduced 47-75% without change in affinity. Administration of the structurally-related partial agonist diprenorphine (2.5-75 mg/kg) failed to alter μ binding. Receptor blockade by residual BP was ruled out by several lines of evidence. B_{max} values of δ (^{3}H-[D-Ser^2,L-Leu^5]enkephalyl-Thr [DSLET]) and κ (^{3}H-U69593) binding were elevated 1.9-4.2-fold by BP treatment. In adult rats BP (0.5-2.5 mg/kg) reduced μ opioid binding to forebrain membranes dose-dependently (25-77%). ^{3}H-DSLET-labeled δ subtype and κ sites in adult forebrain membranes were up-regulated 2-3-fold. The δ subtype that binds ^{3}H-[D-Pen^2,D-Pen^5]enkephalin in neonatal or adult brain membranes was unaffected by 0.5-2.5 mg/kg BP treatment. Down-regulation (70-74%) of μ sites and up-regulation (1.9-6.7 fold) of δ and κ receptors were observed in synaptic plasma membrane-enriched and microsomal fractions from BP-treated adult rat brain.

638.10

OPIOIDS INHIBIT ENDOTHELIN-MEDIATED PHOSPHATIDYLINOSITOL TURNOVER, Ca^{2+} MOBILIZATION AND DNA SYNTHESIS IN RAT C6 GLIOMA CELLS. Z. Vogel*#, M. M. Belcheva†, R. Levy#, R. J. McHale†, F. E. Johnson‡, C. J. Cosciat, J. Barg#† #Dept. of Neurobiol. The Weizmann Inst. of Sci., 76100 Rehovot, Israel, †E.A. Doisy Dept. of Biochem. and Molec. Biol., ‡Dept. of Surgery, St. Louis University School of Medicine, St. Louis, Missouri 63104, U.S.A.

Desipramine (DMI) induces opioid binding in rat glioma C6 cells and opioid agonists inhibit DNA synthesis in DMI-treated but not untreated cells. Endothelin is a known mitogen of C6 cells and in the presence of DMI it increased thymidine incorporation dose dependently up to 1.7 fold. This increase was reversed by the antiidiotypic anti-opiate receptor antibody Ab2AOR, which has opioid agonist properties. The opioid antagonist, naltrexone blocked the inhibition by Ab2AOR. Endothelin also enhanced phosphatidylinositol (PtdIns) turnover and this effect was inhibited by morphine or Ab2AOR (35% and 33%, resp.) in DMI-treated but not in untreated C6 cells. The actions of morphine and Ab2AOR were reversed by naltrexone. Moreover, blockade of PtdIns turnover and thymidine incorporation by Ab2AOR or morphine were insensitive to pertussis toxin. Since PtdIns turnover is known to induce Ca^{2+} mobilization, it was of interest to examine the mechanism by which opioids may alter intracellular Ca^{2+} concentrations. We found that Ab2AOR reversed endothelin-induced Ca^{2+} mobilization in DMI-treated but not untreated C6 cells. The effect of Ab2AOR was again blocked by naltrexone. The results indicate that glial cells can be a target of an opioid receptor-mediated anti-mitogenic action and that an abatement in PtdIns turnover and Ca^{2+} mobilization may be involved in this mechanism. *Supported by the Anti-drug Authority of Israel, the United States-Israel Binational Science Foundation, and the Israel Cancer Research Fund.*

638.11

REGIONAL VARIATION IN THE RATIO OF σ_1 TO σ_2 BINDING IN RAT BRAIN. M. L. Leitner, A.G. Hohmann, S.L.Patrick, and J.M. Walker. Schrier Research Laboratory, Dept. of Psychology, Brown University, Providence, RI 02912.

Recent work has postulated the existence of multiple subtypes of the sigma receptor termed σ_1 and σ_2 (reviewed by Quirion et al., *Trends Pharmacol. Sci.* 13: 85), and behavioral studies have postulated a role of σ_2 sites in motor behavior (Walker et al., *Eur. J. Pharmacol.* 231:61). This study examined the distribution of subtypes of the σ receptor in discrete regions of rat brain and in whole guinea pig brain. Rat brains were dissected into nine regions (hindbrain, midbrain, hypothalamus, thalamus, septum, hippocampus, striatum, cortex, cerebellum), pooled and used to prepare membranes for ligand binding studies. Whole guinea pig brains were prepared in an identical manner. σ_1 Binding was labeled using the selective σ_1 compound [^{3}H](+)-pentazocine and σ_2 receptors were labelled with [^{3}H]1,3 di-o-tolylguanidine (DTG) in the presence of 1 μM dextrallorphan to mask σ_1 sites. Nonspecific binding was determined in the presence of 10 μM haloperidol. Experiments revealed a marked variation in the ratio of σ_1 to σ_2 sites across rat brain regions as well as a consistently higher proportion of σ_2 sites in all areas studied. Guinea pig brain showed a preponderance of σ_1 receptors. These data support prior evidence for a biological role of the σ_2 site in motor function, because areas showing the highest levels of σ_2 binding included the cerebellum, cortex, hindbrain and midbrain. The data also clearly demonstrate a significant difference in receptor abundance between species. Finally, these experiments lend support to the notion that σ_1 and σ_2 sites are biologically distinct entities.

This work was supported by MH48869 and DA04988 (JMW).

638.12

EFFECTS OF SIGMA LIGANDS ON STRIATAL DOPAMINE SYNTHESIS AND ROTATIONAL BEHAVIOR IN RATS. SD Weiser, SL Patrick, SW Mascarella, X Bai, FI Carroll, JM Walker, RL Patrick. Depts of Psychology & Neuroscience, Brown University, Providence, RI 02912, and Research Triangle Institute, Research Triangle Park, NC 27709.

The present studies were undertaken to investigate the biochemical and behavioral effects of sigma ligands on nigrostriatial dopamine activity. We examined the *in vivo* effects of unilateral intranigral injections of two high affinity sigma compounds, 1,3-di-o-tolylguanidine (DTG) and (-)-deoxy-N-benzylnormetazocine (RTI-4612-58), on striatal tyrosine hydroxylase activity and rotational behavior in rats. The rate of dopamine synthesis was determined by measuring the accumulation of L-3,4-dihydroxyphenylalanine (DOPA) following inhibition of dopa decarboxylase with NSD 1015. DTG and RTI-4612-58 dose-dependently increased striatal dopamine synthesis and rodent turning behavior following intranigral administration. These findings provide direct evidence that sigma sites in the substantia nigra play a role in the modulation of dopamine neurotransmission. We also explored the possibility that nigral NMDA receptors mediate the dopaminergic effects of sigma ligands by examining whether the NMDA antagonist, (±)-3-(2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid (CPP), reversed the effects of DTG. CPP, when co-injected with DTG in the substantia nigra, failed to alter DTG- induced increases in striatal DOPA accumulation. CPP also failed to inhibit DTG- evoked rotational behavior. However, this finding is difficult to interpret in view of the fact that CPP elicited vigorous contralateral turning behavior when administered alone. Finally, we determined whether some commonly used anesthetic agents interfered with sigma ligand-induced stimulation of dopamine synthesis. Pre-treatment with halothane and ketamine completely eliminated the effects of DTG, while pretreatment with chloral hydrate did not impede DTG-induced enhancement of dopamine synthesis. These results suggest that chloral hydrate would be the most reliable anesthetic agent to use with *in vivo* neurophysiological experiments.

638.13

SEX DIFFERENCES IN DTG-INDUCED ROTATORY BEHAVIOR: FURTHER EVIDENCE OF A FUNCTIONAL INTERACTION OF *SIGMA* RECEPTORS WITH NIGROSTRIATAL DOPAMINERGIC TRANSMISSION. MK Hemstreet*[1], SL Patrick[2], and JM Walker[2]. [1]Dept. of Neuroscience and [2]Schrier Research Laboratory, Dept. of Psychology, Brown University, Providence, RI 02912.

The influence of physiological and surgical changes in hormonal status upon the behavioral and binding characteristics of DTG were examined in male and female rats. In the first set of experiments, induction of rotatory behavior following unilateral intranigral microinjection of 4nmol DTG was examined in intact cycling female rats and compared with that of male littermates. Estrous stage was determined by daily vaginal lavage and cytological examination. In the second set of experiments, male and female rats gonadectomized or sham-operated as adults were tested behaviorally 30 days after surgery. Intranigral injections of 4nmol DTG were again used to quantify efficacy of the *sigma* ligand. In the third set of experiments, male and female rats were neonatally gonadectomized or sham-operated, then received intranigral DTG (4nmol) as adults. Rotatory behavior was quantified and compared with that of intact littermates. All animals were sacrificed following behavioral studies and their brains processed for both histological confirmation of intranigral injections and radioligand binding studies using [^{3}H]DTG.

Overall, females had a significantly greater rotatory response to intranigral DTG than did males (p=0.0003); this was especially apparent in proestrus/estrus females. Adult gonadectomy had no effect on DTG-induced behavior in either sex, although neonatal gonadectomy significantly potentiated the behavioral effects of DTG in males (p=0.05). This suggests a developmental rather than tonic interaction of steroidal hormones with *sigma* receptors and dopaminergic function. Sham operations were without effect at either age. Binding of [^{3}H]DTG did not differ significantly among the various groups, and multiple regression analysis indicated that the best predictor of DTG-induced rotatory behavior was sex of the animal regardless of *sigma* binding parameters. As *sigma* ligand-induced circling is dopamine-dependent, potentiation in intact females is consistent with the known facilitatory effect of ovarian steroids upon dopamine tone.

638.14

KAPPA$_2$ OPIOID RECEPTORS INHIBIT NMDA RECEPTOR-MEDIATED SYNAPTIC CURRENTS IN THE CA3 OF GUINEA PIG HIPPOCAMPUS. R.M. Caudle*, C. Chavkin[2], R. Dubner. [1]NAB, NIDR, NIH, Bethesda, MD 20892, [2]Dept. Pharmacol., Univ. Washington, Seattle, WA 98195.

The role of the endogenous opioid dynorphin A (1-17) in regulating N-methyl-D-aspartate (NMDA) receptor-mediated synaptic currents was examined in guinea pig hippocampus. Shaffer collateral/commissural fiber-evoked NMDA synaptic currents were recorded using whole cell patch clamp techniques in CA3 pyramidal cells. Dynorphin was found to have dual effects on NMDA synaptic currents: increasing them at low concentrations (10 - 1000 nM) and decreasing them at high concentrations (5 μM). Only the inhibitory action of dynorphin was sensitive to naloxone indicating that this effect was mediated by an opioid receptor. The inhibitory effect was mimicked by bremazocine, but not by U69,593, U50,488, [D-Ala^2, N-Me-Phe^4, Gly-ol]-enkephalin or [D-Pen^{2,5}]-enkephalin. In addition, the inhibitory effect of 1 μM bremazocine was blocked by 1 μM naloxone, but not by the selective kappa$_1$ antagonist nor-binaltorphimine (1 μM) or the selective mu antagonist cyprodime (1 μM). By process of elimination these data suggest that the inhibitory effect of bremazocine and dynorphin was mediated by the kappa$_2$ subtype of opioid receptor. In addition, 1 μM naloxone and anti-sera to dynorphin A (1-17) were found to increase NMDA mediated synaptic currents. Nor-binaltorphimine, cyprodime and anti-sera to met-enkephalin did not increase the NMDA synaptic current. Overall, these findings indicate that dynorphin is an endogenous agonist for kappa$_2$ receptors in the CA3 region of the guinea pig hippocampus and that these receptors regulate NMDA receptor function.

638.15

ROLE OF DELTA- AND KAPPA-OPIOID RECEPTORS IN MODULATING COCAINE-INDUCED BEHAVIORAL SENSITIZATION IN THE RAT.
Christian Heidbreder, Mohammed Shoaib & Toni Shippenberg*. *Behavioral Pharmacology and Genetics Section, Preclinical Pharmacology Laboratory, National Institute on Drug Abuse, P.O. Box 5180, Baltimore, MD 21224, USA.*

The effects of treatment with the selective kappa-opioid receptor agonist U69593 and the delta-opioid receptor antagonist naltrindole upon cocaine-induced changes in locomotor activity and extracellular dopamine levels were examined in rats. Daily injections of cocaine resulted in a dose-dependent sensitization to its locomotor activating effects. In contrast, such sensitized responses failed to develop in animals which had previously received U69593 (0.16-0.32 mg/kg s.c.) or naltrindole (0.3-3.0 mg/kg s.c.) in conjunction with daily cocaine treatment. Interestingly, such pre-treatment was unable to modify the behavioral sensitization induced by another psychostimulant, e.g. nicotine. *In vivo* microdialysis studies indicate that the doses of U69593 which attenuate the behavioral effects of cocaine fail to modify cocaine-induced increases in extracellular dopamine levels within the nucleus accumbens. Taken together, these data thus demonstrate that the selective blockade of delta-opioid receptors or the activation of kappa-opioid receptors prevents the development of cocaine-induced sensitization. In view of the postulated role of sensitization in the maintenance of cocaine abuse, our results suggest that selective kappa agonists and delta-opioid receptor antagonists may be useful in the pharmacological management of cocaine addiction.

638.16

SIGMA INHIBITION OF THE CHOLINERGIC PHOSPHOINOSITIDE RESPONSE: CHARACTERIZATION OF PARTIAL AGONISTS AND ANTAGONISTS AT SIGMA-1 RECEPTORS. K.K. Hsu, J.M. Cutts*, B.R. de Costa, and W.D. Bowen. Lab. Med. Chem., NIDDK, Bethesda, MD 20892.

Our previous work has suggested that activation of sigma-1 receptors results in non-competitive inhibition of the muscarinic phosphoinositide response in rat brain synaptoneurosomes (in: Multiple Sigma and PCP Receptor Ligands: Mechanisms for Neuromodulation and Neuroprotection?, J.-M. Kamenka and E.F. Domino, eds., NPP Books, Ann Arbor, pp. 155-167, 1992). Examination of the "efficacy ratio" [sigma-1 K_i (nM)/PPI inhibitory ED_{50} (uM)] for several sigma compounds allows an approximate ranking in order of intrinsic activity as sigma-1 agonists in this assay, where lower values represent lower efficacy. Sigma compounds tested to date have efficacy ratios in the general range of 0.40 - 1.71. For example, haloperidol, 0.40; (+)-pentazocine, 0.43; dextrallorphan, 0.72; fluphenazine, 0.73; and DTG, 1.71. The novel aryl ethyl(pyrrolidinyl)cyclohexylamine BD737 exhibits an efficacy ratio of 1.3 nM/3.1 uM = 0.42. Removal of the cyclohexane ring from BD737 and opening of the pyrrolidine ring produces the aryl ethylene diamines, BD1008 and BD1139. These compounds maintain high affinity for sigma-1 sites, but have over 10-fold lower efficacy than the parent compound: BD1008, 0.34 nM/10.3 uM = 0.033; BD1139, 5.78 nM/150 uM = 0.039. These data suggest that these compounds may have partial agonist properties. BD1139 produced dose dependent, non-competitive inhibition of oxotremorine-M (oxo-M) stimulated PPI turnover at concentrations above 50 uM. However, at 50 uM and below, BD1139 had little or no effect on oxo-M-stimulated PPI turnover. At 50 uM, BD1139 significantly attenuated the ability of 30 uM (+)-pentazocine and 30 uM SH311 to non-competitively inhibit oxo-M-stimulated PPI turnover. These data appear to confirm that BD1139 is a partial agonist at sigma-1 sites, with antagonist activity in the lower concentration range. Other sigma compounds in this and related series which have low efficacy ratios will also be tested for antagonist activity using this assay.

638.17

[^{3}H](+)-AZIDO-PHENAZOCINE: CHARACTERIZATION AS A SELECTIVE PHOTOAFFINITY PROBE FOR SIGMA-1 RECEPTORS. W.E. Williams, R. Wu, B.R. de Costa, and W.D. Bowen*. Laboratory of Medicinal Chemistry, NIDDK, NIH, Bethesda, MD 20892.

Heterogeneity of sigma binding sites has been suggested on the basis of differences in ligand selectivity profiles using various radioligands and tissues (for reviews, Walker et al., *Pharmacol. Rev.* 42: 355-402, 1990; Quirion et al., *Trends Pharmacol. Sci.* 13: 85-86, 1992). Sigma-1 and sigma-2 receptors differ in their affinity for (+)-benzomorphans, with sigma-1 receptors having much higher affinity for these compounds than sigma-2. These two subtypes have also been differentiated on the basis of photoaffinity labeling using [^{3}H]azido-DTG ([^{3}H]Az-DTG) and determination of molecular weight by SDS polyacrylamide gel electrophoresis (Hellewell and Bowen, *Brain Res.* 527: 244-253, 1990). Sigma-1 receptors have an apparent molecular weight of 25 kDa, whereas sigma-2 receptors have a molecular weight of 18-21 kDa. Since [^{3}H]Az-DTG labels both sigma-1 and sigma-2 sites, we attempted to design a selective photoaffinity probe for sigma-1 sites. The benzomorphan, (+)-phenazocine was found to be highly selective for sigma-1 receptors (sigma-1 K_i = 3.90 nM; sigma-2 K_i = 1,269 nM) (Di Paolo et al., *Soc. Neurosci. Abstr.* 17: 814, 1991). On this basis, we synthesized unlabeled and radiolabeled (+)-azido-phenazocine. Unlabeled (+)-azido-phenazocine exhibited a sigma-1 K_i = 1.34 $\pm$ 0.21 vs. the selective sigma-1 probe, [^{3}H](+)-pentazocine in guinea pig brain. Competition studies (carried out in the dark) revealed that 5 nM [^{3}H](+)-azido-phenazocine ([^{3}H](+)-Az-PHEN) labeled a site in guinea pig brain with a typical sigma-1 profile, similar to that labeled by [^{3}H](+)-pentazocine and [^{3}H](+)-3-PPP: (+)-pentazocine > haloperidol = DTG > (+)-3-PPP > (+)-SKF 10,047 > (-)-pentazocine > (-)-SKF 10,047. Ligands for other receptors were inactive. Thus, [^{3}H](+)-Az-PHEN appears to selectively label sigma-1 receptors, as predicted. Results of studies to determine the ability of this probe to photolabel sigma-1 receptor polypeptides will be discussed.

638.18

ANTAGONISM OF OPIOID ANALGESIA: A FUNCTIONAL ROLE FOR SIGMA$_1$ RECEPTORS. Chih-cheng Chien*#+, Gavril W Pasternak#. #The Cotzias Laboratory of Neuro-Oncology, Memorial Sloan-Kettering Cancer Center and Depts. of Neurology/Neuroscience and Pharmacology, Cornell U. Medical College, NY, NY 10021 and +Cathay General Hospital, Taipei, Taiwan.

Sigma binding sites have been well described. Recent studies have identified several subtypes, including the sigma$_1$ site which is highly selective for (+)pentazocine. However, the functional significance of these sites remains unclear. It has been assumed that (±)pentazocine reverses morphine analgesia due to its partial-agonist action on mu receptors. However, both (+) and (-)pentazocine antagonize morphine analgesia equally well, implying that this antagonism does not involve opioid receptors. Similar antagonism can also be observed with kappa$_1$ and kappa$_3$ analgesia. In additional experiments, DTG [1,3-Di(2-tolyl)guanidine] also significantly attenuates morphine analgesia. Together, these results imply a sigma$_1$ mechanism of action. Haloperidol is a known D_2 and sigma$_1$ antagonist. Haloperidol reverses the action of either (+) or (-)pentazocine on morphine analgesia in doses as low as 0.5 mg/kg. In contrast, the selective D_2 antagonist (-)sulpiride has no effect, implying that haloperidol is acting as a sigma$_1$ antagonist. Haloperidol also enhances analgesia in the absence of pentazocine, particularly for kappa drugs. Our results indicate that sigma$_1$ activity suppresses mu, kappa$_1$ and kappa$_3$ analgesia. The potentiation of opioid analgesia by haloperidol suggests the presence of a tonically active antiopioid system involving sigma$_1$ receptors.

638.19

MODULATION OF I_h BY cAMP-DEPENDENT PROCESSES. S.L. Ingram*[1] and J.T. Williams[2] Department of Pharmacology[1] and Vollum Institute[2], Oregon Health Sciences University, Portland, OR 97201.

Opioid receptors are known to both inhibit adenylate cyclase (AC) and suppress the activity of CNS neurons. It is not known, however, whether these two actions are linked. We have made whole-cell electrophysiological recordings from the soma of primary afferent neurons in culture and looked at the effects of opioids on a cAMP-activated ion current (I_h). Forskolin (10 μM), an AC stimulator, shifts the activation curve for Ih to more depolarized potentials and augments the current. [Met]^5-enkephalin (1-10 μM) can reduce the forskolin-mediated augmentation of I_h in some neurons. It is possible that other compounds linked to AC stimulation can also increase I_h and be depressed through opioid inhibition of AC. Since I_h is a hyperpolarization-activated, mixed Na/K current which seems to be important in stabilizing resting membrane potential, modulation of I_h by nociceptive agents may play a role in the increased neuronal excitability of primary afferents associated with pain and inflammation. Modulation of ion currents suggests a possible mechanism for a peripheral pain-relieving action of opioids.

(Supported by DAO8163.)

638.20

THE EFFECTS OF AGING ON SPINAL OPIOID-INDUCED ANTINOCICEPTION. D.L. Hoskins, J.L. Stafinsky and T. Crisp*. Department of Pharmacology, Northeastern Ohio Universities College of Medicine, Rootstown, OH 44272-0095.

This study compared the effects of aging on the spinal antinociceptive properties of the mu opioid agonist DAMPGO and the delta agonist DPDPE. Young, mature and aged Fischer 344 rats (6,16, and 26 months respectively) were injected intrathecally (i.t.) with different doses of DAMPGO or DPDPE, and the antinociceptive efficacy of these agents was tested on the tail-flick analgesiometric assay. All three age groups responded to i.t. DAMPGO in a dose-dependent manner, but higher doses were required to produce significant elevations in tail-flick latency (TFL) in the aged cohort. The spinal effects of DPDPE were also age and dose-dependent. Whereas the antinociceptive effects of DPDPE were dose-dependent in the 6 and 16 month old animals, even the highest dose tested (300 nmol) failed to significantly elevate TFL in the 26 month old group. The aging process apparently alters the pain-inhibitory function of the delta opioid receptors in the rat spinal cord. The present finding that spinal opioid-induced antinociception is diminished in older animals may be attributable to an age-related decline in the number and/or affinity of spinal opioid receptor sites.

639.1

NGF REGULATION OF $5HT_3$ RECEPTOR EXPRESSION. I.A. Ukhun*†‡, S.G. Holstad†‡, S. Jafri*, I. Uchida*, J. Yang*, & K.E. Isenberg‡. †St. Louis College of Pharmacy; ‡Dept. of Psychiatry, Washington University School of Medicine, St. Louis, MO 63110; *Dept. of Pharmacology, University of Maryland, Baltimore, MD 21201.

PC12 cells show detectable expression of $5HT_3$ receptors upon exposure to NGF. We cloned the rat $5HT_3$ receptor and sought to understand its regulation by NGF. We report electrophysiologic, and polymerase chain reaction (PCR) data supporting regulation of receptor expression via increases in PC12 cell steady-state mRNA levels. Rat $5HT_3$ receptor clones were selected from a rat superior cervical ganglion cDNA library employing an oligonucleotide that detected nicotinic receptor clones; sequence analysis revealed strong homology (95% amino acid similarity) with the previously published mouse receptor subunit. PC12 cells (ATCC) were cultured with and without 50ng/ml of NGF. Whole cell patch clamp recording did not reveal a detectable response to serotonin (10uM) in the absence of NGF; exposure to NGF resulted in steadily increasing peak current in response to serotonin over 8 days. RNA was harvested from PC12 cells after exposure to NGF for 1, 2, 4, 6, and 8 days; PCR was performed quantitatively by varying cycle number over the linear range of the assay. Exposure of PC12 cells to NGF increases the steady-state mRNA levels for the $5HT_3$ receptor in a fashion that precedes and parallels sensitivity to serotonin. The induction of $5HT_3$ receptor expression by NGF involves increasing steady-state mRNA levels and occurs on a time scale suggestive of induction via primary response genes.

639.2

THE SEROTONIN-3 RECEPTOR AGONIST 1-(M-CHLOROPHENYL)-BIGUANIDE INCREASES EXTRACELLULAR DOPAMINE AND SEROTONIN CONCENTRATIONS IN THE NUCLEUS ACCUMBENS OF RATS. A.D. Campbell* and W.J. McBride. Department of Psychiatry, Institute of Psychiatric Research, Indiana University School of Medicine, Indianapolis, IN 46202-4887.

Serotonin (5-HT) and dopamine (DA) neuronal systems projecting to the nucleus accumbens (NA) have been implicated in the rewarding properties of many drugs of abuse, including ethanol. Both systemic and local administration of pharmacologically relevant doses of ethanol have been shown to enhance DA and 5-HT release in the NA. Since this effect is inhibited by $5\text{-}HT_3$ receptor antagonists, the present study was conducted to examine further the role of this receptor subtype in stimulating the release of DA and 5-HT. Application through the dialysis probe of 1-(m-chlorophenyl)-biguanide (CPBG), a $5\text{-}HT_3$ agonist, dose-dependently enhanced the extracellular concentrations of both DA and 5-HT in the NA, as measured by HPLC with electrochemical detection. CPBG appeared to be more potent at enhancing DA efflux, however it was more efficacious in enhancing the 5-HT response. Indeed, the highest concentration used (100 μM) caused an approximately 1500% increase in extracellular DA, whereas the same concentration caused an approximately 3000% increase in 5-HT levels. Corresponding decreases in the major metabolites occurred with the increases in these two monoamines. The CPBG - stimulated release of DA and 5-HT was partially inhibited by the $5\text{-}HT_3$ antagonist ICS 205-930 and was partially Ca^{++} - dependent. The mechanism of CPBG-induced release of DA and 5-HT is not known, however, the results suggest that the effect may be mediated at least in part by $5\text{-}HT_3$ receptors. (Supported by PHS Grants AA 09090 and AA 08553.)

639.3

ELECTROPHYSIOLOGICAL STUDIES OF THE SEROTONERGIC (5-HT) MODULATION OF GLUTAMATE-EVOKED ACTIVITY IN AMYGDALA NEURONS. *E.J. Mah* and K.A. Cunningham*, Dept Pharmacol, Univ TX Med. Branch, Galveston, TX 77555-1031.

Serotonin (5-HT) receptor subtypes designated as $5\text{-}HT_{1A}$ and $5\text{-}HT_3$ have been implicated in the actions of compounds showing anxiolytic activity. The amygdala is thought to be important in mediating anxiogenic, emotional and fear responses; it also has a significant population of both $5\text{-}HT_{1A}$ and $5\text{-}HT_3$ receptors. Little information, however, is available concerning the electrophysiological responses to 5-HT in this region. Previous research has indicated that microiontophoretic application of 5-HT predominantly inhibits the activity of neurons found in subnuclei of the rat amygdala (Callahan & Cunningham, SN Abst 21: 679), although the specific 5-HT receptors which mediate this response are currently uncharacterized. In the present experiment, single unit extracellular and microiontophoretic techniques are being used to study the effects of the $5\text{-}HT_{1A}$ agonist 8-hydroxy-2-(di-n-propylamino)tetralin (8-OHDPAT; 1 mM) and the $5\text{-}HT_3$ agonist methyl-chlorophenylbiguanide (MCPB; 10 mM). Both spontaneously-active and glutamate-stimulated (50 mM;-3.0 to -35.0 nA) neurons in the central, lateral, and basolateral amygdala were studied. Neither 8-OHDPAT (2.5-40 nA; N=4) nor MCPB (2.5-40 nA; N=4) significantly altered spontaneously-active amygdala neurons; however, both 8-OHDPAT and MCPB elicited current-related inhibitions of glutamate-stimulated activity, with a maximal 40-50% inhibition at 40 nA. As glutamate plays a major role in feed-forward transmission in the amygdala, these data suggest that $5\text{-}HT_{1A}$ and $5\text{-}HT_3$ receptor modulation of glutamate-evoked activity may be of important functional significance to information processing in this area. Experiments with additional $5\text{-}HT_{1A}$ and $5\text{-}HT_3$ agonists and antagonists are underway to further characterize the electrophysiological effects of 5-HT on glutamate transmission in the amygdala. Supported by NIDA DA05708 and DA06511.

639.4

A slow excitatory postsynaptic potential mediated by $5\text{-}HT_2$ receptors in guinea-pig central neurons. D.H. Bobker*. Dept of Neurology and Vollum Institute, Oregon Health Sciences University, Portland, OR 97201

Intracellular recordings were made from neurons of the nucleus prepositus hypoglossi in slices of guinea-pig medulla *in vitro*. 5-hydroxytryptamine (5-HT, serotonin) caused a hyperpolarization followed by a late depolarization. The hyperpolarization was mediated by $5\text{-}HT_{1A}$ receptor activation and could be selectively blocked by pindobind-$5HT_{1A}$ (PBD). 5-HT then caused a depolarization only, with an EC_{50} of 21 nM (in cocaine 10 μM). A selective $5\text{-}HT_2$ agonist, (±)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI), also caused a depolarization, with an EC_{50} of 2.6 nM. Ketanserin and spiperone, $5\text{-}HT_2$ antagonists, blocked the depolarization due to both compounds.

Focal electrical stimulation caused an inhibitory postsynaptic potential (IPSP) mediated by 5-HT acting upon $5\text{-}HT_{1A}$ receptors, and a slow excitatory postsynaptic potential (s-EPSP). PBD blocked the IPSP, leaving an isolated s-EPSP. Both spiperone and ketanserin antagonized the s-EPSP, while DOI occluded it. The s-EPSP was from 2-10 mV in amplitude, 35-50 sec in duration and showed voltage-dependence consistent with a potassium-mediated current. Cocaine increased the s-EPSP amplitude by 108%, while having little effect on duration. Both the IPSP and the s-EPSP were presynaptically inhibited by the $5\text{-}HT_{1D}$ agonist sumatriptan. This data indicates that the s-EPSP is mediated by 5-HT acting upon $5\text{-}HT_2$ receptors and expands the role of 5-HT as an excitatory neurotransmitter in the central nervous system.

Supported by grants NS01574-01 from NIH and from the Natl Alliance for Res. on Schizophrenia and Depression.

639.5

REGULATION OF $5\text{-}HT_2$ RECEPTOR BINDING SITES BY PERTUSSIS TOXIN AND PHORBOL ESTER IN CEREBELLAR GRANULAR CELLS. H. CHEN* AND D.-M. CHUANG. Section on Molecular Neurobiology, Biological Psychiatry Branch, NIMH, NIH, Bethesda, MD 20892.

We have documented that persistent stimulation of $5\text{-}HT_2$ receptors in cerebellar granule cells causes an increase in the Bmax of $5\text{-}HT_2$ receptors. (Akiyoshi et al., Mol. Pharmacol. 43:349-355, 1993). To investigate the role of $5\text{-}HT_2$ receptor - mediated phosphoinositide turnover in this paradoxical receptor up-regulation, we examined the effects of suppression of IP_3/DAG production by pretreatment with pertussis toxin (PTX) or phobol dibutyrate (PDBu). Cells were pretreated with PTX (100 ng/ml) for 16 hr before stimulation of $5\text{-}HT_2$ receptors with DOI (10mM) for 24 hr. PTX alone was unexpectedly found to increase [^{3}H] ketanserin binding to $5\text{-}HT_2$ receptors in intact cells. Pretreatment with both PTX and DOI produced a binding increase greater than that elicited by either drug alone, indicating that the DOI induced up-regulation was not inhibited. The effect of PTX was time- and dose- dependent and appeared to be unrelated to a increase in basal cAMP level. Moreover, PTX pretreatment did not influence [^{3}H] QNB binding to muscarinic receptors. Cells were also exposed to PDBu (500 nM) for 15 min or 16 hr before the 24-hr pretreatment with DOI. PDBu alone caused a significant increase in $5\text{-}HT_2$ receptor binding and in conjunction with DOI produced an effect greater than that elicited by DOI alone. Our results suggest that production of IP_3/DAG by $5\text{-}HT_2$ receptor stimulation is not a prerequisite for the delayed up-regulation of $5\text{-}HT_2$ receptor binding sites.

639.6

CHARACTERIZATION OF THE FUNCTIONAL RESPONSES OF THE CLONED RAT $5\text{-}HT_{2B}$ ($5\text{-}HT_{2F}$) RECEPTOR. D. L. Nelson*, M. Baez, L. A. Mercurio, P. A. Hyslop, D. V. Pearson, and V. L. Lucaites. Lilly Research Labs, Eli Lilly and Company, Indianapolis, IN 46285

The $5\text{-}HT_{2B}$ receptor (originally called $5\text{-}HT_{2F}$) belongs to the $5\text{-}HT_2$ subfamily of receptors, which is known to stimulate phosphatidylinositol (PI) hydrolysis. The functional aspects of the $5\text{-}HT_{2B}$ receptor were explored by looking at its effect on the production of inositol 1,4,5-trisphosphate (IP_3). In AV-12 cells expressing the $5\text{-}HT_{2B}$ receptor, 5-HT potently stimulated IP_3 production (EC_{50} = 15.7 ± 3.1 nM, n=16). The IP_3 response to 5-HT was rapid, with peak IP_3 production (6-21 fold increases over basal levels) occurring within 5-10 seconds. The peak response fell to a new plateau (1.6-2.7 fold over basal levels) within 60 seconds. The IP_3 response to 5-HT was paralleled by the 5-HT-induced increase in Ca^{++} flux. Pretreatment of the cells with the potent antagonists 1-napthylpiperazine (1-NP) and methysergide resulted in apparent noncompetitive inhibition of 5-HT-stimulated IP_3 production. For comparison, total PI hydrolysis was examined by measuring the accumulation of inositol monophosphate (IP) in the presence of LiCl. This response was linear for 30 min. Methysergide or 1-NP preincubated with cells before measuring 5-HT-induced IP accumulation also showed noncompetitive antagonism, suggesting that the initial IP_3 peak may be the major contributor to the overall accumulation of IP even at 30 min. The rapid and transient nature of the $5\text{-}HT_{2B}$-mediated IP_3 response precludes the calculation of pA_2 values for antagonists by traditional methods that assume equilibrium kinetics between receptors, agonists, and antagonists.

639.7

EFFECTS OF AGONIST & ANTAGONIST ON SEROTONIN-INDUCED INTRACELLULAR CALUCIUM MOBILIZATION IN 5-HT_{1C} RECEPTOR TRANSFECTED CELL. J. Akiyoshi*[1], R. Kohara[1], H. Ono[1], H. Yamamoto[1], K. Yamada[1] A. Nishizono[2], K. Mifune[2] and I. Fujii[1]. [1]Dept. of Neuropsychiatry, [2]Dept. of Microbiology, Oita Medical University, Hasama-Machi, Oita 879-55, Japan.

The 5-HT_{1C} receptor is one of the major serotonin receptor subtypes and a functional cDNA of this receptor was cloned and characterized. The 5-HT_{1C} receptor is widely distributed in the CNS.

We have studied effects of 5-HT_{1C} receptor agonists (5-HT, mCPP) and antagonists (mianserin, mesulergine) on 5-HT_{1C} receptor transfected cells. 5-HT or mCPP-induced changes in the levels of intracellular Ca^{++} were analyzed in 5-HT_{1C} receptor transfected cells, using the Ca^{++}-sensitive dye fura-2/AM. The fluorescence of fura-2/AM was measured in a fluorescence spectrophotometer with excitations at 340 and 360 nm. 5-HT or mCPP mobilized intracellular Ca^{++} in a dose-dependent fashion from basal level. A second stimulation by 5-HT did not produce a rise in intracellular Ca^{++} after previous exposure to 5-HT in the plateau phase. Mianserin, mesulergine, 5-HT_{1C} receptor antagonists, reversed the 5-HT-induced Ca^{++} increase in a dose-dependent manner.

These results show that 5-HT_{1C} receptor agonists induces 5-HT_{1C} receptor desensitization and 5-HT_{1C} receptor antagonists blocks this response in 5-HT_{1C} receptor transfected cell.

639.8

5HT1A RECEPTORS (5HT1AR) EXPRESSED IN *XENOPUS* OOCYTES CAN COUPLE EITHER POSITIVELY OR NEGATIVELY TO DIFFERENT ISOFORMS OF ADENYLYL CYCLASE (AC) OR PHOSPHOLIPASE C (PLC) THROUGH G-PROTEIN βγ SUBUNITS. Y. Uezono*, C. Min, J. Bradley, C. Chavkin#, N. McCarty, M. Quick, J.R. Riordan@, H.A. Lester, K. Zinn and N. Davidson. Division of Biology, Caltech, Pasadena, CA 91125; #Dept. of Pharmacol. SJ-30, Univ. of Washington, Seattle WA 98125; @Hospital for Sick Children, Toronto, Ontario, Canada.

We have reported that the A-kinase-activated Cl^- channel encoded by the cystic fibrosis transmembrane conductance regulator gene (CFTR) can act as a reporter for changes in cAMP concentration in *Xenopus* oocytes (*Soc. Neurosci. Abstr.* **18**:106, 1992). In oocytes injected with CFTR cRNA, we can differentiate between cAMP changes and PLC activation by measuring currents from CFTR and endogenous Ca^{2+}-activated Cl^- currents, respectively. 5HT1ARs are reported to couple to Gi-proteins and thus inhibit AC. However, in oocytes coinjected with cRNAs for the CFTR, 5HT1AR and adrenergic β_2 receptor, activation of the 5HT1AR enhances, instead of inhibiting, the β_2-induced CFTR current. We believe that this effect is through Gβγ and that oocytes contain one of the isotypes of AC for which Gβγ subunits enhance the stimulation of AC by Gsα subunits. In the present case, the Gβγ subunits are activated by agonist stimulation of the 5HT1AR. The effect is augmented two fold by coinjection of cRNA for AC type II, but not AC type III, as expected given this interpretation. Coinjection of cRNA for the Goα subunit abolishes the 5HT1AR enhancement by scavenging the liberated Gβγ, and an 80% inhibition of β_2-induced CFTR current due to the usual action of Gi is revealed.

PLCβ2 is reported to be activated by Gβγ. In agreement with this, coexpression of PLCβ2 and 5HT1AR results in a 5HT-induced Ca^{2+}-activated Cl^- current. Thus, depending on the effector subtypes present and the network of interactions of Gα and Gβγ subunits, agonist activation of a particular receptor will have different effects. (Support: MH-49176, GM-29836, CA TRDRP).

639.9

SEROTONIN INDUCTION OF AP-1 AND CRE DNA BINDING ACTIVITIES IN RAT C6BU-1 GLIOMA CELLS. M. IKEDA*, C. YAMAZAKI, M. MIKUNI AND K. TAKAHASHI Department of Mental Disorder Res., Natl. Inst. of Neurosci., NCNP, Kodaira, Tokyo 187, Japan

Neurotransmitters regulate the transcription of genes and may be involved in the modulation of cellular functions. Previously, we have found that serotonin induced intracellular Ca^{2+} mobilization in rat C6BU-1 glioma cells through the stimulation of 5HT2 receptors. To better understand the effect of serotonin on transcriptional control of genes, it is important to know its effect on transcriptional factors. Using gel shift assay, we detect the increases of AP-1 and CRE DNA bindings in C6BU-1 cells with serotonin treatment. The affinity and /or levels of protein binding to the AP-1 element was increased in nuclear extracts isolated from C6BU-1 cells treated for 4hr. with serotonin (10 μ M). The CRE DNA binding was also increased after 4hr. of serotonin treatment. The bindings of proteins to the AP-1 and CRE element were shown to be specific in gel shift assays utilizing competitor DNAs. These inductions of DNA bindings were blocked by pretreatment of ketanserine. These findings suggests that the activities of AP-1 and CRE DNA binding in C6BU-1 cells may be modulated by the stimulation of 5HT2 receptors.

639.10

FUNCTIONAL EXPRESSION OF 5-HT_{1A} RECEPTORS IN EMBRYONIC GLIAL CELLS

J. Liu*, R.P. Hellendall and J.M. Lauder, Dept. of Cell Biol. & Anat., Univ. N.C., Chapel Hill, NC 25799-7090.

These studies were designed to determine whether 5-HT_{1A} receptors, expressed by glia in the vicinity of developing 5-HT neurons or their axons, regulate synthesis of the serotonergic growth factor, S-100β. Purified glia (PGLIA) were prepared from embryonic day 14 (E14) rostral raphe (GRR) or substantia nigra (GSN) and cultured in 10% fetal calf serum + BME. At 2 days *in vitro* (DIV), cultures were switched to serum-free medium (BME + ITS + 1%BSA) and grown for another 3-6 days. Immunocytochemistry revealed that both 5-HT_{1A} receptors and S-100β were maximally expressed between 5-8 DIV. Cultures were treated at 5 DIV with 10 nM 8-OH-DPAT to stimulate 5-HT_{1A} receptors. Effects of DPAT on S-100β protein and mRNA were quantified by immunobinding and *in situ* hybridization assays. cAMP was measured by an ^{125}I-radioimmunoassay. Exposure to DPAT elevated cAMP, and differentially regulated S-100β mRNA and protein in GRR and GSN. These results indicate that embryonic glia from RR and SN express functional 5-HT_{1A} receptors, but respond differently to agonist. This raises the possibility that activation of glial 5-HT_{1A} receptors could differentially regulate neurotrophic activity for developing 5-HT neurons in the vicinity of their cell bodies and axons.

639.11

5-HT_{1A} AND $GABA_B$ RECEPTORS COUPLE TO THE SAME PERTUSSIS TOXIN SENSITIVE G PROTEIN IN HIPPOCAMPAL AREA CA3. D. Y. Okuhara* and S. G. Beck, Department of Pharmacology, Loyola University Chicago, Maywood, IL. 60135.

The activated 5-hydroxytryptamine (5-HT) receptor 5-HT_{1A} hyperplorizes hippocampal pyramidal CA3 and CA1 cells through an increase in an inward rectifying potassium conductance. However, 5-HT has a greater efficacy and lower EC_{50} value in area CA3 as compared to area CA1. In area CA1, the activated 5-HT_{1A} and $GABA_B$ receptor increase potassium conductance through the same Pertussis toxin sensitive G protein. An investigation was conducted to determine if the 5-HT_{1A} and $GABA_B$ receptors also couple to the same Pertussis toxin sensitive G protein in area CA3. Male Spague-Dawley rats were bilaterally injected with either saline or 2-4 ug Pertussis toxin into the lateral ventricles. After three to five days, the rats were sacrificed and hippocampal slices prepared for *in vitro* electrophysiological recording. In cells taken from saline treated animals, there was no significant difference in the baclofen and 5-HT elicited hyperpolarizations (5-HT -13.7 ± 1.7 mV, mean ± SEM, n=3; baclofen -13 ± 0.6 mV, n=3). Pertussis toxin treatment significantly ($p < 0.001$) attenuated the 5-HT induced hyperpolarization (-1.3 ± 0.8 mV, n=8) and bacolfen induced hyperpolarization (-1.9 ± 0.5 mV, n=8) as compared to saline treated animals. There was no significant difference between the baclofen and 5-HT elicited hyperpolarization in Pertussis treated cells. Under voltage clamp conditions, the 5-HT and baclofen induced outward currents were not additive. These results suggest the 5-HT_{1A} and $GABA_B$ receptors couple to the same Pertussis toxin sensitive G protein in hippocampal area CA3.

Supported by USPHS NS28512 and MH 00880

639.12

THE EFFECT OF PHORBOL ESTERS ON 5-HT_{1A} RECEPTOR FUNCTION IN RAT HIPPOCAMPAL SLICES. Y. Chen[1], K.A. Berg[2], J.M. Danley[1], and W.P. Clarke[1]*. Departments of Pharmacology[1] and Anesthesiology[2], Mount Sinai School of Medicine, CUNY, New York, NY 10029.

Activation of the 5-HT_{1A} receptor in rat hippocampus elicits two distinct responses: inhibition of adenylyl cyclase activity and an increase in K^+ conductance (which causes cell hyperpolarization). In CHO cells transfected with the human 5-HT_{1A} receptor, activation of protein kinase C (PKC) with phorbol esters phosphorylates and desensitizes the 5-HT_{1A} receptor (Raymond, 1991). To investigate the PKC mediated desensitization of responses coupled to the 5-HT_{1A} receptor in cells which naturally express the 5-HT_{1A} receptor, we have developed a method to measure 5-HT_{1A} mediated inhibition of forskolin-stimulated cAMP accumulation (FScA) in rat hippocampal slices maintained under similar conditions as those used to measure hyperpolarization of pyramidal cells.

Incubation of hippocampal slices (250 μ) with 5-carboxamidotryptamine (5-CT, 15 min) reduced FScA (E_{max} = 30-50%; EC_{50} = 4 nM). The response to 100 nM 5-CT was blocked by spiperone (100 nM) and did not occur in slices incubated in the absence of O_2. 5-CT hyperpolarized CA1 pyramidal cells in hippocampal slices (400 μ) with an EC_{50} of 4 nM and an E_{max} of 11 mV. Treatment (15 min) of hippocampal slices (250 μ) with phorbol 12-myristate 13-acetate (PMA, 100 nM) or phorbol 12,13-dibutyrate (PDBu, 100 nM) produced translocation of PKC activity and potentiated FScA, but did not reduce inhibition of FScA by 5-CT (100 nM). The inactive phorbol ester 4α-phorbol 12,13-didecanoate (4αPDD, 1 μM) did not alter FScA or the inhibition by 5-CT. Treatment with 100 nM PMA or PDBu for up to 1 hr did not reduce the 5-CT (100 nM) induced hyperpolarization, however, larger doses of PDBu (1 μM; 15 min) reduced the hyperpolarization to 5-CT (>90%). These results suggest that activation of PKC in hippocampal slices, as in CHO cells, may desensitize the 5-HT_{1A} receptor. (Supported by USPHS HD 26437; MH 48125; GM 34852).

639.13

G-PROTEIN ACTIVATION BY HUMAN 5-HT_{1A} OR 5-HT_2 RECEPTOR IS RELATED TO ITS EXPRESSION LEVEL F. van Huizen*[1], N.I. Stam[2] and P. Vanderheyden[1]. Dept. of [1]Neuropharmacology and [2]Biotechnology and Biochemistry, Organon Intl, B.V., P.O. Box 20, 5340 BH Oss, The Netherlands.

Swiss 3T3 cells were stably transfected with the human 5-HT_{1A} or 5-HT_2 receptor genes (*Stam et al, Eur. J. Pharmacol.-Mol. Pharmacol. Section, 227:153-162, 1992*). By limiting dilution, single cell clones were obtained, which displayed different levels of receptor number. [^{3}H]GTP binding was performed on frozen cell pellets by incubation in the presence of 5-HT at room-temperature for 5 min followed by centrifugation for 10 min, 18000 rpm, at 4 °C. The pellet was dissolved in 500 µl Soluene and a 400 µl aliquot was mixed with scintillation cocktail. Nonspecific binding was measured by adding 10 µM GppNHp. In the absence of 5-HT, basal binding of [^{3}H]GTP was present in untransfected and, at a higher level, in transfected cells, relative to the number of receptors expressed. Dose-dependent stimulation of [^{3}H]GTP binding by 5-HT was observed in both the 5-HT_{1A}R and 5-HT_2R transfected cells, but the higher the expression level the higher the potency and efficacy of stimulation by 5-HT. No stimulation was observed in the control 3T3 cells. These data indicate that i) in the absence of agonist a spontaneous receptor-mediated G-protein activation is present and ii) potency and efficacy of G-protein activation by agonist is dependent on the number of receptors expressed.

SEROTONIN: OTHER

640.1

INDIVIDUAL DORSAL RAPHE (DR) NEURONS PROJECT BILATERALLY TO THE ROSTRAL RETICULAR THALAMIC NUCLEUS IN RAT. K.L. Simpson, T.M. Fisher, R.C.S. Lin, B.D. Waterhouse, J.K. Chapin, and G.C. Salmoiraghi*. Department of Physiology and Biophysics, Hahnemann University, Philadelphia, PA 19102.

Monoaminergic and cholinergic projections from the brainstem are known to innervate the reticular thalamic nucleus (RT). It has been suggested that the cholinergic and noradrenergic projections control the oscillatory state of the thalamocortical network by modulating the activity of the RT. However, no function has been ascribed to the serotonergic (5-HT) input to the RT. Since the raphe nuclei contain the majority of 5-HT neurons found in the brain, the aim of this study was to characterize the organization of DR projections to the RT in order to gain insight into the function of this probable 5-HT pathway. Specifically, we examined the ipsi- vs contralateral distribution of DR efferents to the RT, the degree of collateralization of individual DR neuron efferents, and topography of cells projecting from the DR to the rostral RT. Bilateral injections (0.7 to 1µl) of different fluorescent retrograde tracers were placed into a functionally identified zone of the rostral RT in anesthetized rats (n=4). Labeled cells observed in the caudal 2/3 of the DR were located in the dorsomedial region of the nucleus and distributed equally on both sides of the midline. This site overlaps a region of the DR previously shown to contain a dense concentration of 5-HT containing neurons. Although sparse labeling was seen in the ventral aspect of the DR, no label was observed in the lateral wing region. In every case studied, the majority (≈85%) of the retrogradely labeled DR cells were double-labeled. In the locus coeruleus (LC) and in cholinergic nuclei, such as the parabrachial (PB), laterodorsal tegmental (LDT), and pedunculopontine tegmental (PPT) nuclei, labeled cells were found predominantly ipsilateral to the injection site. Double-labeled LC, PB, LDT and PPT cells were rarely observed. In conclusion, the projections from individual DR cells to RT are highly collateralized and provide for bilateral innervation of the rostral RT. This pattern is in striking contrast to the predominantly unilateral projection pattern of the LC, PB, LDT and PPT nuclei to RT. Such bilateral input from single raphe neurons to the RT may provide the anatomical substrate for a bilateral, synchronizing role of this probable 5-HT projection in regulating the functional state of the thalamocortical network. *NIDA DA05117 to BDW and NIH NS29161 to RCSL.*

640.2

SEROTONIN TERMINALS FORM EXCITATORY-TYPE SYNAPSES ON NEURONS PROJECTING TO THE NUCLEUS ACCUMBENS OR CONTAINING DOPAMINE IN THE VENTRAL TEGMENTAL AREA. E.J. Van Bockstaele*, D. Cestari and V.M. Pickel, Dept. of Neurol. & Neurosci., Cornell Univ. Med. Coll., New York, N.Y. 10021.

Serotonin activates mesolimbic neurons in the ventral tegmental area (VTA; Guan and McBride, *Brain Res. Bull.*, 23, 1989). Thus, we combined immunoperoxidase labeling for 5-hydroxytryptamine (5-HT; serotonin) with silver enhanced retrogradely transported protein gold particles in the same section of tissue to determine whether 5-HT terminals directly contact ventral tegmental area neurons that project to the nucleus accumbens in the rat brain. By light microscopy, thick and non-varicose or thin and beaded 5-HT-ir processes were interspersed between retrogradely labeled perikarya and proximal dendrites. By electron microscopy, 5-HT immunoreactivity was seen primarily in unmyelinated axons and axon terminals. The 5-HT-ir terminals contained a mixed vesicle population (small, clear and large dense core vesicles) and were 0.3 µm to 1.4 µm in cross sectional diameter. 5-HT-ir terminals preferentially formed asymmetric junctions with retrogradely labeled perikarya and dendrites, but formed symmetric contacts with other unlabeled neurons. A second set of experiments combined immunoperoxidase labeling for 5-HT with immunogold labeling for the catecholamine synthesizing enzyme, tyrosine hydroxylase (TH). This demonstrated that 5-HT-ir terminals also preferentially formed asymmetric junctions with TH-labeled, presumably dopaminergic, neurons in the ventral tegmental area. These findings suggest that formation of the asymmetric, excitatory-type synapses by 5-HT terminals may be limited to a subpopulation of neurons in the ventral tegmental area that project to the nucleus accumbens and contain dopamine. Supported by NS09100-01, MH40342 and 00078.

640.3

ANOREXIC MUTATION CAUSED ABNORMAL DEVELOPMENT OF MONOAMINERGIC NEURONS IN MICE. J. Son*, H. Baker, N. Min, D. Park, T. Houpt, C. Peng and T. Joh. Lab. of Molec. Neurobiol., Cornell Univ. Med. Coll., Burke Med. Res. Inst., White Plains, NY 10605.

In rodents, neonatal suckling is an essential behavior for normal mammalian development and continues until 10 to 15 days postnatally. Subsequent transition to adult ingestive behavior occurs while monoamine neurotransmitter systems are maturing. Pharmacological manipulations have revealed serotonergic influence on suckling behavior. Interestingly, an autosomal recessive lethal mutation in mice was identified which caused starvation in preweanling mice due to suckling dysfunction. In addition, the anorexia mice (*anx/anx*) showed distinct phenotypic characteristics such as, retarded growth, emaciated appearance and behavioral dysfunction including body tremors, hyperactivity, headweaving and uncoordinated gait. The anorexic mutation has been located close to pallid (*pa*) locus on mouse chromosome 2.

We examined catecholaminergic tyrosine hydroxylase (TH) and serotonergic tryptophan hydroxylase (TPH) expression by immunohistochemistry and in situ hybridization to delineate any abnormality in monoaminergic innervation and its expression in anorexia mutant mouse brains. We found strikingly abnormal innervation by serotonergic fibers in various brain regions and apparently normal distribution of TH. In addition, expression and binding affinity studies of serotonin receptors will be presented.

640.4

SYNAPTIC LOSS IN THE CEREBRAL CORTEX FOLLOWING INJECTIONS OF PCPA IN THE RAT. N. Okado*, L. Cheng, and and S. Hamada. Dept. Anat., Inst. Basic Med. Sci., Univ. Tsukuba, Tsukuba, Ibaraki 305, Japan.

To know a role of serotonin fibers in the cerebral cortex, p-chlorophenylalanine (PCPA) was injected in the young rat (6 weeks after birth). Following four times injections of 200 mg, 400 mg or 800 mg PCPA (per kg weight) for a week synaptic structures in the sensory cortex were examined, and the density of synapses was determined in laminae I, IV and V. The density of axodendritic synapses in lamina I decreased in a dose-dependent fashion: 19 %, 25 % and 34 % of synapses were eliminated following injections of 200 mg, 400 mg and 800 mg PCPA, respectively. In lamina IV the density of axodendritic and axosomatic synapses decreased following injections of PCPA, and the magnitude of decrement of synapses was similar to that of lamina I. In lamina V the reduction rate was smaller compared to those found in laminae I and IV. Although fine serotonin positive fibers were frequently found in all the laminae by the use of an immuno-electron microscopic technique, few of them had the synaptic structure. Virtually all the synapses disappeared following injections of PCPA seemed non-serotoninergic synapses. A similar result has shown following injections of p-chloroanphetamine.

640.5

DEVELOPMENT OF 1-METHYL-4-(NAPHTHOTHIAZOLYL)-METHYL-PIPERAZINE DERIVATIVES HAVING IN VITRO 5-HT3 ACTIVITY. R. Perrone, F. Berardi, N. Colabufo, V. Tortorella, E. Daniele#, S. Govoni°, V. Olgiati*^, M. Lograno#. Pharmacochemistry and #Pharmacobiology Depts., Univ. of Bari; ^R&D Dept. Kabi-Pierrel, Milan; °Inst. Pharmacol. Sci. Univ. of Milan, Italy.

The effects of 5-HT3 receptor antagonists in CNS include control of emesis, modulation of the inhibition of K^+ stimulated acetylcholine release, reduction of dopamine release elicited by systemic administration of addictive substances. In particular 5-HT3 receptors appear to modulate mesolimbic dopaminergic systems. These observations have fostered the interest in the development of new 5-HT3 receptor antagonists and of structure-activity studies. In the present investigation the activity of (naphthothiazolyl)-methyl-piperazines on 5-HT3 as well as on other serotoninergic and dopaminergic receptors were investigated by in vitro binding assays. Serotonin receptors were evaluated using the following tritiated ligands and tissues: 5HT1, serotonin, hippocampus; 5-HT2, ketanserin, cerebral cortex; 5-HT3, BRL 43694, cerebral cortex. D2 receptors were studied using rat striatal membranes and tritiated spiroperidol. Among the compounds tested at this time 1-methyl-4-(dihydronaphthothiazolyl)-methyl-piperazine and 1-methyl-4-(benzothiophenthiazolyl)-methyl-piperazine presented an IC50 in the nanomolar range (10-50) against 5-HT3 receptors. The introduction of a methoxy group in the benzene ring of the dihydronaphthothiazolyl moiety abolished the 5-HT3 activity. The compounds appear to be a new class of 5-HT3 ligands.

640.6

KINETICS OF TRYPTOPHAN HYDROXYLASE AND AROMATIC-L-AMINO ACID DECARBOXYLASE IN HUMAN CARCINOID TUMORS. L.A. Gilbert*, L.A. Bates, and M.M. Ames, Div. of Oncology Res., Mayo Clinic & Foundation, Rochester, MN 55905

The carcinoid tumor is a relatively rare neuroendocrine malignancy whose hallmark is excessive production of serotonin (5-hydroxytryptamine; 5-HT). 5-HT is synthesized by conversion of tryptophan (trp) to 5-hydroxy-trp (5-OH-trp) by trp hydroxylase and decarboxylation of 5-OH-trp by aromatic-L-amino acid decarboxylase (AAAD). In our efforts to develop agents selective for the therapy of carcinoid tumors, we have characterized trp hydroxylase and AAAD in individual human carcinoid tumors. Enzyme assay reaction products were quantitated by HPLC with radiometric or fluorescence detection. Kinetics were determined in twelve human carcinoid tumors, five obtained with adjacent normal tissue. Trp hydroxylase was detected in all twelve tumors (K_m=189 μM ± 14.7 [mean ± S.E.]; V_{max}=2.66 nmol/h/mg protein ± 1.02). AAAD was detected in eleven tumors (K_m=47 μM ± 4.8; V_{max}=11 nmol/min/mg protein ± 1.4). The K_m of trp hydroxylase was comparable in carcinoid and corresponding normal tissue (142 μM ± 10 *vs.* 205 μM ± 28; n=4), as was the V_{max} (5.14 nmol/h/mg protein ± 2.72 *vs.* 5.05 nmol/h/mg protein ± 0.49). The K_m of AAAD was comparable in carcinoid and normal tissue (35 μM ± 3.1 *vs.* 48 μM ± 8; n=5); carcinoid V_{max} was 60-fold higher (15 nmol/min/mg protein ± 2.5 *vs.* 0.25 nmol/min/mg protein ± 0.04). The large V_{max} of carcinoid AAAD was the primary difference in 5-HT biosynthetic enzyme kinetics in tumor *vs.* normal tissue. Supported in part by grant CA 31224, NCI, DHHS.

640.7

DIFFERENTIAL ANTAGONISM OF 5-HTP-INDUCED PROLACTIN RELEASE. P. J. Little* and C. M. Kuhn, Dept. of Pharmacology, Duke Univ. Med. Ctr. Durham, NC 27710.

Serotonin (5-HT) is an important regulator of prolactin (PRL) secretion in rats. Studies using specific 5-HT receptor agonists have shown that multiple 5-HT receptors may be involved in the control of PRL release. To characterize the 5-HT receptor subtype(s) involved in physiological control of PRL release we utilized specific 5-HT antagonists to block the release of PRL in rats treated with fluoxetine (Fluox, 5-HT uptake inhibitor) and 5-HTP (5-HT precursor).

Male rats were injected with fluoxetine (10 mg/kg sc) and the specific 5-HT_2 antagonists, ketanserin and LY 53857 or saline 30 min prior to administration of 5-HTP (50 mg/kg, ip). Thirty min after 5-HTP treatment, rats were killed, blood was collected for serum and PRL levels determined by RIA. LY 53857 completely abolished Fluox+ 5-HTP-stimulated PRL release. Ketanserin significantly attenuated, but did not abolish Fluox+ 5-HTP-stimulated PRL release. An even clearer difference between ketanserin and LY 53857 was observed to the blockade of m-CPP (5-$HT_{1B/1C}$)-induced PRL release. LY 53857 significantly inhibited m-CPP-induced PRL release, whereas ketanserin did not block the response of m-CPP.

In conclusion, 5-HT_2 receptors appear to be involved in 5-HT-mediated PRL release. Additionally, the strategy of using selective antagonists rather than selective agonists represents a useful tool in characterizing the nature of 5-HT regulation of PRL release. (Supported by DA-02739 and MH-15177).

640.8

BACTERIAL EXPRESSION OF RABBIT TRYPTOPHAN HYDROXYLASE: THE RECOMBINANT ENZYME IS A SUBSTRATE FOR THE cAMP-DEPENDENT PROTEIN KINASE. K.E. Vrana, P.J. Rucker, S.C. Kumer and S.L. Vrana*. Dept. Physiology and Pharmacology, Bowman Gray School of Medicine, Winston-Salem, NC 27157-1083.

Tryptophan hydroxylase (EC 1.14.16.4; TPH) is the rate-limiting enzyme in serotonin biosynthesis. A full-length cDNA clone for rabbit TPH (Grenett *et al.*, PNAS 84, 5530, 1987) was engineered and subcloned into a bacterial expression vector (under the control of a T7 bacteriophage promoter). Expression of this gene in the protease-deficient strain of bacteria, BL21(DE3), produced TPH-specific immunoreactive protein. A specific radioenzymatic assay for TPH was developed based on the release of ^3H_2O followed by charcoal adsorption of substrates and products. Using this procedure, the recombinant protein displayed significant amounts of TPH activity. The recombinant TPH was expressed at levels representing approximately 1% of the total bacterial protein. Treatment of the recombinant protein (in bacterial extracts) with the purified catalytic subunit of the cAMP-dependent protein kinase and [γ-^{32}P]-ATP resulted in phosphorylation of the TPH. This represents the first bacterial expression system for native TPH and provides a means of generating and purifying large amounts of this important enzyme. Moreover, these experiments establish that TPH is a direct substrate for the cAMP-dependent protein kinase.

This work was supported by NIH GM-38931 (to K.E.V.) and DA-07246 (to S.L.V.).

640.9

SEROTONIN IS RELEASED BY SMALL CELL LUNG CARCINOMA CELLS AND ACTS AS A MITOGEN. L.M.Vicentini[*1], A.Codignola[2], M.G.Cattaneo[1], P.Clementi[1,2] and E.Sher[2]
[1]Dept. of Pharmacology, [2]CNR Center of Cytopharmacology, Univ. of Milano, Milano, Italy.

Small cell lung carcinoma (SCLC) is an aggressive human tumor, often associated with tobacco smoking; it synthetizes and releases hormones and neuropeptides (including cholecistokinin, bombesin, vasopressin, neurotensin) for some of which an autocrine role on cell proliferation has been proposed. Membrane receptors and ion channels possibly controlling hormone release are expressed by SCLC cell lines. In particular, we have recently revealed the presence of nicotinic receptors subtypes and of three distinct voltage-dependent Ca^{2+} channels in GLC8 cells (see Carbone et al, this meeting). We have found that serotonin (5HT) is present at significant levels in three human SCLC cell lines: GLC8, NCI-H-69 and NCI-H-592. Nicotine induces a dose- and Ca^{2+}-dependent 5HT release from these cells which is sensitive to the action of the nicotinic antagonist mecamylamine. Moreover depolarization of the cells with high KCl also induces a Ca^{2+}-dependent 5HT release. Addition of exogeneous 5HT to the culture medium induces a dose-dependent increase in their proliferation rate. 5HT-stimulated cell proliferation is antagonized by methiotepine and metergoline, while sumatriptan possesses a stimulatory effect. In conclusion, we have shown that 5HT is present in SCLC cell lines and is able to act as a mitogen for these cells. Furthermore, 5HT is released in response to depolarization and activation of nicotinic receptors. This "classic" neurotransmitter should be added to the list of autocrine growth factors produced and released by SCLC cells.

640.10

MELATONIN IN AN INSECT NERVOUS SYSTEM: DIEL CHANGES ASSOCIATED WITH PHOTOPERIOD CUES. Charles E. Linn Jr.*, Wendell L. Roelofs, Dept. of Entomology, New York State Agric. Expt. Station, Cornell Univ., Geneva, NY 14456.

Melatonin has been demonstrated to have a prominent role in the regulation of circadian-based activities in many vertebrates. The possible role of this compound in invertebrates is less well known, with only scattered reports of its occurrence and possible action relating to reproductive activities. As part of our program to understand the neuroendocrine control of pheromone perception in male moths (Lepidoptera) we are using HPLC with electrochemical detection to understand the dynamics of biogenic amine metabolism in nervous and non-nervous tissues of the cabbage looper moth. We have shown that octopamine and serotonin can affect male response thresholds for sex pheromone and levels of locomotor activity, and that levels of dopamine, serotonin, and octopamine decrease in dynamic ways in the nervous system of male moths as a function of photoperiod cues and associated changes in locomotor and mating activities during the dark. We now have demonstrated that injection of serotonin leads to increases in melatonin, indicating that the compound can be produced by the insect. We have further demonstrated that melatonin is found naturally in the nervous system of this insect, that its level increases significantly in the protocerebrum and thoracic ganglia, from undetectable levels in the light to levels of 3 to 10 pmols/tissue in the latter half of the 10 hour dark period. These increases parallel decreases in serotonin levels in these tissues. We are investigating the potential physiological and behavioral role of this compound in the circadian-based locomotor and mating activities of this insect.

640.11

SEROTONINERGIC NEUROHEMAL SYSTEMS ASSOCIATED WITH CEPHALIC NERVES IN THE COLORADO POTATO BEETLE.
T. van Haeften[1,*] and H. Schooneveld. Dept. of Entomology, Wageningen Agric. Univ., Binnenhaven 7, 6709 PD Wageningen, The Netherlands.
[1] Present address: Dept. of Anatomy, Vrije Universiteit, Van der Boechorststraat 7, 1081 BT Amsterdam, The Netherlands.

Serotonin has been shown to exert physiological effects on certain visceral muscles of the Colorado potato beetle, *Leptinotarsa decemlineata*. These physiological effects can be explained only by assuming that serotonin is released into the hemolymph as a neurohormone. Release of serotonin in most species occurs through specialized neurohemal organs, usually the corpora cardiaca. However, this is not the case in the Colorado potato beetle. The corpora cardiaca in this species could not be shown to contain serotonin. Instead, another release system is in operation: a diffuse neurohemal system in the head comprising a frontomedial and a lateral neurohemal plexus. The lateral system is formed by axons from two bilateral pairs of neurons in the frontal margin of the suboesophageal ganglion that enter the ipsilateral mandibular nerve, emerge from this nerve at some distance of the ganglion and cover all branches of the mandibular nerve with a dense plexus of immunoreactive axon swellings. The frontomedial system is formed by axons from two large neurons in the frontal ganglion that enter the ipsilateral frontal connectives, emerge from these connectives, and form a network of axon swellings on the labrofrontal, pharyngeal, and antennal nerves and on the surface of the frontal ganglion. Electron microscopy demonstrated that all axon swellings are located outside the neural sheaths of the nerves, and hence in direct contact with the hemolymph. Many swellings are closely associated with the muscles of mandibles, labrum, and anterior pharynx, as well as with the salivary glands. Therefore, it seems likely that these organs are under serotoninergic control.

HYPOTHALAMIC-PITUITARY-GONADAL REGULATION: STEROID RECEPTORS

641.1

FURTHER EXPERIMENTS ON ESTROGEN RECEPTOR-IMMUNOREACTIVITY IN DENDRITES AND AXON TERMINALS IN FEMALE GUINEA PIG BRAIN USING TWO ANTIBODIES.
J.C. Turcotte* and J. D. Blaustein. University of Massachusetts, Neuroscience and Behavior Program and Psychology Department, Amherst, MA 01003.

We have observed estrogen receptor-immunoreactivity (ER-IR) in the female guinea pig hypothalamus within dendrites and axon terminals using the H 222 antibody directed against the ligand binding region of the receptor. In order to confirm the presence of ER-IR in these atypical hypothalamic sites, we used an antibody directed against a distinct epitope on the estrogen receptor (the ER 21 antibody directed against the N-terminus).

At the light microscopic level, immunostaining with ER 21 was similar to H 222-immunostaining with densely labeled nuclei and more lightly labeled cytoplasmic processes. At the ultrastructural level, ER 21-immunoreactivity also was seen in dendrites and axon terminals.

In further immunocytochemical studies using the H 222 antibody, we observed ER-IR in dendrites and axon terminals of a major hypothalamic target site, the midbrain central gray. This suggests that this ultrastructural finding is not limited to the hypothalamus. Furthermore, we have observed ER-IR in axon terminals and distal dendrites within the hypothalamus using two antibodies directed against very different domains on the estrogen receptor. This suggests that the ER-IR that we have observed in extranuclear subcellular locations within the hypothalamus is unlikely to be only a cleaved portion of the receptor.
(supported by NSF BNS 11464 and RSDA MH 00885 from NIMH)

641.2

TISSUE-SPECIFIC CHANGES OF ESTROGEN RECEPTOR PROTEIN IN THE PREOPTIC AREA OF THE BRAIN AND UTERUS DURING THE RAT ESTROUS CYCLE.
Y. Zhou, P.J. Shughrue and D.M. Dorsa*. Departments of Pharmacology and Psychiatry and Behavioral Sciences, University of Washington, and GRECC, VAMC, Seattle, WA 98195.

The regulation of estrogen receptor plays a vital role in female reproduction. Previous studies in our laboratory have shown that the levels of estrogen receptor (ER) mRNA in the preoptic area of the rat brain varied during the estrous cycle, being the highest on estrus and metestrus and low on diestrus and proestrus. The present study examined the changes of ER protein in the preoptic area and uterus during the estrous cycle and compared changes in the female brain with the level of ER in the male. Adult female rats were sacrificed throughout the cycle. Preoptic area and uterine tissue collected. Additional intact male rats were also sacrificed. ER protein was detected by Western blot and measured by densitometric analysis of the resulting autoradiographs. In the preoptic area of the brain, ER protein levels were elevated on estrus, highest during metestrus, and attenuated during diestrus and proestrus. A comparison of the male and female preoptic area revealed that the level of ER protein was significantly lower in the male than at any stage of the female cycle. In contrast, the level of uterine ER was low during metestrus, diestrus and estrus and highest on proestrus. The results suggest that the level of ER protein in the preoptic area changes during the estrous cycle and is sexually dimorphic. These changes of ER protein paralleled previously measured changes of ER mRNA in the preoptic area. While the level of ER protein changed in the uterus during the cycle, the pattern of change was different from the changes detected in the brain. These observations suggest that the changes of ER protein are tissue-specific. The regulatory mechanism involved is under further investigation.
Supported by the VA and NS20311.

641.3

ESTROGEN RECEPTOR GENE EXPRESSION IS NOT REDUCED IN THE FOREBRAIN OF AGING FEMALE RATS.
M.A. Miller*, P.E. Kolb, B. Planas, and M.A. Raskind. Department of Psychiatry and Behavioral Sciences, University of Washington, Seattle, WA 98195.

Aging is associated with an alteration in the sensitivity of the brain to gonadal steroids. Both cell nuclear and cytosolic estrogen binding are reduced in the brain with age suggesting that the age-related loss of steroid responsiveness may result in part from a loss of estrogen receptors. Here we have used in situ hybridization and quantitative autoradiography to test the hypothesis that expression of the estrogen receptor gene is reduced with age. Fischer 344 female rats (3 mo, 11 mo, 20 mo; n=5-7/group) were ovariectomized and implanted for 2 weeks with sham capsules or Silastic capsules filled with estradiol (plasma E levels: 34±1.8, 33±1.5, 36±2 pg/ml for young, middle-aged, and old rats, respectively). Sections through the forebrain were hybridized with a ^{35}S-labeled 850 bp cRNA probe complementary to estrogen receptor mRNA and then apposed to ß-Max Hyperfilm. A computerized image analysis system was used to measure the relative optical densities over five forebrain regions in the film autoradiographs.

GROUP		AVPv	MPO	StHy	BSTL-d	BSTL-v
OVX	3 mo	.88±.02	.80±.05	.64±.03	.49±.04	.42±.04
	11 mo	.89±.04	.83±.06	.69±.04	.57±.04	.38±.03
	20 mo	.80±.05	.80±.03	.60±.06	.44±.03	.34±.02
OVX	3 mo	.56±.03	.48±.06	.30±.04	.25±.04	.14±.02
+ E	11 mo	.56±.07	.57±.04	.44±.05	.41±.03	.23±.03
	20 mo	.54±.07	.53±.04	.35±.04	.30±.04	.19±.03

Estrogen receptor gene expression in ovariectomized animals did not differ with age in any region studied. Estradiol treatment reduced estrogen receptor mRNA levels in all regions measured in all age groups. These results indicate that estrogen receptor gene expression in forebrain regions does not decline with age and suggests that factors other than reduced expression of the estrogen receptor gene account for decreased sensitivity to steroids in aging animals.

641.4

DISTRIBUTION OF THE ANDROGEN RECEPTORS IN VASOPRESSIN AND OXYTOCIN IMMUNOREACTIVE NEURONS IN RAT BRAIN.
L. Zhou, J. D. Blaustein, G. J. DeVries*. Neurosci. Behav. Prog., Dept. of Psychol., Univ. of Mass., Amherst, MA 01003.

Vasopressin immunoreactive (AVP-ir) cells in the bed nucleus of stria terminalis (BST) and medial amygdaloid nucleus (MA) are very responsive to gonadal hormones. These cells loose their AVP immunoreactivity and AVP messenger RNA labeling after gonadectomy. Testosterone treatment can reverse these changes, acting via androgen as well as estrogen receptor-mediated mechanisms. Although AVP-ir cells are also immunoreactive to estrogen receptors, it is unclear whether this is also true for androgen receptors. To answer this question, brains of intact males were stained immunocytochemically for AVP as well as androgen receptors. In the BST and MA, almost all AVP-ir cells contained androgen receptors immunoreactivity (AR-IR). In the supraoptic nucleus and suprachiasmatic nucleus, none of the AVP-ir cells contained AR-IR. In the paraventricular nucleus (PVN), about half of the AVP-ir and oxytocin-immunoreactive (OT-ir) cells in the medial parvocellular part of PVN contained AR-IR, but no AR-IR was found in AVP-ir and OT-ir neurons in other divisions of the PVN. The results suggest that androgens can act directly on AVP-ir cells in the BST and MA to influence AVP expression. Since AVP expression in these cells is more responsive to androgens in males than in females, we also stained the brains of gonadectomized male and female rats that were treated with testosterone to see whether females have a lower proportion of cells that contain AR-IR. However, no sex differences were found in distribution of AR-IR.

641.5

ABSENCE OF ANDROGEN RECEPTORS IN LUTEINIZING HORMONE RELEASING HORMONE (LHRH) IMMUNOREACTIVE NEURONS.
X. Huang* and R. E. Harlan. Dept. of Anatomy, Tulane Med. School, New Orleans, LA 70112.

Androgen has a negative feedback effect on reproductive neuroendocrine functions. Previous studies have suggested that this effect is mediated, at least in part, at the hypothalamic level, to regulate the LHRH pulse generator. However, it is not clear whether the action is mediated directly on LHRH neurons or indirectly on neurons that interact with LHRH neurons. The present study was conducted to answer this question, using double immunocytochemistry to localize LHRH and androgen receptors in the same tissue sections. Three adult male and three adult female rats were used. Androgen receptor immunocytochemistry with the PG21-3 antibody (gift of Dr. G. Prins) was performed first, followed by LHRH immunocytochemistry with the LR1 antibody (gift of Dr. R. Benoit). The chromogen for androgen receptor was Nickel-DAB, while the chromogen for LHRH neurons was DAB. Despite the detection of numerous LHRH neurons and large numbers of neurons with androgen receptors, no double-labelled neurons were observed. We conclude that the negative feedback effect of androgen on the central nervous system is mediated by indirect pathway(s), since the absence of androgen receptors within LHRH neurons would rule out any direct genomic actions. We also attempted to characterize potential androgen sensitive neurons. The preliminary results showed that a small percentage of tyrosine hydroxylase immunoreactive neurons in the arcuate nucleus and ventral tegmental area contain androgen receptor, while no forebrain cholinergic neurons have androgen receptors. Thus, the vast majority of neurons with androgen receptors are uncharacterized. Supported by NS24148 and DA06194 (R. E. H)

641.6

ANDROGEN RECEPTOR IMMUNOREACTIVITY IN BRAIN AND ACCESSORY ORGANS OF REPRODUCTION OF MALE RHESUS MACAQUES,
J.V.A. Choate* and J.A. Resko. Dept. of Physiology, Oregon Health Sciences Univ., Portland, OR 97201

Androgens produce their effects by binding to intracellular receptors in target tissues. We studied the distribution of androgen receptor (AR) in brain and in accessory organs of reproduction from male rhesus macaques (n=2) by immunocytochemistry. Fresh tissues were quickly frozen in dry ice-chilled isopentane. Ten μm sections of brain, prostate (P) and seminal vesicle (SV) were incubated with 5 μg/ml anti-AR antisera (directed against amino acids 201-222 of human AR; Choate and Resko, *Brain Res.* 597:51, 1992) and visualized using a Vector Elite ABC kit with DAB as the chromagen. Immunopositive neurons were found in the medial preoptic and periventricular nuclei of the hypothalamus, the amygdala and in stromal cells of the P and ductular epithelial cells of P and SV. In all tissues, immunostaining was confined to the cell nucleus. This study agrees with similar observations from other species. Supported by NIH grants T32 HD07133 and HD18196.

641.7

REGULATION OF PROGESTIN RECEPTORS IN RAPHE NEURONS OF STEROID-TREATED MONKEYS C. L. Bethea*, Division of Reproductive Sciences, Oregon Regional Primate Research Ctr., Beaverton, OR 97006.

Progesterone (P) administration to estrogen (E)-primed primates increases prolactin secretion. This effect of P is probably mediated through the CNS since lactotropes do not have progestin receptors (PR). Recent work from this laboratory colocalized PR in serotonin (5HT) neurons of macaque with double immunocytochemistry (ICC). 5HT is stimulatory to prolactin and may play a role in mediating the action of P. To perform this function, 5HT neurons should show an induction of PR after E-treatment and PR should be maintained during P treatment. This prediction was examined by counting 5HT+ and PR+ neurons in the raphe nuclei of spay, E-treated and E+P treated monkeys. Macaques (n=12) were spayed and treated with a 3 cm E-filled silastic capsule for 28 days or additionally supplemented with a 6 cm P-filled capsule for the last 14 of 28 days. Controls received an empty capsule. At autopsy, pontine blocks were obtained and processed for ICC. Sections at 100 µm intervals were stained for 5HT (Incstar, 1/2000). The following section was stained with a monoclonal antibody against human PR (B39, 2.5 µg/ml, gift of G. Greene). Stained cells were counted in the same fields at 4 levels using computer assisted image analysis. The mean number of 5HT+ cells and the average ratio of PR+/5HT+ cells was obtained for the left and right dorsal raphe and the ventral raphe. There was no difference in the number of 5HT+ cells with steroid treatment. There was a significant increase in the PR/5HT ratio with E-treatment and this ratio was unchanged with addition of P. Thus, E induced PR in the raphe nuclei and PR were maintained after 14 days of P-treatment. This data is consistent with the possibility that 5HT neurons mediate the effect of P on prolactin secretion. Supported by HD17269, HD18185, and RR00163.

NEUROENDOCRINE REGULATION: MISCELLANEOUS

642.1

GROWTH HORMONE SECRETAGOGUES MODULATE POTASSIUM CURRENTS IN RAT SOMATOTROPHS.
J.F. McGurk*, S.S. Pong, I-Y Chaung, M. Gall, B. Butler and J.P. Arena. Merck Research Laboratories Rahway, NJ 07065.

Growth hormone releasing peptide (GHRP6) is a synthetic six amino acid peptide that induces growth hormone (GH) release in somatotrophs via a pathway independent of growth hormone releasing hormone. Voltage-clamp recordings were done using the perforated patch technique on somatotrophs identified with the reverse hemolytic plaque assay. In normal ringer GHRP6 (1-1000 nM) induced a transient decrease in outward current elicited by depolarizing pulses (c.f. Leonard *et al.* 1991. Biophys. J. 59:254a). Steady-state currents induced by a pulse from -40 mV to 50 mV were reduced by ~36% by 100 nM GHRP6. Reduction in current reached a maximum within 1 min of exposure to GHRP6 but, in most cells, currents recovered to control levels within 5 min even in the continued presence of GHRP6. The non-peptidyl secretagogue L-692,429 (1 µM) also produced a transient reduction in current similar in time-course and magnitude to GHRP6 (100 nM) while the less active isomer L-692-428 (1µM) produced a much smaller reduction. GHRP6-induced reduction in current did not require Ca^{2+} influx, since it was unaffected by Cd^{2+} (500 µM) in the bath. In addition, GHRP6 reduced outward current the when the large conductance Ca^{2+}-activated K^+ channel was blocked in the presence of TEA (1-3 mM) and Cd^{2+} (500 µM). It is probable, therefore, that reduction of potassium current is an initial step in GHRP6 transduction.

642.2

INDUCTION OF FOS-LIKE IMMUNOREACTIVITY IN THE RAT ARCUATE NUCLEUS FOLLOWING INTRACEREBROVENTRICULAR (ICV) ADMINISTRATION OF GROWTH HORMONE-RELEASING PEPTIDE (GHRP-6).
S.L. Dickson, G. Leng and P.C. Emson.* AFRC Babraham Institute, Cambridge CB2 4AT, UK.

In rats, administration of the synthetic hexapeptide GHRP-6 releases growth hormone (GH) from the pituitary gland both *in vitro* and *in vivo* via a direct action at the pituitary (Bowers *et al.* Endocrinol. 114:1537, 1984). Recently, we reported that systemically administered GHRP selectively induces Fos expression in the hypothalamic arcuate nucleus (where the cell bodies of GH-releasing hormone (GRF) neurones are located; Dickson *et al.* Neurosci. 53:303, 1993). We sought further evidence for a central site of action by examining the distribution of Fos-like immunoreactivity within the brain following an i.c.v. injection of GHRP. Conscious adult male rats bearing a chronically implanted i.c.v. catheter were given either 1 µg GHRP (n=5) or an equal volume of artificial cerebrospinal fluid (aCSF; n=4). Ninety minutes following injection, rats were killed and the brains processed for immunocytochemistry using a polyclonal anti-Fos antibody. Dense nuclear staining was observed throught the ventrolateral arcuate nucleus of GHRP-treated rats (mean+SE = 66.0+7.8 nuclei/section/rat) but not in aCSF-treated controls (mean+SE = 0.8+0.1 nuclei/section/rat). Importantly, in GHRP-treated rats, Fos-like immunoreactivity was absent from other hypothalamic structures. Thus, centrally administered GHRP selectively activates arcuate neurones in an identical manner to that described previously for systemic administration of this peptide. (Research supported by Merck & Co., Inc.).

642.3

INTERLEUKIN-2 INHIBITS PULSATILE GROWTH HORMONE SECRETION AT THE LEVEL OF THE BRAIN: MEDIATION IN PART BY SOMATOSTATIN. G.S. Tannenbaum* and A. Beaudet. Departments of Neurology & Neurosurgery and Pediatrics, McGill University, Montreal, Quebec H3H 1P3.

Cytokines have been proposed to mediate cross-talk between the neuroendocrine and immune systems, although the mechanism/site of action remains unclear. In the present study, interleukin-2 (IL-2) mRNA was localized by *in situ* hybridization in the hypothalamus of normal CD-1 mice with moderate to high levels of expression apparent in the medial preoptic/anterior hypothalamic areas as well as in the suprachiasmatic, paraventricular, ventromedial and arcuate nuclei. We subsequently examined the effect of centrally administered IL-2 on pulsatile growth hormone (GH) secretion and assessed the possible involvement of the hypothalamic peptide somatostatin (SRIF). Free-moving adult male rats bearing intracerebroventricular (icv) and intracardiac cannulae were icv administered rat IL-2 in doses of 15 and 30 units, or the vehicle solution. Central administration of IL-2, at both doses, caused a 3-fold suppression in amplitude of the spontaneous GH pulses and significantly ($P<0.01$) reduced mean 6-h plasma GH levels, compared to vehicle-injected controls. Passive immunization of IL-2 icv-treated rats with SRIF antiserum reversed the IL-2-induced inhibition of GH pulses and restored mean 6-h plasma GH levels to values similar to those in vehicle-injected controls. These results demonstrate that: 1) IL-2 is produced in brain in areas of relevance to neuroendocrine function; 2) IL-2 exerts potent inhibitory effects on pulsatile GH secretion at the level of the brain; and 3) the blunting of GH pulse amplitude by IL-2 is mediated, at least in part, by increased release of endogenous SRIF. Such a mechanism of IL-2 action in brain may be important for immune-neuroendocrine communication.

642.4

VOLTAGE-DEPENDENT K^+ CURRENTS IN OVINE SOMATOTROPHS AND THEIR FUNCTION IN GH SECRETION. C. Chen*, J. Zhang, D. Wu and I. J. Clarke. Prince Henry's Institute of Medical Research, P. O. Box 152, Clayton, Vic 3168, Australia

To investigate the role of voltage-dependent K^+ currents in the control of GH secretion, nystatin perforated whole-cell recordings were made on ovine somatotrophs identified by post-recording immunocytochemistry. Using Ca^{2+}-free, TTX containing (1μM) bath solution, inward rectifying (I_{IR}), outward transient (I_A) and outward delayed rectifying (I_K) K^+ currents were recorded under voltage-clamp conditions. I_{IR} was very small at 5mM K^+ bath solution and enhanced by taking the bath solution to 55mM K^+; this was blocked by TEA but not by 4-AP. I_A appeared at -50mV and was selectively diminished by 4-AP (2-4mM). I_K occurred when membrane potential was depolarized to -20mV and was blocked by TEA (2mM) but not 4-AP (4mM). Under current-clamp conditions, 4-AP but not TEA (up to 5mM) depolarized the cell and triggered action potentials when Ca^{2+} (2mM) was in the bath solution and no TTX was used. 4-AP but not TEA also increased intracellular free Ca^{2+} concentration ($[Ca^{2+}]i$) and GH release. Therefore, 4-AP sensitive I_A may be a major component of the voltage-dependent K^+ currents involved in maintaining the resting potential and may play a role in regulating basal $[Ca^{2+}]i$ and GH release. Funded by Australian NH & MRC.

642.5

BLOCKER OF NITRIC OXIDE SYNTHASE ATTENUATES WATER INTAKE IN DEHYDRATED RATS. M. Kadekaro*, M. L. Terrell, P. Harmann, V. Bui*, S. Mantz*, E. Koehler*, J. Y. Summy-Long*. Division of Neurosurgery, Univ. of Texas Medical Branch, Galveston, TX, *Dept. of Pharmacology, Pennsylvania State Univ., Hershey, PA.

Nitric oxide synthase (NOS), the synthetic enzyme of nitric oxide (NO), is present in magnocellular neurons of the supraoptic (SON) and paraventricular nuclei (PVN) and their projections to the neural lobe, as well as in the subfornical organ (SFO). These structures participate in the maintenance of body fluid homeostasis by regulating secretion of vasopressin (VP) and oxytocin (OT). NO could, therefore, modulate water balance by influencing activity of neurons at these sites. Our previous studies showed that intracerebroventricular (icv) blockade of NOS by N^G-monomethyl-L-arginine (NMMA) enhances secretion of OT, but not VP, in 24 h water deprived rats (Summy-Long et al. Neurosci. Lett. 1993, in press). This effect occurred in the absence of increased glucose utilization in the neural lobe, indicating removal of an inhibitory mechanism at nerve terminals. Glucose metabolism decreased in the SFO, suggesting an action of NO on thirst mechanisms. We therefore investigated the effects of icv NMMA (500 μg/5μl) on drinking behavior following 24 h of dehydration in male Sprague-Dawley rats (n=42). NMMA attenuated water intake (ml/60 min; $p<0.05$) in dehydrated (8.7 ± 0.6 vs. 7.0 ± 0.5 ml), but not in water sated rats (0.09 ± 0.09 vs. 0.09 ± 0.09ml; values represent means ± SEM, n=10-11).

These results, in conjunction with our previous studies, indicate that NO promotes rehydration by regulating neural mechanisms controlling drinking behavior and preferential release of VP over OT during dehydration. [Supported by NIH grants NIDS 2R01 NS23055 (M.K.) and HD25498 (J.Y.S-L.)].

642.6

ALTERED GLIAL FIBRILLARY ACIDIC PROTEIN EXPRESSION IN RAT PITUITARY INTERMEDIATE LOBE GLIAL CELLS FOLLOWING ADRENALECTOMY, LACTATION, AND SALT-LOADING. K.A. Gary* and B.M. Chronwall. School of Biological Sciences, University of Missouri-Kansas City, Kansas City, MO 64108.

The majority of cells in rat pituitary intermediate lobe (IL) are melanotropes which produce proopiomelanocortin, the precursor molecule for β-endorphin and α-MSH. A smaller population of cells interspersed among the melanotropes are characterized by stellate cell bodies with long processes and have been described by EM studies and localized immunohistochemically by S-100 protein and glial fibrillary acidic protein (GFAP) antisera. Similarity in morphology to CNS fibrous astrocytes and expression of generally accepted glial-specific protein suggests to some investigators that these cells are glial cells. In light of recent findings regarding expanding functional roles of glia in the CNS and glial-like cells in the pituitary, we have performed an LM level immunohistochemical study of serially sectioned rat pituitaries to characterize the distribution of IL glial cells by localization of cells containing GFAP and S-100 protein. Analysis of GFAP immunoreactivity (IR) performed in male and female rat ILs demonstrated a specific rostro-caudal distribution of IR cells and this distribution began more rostrally in females (900 μm from rostral) than males (1200 μm). Examination of several altered physiological states, e.g. adrenalectomy, lactation, and salt-loading revealed state-specific changes in GFAP-IR. Adrenalectomy and lactation increased GFAP-IR glial cell numbers within the IL, whereas salt-loading decreased the numbers of cells expressing GFAP and abolished the typical pattern of its expression. In contrast, S-100 expressing cells were found evenly distributed throughout the rat IL and its expression was not affected by the experimental conditions. Double-label immunocytochemistry indicated that GFAP-IR cells are a sub-population of S-100-IR cells. These results suggest that S-100 only expressing cells may be induced to express GFAP under altered physiologic conditions.

642.7

GALANIN GENE EXPRESSION AND PEPTIDE SECRETION IN MtTW-10 PITUITARY CELLS. J.F. Hyde*, D.G. Morrison, J.P. Moore, Jr. and G. Howard Depts. Anatomy & Neurobiol. and Pharmacol. & Exp. Ther., Univ. of Kentucky, Lexington, KY 40536.

Estradiol (E2) increases galanin mRNA and peptide levels in the rat pituitary. Moreover, mammotropic pituitary tumors (MtT) are induced by long periods of exposure to E2. These transplantable tumors secrete copious amounts of prolactin (PRL) and growth hormone (GH). Our objectives were to 1) determine if galanin is secreted from MtTW-10 cells *in vitro*, and 2) assess whether galanin gene expression in MtTW-10 tumors is stimulated by E2. MtTW-10 tumors (provided by Dr. U. Kim) were transplanted to female Wistar-Furth rats. Three weeks later, rats were ovariectomized or implanted with E2-containing capsules. E2-treated tumor cells were cultured after trypsin dispersion, and hormone secretion was determined after 4 days in culture by RIA. MtTW-10 cells secreted galanin, PRL and GH in a time-dependent manner. Dopamine, GHRH, CRH, LHRH, and TRH failed to alter hormone release during a 3 hr static incubation. Somatostatin (10 and 100 nM) inhibited the secretion of all 3 hormones in a dose-dependent manner. HPLC fractionation of MtTW-10 culture medium showed one major peak of galanin immunoreactivity which coeluted with synthetic rat galanin. E2 increased galanin mRNA levels ≃50-fold as quantified by solution hybridization. Conclusions: 1) Galanin is secreted from MtTW-10 cells *in vitro* and is inhibited by somatostatin. 2) E2 increases galanin gene expression in MtTW-10 tumors. These data show that MtTW-10 tumors may be a useful model to study the regulation of galanin gene expression, peptide synthesis and secretion. (Supported by DK-45981)

642.8

THE IDENTIFICATION OF AN NF-KB LIKE DNA BINDING PROTEIN AND ITS ACTIVATION IN GH3 CELLS. L. Grandison(+), G. Nolan and D.W. Pfaff* +RW Johnson Med School, Piscataway, NJ; Rockefeller Univ., NY, NY.

The transcription factor NF-KB is present in many tissues in an inactive complex restricted to the cytosol by an inhibitory subunit IKB. Appropriate cell activation induces phosphorylation of IKB releasing NF-KB to translocate to the nucleus where it then binds cognate DNA sequences in responsive genes. Several neuropeptide genes including preproenkephalin and genes for neuroactive cytokines contain NF-KB binding sites within their promoter. To examine the involvement of NF-KB in gene expression within the neuroendocrine system, we investigated the presence and regulation of NF-KB in a neuroendocrine cell model, the GH3. An electrophoretic mobility shift assay employing nuclear extracts from GH3 cells and a 32P labeled oligonucleotide probe corresponding to the NF-KB binding site was used to determine NF-KB like DNA binding proteins. Several protein-DNA complexes were observed. One band showed properties consistent with the presence of an NF-KB like protein in GH3 cells. There was little NF-KB like protein in nuclear extracts of untreated GH3 cells. However exposure to phorbol ester (PMA,100 nM) produced a marked increase in NF-KB like protein. In contrast the releasing factor TRH which generates diglycerides that like PMA activates protein kinase C was ineffective in activating NF-KB. Tumor necrosis factor alpha (TNFa, 10 ng/ml) also induced NF-KB like protein. The combination of TRH and TNFa produced greater stimulation than TNFa alone. Treatments reported to induce NF-KB in other cell types such as okadaic acid (1-100 nM) and hydrogen peroxide (150 uM) were found ineffective in GH3 cells. Combination of PMA with other treatments (TRH, A23187, somatostatin, forskolin) produced no modulation of the PMA-induced response. In summary GH3 cells contain an NF-KB like protein which can be activated by PMA and TNFa. However the receptor ligand TRH which is a strong stimulus for secretion is by itself an ineffective activator of NF-KB in these cells.

642.9

PITUITARY NEUROPEPTIDE FF : RELEASE AND RECEPTORS

C. AKAR, E.A. MAJANE AND H.Y.T. YANG*. LBG, NIMH Neuroscience Center at St. Elizabeths, Washington, D.C. 20032.

Neuropeptide FF (NPFF, FLFQPQRF-NH_2) is an endogenous neuropeptide with opiate modulating activity. NPFF is unevenly distributed in the rat CNS with the highest concentrations in the spinal cord and pituitary. In this study, NPFF binding in the rat pituitary was determined and the NPFF release from the posterior lobe was examined. Using ^{125}I-YLFQPQRFa as the radioligand we detected specific NPFF binding sites in the pituitary. These binding sites were G protein coupled and were localized in both the posterior and anterior lobes. In an in vitro perfusion system we demonstrated that NPFF can be released from the posterior lobe of the pituitary gland by 54 mM K^+ in a calcium dependent manner. In this study the release of AVP was also examined. The peak of AVP release induced by K^+ was found to be 15 min after the peak of NPFF release. Interestingly naloxone can cause a release of NPFF from the pituitaries of rats chronically treated with morphine. These results suggest that hypothalamo-neurohypophyseal NPFF may exert its action at the pituitary gland.

642.10

RELEASE OF JOINING PEPTIDE FROM PERIFUSED RAT HYPOTHALAMIC SLICES.

T.Hamakubo* and T.Inagami. Department of Biochemistry, Vanderbilt University, Nashville, TN 37232.

Joining peptide (JP) is one of the derivatives of the multifactorial precursor molecule proopiomelanocortin (POMC). Although JP is known to be processed by specific enzymes from POMC in several tissues such as pituitary and hypothalamus, the biological relevance of this peptide has not been identified. Recently we reported the cardio-vascular effects of JP in genetically hypertensive rats with central administration (Hamakubo et al., Soc. Neurosci. Abstr. 426.2, 1992). To characterize JP as a neuropeptide, a polyclonol antibody specific to rat JP (rJP) was produced in rabbit. The hypothalamic slices from adult male Sprague-Dawley rats were perifused continuously with Krebs-Ringer bicarbonate (KRB) medium. Fractions were set apart every 5 min and immunoreactive rJP (rJP-ir) level of each fraction was evaluated by RIA. The basal release of rJP-ir in normal KRB was 5pg/5min/8 hypothalami corresponding to 0.05% of the total hypothalamic content. Potassium (50mM) significantly stimulated rJP-ir release about 800% of the basal level in a Ca^{++}-dependent manner. RJP-ir released by K^+ depolarization eluted in one peak at the same elution time as synthetic rJP from C18 reversed phase HPLC column. These results demonstrate that fully processed rJP is released by K^+-depolarization from the hypothalamus.

642.11

C-FOS EXPRESSION IN THE MEDULLA IN RESPONSE TO CHOLECYSTOKININ (CCK) OR HYPERTENSION.

R.G. Mayne*[1], S.L. Bealer[2], W.R. Crowley[3] and W.E. Armstrong[1]. Depts. of [1]Anat.& Neurobiol., [2]Physiol. & Biophys., and [3]Pharmacol.; Univ. Tenn., Memphis, TN 38163.

In rats, elevated blood pressure inhibits vasopressin secretion, whereas systemic injection of CCK enhances oxytocin secretion. This provides an opportunity to examine differences in the responsiveness of nuclei of the medulla that are known to mediate both effects using c-fos as a marker.

Conscious male rats received either CCK (100 µg/kg, i.p.) or were made hypertensive (30-40 mm Hg over controls) by constant intravenous infusion of phenylephrine (5-10 µg/kg/min) for 90 mins, then perfused. Tissue sections were processed immunocytochemically for c-fos and/or dopamine β-hydroxylase (DBH).

As others have shown, CCK injections increased the number of c-fos immunoreactive neurons over controls throughout the rostral-caudal extent of the nucleus of the solitary tract (NTS), including commissural neurons and the medial subnuclei but to a lesser degree dorsolaterally. Some reactive neurons in NTS were also positive for DBH, but most were not, and most DBH-positive NTS neurons were not c-fos-positive. The area postrema (AP) was prominently labeled. In contrast, after hypertension a more restricted distribution of c-fos-positive neurons was observed in the NTS, with most neurons confined to a dorsolateral strip containing few DBH-positive neurons. The medial NTS at the level of the AP and the AP itself were largely unresponsive, but rostral to the AP the medial NTS was labeled, including some DBH-positive neurons. Both treatments produced labeling in the ventral lateral medulla, including many DBH-positive neurons. These results suggest that subpopulations of NTS neurons may mediate the CCK-induced oxytocin release and the hypertension-related inhibition of vasopressin neurons. Supported by NIH grants NS23941 (WEA), HL28577 (SLB) and HD20074 (WRC).

642.12

ETHANOL DISRUPTS STIMULUS-INDUCED SYNTHESIS-SECRETION COUPLING IN TRH NEURONS OF THE PVN.

R.T. Zoeller*, O. Butnariu, A. Simonyi, D.L. Fletcher, I. Stafford-Segert[#], S. McCrone and S.L. Petersen. Depts of Anat. & Neurobiol. and [#]Psychology, Univ. Missouri, Columbia, MO 65212.

TRH neurons of the PVN project to the median eminence and regulate pituitary-thyroid function. Hypothyroidism increases TRH secretion and TRH mRNA levels in PVN. Cold exposure, believed to act through an adrenergic mechanism, increases both TRH release and TRH mRNA levels. Ethanol (E, 3g/kg ip) blocks the cold-induced increase in TRH mRNA, but not the cold-induced increase in TRH release. To examine the mechanism of action, we tested the effects of E on rapid changes in TRH and c-*fos* mRNAs after cold. Using *in situ* hybridization (ISH) with oligonucleotide probes and dual-label ISH with a variety of cRNA probes, we found that cellular levels of TRH mRNA increases in the PVN within 60 minutes of cold exposure, and that E blocks this. In contrast, c-*fos* mRNA is expressed in TRH neurons following cold, and this is not blocked by E. Therefore, E does not block the cold-activation of TRH neurons, but specifically blocks the cold-induced increase in TRH mRNA levels. E alone resulted in a pattern of c-*fos* expression in PVN and somatosensory cortex similar to cold, suggesting that E may affect TRH neurons by modifying afferent activity rather than acting directly. We have found that α1 adrenoceptor mRNA is expressed in TRH neurons of the PVN. To address effects of E on α1-stimulated TRH expression, we are applying agonists directly to the PVN. (Supported by AA08771).

642.13

EFFECT OF PRENATAL PHOTOPERIOD ON REPRODUCTIVE DEVELOPMENT IN SIBERIAN HAMSTER.

D.Shaw, W.D.Chappel*, and B.D. Goldman. Dept. of Physiology & Neurobiology, University of Connecticut, Storrs, CT 06269

Paired testis weight and hormone levels of hamsters were measured at different ages to examine the role of prenatal photoperiod on reproductive development. Breeding pairs were housed either in 16L:8D or 10L:14D. Litters from both groups were reared in 14L:10D beginning on the day of birth. They were bled from the retroorbital capillary bed at 1200 h at various ages, and for males paired testes weights were taken following decapitation.

For male hamsters, testis weight was greater for animals from the short day prenatal environment as compared to those from the long day prenatal environment. This difference was apparent at all times from day 27 to day 52 of life. The males from the short day prenatal environment also had 1) higher serum prolactin (Prl) concentrations at days 42 and 52 and 2) higher serum follicle stimulating hormone (FSH) concentrations at day 18 and 22. In female hamsters, 1) animals from the short day prenatal environment had higher serum Prl concentrations between days 27 and 62 and 2) serum FSH concentrations did not differ between females from long and short day prenatal environments except at day 62, when FSH was higher in the animals from the long day prenatal environment.

These observations indicate sex differences in the effects of prenatal photoperiod on the rate of postnatal reproductive development. These results will be discussed in relation to our observation of a sex difference in the effect of prenatal photoperiod on the pineal melatonin rhythm of juvenile hamsters.

642.14

MOLECULAR CHARACTERISTICS OF LUTEINIZING HORMONE-RELEASING HORMONE IN OPIOID-RESPONSIVE SK-N-SH NEUROBLASTOMA CELLS.

Anna Ratka*, Bruce E. Torian, Becky Flores, James W. Simpkins[1], LeeAnn Berglund. Dept.of Pharmaceutical Sciences, Coll. of Pharmacy, Idaho State University, Pocatello, ID 83209, [1] Dept. of Pharmacodynamics, Coll. of Pharmacy, University of Florida, Gainesville, Fl 32610.

The results of our previous *in vivo* studies strongly implied the functional interactions of Luteinizing Hormone-Releasing Hormone (LHRH) with the opioid systems. To elucidate the nature of this interaction at the neuronal level we have employed an *in vitro* model; μ and δ opioid receptors-containing human SK-N-SH neuroblastoma cells. We have found that LHRH can attenuate the function of opioid receptors in SK-N-SH neuroblastoma cells by preventing the inhibitory effect of morphine on the accumulation of cAMP. Immunocytochemical staining of LHRH revealed antigenic determinants of LHRH in opioid-responsive SK-N-SH cells. A 470 base pair cDNA probe derived from human hypothalamic LHRH positive cells was radiolabelled, and hybridized with genomic DNA isolated from SK-N-SH cells in a slot blot assay. The results of the hybridization study strongly imply the presence of the LHRH gene in opioid-responsive SK-N-SH neuroblastoma cells. Recent efforts are directed at quantitative evaluation of the LHRH mRNA levels. We conclude that, in addition to μ and δ opioid receptors, the SK-N-SH neuroblastoma cells possess the gene to express the LHRH system. Supported by AG 02021 and the Wallace Foundation (ISU's College of Pharmacy).

642.15

NEURAL AND ENDOCRINE MECHANISMS OF COCAINE-INDUCED HSP70 EXPRESSION IN AORTA AND ADRENAL GLAND. M.J. Blake*, A.R. Buckley, D.J. Buckley, K.P. Wikel and T. Bartlett. Dept. Pharm. & Tox., Univ. of N. Dakota Schl. of Med., Grand Forks, ND 58203

Cocaine has properties of a physiologic stressor that are reflected by its ability to activate both the hypothalamic-pituitary-adrenal (HPA) axis and the sympathetic nervous system (SNS). We have previously reported that activation of the HPA axis and SNS by restraint and pharmacologic agents induces expression of a set of highly conserved cellular stress response proteins (heat shock proteins; HSPs) in the adrenal gland and aorta. In the adrenal gland, HSP expression appears to be mediated by stress-induced increases in adrenocorticotropic hormone (ACTH) while expression in the aorta involves noradrenergic neurotransmission. In this report we capitalize on the ability of cocaine to stimulate physiologic stress responses to further define mechanisms regulating HSP70 expression in these tissues. We report the novel observation that cocaine administration induces both adrenal and vascular HSP70 mRNA expression. Elevated HSP70 mRNA was preceded by activation of factors capable of binding to the heat shock transcriptional control element and was followed by an elevation in HSP70 protein. Cocaine significantly increased plasma ACTH while hypophysectomy eliminated cocaine-induced expression in the adrenal gland suggesting that in this tissue, the effect of cocaine on HSP70 expression is also mediated via ACTH. In the aorta, depletion of catecholamines by reserpine pretreatment paradoxically augmented cocaine-induced HSP70 expression. Based on these results, it appears that HSP70 expression in the aorta occurs through direct actions of cocaine on vascular cells that are ultimately transduced to activation of the HSP70 gene rather than indirectly through alterations in catecholamine reuptake and release. The ability to manipulate HSP70 expression in mammalian tissues by hormones and pharmacologic agents suggests that the homeostatic maintenance properties of HSPs are manifest at the physiologic as well as the cellular level.

642.16

THE BOVINE PITUITARY SECRETORY VESICLE PRO-OPIOMELANOCORTIN CONVERTING ENZYME IS STRUCTURALLY HOMOLOGOUS TO THE YEAST ASPARTIC PROTEASE, YAP3. N.X. Cawley, L.P. Pu, and Y.P. Loh*. Section on Cell. Neurobiology, Lab. Devel. Neurobiology, NICHD, NIH, Bethesda, MD. 20892 and Dept. of Biochemistry, Uniformed Services University, Bethesda, MD. 20814.

Pro-opiomelanocortin (POMC) is processed by bovine pituitary intermediate lobe pro-opiomelanocortin converting enzyme (PCE, EC 3.4.23.17): a ~70 KDa aspartic protease with an acidic pH optimum and specificity for paired-basic residues (Loh *et al.*, 1985, *Journal Biol. Chem*, **260**, 7194-7205). A similar protease was found in yeast encoded by the yeast aspartic protease gene, YAP3. In a mutant that lacks the KEX2 gene, which encodes the processing enzyme for pro-α-mating factor, the YAP3 gene product was shown to correctly process pro-α-mating factor (Egel-Mitani *et al.*, 1990, *Yeast*, **6**, 127-137). We have recently overexpressed and purified the YAP3 gene product and characterized it as a ~70 KDa aspartic protease with a pH optimum at 4.0-4.5 and specificity for paired-basic residues (Azaryan *et al.*, 1993, *Journal Biol. Chem*, **268**, in press). To examine the structural similarities between these two enzymes, an antibody to YAP3 was generated. Standard Western blot techniques showed immunocross-reactivity with a ~70 KDa protein in the soluble fractions of purified bovine pituitary intermediate and neural lobe secretory vesicles as well as in a partially purified preparation of PCE. Immunocytochemistry on rat pituitary sections using the YAP3 antibody showed immuno-staining cells in the intermediate lobe and some cells in the anterior lobe where POMC and PCE have been found *in vivo*. These results indicate that the mammalian PCE is structurally homologous to YAP3.

642.17

EFFECT OF GOLD THIOGLUCOSE (GTG)-INDUCED HYPOTHALAMIC LESIONS ON FOOD INTAKE, BODY WEIGHT, AND GLUCOSE REGULATION IN MICE. H.T. Bergen*[1] and C.V. Mobbs[2]. [1]Dept. of Anatomy, University of Manitoba, Winnipeg, MB, Canada R3E 0W3, and [2]Mt. Sinai School of Medicine, New York, NY. 10029

GTG administered to mice induces lesions in the ventromedial hypothalamus and increases body weight. We have previously reported that 6 weeks after GTG injection hypothalamic neuropeptide Y mRNA levels were not increased. Therefore, GTG-induced increase in body weight may occur in the absence of hyperphagia and increased NPY activity. This study was performed in order to examine this possibility and further characterize the physiological impairments associated with GTG lesions. Male CBA mice were injected with GTG (0.7 mg/gm b.w., i.p.) or vehicle. Body weights and blood glucose levels were measured at regular intervals, and food intake was also monitored as mice were fed either rodent chow(d0-d28) or sweet milk (d21-d28). GTG-treated mice initially gained more weight than controls (2.2 ± 0.3 vs. 1.0 ± 0.2 g on day9), and also consumed more food during this time period. Blood glucose levels after i.p. injection if glucose were also elevated in GTG-treated mice as compared to controls. From day 14 to day 21 weight gain was not different between the 2 groups however while on a sweet milk diet (day 21-28) GTG -treated mice gained more weight than similarly fed controls (3.7 ± 0.4 vs. 1.7 ± 0.6 g), in spite of no differences in food intake. These data suggest that hyperphagia is not required for increased weight gain in GTG-treated mice. This increased efficiency may be a result of the previously reported GTG-induced hyperinsulinemia.

642.18

A NOVEL EQUILIBRIUM STEROID HORMONE RECEPTOR ASSAY WITH NEURAL TISSUE. X. Chen, N. G. Simon, J. Nyby*. Psychology Department, Lehigh University, Bethlehem, Pa 18015

Low concentration and instability of steroid hormone receptors in neural tissue pose major difficulties for neuro-biochemical receptor assays. Scatchard analysis, a widely used analytical procedure based on saturation curves, suffers from accuracy problems and tissue needs when receptor concentrations are very low. In addition, total and non-specific binding are measured for each dosage to assess specific binding between steroids and their receptors. Thus two samples are used in order to generate one data point.

We developed a novel assay method that utilizes a single labeled ligand dosage and varying levels of unlabeled competitor. The number of tubes and the amount of tissue required for any assay are therefore substantially reduced (about 50%). In tests of the method no significant difference between Scatchard analysis and the new procedure was found when hypolathamic androgen receptors from CF-1 male mice were assayed. Further support for the utility of the new procedure was obtained from computer simulations.

642.19

NERVE GROWTH FACTOR SUPPRESSES THE TRANSFORMING PHENOTYPE OF HUMAN PROLACTINOMAS. C. Missale*, F. Boroni, M. Losa°, A. Balsari+, P.F. Spano. Div. of Pharmacology, Dept. of Biomed. Sci and Biotech, University of Brescia, Italy, + Chair of Immunology, University of Milano, Italy; ° Chair of Neurosurgery, IRCCS S. Raffaele, University of Milano, Italy.

The therapy of prolactinomas is represented by dopamine (DA) D-2 receptor agonists. There is , however, a population of non responders who require surgical intervention. In this study we report that human prolactinomas refractory to bromocriptine (8 cases), in contrast to the sensitive ones (5 cases), lack DA D-2 receptors, grow in soft agar and form tumors in nude mice. These tumors express the receptors for NGF. Following exposure to NGF for 4 days prolactinoma cells decreased their proliferation rate, lost their capability of forming colonies in soft agar, lost their tumorigenic activity in nude mice and expressed the lactotroph-specific D-2 receptors inhibiting PRL release. These effects were permanent after NGF withdrawal and were reproducible also in vivo in nude mice transplanted with the tumors. NGF administration (1 ug/g, i.v.; 5 days) resulted in fact in the ceasing of tumor growth and the permanent expression of D-2 receptors so that the treated animals recovered the capability to respond to bromocriptine with a decrease of PRL secretion. These data suggest that NGF may modify the transforming phenotype of prolactinomas reverting them to more differentiated, less malignant, DA-sensitive lactotroph-like cells; they also raise the possibility that a short term treatment with NGF by promoting the expression of D-2 receptors may restore the refractory patients to the pharmacological therapy.

643.1

TARGET-DIRECTED SPROUTING OF PRIMARY AFFERENTS IN THE ADULT SPINAL CORD. By Inna A. Belyantseva* & Gary R. Lewin
Neurobiology & Behavior, SUNY at Stony Brook, New York, NY 11794.

We have examined the projection of myelinated primary afferents to the spinal cord after appropriate and inappropriate peripheral nerve regeneration. Transganglionically transported cholera toxin B subunit (CT-B) was detected in synaptic boutons with immunocytochemistry after transport from skin, muscle, or nerve. Somatotopically organized terminals were observed in lamina III in the L5 segment after injection of CT-B into the hairy skin of the lateral ankle. This labelling was presumed to originate from sural nerve (SN) afferents as all other hindlimb nerves were cut. The area of this labelling was around 30% of that seen from the whole SN. Ten weeks after the SN was cut the position and extent of labelling from the reinnervated ankle skin was indistinguishable from control intact animals. In further experiments the medial gastrocnemius nerve GN was redirected to reinnervate the same ankle skin. Under these circumstances myelinated muscle afferents become newly capable of activating dorsal horn neurones (Lewin and McMahon, 1993 Eur. J. Neurosci. in press). When CT-B was injected into ankle skin inappropriately innervated by the GN, labelled terminals were observed in lamina III of the dorsal horn. The labelling was present in a restricted portion of the L5 segment and was symmetrical with labelling from the contralateral ankle reinnervated by the SN. In control experiments it was shown that the central terminals of intact or self-anastomosed GN afferents could be labelled in the intermediate grey and ventral horn without labelling in lamina III. These results suggest that mature muscle afferents can be directed to sprout into the dorsal horn in a somatotopically appropriate manner by a novel cutaneous target. Supported by the APA.

643.2

PERIPHERAL AND CENTRAL MECHANISMS OF NGF-INDUCED HYPERALGESIA IN ADULT RATS. By Alain Rueff*, Gary R. Lewin & Lorne M. Mendell, Dept. of Neurobiology & Behavior, SUNY, Stony Brook, New York, NY 11794-5230.

Acute treatment of adult rats with a single dose of NGF induces a prolonged hyperalgesia to thermal and mechanical stimulation which lasts for at least 3 days (Lewin et al. 1993, J. Neurosci., 13, 2136-2148). In contrast to NGF-induced mechanical hyperalgesia, which develops around 7 hours, thermal hyperalgesia is apparent within 30 minutes after NGF. In addition to its action on sensory neurons, NGF has been shown to induce mast cell degranulation *in vitro* (Mazurek et al. 1987, FEBS Let., 315-320). Here we pretreated adult rats for 4 days with increasing doses of the mast cell degranulating agent compound 48/80 (i.p.) prior to NGF (1µg/g i.p.) administration. This delayed the onset of the thermal hyperalgesia by 3h, as measured by the foot withdrawal latency to a constant radiant heat source. Initial thermal hyperalgesia was also blocked in the presence of ICS 205-930 (100µg/kg, i.p.), a selective 5-HT3/4 receptor antagonist. In addition administration of the non-competitive NMDA-antagonist MK-801 for 14 days (15-30 µg/kg/h via osmotic pumps) limited thermal hyperalgesia after NGF to only 3h. No change was seen in the mechanical hyperalgesia following NGF in 48/80 or MK-801-treated animals (thresholds measured with von Frey hairs). These results suggest that the initial (30 min - 3h) NGF-induced thermal hyperalgesia is due to a peripheral mechanism involving release of serotonin following the acute degranulation of mast cells. The sustained thermal hyperalgesia (7h-3 days), however, appears to involve a central NMDA-dependent mechanism. NGF-induced mechanical hyperalgesia seems to be independent of mast cell degranulation or central NMDA receptor sites. Supported by NIH; NS 14899, NS16996.

643.3

PLASTICITY OF DORSAL HORN CELL RECEPTIVE FIELDS FOLLOWING PERIPHERAL NERVE REGENERATION. H.R. Koerber*, and K. Mirnics. Dept. of Neurobiology, Univ.of Pittsbugh, School of Med., Pittsburgh, PA 15261.

Adult cats were anesthetized with α-chloralose 5-9 months after tibial and sural nerve transection and self union. Microelectrode (3M K-acetate) recordings were made from 95 dorsal horn neurons in 4 animals. Results were divided into two groups based on post-axotomy survival time. Group 1 (5-6 mos.) contained data from 50 cells recorded in 2 animals and Group 2 (9 mos.) from 45 cells. In Group 1 intracellular recordings were made from 20 cells and extracellular recordings from 30. Of these, 19 were characterized as wide dynamic range (WDR) cells, 12 as low threshold only (LT-only) and 2 as high threshold only (HT-only). Of 18 WDR cells examined only 2 had HT-RF components that were larger than their LT ones. The average size of the LT-RFs of cells in this group was 13.04 ± 1.08 cm^2 (SE); (n = 48). In addition, five of these cells had LT-RFs from two separate locations on the hindlimb. The cells with small RFs always had components of their RFs supplied by intact fibers and these RFs were always continuous across the border between reinnervated and intact skin. Group 2 contained data from 45 cells (24 intracellular; 21 extracellular). Of these 28 were classified as WDR; 12 as LT-only: 1 as HT-only and 3 cells had split LT-RFs. In contrast to group 1 these cells had significantly smaller low-threshold RFs (7.52 ± cm^2; n=41; $p < .001$) which is in agreement with results in the rat (Lewin & McMahon; Neurosci. Abstr. '92). Fourteen of the 26 WDR cells examined had HT-RF components that were larger than their LT ones. These findings suggest that DH cells which receive input primarily from regenerated fibers initially have large RFs that usually cover the entire reinnervated area. However, cells that receive substantial input from both intact and regenerated fibers have more restricted RFs at short survival times. With time the large LT-RFs of some DH cells are reduced in size, while at the same time HT-RFs either remain the same or increase in size. Supported by NS 23725 (HRK).

643.4

REINNERVATION OF HINDLIMB MUSCLES BY NONHINDLIMB MOTOR AXONS AND PRIMARY AFFERENTS. R.R. Pindzola*, K. Mirnics, H.R. Koerber. Dept. of Neurobiology, University of Pittsburgh, Pittsburgh, PA 15261.

In this study we have examined the feasibility of rerouting motor axons and their segmental primary afferents to novel targets. Adult rats were anesthetized with a mixture of ketamine and xylazine and the L2-L5 dorsal root ganglia and spinal roots were exposed. The L5 and L4 DRGs were removed and the L4 ventral root cut and ligated. The L5 ventral root was traced rostrally to the level of L2 ganglia where it was cut and the proximal end ligated. The ventral ramus of the L2 spinal nerve was dissected free from surrounding tissue, cut and the proximal end abutted with the distal stump of the L5 ventral root within a polymer cuff. The anastomosed nerve and ventral root were secured in the cuff using fibrin glue. After allowing 3.5-4.5 months for regeneration the animals were anesthetized with the same anesthetic. For anatomical confirmation of successful regeneration the ipsilateral sciatic nerve was exposed, crushed and injected with a mixture of 2% WGA-HRP and 20% HRP in distilled water. The animals were allowed to recover and 3 days later were anesthetized and perfused with 1% paraformaldehyde and 1.25% glutaraldehyde. Labeled L2 motoneurons and DRG cells were visualized using TMB histochemistry. Electrophysiological confirmation of functional reinnervation was determined by stimulating the exposed L2 ventral roots and recording EMGs from hindlimb muscles normally innervated by L5 motor axons and the stimulation of peripheral nerves while recording from the L2 dorsal roots. Motoneuron and DRG cell counts showed that 20-30% (n=2) of the L2 somata were labeled from the sciatic nerve injection. In two other animals EMGs were evoked in hindlimb muscles (e.g. medial and lateral gastrocnemius, soleus, plantaris) in response to electrical stimulation of the regenerated motor axons. In these same preparations EMGs were also evoked in these muscles by stimulation of the L2 dorsal roots (CV=22-26m/s) confirming that the central connections remain functional after regeneration. Furthermore, stimulation of peripheral nerves evoked compound action potentials in the L2 dorsal roots. These findings suggest that regenerating peripheral fibers can successfully reinnervate peripheral targets while maintaining functional central connections. Supported by NS23725 (HRK).

643.5

LARGE CALIBER PRIMARY AFFERENT NEURONS PROJECTING TO THE GRACILE NUCLEUS EXPRESS NEUROPEPTIDE Y AFTER SCIATIC NERVE LESION. X. Zhang, B. Meister, R. Elde, V. M. K. Verge and T. Hökfelt* Departments of Histology and Neurobiology, Karolinska Institute, 10401, Stockholm, Sweden.

Using immunohistochemistry and in situ hybridization, we studied changes in expression of some neuropeptides in large and medium-sized neurons in lumbar 4 and 5 rat dorsal root ganglia projecting to the gracile nucleus, in response to peripheral axotomy. Fourteen days after unilateral sciatic nerve transection, many large neurons and some medium-sized neurons in ipsilateral dorsal root ganglia were strongly neuropeptide Y (NPY)-positive. Galanin (GAL)-, vasoactive intestinal polypeptide (VIP)/peptide histidine-isoleucine (PHI)-like immunoreactivities (LI) coexisted with NPY-LI in some of these neurons. After axotomy numerous large and medium-sized cells contained NPY mRNA in the ipsilateral ganglia, whereas no hybridization was seen in the contralateral or control ganglia. Cross-sectioned, large NPY-positive fibers were observed in a somatotopically appropriate zone within the ipsilateral gracile fasciculus. A dense network of NPY-immunoreactive (IR), large nerve fibers and terminals was seen in the ipsilateral gracile nucleus. A small number of GAL- and VIP/PHI-IR nerve fibers and terminals were also observed in adjacent sections. NPY-LI colocalized with GAL- or VIP/PHI-LI in some nerve fibers. None of these neuropeptide immunoreactivities could be detected in nerve fibers and terminals in the control or contralateral gracile nucleus. These findings suggest that neuropeptides, in addition to their role in small dorsal root ganglion neurons, may have a function in large and medium-sized dorsal root ganglion neurons projecting to laminae III and IV in the dorsal horn as well as to the gracile nuclei, as a part of their response to peripheral axotomy.

643.6

CELLULAR LOCALIZATION OF F8FAMIDE RECEPTORS IN THE SPINAL CORD EVIDENCED BY QUANTITATIVE AUTORADIOGRAPHY FOLLOWING CHEMICAL AND SURGICAL DEAFFERENTATION.

C. Gouarderes[1], S. Kar[2], J.A.M. Tafani[3], J. Martial*[2], R. Quirion[2] and J.-M. Zajac[1]. [1]Laboratoire de Pharmacologie et de Toxicologie Fondamentales, CNRS, Toulouse, FRANCE; [2]Douglas Hospital. Research Center, Department of Psychiatry, McGill University, Montreal, Quebec, CANADA H4H 1R3; [3]Biophysique et Med. Nucl. CHU Purpan, Toulouse, FRANCE.

(1DME)Y8Famide, a synthetic analog of F8Famide resistant to degradation and possessing high affinity for the related receptors was radioiodinated. Binding properties of this ligand were shown similar to that of F8Famide. This peptide offers the possibility to examine the precise localization of F8Famide-receptors in the rat spinal cord (Lumbar4) after sciatic nerve section, dorsal rhizotomy and neonatal capsacin (50 mg/kg) treatment. In control spinal cord, the highest density of [125I]1DME binding sites was noticed in the superficial layers of the dorsal horn whereas low-to-moderate densities were detected in the deeper laminae of the cord. In neonatally treated capsaicin rats, a significant bilateral decrease (25%) in the labelling was observed in laminae I and II of the dorsal horn. Unilateral dorsal rhizotomy and unilateral peripheral axotomy also produced a significant depletion (20-25%) in the same area of the dorsal horn ipsilateral to the surgery. These results suggest that a portion of F8Famide receptors is located on the primary afferent fibers of the superficial layers of the dorsal horn. Thus, F8Famide could play an important role in the modulation of nociceptive transmission by acting directly on primary afferent terminals as well as on receptors located post-synaptically to these terminals. (Supported in part by FRSQ and MRCC).

643.7

DIFFERENTIAL ACTIVATION OF PRESYNAPTIC INHIBITORY CIRCUITS BY PUDENDAL AND PELVIC NERVE AFFERENTS. R.D. Johnson* and V.P. Dugan. Department of Physiological Sciences, University of Florida, Gainesville, FL 32610-0144.

Polysynaptic reflex discharges in pudendal motoneurons regulating bowel, bladder, and sexual function in male rats can be elicited by stimulating myelinated afferents in the pudendal and pelvic nerves. We have shown that these reflex discharges are subject to attenuation by presynaptic inhibition of incoming afferents, although the pelvic nerve circuit is far less vulnerable (SN Abstracts 18:128, 1992). For this study, we used the same paradigm while also monitoring the amount of primary afferent depolarization (PAD). Mature male rats were anesthetized with urethane and acutely spinalized at T8. Stimulating electrodes were placed bilaterally on the dorsal nerve of the penis (DNP), pelvic nerve (PN), and superficial perineal nerve (SP). Recording electrodes were placed bilaterally around the L6 dorsal roots. Dorsal root potentials were recorded in response to electrical stimulation of several two-nerve combinations (20-60ms interval conditioning-test paradigm). By computer subtraction of individual stimuli, the level of PAD produced by each set of afferents was quantitated. Bilaterally, there was significantly greater PAD initiated by incoming DNP and SP afferents than PN afferents. A subset of dorsal root axons, subject to PN induced PAD, is also inhibited by SP afferents. Since SP afferents do not drive pudendal motoneurons or inhibit PN driven reflexes, we suggest that a second subset of PN afferents, unlike most pudendal afferents, does not activate PAD producing neurons. Supported by NS27511.

643.8

INCREASED SUBSTANCE P AND CALCITONIN GENE-RELATED PEPTIDE IMMUNOREACTIVITY IN FELINE KNEE JOINT AFFERENT PROJECTIONS IN CHRONIC ARTHRITIS. D. Homonko*, E.Theriault and K.W. Marshall, Playfair Neuroscience Unit and Dept. of Surgery, University of Toronto, TORONTO, Ontario Canada M5T 2S8.

The level of joint innervation in inflammatory joint disease is a controversial area, with conflicting reports of both increases and decreases in the levels of several peptides in the affected joints and in the central projections of joint afferents. We have recently provided evidence of maintained joint innervation by substance P (SP) and calcitonin gene-related peptide (CGRP) in chronic inflammatory joint disease in the cat (Theriault et al., 93). In the present report we describe statistically significant increases in the distribution and intensity of immunoreactivity for SP and CGRP in areas of the spinal cord known to receive specific knee joint afferent input: the cap of the dorsal horn in L3 and L6, and the intermediolateral cell (Clarke's) column in L3. Commercially available rabbit polyclonal antisera raised against SP and CGRP were used to detect the presence of the peptides in paraformaldehyde-fixed cat spinal cord. Tissues from 3 normal animals and from 5 chronic animals in which methylated bovine serum albumin was used to induce a mono-arthritic inflammatory response (cf, Inman et al., 89) were processed using appropriate methods controls. In addition to increased immunoreactivity, particularly for CGRP, in the dorsal horn, we also found an increase in the numbers of dorsal root ganglion (DRG) cells expressing CGRP from ganglia on the inflammed side, in agreement with previous reports (Hanesch et al., 93). These anatomical data, in conjunction with evolving electrophysiological studies (e.g., Neugebauer and Schaible, 90) may help to delineate the functional pathways subserving joint pain.

643.9

DECREASED DORSAL SPINAL CORD CGRP AND INCREASED HEAT SENSITIVITY IN RATS WITH LOW BACK DYSFUNCTION. J. Hulse Neufeld*. VA Medical Center (151), San Diego, CA 92161.

Low back dysfunction (1991 mtg #120.4) and increased resting levels of corticosterone (1992 mtg #643.1) were documented in rats forced to ambulate in an extreme posture. Now, sensitivity to heat in hot plate testing and concentrations of calcitonin gene-related peptide (CGRP) in the dorsal spinal cord are reported so as to assess the degree of heat hyperalgesia between groups of rats and to determine the correlational relationship between heat sensitiviy and CGRP. Three groups of male rats were compared: rats forced to ambulate on a flat surface (AF, normal posture), rats forced to ambulate in rotating cylinders (AC, extreme posture) and rats not forced to ambulate (NA). After six weeks of forced ambulation, concentrations of CGRP were dependent upon an experience two weeks earlier with a general anesthesia containing ketamine and dependent upon the group: anesthesia increased and forced ambulation decreased CGRP. In repetitive hot plate testing, the AC rats were most sensitve and the NA rats the least sensitive to the second hot plate experience. Also as the CGRP levels decreased, sensitivity to hot plate testing increased and the heat sensitivity behavior was significantly described by CGRP but best described by CGRP, corticosterone levels and interactions between predictors. It is hypothesized that the CGRP levels in the spinal cord reflect the amount of afferent stimulation to the brain so that rats given a general ketamine anesthesia are assumed to have depressed levels of brain stimulation and high levels of CGRP and rats forced to ambulate in an extreme posture are assumed to have the most stimulation and the lowest CGRP. In this hypothesis concentrations of CGRP in exercising animals tend to maintain, in a homeostatic manner, the degree of afferent input via the spinal cord.

643.10

VAGINOCERVICAL STIMULATION INDUCES C-FOS EXPRESSION IN LUMBOSACRAL SPINAL CORD NEURONS THAT PROJECT TO THE DIENCEPHALON - A DOUBLE-LABELING STUDY. S.Chinapen*, J.M. Swann, R. Burstein[1], and B.R. Komisaruk. Inst. Animal Behavior, Rutgers-The State Univ., Newark, NJ 07102 and Harvard Medical School Boston[1], MA 02115.

Vaginocervical stimulation (VS) affects neuronal activity in hypothalamic and thalamic regions that contain neurons that control neuroendocrine and autonomic reflexes or modulate pain. Our hypothesis is that lumbosacral spinal cord neurons that project directly to the diencephalon provide at least part of this input. To determine whether lumbosacral neurons that project to the thalamus and hypothalamus respond to VS, we have combined the immunocytochemical method for the detection of c-Fos (an activity marker) with the retrograde tracer Fluoro-gold (FG) for the localization of projecting neurons. FG was injected into the thalamus (n=3) or hypothalamus (n=5) of ovariectomized rats. 1-2 wk later, VS (400g force) was applied (10 sec on/10 sec off) for 5 min. 2h later, rats were anesthetized, perfused, and the lumbosacral spinal cord removed and, after appropriate processing, sections were examined for neurons expressing c-Fos only, FG only or both. VS induced c-Fos in the superficial (lamina 1) and deep (laminae IV,V,VI) dorsal horn, and in the area around the central canal (lamina X), confirming earlier findings. FG injections into the thalamus or hypothalamus labeled neurons in the same regions of the spinal cord gray matter and in the lateral spinal nucleus. Retrograde labeled neurons expressing c-Fos were located in the superficial and deep dorsal horn, the sacral parasympathetic nucleus, and the area around the central canal. More double-labeled neurons were found to project to the thalamus than to the hypothalamus. These findings indicate that lumbosacral spinal cord neurons that project to the diencephalon respond to VS.

Supported by MBRS-NIH-GM08223-09 and Sigma Xi

643.11

INTRADERMAL CAPSAICIN (CAP) OR COLD INJURY INCREASES FOS-LABELING IN THE SUPERFICIAL DORSAL HORN OF THE RAT SPINAL CORD. C.M. DeLisle[1,3], H.D. Gilchrist[3,4], D.A. Simone[3,4], C.N. Honda[2,4,*], and K.C. Kajander[1,2,4]. Departments of [1]Oral Science, and [2]Cell Biology & Neuroanatomy; [3]Neuroscience Research in Psychiatry; and [4]Graduate Program in Neuroscience, University of Minnesota, Minneapolis, MN 55455.

Immunocytochemical labeling of spinal neurons for Fos-protein increases after nociceptive stimulation, inflammation, and peripheral injuries (Morgan and Curran, 1991). We evaluated Fos-labeling in rats after intradermal injection of CAP (30 µg in 10 µl Tween-saline) into the plantar surface of the hindpaw or after contact cold stimulation (-15°C for 5 minutes, 1 cm^2 area) of the dorsum of the hindpaw. One hour after injury, rats were deeply anesthetized and perfused transcardially with ice-cold normal saline followed by ice-cold 4% paraformaldehyde. Spinal segments L2-L6 were removed, post-fixed, and cryoprotected. Spinal sections (40 µm thick) were processed immunocytochemically using a polyclonal antiserum against a portion of the Fos-protein (M.J. Iadarola, NIH, Bethesda, MD). Sections were evaluated using light microscopy; labeled nuclei were counted using computer-assisted image processing. As compared to the contralateral side, greater Fos-labeling occurred in the spinal gray matter ipsilateral to the injuries. Labeling was restricted primarily to segments L3-L5, and the majority of labeled nuclei were in the superficial dorsal horn (laminae I-II). Few nuclei were labeled in deeper regions of the gray matter. These experiments demonstrate that Fos-labeling increases in the superficial dorsal gray matter of somatotopically appropriate spinal segments one hour after either injection of CAP or cold injury to the rat hindpaw. (Research supported by NIH grants NS29567 & NS31223 and a grant from the Minnesota Medical Foundation)

643.12

Distribution of Fos like immunoreactive cells within the spinal cord of rats with partial sciatic nerve constriction. O.C. Dollberg-Stolik*, C.S. Sehlhorst and M.M. Behbehani. Depts. of Physiology and Biophysics and Anesthesia, Univ. of Cincinnati College of Medicine, Cincinnati, OH 45267-0576.

Partial nerve injury was produced by placing a tight ligature around 1/2 of the sciatic nerve in a proximal location. This procedure caused a behavioral syndrome suggestive of hyperalgesia, hyperpathia, allodynia and spontaneous pain.

Fos like immunoreactivity (FLI) patterns in the dorsal horn (DH) regions in response to mechanical and chemical stimulation were mapped We examined the effect of anesthesia by comparing FLI patterns in awake and anesthetized animals. Fifty male Sprague Dawley rats (200-300gr) were operated. Twenty animals showed symptoms of neuropathy. These animals were divided in 4 groups, 5 animals per group . Animals in group 1 and 2 were stimulated by either injection of 0.1cc of 5% Formalin or by noxious mechanical stimulation applied to both hind paws while awake. Group 3 and 4 animals received similar stimuli while under chloral hydrate anesthesia. The rats were perfused 150 minutes after the stimulation started. FLI cells in the lumbar regions were counted and the data were normalized as the ration of ipsi/contralateral FLI cells. The data is presented as the mean of this ratio ±std (MER).

In awake animals stimulated with mechanical stimulation, MER was 2.4±0.83 The MER using similar stimulation in anesthetized animals was 0.87±0.07. Formalin injection produced MER of 0.32+0.16 in awake and 0.41+0.15 in anesthetized animals respectively.

It is concluded that: (1) hypersensitivity to mechanical stimulation in this model is mediated by A-delta and/or A-beta rather than C fibers, and (2), anesthesia significantly alters the response properties of DH neurons in this animal model.

643.13

IMMEDIATE EARLY GENE EXPRESSION IN THE RAT- PAW FORMALIN MODEL-THE EFFECT OF ENADOLINE. J.A. Poat, E.K.E. Pettersson, J.C. Hunter * and J. Hughes. Parke-Davis Neuroscience Research Centre, Addenbrookes Hospital Site, CAMBRIDGE CB2 2QB.

The kappa opioid agonist, enadoline (CI-977) has a number of actions, it is anticonvulsant, neuroprotective in models of global and focal ischaemia and analgesic in models of pain such as the rat-paw formalin test. In the first two situations immediate early gene (IEG) expression is markedly increased and this increase is unaffected by enadoline. This report examines the effect of enadoline on IEG expression in the formalin model. Injection of 50ul of 5% formalin intraplanter into one hind paw of 70-80g rats caused a pronounced behavioural licking response which lasted up to 60 min. Rapid, transient increases in c-*fos* and NGFI-A mRNA (100 and 200% above saline-treated controls) in the spinal cord followed the injection. The IEG stimulation peaked at 1h and there was a strong correlation between increased expression and the behavioural response The changes in IEG were confined to the lumbar region (lamina II and weaker expression in laminae III-IV) of the cord ipselateral to the side of the injection. Enadoline (100ug/kg) and morphine (10mg/kg) totally abolished the behavioural response to formalin-injection. In contrast morphine reduced the formalin-induced IEG expression (80% inhibited) while enadoline attenuated the response (30% inhibited for c-*fos* and 41% for NGFI-A). The results highlight the importance of IEG expression in this model but demonstrate that analgesia can be obtained in the presence of increased IEG mRNA.

643.14

THE TERMINATION PATTERN AND POSTSYNAPTIC TARGETS OF AXONS ARISING FROM THE NUCLEUS RAPHE MAGNUS IN THE RAT SPINAL CORD M. Antal[1]*, M. Petkó[1] and C.W. Heizmann[2]. [1]Dept. Anat., Univ. Med. School, Debrecen, H-4012 Hungary; [2]Dept. Pediat. Univ. Zurich, Zurich, CH-8032 Switzerland.

The spinal projections of the nucleus raphe magnus were studied in the rat by using the anterograde tracer Phaseolus vulgaris leucoagglutinin (PHA-L). After injecting PHA-L into the nucleus raphe magnus, labeled fibers and terminals were detected at cervical, thoracic as well as lumbar segments of the spinal cord. Descending fibers were located in the dorsolateral funiculus on both sides of the spinal cord. More than 50% of the terminals were revealed in laminae IV-V, while nearly 20% of them were found in laminae I-II_o. Labeled axon terminals, however, were only sparsely distributed in laminae II_i -III and in the ventral horn.

Synaptic contacts of raphe-spinal terminals in laminae I-II_o and in laminae IV-V as well as GABA and glycine immunoreactivities of their postsynaptic targets were investigated in a correlative electron microscopic study.

To obtain a more global view of the relationship between raphe-spinal terminals and spinal interneurons sections were stained for both PHA-L and calbindin-D28k (CaB) or parvalbumin (PV), calcium-binding proteins that have been reported to be markers of certain subsets of islet and stalked cells as well as supraspinally projecting neurons in the spinal dorsal horn. Both CaB- and PV-immunoreactive neurons in laminae I-II_o and IV-V were found to receive contacts from raphe-spinal axons. Terminals of descending fibers impinged mainly upon dendrites of immunostained neurons, but somatic contacts were also revealed.

SOMATOSENSORY CORTEX AND THALAMOCORTICAL RELATIONSHIPS III

644.1

EFFECTS OF DIFFERENT ISOMERS OF PROPRANOLOL ON SOMATOSENSORY TRANSMISSION IN THE URETHANE-ANAESTHETIZED RAT. A.B.A. Majeed*, H.M. Arebi and A.Z. Ibrahim. School of Pharmacy, Universiti Sains Malaysia, 11800 Minden, Pulau Pinang, Malaysia.

We have previously reported that dl-propranolol suppresses somatosensory transmission in the urethane-anaesthetized rat (Proc. IUPS XVII, 1989). We now look at whether this action is due to the membrane stabilizing effects or ß-adrenoceptor blockade of propranolol. Eighteen female Wistar rats (190 - 210g) were anaesthetized with urethane (1.0 - 1.25 g/kg). The mass evoked cortical responses (MECR) to electrical stimulation (0-90V, 1/s) of the forepaw were recorded from the primary somatosensory cortex. Records were divided into the latencies of response (L_i & L_s) and the primary positive (P_i) and negative (N_i) secondary positive (P_s) and negative (N_s) waves. After a good baseline response have been achieved, 10 mg/kg R(+)- or S(-)-propranolol (Sigma) or saline, was injected separately (i.p.). The percentages of change from baseline of the above parameters after injection were recorded and the means of 6 animals per group were generated 10, 20, 30 and 40 min post injection and subjected to unpaired Student's t-test. The S(-)-isomer which is 7-10 times more potent than the R-(+)-isomer in ß-blockade, consistently showed a statistically different response from saline during both the synchronized and desynchronized stage of the EEG. The R(+)-isomer failed to cause significant alterations in any of the recorded MECR parameters. We conclude that suppression of somatosensory transmission by propranolol in the urethane-anaesthetized rat is due to ß-blockade.

644.2

MEMBRANE POTENTIAL OSCILLATIONS UNDERLYING FIRING PATTERNS IN NEOCORTICAL NEURONS. Y. Amitai*, Dept. of physiology, Center for Brain Research, Ben-Gurion University, Beer-Sheva, 84105, Israel.

Rhythmic activity in the neocortex varies with different behavioral states, and may encode sensory information. Oscillations of membrane potential (MPO) might contribute to the generation of these rhythms, but little is known about their prevalence or functional significance in the cortex.

Intracellular recordings were made from cells in layers 2/3 and 5, using brain slices from mature rat SI neocortex. Subthreshold MPOs were observed in a few cells; however, pronounced MPOs appeared in most cells following slow spike inactivation. The MPO frequency was voltage dependent, and ranged between 7 and 40 Hz. In each cell, the actual firing rate was faster than the MPO frequency for comparable membrane potentials. The MPOs were dependent on slow Na^+ and K^+ conductances, and Ca^{+2} seemed to be involved in some cells. The degree of periodicity of the oscillations correlated with the cells' firing patterns; it was highest in a group of layer 5 rhythmically firing neurons (Silva et al., 1991).

From this data I would propose that the oscillations reflect slow ion conductances that operate in the subthreshold voltage range to shape the cells' firing patterns, but which do not dictate its final firing rate.

Supported by BSF 91-00197

644.3

DEVELOPMENT OF SPONTANEOUS GENERALIZED THALAMOCORTICAL ACTIVITY IN RAT THALAMOCORTICAL SLICES IN LOW MG^{2+} MEDIUM.

Douglas A. Coulter* and Yun-Fu Zhang, Department of Neurology, Medical College of Virginia, Richmond, VA 23298-0599

We have been studying mechanisms underlying the development of rhythmic thalamocortical burst activity in mouse thalamocortical slices. To expand these studies, a similar slice is being developed in rat. Thalamocortical slices from 20-30 day-old rats were prepared using the slice angle described by Agmon and Connors (*Neuroscience* 41, 365) for mice. Slices were exposed to a medium containing no added Mg^{2+} to elicit spontaneous thalamocortical bursting, as has been described for mouse slices in our laboratory. Multiple simultaneous field potential recordings from thalamic and cortical sites within the slice disclosed low-Mg^{2+} activated spontaneous generalized thalamocortical burst activity, consisting of 2-10 sec long periods of synchronized 8-10 Hz bursting, reoccurring every 10-60 sec. Synchronized thalamocortical discharges could also be triggered by thalamic or cortical stimulation, either with single stimuli, or brief trains of stimuli at 3-10 Hz. Thalamic activity was abolished by transection of thalamocortical connections, while cortical activity was reduced but not abolished by similar cuts. The principal differences between mouse and rat generalized thalamocortical activity in low-Mg^{2+} medium were: rat slices had a greater tendency to trigger tonic-clonic-like discharges, and had reduced ventrolateral cortical connections to thalamus, evident both through microscopic examination of slices, and in evoked potential studies of thalamocortical responses. We conclude that rat thalamocortical slices may be a promising system in which to study thalamocortical rhythm generation. Supported by NIH grant NS 31000.

644.4

INTRINSIC OSCILLATOR CONTRIBUTIONS TO RESPONSE PROPERTIES OF SI CORTICAL NEURONS. K. Sameshima and M.M. Merzenich*. Keck Center for Integrative Neurosciences and Coleman Laboratory, UCSF, San Francisco, CA 94143.

The discharges of somatosensory cortex neurons in pentobarbital- anesthetized rats are often marked by transient oscillatory bursting in the theta range, initiated by tactile stimulation. To study the significance of this oscillatory variation in cortical excitability for stimulus-evoked neuronal responses, PSTHs were recorded for neurons in trials consisting of pairs of 5 ms stimulus taps, with interstimulus intervals (ISI) varied from 10 to 600 ms in 5 or 10 ms increment steps, and with 3 s long intertrial intervals. Trials with different ISIs were conducted in random order, with stimulation applied at the centers of trunk or hind paw receptive fields by use of a displacement-controlled tactile stimulator.

Following the response to the first stimulus tap, there was a period of response suppression in which no responses were evoked by second stimuli, typically lasting between 50 to 100 ms, depending on the amplitudes of the first and second stimuli. This period of profound response suppression was longer for larger first stimuli and/or smaller second stimuli. Cortical neuronal response magnitude for the second stimulus changed systematically with increasing ISI beyond this suppression period. Within about 80 to 150 ms, the response evoked by an equivalent intensity second stimulus usually equaled or exceeded that evoked by the first. At greater ISIs, the response to the second stimulus was usually *again* suppressed, with maximum suppression recorded at ISIs between 130-200 ms. At still longer ISIs, the response to progressively delayed second stimuli commonly waxed and waned, with changes in response magnitudes following the oscillation frequency revealed by shorter-ISI stimulus pairs.

Response latencies for first and second stimuli were determined by a Gaussian convolution of spikes. Latencies for the first stimulus ranged from 14 to 24 ms, varying with stimulus intensity. For stimulus of equal intensity, response latencies for the second stimulus were almost always longer than for the first. For a given stimulus intensity, second-stimulus response latencies varied by more than 7 ms, with that variation systematically associated with the oscillatory cycle revealed by changes in response magnitude. Thus, shortest latencies were recorded when evoked responses were strongest, and progressively longer latencies for ISIs that resulted in progressively greater response suppression. [Supported by NIH Grant NS-10414, FAPESP Grant 91/2182-8, The Coleman Fund, and HRI.]

644.5

INTRINSIC MEMBRANE PROPERTIES AND SOMATOSENSORY CONVERGENCE IN SI CORTEX OF RACCOON. P. Istvan* and P. Zarzecki, MRC Group in Sensory-Motor Physiology, Department of Physiology, Queen's University, Kingston, Ontario, Canada, K7L 3N6.

Timing and rate of neuronal discharge, important for sensory coding, are regulated by synaptic inputs and by intrinsic membrane properties. Knowledge of both of these regulators is needed to understand how patterns of discharge are created during sensory processing. We investigated intrinsic membrane properties and somatosensory EPSPs and IPSPs in raccoon SI, *in vivo*. Intracellular injection of identical current pulses elicited various patterns of discharge. Regular spiking (RS) neurons (58% of sample) discharged at their highest rate at the beginning of the pulse and this was followed by spike frequency adaptation. Intrinsic bursting (IB) neurons (32%) had reoccurring slow depolarizations that led to bursts. Fast spiking (FS) neurons (10%) had brief spikes, and no frequency adaptation. The RS and IB groups both had well-defined subtypes. All neurons responded with EPSPs or EPSPs/IPSPs to stimulation of digit 4 ("on-focus" digit). Neurons (40%) also responded to stimulation of digits 3 or 5 ("off-focus" digits). Monosynaptic thalamocortical EPSPs from digit 4 were common for RS, IB and FS neurons, but few of the responses from off-focus digits were mediated by monosynaptic thalamocortical connections.

The precise pattern of neural discharge cannot be a reliable estimate of sensory input. Ionic conductances governing patterns of neuronal discharge seem almost identical in intact cortex of rat, cat and raccoon, and in slices of rodent cortex, because matching varieties of intrinsic firing patterns are found. Our studies give new information about thalamocortical connectivity of neurons defined by their firing patterns.

Supported by the MRC of Canada.

644.6

SYNAPTIC PATTERNS ONTO BURSTING VS. REGULAR SPIKING NEURONS. E.L. White*, Y. Amitai and M. J. Gutnick, Center for Brain Research, Faculty of Health Sci., Ben-Gurion Univ., Beer Sheva, ISRAEL.

Regular spiking (RS) and intrinsically bursting (IB) neurons show distinct differences in their morphologies, as assessed at the level of the light microscope (Chagnac-Amitai et al., 1990; Larkman and Mason, 1990; Larkman, 1991a,b,c) and in their inhibitory responses. Under various conditions, the synaptic responses of RS cells display marked IPSPs, whereas most IB cells do not (Silva et al., 1988; Chagnac-Amitai and Connors, 1989a,b; Connors and Gutnick, 1990). This investigation is designed to determine if differences in the inhibitory responses of RS vs. IB cells are reflected in differences in the concentration of inhibitory synapses onto their somata. 4 RS and 5 IB neurons in rat somatosensory cortex were identified using intracellular recording and labeling, examined with the light microscope, and then serial thin sectioned prior to examination with the electron microscope. Synapses onto their somata and proximal dendrites were identified, classified using criteria described by Peters and coworkers (Peters et al., 1990; Peters and Harriman, 1990, 1992), and plotted onto computer-assisted, 3-D reconstructions made from the serial thin sections. Our analysis showed no significant difference in the types and concentration of synapses made onto the cell bodies and proximal dendrites of intrinsically bursting vs. regular spiking neurons. Thus the differences observed in the inhibitory responses of intrinsically bursting vs. regular spiking neurons cannot be explained by differences in the concentrations of synapses onto their somata. Supported by NIH 20149, Israel Acad. of Sciences 236/90 to E.L.W. and G.I.F. SFB 194 to MJG.

644.7

SIMPLIFIED COMPUTATATIONAL MODELS OF NEOCORTICAL NEURONS FOR USE IN ANATOMICALLY REALISTIC NETWORK SIMULATIONS OF INTERAREAL CORTICAL OSCILLATIONS. Mark. E. Jackson* and Larry J. Cauller, GR41 Cognition and Neuroscience Program, University of Texas at Dallas, Richardson, TX 75083-0688.

Pyramidal and nonpyramidal neurons of rat SI neocortex were physiologically studied *in vitro*, filled with biocytin, morphologically reconstructed (NeuroLucida, Glaser, 1991) and simulated using NEURON (Hines, 1992) as in Cauller and Connors (*Single Neuron Computation*, 1992). Models of these complex morphologies were simplified to a few major dendritic compartments that conserved passive electrotonic properties (similar to Stratford *et al.*, 1989). Intracellular R_a was set at 100 Ω-cm and R_m was adjusted to make the somatic input impedance approximately equal to the experimentally observed values (sharp electrodes: 10-50 MΩ). Spine area (1.25 spines / μm) was simulated by simply increasing compartmental C_m because the conductance per spine area (~1 μm^2) corresponding to these R_m values (~0.1-1 pS) is less than established channel conductances. By this assumption, spine area directly increases the time constant independently of impedance or length constant. Models of pyramidal neurons were specifically designed with elaborated apical dendrites including the distal tufts, multiple obliques and the long, thick apical trunk that characterizes these cells. Apical trunk conductances were adjusted to retain the sensitivity to layer I synaptic inputs observed experimentally. The resulting pyramidal neuron involves two dendritic domains, apical and basal, separated by an intervening trunk which serves as a gate for selective modulation of apical inputs. This computationally efficient but realistic model was then incorporated into a network using GENESIS (Wilson and Bowers, 1989) which is organized with reciprocal connections between simulated primary and secondary cortical sensory areas. This network model examines the conditions under which reciprocal interactions can synchronize interareal oscillations.

644.8

MULTIUNIT NORMALIZED CROSS CORRELATION DIFFERS FROM THE AVERAGE SINGLE UNIT NORMALIZED CORRELATION IN RAT SOMATOSENSORY CORTEX P.H. Bedenbaugh* and G.L. Gerstein, *Departments of Neuroscience and Bioengineering, University of Pennsylvania, Philadelphia, PA 19104*

As the technology for simultaneously recording from many brain locations becomes more available, more and more labs are measuring the cross correlation between single units and between multiple unit clusters. The relationship between single unit correlations and unit cluster correlations has not yet been fully explored.

We calculated the normalized cross correlation (NCC) between single unit spike trains, between small clusters of units and between multiunit clusters recorded in the rat somatosensory cortex. The NCC between small clusters of units was 2-3 times larger than between single units, and the NCC between multiunit clusters was and order of magnitude larger than between the small clusters of units. To understand this result we investigated the scaling of the NCC with the number of units in a cluster.

Consider four cells, two recorded on each of two electrodes. Assume that the NCC between cells recorded on the same electrode is ρ_{close} and between cells recorded on different electrodes is ρ_{dist}, both constants. The NCC between the clusters made up of the unit pairs recorded on each of the two electrodes increases linearly as a function of ρ_{dist}, with a slope determined by ρ_{close}. When $\rho_{close} = 0$, the slope is 2. When $\rho_{close} = -\frac{1}{3}$, the slope is $\frac{2}{3}$, and when $\rho_{close} = 1$ (identical spike trains) the slope is 1. In some circumstances it is easier to detect negative NCC between unit clusters than between single units. These results assume perfect spike sorting, even of overlapping waveforms. We are exploring how spike sorter dead time affects single/multiunit scaling.

Supported by NIH MH46428 and DC01249.

644.9

THE SIZE AND LOCATION OF THE TAIL REPRESENTATION AREA IN THE PRIMARY SOMATOSENSORY CORTEX (SI) OF THE RAT. C.-T. Yen* and C.-Y. Lin, Departments of Zoology and Electrical engineering, National Taiwan University, Taipei, Taiwan, 10764, ROC

The size and the location of the tail representation area in the SI region of the rat were studied with evoked potential and unit recording methods. The rats were anesthetized with i.p. pentobarbital initially and with slow continuous i.v. infusion. The medial portion of their parietal cortex were searched with glass microelectrodes for responsiveness to tail stimulations. For evoked potential studies, regular penetrations consisted of rectangular grids separated by either 0.5 or 0.3 mm. Recording depth was 1.0 mm deep in the cortex. The tail of the rat was stimulated electrically. Isopotential maps of the evoked potentials were used to delineate the tail representation area. An average area of 0.51 mm^2 was found if 1/2 maximal response were used as the cutoff threshold. If the cutoff threshold was lowered to twice the height of the baseline noise level, an average area of 1.7 mm^2 was found. For unit recording studies, denser penetrations of 0.2 to 0.3 mm separations were used. Single units whose receptive fields included a part of the tail could be found inside an average 0.35 mm^2 region. When multiunit were studied, the area was larger, averaged 0.78 mm^2. The center of the tail representation area found with all methods was 2.0 to 2.3 mm posterior and 2.5 mm lateral to the Bregma, with the tailtip pointed medioanterially.

644.10

THE ORGANIZATION OF THE FOREPAW REPRESENTATION IN SOMATOSENSORY CORTEX (SI) OF RAT: A COMBINED ELECTROPHYSIOLOGICAL AND MORPHOLOGICAL STUDY. C.A. McCandlish*, C.X. Li, R.S. Waters, Dept. of Anatomy and Neurobiology, Col. of Medicine, UT, Memphis, Memphis, TN 38163.

Few investigators have taken advantage of the forepaw barrel subfield (FBS) in rat SI cortex for studies relating cortical function to the underlying barrel field organization. We describe results of electrophysiological mapping studies where specific parts of the forepaw are associated with individual FBS barrels.

Adult rats were anesthetized with Nembutal (35mg/kg), the head was stabilized in a custom-made holder. An opening was made in the skull overlying SI cortex, a chamber was fashioned out of acrylic and placed around the opening on the skull, the dura was removed, and the cortex was covered with warmed silicon fluid. Carbon fiber electrodes were used to record evoked responses elicited by a hand-held probe, and the FBS was completely mapped, or rows of 3-4 electrode penetrations were made across an entire digit representation. Lesions were placed to identify specific recording sites. Following mapping, the cortex was removed, hemispheres were sectioned coronally or flattened and cut in a tangential plane. Tissue was stained with cytochrome oxidase and/or cresyl violet. The following results are noteworthy:

1. A single representation of the digits was found in FBS.
2. The representation of the glabrous aspect of digits 2-4 is composed of 3-4 barrels, whereas digit 1 is represented by a single barrel.
3. The glabrous digit tip, is represented by the two most anterolaterally located glabrous digit barrels.
4. Other barrel-like structures are associated with the palmar pads, and dorsal digits.

(Supported by USPHS GR NS-25824, NSF Grant BNS 88-02766)

644.11

DISTRIBUTION OF PARVALBUMIN- AND CALBINDIN-IMMUNOREACTIVE NEURONS IN CAT SOMATOSENSORY CORTEX. J. Li, H. Huang and H.D. Schwark*. Dept. of Biological Sciences, University of North Texas, Denton, TX 76203

In many areas of neocortex, the calcium binding proteins parvalbumin and calbindin are contained in separate populations of neurons which differ in their laminar distributions. In the present study, we have compared the laminar distributions of parvalbumin- (PV-IR) and calbindin-immunoreactive (CALB-IR) neurons across the four cytoarchitectonic areas of cat primary somatosensory (SI) cortex. Sagittal sections were cut through SI and stained for parvalbumin, calbindin, cytochrome oxidase activity, or Nissl substance. The locations of all PV-IR and CALB-IR neurons in SI were plotted from three series of six closely spaced sections each.

PV-IR neurons were found in all layers except layer I, with highest densities in layers III and IV. Laminar distributions of PV-IR neurons varied significantly among cortical areas, primarily due to differences in layers II and IV. For layer II, the density of PV-IR neurons was highest in area 2, declined through areas 1 and 3b, and was lowest in area 3a. In contrast, layer IV in area 3b contained over 50% higher density of PV-IR neurons than in the other areas. 97% of the CALB-IR neurons were found in layers II and III, with highest densities in layer II. The laminar distributions of CALB-IR neurons were similar across cytoarchitectonic areas. The results suggest that the laminar distributions of PV-IR neurons in the cytoarchitectonic areas of SI may reflect functional differences between these areas.

Supported by NIH NS25729.

644.12

CALCIUM-BINDING PROTEIN PHENOTYPE AND FUNCTION OF GABA-ERGIC NEURONS IN BARREL CORTEX. J.S. McCasland*, L.S. Hibbard, and T.A. Woolsey. Dept. of Neurology and Neurological Surgery, Washington University School of Medicine, Saint Louis, MO 63110.

GABA-ergic neurons in cerebral cortex are subdivided on the basis of co-localized calcium-binding proteins, parvalbumin and calbindin (e.g. Hendry et al., Exp. Brain Res. 76: 467-72). A high resolution 2DG/immunostaining method was used (McCasland et al., Soc. Neurosci. Abstr. 18:1546) to label a set of large GABAergic neurons at the layer IV-V boundary that have heavy 2DG uptake during active behavior, and which are activated ectopic to zones of direct whisker stimulation in barrel cortex of the behaving hamster. Consistent with reports from other cortical areas, we find that both calbindin and parvalbumin stain mostly nonpyramidal cells. The parvalbumin-stained neurons are larger than those staining for calbindin. Most heavily 2DG-labeled neurons are parvalbumin+, and virtually every parvalbumin+ neuron is moderately to heavily 2DG-labeled. The opposite pattern is observed for calbindin; heavily 2DG-labeled neurons at the IV-V boundary show no calbindin staining, and the numerous calbindin+ cells in layer IV and elsewhere show very light 2DG label. We conclude that functional subtypes of GABAergic neurons in barrel cortex can be defined by the phenotypic expression of a particular calcium-binding protein.

Supported by NIH grant NS17763, The McDonnell Center for Studies of Higher Brain Function and The Spastic Paralysis Research Foundation of the Illinois Eastern-Iowa District of Kiwanis International.

644.13

RELATIONSHIP OF CALBINDIN WITH CORTICOCORTICAL PROJECTION NEURONS IN THE RAT SECOND SOMATOSENSORY CORTEX. K.A. Baskerville*, H.T. Chang, and P. Herron. Dept. of Anatomy and Neurobiology, The Univ. of Tennessee, Memphis, Col. of Medicine, Memphis, TN 38163

Most calbindin-immunoreactive (CaBP+) neurons in the cerebral cortex are GABAergic nonpyramidal neurons. In initial studies, we found that CaBP+ interneurons were located in the same laminae (II/III, V, and VI) as corticocortical projection neurons in the rat second somatosensory cortex (SII). In this study, we investigated the possible synaptic contacts between corticocortical projection neurons and CaBP+ neurons in SII of the rat. To study these relationships, we combined retrograde tracing (Fluoro-Gold) and anterograde tracing (Phaseolus vulgaris-leucoagglutinin or Fluoro-Ruby) with immunocytochemistry for calbindin. The tracers were injected into the forepaw subdivision of the primary somatosensory cortex (SI). At the light microscopic level, CaBP+ neurons and processes were found adjacent to the retrogradely labeled corticocortical neurons in both upper and lower layers of SII. Likewise, anterogradely labeled corticocortical terminals were found in close proximity to CaBP+ cell bodies and dendrites, particularly in the upper layers of SII. Using a combined immunoperoxidase and silver-intensified colloidal gold technique, we observed that anterogradely labeled axon terminals were in close apposition to CaBP+ neurons at the electron microscopic level. (Supported by NIH grant AG05944 and The Center for Neuroscience of The Univ. of Tennessee, Memphis).

644.14

IMMUNOCYTOCHEMISTRY OF THALAMOCORTICAL TERMINALS IN LAYERS I AND IV. V.N. Kharazia*, A.Rustioni and R.J. Weinberg. Dept. of Cell Biology & Anatomy, University of North Carolina, Chapel Hill, NC 27599.

Pharmacologic evidence implicates glutamate as thalamocortical neurotransmitter. Given the inconclusive anatomical evidence available, we have combined anterograde transport with immunocytochemistry to further study this question. Anterogradely-labeled terminals were identified after iontophoretic injections of WGA-HRP in the thalamus of anesthetized rats. Injections were made into the ventroposterior thalamus and the lateral geniculate to label fibers terminating in layer IV of SI and V1. Injections in the ventromedial thalamic nucleus labeled terminals in superficial layer I throughout most of frontal and parietal cortex.

Thalamocortical terminals in layer IV were large, rich in mitochondria, and contained loosely-packed round clear synaptic vesicles. They made asymmetric synapses, mainly with dendritic spines. Terminals in V1 resembled those in SI, though V1 terminals were more elongated, often encapsulated by glia, and with smaller active zones. Thalamocortical terminals in layer I generally resembled those in layer IV. However, they contained fewer mitochondria and contacted apical dendritic shafts more often than terminals in layer IV; in addition to small synaptic vesicles, many layer I terminals contained large dense core vesicles.

Post-embedding immunocytochemistry was performed for glutamate (Glu), aspartate (Asp) and GABA singly or in pairs (using different sizes of gold particles). Thalamic terminals in both layers I and IV were enriched in Glu in comparison to GABA-positive terminals and dendrites postsynaptic to them, random dendrites, and astroglia; layer I terminals contained higher levels of Glu than layer IV terminals. Layer I terminals contained less Asp than layer IV terminals. Dendritic spines postsynaptic to thalamic terminals in both layers were also enriched in Glu and Asp, raising the possibility that released amino acids may be taken up by spines. These observations support pharmacological evidence that thalamic terminals use Glu as neurotransmitter; specific sensory relays projecting to layer IV may also release Asp. Supported by NIH #NS29879 (to RJW).

644.15

DIVERGENCE IN THE PROJECTION OF VENTROBASAL THALAMIC NEURONS TO MONKEY SOMATOSENSORY CORTEX E. Rausell[1*], A. Viñuela[2], M. Molinari[3] and E.G. Jones[4]. Autonoma University, 28029 Madrid, Spain[1,2]; Catholic University, 00168 Roma, Italy[3], and University of California Irvine, CA 92717,[4].

The degree to which thalamocortical axons diverge and innervate separate patches of somatosensory cortex (SI) was examined in *Macaca fuscata* and *Macaca mulatta* monkeys by combining extracellular recording and retrograde tracing. In anesthetized monkeys, two loci 1mm or less apart and containing neurons with the overlapping receptive fields were identified by recording responses to stimulation of the contralateral face and leg, and Diamidino Yellow (DY) or Fast Blue (FB) was injected in each focus. The animals survived for two weeks and were then anesthetized and perfused with 4% paraformaldehyde. Retrogradely labeled neurons in the thalamus were plotted and the sections were re-processed for cytochrome oxidase (CO) and Nissl staining.

The injections involved an area of 400-600 μm^2, were separated by less than 1mm, affected only layers I-V, and never overlapped. No retrogradely double-labeled neurons were found in VB. Single DY- and FB- labeled neurons were generally found to be neighbors and included within elongated anteroposterior groups of neurons or CO-"rods" in VPM or VPL. Usually most of the DY neurons were contained in rods adjacent to but separate from those containing most of the FB neurons, conforming to the well known topography of thalamocortical organization. Invariably, however, a small but consistent number of DY cells were found in the FB rod(s) and vice versa.

Because no double labeled neurons were found, the implication of these results is that a small population of thalamic neurons innervated by lemniscal fibers representing a localized area of the periphery project to "inappropriate" parts of the SI map. In this, there is a possible substrate for plasticity of representational maps under activity-dependent conditions.

Supported by NIH grants NS21377 and 22317.

644.16

PHYSIOLOGICAL PROPERTIES OF HORIZONTAL EXCITATORY SYNAPTIC CONNECTIONS IN THE NEOCORTEX. A.E. Telfeian, H.G. Kim*, and B.W. Connors, Dept. of Neuroscience, Brown University, Providence, RI 02912.

We have compared the length, strength, conduction velocity and divergence of layer 5 and 2/3 horizontal (i.e. parallel to the pial surface) connections in slices of rat SI neocortex. Slices were cut along laminar boundaries to eliminate most vertical connections, and EPSPs were recorded from pyramidal cells in adjacent uncut tissue. When electric stimuli were delivered within the same layer as the recorded cell, EPSPs could be evoked up to 2000 μm away for both layer 2/3 and 5 pyramids. Staining with biocytin showed that some axons of layer 2/3 and 5 pyramids project horizontally over 2000 μm. Estimates of horizontal axonal conduction velocities (~0.4 m/s) and the thresholds for activation also did not differ between layers. However, layer 2/3 cells rarely responded to stimuli delivered to isolated deeper layers, while layer 5 neurons were often excited by horizontal inputs from isolated layers 2/3 and 4. Within layer 5 neurons, the rise-time and half-width of EPSPs evoked by minimal stimuli were not correlated with vertical stimulation distance from the soma; it is likely that the active properties of apical dendrites significantly influence the vertical propagation of EPSPs. We conclude that horizontal excitatory connections in layers 5 and 2/3 are similar in length, strength, and conduction velocity, and that layer 5 neurons can effectively respond to stimuli over a wide vertical distance.

Supported by grants from the NIH and ONR.

644.17

CHOLINERGIC MODULATION OF THE EXTRACELLULAR RESPONSE EVOKED BY ISOLATED HORIZONTAL INPUTS TO LAYER I OF THE PRIMARY SOMATOSENSORY AREA OF THE RAT NEOCORTICAL SLICE. Elizabeth Rich-Bennett*, Hui-Fang. Li, and Larry J. Cauller, Cognition and Neuroscience Program, University of Texas at Dallas, Richardson, TX 75083-0688.

Horizontal inputs to layer I of SI neocortex were isolated *in vitro* by a vertical cut through deeper layers of the cortical slice. Stimuli applied in layer I on one side of the cut evoked a monophasic field potential in layer I on the opposite side associated with EPSPs recorded intracellularly from pyramidal neurons in layers II, III and V. Current source-density (CSD) analysis of this layer I response revealed a current sink restricted to layer I and upper layer II with smaller current sources distributed immediately below down to layer V. This response was reversibly eliminated by bath application of the non-NMDA glutamate channel blocker, DNQX (10 μM). Bath application of the muscarinic blocker, benztropine (20 μM), slightly reduced the amplitude (~10%) of the layer I response, although the muscarinic agonist, carbamyl-beta-methylcholine (Bethanecol, 10-100 μM) had no consistent effect. The acetylcholinesterase inhibitor, physostigmine (Eserine, 10 μM), greatly increased the amplitude (50-100%) and duration of the layer I evoked field potentials and currents. In addition, physostigmine slightly shifted the site of maximal current sink from layer I to upper layer II. Subsequent application of benztropine (20 μM) returned the response to subcontrol levels as when applied alone. These results indicate the layer I response is modulated by endogenous acetylcholine maintained *in vitro* acting upon muscarinic receptors.

SOMATOSENSORY CORTEX AND THALAMOCORTICAL RELATIONSHIPS IV

645.1

EFFECT OF FOCAL COOLING OF AREA 1 ON IPSILATERAL AREA 3b RESPONSES IN FLYING FOXES AND MARMOSETS. J. C. Clarey*, R. Tweedale, L.A. Krubitzer, and M. Calford, Vision, Touch, and Hearing Research Centre, Department of Physiology and Pharmacology, University of Queensland, Australia 4072.

We have shown previously that focal cooling of area 3b in one hemisphere in flying foxes and macaque monkeys leads to an expansion of neuronal receptive fields (RFs) recorded from area 3b in the opposite hemisphere. We inferred that blocking the callosal pathway(s) interconnecting area 3b produces a disinhibition that allows unmasking of large RFs. Since the distal limb representations in area 3b are acallosal in both species, we suggested that this effect was mediated by the interhemispheric connections of area 1 (termed area 1/2 in the flying fox) via its intracortical connections with area 3b. We confirmed that focal cooling of area 1 (1/2) led to expansion of cortical RFs in contralateral area 3b in both species. The present series of experiments reports on the effect of area 1/2 cooling on *ipsilateral* area 3b responses and aimed to confirm the findings in another species, namely the common marmoset (*Callithrix jacchus jacchus*). Experiments performed in adult, ketamine-anaesthetised flying foxes (*Pteropus scapulatus*: n=4) showed that cooling the forelimb digit 1 (D1) representation of area 1/2 resulted in an expansion of multi-unit D1 RFs recorded in area 3b of the same hemisphere. The RF contracted to its original dimensions within the cooling period in all cases. These results indicate that area 1/2 input modulates area 3b responses and that this input is inhibitory. We have demonstrated similar ipsilateral effects in two ketamine-anaesthetised marmosets using controlled brush stimulation and recording from single- and multi-units in area 3b. Area 1 has not previously been described in marmosets; however, electrophysiological maps (n=5) in relation to myeloarchitecture (n=3) and cortical connections (as revealed by flourescent tracers: n=2) have confirmed the existence of this field immediately caudal to area 3b. Mapping experiments have indicated that neurons within area 1 respond to cutaneous stimulation and have a tendency to habituate to repetitive stimulation.

645.2

IMMEDIATE REORGANIZATION IN SOMATOSENSORY CORTICAL AREAS 3B AND 1 IN SQUIRREL MONKEY AFTER COMBINED ULNAR AND MEDIAN NERVE TRANSECTION. C.X. Li, E.F. Johnson, R.S. Waters*, Dept. of Anatomy and Neurobiology, Col. of Medicine, UT, Memphis, Memphis, TN 38163.

The phenomenon of immediate cortical reorganization following nerve transection has been documented in squirrel monkey somatosensory cortical areas 3b and 1 (Merzenich et al. 1983). We reexamined this phenomenon in squirrel monkey by mapping somatosensory cortex (SI), transecting one or more peripheral nerves, and immediately remapping SI cortex.

Adult squirrel monkeys were anesthetized with Ketamine (35mg/kg), the head and left arm were stabilized in custom made holders, and the skin over the ulnar and median nerves was opened, the nerves identified and tagged with suture. Following forepaw preparation, the contralateral SI cortex was exposed, and an acrylic recording chamber was fashioned on the surrounding bone. Carbon fiber electrodes were used to record evoked responses elicited by mechanical and/or hand held stimulators, and the forepaw region of SI cortex was identified and mapped. Following complete mapping of the forepaw representation, the ulnar and/or median nerve was cut and the cortex remapped. Using these techniques the following results are noteworthy:

1. Ulnar nerve transection alone was not sufficient in eliminating digit 5 glabrous input into SI cortex.
2. When ulnar and median nerve transections were combined, glabrous digit stimulation was ineffective in driving SI cortex.
3. In those cases where immediate cortical reorganization was observed the region of SI cortex formerly served by stimulation of the glabrous skin was driven by stimulation of the corresponding hairy skin.

(Supported by USPHS GR NS-25824, NSF Grant BNS 88-02766)

645.3

INTRACORTICAL CONTRIBUTIONS TO SHORT-TERM NEOCORTICAL PLASTICITY: CORTICAL AND SUBCORTICAL EFFECTS OF MICROSTIMULATION IN SI-BARREL CORTEX. D. Sinis(*) and P. Hand, Animal Biology, Sch.Vet.Med. and Neurology, Sch.Med., Univ. of Penna., Phila., Pa. 19104

We previously showed that 3-6 hours of electrical microstimulation (MS) confined to a layer IV "barrel" with a clearly defined principle-whisker (PW) receptive field resulted in an expansion of that whisker's cortical functional representation using 2-deoxyglucose (2DG) and electrode mapping techniques. In this study, we used a chronic electrode array (up to 4 electrodes) to simultaneously record multi-unit activity prior to, during, and following cortical MS; in many of the same animals the 2DG technique was also used to determine spatial representational changes of the PW throughout the central trigeminal neuraxis following MS.

A micro-electrode array with tip separations of 500um was positioned over the barrel cortex and lowered to a depth of 800um; the assembly was attached to the skull. 3 weeks later the animal was lightly anesthetized and the receptive field of neurons at each electrode tip was determined. One electrode with a non-overlapping PW-receptive field was chosen as the MS site. The response latency and magnitude (# spikes/deflection) of cortical neurons to deflection of the PW was determined for each electrode position. MS began and consisted of a 40ms train of charge-balanced 7uA monophasic pulses @330Hz; 1 train per second. MS continued for 3-6 hours and was interrupted at 1-hour intervals to re-examine the response properties of neurons at each electrode position to deflection of the PW. The 2DG technique was then used in awake animals that received MS to determine the distributed CNS metabolic (functional) representation of the PW.

Response latency (RL) in the PW-stimulated barrel pathway was significantly shortened following MS (5.4ms ±0.27 vs. 7.2ms ±0.2; $p<0.001$). The RL in the PW-unstimulated barrel pathways was also significantly shortened following MS (11.3ms ±0.36 vs. 13.8ms ±0.26; $p<0.001$). The # spikes/whisker deflection did not change with MS (PW-1.2 spks/deflection; nonPW-0.7 spks/deflection). 2DG also showed a significantly increased spatial representation of the PW in the barrel cortex following MS; 2DG labeling in the thalamus and brainstem was unchanged (ie, NO expansion). We conclude that short-term cortical plasticity following MS is mediated in large part by intrinsic cortical network properties; other possibilities are discussed.

Supported by NIH Grants DE-00227 and NS-22283.

645.4

PARVALBUMIN AND CALBINDIN IMMUNOREACTIVITY IN THE SOMATOSENSORY BARREL CORTEX AND EFFECT OF TACTILE EXPERIENCE. M.H. Cohen, R. Zatezalo and P.W. Land*. Dept. of Neurobiology, Univ. of Pittsburgh, Pittsburgh, PA 15261.

Calcium-binding proteins parvalbumin (PV) and calbindin D-28K (CB) differentiate populations of neocortical GABAergic neurons. We used immunochemical methods to study the distribution of PV-immunoreactive (IR) and CB-IR neurons and processes in the somatosensory barrel cortex of rats. PV-IR and CB-IR neurons and processes occur in all cortical laminae, except lamina I which contains processes only. Lamina IV has the highest density of PV-IR neurons and processes, where they define the sides and hollows of cortical barrels. PV-IR puncta nevertheless surround unreactive somata in all laminae below lamina I. By contrast with PV-IR neurons, CB-IR neurons are least numerous in lamina IV, and fall within the septae between barrels. The density of CB-IR processes parallels the distribution of CB-IR somata, being particularly robust in lamina Vb. Interestingly, we observe no CB-IR puncta in any laminae.

Tactile deprivation by simple whisker trimming has no visible effect on immunostaining for either protein, though GABA levels are reduced by this procedure. However, destroying vibrissa follicles by electrocautery leads to decreased PV-IR in corresponding lamina IV barrels. The data indicate that neurons containing different calcium binding proteins are segregated according to structural and functional subdivisions of the barrel cortex. Further, within barrel hollows PV synthesis can be modulated by peripheral deafferentation. (Supported by NSF grant BNS 9110731)

645.5

LESION-INDUCED MODULATION OF α_7 NICOTINIC RECEPTORS IN DEVELOPING RAT SOMATOSENSORY CORTEX. R.S. Broide*, R.T. Robertson and F.M. Leslie. Depts. of Pharmacology; Anatomy and Neurobiology, Univ. of California, Irvine, CA 92717.

The rat somatosensory cortex (SS1) displays a unique cytoarchitechture which is developmentally influenced by afferent neuronal activity. Several cholinergic markers have been found to label the barrel field within the SS1 during a critical period of development. [^{125}I]α-Bungarotoxin (BTX) exhibits a unique, transient developmental binding pattern that delineates columnar bands, extending through all layers of the SS1. These bands, which correspond to the barrel field, are most clearly distinguished in tangential cortical sections of laminae IV and VI. Studies have indicated that, in the CNS, the toxin binds to the α_7 neuronal nicotinic receptor subunit and have suggested that this site may be involved in the formation of neural connections. In order to elucidate the potential mechanism, it is important first to determine whether this site is found on thalamic terminals or on their cortical targets during the early stages of synaptogenesis. We placed unilateral electrolytic lesions in the ventrobasal thalamic nucleus of P6 rat pups. Pups were then sacrificed at P7, 8, 9, and 10. Both cortical hemispheres were sectioned in the tangential plane and processed for acetylcholinesterase (AChE) histochemistry, receptor autoradiography, and *in situ* hybridization. While there was no change to the contralateral side, the ipsilateral cortex showed a marked decrease in both [^{125}I]α-BTX binding and α_7 mRNA distribution levels within barrel-like structures in laminae IV and VI. This decrease was apparent as early as P7 and correlated well with a loss in AChE staining within lamina IV. These findings suggest that the α_7 nicotinic receptor may be localized on presynaptic thalamocortical terminals, although this may also reflect a dynamic transynaptic modulation of receptors.

Supported by PHS grant #DC00450 and NS30109.

645.6

MECHANISMS OF SOMATOSENSORY PLASTICITY IN ADULT PRIMATES. S. Seto*, C.E. Schroeder, J.P. Noonan, J.C. Arezzo and P.E. Garraghty. Depts. Neurosci. and Neurol., Albert Einstein Coll. Med., Bronx, NY 10461 and Dept. Psych., Indiana Univ., Bloomington, IN 47405.

The time course and underlying physiology of reorganization in the somatosensory cortex of squirrel monkeys were examined by sampling both prior to, and at biweekly intervals after, median nerve transection and ligation. Linear array, multicontact electrodes were chronically implanted in the median nerve representation of Area 3B, as defined by somatic stimulation. Laminar current source density (CSD) and concomitant multiunit activity (MUA) profiles were recorded from anesthetized monkeys using electrical stimulation applied to the median, radial and ulnar nerves near the elbow. Isolation of peripheral nerve stimulation was confirmed by simultaneous recording of EMG from appropriate distal muscles. Prior to nerve section, no ulnar nerve cortical response was evident, but radial nerve stimulation produced a "nondominant" response in median nerve territory (see also Schroeder et al., *Neurosci. Abst.*, 1992). The initial (10-30 ms) epoch of this response was characterized by small current sources and sinks without clear MUA correlates, suggestive of subthreshold activation. Acute recordings after the nerve cut did not reveal any changes. By the next sample point (day 5), this initial epoch of the radial nerve response had undergone a two-fold increase in CSD amplitude that was accompanied by the emergence of a large increase in MUA. These changes progressed over time and appeared to stabilize by the 3rd week. Qualitatively, the post-section radial response greatly resembled the pre-section median response. These findings support the view that reorganization of somatic representation is based on the unmasking of pre-existing, but "silent", radial nerve inputs. (Supported by MH06723, MH47939 and DC00657).

645.7

SELECTIVE ISCHEMIC BLOCK OF THE SURAL NERVE: EFFECTS ON SCALP POTENTIAL TOPOGRAPHIES EVOKED BY NOXIOUS STIMULATION. R. Dowman*, Dept. Psychology, Clarkson University, Potsdam, N.Y., 13699-5825.

Scalp potential topographies evoked by electrical stimulation of the sural nerve were obtained in 10 human subjects. Two different stimulus levels were used: innocuous (elicited a just-maximal sural nerve compound action potential and was rated as non-painful) and noxious (elicited a spinal nociceptive withdrawal reflex and was rated as painful). At 30-50 min following ischemic block of the lower leg none of the subjects could detect the innocuous stimulus. No scalp potentials were evident at this level. All subjects detected the noxious stimulus. In 5 subjects there were no scalp potentials at the noxious level. These subjects described the noxious stimulus as having a burning quality. A positive scalp potential occurring 280-400 ms following the stimulus was evident at the noxious level in the remaining subjects. Most of these subjects described this stimulus as having a sharp, prickling quality. These results suggest that the pain-related scalp potentials are elicited by the smaller diameter myelinated afferents. A quantitative analysis of the topographic patterns will be performed to determine whether the configuration of the sources generating the pain-related positive potential changed during the selective block.

645.8

NEURAL ACTIVITY AFTER NOMINAL DEPRIVATION OF THE SOMATIC-SENSORY CORTEX: ^{3}H 2-DEOXYGLUCOSE EVALUATION IN THE RAT. G. Gutierrez[1], D. Riddle* and D. Purves. Dept. of Neurobiology, Duke University, Durham, NC 27710.

Deprivation paradigms have often been used to explore the role of neural activity in the development of the sensory cortex. The actual acute and long-term effects of such manipulations on cortical activity, however, are not usually known. We have therefore used ^{3}H 2-deoxyglucose (2-DG) to evaluate neural activity in the primary somatic-sensory cortex (SI) of the rat after deprivation of input from peripheral sensors. Juvenile rats (7 days old) were unilaterally deprived by clipping, plucking or cauterizing the 36 mystacial vibrissae represented in the posteromedial barrel subfield of the contralateral SI. After 10 weeks, a femoral catheter was implanted and 3uCi/gr of 2DG injected in the awake behaving animal. The rats were sacrified 45 minutes later by an overdose of pentobarbital; the brains were then removed, hemisected, flattened and frozen. Tangential cryostat sections (20μm) through layer IV of each cortex were overlaid with autoradiography film and maintained at 4°C for 12 days. The film was developed and analyzed by video densitometry. We found no differences in 2DG uptake between the deprived and non-deprived PMBSF in any of the experimental groups. Nor did comparison of experimental and normal (control) animals show any difference of 2DG accumulation in this representation. Evidently, neural activity in the PMBSF is little diminished following peripheral deprivation, at least after few weeks. This ongoing activity may explain the absence of appreciable morphological changes in the somatic-sensory system following peripheral manipulations performed after the critical period.

[1] Supported by the Latin American Fellows Program of the Pew Foundation.

645.9

NMDA RECEPTOR BLOCKADE PREVENTS MOST CORTICAL REORGANIZATION AFTER PERIPHERAL NERVE INJURY IN ADULT MONKEYS. P.E. Garraghty*, N. Muja, and R. Hoard, Dept. Psychology, Indiana University, Bloomington, IN 47405.

After median nerve transection in adult New World monkeys, neurons in the part of somatosensory area 3b deprived of their normal inputs eventually regain responsiveness to inputs from skin surfaces with intact innervation. Merzenich et al. (Neuroscience, 10:639, 1983) showed this reorganizational process to be essentially a two-stage process, one immediate, and one more protracted, with the recovery of cortical responsiveness taking no more than 3-4 weeks. In the present experiments, we have investigated the role of the NMDA receptor system in these two phases of injury-induced reorganization. In 6 monkeys (5 squirrel monkeys, 1 owl monkey), we studied the immediate, or more protracted cortical response to median nerve transection in area 3b. In 2 monkeys (1 pre-treated with CPP, an NMDA receptor blocker), the immediate effects of median nerve transection were assessed. In 3 monkeys, NMDA receptor blockade and nerve transection were contemporaneous, with terminal recording at 11, 25, or 28 days after nerve injury. In a final monkey, cortical mapping 30 days after median nerve transection (with no NMDA receptor blockade) revealed virtually complete reorganization. Acute post-nerve injury mapping revealed immediate unmasking within approximately 20% of the deprived cortex whether NMDA receptors had been blocked or not. In the 3 monkeys with longer survival times and NMDA receptor blockade, there was no indication that any of the protracted recovery had occurred. In each case, approximately 20% of the presumed deprived cortex showed reorganization while the remainder remained unresponsive to cutaneous stimulation. These results suggest that the immediate "unmasking" phase of cortical recovery is not dependent on NMDA receptor activation while the more protracted phase can be prevented by NMDA receptor blockade. (Supported by Indiana University 22-314-31)

645.10

LAMINAR COMPARISON OF PLASTICITY IN ADULT RAT BARREL CORTEX. M.E. Diamond*, W. Huang, and F.F. Ebner, Inst. for Developmental Neurosci., Vanderbilt Univ., Nashville, TN 37203.

Previous experiments have shown that the receptive fields (RFs) of neurons in layer IV of barrel cortex are significantly altered by a 3-30 day period of "whisker pairing." The present study compared the modifiability of different cortical layers, with the aim of identifying the sites in barrel cortex that first express RF changes. Whisker D2 and one neighbor, "D-paired" (either D1 or D3), were spared and all other whiskers on one side of the face were clipped to a length of 3 mm. Rats were anesthetized 24 hours later and the response of single units in barrel-column D2 to stimulation of whiskers D2, D-paired, and 3 surrounding cut whiskers ("D-cut," C2, and E2) was measured. In control rats, neurons in all layers of barrel-column D2 gave equal responses (on average) to the 2 neighboring whiskers, D1 and D3. After 1 day of whisker pairing, the RFs of layer IV barrel D2 cells (n=39) did not yet show any significant bias (**bias** = number of spikes evoked by D-paired / number of spikes evoked by D-cut = .96). In contrast, cells located above layer IV (n=30) had a significant RF shift in favor of the paired whisker (D-paired / D-cut = 1.90). Cells located below layer IV (n=20) also showed an RF bias (D-paired / D-cut = 1.29), though not as pronounced as above-barrel cells. These findings suggest that the initial cortical modifications induced by pairing whiskers arise from changes in the linkage between cortical barrel-columns, rather than from the relay of altered sensory information directly from VPM to layer IV. The earliest experience-dependent plasticity seems to occur among cells located above and below the barrel, and is subsequently relayed to layer IV.

(Supported by NS-25907 and P30-HD15052).

645.11

BCM MODEL OF EXPERIENCE-DEPENDENT PLASTICITY IN RAT BARREL CORTEX. L. Benuskova, M.E. Diamond, M. Armstrong-James, M. Ebert* and F.F. Ebner Institute for Developmental Neuroscience, Vanderbilt University, Nashville, TN 37203.

Previous experiments showed that the receptive fields of neurons in adult rat barrel cortex are modified by 3-30 days of "whisker pairing". Whiskers D2 and either D1 or D3 were spared while all others on one side of the face were clipped daily. Physiological recordings from barrel D2 suggested 3 types of cortical plasticity: (1) monotonic increase in the strength of thalamocortical input, (2) initial potentiation (during 3-10 days) followed by a progressive depression (during 10-30 days) of the intracortical inputs from whisker D2 and its paired neighbor, (3) monotonic depression of the intracortical inputs from clipped whiskers. In the present study we used the Bienenstock, Cooper and Munro (BCM) theory to address these observations. The BCM rules, according to which a neuron's threshold for synaptic modification is a dynamic function of its average firing rate, were applied to a model barrel D2 neuron with input from VPM barreloid D2, from within barrel-column D2, and from surrounding barrels. Computer simulations show that the threshold for synaptic modification decreases during the first 10 days of whisker pairing, allowing thalamocortical input and intracortical inputs related to the paired whiskers to potentiate; however, the threshold remains sufficiently high that purely 'noisy' inputs from the barrels of cut whiskers become depressed. After 10 days of whisker pairing, the thalamocortical input continues to potentiate. As a result, the modification threshold now begins to increase, causing the intracortical inputs from both paired and unpaired whiskers to become depressed. The sequential decrease and increase in the BCM synaptic modification threshold provides a possible mechanism for experimental observations of experience-dependent cortical plasticity. (Supported by NS-25907 and P30-HD15052)

645.12

CHANGES IN WHISKER USE MODIFY RECEPTIVE FIELDS IN ADULT RAT BARREL CORTEX. M. Armstrong-James*, M. Diamond and F. Ebner. Institute for Developmental Neuroscience, Vanderbilt University, Nashville, TN 37203.

In adult rats all but an adjacent pair of whiskers (D2 + D1 or D3) were trimmed for 3, 7 or 30 days. Then, under controlled urethane anesthesia, all whiskers were trimmed to equal length and receptive fields of cells in the D2 cortical barrel were quantitatively compared with controls. For control animals responses to the surround receptive field (SRF) inputs to the D2 barrel (D1 + D3 and C2 + E2) were symmetrical. With paired whisker experience a profound bias developed in response to the paired SRF D-Row whisker relative to the trimmed SRF D-Row whisker, which was maximal at 30 days. D2 barrel neurons responded decrementally to trimmed whiskers in the arc (C2 + E2). PSTH analysis revealed major changes in long latency discharges at 3 & 7 days consistent with intracortical modification. At 30 days of pairing shortest latency discharges to both paired whiskers increased, commensurate with potentiation of thalamo-cortical transmission. The results suggest that novel sensory experiences, in this study paired whisker use, produce changes in RF configuration, with early alterations in synaptic strength in intracortical circuits and later changes in thalamocortical relays. (Supported by NS-25907 and HD-15052, U.S.A., and the Wellcome Trust, U.K.).

645.13

ACQUISITION OF DIGITAL DEXTERITY AND PARALLEL REMODELING IN AREA 3B REPRESENTATIONS IN ADULT OWL AND SQUIRREL MONKEYS. C. Xerri*, W. M. Jenkins, B. Peterson, S. Santucci and M. M. Merzenich. Keck Center, UCSF, San Francisco, CA 94143-0732, USA.

The capacity for representational changes within the primary somatosensory cortex (S1) following behaviorally controlled tactile stimulation has been demonstrated in a series of studies conducted in our laboratory. These studies examined post training reorganization of the area 3b cortical map within the zone of cutaneous representation of hand digits. The present experiments were designed to document representational remodeling in area 3b resulting from a sensorimotor training requiring digit use. Seven owl and 2 squirrel monkeys were trained to retrieve banana-flavored food pellets from different size wells on a Klüver board. Animals were tested on pellet retrieval from each well according to a random sequential order (20 pellet presentations for each well size/session). A camera and video tape recorder were used to quantitatively determine and analyze the number of grasping movements, and to identify the digits and specific skin surfaces stimulated in the retrieval task (RT). A map of the cortical representation of the hand used in the RT was derived on the basis of multiunit recordings at the completion of testing. As a consequence of successive attempts, the most effective digit patterns were progressively selected, depending on the well size, i.e., on the degree of difficulty of the RT. Acquisition of digital dexterity was found to occur over training sessions. This sensorimotor learning was characterized by: 1) A gradual decrease in the average number of digit grasps for pellet retrieval (this held true for all well sizes); 2) A progressive reduction in the variability of the RT performance; 3) A continuous increase in the percentage of correct retrievals (pellet neither released nor ejected from the wells). Analysis of the cortical maps showed: 1) A greater cortical magnification for the skin territories of the distal phalanges of the digits most heavily engaged in the RT; 2) smaller receptive fields located on the trained skin surfaces; and 3) a decrease in the percent overlap of receptive fields in the cortical zone of representation of the trained phalanges. Some implications of these findings will be summarized.

Supported by NIH Grant NS-10414, The Coleman Fund, HRI, NSF and the CNRS.

645.14

PERCEPTUAL CORRELATES OF SOMATOSENSORY PLASTI-CITY IN MAN. V. S. Ramachandran and D. Rogers-Ramachandran*, R. Grush, UCSD, La Jolla, CA 92093.

Twelve years after deafferentation of one upper limb in adult monkeys, the area in S1 corresponding to the hand gets "taken over" by input from the face. In human patients we found that 4 weeks after amputation of one arm, touching the face elicits referred sensations (RS) to the missing (phantom) hand. Two "maps" were found, one on the face, and one just proximal to the amputation line -- as one might predict from the Penfield map (Ramachandran, 1992, *Science*). We now report that: a) The map on the face is modality specific; when water trickles on the face, it is felt to trickle down the phantom. b) Only a subset of the patients have a face map, but almost all have the map (or set of maps) near the line of amputation. c) After finger amputation, topographically organized referred sensations from adjacent fingers were seen in 2 out of 8 patients. Perhaps the other patients had learned to "ignore" the RS. d) When a normal volunteer stimulated his left forearm for 2 months (6 hrs a day) using a TENS, there was a 50% improvement in 2-point discrimination and point localization. Furthermore, intermittent muscular twitching was seen in the fingers of both hands and persisted for a week after the unit was removed! e) In one patient we saw a beautifully organized map 6" above the amputation line. When the patient imagined he was pronating the phantom, the entire map shifted by 1 cm and returned again only when he supinated the phantom. Thus the maps in S1 (or their subsequent "readout") might be modulated by reafference signals from motor commands sent to the phantom.

PAIN: PATHWAYS III

646.1

IRRITATION OF THE LOWER AIRWAYS: NEURONAL RESPONSES IN THE THORACIC SPINAL CORD. T. Hummel*, J.N. Sengupta, S.T. Meller, G.F. Gebhart, Dept. of Pharmacology, Univ. of Iowa, Iowa City, IA 52242

Numerous studies have investigated the response properties of afferents from the lower airways after application of irritants. However, little is known about integration of these responses in the spinal cord. The aim of this study was to analyze responses of T2-T3 neurons after stimulation with ammonia or smoke. Experiments were performed in 35 pentobarbital anesthetized, pancuronium paralyzed male Sprague-Dawley rats (430-530 g). Ammonia or smoke (5 or 15 respiratory cycles, respectively; interval > 8 s) were applied in 2 concentrations each via the tracheal canula. The irritants produced excitatory, inhibitory and biphasic responses in a concentration-related manner (ammonia, 40/40; smoke, 23/39). Latencies of typical responses ranged between 4 and 10 s. Almost all neurons (39/40) responded to distension of the esophagus which was used as the search stimulus. Tracheal distension elicited responses in only 3/39 neurons. Cutaneous receptive fields, sensitive predominantly to pinch, were located on the thorax, axillary region or the forearm. Since the irritants generally produced changes in arterial pressure, only neurons were tested in which sodium nitroprusside (10 µg/kg i.v.) induced no or only marginal activity. Most neurons (20/23) excited by ammonia/smoke also responded to bradykinin (20 µg/kg i.v.) while phenyl diguanide (40 µg/kg i.v.) produced responses in 13/29 neurons. Responses to irritation could still be obtained after both reversible or irreversible block of the thoracic vagi, and reversible or irreversible spinalization at C2. The current data suggest that, after irritation of the lower airways, the thoracic spinal cord receives its major input via pathways different from the vagal nerves or fibers descending from supraspinal centers. This study was supported by NS19912, NS29844, and a fellowship of the Feodor-Lynen-Stiftung, Germany.

646.2

NITRIC OXIDE, NMDA RECEPTORS, AND TRIGEMINOTHALAMIC NEURONS IN THE SPINAL TRIGEMINAL NUCLEUS. C.S. Dohrn,* L. Kus and A.J. Beitz, Department of Veterinary PathoBiology, University of Minnesota, St Paul, MN 55108

The spinal trigeminal nucleus (STN) is involved in the transmission of orofacial sensory information and is divided, both functionally and anatomically, into three subnuclei: oralis, interpolaris, and caudalis. Neither the distribution of the neuromessenger, nitric oxide (NO), within the trigeminal system nor the possible relationship of this simple gas with trigeminothalamic neurons has been carefully studied to date. Using both immunocytochemical (against nitric oxide synthase) and histochemical (i.e., NADPH-diaphorase staining) techniques, we have found that NO neurons are more predominant in the subnucleus caudalis than in the other two nuclei (n=8). To study the relationship of NO to trigeminothalamic neurons (TTN), STN neurons were retrogradely labeled by thalamic (VPM/VPL/Po) injections of fluorogold (FG;1%). NOS-stained cells were not colocalized with TTN in any of the subnuclei (n=8), but some NOS positive processes were found in close proximity to TTN. Additional *in situ* hybridization studies of NMDA NR1 receptor mRNA revealed that TTN in the subnucleus caudalis of the STN contained a significantly greater amount of NR1 receptor mRNA than retrogradely-labeled neurons in either of the other subnuclei (n=3; $p<0.001$, unpaired *t*-tests). These data suggest that TTN may be influenced by NO and further indicate that TTN in subnucleus caudalis have more NMDA receptor mRNA than the subnucleus oralis or interpolaris, which implies that the NMDA receptor may play a greater role in TTN nociceptive neurons.

646.3

EXPRESSION OF EGR-1 IN SPINAL CORD CORRELATES WITH PAW INFLAMMATION IN SLEEP DEPRIVED RATS C.A. Landis*, Dept of Physiological Nursing SM-28, Univ. of Washington, Seattle, WA 98195; Sleep Research Lab, Univ. of Chicago, Chicago, IL 60637.

We have previously reported (*Sleep Res 21*, 1992) that chronic sleep deprived (SD) rats had more intense immunocytochemical staining of Egr-1 protein in specific regions of brain and in the spinal cord dorsal horn compared to control rats. Since SD rats develop lesions on the plantar paw surface (Kushida et.al., *Sleep 12*, 1989), increased immunoreactivity localized in cells of the dorsal horn could reflect increased neural activity from persistent sensory stimulation. In this analysis, we compared extent of Egr-1 expression in cells in the dorsal horn of the spinal cord with paw lesion severity scores in SD and in their yoked control rats.

Sprague-Dawley rats were subjected to SD by the disk-over-water method for 10 days (experimental procedures were approved by the University of Chicago Animal Care and Use Committee). As expected, severity of lesions in 4 SD rats for right (1.9 ± 0.25, $X \pm$SD) and for left (1.75 ± 0.5) hind paws were significantly (both $p < 0.02$) greater than yoked control rats (right, 0.88 ± 0.5; left, 0.38 ± 0.5; ordinal scale range 0 to 3). For this analysis, mean cell counts from the right and from the left dorsal horn were obtained from camera lucida drawings of 4 randomly chosen sections through the lumbar enlargement (L4-5) for each rat, and compared to mean paw lesion severity scores. For all rats, there was a significant ($p < 0.01$) correlation, $r_s = +.89$, between lesion severity of the left hind paw and mean Egr-1 cell counts from superficial lamina of the left dorsal horn. For the right paw lesions and right superficial dorsal horn Egr-1 cell counts, the corresponding correlation (+.35) was not significant. Thus, there is evidence for a relationship between severity of inflammation and extent of Egr-1 expression, possibly reflecting chronic nociceptive stimulation.

Support: NIMH, T32 MH18825; School of Nursing IRS grant.

646.4

JAW ELECTROMYOGRAPHIC (EMG) ACTIVITY INDUCED BY APPLICATION OF ALGESIC CHEMICALS TO THE RAT TOOTH PULP. M.Sunakawa, C.Y.Chiang, D.A.Haas, J.W.Hu* and B.J.Sessle, Faculty of Dentistry, University of Toronto, Toronto, Ontario M5G 1G6, Canada.

The aim of this study was to test for jaw EMG activity induced by application to the rat maxillary molar pulp of various algesic chemicals which especially activate C-fibre afferents. Experiments were performed in rats anaesthetized with 1/3 O_2, 2/3 N_2O, and 0.5-0.8% halothane. EMG activity was recorded from the masseter muscle (Mass) bilaterally and digastric muscle (Dig) ipsilaterally, before and after application to the exposed pulp of 9.88M mustard oil, 1.64×10^{-1}M capsaicin, 10^{-4}M capsaicin, 9.43×10^{-5}M bradykinin or mineral oil (as control). Mustard oil evoked a significant increase in ipsilateral EMG activity, often with early and late phases. Mean ($\pm$S.E.) latency, EMG increase magnitude, and duration of early and late responses respectively were 50 $\pm$ 13s, 330 $\pm$ 73%, 87 $\pm$ 16s and 933 $\pm$ 166s, 188 $\pm$ 50%, 152 $\pm$ 83s (Mass) and 29 $\pm$ 11s, 160 $\pm$ 12%, 100 $\pm$ 36s and 895 $\pm$ 447s, 155 $\pm$ 17%, 143 $\pm$ 75s (Dig). Capsaicin (10^{-4}M) evoked no noticeable responses, but capsaicin (1.64×10^{-1}M) evoked a prolonged 120 - 150% EMG increase lasting >20 min in all three muscles. Bradykinin elicited only an early response (latency: 6 $\pm$ 5sec, EMG increase: 169 $\pm$ 41%, and duration: 20 $\pm$ 10s) in Dig, and no responses were observed in either Mass. No EMG changes occurred following mineral oil application to the pulp or mustard oil application to pulpectomized teeth. While these data demonstrate that algesic chemical excitation of pulp afferents can evoke jaw muscle activity, the variations in EMG responses between the different algesic chemicals suggest differences in their efficacy in exciting pulpal afferents or in the CNS connectivity of the afferents. (Supported by NIH grants DE04786 and DE09559).

646.5

ENHANCED VENTRAL ROOT REFLEX RESPONSES IN THE NEONATAL RAT SPINAL CORD *IN VITRO* FOLLOWING UV-INDUCED PERIPHERAL INFLAMMATION Dray,A*, Urban,L, and Thompson,S.W.N, Sandoz Institute for Medical Research. London WC1E 6BN, UK.

Brief exposure of the plantar surface of the rat hindpaw to UVA irradiation induces a prolonged period of mechanical and thermal hyperalgesia (Perkins et al.,1993 J.Physiol.,*459* 205P). Here we report changes in reflex activity, recorded *in vitro* following the induction of a behavioural hyperalgesia in 12-14 day old rat pups.

Hemisected spinal cords were prepared and maintained *in vitro*, from naive animals and from animals in which both a mechanical and thermal hyperalgesia had been established following UVA irradiation of the left hindpaw. Synaptic activity was recorded as a ventral root potential (VRP) from the L5 ventral root following electrical stimulation of the ipsilateral L5 dorsal root (DR).

Single shock low intensity electrical stimulation (5V, 20μs) evoked a brief VRP, which lasted significantly longer in UV treated animals than in naive (naive, 1.64±0.31s n=11; UV, 4.28±0.65s, n=11, $p<0.01$, *t* test). High intensity single shock electrical stimulation (50V, 200μs) evoked a long duration slow VRP which was also significantly longer in UV treated animals (15.6±1.5s n=8) than in naive (9.6±0.78s n=10, $p<0.05$, *t* test). Low frequency (1.0Hz) short duration (20s) high intensity DR stimulation evoked a cumulatively depolarizing VRP (CDVRP), indicative of windup, which was significantly greater in UV treated animals 0.65±0.1mV n=11) than in naive animals (0.36±0.05mV n=10). Repetitive low intensity DR stimulation rarely evoked a sustainable CDVRP in naive animals. In contrast, a substantial CDVRP was evoked following repetitive low intensity stimulation in UV treated animals (0.41mV±0.08mV n=8).

These data indicate that increased central excitability occurs during UV-induced peripheral inflammation which may contribute to the hyperalgesia observed .

646.6

COMPARISON OF NEURAL ACTIVITY IN THE LUMBAR DORSAL HORN WITH LATENCY FOR HEAT-EVOKED HINDPAW WITHDRAWAL. M. M. Morgan*. Dept. Neurology, Univ. California, San Francisco, CA 94143-0114.

Noxious cutaneous stimuli evoke activity in wide dynamic range (WDR) and high threshold (HT) neurons in the spinal dorsal horn. Although the sensory coding properties of these neurons have been well studied, little is known about the role these neurons play in nociceptive reflexes.

Single unit activity from neurons in the lumbar enlargement (primarily laminae V and VI) and the latency for hindpaw withdrawal were measured simultaneously in lightly halothane-anesthetized male rats (Sprague-Dawley). Light mechanical stimulation and noxious pinch were used to characterize neurons as WDR or HT and to map the receptive field (RF). Hindpaw withdrawal and the evoked activity of a WDR or HT neuron to noxious radiant heat applied to the RF were measured every 3 min.

The activity of 112 WDR and 58 HT neurons was studied in 76 rats. Only those rats in which noxious heat evoked a hindpaw withdrawal reflex (82% of the experiments) were used in data analysis. WDR neurons (78%) were more likely to fire prior to the withdrawal reflex than HT neurons (57%). In addition, neural activity preceded the reflex in 81% and 84% of the WDR neurons with medium and large RFs and 62% with small RFs. Likewise, 69% and 62.5% of the HT neurons with medium and large RFs fired prior to hindpaw withdrawal compared to only 30% with small RFs.

Since dorsal horn neural activity must precede spinal reflexes, the present data suggest that WDR neurons with medium or large RFs are more likely to underlie heat-evoked withdrawal reflexes than HT neurons or neurons with small RFs.

Supported by DA01949, DA05399, and Bristol-Myers Squibb Co.

646.7

MORPHOLOGICAL TYPES OF RETROGRADELY LABELED LAMINA I SPINOTHALAMIC NEURONS IN THE CAT. E.-T. Zhang*, Z.-S. Han and A.D. (Bud) Craig, Div. Neurobiology, Barrow Neurological Institute, Phoenix, Arizona 85013

Spinothalamic tract (STT) neurons in the marginal zone (lamina I) of the cervical (C5-8) and lumbosacral (L5-S1) spinal enlargements were studied after large thalamic injections of cholera toxin subunit B and development with the ABC technique, which produced excellent dendritic staining. Morphological examination of a total of 1495 labeled cells (3 cats) in serial horizontal sections disclosed at least 3 cell types: 1) fusiform (F) cells with longitudinal perikarya and bipolar dendritic arbors within superficial lamina I; 2) pyramidal (P) neurons with triangular somata and dendritic arbors oriented mainly rostrocaudally; and, 3) multipolar (M) cells with larger multiangular perikarya and dendritic arbors oriented rostrocaudally as well as mediolaterally. In addition, a small number of cells with round or oval perikarya were included with others as unclassified (U) cells. Overall, the F (39%) and P (35%) cells formed the majority, followed by M (18%) cells and the unclassified cells (8%).

The labelled lamina I STT cells were not distributed uniformly. Different proportions of F cells and P cells were found in the cervical (41% F, 33% P) and lumbosacral segments (33% F, 40% P). Cells were more numerous in the caudal portions of both enlargements. In addition, the presence of clusters of F and P cells was obvious in this material.

The lamina I STT cells, which form half of the STT, are considered to transmit pain and temperature activity in several species. Several different physiological cell types are thought to be included: nociceptive-specific, cold-specific, multireceptive (responsive to heat, pinch, and cold), and wide dynamic range. The hypothesis that the morphological types of lamina I STT cells observed in this study may correspond to these functional classes must be considered. (Supported by NS 25616)

646.8

GLUTAMATE-LIKE IMMUNOREACTIVITY IN PRIMATE SPINOTHALAMIC TRACT TERMINALS. A.-C. Ericson*, J. Broman and A. Blomqvist. Department of Cell Biology, Faculty of Health Sciences, University of Linköping, S-581 85 Linköping, Sweden.

In order to identify the neurotransmitter content of primate spinothalamic tract (STT) terminals, an antiserum to glutaraldehyde-coupled glutamate was used in a postembedding immunogold procedure at the ultrastructural level. In adult owl monkeys (*Aotus trivirgatus*) WGA:HRP was injected into the dorsal horn of the cervical or lumbar spinal cord. STT terminals in the posterior nuclei (Po) of the thalamus that included the lamina I STT termination zone, were identified by tetramethyl benzidine histochemistry. The density of gold particles over the STT terminals was compared with that over peroxidase-negative terminals of the same type as the STT terminals (RL terminals), terminals of presumed cortical origin (RS terminals), presynaptic dendrites (PSDs), large cell bodies, dendrites of presumed relay cells (RCDs) postsynaptic to STT terminals, and randomly selected areas throughout the EM section. The density of gold particles over STT terminals, RL terminals and RS terminals was significantly higher than that over PSDs, cell bodies, RCDs and tissue average. Since many of the STT neurons that project to Po respond to noxious stimulation, the present results indicate that glutamate may serve as a neurotransmitter for ascending nociceptive information in primates.

Supported by the Swedish Medical Research Council, project # 7879.

646.9

ANTIDROMIC IDENTIFICATION OF NOCICEPTIVE LAMINA I CELL TERMINATIONS IN THE CAT THALAMUS. J.O. Dostrovsky* and A.D. (Bud) Craig. Dept. of Physiology, University of Toronto, Toronto, Canada, M5S 1A8 and Div. of Neurobiology, Barrow Neurological Inst., Phoenix, AZ 85013.

The anatomically identified thalamic projections of lamina I cells in the cat occur most prominently in the medial thalamic n. submedius (Sm), the ventral aspect of the basal ventral medial n. (vVMb), the dorsomedial aspect of the ventral posterior medial n. (dmVPM), and in the ventral posterior inferior n. (VPI) and laterally adjacent paralaminar zone (pl). In previous antidromic mapping work we found that nearly all thermoreceptive-specific lamina I spinothalamic and trigeminothalamic cells projected to dmVPM and vVMB but only about half projected to Sm. In this study, we have used the antidromic method to map the projections of single nociceptive lamina I neurons.

A roving array of 8 concentric bipolar electrodes was initially positioned such that an electrode was in Sm or vVMb. Antidromic maps with 0.25 mm vertical and 0.5-1.0 mm horizontal resolution were made for 19 neurons (12 trigeminal and 7 spinal) recorded with tungsten microelectrodes in 17 barbiturate-anesthetized cats. Of these neurons, 9 were nociceptive-specific (NS), 9 were multireceptive (HPC: heat, pinch, and cold), and 1 was wide dynamic range. The units' projections were mapped in 1-8 planes. Nearly all (16/19) projected to Sm, vVMb, and VPI/pl. Only 6/15 cells tested projected to dmVPM, and fewer projected to other sites (eg, PO, Pf, CL). The projection patterns of NS and HPC cells were not different.

We conclude that (1) nociceptive and thermoreceptive lamina I cells have different though not exclusive projection patterns, (2) dmVPM may be involved primarily in thermoreception, and (3) vVMb is the only thalamic target common to all afferent lamina I cell axons. Finally, the dense nociceptive input to Sm, vVMb, and VPI/pl from trigeminal and lumbar lamina I cells supports their involvement in pain. (Supported by NIH grants NS25616, DE05404)

646.10

Sensations Evoked by Microstimulation in the Area of the Ventrocaudal Nucleus of Thalamus (Vc) in Patients with Chronic Pain. Y.C.Lin,* R.H.Gracely[1], F.A.Lenz, F.H. Baker, L.H.Rowland, P.M.Dougherty, R.T.Richardson. Departments of Neurosurg. and Neurosci. Johns Hopkins University, Baltimore, MD, USA 21287-7713 and NAB-NIDR-NIH[1].

It has often been suggested that the sensations evoked by stimulation of the CNS are altered in patients with neuropathic pain. We now report results of microelectrode recording and threshold microstimulation (TMS) prior to thalamic procedures for treatment of chronic neuropathic pain (n=12) or movement disorders (n=5). Patients were trained to use a questionnaire to describe the location (projected field - PF) and quality of sensations evoked by cutaneous stimulation preoperatively and by TMS intraoperatively. TMS evoked pain at 17% (22/127) of stimulation sites in the region of Vc representing the 'painful' part of the body but only 4% (10/253) of those in the representation of 'non-painful' part of the body ($p<0.001$, Chi square). Non-painful thermal sensations were evoked by stimulation at 5% (7/127) of sites representing the 'painful' part of the body and 16% (36/242) of sites in areas representing the 'non-painful' parts of the body. Therefore, the increase in the proportion of sites where pain sensations are evoked is accompanied by a decrease in sites where non-painful thermal sensations are evoked, in 'painful' body parts.

The quality of the patient's spontaneous pain and TMS evoked pain was assessed by overlap between ideal type pain descriptors of these two sensations. Thalamic stimulation produced pain sensations in 7 of 12 patients with chronic pain, all of whom had pain evoked by stimuli which are normally non-painful (hyperalgesia). Quality of the evoked sensation was similar to the patients pain at 83% (20/24) of sites where the PF was located in the 'painful' part of the body. These results suggest that activity of neurons signalling thermal sensations is related to ongoing pain in patients with hyperalgesia.
(Support: E Lilly Corp, NIH NS28598 and K08-NS1384).

646.11

Microstimulation in the Area of Human Vc can evoke the Sensation of Angina. F.A.Lenz*, R.H.Gracely[1], E. Hope, F.H. Baker, L.H.Rowland, P.M.Dougherty, R.T.Richardson. Departments of Neurosurg., Neurosci. and Cardiology, Johns Hopkins University, Baltimore, MD, USA 21287-7713 and NAB-NIDR-NIH[1].

The previous abstract suggests that Vc may mediate some of the symptoms of chronic pain syndromes of somatic origin.

We report results of microelectrode studies (see preceding abstract) prior to implantation of deep brain stimulating electrodes for treatment of pain secondary to arachnoiditis in a patient with a history of angina effectively treated by transluminal coronary angioplasty. Along a trajectory through Vc, cells were encountered with receptive fields to innocuous cutaneous stimulation of the chest wall. At and postero-inferior to the location of these cells TMS evoked pain identical to her angina, as described using the questionnaire. However, unlike her angina, the TMS evoked pain started suddenly, stopped suddenly and completely, and was not associated with dyspnea, diaphoresis or aftereffects. In addition to the chest pain stimulation at this site evoked a sensation of non-painful tingling in the left leg. The onset and termination of the sensation in the leg was instantaneous and identical to that for the chest pain. During previous procedures, TMS evoked sensations referred to the chest wall at 10 sites in 7/50 patients, none with angina. The sensations were described as paresthesiae at 9 sites and sharp pain at one site.

This report demonstrates that stimulation in Vc may provoke the sensation of angina in a patient who has previously experienced angina. Since stimulation of cardiac afferents evokes activity of spinothalamic tract cells and cells in the area of the thalamic principal sensory nucleus the present result may suggest that Vc mediates the sensation of angina. (Support: E Lilly Corp, NIH NS28598 and K08-NS1384).

646.12

ALTERATIONS IN HUMAN SOMATOSENSORY THALAMUS IN VARIOUS DEAFFERENTATION SYNDROMES. Z.H.T. Kiss, J.O. Dostrovsky, R.R. Tasker, K.D. Davis, W.D. Hutchison and A.M. Lozano*. Division of Neurosurgery and Department of Physiology, University of Toronto, Toronto, Ontario, Canada M5S 1A8

Experimental studies indicate that deafferentation results in reorganization of the somatosensory map at various levels of the CNS, such that the representation of a body part adjacent to a region that is denervated expands into the deafferented area. Recent data suggest that in the human this occurs at the cortical level, but subcortical structures have not been systematically investigated.

To test the hypothesis that the human thalamus is capable of significant reorganization as a result of changes in afferent input, microelectrode recording and stimulating techniques were used to define thalamic somatotopy in 33 patients undergoing stereotactic procedures. Five groups were compared: those with pain in the deafferented body part, face (n=9), upper limb (n=2), lower limb (n=4) and hemibody (n=3) and those with neither pain nor deafferentation, i.e., movement disorder (n=15).

Microstimulation induced paresthesia in the face (projected field) from a significantly larger region of thalamus in the facially denervated group compared to the movement disorder group ($p<0.001$). Similarly, in patients with hemibody hypesthesia, stimulation produced paresthesia in the entire hemibody from a significantly greater region of thalamus ($p<0.001$). However, there were no significant differences in body representation based on neuronal recordings (receptive fields) or in sampling of ventrobasal thalamus in the five groups.

These results suggest that deafferentation results in alterations in thalamocortical processing of somatosensory information from the affected region. These changes may be involved in the pathophysiology of this type of deafferentation pain.
[Supported by the MRC and Parkinson Foundation of Canada.]

646.13

INCREASED INCIDENCE OF PAIN EVOKED BY THALAMIC STIMULATION IN POST-STROKE PAIN PATIENTS. K.D. Davis*, J.O. Dostrovsky, R.R. Tasker, Z.K. Kiss and W.D. Hutchison, Department of Physiology and Division of Neurosurgery, University of Toronto, Toronto, Ontario, Canada M5S 1A8

The ventrobasal complex (VB) has been implicated in mediating the sensory aspect of pain and yet stimulation in this region only evokes pain in some patients and at limited sites. The present study examined whether the incidence of stimulation-evoked pain is similar in patients with different neurological disorders.

Data were obtained during stereotactic exploration of the thalamus in three groups of patients with: 1) post (supra)thalamic stroke pain (PSP), n=10, 2) non-stroke pain (NSP) (e.g., peripheral deafferentation), n=23, 3) movement disorders, n=24. Microelectrodes were used to record neuronal activity to identify VB and for microstimulation at regular intervals within VB.

In the PSP patients, 31% of the VB sites stimulated evoked painful sensations often described as burning. At 61% of the VB sites, stimulation elicited paraesthesia. The thresholds to elicit paraesthesia ($20 \pm 3\mu A$) or pain ($26 \pm 3\mu A$) were similar in these patients. However, threshold stimulation never evoked pain in the NSP patients and evoked pain at only 2% of VB sites in the movement disorder patients. In these latter 2 groups of patients stimulation at >98% of VB sites evoked paraesthesia. The thresholds to evoke paraesthesia in the NSP ($13 \pm 2\mu A$) and movement disorder ($9 \pm 1\mu A$) patients were significantly less ($p<.05$) than the thresholds in the PSP patients.

The increased incidence of thalamic-evoked pain in PSP patients may be due to 1) a selective loss of low threshold mechanoreceptive thalamic neurons such that nociceptive neuronal output is now prominent, 2) reduced tonic inhibitory control of thalamic or cortical nociceptive neurons, and/or 3) unmasking or strengthening of nociceptive pathways.
Supported by the Canadian MRC and Parkinson Foundation of Canada.

646.14

FEMALE MICE DISPLAY LESS PAIN BEHAVIOR THAN MALES FOLLOWING ACETIC ACID OR FORMALIN ADMINISTRATION. W.F. Sternberg, B. Kest, J.S. Mogil, and J.C. Liebeskind. Department of Psychology, University of California, Los Angeles, CA 90024.

Several investigators have previously reported that female rodents display shorter tail-flick, hot-plate, and jump test latencies than do males. These algesiometric tests all assess thresholds for brief, phasic noxious stimuli. These models of acute, cutaneous pain have been shown to differ from models of tonic, continuous pain, in both neurochemical and neuroanatomical substrates. Thus, sex differences previously noted in studies assessing pain thresholds may not be indicative of sex differences in tonic pain states. The formalin test and the acetic acid writhing test are two such models of tonic pain. Male and female Swiss- Webster mice were tested for nociceptive responses on formalin and writhing tests. In one experiment, animals were injected with 0.6% acetic acid (10 ml/kg; i.p.), and 5 min later, the number of abdominal constrictions was assessed during a 6 min period. In the second experiment, animals were injected with .02 ml of 5% formalin in the plantar surface of the left hindpaw. Time spent licking the injected paw was asessed for a 10 min period following injection. In both experiments, females exhibited less pain behavior than males. Contrary to previous reports of females displaying lower nociceptive thresholds for phasic stimuli, these findings demonstrate that females exhibit less pain behavior than males in response to tonic pain conditions. These findings have potential clinical implications since tonic pain models, because of their longer duration and association with tissue damage, provide a more accurate representation of clinical pain states. This research was supported by NIH grant NS07628 and an Unrestricted Pain Research Grant from the Bristol-Myers Squibb Company.

646.15

CEREBRAL BLOOD FLOW (CBF) DURING TONIC PAIN IN MAN. R.C. Coghill*, C. Morin, A. Evans, E. Meyer, A. Gjedde, G.H. Duncan and M.C. Bushnell. Univ. Montréal and Montreal Neurological Inst., Montréal, Canada.

Experimental models using continuous or tonic noxious stimulation have been proposed to study cerebral processes of pain in man. However, initial imaging studies of tonic or chronic pain (Apkarian et al, 1992; Di Piero et al, 1991) have revealed only decreases in CBF, while those using phasic pain (Talbot et al, 1991; Jones et al, 1991; Casey et al, 1992; Coghill et al, 1992) show primarily pain-related increases in CBF. We now examine in greater detail CBF changes during tonic pain in 10 males, using dynamic PET, with $H_2{}^{15}O$ and arterial sampling for quantitative CBF measurements, and correlated 3-D MRI/PET analysis.

The effect of stimulus intensity (70-s immersions of the index finger in 35-49°C circulating water) on CBF was examined in primary and secondary somatosensory cortices (SI and SII), anterior cingulate (AC) and thalamus--regions known to be activated by phasic noxious stimuli. Although subjects showed monotonic increases in ratings of intensity and unpleasantness (ANOVA, p's<0.001), these tonic stimuli did not evoke detectable CBF changes in SI, SII or AC; in thalamus, only 49°C produced an increased CBF relative to 35°C ($p<0.05$). Whole brain CBF (K_1) was not influenced by stimulus intensity, indicating that global CBF changes did not mask regional activation. A comparison with previous data (Coghill et al, 1992) shows that tonic 48°C stimuli evoke smaller CBF increases than did phasic 48° stimuli (ANOVA, $p<0.01$), although similar tonic stimuli generate *higher* ratings of pain (Morin et al, 1993).

Since human pain perception has been shown to correlate with both tonic and phasic noxious stimulus intensity, and with nociceptive single-unit activity recorded in primate SI and thalamus, our findings suggest a possible decoupling of CBF from neuronal mechanisms underlying pain perception during tonic stimulation. Supported by Canadian MRC and FRSQ.

646.16

PAIN INTENSITY DURING PHASIC AND TONIC HEAT STIMULATION. C. Morin, R.C. Coghill, B. Lisak, M.C. Bushnell and G.H. Duncan*. Université de Montréal, Montréal, Québec, Canada. H3C 3J7.

Imaging studies of pain perception, using phasic noxious stimuli (Talbot et al 1991; Jones et al 1992; Coghill et al 1992), have shown both cortical and subcortical regions of increased cerebral blood flow (CBF), while those using tonic noxious stimuli (Apkarian et al 1992; Coghill et al 1993) have not. This study tests a number of hypotheses that might account for this discrepancy in ΔCBF evoked during phasic and tonic pain, including differences in evoked sensations of pain intensity or unpleasantness, the habituation of the pain sensation, and the total fluctuation in perceived pain during the testing period.

Subjects (11 males) evaluated phasic and tonic presentations of noxious and innocuous heat stimuli; each paralleled procedures used in PET studies of ΔCBF performed in our labs. Phasic stimuli were six 6-s presentations (4-s ISI) of 47-48°C and 34°C heat pulses delivered to the left arm by a 1-cm contact thermode; tonic stimuli were 70-s immersions of the right index finger in 49°C and 35°C circulating water. Subjects gave continuous ratings of pain intensity on a computerized visual analog scale (VAS), and summarized rating of pain intensity and unpleasantness following the sessions.

Mean ratings of pain intensity (continuous VAS) were higher in the tonic than in the phasic condition ($p<0.001$), a finding confirmed by summary ratings of pain intensity ($p<0.001$) and unpleasantness ($p<0.01$). Neither tonic nor phasic paradigms showed habituation of evoked pain (p's>0.5). Only the total fluctuation in pain intensity proved greater for phasic than for tonic pain ($p<0.005$), suggesting that increases in regional CBF during studies of phasic pain may reflect total variation in pain perception better than maximum intensity of perceived pain. Supported by FRSQ and Canadian MRC.

646.17

THE THERMAL GRILL ILLUSION: A UNIQUE TOOL FOR STUDYING PAIN AND TEMPERATURE SENSATION. A.D. (Bud) Craig and M.C. Bushnell*. Barrow Neurol. Inst., Phoenix AZ and Univ. Montreal, Canada.

In 1896 Thunberg reported that simultaneous warm and cool stimuli applied to the skin elicit an unusual sensation of strong heat or pain similar to the burning sensation of cold pain. We are exploiting this remarkable, unique illusion by examining its psychophysical characteristics and by applying the same stimulus paradigms in physiological single unit studies.

Five subjects used VAS and verbal descriptors to rate the sensations in their right hand, which they placed on a 20x14 cm thermal grill made of 1 cm wide silver bars on computer-driven Peltier elements. Using randomized, double-blind procedures, either a warm (40°C) step, a cool (20°C) step, or an interlaced (alternate bars) warm and cool stimulus ("W+C") was presented from a base of 34°C. Subjects rated W+C more painful and hotter than warm or cool ($p<0.05$, ANOVA), more pain descriptors were assigned to W+C ($p<0.05$), and words such as "burning", "stinging", and "smarting" were used exclusively for W+C.

A smaller thermal grill was applied to the hindpaw of anesthetized cats during recordings from lamina I spinothalamic tract (STT) cells. Nociceptive-specific cells (n=3) were unresponsive to these stimuli. The average response of cold-specific cells (n=7) to W+C was only 50% of their response to cool alone. In contrast, the average response of multireceptive cells (MR-HPC: heat, pinch, and cold; n=4) to W+C remained high at 82% of their response to cool alone ($p<0.003$). A detailed hypothesis will be presented, based on this shift in the ratio of the activities of MR-HPC and cold cells, that offers an explanation for the thermal grill illusion and a substrate for the sensation of cold pain.

We conclude that the Thunberg thermal grill illusion is a robust phenomenon useful for studying the central integration of pain and temperature. (Supported by NIH grants NS 25616 and DA 07402)

646.18

PAIN AND FLARE IN HUMAN SUBJECTS: EVIDENCE THAT SEPARATE GROUPS OF NOCICEPTIVE AFFERENT FIBRES ARE INVOLVED. F. Cervero, M.D. Gee & A. Hill (SPON: European Neuroscience Association). Department of Physiology, University of Bristol Medical School, Bristol, U.K.

An injury to the skin evokes pain and induces an area of vasodilatation (flare) around the injury site. These two phenomena are commonly interpreted as being mediated by a single class of nociceptive afferent that evokes pain centrally and flare peripherally via an axon reflex mechanism. This interpretation has recently been questioned; therefore we decided to investigate if flare and pain could be dissociated, and thus whether several categories of nociceptive afferent fibre are involved, some mediating mainly pain and some mediating mainly flare.

In seven normal human volunteers three types of thermal and chemical noxious stimuli were applied to the skin of their forearms: i) short duration ramps of radiant heat raising skin temperature from 30 to 54°C at a rate of 0.4°C/sec, ii) 30 seconds applications of mustard oil at concentrations from 15 to 40% and iii) prolonged heat stimuli that evoked pain sensations with profiles similar to those produced by mustard oil applications.

No differences in pain sensation or flare areas were detected between the right and left arms of the subjects with the exception of a consistently lower heat pain threshold in the right arm. Heat stimuli evoked flare and pain that correlated with the stimulus intensity whereas mustard oil induced dose dependant flares but similar pain sensations regardless of concentration. In six of the seven subjects the prolonged heat stimuli produced significantly different areas of flare than mustard oil applications with the same pain profile. These results are consistent with the hypothesis that pain and flare are mediated by different categories of nociceptive afferent fibre, all of which are sensitive to both thermal and chemical noxious stimuli.

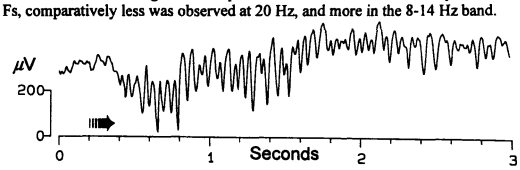
STRIATE CORTEX: RESPONSE PROPERTIES II

647.1

MOTION DRIVEN 20 HZ OSCILLATIONS IN TURTLE VISUAL CORTEX. J.C. Prechtl* & T.H. Bullock. Dept. Neurosciences, UC San Diego, CA. 92093-0201.

We previously reported that abrupt visual stimuli such as single flashes and flashes omitted after a series can evoke 17-23 Hz oscillations in the visual cortex of the turtle. The oscillations typically last a few hundred milliseconds and are not phase-locked to the stimulus. Recently, we observed similar but longer-lived oscillations that spontaneously occur with shifts in gaze and/or the presence of moving stimuli.

To further characterize these oscillations awake, attentive turtles (*Pseudemys scripta*; N=6) were stimulated with a horizontally moving black bar (9 ° wide, 11 °/s, 7 s movement on white video) while field potentials were recorded bilaterally from 8 cortical electrodes, plus an electro-oculogram. Individual electrode depths varied but in each specimen 2-5 electrodes showed stimulus-bound "high" frequency (F; ~20 Hz) oscillations with and without eye movements; the plot below is an example. All Fs sampled from the power spectrum (8, 14, 20, 26 Hz) were maximally elevated in the 1st second and declined over the 7 s stimulus period. 20 Hz activity was 432 ± 80% greater than the control period and the other Fs were 230-290% greater. The 20 and 26 Hz activity remained significantly elevated (2-tailed, paired t-test, $Ps < 0.05$) for 6 of the 7 seconds, whereas, the 8 and 14 Hz activities were not different after 2.5 and 4 s, respectively. Complex stimuli, such as moving fingers at 15 cm, evoked the greatest amplitude 20 Hz discharge for tens of seconds. Although arousing cutaneous stimuli under lights or complete darkness also evoked activity increases in all Fs, comparatively less was observed at 20 Hz, and more in the 8-14 Hz band.



647.2

HIGH FREQUENCY OSCILLATIONS IN CAT VISUAL CORTEX: INFLUENCE OF INCOHERENT STIMULI ON CORRELATIONS BETWEEN NEURONS WITH NON-OVERLAPPING RECEPTIVE FIELDS. M. Brosch, R. Bauer, R. Eckhorn and H.J. Reitboeck*. Deptm. Biophysics, Philipps-University, Renthof 7, D-3550 Marburg, Germany.

The finding of stimulus related synchronized oscillations (40-80 Hz) in cat visual cortex supported the hypothesis that correlated neural activity can define the continuity of a visual object while uncorrelated activity is indicative for different objects in a visual scene (Eckhorn et al., Biol. Cybern. 60:121-130, 1988). The present study concentrated on the potential contribution of oscillations for such scene segmentation. Multi-unit activities (MUA) and local field potentials (LFP) were pairwise recorded at spatially distant sites in areas 17 and 18 of anaesthetized cats. The correlations of oscillatory signals of neurons with non-overlapping receptive fields (RFs) were compared under 3 different stimulus conditions (1 vs 2 and 1 vs 3) : 1. Coherent stimuli: whole field grating or long bars moving slowly across the RFs in both recording positions. Incoherent stimuli: 2. Masking of the region between the RFs while stimulating the remaining visual field as in (1); 3. Two separate bars or gratings moving in opposite directions (anti-phase). In both incoherent stimulus paradigms the correlation strengths and the incidence of significantly correlated pairs was reduced compared to coherent stimulation. In the masking paradigm the correlation index of LFP oscillations dropped from 0.22 (± 0.12) to 0.15 (± 0.11) (43 pairs, U= -4.71, $p<0.001$) and in the anti-phase paradigm from 0.20 (± 0.12) to 0.14 (± 0.12)(25 pairs, U=-4.71, $p<0.001$). Similar results were found for MUA: 8 of 10 pairs were weaker correlated in the anti-phase condition than during whole-field stimulation (10/15 in the masking paradigm). Our results are consistent with previous findings for neurons with overlapping RFs (Engel et al., PNAS 88:6048-52, 1991), and, in conclusion, compatible with the hypothesis that synchronized activity can support scene segmentation and feature linking.

(Support by the DFG is greatly acknowledged: Re 547/2 and Ec 53/6).

647.3

HIGH FREQUENCY (50 - 90 Hz) OSCILLATIONS IN VISUAL CORTICAL AREAS V1 AND V2 OF AN AWAKE MONKEY ARE PHASE-LOCKED AT ZERO DELAY. R. Eckhorn*, A. Frien, R. Bauer, H. Kehr, T. Woelbern and W. Kruse, Deptm. Biophysics, Philipps-University, Renthof 7, 3550-Marburg, Germany.

The representation of a visual object is distributed over several visual cortical areas. It was proposed that synchronization of the distributed activities, evoked by an object, is the basis for its coherent perception /1/. This hypothesis was supported by the discovery of stimulus-dependent synchronization between different cortical areas of anesthetized cats /1-3/. We now demonstrate the presence of and the phase-locking between high amplitude oscillations in V1 and V2 of an awake monkey. For this we recorded spikes (single and MUA) and local field potentials (LFP) with a 7-electrode array /4/. Visual stimuli were a small fixation spot and a moving light bar on a computer screen (94 Hz). The latter stimulated the receptive fields in V1 and V2 simultaneously. A synchronization index SI was calculated for LFP (interareal: SI(V1-V2) = 0.40 ± 0.16 (N=114); intraareal: SI(V1) = 0.54 ± 0.15 (N=21) and SI (V2) = 0.50 ± 0.15 (N=48)). The average phase difference of oscillatory events of all V1-V2 recording pairs was zero, while individual pairs were distributed at SD = 3.2 ms. Zero phase difference is of particular interest, because V1 and V2 are serially arranged with respect to the afferent visual stream and V1-V2 conduction delays are several milliseconds. The present and previous data suggest that V1-V2 synchronization is mainly due to cortico-cortical feedback interactions and not to a common subcortical "oscillator". Our discovery of synchronized high frequency oscillations between two visual cortical areas of an awake monkey at high amplitudes and zero phase lag implies that synchronization supports the combined processing of common visual features and it supports the "feature-linking-hypothesis".

1. Eckhorn et al. (1988) Biological Cybernetics 60:121-130. 2. Eckhorn et al. (1990) In: Brain and Perception, Elsner N, Roth G (eds), Thieme Stuttgart New York, p 237. 3. Engel et al (1991) Science 252:1177-1179. 4. Eckhorn et al (1993) NeuroReport 4:243-246. (Supported by DFG Ec 53/6 and Ec 53/7)

647.4

20 Hz BURSTS OF ACTIVITY IN CORTICO-THALAMIC PATHWAY DURING ATTENTIVE PERCEPTION **A. Wróbel[1], M. Bekisz[1] and G.L. Gerstein[*]. [1]Dept. of Neurophys., Nencki Institute of Exp. Biol. 02-093 Warsaw, Poland and [*]Dept. of Physiol., Univ. of Penna., PA 19104**

Cats were taught to attend to the location of disappearance of either visual or acoustic moving stimuli which signaled reward in the left or right foodwell. Simultaneous frequency analysis of EEG activity, recorded from the primary visual cortex (VCx) and lateral geniculate nucleus (LGN), revealed a significant elevation of the 20 Hz band only during visual trials that ended with successful response. Such elevation was not observed either during acoustic or erroneously ended visual trials. This specific activity was characterized by 0.1 - 1 s bursts of oscillations appearing coincidentally in VCx and LGN. The mean frequency of their appearance, amplitude and average duration observed during visual trials exceeded the relevant values for acoustic trials. The calculation of directed transfer function suggested that this frequency was propagated by descending visual pathway. Finally, during visual trials we observed also the relevant changes of potentials evoked by paired electrical stimulation of the optic chiasm, as recorded in both LGN and VCx.

We hypothesize that short synchronized bursts of 20 Hz frequency in the cortico-thalamic system might set the gain of geniculate relay during attentive perception.

647.5

STIMULUS-DEPENDENT CHANGES IN THE CORRELATION STRUCTURE OF THE ACTIVITY OF NEURONAL POPULATIONS IN PRIMARY VISUAL CORTEX OF THE MACAQUE. J. Victor*, A. Canel, and K. Purpura, Dept. of Neurology and Neuroscience, Cornell University Medical College, New York, NY 10021.

Standard quantitative approaches to the analysis of the responses of visual neurons (peri-stimulus histograms, Fourier analysis, and cross-correlations) are fundamentally averaging methods. Since these methods are based on the notion that activity which is not temporally locked to the stimulus represents "noise", they aim to remove this "noise" from the response characterization. However, visual stimuli may affect the ongoing activity of neurons in a manner which is not synchronized to the stimulus -- e.g., modulation of the overall level of spontaneous activity or induction of oscillations. Since these phenomena are suppressed by standard averaging methods, we developed a new approach, phase-locked spectral analysis (PLSA), to examine responses to periodic visual stimuli.

In essence, PLSA has two stages: (i) estimation of the average response to a periodic stimulus, and (ii) spectral analysis of the difference between individual sweeps and the estimated average response. PLSA goes beyond power spectral estimation, by exploiting covariances of components whose frequencies differ by multiples of the stimulus frequency. PLSA has a time-domain counterpart, phase-locked cross-correlation (PLCC), which describes how auto- and cross-correlations vary over the stimulus period. We have applied these methods to multichannel recordings of local field potentials in the striate cortex of the anaesthetized macaque monkey. Modulation of auto- and cross-spectra elicited by checkerboard stimulation occurs over a broad range (at least 3 to 25 Hz), and is not confined to a narrow frequency range, as would have been expected for oscillations.

647.6

ACCURATE FIXATION DOES NOT SUBSTANTIALLY REDUCE THE NOISE OF COMPLEX CELLS IN PRIMARY VISUAL CORTEX. T.W. Kjaer, T.J. Gawne, J.A. Hertz and B.J. Richmond*. Laboratory of Neuropsychology, National Institute of Mental Health, Bethesda, MD 20892.

When a primate fixates on an object many times in succession the actual eye position has substantial scatter. These small changes in eye position could make a major contribution to the variability in the responses of neurons in primary visual cortex. It is essential to know how much of the observed variation in neuronal recordings from awake animals can be attributed to these fluctuations and how much is intrinsic to the functioning visual system. We have investigated how much of the variance in the neuronal response of V1 neurons is due to variability in eye position. 13 neurons with peripheral receptive fields were recorded from V1 of fixating monkeys while black and white patterns were presented on a projection screen. Eye position was measured to a resolution of 3' using a magnetic search coil. The typical observed shifts were of about the same size as the smallest features in the patterns, so one might expect substantial related changes in the neuronal responses.

We have been unable to detect such changes. We have quantified this result in two ways. First we calculated the information about the stimulus set available in the response from two sets of data: the most central 50% of fixations (these were within 40' of the mean fixation position), and the remaining 50% of the data. For 9 neurons, restricting the eye position to the central region did not increase the transmitted information. For the other 4 neurons, restricting the eye position did increase the information, but by less than 5% of the maximum possible. Second, linear regression showed no correlation between eye position and response variance.

We conjecture that the circuitry of V1 is able to compensate for these small shifts of fixation by some as-yet unknown mechanism.

647.7

SPONTANEOUS ACTIVITY AND RESPONSE PROPERTIES OF NEURONS IN STRIATE CORTEX OF TRAINED MONKEYS. D. M. Snodderly* and M. Gur[1] Schepens Eye Res. Inst. and Harvard Med. Sch., Boston MA, 02114; [1] Biomed. Eng., Technion, Haifa, Israel.

In awake monkeys, zones of strong spontaneous activity in striate cortex correspond closely to the regions of high cytochrome oxidase activity, laminae 4A, 4C, and 6. Spontaneous activity in layer 6 implies a tonic corticofugal influence on subcortical centers. This activity is suppressed by sleep or anesthesia. In combination with dye-marking of the electrode track, the spontaneous activity facilitates assignment of cells to individual cortical laminae. DiI marks have remained localized for at least 5 weeks and they correspond well to the electrode depth. Among the easiest cells to determine their distribution among the layers are the directionally selective (DS) neurons. With one exception, they were confined to layers 4B, 4C (mostly 4C alpha), or 6. This agrees well with the laminar distribution reported for anesthetized animals and it justifies our combination of physiological and anatomical criteria The width of the activating region (AR) of the receptive field does not simply increase with distance from the main geniculorecipient layer 4C but has a profile such that neurons in the more spontaneous layers 4A and 6 often have larger AR's while neurons in the quieter layers tend to have smaller AR's. DS neurons in layer 4 have AR's that are among the smallest in striate cortex, whereas those in layer 6 that are spontaneously active can have much larger AR's. This raises the question how cells in MT that receive inputs from the DS cells in V1 utilize those inputs within the large receptive fields reported in MT.

647.8

PAN: A NEURAL NETWORK MODEL OF MAMMALIAN VISUAL CORTEX RECEPTIVE FIELD AND COLUMN FORMATION

Frank W. Grasso.* Neuroscience and Behavior Program
University of Massachusetts, Amherst, MA 01003

The Primitive Acquisition Network (PAN) models layer IV of V1 as a 2^d sheet of neuron-like elements with local lateral inhibitory connections and excitatory local retinotopic input connections from 'on' and 'off' pre-processing layers. PAN networks self-organize representations of the input 'world' by means of a Hebbian learning rule and a modification threshold that increases in each element. The learning rule permits an evolution of receptive fields (RFs) via modification of the input connections. PAN does not favor a specific RF organization *a priori*. The modification threshold induces a stochastic experience-dependent, critical-period for the evolution of network connections. In computer simulations, separate PAN networks with initial random weights were trained with oriented sinusoidal gratings, digitized natural images of forest scenes or pseudo-random images. All three training sets produced aggregates of elements in the layer with like orientation selectivities (iso-orientation domains). Psuedo-random image sets led to PAN elements with weaker orientation selectivity compared to those from nets trained with the other sets. Natural image sets produced aggregates of elements with selectivity for specific natural images that exist in parallel with the orientation selective aggregates. These results suggest multiple representations that can be dynamically evoked by input context might be stored in the synapses of a 2^d patch of layer IV in primary visual cortex.

647.9

ADAPTING RETARDS RESPONSE TIMING IN SINGLE NEURONS OF CAT VISUAL CORTEX. A.B. Saul*. Dept. of Neurobiology, Univ. of Pittsburgh Sch. of Med., Pittsburgh PA 15261.

Previous studies have demonstrated the existence of adaptation aftereffects in single cells in cat visual cortex. Reports of aftereffects have heretofore discussed reduced response amplitudes, or, equivalently, shifts of the contrast response function toward higher contrasts.

This study extends the concept of aftereffects in single neurons to changes in *timing* as well as in amplitude. Simple cells were recorded in anesthetized, paralyzed cats. Stimuli were sinusoidally-modulated gratings and bars. Responses in all cells were therefore modulated at the stimulus temporal frequency, and had phase as well as amplitude values. Response phase measurements are extremely reliable. Adapted response phase values were consistently and significantly later than control values. Thus, adaptation aftereffects in single cells consist of reduced amplitudes *and* delayed responses. The change in response timing was not due to transient effects from the switch between adapting and test stimuli; 2 seconds after the end of adapting the effect was still present. The retarded response phase reflects a delayed onset of the excitatory discharge. Offsets were similar in control and adapted histograms.

Like amplitude aftereffects, the tuning of these timing aftereffects was related to the adapting stimulus. Nonetheless, the amplitude and phase aftereffects were poorly correlated.

These results are consistent with the hypothesis that adapting potentiates inhibitory inputs. Inhibition can affect timing as well as amplitude. These physiological data may also be related to Menees' psychophysical finding that adapting increases reaction time (ARVO 92).

Supported by BNS-9021495 and EY-08098.

647.10

ORIENTATIONS TUNING OF CELLS IN AREA 18 DURING LOCAL BLOCKADE OF AREA 17, IN CATS. A. Chabli* and S. Molotchnikoff. Dept. of Biology, University of Montreal, Montreal, Quebec, Canada, H3C 3J7.

The hierarchical model of Hubel and Wiesel proposes that complex cells of area 18 are mostly contacted by simple units of area 17. This model has been challenged, and it is still unclear what is the role of long-horizontal cortico-cortical connections (LHC)? Specifically are LHC organized along iso-orientational or cross-orientational lines of connectivity? As we recently suggested that LHC can be subdivided along simple and complex cell types, the present experiments intend to further clarify the role of LHC. Cats were anesthetized and prepared for single cell recordings. In area 17, a pressure injecting-recording pipette filled with GABA depresses neuronal activity. The same pipette allowed recording of multiunit activity and the determination of the orientational bias of the column under GABA injection site. Cells of area 18 were stimulated with drifting sinusoïdal gratings (optimal spatial and temporal frequencies). Orientation tuning curves were plotted for each neuron of area 18 prior to, during and after blockade of area 17. Thus we could analyze the modifications of the tuning curves of units in area 18 after the depression of specific orientational columns in area 17. Under stable conditions the same cell was tested with the successive depression of two columns whose orientations were 90° apart. Although results are preliminary it appears that simple cell responses to optimal orientation are depressed when both orientations (in 17 and 18) are similar, while the responses are augmented if the depressed column exhibit orthogonal orientation relative to the tested cells. The converse situation appears to hold for complex cells.

647.11

THE LATERAL POSTERIOR-PULVINAR COMPLEX INFLUENCES OF THE FAR SURROUND ON CELLS IN AREAS 17 AND 18 OF CATS. S. Shumikhina, S. Molotchnikoff* and C. Casanova, Dépt. Sc. biologiques, Université de Montréal, Montréal, Québec, Canada, H3C 3J7.

Although the lateral-posterior-pulvinar complex (LP-P) sends fibers to visual areas 17 and 18 of cats, the role of this thalamic input is not yet understood. It has been proposed that this complex is involved in the perception of saliant features of visual targets. The aim of the present investigation is to look at possible functions of the LP-P afferents to cortex. Cats were anesthetized and prepared for single cell recordings in areas 17 and 18. In parallel a micro-injecting-recording pipette filled with GABA was introduced into the LP-P. In all experiments the position of this electrode was histologically verified. Cells were stimulated either with slits or drifting sinusoïdal gratings centered in the receptive field. These targets were presented either with low (20%) or high (80%) contrasts. The far surround of the receptive field was masked or invaded by both types of stimuli. Out of 37 cortical cells which were tested during LP-P blockade, 67% modified their responses. With high contrast stimuli the difference (> 25%) in response magnitude between masked and unmasked far surround was suppressed or inverted when LP-P was injected. This effect appeared to be more pronounced in complex cells. With low contrast stimuli LP-P injections increased the response difference between the two conditions of stimulation (mask-no-mask). Thus it appears that the LP-P modulates the contribution of the far surround to responses of targets located within the receptive-field. This contribution may be one mechanism through which the LP-P is associated to the saliance of the visual targets.

STRIATE CORTEX: RESPONSE PROPERTIES III

648.1

Y-CELL SIGNATURE IN MONKEY V1 NEURONS.

S. Hochstein[*,2] *E. A. Stern*[1,2], *V. Yakovlev*[1,2] *and E. Vaadia*[1,3].
[1]*Center for Neural Computation,* [2]*Dept. of Neurobiology and* [3]*Hadassah Medical School, Hebrew University, Jerusalem, Israel.*

Cat retinal and geniculate Y-cells show a characteristic response to sinusoidal stimulus gratings, which has been termed "the Y-cell signature". The response is spatial-frequency (SF) dependent, so that its Fourier transform is dominated by the odd portion (especially the fundamental component) at low spatial frequencies, and the even portion (DC and second harmonics) at high spatial frequencies. The ratio of even:odd spatial frequency cut-off is generally about 3:1. The Y-cell signature has not been demonstrated in cat area 17 neurons.

We recorded from supergranulate layers of V1 in an awake monkey performing a fixation task, and presented drifting sinusoidal grating stimuli. Many cells showed sustained and modulated responses under different stimulus conditions. As in most cat area 17 neurons, the SF dependences of the even and odd portions of the response were often similar. However, at least 20% of the cells showed the 3:1 even:odd spatial-frequency cut-off, i.e. the Y-cell signature. This characteristic could only be uncovered by using tests at a full range of SFs. In addition, sometimes the tuning of the modulated and the DC components to other stimulus dimensions also differed considerably.

The source of this activity could be from Y-like input to the cortex from subcortical areas or *de novo* from cortical processing. We propose the latter as we only found this type of activity in the upper cortical layers.

Supported by US-Israel Binational Science Foundation & Israel Academy of Sciences.

648.2

INFLUENCE OF STIMULUS SIZE ON RESPONSES OF CELLS IN CAT'S AREA 17 AND IN LPI TO MOVING RANDOM DOT PATTERNS. C. Casanova*, T. Savard, D. Arseneault,. Department of Ophthalmology and Department of Physiology and Biophysics, Fac. Med, University of Sherbrooke, Sherbrooke, Quebec, Canada, J1H 5N4.

We reported that most simple cells in the cat's striate cortex respond to the motion of a random dot pattern (Casanova, 1992, Soc. Neurosci. Abstr. 18, 1033) supporting then the claim that both simple and complex cells in area 17 could be driven by visual noise (Skottun et al. 1988, J. Neurophysiol. 59, 1719). We are now investigating the cells' responses as a function of the size of the stimulus i.e. the area of the visual field stimulated with noise. Experiments were performed with anesthetized normal adult cats. Receptive fields (RFs) in area 17 and in its thalamic target (LPI) were characterized with drifting sine-wave gratings and moving bars. Under computer control, a random texture field (256x256 elements subtending each 0.08°) moving at optimal direction and speed was presented in a circular window with varying diameter. Results indicated that 1) many cells responded poorly when the noise only covered the classical RF. 2) for most units, the response increased with diameter and was either saturated or did not level off. In the cortex, the mean size of the stimuli necessary to evoke half-maximal cell responses was respectively 7.4° and 3.8° for noise and grating (t-test: $p<0.05$). When stimulated with the noise, inhibition from the periphery of the classical RF does not manifest itself as strongly as for the bars of a grating. Overall, our results suggest that the minimal excitatory field for noise may be larger than the classical RF.

Supp. by Grant MT-10962 of MRC of Canada, FRSQ, and FCAR.

648.3

INHIBITATORY SPATIAL INTERACTIONS OF LOCAL FIELD POTENTIALS IN CAT VISUAL CORTEX. K. Niiyama, T. Kasamatsu*, E. E. Sutter and A. M. Norcia The Smith-Kettlewell Eye Research Institute, San Francisco, CA, 94115

Cortical neurons in area 17 have dendritic and axon collateral arborizations which spread well beyond the limit of hypercolumns. We have found a local field potential whose receptive field extends far beyond the range of classical receptive fields of single neurons. Here, we studied the spatial organization of these large receptive field by simultaneously stimulating multiple areas within the field. A pair of tungsten-in-glass microelectrodes (tip separation, ~300μm) was placed in area 17 of anesthetized and paralyzed cats. Receptive-field properties of single cells were characterized by an oriented moving light slit. At the same cortical location, field potentials were evoked by contrast reversal of bar gratings (1.0 c/deg) in a 3-deg hexagonal patch. The gratings were presented at one, two or all of the 61 hexagons that covered 25-deg of the central visual field. Contrast reversal was modulated in time using a pseudorandom, binary m-sequence and responses were extracted by a multi-input nonlinear systems analysis method (Sutter, 1992). The stimulus array was centered on the classical receptive field. With single-area stimulation, a slow component (SC) of field-potential showed a very large receptive field. With simultaneous stimulation at two or all of the 61 patches, the SC decreased in amplitude. Amplitude reductions in two-area stimulation extended over a considerable distance and were largest when the two areas were close together. This nonlinear inhibitatory interaction within the non-classical receptive field is interpreted as being due to the presence of long-range intercortical connections within visual cortex.

(USPHS Grants BRSG-05981, Core Grant EY-06883)

648.4

COMPUTING STEREO DISPARITY WITH KNOWN BINOCULAR CELL PROPERTIES. N. Qian*, B. J. Geesaman and R. A. Andersen, Dept. of Brain and Cognitive Sciences, MIT, Cambridge, MA 02139.

Cells sensitive to binocular disparity have been found in the visual cortex, but it is not clear how these cells might be used collectively to compute disparity maps from stereograms. Although many models for stereo disparity computation have been proposed, few, if any, adhere rigidly to physiological data. Here we propose a model for biological stereo vision based on known receptive field profiles of binocular cells described by Freeman and Ohzawa (1990, *Vision Res.*, 30:1661-1676), and provide the first demonstration that filters similar to real binocular cells can be used to effectively solve random dot stereograms. More specifically, we found through analysis and computer simulations that binocular simple cells do not have consistent disparity tuning behavior because their responses are also dependent on the Fourier phases of the stimuli, and that reliable tuning can be achieved by properly combining simple cell outputs to construct model complex cells. A family of such complex cells can then form a distributed representation of disparity and the actual disparity values of the stimuli can be easily estimated from such a distribution. Our model also allows a natural integration of stereo vision and motion detection. One attribute of the unified model is that motion along fronto-parallel planes is favored over motion in depth. To test this prediction we generated displays containing ambiguous stereo matches that could potentially give rise to both types of motion. Perceptually, motion was perceived only along the fronto-parallel plane, and ambiguities in stereo correspondence were resolved. Another prediction of the model, verified by our psychophysics, is that disparity cues are found to facilitate representation of more than one motion vector at a given spatial location. Thus, the model is not only physiologically plausible, but it also explains several novel psychophysical phenomena.

648.5

NEURONS IN PRIMATE VISUAL CORTEX MULTIPLEX INFORMATION ABOUT RED/GREEN, BLUE/YELLOW, AND BLACK/WHITE OPPONENCIES USING TEMPORAL CODES. J.W. McClurkin*, J.A. Zarbock, and L.M. Optican. Laboratory of Sensorimotor Research, National Eye Institute, Bethesda, MD 20892.

Last year we reported that visual cortical neurons represented stimulus *pattern* with a temporal code that was common to all neurons within an area, and similar across visual areas. This year we report that visual cortical neurons also represent stimulus *color* with a temporal code that is common to all neurons within an area, and similar across visual areas.

Responses of neurons in cortical areas V1, V2, V3 and V4 were recorded in a monkey trained to fixate a spot while red, green, yellow, and blue bars, isoluminant with the background, or black and white bars were flashed on their receptive fields. The bars were presented at eight different orientations. The amount of information the neurons could transmit about the color or the luminance of the bars, independent of orientation, was measured assuming codes based on either the number of spikes, or the temporal distribution of spikes in the responses. The temporal codes enabled neurons to transmit six times as much information about color and three times as much information about luminance as did the spike count codes. This suggests that these neurons used temporal codes for color and luminance. We expected to find neurons with codes for either red/green, blue/yellow, or black/white opponencies. However, we were able to extract codes representing all three opponencies from the responses of each neuron.

These results suggest a change in the color and luminance encoding mechanisms from LGN to cortex. In LGN, different opponencies are represented by different neurons. However, in cortex different opponencies are represented by different temporal codes within the same neuron. This is consistent with our hypothesis that, as you proceed into the visual system, information shifts from strength codes carried by different neurons, to temporal codes shared by all neurons.

648.6

CORTICO-GENICULATE AND CORTICO-CLAUSTRAL CELLS OF LAYER VI - OPPOSITE ENDS OF A RECEPTIVE FIELD LENGTH SPECTRUM? K.L. Grieve* and A.M. Sillito. Dept. of Visual Science, Inst. of Ophthalmology, Bath St., London EC1V 9EL, UK.

We have now shown that the majority of cells in layer VI of the cat striate cortex have relatively short receptive field (RF) lengths, in contradistinction to the traditional view of a layer dominated by cells with extremely long RF's. Layer VI has also been shown to play a critical role in the production of length selectivity at the level of the dLGN, and its maintenance within the cortex, by enhanced intragenicular inhibition and intracortical facilitation respectively. This dual nature is in keeping with morphology and synaptology of corticogeniculate layer VI cells, which also have extensive intrinsic collateral axons within cortex - cells lacking such collaterals project to other targets, such as the visual claustrum. Although evidence suggested that the influence of the corticogeniculate cells was probably restricted in the length domain, our previous study could not directly identify cells projecting to the dLGN, and a small but significant proportion of cells with very long RF's were found, leaving open the question of RF length vs axonal target. Here we report length response curves of cells shown to project to the dLGN or claustrum, identified by antidromic activation. We recorded from single cortical cells of anaesthetized, paralysed cats, electrically stimulating via electrodes in the dLGN and visual claustrum. Length tuning curves were derived from responses to randomly interleaved bar stimuli of varied length drifted over the RF at the optimum orientation. For the vast majority of the corticogeniculate projecting cells, RF lengths were short, with a mean value of around 3°. On the other hand, corticoclaustrally projecting cells were exclusively of the very long RF type, very similar to the known properties of claustral cells themselves, which also have very long RF's. Our data therefore supports the view that the short field layer VI cells provide both corticogeniculate feedback and intracortical interlaminar information transfer.

648.7

NEURONAL ELEMENTS ACTIVATED BY ELECTRICAL STIMULATION IN THE CORTICAL GRAY MATTER. L.G. Nowak* and J. Bullier,. Inserm U371, 69500 BRON/LYON, FRANCE.

Despite its extensive use in neurobiology, the effects of electrical stimulation are not well understood. In particular, the neuronal elements activated in the cortical gray matter are not clearly identified. To identify these elements, we have conducted two sets of experiments on slices of rat visual cortex, using monopolar, cathodal electrical stimulations through tungsten in glass micro-electrodes. In the first series of experiments, we made chronaxie measurements, which give an indirect estimate of the time constants of the activated elements (Ranck 1975, Brain Res. 98:417-440). Using intracellular recordings, we found that the chronaxies for eliciting small, presumably unitary, EPSPs have an average value of 0,424 msec (SD = 0,178 msec). This is not different from values corresponding to the activation of axons, as determined by recording antidromic action potentials (0,363 ± 0,196 msec). These results suggest that the unitary EPSPs observed after electrical stimulation did not result from activation of cell bodies, which have time constant of at least 10 msec.

It was then important to determine whether the axonal elements activated by electrical stimulation were initial segments or axonal branches. To clarify this point, we applied glutamate in the close vicinity of the stimulating electrode to produce a depolarization block in dendrites, cell body and axon initial segment. In that situation, electrical stimulation cannot lead to spike initiation in these elements. On the other hand, axons, which lack glutamate receptors, should not be affected. We compared the amplitudes of field potentials obtained by electrical stimulation before, during and after the glutamate-induced depolarization block. So far, in 4 out of 4 cases, we found that the field potentials were not modified.

These results, together with the results from chronaxie measurements, suggest that the large majority of neuronal elements activated by electrical stimulations are *en passant* axons. Given that axons can come from, or project to, many different cortical and subcortical structures, results obtained with electrical stimulation in the cortical gray matter should be interpreted with caution.

648.8

Synaptic potentials in visual cortical neurons: temporal variability and spatial structure. L.J.Toth*, S.B.Nelson, S.C.Rao, M.Sur. Department of Brain and Cognitive Sciences, MIT, Cambridge MA.

Using *in vivo* whole–cell recording from area 17 of cats, we examined the intracellular responses of cells to high contrast stationary and moving bars of light. These stimuli evoked a complex combination of excitatory and inhibitory potentials which underlied the spiking response. In general, responses to flashed bars were phase locked to stimulus onset or offset and highly repeatable (consistently present from trial to trial). Some responses to moving bars showed temporally locked onsets and offsets; other responses were less tightly locked to the stimulus and showed more gradual onsets and offsets. One interpretation of these data is that flashed bars evoke temporally correlated responses from cells earlier in the visual pathway than those we recorded, and such inputs lead to better phase locked responses in our cells than do inputs activated by moving bars.

These data were collected to study the feasibility of measuring receptive fields inclusive of subthreshold potentials. Preliminary results with moving bars indicate that the region generating a cell's spike response is only a subset of the larger region over which it sums inputs. It is possible that inputs from outside an extracellularly measured receptive field produce the temporal variability in intracellular responses to moving stimuli. Supported by EY07023.

648.9

THE DISTRIBUTION OF NMDA RESPONSES ALONG THE APICAL DENDRITES OF PYRAMIDAL CELLS IN RAT VISUAL CORTEX. S.N. Currie*, X.F. Wang and N.W. Daw. University of California, Riverside and Yale University, New Haven.

In adult cortex NMDA receptors are concentrated in layers II and III. This raises the question: do pyramidal cells in layers V and VI, that send apical dendrites to layers II and III, have a substantial response to NMDA iontophoresed along their apical dendrites away from the cell body, but not near the cell body? We recorded 40 cells by the whole cell technique in slices of rat visual cortex; electrodes contained 1% biocytin. NMDA was iontophoresed at a series of positions, then TTX was introduced into the solution and a second set of records taken. In general, the response to NMDA increased as the electrode was moved closer to the cell body. One cell had a response in layers II and III that was as large as in layer IV. When reconstructed, this cell had an exceptionally large arborization in layer II, suggesting that the large NMDA response was due to the anatomy of the cell, rather than the density of NMDA receptors. Several cells that had little response to NMDA in layers II and III after infusion of TTX responded with action potentials before infusion of TTX. We conclude that the NMDA receptors found in layers II and III reside primarily on the bodies of cells in layers II and III, rather than on the apical dendrites of cells in layers V and VI.

649.10

SYNAPTIC ORGANIZATION OF LATERAL PYRAMIDAL CELLS IN VISUAL CORTEX: SPATIAL DISTRIBUTION OF $GABA_A$ RECEPTORS. M. Fowler* and P. S. Ulinski. Departments of Psychology and of Organismal Biology and Anatomy, University of Chicago, Chicago, Il 60637.

GABAergic neurons in visual cortex access both $GABA_A$ and $GABA_B$ receptors, but the spatial distribution of the two types of receptors upon cortical neurons is not known. This study uses an *in vitro* brain-eye preparation of freshwater turtles (*Chrysemys picta*) to characterize the spatial distribution of $GABA_A$ receptors upon pyramidal cells in lateral visual cortex. Conventional recording methods were used to record postsynaptic potentials from neurons characterized as lateral pyramidal cells. Spontaneous IPSPs with shape parameters and reversal potentials characteristic of $GABA_A$-mediated IPSPs occur when the retina is in the dark. The electrotonic lengths of the cells were estimated using the method of peeled exponentials and an equivalent cable model for each cell was constructed. IPSPs were simulated using this cable model and parameters for $GABA_A$-mediated IPSCs. Amplitudes of simulated IPSPs generated for known distances from the soma were used to estimate the positions of the real IPSPs. $GABA_A$-mediated IPSPs are generated with low frequency on the soma and then increase to a maximum frequency between 150 and 250 μm from the soma. Supported by PHS Grant EY 08352.

649.11

SYNAPTIC ORGANIZATION OF LATERAL PYRAMIDAL CELLS IN VISUAL CORTEX: GEOMETRY OF PYRAMIDAL CELLS. Matthew T. Calef and Philip S. Ulinski*. Department of Organismal Biology and Anatomy, Univ. Chicago, Chicago, IL 60637.

The visual cortex of freshwater turtles is divided into cytoarchitecturally distinct lateral and medial parts. Pyramidal cells in these two parts differ significantly in their morphology. Since an understanding of the integrative physiology of a neuron depends upon a knowledge of its geometry, lateral pyramidal cells were studied in Golgi preparations of turtle forebrain. Lateral pyramidal cells have a total surface area of about 19,500 μm^2, of which about 7 % is on the fusiform somata. Apical and basal dendritic trees originate from a primary dendrite about 2.6 μm in diameter and bear 1 to 4 secondary and tertiary dendrites with diameters that taper from 1.7 μm to 1.2 μm. The ratio $(\Sigma d_{br}{}^{3/2})/(d_p)^{3/2}$ varies from 1.4 proximally to 1.2 distally. The total path lengths of dendrites are about 320 μm from the soma to their tips. Dendrites bear about 260 spines per dendrite that vary in shape from stubby, to mushroom-like to pedunculated. The spine density increases along the dendrites from about 0.3 spines/10 μm near the soma to a peak of about 12 spines /10 μm about 170 μm from the soma and then gradually declines. Spines contribute about 32 % of the total surface area of the cell. These data are being used to construct compartmental models of lateral pyramidal cells. Supported by PHS grant EY 08352.

AUDITORY, VESTIBULAR, AND LATERAL LINE: HAIR CELLS

649.1

ACTION OF ACETYLCHOLINE ON COCHLEAR OUTER HAIR CELLS C. Erόstegui[1,2], C.H. Norris[1]* and R.P. Bobbin[2], [1] Dept. Otolaryngology, Tulane University School of Medicine, and [2]Kresge Hearing Research Laboratory, Dept. of Otolaryngology, LSU Medical Center, New Orleans, LA 70112 USA

Acetylcholine (Ach) hyperpolarizes outer hair cells (OHCs) by increasing an outward K^+ current (Housley & Ashmore, Proc. R. Soc. Lond. B 244, 1991; Erόstegui et.al., ARO Abstr. 83, 329, 1993). Ach may induce Ca^{2+} entry which activates a $K_{(Ca)}$ channel. However the mechanism of action of Ach is unclear. Thus we investigated the conductances involved in Ach induced current. OHCs were isolated from guinea pig cochleae and placed in Ringer containing 10 mM Cs^+. Recordings from cells were made with the whole-cell configuration of voltage-clamp technique. Pipettes were filled with intracellular solutions containing 145 mM Cs^+. Cs^+ shifted the I-V plot of OHCs decreasing the outward and inward currents. This indicates that Cs^+ blocked both the K^+ conductance at rest and $K_{(Ca)}$ conductance. Application of 100 μM Ach induced an outward current which reversed about -60 mV. As the cell was depolarized the current increased. This outward current was blocked by 100 μM Cd^{2+}. We conclude that Ach activates an outward current through a channel that is different from the $K_{(Ca)}$ channels described in these cells, but similar to the non-specific cationic channel in chick short hair cells (Fuchs & Murrow, J. of Neurosc. 12 1992). Supported by NIH research grant DC00722.

649.2

VOLTAGE-DEPENDENT CURRENTS IN HAIR CELLS OF THE CULTURED NEONATAL MOUSE UTRICLE. A. Rüsch and R.A. Eatock*; Dept. of Otolaryngology, Baylor College of Medicine, Houston, TX 77030

Using the whole-cell, voltage-clamp configuration of the patch-clamp technique, we recorded voltage-activated membrane currents from utricular hair cells, cultured on postnatal day 1 to 4 and maintained in vitro for up to 8 days. Cells were grouped in three classes based on their conductances.

In one class of hair cells (resting potential, RP: -64 ± 5.4 mV (mean ± S.D.; n=33 cells)), depolarizations from a holding potential of −83 mV to potentials above −63 mV activated large outward currents with a sigmoidal onset (peak values ≤ 7 nA at 0 mV). These currents inactivated partially, reversed close to the potassium equilibrium potential, and were reversibly reduced by about 90% by 5 mM 4-AP. We assume these currents to be of the delayed rectifier type. In all cells of this class an inward current through an inwardly rectifying conductance was recorded upon hyperpolarization below −83 mV (up to −250 pA at −123 mV). In 12 cells fast transient inward currents (up to −800 pA) were recorded after the end of hyperpolarizing voltage steps.

A second group of hair cells (RP: -76 ± 4.3 mV, n=24) also had potassium currents similar to those described above (≤ 16 nA at 0 mV). In the absence of drugs this conductance was masked by another large outwardly rectifying conductance (≤ 1 nA at −63 mV) which activated with slow kinetics above −93 mV after voltage steps to −103 mV. This conductance was completely and reversibly blocked by 5 mM 4-AP, but less effectively reduced by 25 mM TEA. Its reversal potential, close to the potassium equilibrium potential, identified it as a potassium conductance.

In a third group of hair cells (RP: -66 ± 5.2 mV, n=5), outward currents activated above −73 mV from a holding potential of −83 mV.

649.3

THE EFFECTS OF EXTERNAL CESIUM ON THE VOLTAGE-DEPENDENT CONDUCTANCES INVOLVED IN TUNING OF TURTLE HAIR CELLS. M. B. Goodman* & J. J. Art, Comm. on Neurobiology, Univ. of Chicago, Chicago, IL 60637.

In the basilar papilla of the turtle, hair cells are tuned to different frequencies in an acoustic stimulus by voltage- and ion-sensitive conductances whose magnitude and kinetics vary systematically (Art & Fettiplace, 1987, *J. Physiol.* **385**: 207). Calcium and calcium-activated K^+ currents have previously been implicated in this resonant behavior. These cells also possess an inwardly rectifying current (I_{IR}) that is readily observed at potentials negative to E_K, and is most prominent in cells tuned to lower frequencies. The significance of this current and its contribution to frequency selectivity and sensitivity remains obscure.

The reversal potential of I_{IR} is insensitive to removal of external Na^+ and shifts with external K^+ in a manner predicted for a K^+ conductance. The slope conductance is also increased with elevation of external K^+. For hair cells tuned to frequencies between 9 and 260 Hz, the maximum chord conductance of the inward rectifier is inversely proportional to the characteristic frequency of the cell. In the lowest frequency cells, the maximum chord conductances of the inward and outward K^+ currents are comparable. Superfusion of 5 mM CsCl in voltage clamp blocks the inward rectifier and decreases the apparent voltage-sensitivity of the net whole-cell current near the cell's resting potential. The same manipulation in current clamp depolarizes the cell slightly, decreases the resonant frequency and dramatically reduces the quality of resonance. These results suggest that currents sensitive to external Cs^+ contribute significantly to electrical resonance in the turtle cochlea, particularly in low frequency cells. [Supported by Howard Hughes Predoctoral Fellowship to MBG & NIH DC00454 to JJA].

649.4

SPEED OF SOMATIC SHAPE CHANGES OF COCHLEAR OUTER HAIR CELLS. B.N. Evans, B. Clark, D.Z.Z. He, I. Sziklai, P. Dallos*. Auditory Physiology Laboratory (Hugh Knowles Ctr.), Dept. of Neurobiology, Northwestern University, Evanston, IL 60208.

Somatic shape changes of mammalian outer hair cells may participate in a local mechanical feedback process in the cochlea. It is likely that these shape changes are produced by voltage-sensing molecular motors located in the plasma membrane. While the speed of the motile response *in vivo* is likely limited by the low-pass filter properties of the cell membrane, it is of interest to determine the speed with which the ensemble of molecular motors may act, when not limited by membrane properties. Using the partitioning microchamber technique [Evans et al., *Hearing Res.* 52: 288 (1991)], it is possible to deliver constant amplitude ac stimuli to the isolated outer hair cell up to approximately 30 kHz. With the electrical partitioning, the two cell segments form a series-parallel combination of filters, reducing frequency-dependence, and thus producing a quasi all-pass system. Using low-level, wide-band pseudorandom ternary electrical noise stimuli or sequences of sinusoidal voltage commands, we show that the motile response persists up to at least 24 kHz, only limited by our system. The frequency following motile response is accompanied by a tonic contractile response component. This dc response is also maintained up to the highest frequencies tested. Furthermore, above ~100 Hz, and at low and modest levels, the dc response changes linearly with signal amplitude. This dc component could be of significance *in vivo*. [Supported by the NIDCD and the Amer. Hrg. Res. Foundation.]

649.5

REGIONAL VARIATIONS IN SYNAPTIC INNERVATION OF THE SQUIRREL MONKEY CRISTA. A. Lysakowski* and J.M. Goldberg, Committee on Neurobiology, The University of Chicago, Chicago, IL 60637

There are regional variations in synaptic innervation of the chinchilla crista (Lysakowski and Goldberg, SNA, 1989). Here, we apply similar methods to the squirrel monkey crista. We had previously shown that the ratio of type I to type II hair cells was higher in the monkey (3:1) than in the chinchilla (1:1) (Fernandez et al., SNA, 1991). We were interested in how the difference in hair-cell ratios would be reflected in the synaptic organization of the crista in the two species. Results are based on 5 samples from 2 cristae in 2 monkeys.

In the monkey, there are 6-10 ribbons per type I hair cell and some indication that central type I hair cells have more ribbons than peripheral type I hair cells. In addition, each central type I hair cell had ≈20 calyceal invaginations, whereas each peripheral type I hair cell had ≈2 invaginations. Although qualitatively similar gradients were observed in the chinchilla, the numbers of ribbon synapses and calyceal invaginations were smaller in the monkey.

Type II hair cells in the monkey had similar numbers of ribbons and afferent boutons; there were fewer boutons and ribbons centrally, than peripherally (≈13 vs ≈40 per type II hair cell). Very few ribbons from type II hair cells onto the outer surface of calyces were seen in the monkey as compared to the chinchilla. In the latter species, outer-face synapses are so common that most calyx afferents could be functionally dimorphic; this is not the case in the monkey.

The number of efferent boutons per hair cell differs between the central and peripheral zones in the monkey with ≈2 efferent boutons per type I hair cell in the central zone, and ≈0.5 per hair cell in the periphery. Type II hair cells had ≈4 in the central zone and ≈10 in the periphery. In contrast, the efferent innervation of type I and type II hair cells was more uniform in the chinchilla.

(Supported by NIDCD DC-0070 and the American Otological Society.)

649.6

LOCALIZATION OF $GABA_A$ RECEPTOR-LIKE IMMUNOREACTIVITY IN MAMMALIAN VESTIBULAR END ORGANS. J. D. Foster[1,2*], M. J. Drescher[1,2], and D. G. Drescher[1,2,3]. [1]Lab. of Bio-otology, Depts. of [2]Otolaryngology and [3]Biochemistry, Wayne State University, Detroit, MI 48201.

Immunohistochemical analyses by several investigators have indicated that γ-aminobutyric acid (GABA) and its enzymes of synthesis and metabolism, GAD and GABA-T, respectively, are present in mammalian vestibular end organs. The localization of GABA-like immunoreactivity to the receptor cell or hair cell has been cited as evidence that GABA may act as a hair cell transmitter. However, immunoreactivity for this amino acid in the vestibular end organ has been associated with several, and in some investigations, multiple cell types, giving rise to a number of possible interpretations. In the current study, we demonstrate the presence of $GABA_A$ receptor-like immunoreactivity in vestibular end organs of the hamster by use of immunohistochemical methods (immunofluorescence and immunoperoxidase), incorporating a monoclonal antibody specific for the β_2/β_3 subunits of the $GABA_A$ receptor protein.

In the crista ampullaris and utricular and saccular maculae, $GABA_A$ recepfor-like immunoreactivity was observed in close association with afferent nerve calyces surrounding vestibular type I hair cells. The cytoplasm of vestibular type I hair cells did not appear to be immunoreactive. Some nerve fibers entering the epithelium were positive for $GABA_A$ receptor-like immunoreactivity. There was no clear indication that vestibular type II hair cells or nerve fibers innervating them were immunoreactive. In the crista ampullaris, immunoreactivity appeared to be preferentially localized to calyces around vestibular type I hair cells located in the crest of the crista.

The localization of $GABA_A$ receptor-like immunoreactivity to afferent nerve calyces is compatible with a receptor that is post-synaptic to the hair cell or to efferent neurons utilizing GABA as a transmitter, or alternatively, a presynaptic autoreceptor for the afferent nerve releasing GABA as a neuromodulator.

(Supported by NIH Grants DC00026 and DC00156.)

649.7

HAIR BUNDLES CONTAIN A FORM OF MYOSIN I. P. G. Gillespie* and A. J. Hudspeth. Department of Cell Biology and Neuroscience, University of Texas Southwestern Medical Center, Dallas, TX 75235-9039.

Although current models suggest that a myosin isozyme mediates adaptation of mechanoelectrical transduction in hair cells, identification of hair-bundle myosins has been hampered by small numbers of bundles. Using purified hair bundles and photoaffinity crosslinking of vanadate-trapped [α-^{32}P]UTP, we characterized hair-bundle myosin candidates of molecular masses 120, 160, and 230 kD (Gillespie & Hudspeth, *Soc. Neurosci. Abstr.* **18**: 1398, 1992). Because the 120-kD protein was observed most consistently, and because Ca^{2+} affects its ATPase cycle as anticipated for an adaptation motor, we examined this protein in more detail.

To identify the 120-kD protein, we employed two monoclonal antibodies, M2 and M3, that were raised against bovine adrenal myosin I and that display cross-reactivity between species (Wagner *et al., J. Cell Biol.* **119**: 163-170, 1993). Protein immunoblot analysis indicated that hair bundles purified from the bullfrog's sacculus contain a 120-kD protein recognized by both antibodies. Assuming that the immunoreactivity of the bundle myosin matches that of bovine adrenal myosin I, the bundles in a frog's sacculus together contain about 2.5 pg of myosin I. As observed with the photolabeled protein, the immunoreactive 120-kD protein resisted extraction by high salt, detergent, or ATP. An immunoreactive 120-kD protein was also present in the hair- and supporting-cell somata, where it again resisted extraction by conditions that solubilize many other myosin isozymes. Both antibodies labeled isolated hair cells or hair bundles in a punctate pattern focussed towards the stereociliary tips. These results indicate that the 120-kD myosin is a strong candidate to be the hair cell's adaptation motor.

This research was supported by NIH grant DC00241.

649.8

A CALMODULIN INHIBITOR SLOWS CHANNEL GATING AND ADAPTATION IN MECHANOELECTRICAL TRANSDUCTION BY HAIR CELLS. R. G. Walker, P. G. Gillespie, and A. J. Hudspeth*. Department of Cell Biology and Neuroscience, University of Texas Southwestern Medical Center, Dallas, TX 75235-9039.

When a hair cell is stimulated by deflection of its hair bundle, mechanically sensitive ion channels rapidly open. If the stimulus persists, some of the channels then reclose during an adaptation process. The rate and extent of adaptation are influenced by the concentration of Ca^{2+} in the hair bundle. Calmodulin, a cytoplasmic receptor for Ca^{2+} in many cellular processes, occurs in the bundle in association with several receptor proteins (Walker *et al., Proc. Natl. Acad. Sci. USA* **90**: 2807-2811, 1993). To investigate the possibility that calmodulin mediates the effects of Ca^{2+} on transduction, we sought to perturb calmodulin's actions by dialyzing cells with a potent peptide inhibitor of calmodulin binding. This peptide, which corresponds to the calmodulin-binding domain in Ca^{2+}/calmodulin-dependent protein kinase II, inhibits several calmodulin-dependent enzymes at nanomolar concentrations. Upon including this peptide at a concentration of 250 nM in tight-seal, whole-cell recording pipettes, we observed slowing of mechanoelectrical-transduction currents; the opening time constant increased from less than 1 ms to ~6 ms and the closing time constant to ~17 ms. In addition, adaptation to sustained stimuli was virtually abolished several minutes after rupturing the cell's membrane. The effects of the inhibitory peptide resembled those of treatments that lower the concentration of free Ca^{2+} in the hair bundle, including cellular depolarization and dialysis with a Ca^{2+} chelator.

This research was supported by NIH grant DC00241.

649.9

FINITE ELEMENT ANALYSIS OF CILIARY BUNDLE STIFFNESS. R. K. Duncan[1], J. W. Grant[1], and E. H. Peterson[2*]. [1]Biomedical Engineering Program, Virginia Polytechnic Institute, Blacksburg, VA 24061 and [2]Neurobiology Program, Ohio U., Athens, OH 45701.

We are using finite element analysis to help understand mechanisms of mechanotransduction in hair cells. This method uses information about the dimensions and material properties of a structure to estimate the structure's mechanical response to deformation. Previous work dealt with the effects of ciliary numbers and dimensions (DiCaprio, Duncan, Grant, and Peterson, ARO abs., 1993) and interconnections (Duncan and Grant, ARO abs., 1993). Here we focus on the material properties of stereocilia.

Stereocilia are bundles of cross-linked actin filaments surrounded by membrane. Estimates are available in the literature for material properties for filamentous actin and actin gels, but not for actin bundles. We first modeled the ciliary shaft material as isotropic (material properties not directional; Poisson's ratio estimated at 0.4). Over a modulus range of 10^6 to 10^{10} N/m^2 there was a distinct change in the slope of Bundle Stiffness vs. Cilium Modulus. This change in slope was associated with a change in the mode of deformation. Above modulus values of 10^8, stereocilia bend at the base but the shaft remains straight, as observed experimentally, but the resulting bundle stiffness is higher than is generally observed experimentally. Below this modulus value, overall bundle stiffness falls within physiologic range, but the ciliary shafts buckle in response to an applied force. These observations, coupled with ultrastructural data on the internal structure of stereocilia (Tilney and Tilney, 1988) indicate that the ciliary material should be modeled as transversely isotropic.

649.10

CILIA OF HAIR CELLS FROM THE MACULAE LAGENAE OF *Gallus gallus*. A. Illescas* and S. Gómez. Departamento de Anatomía, Facultad de Medicina y Unidad de Microscopía Electrónica. Facultad de Medicina Veterinaria y Zootécnia. UNAM, México, D. F. 04510.

In certain number of species, the existence of cilia in hair cells has been widely reported. However important questions still are open over the characteristics of this structures. The objective of the present study was to analize the structural pattern of the cilia in the maculae lagenae of *Gallus gallus*. Anaesthetized with sodic penthobarbital (ip), 3mg/100g of body weigth. Beheaded internal ear was processed for electron microscopy transmission. We found in the apical end a bundle of stereocilia and one quinocilium on the periphery of the hair cells Type I and II. The maculae lagenae presents a morphological polarization in the saccular type. This bundle of sensory hair face a significative likeness with the pattern of cilia of vestibular hair cell of the avian group. This fact agrees with the point of view that the *maculae lagenae* functions as a vestibular organ. All these data suggest that in this birds with flying habits, they were structurally involutionated.

649.11

A PROTEIN WITH TGFα-LIKE IMMUNOREACTIVITY IS EXPRESSED IN THE COCHLEA AND MAY BE UP-REGULATED DURING HAIR CELL REGENERATION. X.-M. Xu and J.T. Corwin*. Dept. of Otolaryngology-HNS and Dept. of Neuroscience, University of Virginia, Charlottesville, VA 22908.

Transforming growth factor alpha (TGFα) is a 50 amino acid protein that shares homology with EGF and has mitogenic effects on ectodermally derived tissues. Variants of TGFα range from roughly 5 to 20 kDa depending on differential processing. We have used western blots to look for TGFα-like proteins in the hair cell sensory epithelia of the inner ear. Eleven groups of 6 juvenile white leghorn chicks were each given an injection of gentamicin at 100 mg/kg body weight and then exposed to tonal sound stimulation at 1.5 kHz and 120 dB SPL for 24 hr, so as to damage hair cells and trigger regeneration in the cochlea. Immediately after the treatment, the chicks were euthanized and their cochleae were removed. The sensory epithelium was collected from each cochlea and homogenized in a detergent buffer together with other cochleae from the same treatment group. After centrifugation, supernatants were run through SDS-PAGE along with samples from age-matched controls. Nitrocellulose blots were reacted with a commercial mouse monoclonal antibody against recombinant human TGFα.

A single protein band that runs at approximately 12 kDa was labeled by the anti-TGFα in both experimental and control cochlear samples. In the majority of the experiments the labeling of this band was stronger in samples from the experimentally damaged cochleae than the labeling in samples from control cochleae. The increased expression of this protein is detectable after a treatment which causes extensive hair cell loss and which evokes hair cell regeneration. The results suggest that a protein that contains a potentially mitogenic TGFα-like domain may have a role in triggering cell divisions during the regeneration of hair cells in the cochlea.

(Supported by grants from the NIDCD and the NOFHR)

649.12

SPATIAL GRADIENT OF CELL PROLIFERATION IS GUIDED BY HAIR CELL DEGENERATION IN THE CHICK COCHLEA. E. Hashino*, E.K. TinHan and R.J. Salvi. Hearing Research Lab., SUNY at Buffalo, NY 14214.

Sensory hair cells in the avian cochlea regenerate after being destroyed. The replacement of new hair cells is more protracted after aminoglycoside ototoxicity (2-20 weeks) than after acoustic trauma (7-10 days). It is possible that different cellular events are involved in triggering cell proliferation following the different types of hair cell degeneration. In order to understand the cellular mechanisms underlying cell cycle regulation in the avian cochlea, we investigated the relationship between hair cell degeneration and cell proliferation after aminoglycoside ototoxicity.

Hair cell lesions were produced in neonatal chicks using kanamycin (KM; 400 mg/kg/d x 10d). Hair cell damage was monitored by phalloidin staining or SEM and cell proliferation was evaluated by bromodeoxyuridine (BrdU) pulse-fix analysis. Hair cell loss began 6d after the start of KM-injections, whereas cell proliferation was first observed between days 6-8 of KM injections. Thus, the latency of cell proliferation after the hair cell loss is less than 48 hrs. Both the hair cell loss and cell proliferation were first identified in the basal region and moved toward the apical region of the basilar papilla in a parallel manner during the KM treatment. The direction of cell proliferation appeared to be guided by hair cell loss, suggesting that some aspect of hair cell degeneration provides trigger signals for cell proliferation. TEM studies are currently underway to describe the early events of hair cell degeneration. (Supported by NSF BNS9007822 and NIH DC01685)

649.13

VESTIBULAR HAIR CELL ABNORMALITIES IN CONGENITALLY HEARING IMPAIRED CANARIES. T. J. Park*, Y. Lu, and P. Weisleder. Goodhair-Day Laboratories, Dept. of Zoology, Univ. of Texas, Austin, TX 78712.

Recent reports have demonstrated that the hearing impairment of the Belgian Waterslager canary (BWC) strain is caused by severe hair cell anomalies (Dooling & Gleich, 1993 ARO Abs 16:88). Given that the auditory portion of the inner ear develops as an outgrowth of the sacculus (Streeter, 1906 Am J Anat 6:139), we were interested in ascertaining if the vestibular parenchyma of BWCs displayed abnormalities similar to those observed in the basilar papilla. The inner ear of adult BWCs and non-BWCs were examined by scanning electron microscopy. Hair cells in the basilar papilla and vestibular organs of non-BWCs had normal appearance. In BWCs, the hair cells in the basilar papilla displayed the same abnormalities previously reported by Dooling & Gleich. Hair cells in the sacculus and utricle were also abnormal. The severity of the abnormalities varied among animals and organs. Aberrant hair cells exhibited the following: 1) absence of kinocilium, 2) shorter than normal stereocilia, 3) stereociliary bundles not arranged in a staircase pattern, and 4) stereociliary bundles without a distinct orientation. In contrast, hair cells in ampullary organs had normal morphology. Given that all inner ear sensory structures develop from the otic placode, it is not surprising that otolithic and auditory hair cells in BWCs display similar abnormalities. For the same reason, it is puzzling that the surface morphology of semicircular canal hair cells appeared normal. (Supported by the Deafness Research Foundation, NIH DC 01522 & DC 20068, Dr. L. Popejoy, and the Cell Research Institute at U. of Texas)

649.14

INCREASE IN HAIR CELL NUMBER DURING DEVELOPMENT OF THE *XENOPUS LAEVIS* SACCULUS. M. E. Díaz de Lodrón*, D. López, A. Varela-Ramírez, and E. E. Serrano. Dept. of Biology, New Mexico State University, Las Cruces, New Mexico, 88003.

This research focuses on a quantitative analysis of the development of inner ear sensory hair cells, using the *Xenopus laevis* sacculus as an experimental model. Embryonic animals (developmental stages 47, 52 and 56 according to Nieuwkoop & Faber, 1975) and adults (3.5 to 60 gms) were utilized. Sacculi were dissected, fixed by immersion in a dialdehydic mixture (2.5% glutaraldehyde, 1.5% paraformaldehide in 0.1M sodium cacodylate buffer, pH=7.4) and postfixed in 2% osmium tetroxide. The sacculi were critical point-dried with CO_2, sputter-coated with gold and examined with a Phillips 501B scanning electron microscope. The entire saccular epithelium was photographed in serial sectors at 1250X and posteriorly reconstructed in order to count all the hair cells. The results show that the youngest animals had about 600 saccular hair cells, and this number increased to 2500 in the adults. The increase in hair cell numbers was most pronounced during embryogenesis and reached a stationary number in the postembryonic stages. This research was supported by grants to E.E.S. from NIH (S06-GMO8136-18, RO3 DC01460-01) and the Whitehall Foundation.

649.15

ONGOING AND GENTAMICIN-INDUCED HAIR CELL REGENERATION IN THE BULLFROG VESTIBULAR OTOLITH ORGANS. R.A. Baird*, M.A. Torres, and N.R. Schuff, R.S. Dow Neurological Sciences Institute, 1120 NW 20th Ave., Portland, OR 97209.

Hair cells in the bullfrog otolith organs have been shown to regenerate following exposure to the aminoglycoside antibiotic gentamicin sulfate (*Baird et al., Hear. Res. 65:164-174, 1993*). To examine the role of mitotic division in this recovery, the thymidine analogue bromodeoxyuridine (BrdU) was used to measure ongoing and gentamicin-induced cell proliferation in the otolith organs. Cell proliferation, measured with BrdU immunocytochemistry, was seen throughout the sensory maculae of both normal and gentamicin-treated animals and was significantly higher in the saccular than in the utricular macula. The number of proliferating cells in gentamicin-treated animals was initially lower than in normal animals, presumably because of the death of many BrdU-labelled cells. The *rate* of cell proliferation, however, was larger in gentamicin-treated than in normal animals. At early times, BrdU-labelled cells were seen adjacent to the peripheral macular margin and, within the maculae, in a subset of supporting cells immediately adjacent to the basement membrane. These cells were the first cells to incorporate BrdU labeling, suggesting that they are the progenitors of new hair cells. At later times, BrdU-labeled cells within the maculae were smaller and located further from the basement membrane, suggesting that the progeny of these cells had migrated to more apical positions. Our results also indicate that the rate of mitotic division is too low to explain the rapid replacement of hair cells in the otolith organs, suggesting that hair cell recovery in these organs is due largely to the migration of undamaged hair cells or the transdifferentiation of supporting cells.

(Supported by NASA and the Medical Research and Oregon Lions Sight and Hearing Foundations).

649.16

DENERVATION AND SUBSEQUENT RECEPTOR CELL DEATH INCREASE BASAL CELL PROLIFERATION IN TUBEROUS ELECTRORECEPTOR ORGANS OF A WEAKLY ELECTRIC FISH. P. Weisleder*, Y. Lu, and H. H. Zakon. Dept. of Zoology, Univ. of Texas, Austin, TX 78712.

Weakly electric fish generate electric fields for the purposes of electrolocation and communication. The fields are detected by specialized receptor organs: the tuberous organs. Tuberous organs contain four different cell types: electroreceptor cells (sensory cells), basal cells, plug cells, and capsule cells. Previous studies have shown that basal cells are the progenitors of electroreceptor cells. In the present study we investigated the effect of denervation on the rate of proliferation of basal cells. The left, infraorbital, anterior lateral line nerve of sixteen brown ghosts *(Apteronotus leptorhynchus)* was sectioned, and the proximal stump was dipped in ricin to prevent regrowth. In groups of four, the animals were given twice daily injections of the cell proliferation marker bromodeoxyuridine (BrdU) at one, two, three, or four weeks following denervation. Following two days of BrdU injections, a piece of cheek skin, ventral to the eye, was removed from the left (denervated) and the right (innervated) sides and processed for immnocytochemistry. Our results show: 1) there is progressive receptor cell death and tuberous organ degeneration following denervation; 2) at all survival times the number of labeled basal cells is greater in denervated organs; 3) the number of labeled basal cells increases steadily with survival time and tuberous organ degeneration. These results suggest that innervation is essential for electroreceptor cell survival, and that receptor cell death is a plausible trigger for basal cell proliferation.

(Supported by NIDCD and the Deafness Research Foundation)

649.17

MORPHOLOGY, DISTRIBUTION AND INNERVATION OF CEPHALIC SUPERFICIAL NEUROMASTS IN GOBIES (TELEOSTS). J. Song*, Div. of Fishes, NMNH, Smithsonian Institution, Washington D.C. 20560.

Several lateral-line head canals of gobiid fishes are replaced by superficial neuromasts (SNM) in a great variety of patterns. This study compares two topographic patterns of cephalic SNM in two goby genera (Bathygobius and Gobiosoma). The distribution and hair cell orientation of the neuromasts were examined by using scanning electron microscopy, and the innervation patterns were observed on specimens cleared and triple stained (alizarin, alcian, Sudan Black B). All the specimens were from museum collections. The results indicate that orientation of the neuromasts are determined by their innervation rather than their topographic location. A set of SNM lines innervated by the same ramule possesses the same orientation. The primary SNM (pit-organs) orient perpendicular to the axis of their line. The canal replacement SNM orient parallel to the axis of their line. For example, the segments of the horizontal primary SNM line are distinguished from infraorbital canal replacement lines in a SNM complex of the cheek region by their innervation and orientation. The segments of vertical primary SNM and mandibular line are distiguished from the preopercular and mandibular canal replacement at the preopercular-mandibular region by their innervation, orientation and hair cell morphology. The innervation pattern is also a criterion for homologizing individual SNM lines in different patterns among gobies and other fishes. This study was supported by a Smithsonian Postdoctoral Fellowship to the author.

649.18

ULTRASTRUCTURAL CORRELATES TO PHYSIOLOGICALLY DISTINCT HAIR CELL TYPES IN THE GOLDFISH SACCULE. P.J. Lanford and A.N. Popper* Dept. of Zoology, Univ. Maryland, College Park, MD 20742.

Dissociated hair cells from rostral vs. caudal regions of the goldfish saccule are physiologically and morphologically distinct (Sugihara & Furukawa, J. Neurophys. 1989, 62:1330). We present ultrastructural evidence that confirms these regional variations in situ and provides further morphological distinctions between cell types.

Cells in the rostral half of the saccular epithelium are pear-shaped, with round nuclei, small synaptic bodies, and evenly distributed mitochondria. Rough endoplasmic reticulum (RER) is generally scattered, with occasional subnuclear layering. This morphology continues through the central to mid-caudal regions of the epithelium, with subnuclear RER layering becoming more prominent and well organized caudally. In these areas, cells have some resemblance to striolar cells found in the utricle of the oscar, *Astronotus ocellatus* (Chang et al., 1992, J. Comp. Neurol. 302:629). At the extreme caudal end, however, cells exhibit a taller, cylindrical morphology with elongate nuclei, no RER subnuclear layering, large synaptic bodies, and mitochondria clustered at the basal region of the cell. These resemble the oscar utricular extrastriolar hair cells.

Our data demonstrate substantial differences in hair cell ultrastructure along the length of the goldfish saccule. This variation may provide an ultrastructural basis for functional differences within this endorgan.

[Supported by NASA and ONR]

649.19

LOCATIONS OF VESTIBULAR HAIR CELLS IN DEVELOPING ZEBRAFISH EMBRYOS VISUALIZED WITH A FLUORESCENT VITAL DYE. Barbara Chapman* and Scott E. Fraser. Division of Biology, Mail code 139-74, Caltech, Pasadena CA 91125.

To study the development of vestibular sensory receptors, we utilize a fluorescent dye injected into the lumen of the otocyst in living zebrafish at different stages. We have found that 4-(4-diethylaminostyryl)-N-methylpyridinium iodide (4-Di-2-ASP), which has previously been used to visualize nerve terminals, selectively labels hair cells in the zebrafish inner ear. Confocal microscopy was used to localize the labeled hair cells within the complex three dimensional structure of the developing ear.

We began 4-Di-2-ASP injections at 19 hrs (development at 28.5°C), when two otoliths are clearly visible. The first specific labeling was seen at 26 hrs when one or a few brightly labeled cells are seen in epithelial thickenings adjacent to each otolith. By 36 hrs the maculae of both otoliths are well formed, with brightly labeled hair cells forming a shallow cup closely opposed to each otolith. Hair cells in the rostral macula are oriented vertically and located ventral to the otolith, while those in the caudal macula are oriented horizontally and located medial to the otolith.

The hair cells of the semicircular canal cristae are not visible until 48 hrs when one brightly labeled cell is seen within each of three epithelial thickenings on the lateral wall of the otocyst. At this stage the cranial and lateral projections, which will fuse to create the first of the canal walls, are just touching. Thus the sensory receptors of the semicircular canals begin to develop before the canals themselves are formed. More hair cells are gradually added in each of the cristae over the next several hours. The otoliths and their maculae move to a more medial position in the otocyst, while the cristae move ventrally. During this period, ending around 60 hours, the epithelial projections forming the walls between the semicircular canals fuse. At about this time the zebrafish begin to exhibit a dorsal-up attitude and righting reflex.

Over the next several weeks of development the morphology of the semicircular canals continues to change, with the canals becoming more elongated. No major changes in the maculae or cristae are seen during this period.

650.1

MUSCLE ACTIVITY EVOKED BY PAIRED-PULSE STIMULATION OF SUBSTANTIA NIGRA PARS RETICULATE: THE EFFECT OF TECTAL, PONTINE, & RETICULAR FORMATION LESIONS. J.A. Moretti, & D. Asdourian*. Dept. of Psychology, Wayne State Univ., Detroit, MI 48202

The substantia nigra pars reticulate (SNr) is a major output for information processed by the basal ganglia. Multiple ascending and descending SNr efferents have been identified, indicating that SNr exerts control over motor behavior via many pathways. Previous work from our laboratory has shown that unilateral electrical stimulation of SNr elicits bilateral activity from neck muscles in the rat. It was also observed that antecedent stimulation of one SNr differentially affected the amplitude of the muscle response elicited by stimulation of the other SNr, depending upon the delay between the first and second stimulus. The present study attempts to locate the area(s) responsible for the differential effect on the amplitude of muscle activity resulting from bilateral stimulation of SNr by making lesions in either SNr output pathways or target nuclei. The lesion targets were PPN, the tecto-and rubrospinal tracts, VA/VL, and the ponto-medullary RF. The only significant lesion effect on overall response amplitudes was seen following PPN lesions. None of the lesions influenced changes in muscle amplitude resulting from the delays between the first and second stimulus during bilateral SNr stimulation.

650.2

STIMULATION OF DOPAMINERGIC D1 RECEPTORS IN THE SUBTHALAMIC NUCLEUS ELICITS ORAL DYSKINESIA IN RATS. T.J.Parry*, I. Lucki[1], M.F. Chesselet. Dept. of Pharmacology and Psychiatry[1], Univ. of Pennsylvania , Philadelphia, PA 19104.

The subthalamic nucleus (STN) plays a critical role in regulating basal ganglia function. Although the STN receives dopaminergic input from the substantia nigra pars compacta and contains dopaminergic D1 binding sites, the role of dopaminergic stimulation of the STN on motor behavior is unknown. We tested the hypothesis that dopaminergic stimulation of the subthalamic nucleus induces motor behaviors in the conscious rat. Unilateral injections of apomorphine (0.1 to 3.2 µg in 100 nl) into the STN caused a dose-dependent increase in abnormal orofacial movements and no change in turning, sniffing, grooming, or rearing behaviors. Peripheral administration of SCH 23390, a D1 receptor antagonist, markedly reduced the number of orofacial movements elicited by STN injections of apomorphine at a dose of 0.1 mg/kg sc while sulpiride, a D2 selective antagonist, failed to block these oral movements at a dose of 50 mg/kg. Further, simultaneous injection of SCH 23390 (1 µg) with apomorphine (1 µg) within the STN prevented oral movements normally observed with apomorphine alone. In contrast, a similar experiment using the D2 antagonist sulpiride (5 µg) did not reduce abnormal perioral movements normally observed with apomorphine alone. Finally, lesions of the STN by local injection of kaininc acid prevented orofacial dyskinesia induced by local administration of apomorphine These results suggest that local dopaminergic D1 receptor stimulation of the STN induces abnormal orofacial movements and highlights the importance of dopaminergic input to the STN in the regulation of motor function. This work was supported by PHS grants MH-44894, GM-34781 and MH-14654.

650.3

REGIONALLY SPECIFIC, NMDA-RECEPTOR MEDIATION OF ORAL MOTOR CONTROL IN THE RAT'S STRIATUM AND ITS INTERACTION WITH LOCAL CHOLINERGIC SYSTEMS. M. Pisa* and D. Bosiljevac. Dept. Biomed. Sci., McMaster Univ., Hamilton, Ont., Canada, L8N 3Z5.

In rats, bilateral injections of the wide-spectrum glutamate receptor antagonist kynurenic acid (12-72 nmol) in the lateral striatum induce an increase of nondirected (in vacuo) oral movements (NDOM), suggesting a role of corticostriatal glutamatergic transmission in oral motor control. In the present study, we examined the roles of both striatal subregions and distinctive types of glutamate receptors in this effect. Injections (0.2 - 0.5 µl) of the competitive NMDA-receptor antagonist CPP (0.4 - 10 nmol) into the ventrolateral striatum (VLS) produced a powerful, dose-dependent increase of NDOM (threshold dose 1 nmol) that could be attenuated by co-injections (0.5 µl) of the muscarinic receptor antagonist atropine sulphate (1 - 10 µg). The effect was regionally specific because similar injections of CPP into the dorsomedial striatum did not increase NDOM. Injections (0.5 µl) of either the noncompetitive NMDA-receptor antagonist MK-801 (10 nmol) or the QUIS/KA receptor antagonist DNQX (10 nmol) into the VLS failed to increase NDOM. The results indicate regionally selective roles of NMDA and muscarinic receptors of the VLS in the control of oral behavior (Supported by the Natural Sciences and Engineering Research Council of Canada and the Scottish Rite Schizophrenia Research Program, N.M.J., U.S.A. M. Pisa is a Research Associate of the Ontario Mental Health Foundation).

650.4

GABAERGIC RECEPTORS IN THE ANTERIOR AND POSTERIOR SUBSTANTIA NIGRA PARS RETICULATA ARE DIFFERENTIALLY INVOLVED IN EXPRESSION OF AMPHETAMINE-INDUCED OROFACIAL STEREOTYPY. P. R. Dickson* and A. E. Kelley. Dept. Psychology, Northeastern Univ., Boston, Ma, 02115.

Microinjection of amphetamine into the ventrolateral striatum (VLS) induces intense orofacial stereotypy (licking, biting, and self-gnawing) in rats. GABAergic striatonigral neurons may be involved in the expression of VLS-mediated oral stereotypy. To test this hypothesis, several experiments were carried out. In the first experiment, animals were implanted with bilateral cannulae aimed at either the anterior (ant) or posterior (post) substantia nigra pars reticulata (SNR). Behavior was recorded via observation in the home cage following infusions of either saline (0.5 µl), muscimol (75 ng/0.5 µl), or picrotoxin (75 ng/0.5 µl). In the antSNR, muscimol induced a strong oral stereotypy, including intense self-gnawing, at 30, 60, and 90 minutes post-injection. Picrotoxin had no behavioral effects at this site. In the postSNR, picrotoxin induced strong oral stereotypy which was apparent in the first 30 minutes and subsided thereafter. Muscimol induced no alteration of oral behavior in the postSNR, although there was significant general behavioral activation. These results suggest that striatonigral fibers terminating in the antSNR (presumably on nigrotectal neurons) mediate amphetamine-induced orofacial stereotypies, and that these nigrofugal neurons are inhibited during oral stereotypy. Nigrofugal neurons in the postSNR are also involved in orofacial stereotypy, but appear to be disinhibited during this behavior. Moreover, neurons in the postSNR may be preferentially influenced by striato-pallido-nigral input, rather than direct striatonigral input.

650.5

THE PEDUNCULOPONTINE TEGMENTAL NUCLEUS AS A STRIATAL OUTPUT STATION. I. EFFECTS OF PPTg OR CUNEIFORM NUCLEUS LESIONS ON LOCOMOTION ELICITED BY STIMULATION OF NUCLEUS ACCUMBENS. P. Winn*, M.P. Latimer, W.L. Inglis, J.S. Dunbar and L.F. Allen Dept. Psychol., Univ. St Andrews, Fife, Scotland KY16 9JU.

The role of the PPTg in mediating locomotion stimulated from nucleus accumbens (NAS) is controversial. Some authors have shown that PPTg interference attenuates NAS locomotion; others have not. We used excitotoxins to make complete lesions of PPTg to examine this further. Ibotenate (IBO) and quinolinate (QUIN) destroy cholinergic PPTg neurons but QUIN has less effect on non-cholinergic neurons (Rugg E.L. *et al.* 1992 Brain Res. 589:181-193). The following groups were tested: bilateral IBO or QUIN PPTg lesions (0.12M: 2 X 0.2µl injections/hemisphere) and bilateral sham lesions (2 X 0.2µl PB). Bilateral guide cannulae were aimed at the NAS; each rat received (randomized order) bilateral infusions of 10, 20 and 30µg AMPH and vehicle control (all 0.5µl per hemisphere via 30ga cannulae, 0.25µl/60sec). Spontaneous locomotion (measured in photocell cages) was unaffected by either IBO or QUIN lesions of PPTg; AMPH increased locomotion dose-dependently in all rats regardless of lesion, suggesting that the PPTg does not mediate locomotion induced by intra-NAS AMPH. One possibility however is that the adjacent cuneiform nucleus (CNF), identified by some authors as an essential component of the mesencephalic locomotor region, mediates NAS locomotion. This hypothesis was tested by making sham control and IBO lesions of the CNF (0.12M in 0.2µl phosphate buffer/hemisphere) in rats equipped with guide cannulae aimed at the NAS (as above). Neither spontaneous locomotion nor that elicited by intra-NAS AMPH was affected by CNF lesions (which did not encroach into the PPTg). Overall these data suggest that locomotion induced by NAS AMPH is not mediated solely (if at all) by these pontine nuclei.

650.6

THE PEDUNCULOPONTINE TEGMENTAL NUCLEUS AS A STRIATAL OUTPUT STATION. II. EFFECTS OF PPTg LESIONS ON RESPONDING FOR CONDITIONED REINFORCEMENT ELICITED BY STIMULATION OF NUCLEUS ACCUMBENS. W.L. Inglis* and P. Winn Dept. Psychol., Univ. St Andrews, Fife, Scotland KY16 9JU.

Despite suggestions that the PPTg is an output station for the nucleus accumbens (NAS) we found that excitotoxic lesions of the PPTg did not block spontaneous locomotion or that induced by intra-NAS *d*-amphetamine (AMPH). Other behaviours stimulated by intra-NAS AMPH may however be affected by PPTg loss. We have examined responding for conditioned reinforcement stimulated by intra-NAS AMPH in rats with either ibotenate (IBO) or quinolinate (QUIN) lesions of the PPTg. IBO and QUIN both destroy cholinergic PPTg neurons but QUIN has less effect on non-cholinergic neurons (Rugg E.L. *et al.* 1992 Brain Res. 589:181-193). Rats were trained to associate food in a standard operant chamber with a compound visual/auditory stimulus after which bilateral IBO or QUIN PPTg lesions (0.12M: 2 X 0.2µl injections/hemisphere) or sham lesions (2 X 0.2µl PB) were made. Rats were also implanted with bilateral guide cannulae aimed at NAS and were retrained for 8 sessions after surgery. In test trials one lever produced CR, while the other did not (NCR). Sham lesioned rats showed dose-dependent increases in CR lever pressing, but there was no substantive effect of NAS AMPH on the NCR lever or the food hopper panel. QUIN lesioned rats showed a strikingly similar pattern of responding to controls. IBO lesioned rats showed relatively normal responding on the CR lever but exaggerated responding on the NCR lever in response to intra-NAS AMPH. Panel pressing was low in these rats. These data suggest that IBO lesioned rats had not associated the presence of the CR with a particular lever. The data also indicate that the non-cholinergic neurons of the PPTg affect NAS outflow: but why should they affect CR but not locomotion?

650.7

THE PEDUNCULOPONTINE TEGMENTAL NUCLEUS AS A STRIATAL OUTPUT STATION. III. EFFECTS OF PPTg LESIONS ON BITING ELICITED BY STIMULATION OF VENTROLATERAL CAUDATE-PUTAMEN. L.F. Allen*, M.P. Latimer and P. Winn Dept. Psychol., Univ. St Andrews, Fife, Scotland KY16 9JU.

We have previously observed that bilateral ibotenate (IBO) lesions of the PPTg increase the incidence of biting in response to systemic *d*-amphetamine (AMPH). In the present experiment, biting induced by microinjection of AMPH into the ventrolateral caudate-putamen (VLCP) was examined in rats with either quinolinate (QUIN), IBO or sham lesions of the PPTg. Both IBO and QUIN destroy cholinergic PPTg neurons but QUIN has less effect on non-cholinergic neurons (Rugg E.L. *et al.* 1992 Brain Res. 589:181-193). The following groups were tested: bilateral IBO or QUIN PPTg lesions (0.12M: 2 X 0.2μl injections/hemisphere) and bilateral phosphate buffer sham lesions (2 X 0.2μl). All rats were also equipped with bilateral guide cannulae aimed at the VLCP; each rat received (in a randomized order) bilateral infusions of 5, 10 and 20μg AMPH and a vehicle control injection (all injections 0.5μl per hemisphere via 30ga cannulae, 0.25μl/60sec). Sham lesioned rats showed a dose response effect to AMPH microinjected into the VLCP. Biting (assessed by observation of video recordings) was principally directed at the cage floor and paws. All doses of AMPH elicited biting (20>10>5μg). QUIN lesions had no substantial effect on the response to VLCP AMPH but IBO lesions produced a significant shift to the left in the dose response curve. These data suggest that the non-cholinergic neurons of the PPTg are involved in the mediation of biting elicited from the VLCP by AMPH. Under normal circumstances these neurons presumably work to inhibit orofacial activity. How information is transmitted from the VLCP to PPTg (through the pallidum? through SNr? through both?) is unclear. Further experiments will clarify this issue.

650.8

NEURONAL ENSEMBLES IN RAT NEOSTRIATUM COACTIVATED ACROSS DIFFERENT BEHAVIORS. A.B. Kirillov, S.F. Sawyer and D.J. Woodward*. Department of Physiology and Pharmacology, Bowman Gray School of Medicine, Winston-Salem, NC 27157.

The aim of this work was to study the characteristics of ensemble activity of neostriatal neurons in awake rats which emerge across behaviors. Up to 24 neostriatal neurons were concurrently recorded from chronically implanted arrays of microwires. Rest and treadmill walk was employed to generate the substantial activity needed to detect groups. Crosscorrelation analysis with standard parameters (1 ms bin within 200 ms window) did not reveal peaks or dips, characteristic to monosynaptic interactions. On the other hand, a considerable fraction of neurons showed statistically significant positive or negative offshoots in crosscorrelograms, calculated with a large bin size (10 ms) and large window size (2 sec). Wide central peaks of these crosscorrelograms (100-500 ms) suggest that groups of neurons may receive some common excitatory inputs. The expression of crosscorrelations was increased during the 30 sec treadmill walk periods as compared with the 30 sec resting periods between treadmill walks. Based on pairwise cross-correlograms, in one experiment two groups of coactivated neurons (5 and 6 units of 24) were identified. The groups of neurons that showed pairwise correlations also exhibited significant increases in the frequency of instantaneous coactivation patterns in which 80% of neurons fired in the same time bin, as compared to the expected frequencies of such patterns for independent spike trains. Perievent histograms around coactivation times in one group revealed existence of simultaneous inhibition of the other group. The demonstration that coherent patterns vary across behavioral states provide an initial analysis of coordinated neural activity within neostriatum.

Supported by AFOSR 90-0146, DA 02338, NIAAA 3901.

650.9

GLUTAMATE IN THE VENTRAL STRIATUM CAN AFFECT PSYCHOMOTOR FUNCTIONS IN OPPOSITE DIRECTIONS DEPENDING ON THE DOPAMINERGIC TONE. A. Svensson*, M.L. Carlsson and A. Carlsson. Department of Pharmacology, University of Göteborg, S-413 90 Göteborg, Sweden.

The psychomotor effects of glutamate blockade in the nucleus accumbens were studied in male NMRI mice with different tones in the dopaminergic system.

A unilateral injection of the competitive N-methyl-D-aspartate (NMDA) receptor antagonist AP-5 into the nucleus accumbens caused the animals to rotate. The rotation was predominantly *ipsilateral* in animals with intact monoaminergic systems. In contrast, in monoamine-depleted mice, pretreated with reserpine and α-methyl-tyrosine, the rotation was consistently *contralateral*.

This shift in the direction of rotation was apparently due to the lack of dopamine D-2 receptor stimulation, because in monoamine-depleted mice systemically treated with the the selective D-2 receptor agonist quinpirole, AP-5 caused the animals to rotate ipsilaterally. Pretreatment with the D-1 agonist SKF 38393 failed to induce this shift in the direction of rotation. The crucial involvement of the dopamine D-2 receptor was also confirmed in experiments with intact mice pretreated with either the selective D-1 receptor antagonist SCH 23390 or the selective D-2 antagonist raclopride.

These results show that, depending on the dopaminergic tone and the stimulation of dopamine D-1/D-2 receptors, the glutamatergic neurons projecting to the ventral striatum can affect psychomotor functions in opposite directions.

650.10

CHARACTERIZATION OF NEURONAL ACTIVITY IN THE PATCH-MATRIX COMPARTMENTS OF THE STRIATUM IN FREELY MOVING RATS. E.S. Trytek*, I.M. White, D.M. Schroeder, and G.V. Rebec. Prog. Neural Science, Depts. Anatomy and Psychology, Indiana University, Bloomington, IN 47405.

Sensorimotor and limbic cortices provide inputs to both patch and matrix compartments of the rat striatum, but in terms of their relative contributions, sensorimotor inputs dominate the matrix, whereas limbic inputs project primarily to patch. This differential distribution of afferents implies important functional distinctions between these compartments (Gerfen, Annu. Rev. Neurosci., 15:285-320, 1992). To assess such distinctions, we recorded from neurons in either the patch or matrix compartment in awake, behaving rats. All animals were prepared for single-unit recording and allowed 7-10 days for recovery. On the recording day, a glass electrode (2-7 MΩ), filled with 2M NaCl and pontamine sky blue (PSB) dye, was lowered into the striatum. After the sensorimotor responsiveness of individual neurons was characterized, the recording site was marked by ejection of PSB. Immunostaining for calbindin by the avidin-biotin-peroxidase method (Hsu et al., J. Histochem. Cytochem., 29:577-580, 1981; Vector Laboratories) was used in conjunction with microscopic examination to assign the recording site to the patch or matrix compartment. Preliminary findings indicate that neurons increasing activity in close association with movement are located either entirely within the matrix or along the patch-matrix border. Neurons responding to tactile stimulation of specific body parts also are located in the matrix. These results, which link the processing of sensorimotor information with the striatal matrix compartment, demonstrate the feasibility of combining electrophysiological and neuroanatomical procedures for elucidating patch-matrix function.

Supported, in part, by the National Institute on Drug Abuse, DA 02451.

650.11

LOCOMOTOR RESPONSE TO MICROINJECTION OF PICROTOXIN INTO VENTRAL PALLIDAL REGION DOES NOT SHOW LATERALIZATION. N.A. Otmakhova, C.H. Woodworth and R.G. Robinson*. Dept. Psychiatry, Univ. of Iowa Coll. Med., Iowa City, IA 52242.

The nucleus accumbens (NAS), a locus for interactions between limbic and motor processes, plays a role in mediating locomotion in rodents. A major mesolimbic efferent pathway arising within the NAS is a dense GABAergic projection into the substantia innominata/ventral pallidal region (SI/VP). Previously we have demonstrated an asymmetrical locomotor response to discrete, unilateral lesions of either parietal cortex (PC) or NAS: only right-hemisphere lesions resulted in a significant change in locomotor activity. The goal of the present study was to ascertain whether locomotion elicited from the SI/VP is also characterized by behavioral asymmetry.

Unilateral microinjections of the GABA antagonist picrotoxin (PTX), a chloride-channel blocker, were used to produce locomotor activation. Male Sprague-Dawley rats were implanted bilaterally with cannulae in the SI/VP and injected (0.5 μl over 1 min) every other day with either saline (SAL) or PTX in either the right or left hemisphere (4 injections in all). Each animal was treated with one of 7 doses of PTX (0.005, 0.010, 0.015, 0.020, 5, 15, and 25 ng in saline vehicle; n = 6-9 each dose group). Rats were placed in Omnitech activity chambers immediately following injection, and horizontal and vertical activity monitored at 20-min intervals for 2 hours.

Results were analyzed separately for each group, using a 3-way MANOVA with treatment (SAL vs PTX), injection side (right vs left), and time (successive intervals) as within-subject factors. Injection side was found to be either insignificant or inconsistent, leading to the conclusion that the SI/VP does not contribute to the locomotor asymmetry elicited from PC or NAS.

650.12

SINGLE CELL RECORDING IN THE FREELY MOVING, UNILATERALLY 6-OHDA LESIONED RAT. J. CHO, L. MANZINO*, P.K. SONSALLA, and M.O. WEST. Dept. of Psychology, Rutgers Univ., New Brunswick, NJ 08903., Dept. of Neurology, UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ 08854*.

The basal ganglia (BG) are essential for initiating movements, and for processing sensory (e.g., proprioceptive) information in order to guide ongoing movements. In particular, the role of the BG in movement depends upon dopaminergic innervation of these structures. The depletion of striatal dopamine can produce severe behavioral deficits such as hypokinesia, akinesia, tremor, rigidity, adipsia, hypodipsia, and sensory inattention. This study examined the effects of unilateral depletion of striatal dopamine on single neurons in the lateral striatum of awake, unrestrained rats (N=5). Injection of 4μL of 6-OHDA (6-OHDA HBr Salt 3 mg/mL) into the medial forebrain bundle (and desmethylimipramine intraperitoneally to protect NE projections) resulted in >98% depletion of dopamine unilaterally in all cases (N=5). Two months after the lesion, animals exhibited robust rotational responses to L-DOPA (10 mg/kg plus carbidopa, 10 mg/kg), i.e., at least 500 turns contralateral to the lesioned side during the 3 hr post-injection period. Animals were then prepared for chronic single-cell recording. The striatal neurons that were studied were of the type that fire phasically in relation to active movement, passive manipulation or cutaneous stimulation of particular body parts. No changes were observed in clustering or somatotopic organization after the lesion. However, the main effect on the lesioned side was that in all cases, a percentage of these striatal neurons (mean=17%) fired in relation to sensorimotor activity of multiple body parts, as opposed to the normal relation of such neurons to only one body part. It is possible that sensorimotor deficits observed in dopamine-depleted animals, such as the failure to orient to discrete somatosensory stimuli, may involve a loss of selectivity by some striatal neurons, resulting in disrupted sensorimotor integration.

650.13

SINGLE NEURONS IN THE VENTROLATERAL STRIATUM OF THE RAT RELATED TO LICKING. T.Mittler, J.Cho, L.L.Peoples and M.O.West*. Dept. of Psychology, Rutgers Univ., New Brunswick, NJ 08903.

Recent evidence indicates that the ventrolateral subregion of the striatum (caudate-putamen) is a component of the circuitry controlling oral behavior. This study examined the relationship of single-neuron activity (n=728), recorded from the striatum of awake, unrestrained rats (n=4), to oral movements involved in licking. Neuronal firing was evaluated during licking of single drops of water or sucrose solution, and during cutaneous stimulation and motor activation of the mouth and perioral area. Peri-event histograms were constructed around licking, by using frame by frame analysis (30 frames/sec) of computer-synchronized videotape recordings. Firing rates of 75 neurons ("lick-related") increased specifically during licking but showed no change during a full sensory examination and motor activation of all other body parts, including other orofacial movements. 15 additional neurons ("orofacial-combination") fired during licking and also fired in the absence of licking, during one or more other orofacial sensorimotor functions. Lick-related neurons were located in the lateral striatum, throughout the entire anterior-posterior range studied (from +1.5 mm to -1.5 mm A-P). Initial analysis showed that lick-related neurons were located ventral to representations of the body and limbs. These findings extend the characterization of the somatotopic map in the striatum of the rat to include representations of oral sensorimotor functions by single neurons, and provide the potential for using this preparation to study oral motor dysfunctions (e.g. tardive dyskinesia) at the single cell level. Supported by grant DA 04551.

650.14

TWO-DIMENSIONAL AND THREE-DIMENSIONAL VISUALIZATION OF FUNCTIONAL REPRESENTATION IN RAT NEOSTRIATUM.
E. Lubin†, L.L. Brown†, D. Smith† and S. M. Feldman‡.* †Department of Neurology, Albert Einstein College of Medicine, Bronx, NY 10461 and ‡Center for Neural Science, New York University, New York, NY 10003 .

We previously reported the use of feature detection procedures in conjunction with deoxyglucose autoradiography to reveal a combinatorial map of somatosensory activity in rat neostriatum (Brown, *PNAS*, 1992). We here report the use of *Voxel View* software for 3D image analysis, to further examine functional activity in the region of rat neostriatum that receives input from sensory and sensorimotor cortex.

Tactile stimulation was applied to awake rats during iv injection of radiolabelled deoxyglucose. Image analysis was carried out on digitized autoradiograms of coronal brain sections, with optical density initially coded as grey level. Subsequent color coding was used to identify the areas of maximal and minimal glucose utilization in striatal regions of interest. Textural characteristics of each image were demonstrated by use of a seed function, image enhancement and boundary extraction procedures.

The creation of a series of 2D images that display bounded areas of maximal activation revealed a patchy array that corresponds both to known neuroanatomic patterns of corticostriate terminal distribution and to clustered regions of neural activity; we previously identified these regions as *features*, using different image processing algorithms (Brown, 1992). The images have the advantage of obviating the user-interactive thresholding procedures required in our earlier studies. In addition, for each rat we rendered the series of 2D images as a 3D volume, which showed the shapes and spatial arrangements of the features. The technique affords an opportunity to view in three dimensions the regional metabolic activity produced under physiological conditions.

Supported by USPHS Grant NS21356.

650.15

REPEATED L-DOPA ADMINISTRATION INCREASES THE NUMBER OF SPONTANEOUSLY ACTIVE A9 DOPAMINE NEURONS IN THE PARTIAL DOPAMINE-DEPLETED RAT. D. G. Harden* and A. A. Grace. Depts. of Behavioral Neuroscience and Psychiatry, Center for Neuroscience, University of Pittsburgh, Pittsburgh, PA 15260

Using 6-OHDA-induced dopamine (DA) depletion as a model for Parkinson's disease, we investigated the effect of repeated L-DOPA treatment on the proportion of spontaneously active A9 DA neurons. One week following i.c.v. injection of a sufficient level of 6-OHDA to deplete approximately 70-90% of striatal DA, animals were divided into control and L-DOPA treatment groups. L-DOPA administration consisted of two daily i. p. injections of 50mg/kg of L-DOPA and 50mg/kg of benserazide dissolved in isotonic saline. In parallel, control depleted animals were injected with isotonic saline. Administration was between the hours of 8:30 and 10:30 A.M. and 4:30 and 6:30 P.M.. Following at least 4 weeks of treatment, nine electrode tracks were made through a predefined region of the substantia nigra . In saline-treated, depleted animals, the average number of active DA neurons per track was 0.22 whereas in animals treated with L-DOPA there were substantially more DA cells found (0.72 neurons/track). The results of this study indicate that repeated L-DOPA treatment increases the number of active DA neurons following partial 6-OHDA-induced DA depletion. This increase in active DA neurons may contribute to some of the therapeutic actions and side effects observed upon the initiation of L-DOPA treatment in humans with Parkinson's disease.

650.16

LONG-TERM ENHANCEMENT OF PRESYNAPTIC EXCITABILITY OF GLUTAMATE (GLU) AFFERENTS TO ACCUMBENS. M. Garcia-Munoz, P. Patino, S.J. Young and P.M. Groves. Dept. of Psychiatry., Sch. of Medicine, University of California San Diego, La Jolla CA 92093-0603.

We have reported that tetanic stimulation of cortical afferents can induce an enduring modification in striatal terminal excitability. The induction does not appear to depend on postsynaptic actions, but on alterations occurring in the presynaptic membrane since these changes in excitability can be obtained in animals in which most postsynaptic neurons are destroyed with kainic acid. We now report that long-term changes in presynaptic excitability can also be induced in GLU afferents to accumbens following tetanic stimulation of prefrontal cortex (PC), amygdala (Amyg) or hippocampal subiculum (Hipp). Extracellular action potentials were recorded either in PC, Amyg or Hipp in urethane anesthetized, male Sprague-Dawley rats. Excitability was assessed by determining the threshold current for antidromic activation of the terminal field before and after tetanization. Tetanic stimulation (100 Hz/ 250ms/ 6s, 5 times) to the ipsilateral Amyg or the contralateral PC produced a long-lasting (>1h) increase in the excitability of their respective terminal fields in accumbens. In contrast, similar to corticostriatal terminals hippocampal high-frequency stimulation produced a transient increase followed by an enduring decrease in excitability, and an enduring increase if a second tetanus was administered at the time of the occurrence of the initial transient increase. Experiments are underway to examine these effects in animals with kainate lesions in accumbens. We propose that the induction of striatal and accumbens long-term changes in presynaptic excitability occurs via a positive feedback system in which increases in firing rate increase GLU release, which in turn increases the activation of presynaptic autoreceptors (KA, NMDA or *t*-ACPD), to locally modify terminal membrane conductance and/or polarization and thereby increase electrical excitability and release.

650.17

DEFICIENT STARTLE GATING AFTER QUINOLINIC ACID (QA) LESIONS OF THE RAT STRIATUM MIMICS DEFICITS IN HUNTINGTON'S DISEASE (HD). M.H. Kodsi * and N.R. Swerdlow. Depts. Neuroscience and Psychiatry, UCSD Sch. of Med., La Jolla, CA 92093.

The acoustic startle reflex is normally inhibited, or "gated", when the startling noise is preceded by a weak "prepulse". "Prepulse inhibition" (PPI) is impaired in patients with neuropsychiatric disorders associated with limbic and basal ganglia dysfunction, including Schizophrenia, OCD and HD. This failure of sensorimotor gating in HD patients may reflect striatal pathology that underlies their inability to inhibit involuntary movements.

Previous studies in rats suggest that striatal modulation of PPI is effected through striato-pallidal GABAergic efferents; degeneration of these same projections is implicated in the etiology of HD. In the present study, we assessed whether PPI deficits in HD patients can be modeled in rats by QA lesions of the striato-pallidal projection. QA, an endogenous neurotoxin, has been implicated in striatal neurodegeneration in HD.

Startle was measured in rats after QA-induced lesions of the ventral striatum. Lesioned animals had elevated startle amplitude and significantly reduced PPI. Similar deficits were noted after QA lesions of the posterior dorsolateral striatum. In addition, reduced PPI after QA lesions of the ventral striatum was significantly reversed by muscimol infusion into the ventral pallidum. These findings suggest that a loss of PPI in HD patients may reflect neuronal degeneration within striato-pallidal GABAergic circuitry.

650.18

DEPLETION OF DOPAMINE IN THE CAUDATE NUCLEUS BUT NOT DESTRUCTION OF VESTIBULAR INPUTS IMPAIRS SHORT-INTERVAL TIMING IN RATS. Nancy L. Dallal* and Warren H. Meck. Department of Psychology, Columbia University, New York, NY 10027.

Impairment of the dopaminergic system in the striatum induced by dopamine-receptor antagonists or by specific neurotoxin terminal lesions results in both motor and cognitive disturbances in rats trained in psychophysical tasks involving the discrimination of durations in the seconds to minutes range. In order to specify further the role of the striatum in timing and time perception, the performances of rats trained in two sets of operant peak-interval (PI) timing procedures (10s & 60s or 20s & 80s) was examined after specific destruction of dopamine neurons by 6-hydroxydopamine perfusion into the caudate nucleus or by destroying vestibular inputs to the striatum with intratympanic injections of sodium arsenate. Destruction of vestibular inputs to the striatum did not affect sensitivity to time but did result in a higher response rate in the PI timing task that was associated with identical start times for responding in lesioned and control rats, but significantly earlier stop times for the rats with vestibular lesions. In contrast, the effects of dopamine depletion from the caudate resulted in a severe impairment in the sensitivity to time. Rats with caudate lesions were unable to exhibit temporal control of their behavior, but were able to show discrimination of the relative reward value of a signal by differentially modifying their response rates. These results suggest that areas of the basal ganglia receiving vestibular input influence the determination of response thresholds for timing, whereas the dorsal striatum may serve an accumulator function for temporal integration.

650.19

SYNCHRONIZATION AMONG GABAERGIC NEURONS IN A GLOBALLY COUPLED NETWORK. D. Golomb,[1] X.-J. Wang[2] and J. Rinzel[1]*. [1]Mathematical Research Branch, NIDDK, NIH, Bethesda, MD 20892; [2]Depart. of Mathematics and James Franck Inst., Univ. of Chicago, Chicago, IL 60637.

We explore computationally, with a globally-coupled network of conductance-based inhibitory model neurons, the hypothesis that the reticular thalamic nucleus (RTN) is a synchronized pacemaker for sleep spindle rhythms. A single cell shows post-inhibitory rebound due to a T-type calcium current that deinactivates with hyperpolarization. We showed previously (Wang & Rinzel, Neuroscience 53:899-904,1993) that such a network can oscillate *synchronously*, if the synaptic conductance decays slowly (as do $GABA_B$ synapses) and if the synaptic strength is large enough. Here we consider the separate effects on synchronization of conductances G_A and G_B (for $GABA_A$ and for $GABA_B$ synapses). G_A is fast, while G_B decays slowly. If, with only G_B present, our homogeneous network oscillates synchronously, adding a sufficiently strong G_A leads to desynchronization: the population subdivides into several groups which burst alternately. For moderately strong G_A, the oscillation's frequency increases with G_A strength. This effect is from slowing of T-current de-inactivation with deeper hyperpolarization. Increasing G_A, for which the reversal potential is less negative than for G_B, decreases the level of post-burst hyperpolarization, thereby avoiding the voltage region of slower de-inactivation. We also study the effects of heterogeneous intrinsic properties amongst the neurons, by assuming that the T-type calcium conductance density varies from cell to cell. The level of synchrony decreases as the variability increases. When the neurons are nearly identical they still synchronize, with modest phase shifts. With large variability, the network is totally asynchronous; individual cells are either periodic or quiescent. For intermediate variability, there are two regimes of complex behavior. In one, some neurons burst every cycle, while others burst every few cycles or not at all. In the second, there are two groups of neurons bursting alternately, while other neurons burst at lower rate or are quiescent.

BASAL GANGLIA AND THALAMUS IX

651.1

INCREASE OF GLUCOSE CONSUMPTION IN BASAL GANGLIA AND THALAMUS OF PATIENTS WITH SPASMODIC TORTICOLLIS.
D.Perani*, G.Galardi, F.Grassi, S.Amadio, L.Maderna, GC.Comi, N.Canal, F.Fazio.
INB-CNR, Scientific Institute H S Raffaele, University of Milano, Italy.

The pathophysiology of spasmodic torticollis, a focal dystonia involving neck muscles, is still unclear. In the present study, 2-[18F]fluoro-2-deoxy-D-glucose ([^{18}F]FDG) positron emission tomography (PET) was used to compare rCMRglu in 7 patients with spasmodic torticollis (mean age 50.37 ± 11.47) and 10 age matched controls. All cases with a short disease duration, were untreated, except one patient with 15 years history, treated with anticholinergic drugs. An inter-groups analysis (patients vs controls) using Statistical Parametric Mapping (Friston et al.1989) revealed a significant bilateral increase of glucose consumption in putamen and thalamus ($p<0.001$). An individual analysis, with mean ± 2 SD of normal rCMRglu values as the cut-off scores, demonstrated a significant increase of rCMRglu in putamen (6 cases bilaterally), caudate (4 cases bilaterally, 2 unilaterally) and thalamus (5 cases bilaterally, one unilateral). The patient treated, with a long history, had a hypometabolism in caudate and putamen. Enhanced glucose metabolism in basal ganglia and thalamus is possibly the functional correlate of focal dystonia. A recently proposed model suggests that dystonia would be the consequence of a putaminal hyperactivity, leading to the breakdown of the pallidal inhibitory control on thalamus and thalamo-cortical projections.

651.2

LESIONS IN THE SENSORIMOTOR REGION OF THE INTERNAL SEGMENT OF THE GLOBUS PALLIDUS (GPi) IN PARKINSONIAN PATIENTS ARE EFFECTIVE IN ALLEVEATING THE CARDINAL SIGNS OF PARKINSON'S DISEASE M. Baron, J. Vitek, R. Turner, Y. Kaneoke, R. Bakay, M. DeLong. Dept. of Neurology. Emory University Sch. of Med., Atlanta, GA. 30322

Previous studies have reported that lesions placed in GPi are effective in reducing or abolishing many of the cardinal signs of Parkinson's disease. These studies, however, did not directly correlate lesion location to outcome nor were standard clinical rating scales or quantitative motor assessments performed.

In patients (n=7) undergoing microelectrode-guided pallidotomy, we performed (pre- and post-operatively) a series of standard clinical assessments [United Parkinson's Disease Rating Scale (UPDRS), Schwab and England (S & E)], as well as quantitative measures of motor function, including reaction and movement time. Using microelectrode mapping for guidance radiofrequency lesions (65-80 degrees F for 1 minute) were placed in the caudal sensorimotor territory ofd GPi. Immediate improvement in parkinsonian signs was often observed in the contralateral limbs. At 1 and 3 months we noted substantial improvement in the UPDRS with patients' total scores decreasing from 102 to 84. The S & E scores improved from 41 to 67 % independence. Improvement was reflected in decreased reaction and movement times, with some patients showing bilateral improvement. In addition, tremor and rigidity were reduced and gait was improved. Furthermore, drug-induced dyskinesias were markedly diminished, in some cases bilaterally. Lesion locations were confirmed by high-resolution MRI to lie within the caudal and lateral portion of GPi in regions where neurons intra-operatively responded to passive or active movement of the limbs.

These studies provide direct evidence that lesions of GPi within the motor territory are effective in reducing parkinsonian motor signs. Mapping boundaries of GPi and assessing neuronal responses to limb movement are helpful means of guiding the placement of lesions and improving the surgical results of pallidotomy.

651.3

NEURONAL ACTIVITY IN THE INTERNAL (GPi) AND EXTERNAL (GPe) SEGMENTS OF THE GLOBUS PALLIDUS (GP) OF PARKINSONIAN PATIENTS IS SIMILAR TO THAT IN THE MPTP-TREATED PRIMATE MODEL OF PARKINSONISM. J. Vitek*, Y. Kaneoke, R. Turner, M. Baron, R. Bakay, M. DeLong. Dept. of Neurology, Emory University, of Medicine, Atlanta, GA, USA.

Previous studies of neuronal activity in the GP of MPTP-treated monkeys have demonstrated increased tonic discharge rates in GPi and decreased rates in GPe as well as widened receptive fields to proprioceptive input. These findings have lent support to a current model whereby increased (inhibitory) output from GPi results in the cardinal signs of parkinsonism. In patients with Parkinson's disease (PD) undergoing micro-electrode guided pallidotomy, we recorded the spontaneous discharge of cells in GP and surrounding structures and examined the response of cells to active and passive limb movements.

The discharge pattern of cells in the striatum, GPe, GPi, and the basal forebrain were similar to those reported previously in the monkey. Most striatal neurons exhibited very low discharge rates (< 1 Hz). A small number of tonically active cells discharged at low rates (3.5 ± 1.4 Hz, n=7). The mean discharge rate of high frequency discharge cells in GPe (n=66) was 49.9 ± 20.6 Hz. In GPi the mean discharge rate was 81.9 ± 23.7 Hz (n=54). Nucleus basalis neurons located in the laminae of the pallidum (border cells) discharged at a rate of 34.1 ± 18.6 Hz (n=13).

Of 105 cells examined in GPi, 18% responded to passive and/or active movements of the arm and 16% to the leg. In general, leg cells were located dorsal and medial to arm cells. Receptive fields were confined to movements about a single joint in the contralateral limbs and responses were strongly directional in all cases.

These findings indicate that the neural discharge patterns and somatotopic organization is similar in man and monkey. Furthermore the finding that discharge rates in GPi neurons are significantly ($p < 0.001$, t-test) higher than in GPe neurons, as observed in the MPTP-primate model of PD, lends strong support for the current pathophysiologic model of Parkinson's disease.

651.4

ACTIVITY OF PALLIDAL NEURONS DURING SEQUENTIAL MOVEMENTS. H. Mushiake* and P.L. Strick. Research Service, VAMC and Depts. of Neurosurgery & Physiology, SUNY-HSC@Syracuse, NY 13210

We recorded the activity of single neurons in the globus pallidus (GP) while a monkey performed sequential pointing movements under two task conditions. The monkey faced a panel with 5 touch pads and started the task with his hand on a hold key in front of him. In the Remembered Sequence Task (SEQ task), LEDs over 3 touch pads were illuminated in a pseudo-random sequence as an instruction to the monkey. After a delay period, an auditory 'Go' signal told the monkey to release the hold key and press the touch pads according to the instructed sequence. In the Tracking Task (TRAC task), the monkey was required to press 3 touch pads immediately after the LED over each of them was illuminated. We recorded from over 100 GP neurons that displayed activity related to the tasks. For approximately one-third of the related neurons, activity changes occurred only during the SEQ task or were more pronounced during the SEQ task than during the TRAC task (SEQ neurons). SEQ neurons tended to be in regions of GP that were dorsal to the other task related neurons. For some SEQ neurons the activity changes were limited to one phase of the movement sequence (e.g., a phasic decrease only between the second and third button presses). These observations raise the possibility that the neural mechanism which solves the 'serial order of motor behavior' problem involves a portion of the GP. Supported by the VA Medical Research Service.

651.5

ACTIVITY OF DOPAMINE NEURONS IN MONKEY PERFORMING AN ACTIVE AVOIDANCE TASK. J. Mirenowicz* and W. Schultz* (SPON: European Neuroscience Association). Institut de Physiologie, Univ. Fribourg, CH-1700 Fribourg, Switzerland.

Dopamine (DA) neurons of the substantia nigra of the monkey show phasic electrophysiological responses to external stimuli with particular behavioral significance. Previously tested stimuli included reward, reward-associated stimuli and unexpected novel stimuli, all being appetitive. A more comprehensive account of the activity of DA neurons requires the assessment of their responsiveness to aversive stimuli. Furthermore, numerous studies using dialysis or voltammetric techniques revealed an increase in DA turnover in the prefrontal cortex and the striatum of animals submitted to stressful situations or to aversive stimuli, which suggests the activation of DA neurons in such situations.

We investigated the activity of DA neurons in an active avoidance task. The monkey withdrew its hand from a resting key in response to a light in order to avoid a mild air puff to the hand. Of 52 tested DA cells, 40% responded phasically to the conditioned light. On the rare instances where the monkey did not succeed in withdrawing its hand from the key when the light appeared, most DA neurons were activated phasically by the air puff. For reasons of comparison, the animal was presented with appetitive stimuli in randomly alternating trials. Here, the monkey released the resting key on hearing a conditioned sound and pressed a lever in order to receive a drop of apple juice. Of the tested DA neurons, 75% responded phasically to this conditioned stimulus. Almost all neurons activated by the aversive conditioned stimulus also responded to the appetitive conditioned stimulus.

This study suggests that DA neurons respond to aversive stimuli. Thus, DA neurons are activated by the two most important classes of external stimuli with behavioral significance, appetitive and aversive stimuli. The fact that most neurons activated by aversive stimuli also responded to appetitive stimuli suggests that there is no segregation within the population of DA cells activated by motivationally significant stimuli.

651.6

ACTIVITY OF MONKEY DOPAMINE NEURONS IN A LEARNING SET PARADIGM. J. Hollerman* and W. Schultz. Institut de Physiologie, Univ. Fribourg, CH-1700 Fribourg, Switzerland.

Previous studies have shown that dopamine neurons respond to reward presentation during early stages in the learning of an appetitive task but this response is transferred to the conditioned stimuli after the task is well learned. These studies involved comparisons of the responses of different dopamine neurons recorded at different stages of task acquisition across weeks of learning. In order to investigate the activity of individual dopamine neurons during the course of learning, we attempted to use a more circumscribed learning situation, namely, a learning set paradigm. We used a conditional motor task in which the animal performed, or refrained from performing, an arm movement in response to a trigger signal depending on a preceding instruction (go-nogo task). Responses of dopamine neurons were recorded in two basic conditions: 1) a standard condition, in which the instruction stimuli were previously well learned (stimulus-response associations previously formed), and 2) a learning condition in which the instruction stimuli consisted of previously unseen stimuli (new stimulus-response associations forming). Responses of dopamine neurons to reward were compared in the two conditions.

Consistent with previous studies, a very small percentage of dopamine neurons responded to reward in the well established standard condition. However, there were very few individual cells which exhibited greater reward responses in the "new learning" than in the standard condition. This seems to indicate that dopamine neurons are not actively involved in the learning of new stimulus-response associations in the context of a previously learned task. This may be due to the fact that in the context of the task, the delivery of reward is reasonably well predicted, even in the learning condition. This is supported by the observations that the dopamine neurons did respond to unexpected reward presentation, delivered either outside the task or within the task (on correct trials) at other than the usual post-response delay. Thus dopamine neuron responses to reward appear to be highly sensitive to the predictability or expectedness of the reward.

651.7

PREPARATORY AND MOVEMENT-RELATED ACTIVITY OF NEURONS IN PALLIDAL-RECEIVING THALAMUS. J.A. Buford* and M.E. Anderson. Depts. of Rehabilitation Medicine and Physiology & Biophysics and the Regional Primate Research Center, RJ-30, University of Washington, Seattle, WA 98195, U.S.A.

Prior knowledge of target location or movement direction may elicit preparatory activity in striatal, cortical, and pallidal neurons. We have now examined the effect of a precue on neural activity in pallidal-receiving areas of the thalamus.

Two monkeys performed arm movements to one of 8 target lights that were either lit coincident with a trigger tone and kept visible (V task) during the movement or flashed briefly as a precue (P task) before the tone triggered the movement. Forty-four neurons were studied in arm-related, pallidal-receiving areas of thalamus. Seventy-five percent had early (Q) responses following the precue in the P task and movement-related (M) responses in both tasks; 2/3 of those also had more sustained, set-related (S) activity between the precue and trigger in the P task. For 60% of the cells, the M response was different for the P and V tasks. However, the M response in the P task could be either an enhancement (27%) or a depression (33%) of that in the V task. Directional tuning of Q and S responses for the P task as well as M responses for both tasks was common (50%). Only 10% of the neurons studied had no directional tuning for either task.

Task-related neurons in premotor areas of cortex or in striatum may have instruction-related or movement-related activity, but few cells have both (Wise and Tanji 1985, Alexander and Crutcher 1990, Schultz and Romo 1992). Pallidal neurons, in contrast, often have both instruction-related and movement-related activity (Turner and Anderson 1989). Our data imply some integration of preparatory and movement-related signals in the pallidum that is reflected in the discharge of pallidal targets in the thalamus.

Supported by NS15017, NS07097, and RR00016.

651.8

ALTERED SOMATOSENSORY RESPONSE PROPERTIES OF NEURONS IN THE CEREBELLAR RECEIVING AREA OF THE MOTOR THALAMUS IN MPTP TREATED PARKINSONIAN MONKEYS. Y. Kaneoke, J. L. Vitek, J. Ashe, M. R. DeLong, M.D. Crutcher*. Dept. of Neurology, Emory University. Atlanta, GA. 30322.

Previous studies in monkeys rendered parkinsonian by injections of the neurotoxin MPTP have found that neurons in the globus pallidus, pars interna (GPi) show increased sensitivity and decreased specificity of somatosensory responses. We have previously described similar alterations in the pallidal receiving portion of the motor thalamus, ventralis lateralis, pars oralis (VLo). In order to determine whether alterations in the somatosensory responses of thalamic neurons are restricted to the pallidal portion of the motor thalamus or are more widespread we examined the sensorimotor response properties of neurons in the cerebellar receiving portion of the motor thalamus, ventralis posterior lateralis, pars oralis (VPLo) and ventralis lateralis, pars caudalis (VLc) in African Green monkeys both prior to and following treatment with MPTP (0.5 mg/kg/day x 3 days i.m.). African Green monkeys were conditioned to permit a detailed somatosensory examination consisting of passive joint rotation, muscle palpation, tactile stimulation and the elicitation of active movements of the limbs and orofacial structures. The same electrode was used to map the sensorimotor response properties of neurons in VPLo and VLc both before and after MPTP treatment, thus it was possible to keep the physiologic maps in precise register. Treatment with MPTP resulted in severe parkinsonism, including akinesia, bradykinesia, rigidity, tremor and postural instability. Prior to MPTP treatment neurons in VPLo and VLc typically responded to somatosensory stimulation to passive movements about a single joint of the contralateral limb. Neuronal responses were directionally specific. Following treatment with MPTP somatosensory responses in VPLo and VLc were less specific and it was common to find neurons responding to movements of multiple joints, in more than one direction, and often more than one limb. Although ipsilateral responses were never seen prior to MPTP treatment, such responses were commonly observed following treatment with MPTP. We conclude that proprioceptive responses are altered in both the pallido- and cerebello-thalamocortical pathways and suggest that dysfunction in *both* these circuits may play a significant role in the pathogenesis of parkinsonian signs. Supported by NIH grant NS01328.

651.9

TONICALLY ACTIVE NEURONS IN THE PRIMATE STRIATUM ACQUIRE RESPONSES TO SENSORY STIMULI DURING BEHAVIORAL CONDITIONING. T. Aosaki*, H. Tsubokawa, K. Watanabe, A. M. Graybiel and M. Kimura. Jichi Med. Sch. Dept. of Physiol., Tochigi 329-04, Japan; M.I.T. Dept. of Brain and Cog. Sci., Cambridge, MA 02139; Osaka University, Osaka 560, Japan.

Tonically active neurons (TANs) in the primate striatum are known to respond to conditioned sensory stimuli that elicit behavioral reactions. We have studied how these unit responses develop during the acquisition of a sensorimotor association. The activities of 682 TANs were recorded extracellularly in alert behaving monkeys before, during and after conditioning. Two monkeys were trained to lick reward juice delivered simultaneously with the presentation of a click. Before conditioning, the animals made almost no licking movements. During conditioning they learned to lick after the clicks sounded, and by three weeks, the onset of the licking movements became time-locked to the clicks. Population peristimulus-time histograms of TANs showed almost no response to the clicks. The average activity of TANs followed daily in one monkey during conditioning showed initially (1-4 da, n=51) a small transient suppression response to the click followed by a rebound activation, then (by 5-8 da, n=53) an initial activation and stronger transient suppression/rebound, and finally (by 3 wk, n=91) initial activation with strong ca. 100 msec suppression and rebound. By prolonged recording from single TANs (n=4), we found that by ca. 2 weeks, individual TANs can acquire the response to the click within 10 minutes. Histological reconstruction showed that the TANs that became responsive were widely distributed through the striatum, suggesting that, after conditioning, the activity of TANs can become synchronized in widespread parts of the striatum. These results suggest that TANs of the striatum may serve to organize striatal activity during conditioned movements. Supported by Human Frontier Science Program.

651.10

DIFFERENTIAL LOCALIZATION OF TONICALLY ACTIVE NEURONS OF PRIMATE STRIATUM IN THE MATRIX AND AT STRIOSOME/MATRIX BOUNDARIES. M. Kimura*, T. Aosaki, and A. M. Graybiel. Osaka University, Faculty of Health and Sport Sciences, Osaka 560, Japan; M.I.T. Dept. of Brain and Cognitive Sciences, Cambridge, MA 02139, USA.

Primate striatal neurons can be classified into at least two types in terms of wave form, spontaneous activity and behavioral response relationships. Numerous slowly firing cells (type II), presumed medium spiny projection neurons, show phasic discharges prior to or during movements. Rarely encountered tonically active neurons (TAN or type I), presumed striatal interneurons, do not exhibit burst discharges in relation to behavioral events, but acquire conditioned responses to sensory stimuli that trigger conditional motor behavior during behavioral training. They are activated synchronously across widespread regions of the striatum. The present study was carried out to determine whether the TANs are located in one or in both of the neurochemically distinguished compartments, striosomes and matrix.

TANs were electrophysiologically identified at 151 sites in 5 anesthetized squirrel monkeys, and extracellular marks were made at these sites by injecting the dye, Chicago sky blue, through a glass microelectrode. We encountered TANs at ca. 400 μm intervals and held the neuronal discharges of the same cell for ca. 100 μm. Brains were processed for enkephalin immunostaining to distinguish striosomes from matrix. Some marks were as large as 200 μm, but most were less than 100 μm wide. None of the marks recovered (n=82) was in a striosome. However, 44 out of 82 marks were either close to or precisely on striosome/matrix borders. Of the 38 marks in the matrix beyond the borders, 14 were found at visible borders between inhomogeneous zones of staining in the matrix. These results suggest that the distribution of TANs is not random, and that the conditional activation of many TANs during learning may depend in part on their differential distribution at borders of striosomes. Supported by H.F.S.P. and NIH Javits NS25529.

651.11

EFFECTS OF BICUCULLINE INJECTION INTO THE SUBSTANTIA NIGRA PARS RETICULATA ON ELECTRICALLY INDUCED VOCALIZATION IN KETAMINE ANESTHETIZE CATS. T. Sakamoto*, K. Shiba and Y. Nakajima. Dept. of Physiol., Sch. of Med., Chiba Univ., Chiba, JAPAN 260.

To examine whether the substantia nigra pars reticulata (SNr) is the plausible origin of the inhibitory GABAergic projection to PAG vocalization area or not, a GABA antagonist, bicuculline methiodide (BIC) was microinjected into SNr.

Vocalization was induced by electrical stimulation (0.2 ms pulses, 20 to 100 μA, 100 Hz, for 5-10 s) to the ventrolateral periaqueductal gray (PAG). After injection of 0.1 μl of 2 mM BIC into SNr (n=8), the stimulus threshold of PAG area for vocalization increased for more than 60 min, and it returned to the preinjection level after 2 hrs. Saline injection induced slight increase of the stimulus threshold (less than 0.2 T), which lasted for about 5 min. Effective sites were localized within SNr. After injection of BIC, respiration cycle of spontaneous breathing became longer. However, PAG stimulation induced vocalization with the similar respiration cycle to that of before injection, although the higher stimulus intensity was necessary.

These results suggested that the GABAergic inhibition from SNr regulated the excitability of PAG neurons which send the motor command for vocalization to respiratory and laryngeal control systems within the pons and medulla.

651.12

NEOSTRIATAL LESIONS IN THE CAT PRODUCE APRAXIA AND CHANGES IN PALLIDAL NEURONAL ACTIVITY. J.W. Aldridge*, J.F. Thompson, R.C. Meyer and S. Gilman. Dept. of Neurology, Univ. of Michigan, 1103 E. Huron, Ann Arbor, MI 48104.

We studied the effects of a unilateral excitotoxic lesion of the neostriatum on motor behavior and neuronal activity in the pallidum (globus pallidus and entopeduncular nucleus). Cats were trained to make a forelimb reaching movement in response to a visual cue (GO) and to withhold movement with a different (NO-GO) cue. Animals were prepared for chronic recording of neuronal activity. After control data were collected, large lesions of the caudate nucleus and putamen were made by multiple (15-17) small injections (.25-1.25 μl) of 200 nM quinolinic acid. Short term lesion effects consisted of disturbed locomotion, persistent circling and sensory inattention. These effects peaked within hours of the lesion and subsided in 1 to 3 days. Although there were no noticeable long term deficits in free range behavior, in the context of the GO/NO-GO reaching task, a severe, permanent apraxia of movement appeared. An inability to complete the reaching movement sequence in GO trials was most conspicuous even though initial postural movements were triggered by the cue and the animal could perform the reaching movement in other contexts. Over time, limited recovery occurred, however, inability to withhold movements in NO-GO trials became more noticeable. Pallidal neuronal activity correlated to the motor task persisted in spite of striatal deafferentation. Indeed, the proportion of neurons exhibiting increases of activity rose from 31% to 61%. The proportion of neurons with decreases of activity fell from 13% to 4%. Thus, the basal ganglia appears to have a critical role in performance of skilled movements. Striatal damage produces an apraxia in which the animal has the capabilities to make a movement but fails to do so in specific behavioral contexts. The loss of GABAergic afferents to the pallidum appears to unmask excitatory inputs; possibly these arise from the subthalamic nucleus. Support: NIH grant NS19613 and United Cerebral Palsy Foundation.

651.13

HALOPERIDOL-INDUCED MORPHOLOGICAL ALTERATIONS ARE ASSOCIATED WITH CHANGES IN CALCIUM/CALMODULIN KINASE II ACTIVITY AND GLUTAMATE IMMUNOREACTIVITY. C.K. Meshul*, and S.E. Tan. V.A. Medical Center and Oregon Health Sciences University, Portland, OR. 97201.

Subchronic treatment with haloperidol (HAL)(0.5 mg/kg/d) for 14-30d results in an increased density of synapses within the striatum containing a perforated or discontinuous postsynaptic density (PSD). These 'perforated' synapses are asymmetric and show immuno-gold labelling for glutamate. Thus, the HAL-induced increase in perforated synapses may involve glutamatergic neurons. Co-administration of MK-801, a NMDA antagonist, and HAL blocks the HAL-induced increase in striatal perforated synapses. Since calcium/calmodulin kinase II (CaMK II) is the major protein constituent of the PSD, the activity of this enzyme was determined within the caudate nucleus following 30d treatment with HAL or saline (SAL). Glutamate immunoreactivity was evaluated within the striatum from another group of treated rats. There was a 75% increase in the activity of CaMK II in the HAL-treated animals compared to the SAL controls. This was associated with a significant decrease in calmodulin binding to CaMK II in the drug treated group. There was a concomitant 50% decrease in the density of immunogold labelling for glutamate within perforated synapses of the HAL-treated group compared to the SAL controls. The increase in CaMK II activity and the decrease in glutamate immunoreactivity would be consistent with the hypothesis that there is an increase in release of neurotransmitter at glutamate synapses, resulting in greater CaMK II activity. Increased CaMK II activity could lead to a conformational change of the PSD and the formation of a perforated PSD. Supported by the Dept. of Veterans Affairs and NIH.

651.14

CHRONIC EXPOSURE TO NEUROLEPTICS ALTERS EXCITATORY AMINO ACID TRANSMISSION (EAA) IN SPECIFIC REGIONS OF THE RAT BRAIN. A. E. Johnson*, U. Liminga, L. Källström, L. Gunne and F. -A. Wiesel. Department of Psychiatry, Uppsala University, S-75017 Uppsala, Sweden.

The treatment of schizophrenia often involves chronic administration of neuroleptics (NL). While NL act primarily on the dopaminergic system, several other transmitter systems are also affected by these compounds. In the following experiments, we examined the consequences of long-term exposure to fluphenazine decanoate (FLU) on EAA transmission as indicated by changes in [^{3}H]AMPA and [^{3}H]MK-801 binding. Adult female Sprague-Dawley rats received 6 monthly depot injections of FLU (30mg/kg/month i.m.) or vehicle (N=7/group). Animals were killed 60 days after the last injection and their brains were processed for receptor autoradiography. AMPA binding sites were labelled with 10nM [^{3}H]AMPA and nonspecific binding was defined with 100μM quisqualate. The noncompetitive binding site of the NMDA receptor was labelled with 10nM [^{3}H]MK-801 and 200μM Ketamine HCl was used to define nonspecific binding. Densitometric analysis of [^{3}H]AMPA autoradiograms showed that FLU exposure significantly decreased binding relative to controls in the entopeduncular n. and in the ventrolateral thalamic n. by 20% and 14% respectively. Small but significant decreases in [^{3}H]MK-801 binding were detected in the medial geniculate n. Binding in striatum, substantia nigra and other thalamic nuclei were not affected by FLU treatment. These data suggest that changes in EAA transmission occur after chronic FLU exposure. Changes in some of these areas may be related to the increased display of the extrapyramidal side effects that often are associated with long-term NL exposure. Supported by grant #8318 (F.-A.W.) and #4546 (L.G.) from the Swedish Medical Research Council.

651.15

EFFECTS OF CHRONIC LEVODOPA ON DOPAMINE, EXCITATORY AMINO ACID AND GABA RECEPTORS IN BASAL GANGLIA. S.M. Papa*, T.N. Chase and T.M. Engber. Experimental Therapeutics Branch, NINDS, NIH Bethesda, MD 20892.

Chronic levodopa treatment of Parkinson's disease is usually associated with motor response complications whose pathogenesis remains obscure. We examined the effect of chronic levodopa treatment (25 mg/kg i.p., b.i.d.) on binding to dopamine (D1 and D2), excitatory amino acid (NMDA and AMPA) and GABA receptors in the basal ganglia of 6-hydroxydopamine lesioned rats using receptor autoradiography. Binding to both D1 and D2 dopamine receptors in striatum increased due to the lesion (20% and 27%, respectively). Levodopa treatment increased D1 binding by 32% and reduced D2 binding by 9%; D1 binding in substantia nigra pars reticulata (SNr), which was unaffected by the lesion, increased by 19% in response to levodopa treatment. Striatal NMDA binding was not significantly affected by dopamine denervation, but was increased by 39% by levodopa treatment; binding to both NMDA and AMPA receptors in SNr was decreased by the lesion (by 16% and 18%, respectively), but levodopa treatment did not affect either receptor in this region. GABA receptor binding decreased by 17% in globus pallidus (GP) but increased by 14% in subthalamic nucleus (STN) and 28% in SNr following dopamine denervation; chronic levodopa reduced binding to nigral GABA receptors but had no effect in other regions. Levodopa treatment thus reversed the effect of the lesion on GABA binding in SNr but not in GP and STN, nor on NMDA and AMPA receptors in SNr. These finding suggest that chronic levodopa treatment differentially affects striatal output through the direct and indirect pathways.

651.16

STIMULATION OF LOCAL DOPAMINE D1 RECEPTORS ENHANCES RELEASE OF STRIATAL ACETYLCHOLINE IN 6-HYDROXYDOPAMINE LESIONED RATS. J.J. Anderson*, S. Kuo, T.N. Chase, and T.M. Engber. Experimental Therapeutics Branch, NINDS, NIH, Bethesda, MD 20892.

Systemically administered dopamine D1 receptor agonists stimulate release of striatal acetylcholine (ACh) in vivo. It is not clear, however, if D1 receptors which modulate striatal ACh release are intra- or extra-striatal. We, therefore, examined the effects of local perfusion of the D1 agonist SKF 38393 on striatal ACh release using in vivo microdialysis in freely moving rats with a unilateral 6-hydroxy-dopamine lesion of the nigrostriatal pathway. Lesioned rats were implanted with chronic guide cannulae and after at least a three day surgical recovery period, microdialysis probes were inserted through the guide into the dorsal striatum. The reversible AChesterase inhibitor neostigmine (1 μM) was included in the perfusion solution to improve recovery of ACh. Perfusion for 20 min with 1 and 10 μM (±)SKF 38393 increased extracellular ACh by 37 and 67%, respectively. Perfusion with the less active enantiomer S(-)SKF 38393 (10 μM) did not appreciably influence extracellular ACh levels. Co-perfusion of the D1 antagonist R(+)SCH 23390 (100 μM) prevented the increase in extracellular ACh induced by (±)SKF 38393 (1 μM). These results suggest that activation of local intra-striatal D1 receptors stimulates release of ACh in the striatum and point to the important role of local D1 receptors in regulating striatal cholinergic transmission.

651.17

INCREASED CALBINDIN-D28K IMMUNOREACTIVITY IN STRIATAL SPINY NEURONS IN HUNTINGTON'S DISEASE BRAINS IS ALSO SEEN IN RAT STRIATAL CELLS TREATED WITH QUINOLINIC ACID. Q. Huang*, D. Zhou, E. Sapp, P. Ge, H. Aizawa, E. Bird, J-P. Vonsattel and M. DiFiglia. Lab. of Cell. Neurobi., Mass. General Hospital, Boston, MA 02114.

During the process of cell death in Huntington's disease (HD), the dendrites of medium spiny neurons undergo marked regenerative and degenerative changes (Graveland, *et al.*, 1985, Science, 227:720-723) which are accompanied by an increase in immunoreactive calbindin-D28K (iCalb) (Ferrante, *et al.*, J. Neurosci. (1991) 11:3877-3887), a calcium binding protein enriched in striatal spiny neurons. Study of grade 1-3 HD brains in our laboratory indicates that increased iCalb in dendritic spines can be detected more ubiquitously in higher grades (grades 2 and 3) where neuronal loss is more severe. Since excitotoxic injury has been proposed as a potential cause of cell death in HD, we examined in rat striatal neurons, *in vivo* and *in vitro*, the effects of quinolinic acid (QA) on iCalb. Results showed that 2-3 weeks following intrastriatal injection of QA (6-20 ng) in rats (n=3), striatal neurons in the transition zone around the lesion core exhibited a marked increase in iCalb within dendrites and spines. *In situ* hybridization with a ^{35}S-labeled oligonucleotide probe revealed a slight reduction or no change in Calb mRNA levels in neurons within the transition zone. In 12-day-old striatal cultures derived from neonatal rats, a significant increase ($p<0.05$) compared to untreated controls was seen in iCalb cells and dendrites following exposure for 2-6 hours to 500 μm QA. This effect was blocked by treatment with the NMDA receptor antagonist, AP-5. Results in rat suggest that an excitotoxic mechanism may account for the marked increase in iCalb in dendrites in HD and that the increase may involve a post-translational change in protein turnover and/or transport. (Supported by NIH grants NS09349 to QH and NS16367 to MD).

651.18

DEGENERATION OF STRIATONIGRAL NEURONS FOLLOWING VOLKENSIN INJECTION IN THE SUBSTANTIA NIGRA. M.B.Harrison[1], G.F.Wooten[1]*, R.G.Wiley[2] and L.Zaborszky[1]. [1]Univ. of Virgina, Charlottesville VA 22908; [2]DVAMC and Vanderbilt Univ., Nashville TN 37212.

Volkensin is a suicide transport agent which produces retrograde degeneration of neurons projecting to injection sites in the CNS. Previous studies in the striatum using receptor autoradiography, in situ hybridization and immunocytochemical techniques suggest that the initial effects of nigral injection of volkensin are selective for striatonigral neurons. Neuronal degeneration has not been directly demonstrated in suicide transport lesions of the CNS. In this study, the silver method of Gallyas et al. (1990) was used to evaluate time course and distribution of neuronal degeneration following nigral injection of volkensin. This method produces selective, Golgi-like staining of neurons whose cytoskeletal system is damaged by various pathologic events. 2.5 ng of volkensin was injected stereotactically into the left SN of adult rats perfused at 5 (n=5), 10 (n=4) or 20 (n=5) days postlesion. At 5 days, the ipsilateral striatum contained numerous Golgi-like impregnated medium-sized spiny neurons, located according to the exact topography of the injection site. Many neurons showed pathologic alterations (corkscrew-like or beaded dendrites). Degeneration of axon terminals was also seen in the striatum. At later times, there was marked gliosis in the striatum and progressive loss of impregnated neurons. Other regions of the CNS occasionally showed neuronal degeneration based on variations in lesion size and both anterograde and retrograde connections. This study confirms that nigral injection of volkensin produces an early, consistent and pronounced retrograde effect in the striatum and demonstrates the utility of the Gallyas silver method for direct visualization of degenerating neurons in this and other models of neurodegenerative disease.

651.19

PREFRONTAL CORTEX INJURY ALTERS AMPHETAMINE-INDUCED *c-fos* EXPRESSION IN STRIATUM. J.M. Vargo and J.F. Marshall. Department of Psychobiology, University of California, Irvine, CA 92717.

An important issue in understanding the effects of neocortical injury is the determination of the impact of that injury on subcortical functions. *C-fos* expression induced by dopamine agonists has been used as one marker of neuronal response within the caudate-putamen (CPu). In this study, Fos immunohistochemistry was used to determine whether a circumscribed cortical lesion would alter neuronal functioning in those regions of the CPu known to receive afferents from the lesioned site. Rats received unilateral aspiration lesions of the medial agranular (AGm) region of the prefrontal cortex. Five days later, 2 hours after 5 mg/kg d-amphetamine (i.p.), they were perfused, the brains were sectioned and reacted with antisera to Fos, and the numbers of Fos immunoreactive nuclei (ir-nuclei) in the CPu were determined through image analysis. The numbers of amphetamine-induced ir-nuclei in the CPu ipsilateral to the cortical lesion were reduced 25-40% compared to those in the contralateral CPu. These reductions were seen in the dorsolateral CPu, the region receiving afferents from AGm. The numbers of ir-nuclei were similar in both hemispheres in the ventral CPu, an area receiving little or no AGm input. In surgical controls, the numbers of ir-nuclei were symmetrical in both dorsal and ventral regions of CPu. These findings suggest that prefrontal injury affects striatal neuronal response to dopamine agonists in a regionally specific manner, i.e., in those regions partially deafferented by the cortical injury.

651.20

THE SYNAPTIC ORGANIZATION OF THE STRIATUM FOLLOWING VOLKENSIN INJECTIONS IN THE SUBSTANTIA NIGRA OR OX7-SAPORIN INJECTIONS IN THE GLOBUS PALLIDUS. R.C. Roberts*1 and R.G. Wiley[2]. Maryland Psychiatric Research Center[1], Baltimore, MD 21228, and DVAMC and Vanderbilt University[2], Nashville, TN 37212.

Suicide transport agents and retrograde immunotoxins produce degeneration of neurons projecting to an injection site. Injections of the substantia nigra (SN) or globus pallidus (GP) with these agents produces selective and profound lesions of the striatonigral and striatopallidal pathways based on receptor binding and neuropeptide mRNA studies. Also, anatomical analysis indicates a moderate and selective cell loss of projection neurons. In the present study, we sought to determine the events occurring at the electron microscopic (EM) level. Thus, the neostriata of adult rats were examined at the EM level 10 days after unilateral injections of volkensin into the SN (n=4) or of OX7-saporin into the GP (n=4). Blocks were taken from the striata on both the ipsi- and contralateral sides to the injections. EM pictures of striatal neuropil were analyzed for synaptic density and type. Both injections of the SN and GP produced similar degenerative changes in the ipsilateral striata. Synaptic density in the striatum was reduced to 70% of contralateral values in both injection paradigms. Dark degenerating profiles, though very rare in the contralateral striata, were present throughout the neuropil in the ipsilateral striata. Moreover, approximately 12% of the degenerating profiles were forming synapses and were located in both pre- and postsynaptic locations. In spite of the damage to the striata, the proportion of axospinous and axodendritic synapses remained unchanged ipsilateral to the injections. These data show that the damage to the striatal neuropil (30% loss of synaptic density and accompanying degeneration) is greater than the neuronal cell loss (12-14%) observed in a previous study using the same experimental paradigms and suggests a pruning of processes in response to loss of projection neurons. Supported by DRIF, Univ. of MD.

CEREBELLUM V

652.1

INDEPENDENCE OF CEREBELLAR AND OTHER PROJECTIONS TO SUPERIOR COLLICULUS AND MESOPONTINE TEGMENTUM Grunwerg,B.S.*,Krein,H. and Krauthamer,G.M.
Neurosci.& Cell Biol. UMDNJ-R.W.Johnson Med. Sch., Piscataway, NJ 08854

Neurons of the substantia nigra, pars reticulata (SN-r) terminate in the pedunculopontine tegmental nucleus (PPTn) and the deep layers of the superior colliculus (SC-d). The deep cerebellar nuclei (DCN) project to SC-d but a definite projection to PPTn has not been established. Converging projections to PPTn and SC-d from the basal ganglia and the cerebellum may be important for the organization of orienting responses. The extent to which these projections are provided by axon collaterals has important bearings on the nature of information transmission in this system. The present study addresses these two issues. Eight rats received double injections of 0.1-0.5μl diamidino yellow (DY) and true blue (TB) in PPTn and SC-d. Four additional rats received single dye deposits. Brain sections were examined for retrogradely labeled cells after a 48 hr. survival period. The contralateral DCN were labeled from PPTn and SC-d. Labelling was heaviest ventrally in the intermediate nucleus and much less in the medial and lateral nuclei. As expected, DY and TB labeled many neurons in SN-r neurons and zona incerta (ZI) bilaterally with an ipsilateral concentration. In all 3 structures DY and TB labeled neurons were intermingled but no double labeled cells were seen. In agreement with earlier reports, numerous other structures were also labeled. Thus, PPTn deposits labeled SC-d and the entopeduncular n. ipsilaterally. SC-d deposits labeled PPTn bilaterally with a contralateral preponderance. Although the presence of a small number of SN-r, DCN and ZI projection neurons with axon collaterals to PPTn and SC-d cannot be ruled out because the injection sites were small, the results strongly favor the hypothesis of largely independent cerebellar and nigral projections to PPTn and SC-d. (Supported by NIH DE00203 and Dystonia Med. Res. Found.)

652.2

DENDRITES OF PROJECTION NEURONS IN THE RAT PONTINE NUCLEI RESPECT BORDERS OF CORTICAL AFFERENT FIELDS. C. Schwarz* and P. Thier. Neurologische Universitätsklinik, Hoppe-Seyler Str. 3, W-7400 Tübingen, Germany.

The pontine nuclei (PN) are the major relay for information originating in the cortex destined for the cerebellum. As it is known that cortical afferents terminate in the PN in a columnar fashion, we posed the question of whether the dendritic fields of pontine projection neurons are related to that columnar structure. In order to reveal the dendritic morphology of identified projection neurons in the rat PN, we used a combination of retrograde labeling (large injection of fluorogold into the cerebellar cortex) and intracellular fills (lucifer yellow) in slightly fixed slices of pontine brainstem. Cortical afferent fields in the PN were visualized by small injections of the anterograde tracer DiI into the primary visual (A17) or somatosensory (SI) cortex. Projection neurons (n = 85) in the PN shared common characteristic features in the morphology of their dendrites. Proximal dendrites were relatively thick and often branched abruptly into 2 to 4 thin daughter branches. Typically, these thin distal dendrites branched profusely and were irregularly covered with spinelike processes and dendritic appendages. In contrast to the uniform morphology of the dendrites, a striking variability of dendritic field shapes was shown by the PN projection neurons. Dendritic fields displayed circular or elliptic as well as highly asymmetric forms. 43 somata of projection neurons were located within 200 μm of the border defined by cortical afferent fields. Most of the dendritic trees of these neurons respected the border. Proximal dendrites which were oriented towards the border often bent in order to avoid the boundary. In only 6 cases we observed proximal dendrites clearly crossing it. These results suggest that the PN is organized in modules which process different sets of cortical information independently from each other. *Supported by DFG SFB 307-A1*

652.3

MOSSY FIBER BRANCHES OF NEURONS IN THE PONTINE NUCLEUS AND NUCLEUS RETICULARIS TEGMENTI PONTIS PROJECTING TO THE CEREBELLAR NUCLEUS RECONSTRUCTED IN THE CAT AND RAT.
I. Sugihara*, H. Wu, T. Futami, J. Na and Y. Shinoda Dept. of Physiology, Tokyo Medical and Dental Univ. School of Med., 1-5-45 Yushima, Bunkyo-ku, Tokyo 113, Japan.

Previous studies using intracellular recording and HRP-labelling demonstrated a collateral projection to the cerebellar nucleus of the mossy fiber originating from the pontine nucleus (PN) in the cat (Shinoda et al., 1992, J. Neurophysiol. 67, 547-60). Similar intracellular labelling was done for axons from the nucleus tegmenti pontis (NRTP) in the cat. Labelled axons projected to the cerebellar cortex as mossy fibers, some of which were found to give rise to collaterals terminating in the cerebellar nucleus. These intracellular labelling showed that virtually all axon terminals found in the cerebellar nucleus were axon collaterals of mossy fibers projecting to the cerebellar cortex. To further examine the possibilitity that there might be PN and NRTP neurons that project only to the cerebellar nucleus, *Phaseolus vulgaris* leucoagglutinin (PHA-L) was injected ionophoretically into the right PN (n=1) and NRTP (n=2) in the rat. After immunohistochemical visualization of PHA-L, labelled arborized terminal branches were seen in the bilateral dentate (DN) and posterior interpositus nuclei for the NRTP injections and in the bilateral DN for the PN injection. Labelled fibers terminating in the cerebellar nucleus were identified and traced backward from serial sections. So far six NRTP (one contralateral and five ipsilateral to the injection site) and one PN (contralateral) fibers were traced and reconstructed. All of these fibers in the cerebellar nucleus were found to be axon collaterals of mossy fibers terminating in the cerebellar cortex.

652.4

MODULATION OF ASCENDING INPUTS TO CEREBELLAR CORTICAL NEURONS DURING FICTIVE SCRATCHING IN THE CAT.
K.V. Baev*, J.R. Bloedel, M.S. Milak, Yu.P. Shimansky. Barrow Neurological Institute, Phoenix, Arizona 85013.

Both field potentials and responses of single neurons of the cerebellar cortex were recorded in cats in response to stimulation of different peripheral nerves during periods of fictive scratching potentiated by the application of bicuculline solution to the cord dorsum at C_1-C_2. The amplitude of field potentials activated from the ipsilateral hindlimb as well as other sites on the body surface diminished significantly or even disappeared during spontaneous scratching or scratching evoked by stimulation of the periauricular area, the natural target for the limb tip during cat scratching. This effect also could be observed even before bicuculline application in the absence of fictive scratching. In addition, oscillatory field potentials in the rhythm of fictive scratching were observed in some regions of the cerebellar cortex. These data together with the data obtained in experiments with lidocaine microinjections in different structures and results of single unit recordings suggest that cerebellar inputs evoked from afferents of the scratching limb are substantially modified by the central pattern generator for scratching and by interactions evoked by stimulating the target of the scratch reflex which are not dependent exclusively on the rhythmic activity. Supported by NIH grants NS 21958 and NS 30013.

652.5

RAT CEREBELLAR GRANULE CELL ACTIVITY MONITORED WITH CHRONIC IMPLANTS.
M.J. Hartmann* and J.M. Bower, Division of Biology, Caltech, Pasadena, CA 91125.

Experiments on cerebellar cortex in the anesthetized animal are inadequate to determine the nature of cerebellar responses in a natural context. Here we describe the results of experiments in which activity from populations of cerebellar granule cells has been recorded in awake, freely moving rats using a lightweight microdrive developed in our lab. The microdrive allows up to eight electrodes to be independently positioned, and can remain implanted for several months. Granule cell activity was recorded from multiple sites in Crus IIa, a region known to contain representations of the perioral surfaces. Implanted rats were trained to elicit a tactile stimulus which was followed by a water reward. Activity was also recorded during some natural, untrained behaviors, including grooming, chewing, and exploration. Using these procedures we have investigated the temporal and spatial patterns of granule cell activity that immediately follow self-elicited tactile stimulation.

We compare these granule cell responses to those seen during tactile stimulation of the anesthetized rat. Both the characteristic granule cell bursts and double-peaked field potential are present. However, we find significantly more spontaneous activity in the awake animal; this activity is not uniform, but rather has distinct, burst-like increases. During natural behaviors, self stimulation of perioral regions leads to clear and distinct granule cell responses. Short stimulations give short bursts, but longer stimulations (e.g. dragging the lip along a surface) give prolonged bursts of granule cell activity. Supported in part by a grant from Medtronic Corporation and by NIH grant GM07737-14

652.6

PROLONGED DEPOLARIZATION OF CEREBELLAR PURKINJE CELLS FOLLOWING PERIPHERAL STIMULATON: CONVERGENCE AND TIMING OF INPUT FROM BRAIN STEM AND CEREBRAL CORTEX.
D. Jaeger* and J.M. Bower. Div. of Biology 216-76, California Institute of Technology, Pasadena, CA 91125

Previously we showed that Purkinje cells react to brief excitatory input with prolonged depolarizations and increases in spike rate both *in vitro* (Jaeger and Bower, Soc. Neurosci. Abstr. 17, 552.10) and *in vivo* (Jaeger and Bower, Soc. Neurosci. Abstr. 18, 438.12). In the present study we investigated how this intrinsic property of Purkinje cells contributes to the integration of input from brain stem and cerebral cortex. This question was addressed by *in vivo* intracellular recordings from Purkinje cells of rat crus IIa, which has a known fractured somatotopic representation of perioral areas. Cerebral cortical input to crus IIA following peripheral stimulation was transiently suppressed by lidocaine injection into S1, or permanently by decerebration.

The results show that a burst of brain stem input following peripheral stimulation can trigger prolonged depolarizations in Purkinje cells located in a small region directly overlying the matching granule cell receptive field. This activation is enhanced by a subsequent burst of cortical input present in most trials with intact cortex. Therefore, the prolonged activation following brain stem input leads to an interaction of brain stem input with temporally non-overlapping cortical inputs. Strong IPSP's, likely derived from basket cells, were often seen to transiently interrupt prolonged depolarizations, or to abolish them completely. We suspect that this modulation of prolonged activation by inhibitory interneurons plays an important functional role in creating a pattern of interaction between input from brain stem and cerebral cortex.

Supported by a Del Webb postdoctoral fellowship and NINDS - NS31378

652.7

INTERACTIONS BETWEEN PARALLEL AND ASCENDING FIBER INPUTS IN A PURKINJE CELL MODEL.
E. De Schutter* and J.M. Bower.
Div. of Biology 216-76, Caltech, Pasadena, CA 91125.

We have constructed a detailed compartmental model of a Purkinje cell, based upon anatomical and physiological data. We used this model to explore the response to synchronous synaptic inputs, expected to result from activation of synapses from the ascending branch of the granule cell axon, during continuous background asynchronous inputs from the parallel fibers.

We have previously shown that dendritic P-type Ca^{2+} channels amplify synaptic inputs, so that the somatic response is independent of the dendritic location of synchronous inputs. This response depended strongly on the preceding pattern of asynchronous inputs. The amplitude of somatic EPSPs to identical synchronous stimuli had an average standard deviation of 52% and sometimes there was no response at all. This modulation of EPSP size by parallel fibers was independent of the location of the synchronous inputs. For identical patterns of random inputs, the size of EPSPs generated from different synchronous input locations was correlated by 78 to 98%. The modulation effect was also prolonged, i.e. a particular pattern of asynchronous inputs affected EPSP amplitudes for inputs up to 20 ms afterwards. The responsiveness of the Purkinje cell dendrite to small synchronous stimuli thus seemed to be controlled globally over time periods of at least several ms by the parallel fiber inputs. This responsiveness was determined by the voltage-dependent activation of the P-type Ca^{2+} channel and the Ca^{2+}-activation of Ca^{2+}-activated K^+ channels in the model.

Supported by NINDS, NS31378 and NSF, BIR-9017153.

652.8

PATTERNS OF CEREBELLAR PURKINJE CELL ACTIVATION TO MULTIMODAL SENSORY INPUTS.
L.J. Larson-Prior*, Penn State College of Medicine, M.S. Hershey Medical Center, Hershey PA 17033.

The cerebellar cortex is known to be a site of sensory-to-motor signal transduction. Incoming sensory signals from all modalities are carried by mossy fiber inputs to the cortex while the output neurons, the Purkinje cells, show discharge patterns which are correlated to movement features. Thus, the cerebellar Purkinje cell has been assumed to be the final site of integration and transformation of incoming sensory signals. In order to perform this task, Purkinje cells must receive multimodal inputs. To examine the ability of incoming sensory information to access specific subsets of Purkinje cells, the activity dependent marker, sulforhodamine 101 (Keifer *et al., J. Neurosci.* 12:3187,1992) was applied to an *in vitro* turtle brainstem-cerebellar preparation during stimulation of either the spinocerebellar tract, the vestibular nuclear complex, or the anterior division of the vestibular nerve. In this preparation, the inferior olivary nucleus was removed in order to prevent activation of the climbing fiber system.

Stimulation of each of these sites resulted in a circumscribed pattern of Purkinje cell activation. No Pukinje cells in the caudal 25% of the cerebellar cortex were activated by stimulation of any of these sensory systems. In response to stimulation of the vestibular system, by far the greatest activity was observed in the most rostral 14% of the cerbellum. Spinocerebellar mossy fiber inputs activated two populations of Purkinje cells: one overlapping those in the rostral cortex which were activated with vestibular inputs, and the second in the intermediate zone of the middle 20% of the cortex. Thus, Purkinje cells show a pattern of activation that is dependent upon incoming sensory signals. Supported by NS 30759.

652.9

SPECIFIC RESPONSES OF RAT CEREBELLAR CORTEX TO MOVING INPUT: DEPENDENCE ON DISTANCE COVERED BY THE MOVEMENT. D. Heck and V. Braitenberg.* Max-Planck-Institute for Biological Cybernetics, 72076 Tuebingen, FRG.

It has been suggested that the anatomical arrangement of parallel fibers and Purkinje cells enables the cerebellar cortex to act as a movement detector (1). This idea received strong support from experimental data (2). In those experiments a multi-electrode array (consisting of 11 tungsten stimulating electrodes regularly placed at distances of about 140 μm) was inserted into the granular layer of horizontally cut slices of rat cerebellar cortex. The mass parallel fiber activity was recorded from the molecular layer. A "moving" stimulus was produced by switching the current in an orderly fashion from one electrode to the next (e.g. 1,2,3,...,11). We found that amplitudes of parallel fiber responses depend on direction and velocity of the "movement" in the way predicted. The largest amplitudes were found when the stimuli "moved" towards the recording site with velocities being in the range of the parallel fiber conductance velocity.

Using the same experimental setup as described above we investigated the dependence of the response amplitudes on the distance covered by the "movement" (number of neighbouring electrodes used to produce a "moving" stimulus). Preliminary results show that the amplitudes become larger when the movement distance is increased, e.g. responses to stimulation sequence 4,3,2,1 were larger than those to sequence 3,2,1. Surprisingly, saturation often occurred already when the distances covered by the "movement" were even shorter than 0.5 mm.

(1) Braitenberg,V., J.Theoret.Neurobiol. 2 (1983) 237-241; Braitenberg,V., in: Cerebellum and Neuronal Plasticity, Glickstein et al. (Eds.), Plenum (1987) 193-207

(2) Heck,D., Neurosci.Lett. (in press)

652.10

DISTRIBUTION OF VARICOSITIES ALONG CEREBELLAR PARALLEL FIBERS IN THE CAT POSTERIOR VERMIS. C.Huang* and R.Huang. Univ. Missouri-Kansas City, Kansas City, MO 64110.

A point-to-point but fractured somatotopic representation has been found in the granule cell layer of the posterior cerebellum in the rat and the cat. However, the functional consequence of this precise somatotopy is uncertain considering the orthogonal relationship between the parallel fibers, which are the axons of the granule cells, and the Purkinje cells, which are the output neurons of the cerebellar cortex. Could Purkinje cells retain response specificity when each Purkinje cell is innervated by 80,000 parallel fibers? In this preliminary morphometric analysis, we made the assumption that varicosities along the parallel fibers are the primary sites of parallel-fiber-to-Purkinje-cell synapses. We examined the distribution of varicosities along 300 HRP-labeled parallel fibers in a restricted region (500X500μ) in the posterior vermis of the cat with light microscopy. (1) Varicosities were classified as either large or small, (2) the number of small varicosities in a given axon appeared to be Gaussian, and (3) there was a bimodal pattern in the number of the large varicosities and the total number of varicosities among parallel fibers. Hence, different parallel fibers may deliver information differently along their length, thereby generating zones of Purkinje cells with unique response characteristics. (supported by UMKC FRG)

652.11

AUTOMATED SEGMENTATION OF CEREBELLAR PURKINJE CELL DENDRITES. FW Prior, RM Bushey, RJ Milner* and LJ Larson-Prior. Penn State College of Med, MS Hershey Med Center, Hershey, PA 17033 USA

Computational models of single cell response properties require analysis of the diameter distribution and branch patterns of dendritic arbors. As a precursor to automated compartmentalization, an algorithm has been developed that extracts this information from high resolution confocal micrograms.

A Zeiss Laser Scan Confocal Microscope was used to acquire 512 x 512 pixel images from 50 m sections of turtle cerebellar Purkinje cells which had been intracellularly filled with Biocytin (Sigma). Images were transferred to a Sun workstation and processed to accentuate the boundary between the dendrite and the extracellular matrix. The coordinates of the origin of the dendritic tree were determined visually and input as the starting point for the segmentation algorithm. The center of the primary dendrite was determined by scanning the indicated image row. An iterative search technique was used to determine the minimum diameter of the dendrite at the center point. This diameter(D) was stored and the center of action moved a distance ΔL along a line perpendicular to the diameter at the center point. The algorithm was repeated at each new center of action until the cell boundary was encountered. A threshold was applied to the resulting function D(L) to locate spikes of amplitude $\geq \varepsilon$ and the coordinates of each spike used as a new origin. Each potential branch was explored by recursive application of the algorithm. The parameters ΔL, ε and δ (the cell boundary search limit) were determined empirically. Output data was stored in a linked list data structure to facilitate future processing. Supported by NS30759.

652.12

INVOLVEMENT OF INSULIN-LIKE GROWTH FACTOR-I (IGF-I) IN CEREBELLAR LONG-TERM DEPRESSION OF GLUTAMATE INDUCED GABA RELEASE. **M.A. Castro-Alamancos and I. Torres-Aleman (SPON: European Brain and Behaviour Society). Cajal Institute, CSIC. Avda. Dr. Arce 37, 28002-Madrid. Spain.**

The Purkinje cell is a target for the trophic effects of IGF-I during development. Thus, we became interested in the possibility that IGF-I could act as a neuromodulator in the adult cerebellar cortex. By using microdialysis techniques we fist determined that IGF-I is released in the cerebellar cortex during electrical stimulation of the inferior olive. Using a microdialysis probe implanted into the cerebellar cortex and deep cerebellar nuclei we further determined that infusion of glutamate induces the release of gamma-aminobutyric acid (GABA). Moreover, the conjoint infusion of glutamate with IGF-I induced a depression in the release of GABA, and to subsequent pulses of glutamate alone, while the separate application of glutamate and IGF-I did not. Furthermore, the release of GABA by KCl was not affected by IGF-I, nor was glutamate-induced GABA release affected by another cerebellar trophic factor (bFGF). Furthermore, conjoint electrical stimulation of the inferior olive and infusion of glutamate in cerebellum produced a depression in the subsequent release of GABA by glutamate pulses in a manner that resembled the conjoint application of IGF-I and glutamate. Recent results indicate that this IGF-I induced depresion of GABA release is not mediated through the activation of the metabotropic receptor by glutamate. In conclusion, these findings indicate that IGF-I may be involved in the long-term depression of the parallel-fiber-Purkinje cell synapse efficacy which has been described electrophysiologically in the cerebellar cortex, and which is believed to mediate motor learning processes.

652.13

MUSCARINIC AGONISTS MODULATE PURKINJE CELL RESPONSES TO GLUTAMATE AND GABA. Q. Pompeiano*, P. Andre, and S.R. White. Dept. of Physiol., Univ. of Pisa, Pisa, Italy and Dept. of VCAPP, Washington State Univ., Pullman, WA, USA 99164.

The cerebellar cortex contains diffusely distributed cholinergic fibers and both muscarinic and nicotinic receptors. The present study used microiontophoretic techniques to examine the effects of the muscarinic agonist bethanechol (Beth) on Purkinje cell (PC) excitability in urethane-anesthetized Sprague-Dawley rats. The effects of Beth (10-60 nA for 300 sec) on the spontaneous firing rates of PC were compared to effects on responses of PC to the excitatory transmitter glutamate and the inhibitory transmitter GABA. Beth increased the glutamate responses of 22/33 PC regardless of whether it increased (4 cells), decreased (3 cells) failed to alter (10 cells) or had a bimodal effect (5 cells) on the basal firing rate of the cells. In other experiments, Beth increased the GABA-induced inhibition of 22/25 PC. The facilitatory effects of Beth on the PC responses to glutamate or GABA lasted 15-30 minutes after the offset of the muscarinic agonist and were prevented by simultaneous application of the selective antagonist scopolamine. Thus the cholinergic muscarinic system appears to exert a prominent modulatory effect on PC activity by increasing responses to both excitatory and inhibitory neurotransmitters.

652.14

EFFECTS OF SUPERSMALL DOSES OF ETHANOL ON THE ACTIVITY OF CEREBELLAR PURKINJE CELLS IN RATS. R.A.Grigorian* G.Zernova, A.D.Khorkov. Sechenov Institute, 194223,St.Petersburg,Russia.

In the experiments on adult Wistar rats the effects of intravenous injections (for 1 min) 0.1, 0.25, 1.0 g/kg of ethanol on Purkinje cell (PC) discharges were studied. The ethanol level in blood was determined using gas chromatography method. The next statistical parameters were studied: mean interspike interval (ISI) of PC discharge, standard deviation (SD), current frequency of PC discharges. The mean ISI of spontaneously active PC fluctuated from 9.77+0.1 up to 14.+ 9.07 ms. However, for 70% of PC it was in range 20-45 ms. This means that a mean discharge frequency of PC was within 50-20 imp/s respectively. After injection of 0.1-0.5 g/kg ethanol the frequency of PC discharges did not changed. At the same time, regularity of PC discharge sharply increased and SD was 1.5-2.0 times lower than in spontaneously active PC before the ethanol injection. It should be pointed out that unlike the moderate doses (over 1.0g/kg), the doses of ethanol lower than 1.0g/kg usually do not increased the rate of PC activity. In conclusion, the main effect of supersmall doses of ethanol is an increase of the excess (mode) of PC discharges, but not its frequency.

652.15

IPSPs IN RAT DEEP CEREBELLAR NUCLEI EXHIBIT A POST-TETANIC LONG-TERM DEPRESSION. W. Morishita* and B. R. Sastry. Neuroscience Research Laboratory, Dept. of Pharmacology & Therapeutics, The University of British Columbia, Vancouver, Canada, V6T 1Z3.

Repetitive activation of certain synapses in the CNS produces long-term changes in synaptic efficacy. In the cerebellar cortex this is characterized by a long-term depression of excitatory synaptic transmission. We report that synaptic plasticity can also occur following repetitive activation of the cerebellar Purkinje axon-deep nuclear cell synapses. Intracellular recordings were obtained from the deep nuclei in cerebellar slices. Stimulation of the white matter produced IPSPs that reversed around a membrane potential of -75 mV and were blocked by 50 µM picrotoxinin. Paired-pulse depression of the IPSPs was observed over inter-pulse intervals of 100-400 ms with maximal suppression occurring around 100 ms. When the stimulation frequency was changed from 0.033 to 0.2 or 5 Hz the amplitude of the IPSP was decreased. Recovery from the depression occurred 3-5 min after the stimulation frequency was returned to 0.033 Hz (IPSP 1 min after a tetanus [5 Hz, 1 s] as a % of control: 58.6 ± 10.3%; n=5 neurons). When the afferents were tetanized at 100 Hz for 1 s, a post-tetanic long-term depression (LTD) of the IPSPs occurred (IPSP 30 min post-tetanus as a % of control: 53.7 ± 3.4%; n=7 neurons). No changes in input resistance, resting membrane potential or the reversal potential for the IPSP were observed during LTD.

These results suggest that deep nuclear IPSPs are suppressed by repeated activation. In addition, LTD can be induced when the IPSPs are tetanically stimulated. Supported by a grant from the NIH (NS 30959) to B. R. S. and a Ciba-Geigy/MRC studentship to W. M.

652.16

RETROGRADE TRANSNEURONAL TRANSPORT OF HSV-1 FROM PRIMARY MOTOR CORTEX TO CEREBELLAR DEEP NUCLEI AND PURKINJE CELLS. J.E. Hoover[1]* and P.L. Strick[2]. Dept. of Biology, Millersville Univ., Millersville, PA 17551[1]; Research Service, VAMC and Depts. of Neurosurgery & Physiology, SUNY-HSC, Syracuse, NY 13210[2].

We injected the McIntyre-B strain of herpes simplex virus type 1 (HSV-1) into the arm area of the primary motor cortex in cebus monkeys (n=7). Animals were allowed to survive for 2-7 days post-inoculation. In animals that survived less than 3 days, virus transport was limited to **first order neurons** in regions known to project to the cortical injection sites. For example, labeled cells were observed in VPLo of the ventrolateral thalamus. **Second order neurons**, labeled by retrograde transneuronal transport, were only observed in animals that survived longer than 4 days. For example, labeled neurons were found in a localized region of the dentate nucleus known to project to VPLo and to be active during arm movements. In animals that survived 7 days, we observed an additional stage of transneuronal transport to **third order neurons** in cerebellar cortex. Patches of labeled Purkinje cells were found at sites in paravermal and lateral cerebellar cortex known to innervate the dentate nucleus. These observations provide further evidence for retrograde *transsynaptic* transport of the McIntyre-B strain of HSV-1 through projection-specific neural circuits. Furthermore, our results indicate that careful adjustment of survival time can be used to reveal different stages in cerebello-thalamocortical circuits.

Support: VA Medical Research Service and USPHS 2957 & 24328 (PLS).

652.17

HIGH-RESOLUTION IMAGING OF ELECTRICAL SIGNAL SPREAD WITHIN THE GRANULE CELL LAYER OF THIN RAT CEREBELLAR SLICES USING A VOLTAGE-SENSITIVE DYE

I. Vranesic*, T. Iijima, M. Ichikawa, G. Matsumoto and T. Knöpfel[1]
Electrotechnical Laboratory, 1-1-4 Umezono, Tsukuba, Ibaraki 305, Japan and [1]Pharmaceuticals Division, R&D, ciba, CH-4002 Basel, Switzerland

Optical recordings using voltage-sensitive dyes provide a tool for monitoring the propagation of electrical signals along the membrane of single cells as well as within neuronal circuits. We used a low-noise imaging system (frame rate 1.7 kHz, 64 x 64 pixels) to record absorption signals in thin slices of rat cerebellum stained with the voltage sensitive dye RH-155.

Brief electrical stimuli applied to the granule cell layer of slices cut in parasagittal planes gave rise to a decrease in light transmission, corresponding to membrane depolarization. From the site of stimulation these optical signals propagated within in the granule cell layer over considerable portions of single folia. The signals were abolished by tetrodotoxin and the spread of excitation in the granule cell layer was abolished in nominally Ca-free medium.

These observations indicate that there are calcium-dependent mechanisms that can mediate a spread of excitation in the granule cell layer over appreciable distances.

Supported by the Human Frontier Science Program (HFSP). I.V. is a recipient of a Science and Technology Agency (STA) Fellowship (Japan).

652.18

NITRIC OXIDE SYNTHASE IS INDUCED IN PURKINJE CELLS AND NEURONS OF THE INFERIOR OLIVE, NUCLEUS X AND EXTERNAL CUNEATE NUCLEUS FOLLOWING CEREBELLAR DAMAGE. D.W. Saxon* and A.J. Beitz. Department of Veterinary PathoBiology, Univ. of Minnesota, St. Paul, MN 55108. Nitric oxide synthase (NOS) has two distinct isoforms based on molecular and immunocytochemical techniques. The constitutive isoform is Ca^{2+} dependent, while the induced isoform is Ca^{2+} independent. NADPH-diaphorase histochemistry identifies both NOS isoforms while antibodies to constitutive neuronal NOS are specific and do not recognize the induced isoform found in non-neuronal cell types. Glass micropipettes were used to make unilateral stereotaxic stab wounds in the cerebellum of 14 rats. A further 10 rats received stereotaxic injections (0.05-1.0 µl) of Fluoro-gold. Following treatments the rats were sacrificed at various time intervals ranging from 6 hours to 21 days. Variable numbers of NOS positive Purkinje cells were initially found in the immediate vicinity of the pipette wound between 72-96 hours. NOS positive Purkinje cells were found to persist in the cerebellum at 21 days and these cells were morphologically similar to those identified at shorter times. Cerebellar afferent cell groups including the contralateral inferior olive and ipsilateral external cuneate nucleus and nucleus X also showed marked increases in the number of NOS positive neurons. In cases where Fluoro-gold was injected some of the NOS neurons in the brainstem nuclei mentioned above contained the retrograde tracer. Immunocytochemical identification of neuronal NOS in adjacent sections to those processed for NADPH-diaphorase revealed Purkinje cells positive for neuronal NOS at the site of damage. In light of this evidence two possibilities exist; 1) Purkinje cells contain an inducible form of neuronal NOS. or 2) neuronal NOS exists in undetectable amounts under normal conditions but can be increased in response to injury. (NIH DA06687 DE06682 and DC01086).

652.19

VINCRISTINE-INDUCED DEGENERATION OF PURKINJE CELLS FOLLOWING SYSTEMIC ADMINISTRATION. G.R. Stewart*, S.M. Little, J.R. Bringas and D.G. Fairchild. Dept. of Neurosciences, Inst. of Pharmacology, Syntex Discovery Research, Palo Alto, CA 94303, USA.

The clinical use of vincristine sulfate (VCR) in the treatment of cancer is often associated with the presence of a mixed sensory and motor neuropathy that is usually attributed to peripheral nerve damage or dysfunction. However, we have observed that intraperitoneal (ip) administration of VCR causes a selective degeneration of Purkinje cells within the cerebellum of mice.

Adult male CD-1 mice were treated ip with VCR (Oncovin®, Lilly, Indianapolis, IN) at doses ranging from 0.0 (Vehicle) to 2.0mg/kg, 2x/wk for 4 weeks. VCR-treated animals displayed abnormal gait, impaired sensorimotor function and decreased locomotor activity. After animals were sacrificed and transcardially perfused with formalin fixative, nervous tissues were processed for paraffin histology. In sections through the cerebellum there was a dose-dependent loss of Purkinje cells ranging from 5 to 80% relative to vehicle-treated animals. Cell loss was primarily restricted to the medial aspects of the anterior lobe. Throughout this region were numerous hyperchromatic cells at the base of the granule cell layer that appeared to be Purkinje cells undergoing degeneration. In contrast, granule cell numbers were not significantly reduced by VCR treatment, nor was there evidence of degeneration elsewhere in the brain. Neuropathy, characterized by vacuolization of axons and/or myelin, was present to only a minimal degree in spinal cord and peripheral nerves (sciatic and brachial plexus).

These studies indicate that VCR can directly cause CNS damage. The selective action of VCR on Purkinje cells in the cerebellum and the clinical significance of this observation remains to be determined.

653.1

NEUROANATOMICAL EVIDENCE FOR GLYCINE AS THE TRANSMITTER OF SACCADIC OMNIPAUSE NEURONS

A.K.E. Horn*, J.A. Büttner-Ennever, P. Wahle, Inst. Neuropathology, LMU Munich, 8 Munich 2; Dept. Zoology & Neurobiology, Ruhr-University Bochum, 4630 Bochum; F.R.G.

Omnipause (OPNs) are considered to participate in the gating of saccadic eye movements. They have been identified cytologically as the nucleus raphe interpositus (rip) in the monkey, and shown to project to the burst neuron area in the paramedian pontine reticular formation (PPRF) and rostral interstitial nucleus of the medial longitudinal fascicle (riMLF), where they are thought to provide a tonic inhibition of the burst neurons. A saccade would be induced by the inhibition of the OPNs, thereby disinhibiting burst neurons. In the present study we used several different neuroanatomical methods to investigate the transmitters of OPNs in the monkey. We found:

1. The somata of OPNs are glycine-immunoreactive, but they are devoid of GABA- and 5-HT-immunostaining.
2. In situ-hybridisation revealed no detectable levels of the GABA-synthetizing enzyme glutamate decarboxylase (GAD) mRNA within OPNs.
3. OPNs were selectively labelled after ^{3}H-glycine injection into the riMLF.
4. The somata and dendrites of putative burst neurons in the riMLF were contacted by numerous glycine-immunoreactive terminal-like puncta.
5. OPN somata and dendrites receive a strong input from glycine- and GABA-positive terminals, whereas glutamate-immunostained terminals are mainly confined to the dendrites. Very few 5-HT- and catecholamine-positive puncta contacted OPNs. Our results provide strong evidence for the fact that OPNs use glycine as an inhibitory transmitter. The results contrast with iontophoretic studies where glycine and GABA had little or no effect on OPN firing rates.

Supported by the DFG in SFB 220/D8 and "Neurovision" Bochum

653.2

SUPRAOCULOMOTOR AREA AND OCULOMOTOR INTERNUCLEAR NEURONS IN THE CAT. Paul J. May*. Departments of Anatomy and Neurology, University of Mississippi Medical Center, Jackson, MS 39216.

The supraoculomotor area has been implicated as the anatomical correlate of the near response region. However, one of its inputs is a conjugate eye movement center, the superior colliculus. A combined light and EM study of the tectal input to this region has been initiated in the cat. Fine, biocytin labelled, tectal axons enter the ipsilateral supraoculomotor area and extend parallel to the oculomotor nucleus border. Small enlargements are scattered along the axon. In addition, numerous terminal puncta are found on single and branched, very thin collaterals that emerge from the main axon at right angles. Some axons extend across the midline and similar, but fewer, labelled axons are observed contralaterally. Ultrastructurally, the biocytin labelled profiles are small (long axis = 0.7-1.2 um), contain spherical vesicles, and make asymmetric synaptic contacts. They terminate along the entire somatodendritic membrane with a distal preference. Preliminary experiments, in which the supraoculomotor area neurons projecting to the abducens and medial pons were retrogradely labelled with WGA-HRP, suggest these cells are not the target of the tectal input. However, these supraoculomotor area cells do receive reciprocal input from the pons; for WGA-HRP labelled terminals containing pleomorphic vesicles contact their somata. These neurons displayed few axosomatic contacts, their distal dendrites were primarily contacted by small profiles with numerous spherical vesicles that made asymmetric synapses, and their proximal dendrites receive multiple short contacts with postjunctional bodies from large, dark-matrix terminals. Cells with this ultrastructure are present bilaterally in the caudal supraoculomotor area with an ipsilateral bias. Within the oculomotor nucleus, however, the labelled neurons are concentrated within the contralateral medial rectus subdivision. This result suggests that the oculomotor internuclear neurons and supraoculomotor area neurons may be two distinct populations. Supported by NEI grant EY07166.

653.3

HETEROGENEOUS CHEMICAL PHENOTYPES IN THE INTERSTITIAL NUCLEUS OF THE MEDIAL LONGITUDINAL FASCICULUS OF THE RAT. X.J. Hu*, W.J. Crossland, and J.A. Rafols, Dept. Anatomy & Cell Biology, Wayne State Univ. Schl. Med., Detroit, MI 48201.

The putative transmitters of the morphologically diverse types of neurons found in the rat interstitial nucleus of Cajal (IC) and the adjacent nucleus of the medial longitudinal fasciculus (IMLFg) are unknown. We used polyclonal antibodies and postembedding immunocytochemical methods at light microscopic levels to detect the presence of GABA-, glutamate (GLU)-, and glycine (GLY)-like immunoreactivity in these nuclei. In addition, a monoclonal antibody for choline acetyltransferase (ChAT) was used on midbrain cross-sections to localize cholinergic neurons. Adjacent 1-2 µm, serial sections were stained for GLU or GABA to show colocalization of amino acids, and with toluidine blue for normal morphology.

Medium and small cell bodies show GLU-like immunoreactivity (GLU+) in both IC and IMLFg, although IC contains greater numbers of reactive neurons. A few, large (37-40 µm) GLU+ cell bodies are also found in IMLFg. Most GABA+ cells in both nuclei are medium to small, the latter having similar features of local circuit neurons. Colocalization of both amino acids appears to be restricted to a few medium to small cells in IC. GLY immunostaining failed to show the presence of GLY+ cell bodies in these nuclei although GLY+ myelinated axons and terminals were found in relation to nonlabeled somata. ChAT+ staining was restricted to dendrites of oculomotor neurons, a few fine caliber axons and terminals, and an occasional cell body in IC.

The findings reveal a variety of chemical phenotypes, some of which can be correlated with the morphological types in these nuclei.

(Supported in part by NIH grant GM08167 to J.A.R.)

653.4

MORPHOLOGICALLY DISTINCT CELL TYPES IN THE RAT INTERSTITIAL NUCLEUS OF CAJAL PROJECT TO NUCLEI OF THE OCULOMOTOR SYSTEM. D. Liu, X.-X. Hu, J. A. Rafols*, and W. J. Crossland, Dept. Anatomy & Cell Biology, Wayne State Univ. Schl. Med., Detroit, MI 48201

The interstitial nucleus of Cajal (IC) is an important center for the control of eye and head movements, however the functional circuitry underlying this control is unknown. We used the Golgi method to determine the morphological types of neurons present in the rat IC and related them to previously characterized neuron types in the primate IC. We also used DiI labeling to identify which morphological types projected to the oculomotor system.

Based on Golgi-stained characteristics of the cell body and dendrites 6 morphological types (I-VI) of neurons were distinguished in the IC and the adjacent midbrain tegmentum (IMLF). Types I-IV in the monkey have been described previously by us (Soc. Neurosci. Abstr. 17: 859). Type V is a large multipolar neuron with long, tapering dendrites found in the lateral IMLF. Type VI has a large multipolar body issuing short, stout, rapidly tapering dendrites. Placements of DiI in the oculomotor and trochlear nuclei revealed that Type Ia, Ib, II, and III project to these centers.

Our results show that the rat IC contains similar morphological types of neurons to those found in the primate, plus at least 2 additional types. Furthermore, at least 3 of these types project to the oculomotor system and are presumed to function in the control of vertical eye movements. The combination of Golgi and DiI techniques will extend data obtained from other techniques in order to build functional circuit models of eye and head movement control.

(Supported in part by NIH grant GM08167 to J.A.R. and by a Summer Fellowship to D.L. from Wayne State Univ. Schl. Med.)

653.5

INTERLAMINAR CONNECTIONS IN THE SUPERIOR COLLICULUS OF THE TREE SHREW. P. Lee and W. C. Hall*. Department of Neurobiology, Duke University, Durham, N. C. 27710

In the tree shrew, the superficial grey layer gives rise to a prominent pathway that terminates in the zone of optic fibers, stratum opticum. We have attempted to identify the cells of origin for this pathway by tracing axons from single cells in the superficial grey layer that were labelled by small extracellular injections of biocytin in living brain slices. Cells located in the deep half of the superficial grey layer have axons that contribute a prominent terminal arbor in the subjacent zone of stratum opticum. These cells have narrow, vertically oriented dendritic fields that ascend through the upper half of the superficial grey layer; they resemble, in soma size and location, the cells that project to the pulvinar in the dorsal thalamus. They may also be the source of an intracollicular pathway to the deep, premotor layers, either by terminating on stratum opticum cells that project in turn to the deeper layers or by contacting the apical dendrites of deep layer cells that ascend to the stratum opticum. Supported by NIH Grant EY08233.

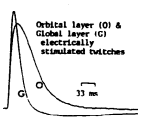
653.6

CONTRACTILE PROPERTIES OF ORBITAL AND GLOBAL LAYERS OF EXTRAOCULAR MUSCLE. J.J. Jacoby* and K. Ko. Dept. of Ophthalmology, NYU Med. Ctr, NY, NY 10016

Extraocular muscles (EOM) are composed of two major layers, the orbital and global, with fiber populations that differ structurally and with respect to patterns of myosin heavy chain expression. The isometric contractile properties of each layer were investigated in vitro. The inferior rectus was dissected from euthanized adult S-D rats and either the global or orbital layer was isolated.

Electrically stimulated orbital strips produced twitches with slower rise and 1/2 relaxation times than global strips (figure), and slower tetanic rise times. Fatigability was similar. 0-Ca^{2+} saline reduced baseline tension in both layers, and dramatically so in orbital strips (1.5 x twitch amplitude).

In high K^+, tonic contractures were generated in orbital strips, although the orbital MIF population expresses fast myosin along part of its length. Supp. by EY06232, Eye Surgery Fund and an unrestricted grant from RPB.



653.7

DIFFERENTIAL TOPOGRAPHY OF THALAMIC PROJECTIONS TO PRIMATE FEF. J.Yin*, M.Schlag-Rey and J.Schlag. Dept. of Anatomy & Cell Biology, UCLA, Los Angeles, Ca. 90024 .

The projections of the arcuate frontal eye field (FEF) to the superior colliculus (SC) have been shown to be topographically organized according to saccade amplitude (Stanton et al., 1988). The intermediate layer of SC projects to the central thalamic region relaying saccade related signals to the FEF. Is there evidence for a topographical organization of the thalamic projections to the FEF regions of large (lFEF) and small (sFEF) eye movements?

WGA-HRP was injected in the sFEF region, electrophysiologically defined, in a trained monkey. The resulting thalamic labeling was compared to that previously obtained by similar methods (Shook et al. '91) after injecting WGA-HRP in the lFEF. The boundaries of nuclei were determined by Nissl staining and AChE histochemistry.

In the 2 cases, the injections yielded dense anterograde and retrograde label in lateral parts of n.medialis dorsalis (MD) and in medial aspects of the ventral anterior complex (VA), bordering n. paracentralis (Pcn). Patches of lFEF and sFEF labeled cells appeared to overlap at some thalamic levels.But, whereas the densest label produced by the lFEF injection was found dorsally, in MDpc, the densest label yielded by the sFEF injection was found more ventrally located,in MDmf.

The dorso-ventral trend in the organization of MD afferents to lFEF and sFEF fits with that of efferents (Stanton et al.,88) and with the observation that electrically evoked saccades progressively decrease in amplitude at successivsly deeper levels in penetrations along MD/Pc border. *(Supported by USPHS grants EY02305 & EY05879)*

653.8

TOPOGRAPHY OF SUPERIOR TEMPORAL SULCUS (STS) AFFERENTS TO FRONTAL EYE FIELD (FEF) IN MACAQUE J.D. Schall*, D.J. King & A. Morel. Dept. Psychology, Vanderbilt University, Nashville, TN 37240

FEF maps saccade amplitude with shorter saccades ventrolateral, and longer saccades, dorsomedial. We investigated the topographic organization of the afferents to FEF from STS in macaque monkeys. Distinct retrograde tracers (FB, DY, FG, WGA-HRP, NY) were injected into medial and lateral FEF; the distributions of labeled cells in the STS were analyzed in two-dimensional reconstructions. The density of afferents to FEF decreased from caudal to rostral STS. Afferents from MT were topographic; the MT representation of the central visual field projected to lateral FEF and the peripheral field representation projected more medially in FEF. Whereas MSTl projected throughout FEF, the densely myelinated MSTd projected predominately to the long saccade representation in medial FEF. Retrogradely labeled neurons in FST, PGa and IPa were commonly intermingled from both medial and lateral FEF injections. Cells in TEO and TEa on the ventral bank were labeled by the lateral FEF injections. Neurons in TPO and TAa on the dorsal bank were labeled by medial FEF injections. Thus, the region of FEF responsible for generating short saccades and pursuit eye movements receives visual motion information representing the central visual, and the longer saccade representation receives motion information from peripheral visual field. Moreover, areas with distinct functional properties project differentially to medial and lateral FEF. Whereas TEO and TEa, which subserve object recognition, project only to the shorter saccade representation in FEF, MSTd which responds to optic flow of the whole field projects to the longer saccade representation in FEF. These findings suggest that qualitatively different visual information is utilized in guiding saccades of different amplitudes. (Supported by R01-EY08890 and the Alfred P. Sloan Foundation.)

OCULOMOTOR SYSTEM: VERTICAL MOVEMENTS, INTEGRATION, TORSION

654.1

RESPECTIVE CONTRIBUTIONS OF THE PREPOSITUS HYPOGLOSSI AND MEDIAL VESTIBULAR NUCLEI TO THE OCULOMOTOR NEURAL INTEGRATOR. P. Mettens*, E. Godaux, G. Cheron and H.L. Galiana. Lab. of Neurophysiology, Univ. of Mons, 7000 Mons, Belgium.

For horizontal eye movements, previous observations led to the hypothesis that the legendary integrator necessary for correct gaze holding and adequate vestibulo-ocular reflex (VOR) was located in the region of the complex formed by the nucleus prepositus hypoglossi (NPH) and the medial vestibular nucleus (MVN). In the present study we studied whether the oculomotor neural integrator was affected by an injection of 0.1-0.2 µl of muscimol into three regions of the brain stem of the alert cat: (1) the NPH, (2) the rostral part of the MVN and (3) the central part of the MVN.

The search coil technique was used to record (1) spontaneous eye movements and (2) the VOR induced by a constant-velocity rotation (50 deg/sec for 40 sec). Recording of the antidromic field potentials evoked by stimulations of the abducens nerve helped to locate the aimed sites.

A leakiness of the neural integrator was assessed by the time constant of an eventual postsaccadic drift and by the time constant given by the transient analysis of the VOR.

Muscimol caused a failure of the oculomotor neural integrator when it was injected into the NPH or into the adjacent central MVN, but not when it was injected into the rostral MVN. Furthermore, a vestibular imbalance at the expense of the injected side also appeared: it was severe after injection into the rostral MVN or the central MVN, but moderate after injection into the NPH.

We conclude that both the NPH and the adjacent central MVN are key sites of the horizontal neural integrator, whereas the rostral MVN is not.

654.2

AN ASYMMETRY ADAPTATION MECHANISM IN THE NEURAL INTEGRATOR OF THE OCULOMOTOR SYSTEM. T. Ogawa* and T. Kasai. Communications Res. Lab., Koganei-shi, Tokyo 184, Japan.

It is thought that some kinds of eye commands are generated as an eye velocity signal and that these commands are integrated mathematically by the neural integrator (NI) to supply an eye position signal to the eye-muscle plant. It is also thought that the horizontal NI consists of the nucleus prepositus hypoglossi and the medial vestibular nucleus, so we have modeled the structure of NI with a neural network based on the anatomical and physiological data of those nucleus. As input signals to NI, we consider an eye velocity signal from the burst neurons and a resting rate signal from the canal. This NI model can integrate only the eye velocity signal without integrating the resting rate signal. To give the proper time constant to NI, we proposed a simple learning rule. The learning is done during saccadic eye movements. The synaptic weights are divided into two types, belonging to the right or the left half-NI, and the synaptic weights of each half-NI are modified uniformly, one being modified more and the other being modified less, according to the postsaccadic retinal slip signal. This learning rule directly uses the retinal slip signal in modifying the synaptic weights, and requires no complex calculations. If saccadic targets are presented at random in the oculomotor range, this learning rule tends to keep the resting eye position at the center of the oculomotor range. We theoretically analyze the proposed NI model and the learning rule and present simulation results. Our adaptation mechanism model can give the NI a sufficiently large time constant and the proper resting eye position using only the retinal slip signal, without complex calculations.

654.3

VERTICAL AND HORIZONTAL COMPONENTS OF VERTICAL PHORIA ADAPTATION COMBINE LINEARLY. J.S Maxwell* AND C.S. Schor. University of California at Berkeley, Berkeley, CA 94720

Phoria is often defined as the relative alignment of the eyes when one eye is occluded and the other is fixating a distant target, i.e., the open loop alignment of the eyes. Phoria adaptation is the process by which alignment of the eyes is maintained despite changes in extraocular muscle stiffness, orbital fascial tissue, etc. In previous experiments we demonstrated that changes in vertical phoria following training to a single disparity presented at a single locus in space spread uniformly to all eye positions. Following training to two disparities of opposite sign presented at two sites along either the primary vertical or primary horizontal meridians, the change in vertical phoria conformed to the demands of the stimulus at the eye positions at which the training was given with a graded spread in between. The change was uniform at eye positions orthogonal to the one containing the training sites. We predicted that phoria adaptation to two different disparities presented at tertiary eye positions would follow the same pattern, conforming to the stimulus demands along a line containing the two sites and spreading uniformly in the orthogonal direction. A 1 deg right hyperdisparity was presented in the upper right visual field and a 1 deg right hypodisparity was presented in the lower left field. The subject alternated between these two stimuli every 10 sec for 40 min after which his phoria was measured at 45 different eye positions. The results were compared to those obtained when the subject trained to two disparities presented on the primary horizontal and vertical meridians. It was found that the response to training at tertiary positions could be predicted by a linear combination of the phoria produced in the horizontal and vertical separation trials. *Supported by EY03532*

654.4

SHORT-LATENCY VESTIBULO-OCULAR REFLEX CANCELLATION IN NORMAL HUMANS. J.L. Johnston* and J.D. Miller. Section of Neurology, Depts. of Medicine, Otolaryngology and Ophthalmology, University of Manitoba, Winnipeg, Canada R3E 0Z3

We measured vestibulo-ocular reflex cancellation (VORc) in normal humans using magnetic search coil oculography and unpredictable head and target accelerations (82-644 deg/s/s). Accelerations occurred while tracking a head-fixed target 1) from 0 velocity onset, or 2) while moving at constant velocity (30 deg/s) or 3) constant acceleration (300 deg/s/s). In some subjects, VORc gains from 0 velocity onset were reduced compared to VOR gains in darkness, when measured over the first 60 ms after head and target motion. Similar reductions in VOR gain occurred in darkness without a target, if the subject was instructed to track an imaginary head-fixed target. In all subjects, VORc gains were reduced at short-latency (<60ms) when accelerations were made during constant velocity rotations and established tracking of the head-fixed target. When the accelerations were superimposed on a constant acceleration, VORc gains were again reduced, even though effective tracking of the target had not been achieved prior to the accelerations. These results suggest that 1) parametric modulation may plan an important role in VOR cancellation from 0 velocity onset; 2) short-latency VOR cancellation occurs in humans when accelerations are made during established eye-head tracking; and 3) retinal slip prior to head and target acceleration may be important in improving initial VOR cancellation.

654.5

MODEL OF THREE DIMENSIONAL VELOCITY-POSITION TRANSFORMATION IN OCULOMOTOR CONTROL. C. Schnabolk* and T. Raphan, Inst. for Neural and Intell. Systems, Department CIS, Brooklyn College of CUNY, 2900 Bedford Avenue, Brooklyn, N. Y, 11210.

We developed a three dimensional dynamical eye movement system model to study velocity-position transformation in oculomotor control. The main point of the model is that the eye muscles generate torque to rotate the eye. When the eye reaches an orientation where the restoring torque of the orbital tissue counterbalances the torque applied by the muscles, a unique equilibrium point is reached. The eye trajectory followed to reach equilibrium, depends on the starting eye orientation and eye velocity. According to Euler's theorem, eye orientation at any instant of time can be represented as an angle of rotation about some axis from a primary position. The restoring torque was approximated as proportional to the product of this angle and a unit vector along this axis. Thus, the dynamical system representing the eye and its orbital tissue gives the torque-orientation transformation associated with eye rotations from the primary position. *The velocity-position integrator can be modelled as a dynamical system such that a linear combination of the integrator state and a direct pathway signal is converted to a torque signal that activates the muscles to rotate the eyes.* Because torque signals are vectors, they commute. Thus, our model indicates that the signals in the central nervous system (CNS) can be treated as vectors. The non-vector orientation properties of the eye result from the dynamic torque-orientation properties of the globe and its underlying tissue. Listing's law is explained by our model as being a property of the vector nature of the signals in the CNS driving the eyes, and not localized to any specific location within the CNS. If a neural vector command is confined to Listing's plane, then eye orientation will obey Listing's law. Model simulations were run to show that Listing's law is obeyed for both saccades and smooth pursuit eye movements, consistent with experimental findings.

Supported by NIH grant EY04148 and PSC-CUNY award #663291.

654.6

EFFERENT NEURONS IN THE INTERSTITIAL NUCLEUS OF CAJAL (INC) MEDIATING VERTICAL EYE POSITION SIGNALS. Y. Iwamoto*, S. Chimoto, E. Nambu, K. Yoshida Dpt. Physiology, Inst. Basic Med. Sci., Univ. of Tsukuba, Tsukuba, Ibaraki, 305 Japan

The behavior, projection and vestibular response of vertical eye position-related INC neurons were studied in alert cats. Efferent neurons were identified by their antidromic response to stimulation of the ipsilateral (i-) vestibular nucleus (VN) and/or contralateral (c-) INC. Thirty of 102 downward-on neurons were antidromically activated from i-VN. These neurons received disynaptic excitation from c-VIIIth nerve and inhibition from i-VIIIth nerve. Their firing rate was best modulated for rotation in c-posterior canal (PC) plane, increasing in the nose-up direction. Fifteen of 39 downward-on neurons examined were antidromically activated from c-INC. These neurons were also modulated by rotation in c-PC plane but received very weak, if any, short-latency effects from VIIIth nerves. No neurons were activated from both i-VN and c-INC. Eight of 17 upward-on neurons tested showed antidromic responses from c-INC. Some of these upward-on neurons were disynaptically excited from c-VIIIth nerve. None of 50 upward-on neurons tested were activated from i-VN. For 11 downward-on neurons with antidromic activation from i-VN, depth profiles of threshold were obtained by tracking in VN. Double low-threshold peaks and variation in latency were observed for 8 neurons, suggesting the existence of branches. Results show that vertical eye position signals are sent from INC to both i-VN and c-INC and that different groups of neurons contribute to these two efferent pathways.

654.7

TORSIONAL EYE POSITION AND MOVEMENTS AFTER LESION OF THE ROSTRAL INTERSTITIAL NUCLEUS OF THE MLF.

Y Suzuki*, D Straumann, BJM Hess, V Henn. Neurology Department, University Hospital, CH-8091 Zürich, Switzerland.

The rostral interstitial nucleus of the MLF (riMLF) contains premotor burst neurons for torsional and vertical rapid eye movements. In three rhesus monkeys, we have investigated the effects of unilateral and bilateral riMLF kainic acid lesions (0.3-1.2μl, 8-16μg/μl) during spontaneous eye movements in upright and in different static roll positions which induce ocular counter-rolling.

After a unilateral lesion, all monkeys showed an increased thickness of Listing's plane together with a shift to the counter-torsional direction, e.g., in a positive direction after a left-sided lesion. The torsional shift in the light was 8.2 to 16.2 deg, but always less in the dark. After the second lesion was placed on the other side, Listing's plane displaced to the opposite torsional direction. On average, the residual torsional shift reduced to 22% of the shift after the first lesion, and the thickness of Listing's plane decreased to the level of the pre-lesion state. Counter-rolling in response to static tilt was well preserved after bilateral lesions.

The results suggest that symmetrical activity of burst neurons in the riMLF is essential to keep the torsional component of eye movement around zero and to minimize its scatter. Yet, after bilateral riMLF lesions, Listing's law is still preserved, suggesting its neural implementation downstream of the riMLF.

Supported by Swiss National Foundation 3100-28008.89

654.8

CHARACTERISTICS OF RAPID EYE MOVEMENTS AFTER LESIONS OF THE ROSTRAL INTERSTITIAL NUCLEUS OF THE MLF IN THE RHESUS MONKEY.

V Henn*, Y Suzuki, D Straumann. Neurology Department, University Hospital, CH-8091 Zürich, Switzerland.

The rostral interstitial nucleus of the MLF (riMLF) contains premotor burst neurons for torsional and vertical rapid eye movements. In four rhesus monkeys, we have investigated the dynamics of saccades after unilateral and bilateral riMLF kainic acid lesions. Fast phases of nystagmus were collected during animal oscillation in the light (+/-10deg 0.1-0.8Hz, +/-40deg 0.2Hz).

After bilateral lesions, all torsional and vertical rapid eye movements were permanently lost. After unilateral lesions, torsional rapid movements were lost to one side only, while vertical movements were still possible. The velocity and amplitudes of downward movements were decreased in all four monkeys. Upward rapid eye movements were decreased in amplitude, but velocity was slowed in only two monkeys. The velocity of vertical compensatory movements was not affected.

Output information of riMLF neurons seem to be redundant enough that vertical movements can still be generated, even if about 50% of the burst neurons are inactivated. We speculate that the asymmetry of deficits in vertical rapid eye movements can be attributed to a partially segregated anatomical localization of neurons.

Supported by SNF 3100-28008.89

654.9

Dynamical Properties of Torsional Quick Phase Eye Movements in Rhesus Monkeys

D Straumann*, Y Suzuki, V Henn. Neurology Department, University Hospital, CH-8091 Zürich, Switzerland

In humans and monkeys, eye movements have a position range which is about three times smaller in the torsional than in the horizontal direction. We have investigated whether this anisotropy in range is associated with dynamical differences of rapid eye movements in the torsional and horizontal directions. Rhesus monkeys were given velocity steps about an earth-vertical axis in the dark. Body position was upright for horizontal and supine for torsional nystagmus. Eye movements were recorded with the 3D search coil technique. Quick phases of vestibular nystagmus were collected and sorted according to amplitude. Although torsional quick phases on average had lower amplitudes than horizontal quick phases, a considerable number of both horizontal and torsional movements were obtained in the 10-15 deg bin and further analyzed: Torsional quick phases generally showed a higher peak velocity and a greater peak/mean velocity ratio than horizontal quick phases, reflecting differences in the shape of velocity profiles. We hypothesize that these dynamical differences could be a consequence of different mechanical properties of the eye plant for horizontal and torsional movements.

Supported by SNF 3100-28008.89.

654.10

LESION OF THE CEREBELLAR NODULUS AND THE VENTRAL UVULA MODIFY THREE-DIMENSIONAL VESTIBULO-OCULAR AND OPTOKINETIC REFLEX

B.J.M. Hess* and D.E. Angelaki, Neurology Department, University Hospital CH-8091 Zürich, Switzerland

3D eye movements were studied following lesions of the nodulus and ventral uvula in rhesus monkeys. A spontaneous horizontal and vertical nystagmus, present acutely after the lesion, recovered largely within the first 4 weeks. Abnormal torsional eye movements persisted, and a torsional pendular nystagmus developed in light and dark consisting of slow phase oscillations of about ± 5° at about 0.05 Hz. Torsional pendular nystagmus was suppressed in ear-down but not in prone or supine positions. The vestibulo-ocular reflex (VOR) and the optokinetic nystagmus (OKN) were altered as follows: (i) The dynamics of the horizonal VOR became underdamped, exhibiting periodic alternating nystagmus with period of more than one minute during the per- and postrotatory response phase as well as during optokinetic afternystagmus. (ii) The time constant of torsional VOR decreased to a value similar to that characterizing the semicircular canal afferent responses. No torsional OKN and OKAN could be elicited. (iii) During constant velocity off-vertical axis rotation, there was no steady-state reponse component suggesting a loss of the ability to detect head angular velocity from dynamic otolith information. (iv) Transformation of postrotatory eye velocity from head- to gravity-centred coordinates was abolished following passive head tilts. The results suggest that the cerebellar nodulus and ventral uvula play a crucial role in processing otolith information and in the proper function of the velocity storage network.

Supported by the SNF 31-32484.91 and the NIH F32 DC-00092.

655.1

GENERALIZATION OF VISUO-MOTOR LEARNING. H. Imamizu*, Y. Uno and M. Kawato ATR Human Information Processing Research Labs, Kyoto, Japan.

Some recent computational studies propose that the biological motor system acquires internal models of the inverse kinematics and/or inverse dynamics transformations by learning (Kawato & Gomi, 1992, *TINS*, 15, 445-453). Computationally, internal models can be characterized by two alternative representations, i.e., parametric and tabular (Atkeson, 1989, *Ann. Rev. Neurosci*, 12, 157-83). Tabular models do not need prior knowledge about the structure of the musclo-skeletal system but it lacks the capability of generalizing learned movements. Parametric models can generalize movements but requires the analytical description of the system. To investigate which type of representation the human motor system might use, we examined spatio-temporal characteristics of aiming behavior of normal human subjects at various target locations before and after excessive training at one fixed location. A marker was attached to the subject's hand and its current position was displayed as a cursor on the CRT screen. Subjects moved their hands within a horizontal plane so as to move the cursor from the starting position to the target. The visual feedback displayed on the CRT was rotated by 75 deg. around the center of the screen and the subjects learned how to achieve the target under this rotated condition. The effect of learning was most prominent when the subjects aimed at the target used during the training session but it was also obtained when they aimed at different targets not used during the training session. These results suggest that the internal representation corresponding to the inverse kinematics is basically parametirc, but not so completely as represented by mathematical equations.

655.2

SEQUENTIAL MOVEMENT PATTERNS DURING THE LEARNING OF NOVEL TYPING RESPONSES. A.M. Gordon', A. Casabona, and J.F. Soechting. Dept. of Physiology, University of Minnesota, Minneapolis, MN 55455 and Inst. of Human Physiology, University of Catania, Italy

We previously have shown that typing movements normally involve characteristic patterns of movement for each keystroke (flexion-extension and rotation in the horizontal plane) and that sequences of key presses entailing both hands occur in parallel (i.e., the movements of each hand overlap). The present study characterizes the execution of a novel bimanual sequence of movements (typing) and the changes that occur during learning. Pairs of letters on the keyboard were transposed (e.g., the letters 'n' and 'u,' which are normally typed with the right index finger) while the time of each keypress and the kinematics of the hands and fingers were recorded. Skilled typists typed words in which all of the letters required the use of only one hand, except the transposed letter, which required the use of the other (e.g., 'sadness'). Temporal delays were seen for words containing transposed keys immediately before the first letter of the word and directly before and after the transposed key compared to words without transposed keys. Initially the movements were highly sequential, with initiation of the keystroke to the transposed key beginning only after completion of the previous keystroke with the other hand. However, once the movement to the transposed key was initiated, the wrist and finger movement trajectories and the movement durations were in most instances similar to those used for the letter normally corresponding to the given key location. With practice the movement of the two hands began to overlap (i.e., they were performed more in parallel) and the temporal delays decreased. The results suggest that typing movements are organized at both the word and individual keystroke level and that novel bimanual sequences of movement are initially performed sequentially but are performed more in parallel following practice.

655.3

FUNCTIONAL CHANGES WITH STRETCH REFLEX CONDITIONING. S.L. Wolf, R.L. Segal, T.R. Nichols, J.L. Vitek, and R.L. Watts*. Departments of Rehab Medicine, Physiology and Neurology, Emory University School of Medicine, Atlanta, GA 30322

Eighteen normal subjects, were randomly assigned by pairs to receive conditioning designed to reduce the magnitude of the biceps brachii spinal stretch reflex (N=9) or as controls (N=9) who were not provided feedback about reflex responses. All subjects were given 6 baseline sessions followed by an additional 24 training or extended baseline trials. Stretches (250 per session) were delivered through a motor which provided a brief extension torque when specific joint angle and background EMG criteria were met. Measurements of passive joint stiffness and speed of upper extremity, task-specific movements were also made. Training subjects reduced their median biceps reflex sizes by 30% while controls showed virtually no change. Brachioradialis reflex responses covaried with those of biceps for the training group while the antagonist triceps declined by about 40% in both experimental and control subjects. There were no noticeable changes in elbow joint stiffness but speed of upper extremity movements, especially those specifically related to the elbow joint improved. The entire procedure may be "relaxing" to all subjects, but specific training may reduce cocontraction, facilitate the recruitment of elbow extensor motoneurons, or produce greater inhibition of elbow flexor motoneurons, thus enabling faster movements.

This study was supported by NINDS Grant R01 NS28784

655.4

INFLUENCE OF INCREASING THE NUMBER OF UNPREDICTABLE PERTURBATION FEATURES ON EVOKED POSTURAL RESPONSES. B.E.Maki, W.E.McIlroy and J.D.Brooke*. Sunnybrook Health Science Centre, University of Toronto, Toronto, Ontario, CANADA

Prior expectation of perturbation characteristics has the potential to greatly influence evoked postural responses. However, only certain features of perturbations are typically varied (ie. duration, magnitude and directional "polarity"). The present study examines the effect of expectation on postural responses under a wider range of unpredictability, by including: 1) different perturbation waveforms (biphasic vs tri-phasic acceleration) and 2) multiple perturbation directions. Perturbations were applied as horizontal platform translations, and ground reaction forces and EMG responses were compared across trial blocks involving different levels of unpredictability, as determined by the number of perturbation features which were randomized. In the first series, 10 subjects were tested over a range of trial blocks involving various degrees of randomization of perturbation velocity, displacement, duration, directional polarity (forward vs. backward) and waveform (biphasic vs. triphasic). In the second series, multiple horizontal perturbation directions (antero-posterior, medio-lateral and oblique axes) were also tested. In 6 of the 10 subjects, changes in the number of unpredictable features had a significant influence on center-of-pressure (COP) and EMG responses. These effects were most commonly seen as changes in later response characteristics (ie. after peak COP, ~300ms), but the direction and magnitude of change was strongly dependent on the individual subject. The results of our study suggest that contributions of central processes to postural control may be overestimated in experimental paradigms that lack sufficient unpredictability.

655.5

ERROR CORRECTION STRATEGIES: IMPLICATIONS FOR ONE-TRIAL LEARNING. A.G. Feldman*, M.F. Levin, Y. Lamarre. Neurological Research Centre, University of Montreal, Montreal, Quebec. Canada H3C 3J7

The question of how subjects attain the same final joint position under varying load conditions was studied. Subjects trained to make fast wrist flexion movements with no load to a target. They then repeated the same movements (like-training trials) but in random trials (test trials), movements were either opposed or assisted by a load generated by linear position feedback to a torque motor. In two other experiments, subjects initially trained to reach the target with opposing or assisting loads respectively, while on random test trials, the load was not presented. Subjects were instructed to correct errors as soon as possible. In most of the test trials, the wrist arrived initially in a static position outside the target zone and then subjects made corrections by secondary movements. These errors were either positional undershoots or overshoots. EMG signals and movement kinematics were load-dependent. However, these reflex EMG changes did not, in themselves, prevent positional errors. Subjects made errors and corrections not only in the test but also in a significant number of like-training trials. When two test trials occurred in a row, the second movement usually had an error similar to the first or was accurate. The findings were interpreted in the framework of the model showing how control variables for the upcoming movements may be formed based on the previous movement. The model predicts movement errors and may also explain one-trial learning.

655.6

SPEED SCALING AND ADAPTIVE CEREBELLAR CONTROL OF RENSHAW CELL AND MOTONEURON GAIN.
D. Bullock*, J.L. Contreras-Vidal, and S. Grossberg. Cognitive and Neural Systems Department, Boston University, Boston, MA 02215, USA.

Research has established that interpositus-receiving areas of the Red Nucleus often show velocity-like responses during limb movements in well-trained subjects, and that stimulation of rubral sites can facilitate motoneurons while simultaneously inhibiting Renshaw cells. We have conducted large-scale neural network simulations to assess how best to incorporate these data into a comprehensive movement generation model. Specifically, we tested ideas about how cerebellar processing may cooperate with spinal processes to produce desired limb dynamics during rapid voluntary arm movements. Simulation results supported three functional hypotheses: (1) Mossy fiber conduction of movement velocity commands to both cerebellar cortex and related deep nuclear cells would allow generation of phasic deep nuclear outputs whose feedforward action can greatly improve upon spinal feedback control of tracking. (2) Phasic cerebellar excitation of motoneurons must be coupled with phasic inhibition of Renshaw cells to achieve good tracking. (3) Cerebellum mediated learning generalizes better across movement speeds if motoneuron pools obey the size principle. Both monoarticular and biarticular arm simulations are described, and modeled cell activation patterns compare well with activation history recordings from various labs. Simulated motoneuron pool activations also compare well with parametric EMG data. These results show that accurate rapid movements can be explained without recourse to the concept of a virtual trajectory that deviates markedly from the observed trajectory.

655.7

HEIGHT AND SURFACE SPECIFIC ADAPTATIONS WHEN LANDING FROM A JUMP. M.Cincera[1,2], P.McKinley[2]*, R. Rodano[1], & A. Pedotti[1] C. Bioingegneria, I. Politecnico, Milano[1], Italia & McGill University[2], Montreal, Canada H3G 1Y5

EMG, kinematics and kinetics of the landing phase of jump downs were analyzed in two groups (n=4) of adolescent females: trained sprinters, and active, but untrained, using the ELITE motion analysis system and a force plate (Kistler 9261A). Subjects jumped from 2 heights (45 & 60cm) onto both rigid and foam surfaces (n=5/condition). The major findings of this study were that as a group, the subjects trained as runners absorbed more energy at the hip than the untrained, and were highly individualized, but consistent in the pattern of energy absorption across conditions. By contrast, the untrained individuals showed adaptation in their pattern of energy absorption, with increased energy absorption at the hip or decreased ankle absorption when landing on a foam as compared to rigid surface and with increased height. Differences in joint adjustments and Range Of Motion post-landing were parallel kinematic adaptations. Both groups showed kinematic adjustments at the ankle related to surface effects, but little adjustment related to height. Time to stabilization was also significantly less in trained subjects, when landing on a rigid surface. Results are attributed to the way in which sprinters are trained to land and push off almost simultaneously and to the stereotypic way in which they are encouraged to land regardless of external conditions. *supported by NSERC (PMc), Telethon (M Cincera) and Fondazione pro Juventute, Don Gnocchi*

655.8

TRANSFER OF ADAPTATION TO CORIOLIS FORCE PERTURBATIONS FROM THE EXPOSED TO THE NON-EXPOSED ARM. P. DiZio* and J.R. Lackner. Ashton Graybiel Spatial Orientation Laboratory, Brandeis University, Waltham, MA 02254.

When subjects reach forward during body rotation (10 rpm), movement endponts are deviated in the direction of the transient Coriolis forces on the arm. Endpoint accuracy is regained within 40 per-rotation reaches made without visual feedback. Mirror-image trajectory and endpoint errors occur when subjects are again stationary.

We studied whether adaptation to Coriolis force perturbations achieved with the right arm (RA) transfers to the left arm (LA). Subjects (n=10) reached to a midline visual target without ever receiving visual or tactile error feedback about accuracy. The RA endpoint was deviated 25mm rightward initially during body rotation (10 rpm CCW) but returned within 4mm of baseline after 48 reaches. Post-rotation, LA endpoints were significantly deviated 12mm leftward of baseline value but returned to within 5mm after 8 reaches. RA movement endpoints when first measured after 8 post-rotation LA reaches, were deviated 9mm left of pre-rotation baseline, and returned to baseline after 24 reaches.

These results indicate significant intermanual transfer of adaptation to Coriolis forces, and transfer of readaptation. They point to adapted central motor planning partly independent of effector appendage.

Supported by NASA Grant NAG9-515.

655.9

FACTORS CONTRIBUTING TO INITIAL REACHING ERRORS AND ADAPTATION TO CORIOLIS FORCE PERTURBATIONS. J.R. Lackner* and P. DiZio. Ashton Graybiel Spatial Orientation Laboratory, Brandeis University, Waltham, MA 02254.

We studied (1) whether reaching errors made in the center of a rotating room (DiZio & Lackner, 1992. Soc Neurosci Abst, 18(1):516) are caused by Coriolis (F_{Cor}) or centrifugal (F_{cent}) forces and (2) whether information about endpoint errors contributes to adaptation (Lackner & DiZio, 1992. Soc Neurosci Abst, 18(1):515).

We used the DiZio & Lackner, 1992, paradigm but reversed rotation direction. This reversed F_{Cor} but kept F_{cent} constant. Opposite endpoint and trajectory errors resulted, thus F_{Cor} was responsible. F_{Cor} is zero once a movement ends, so the results are inconsistent with current equilibrium point models of movement control.

We used the cited paradigm and allowed subjects (N=8) to correct for any proprioceptively perceived endpoint errors following each reach. Prior to correction, the pattern of F_{Cor}-induced errors, adaptation and aftereffects were quantitatively the same as in our cited work. Corrective attempts were no more frequent while subjects were adapting to rotation or readapting to being stationary again than pre-rotation, and only partially eliminated endpoint errors. The lack of accurate proprioceptive appreciation of endpoint errors means that dynamic information about trajectory errors is essential for adaptation. Supported by NASA Grant NAG 9-515.

655.10

MOTOR LEARNING OF A SEQUENTIAL AIMING TASK IN PARKINSON'S DISEASE. A.L.Smiley-Oyen, C.J.Worringham*, & C.L.Cross. Center for Human Motor Research, University of Michigan, Ann Arbor, MI 48109-2214.

Motor learning in patients with Parkinson's Disease (PD) was studied using a three-dimensional sequential aiming task. Six PD and six healthy age-matched control subjects contacted a series of five targets with a hand-held stylus. The targets ranged in size from 1.5 to 6 cm in diameter and in center-to-center distance from 9.5 to 21 cm. A direction change was required at each target. Response time (reaction time plus movement time) was to be minimized while maintaining a 90% accuracy rate per target. A total of 90 trials were practiced for each of two days. Reaction times, execution times and movement kinematics were measured to determine if learning took place. Results indicated that both groups improved performance with practice. While, in general, the rate of improvement did not differ significantly between groups, specific components of the movement indicated that the improvement in PD relative to control subjects occurred earlier in the practice trials. These results extend earlier findings that PD patients can improve their motor performance with practice.

(Supported by NINDS Grant 1R29 NS27761-01)

655.11

EFFECTS OF CLONIDINE AND YOHIMBINE ON STEPPING IN CHRONIC SPINAL CATS. R. D. de Leon, W. Seto, S. Bautista, A. Soliman, J. A. Hodgson, R. R. Roy, and V. R. Edgerton*, Department of Physiological Science and Brain Research Institute, UCLA, L.A., CA 90024.

The effects of clonidine and yohimbine on stepping in cats spinalized at T12-T13 were studied and compared to pre-spinal stepping. EMG from 3 flexors and 3 extensors were recorded during bipedal stepping before and at various times following spinalization. Three cats were trained to step on a treadmill at various speeds (0.2-1.0 $m \cdot s^{-1}$) and 3 other cats were trained to stand. Each cat was trained for 30 min/day, 5 days/week beginning 1 week after spinalization. Approximately six months later, the training regimens for the two groups were switched. Two of 3 cats initially trained to walk were able to step with full weight support after 3 weeks of training. In contrast, the stand-trained cats were unable to walk throughout the training period. The effect of clonidine (15-75 μg) and yohimbine (0.25-4 mg) on stepping were studied. In two cats undergoing standing training, clonidine enabled stepping (0.2-0.8 $m \cdot s^{-1}$) that resembled pre-spinal stepping, including full-weight support on the plantar surface of the paw. The step-trained cat that was unable to walk 3 weeks after spinalization walked (0.2-0.8 $m \cdot s^{-1}$) after clonidine. Another cat trained to step for 5 months walked more consistently and reached a higher maximum speed (0.8 vs 1.0 $m \cdot s^{-1}$) after clonidine. Clonidine increased extensor and flexor EMG burst durations near to or sometimes greater than pre-spinal durations. Three cats that could perform excellent stepping during the step trained period were unable to step after administration of yohimbine. The stand-trained cats remained unable to walk after yohimbine. These data suggest that at least some of the acquired plasticity in the spinalized, stand- and step-trained cats involved a modulation of noradrenergic dependent spinal networks.

(Supported by NIH Grant NS16333)

655.12

CONTRALATERAL EFFECTS OF CONDITIONING THE BICEPS BRACHII SPINAL STRETCH REFLEX. P.A. Catlin, S.L. Wolf*, L. Gabrielson, T. Kassel, and S. Martin. Div. Physical Therapy, Dept. Rehab. Med, Emory University, Atlanta, GA 30322 Supported by NINDS Grant R01 NS28784.

This study examined the effects of conditioning the biceps brachii spinal stretch reflex (BBSSR) on the contralateral homologous reflex. Ten healthy subjects between the ages of twenty to thirty years participated in this study. The method of conditioning the BBSSR included the use of a torque motor to elicit a stretch from the biceps brachii muscle and feedback to shape the response in the direction of training. Each subject underwent six baseline sessions without feedback. Subjects were randomly assigned to either an uptraining group (N=5) or a downtraining group (N=5). Each subject participated in ten training sessions receiving feedback based on assignment to groups.

The percent change in EMG amplitude between baseline and training sessions was statistically significant for the ipsilateral M_1 response in the uptraining group and the ipsilateral M_2 response in the downtraining group. With the elimination of data from one unsuccessful downtrainer and one unsuccessful downtraining session for another subject, the percent reduction in EMG amplitude of the BBSSR was also significant for the ipsilateral M_1 response. Although statistical significance was not found for the M_1 or M_2 response on the contralateral side for either group, a trend was observed in the appropriate direction for all data according to the training regimen. The results support the concept that uptraining or downtraining of the ipsilateral BBSSR can occur and that contralateral reflex responses show parallel changes of lesser magnitude. Although changes in the contralateral BBSSR responses found in this study were not statistically significance, an actual carry-over effect in the clinical setting or with functional activities among patients with either hypo- or hyperactive reflexes remains to be demonstrated.

655.13

REVERSIBLE MOTOR LEARNING IN A SPATIALLY ROTATED, TWO-DIMENSIONAL REACHING TASK. L. Shen*, G. E. Alexander. Dept. of Neurology, Emory Univ. Sch. Med., Atlanta, GA 30322.

Two macaque monkeys were trained to position a cursor presented on a video display by moving a two-dimensional joystick with the right upper extremity. The subjects were prevented from viewing either the joystick or their limbs; they were given only indirect visual feedback about hand/joystick position via the cursor. On each trial, the subject was required to move the cursor from a starting position, indicated by a fixation target located in the center of the display, to one of four possible peripheral targets located at 0/360°, 90°, 180° or 270°. Once each subject had learned the basic task, the two-dimensional mapping between the joystick and the cursor was rotated by + or - 90°. Each subject was gradually able to adapt to this transformation in the course of a single training session. Prior to the spatial rotation, all hand trajectories had been nearly straight. Immediately after the rotation was imposed, the initial component of each trajectory was the same as it would have been before the transformation; this required a second, corrective component in order for the subject to acquire the target with what then became a curvilinear trajectory. During the process of adaptation, each of the target-directed trajectories became gradually more straightened, with the behavior stabilizing when the trajectories again approached straight lines. Reaction times were unaffected. When the joystick/cursor mapping was rotated back to the original condition, the subjects required the same amount of time to adapt to this as to the 90° transformation. The time course for adaptation remained quite stable across numerous such reversals. The adapted behavior remained stable over several days until the next reversal. The rate of adaptation varied systematically across the four quadrants of the hand's work space, irrespective of the target location or the position of the cursor on the screen. Taken together, these results suggest that the observed adaptation represents a readily reversible form of task-specific motor learning.

655.14

PARTICIPATION OF CHOLINERGIC SYSTEMS OF NEOSTRIATUM AND NUCLEUS ACCUMBENS IN INSTRUMENTAL BEHAVIOUR IN DOGS. K. B. Shapovalova, E. V. Pominova & M. Robinson*. I.P. Pavlov Inst. of Physiol., St. Petersburg, 199034, Russia & Dept. of Physiol. Univ. of Western Ont., London, N6A 5K8, Canada.

The chronic experiments were carried out on seven dogs with defensive instrumental reflex (IR) associated with the maintenance of flexor posture. The influence of carbachol (CAR) microinjections (0.05 to 0.1 μg) in the left and right caudate nucleus head (HNC) and in the nucleus accumbens (NAC) was studied for the occurrence of IR and for behavioral differentiation of acoustical signals under defensive situation. These microinjections showed that the cholinergic systems of HNC and NAC participate in both the motor and sensory mechanisms connected with the occurrence of motor responses to defensive and differentiation signals. This participation has common and specific features. The dorsal striatum (HNC) is structurally involved in the initiation and occurrence of IR. Its cholinergic structures have complex and unequal influences on the components of postural adjustment and instrumental movement. The ventral striatum (NAC) also participated in regulation of voluntary movement, but this influence is mainly excitatory and is of a nonspecific and prolonged character. Activation of cholinergic systems of HNC or NAC leads to "improvement" of signal differentiation due to better sensory attention. In comparison to ventral striatum the participation of cholinergic structures of dorsal striatum is more complicated in this process.

CIRCUITRY AND PATTERN GENERATION IV

656.1

CENTRAL PATTERN GENERATION WITHOUT CENTRAL PATTERN GENERATORS. J.L. Leonard*, Mark O. Hatfield Marine Science Center, Oregon State Univ., Newport, OR 97365.

A major question in neuroethology is whether or not the existence of a FAP/MAP implies the existence of a central pattern generator or whether have suggested behavior patterns can also be "self-organized" i.e, arise through network properties of the nervous system (Mpitsos and Cohan 1986; Mpitsos et al. 1988). Current ideas as to the organization of behavior patterns by neural circuits consisting of unique combinations of neurons are summarized in the "alphabet" model, whereby a neuron may participate in many different behaviors but each behavior can be produced by only one combination of neurons. The "equation model" is a simple, explicit model of the self-organization of stereotyped behavior which makes falsifiable predictions as to anatomical and physiological properties of the system. In this model neurons make quantitative rather than qualitative contributions to the behavior and the same value may be attained by any of several combinations of neurons. This system will produce a set of discrete behaviors (rather than a continuum), if the set of neurons is small, with discrete values, and/or the structure of the periphery is such that only a limited set of behavioral outputs is possible. The peripheral filter might consist of a skeletal system that limits the degrees of freedom of movement of a limb (i.e. hinge joint, etc.) or it might represent the limited range of receptors or ion conductances at muscle. The organization of motor units would, in vertebrates, represent part of the peripheral filter. The values of the neurons may or may not be variable and they may interact according to a variety of rules, "the equation": Inhibitory neurons might (but need not) be included in the network. This "equation" model is conceptually distinct from the "alphabet model" and represents one possible form of "self-organization" of behavior by the nervous system, in that the role of a given neuron in a specific behavior depends on the activity of the other neurons in the network. Supported by NIMH and NSF.

656.2

ATTRACTORS IN NEURAL ACTIVITY AND A CONJECTURE REGARDING THEIR ROLE IN FORMING NEURONAL AND NETWORK ARCHITECTURE. G.J. Mpitsos*, M. O. Hatfield Marine Science Center, Newport, OR 97365.

For a number of years now, we have proposed and examined the ability of networks to sustain attractors. These phase-space structures may help to explain how high-dimensional networks, arising from widely converging and diverging connections, can often exhibit low-dimensional activity. Less persistent "attracting states" may underlie high-dimensional activity (akin to turbulence) representative of motor pattern blending and of one mechanism by which neurons may act variably with one another in contextual groups.

In simulation studies reported here we examine the ability of synaptic strengths (as well as of the firing frequency of neurons that have inputs to the network) to act as bifurcation parameters to cause the network to exhibit multiple patterns of activity.

Additionally, we propose that attractor gradients (basins) and, to a lessor extent, gradients relating to attracting states, act as global organizing forces that set synaptic strengths (and firing thresholds) "optimally" with respect to one another in developing and trainable synapses, and impose a variety of other network characteristic. Thus, the real architect of the structure of individual neurons and of the network as a whole may be the dynamics that a network can increasingly sustain, rather than, for example, whether a neurotransmitter evokes particular electrical changes in postsynaptic cells or whether it effects the movement of calcium ions which in turn affect growth. That is, there may be an inseparable dialectic between dynamical state and the cellular elements that become used by such states. Exclusion of dynamics may lead to misinterpretations of the causative factors that one might attribute to cellular elements examined in isolation.

Ref: Mpitsos, G. J., Soinila, S. (1993) In search of a unifying theory of biological organization: What does the motor system of a sea slug tell us about human motor integration? In *Variability and Motor Control*, (K. M. Newell, and D. Corcos, eds), Champaign: Human Kinetics. 225-290. Supported by AFOSR-92J0140

656.3

GENETIC ALGORITHMS THAT OPTIMIZE MODELS OF RHYTHMIC FEEDING BEHAVIOR IN *APLYSIA*: THE EFFECTS OF VARIATIONS OF THE PARAMETERS. D. Deodhar*, I Kupfermann, S.R. Rosen, and K.R. Weiss. Center for Neurobiology and Behavior, Columbia University, 722 W. 168 St. New York, NY 10032 and Fishberg Center, Mt. Sinai School of Medicine, New York, NY 10029.

We have continued our studies designed to explore how behavior is optimized. We use simple neural models, in which the effective parameters are discovered by genetic algorithms (Goldberg, 1989) that roughly simulate a process of natural evolution. As previously reported, we have attempted to define the simplest model of the process by which food is rhythmically drawn into the buccal cavity and swallowed. The operation was determined using an integrate-and-fire model. As a first approximation, the fitness of our system was defined as the difference between the energy expended by the muscles and the energy gained. The defined systems lack any modulatory mechanisms, and therefore, as predicted, their fitness dramatically fell when the system was challenged by even small variations of the evolved parameters. For example, after a "good" solution was reached, we tested the function of the network when the force of contraction was systematically varied by altering the excitatory drive to the muscle.

656.4

PATTERN RECOGNITION AND GENERATION IN SENSORIMOTOR NEURAL ASSEMBLIES: SYNAPTIC CHANGE AND TEMPORAL DYNAMICS. J. E. Dayhoff* and D. T. Lin. Institute for Systems Research, University of Maryland, College Park, MD 20742.

We have examined alternative models for neural circuits that recognize patterns, generate patterns, and respond to stimuli with dynamic activity traces. We have modeled a neural circuit that recognizes temporal patterns - stimuli that occur over a period of time, such as a spoken word. This model uses time delays on interconnections, and recognizes spatiotemporal stimulus patterns. Training involves adjustment of synaptic weights and time delays, and we propose how intracellular signals could mediate these adjustments. Circuits that generate temporal patterns such as spatiotemporal activity traces or spatial trajectories have also been examined. A neural network model has been trained to produce a spatial trajectory that is pre-chosen, and the network utilizes time delays along interconnections. Another model generates complex spatiotemporal variations in assembly activity stimulated by static patterns as input. This model relies on adaptive thresholds to cause the network to activate in complex ways.

656.5

DYNAMIC BEHAVIOR OF A BINEURAL NETWORK MODEL. Budelli, R.*, Catsigeras, E. and Gómez, L. Sección de Biomatemática, Inst. de Biología, Facultad de Ciencias and Inst. de Matemática, Facultad de Ingeniería, Universidad de la República, Montevideo, Uruguay.

Two synaptically interacting pacemaker neurons were studied mathematically. Each neuron was modelled as a general relaxation oscillator; the synaptic action was simulated as jumps in the postsynaptic membrane potential. The model indicates that the network behaves as a discontinuous nonlinear dynamical system on the torus.

We prove that generic noninhibitory systems necessarily exhibit a periodic behavior, a finite number of limit cycles, and two (at most) binary codes. In the particular case of the leaky integrator model, the number of limit cycles cannot be more than two.

Our study also showed that when both neurons are inhibitory, either the system presents a periodic asymptotic behavior or it has a non trivial compact set attracting all the orbits. For the leaky integrator model, the first behavior occurs for almost any set of parameters.

In addition, our study on the structural stability of these bineural networks indicates that the system may act as a stable memory. Supported by the Universidad de la República and the CCE [contract C.I. 1.10165 U (H)].

656.6

MODELLING CENTRAL PATTERN GENERATION FOR MAMMALIAN LOCOMOTION WITH ARTIFICIAL NEURAL NETWORKS. C.J. Brown, D.J. Kriellaars*, and D. Scuse. Department of Computer Science, University of Manitoba, Winnipeg, Manitoba, Canada, R3T 2N2.

In order to provide further insight into the potential structure of the network of neurons that constitute the spinal central pattern generators (CPG's) involved in locomotor activity in mammals, a developmental model CPG was designed that has half-centre or bipartite characteristics, has distinct frequency and amplitude control, and responds to descending spinal control signals and muscle length afferents. To explore the behaviour of the model and to facilitate future enhancement, the model was implemented in software as a system of interconnected feedforward and recurrent Artificial Neural Networks interacting with another subsystem that simulates a pair of opposing muscles. Data from computer simulations will be presented to demonstrate how the CPG model is able to imitate its biological counterpart while driving and receiving feedback from muscles during "normal" locomotion, while involved in "fictive" locomotion, and while being "entrained" by external manipulation of muscle length.

656.7

LARGE-SCALE SIMULATION OF SPINAL MOTOR CIRCUITS. D. P. Bashor* and G. C. Alig. Dept. of Biology, University of North Carolina at Charlotte, Charlotte, NC 28223.

The dynamics of interacting spinal motor cell populations were investigated with a computer simulation composed of twelve interacting cell populations driven by five or more fiber populations. The simulation was based on R. J. MacGregor's SYSTM11 and SYSTM20 programs (MacGregor, 1987). Two motoneuron populations represented a single agonist and antagonist. The associated interneurons were organized as 5 pairs of populations, including Renshaw cells, IA and IB interneurons, and excitatory and inhibitory interneurons. About 2300 cells (fewer than 400 of them motoneurons) were used, interconnected by about 600,000 terminals. Four types of synapses represented short- and long-time constant excitation and inhibition. Five or more input fiber populations (more than 680 fibers and 130,000 connections) drove the cell populations at selected times and rates. These represented, for example, IA and IB afferent input, and general excitatory drive. Simulation results were dependent on the properties of the interneurons and their connections, since they comprised the majority of cells. The addition of a small fraction of long-time-constant synapses had a profound effect on network dynamics. Their presence dramatically altered the number of terminals in the network required to maintain (or quench) internally-sustained activity, when that was used as an index of effect.

656.8

FREQUENCY CONTROL IN RECIPROCAL INHIBITORY NEURAL NETWORKS. F.K. Skinner*, A.A. Sharp, N. Kopell, and E. Marder. Ctr. for Complex Systems and Dept. of Biology, Brandeis University, Waltham MA 02254, Dept. of Mathematics, Boston University, Boston MA 02215.

Four mechanisms describe oscillatory behaviour that occurs in two cells connected by reciprocal inhibitory synapses: (a) Intrinsic Release (I.R.); inhibited cell released from inhibition by free intrinsically oscillatory cell, (b) Intrinsic Escape (I.E.); intrinsically oscillatory inhibited cell escapes from inhibition, (c) Synaptic Release (S.R.); free cell stops liberating transmitter allowing inhibited cell to be released, (d) Synaptic Escape (S.E.); inhibited cell starts liberating transmitter thus inhibiting free cell. If synaptic activation is described by a step-like function and oscillations are in relaxation mode, then there are well-defined boundaries between the mechanisms as the synaptic threshold changes. Network frequency does not change with synaptic threshold for I.R. and I.E. mechanisms, and increases and decreases with increasing synaptic threshold for S.R. and S.E. mechanisms respectively. Minimum network frequency occurs during intrinsic mechanisms. As oscillations become less relaxation-like and approach sine-waves, the dependence of the network frequency on synaptic threshold for the different mechanisms does not change significantly. As synaptic activation slope decreases, boundaries between the different mechanisms as the synaptic threshold is changed become less distinct. These mechanisms are investigated theoretically with model simulations, and experimentally with the dynamic clamp in stomatogastric neurons. Supported by NIMH MH46742 and NSF BNS9009251.

656.9

A DYNAMIC COMPUTATIONAL MODEL OF THE NEMATODE TAP-WITHDRAWAL REFLEX. B. Hutcheon*[1], C. Roehrig[2], and S. Wicks[1]. [1]Program in Neuroscience and [2]Dept. of Computer Science. University of British Columbia. Vancouver, B.C., Canada.

The nematode worm *C. elegans* exhibits a surprisingly broad behavioural repertoire despite possession of only 302 neurons. Several characteristics of the worm's neurobiology make it a candidate for behavioural based computational modeling: 1) the neurons are simple processes with at most three bifurcations 2) the circuitry is defined accurately to synaptic levels 3) the modeled behaviour can be manipulated by laser ablation of individual neurons which underlie the behaviour.

A computational model of the nematode tap-withdrawal reflex was formulated using simplified biological assumptions. Neurons were assumed to be isopotential and non-spiking. Groups of like synapses with the same pre- and post-synaptic neurons were modeled as a single functional synapse.

Previous reports (Wicks and Rankin, 1992) have defined the circuitry underlying the tap-withdrawal reflex using single-cell laser microsurgery. These ablation results were used to fit the models parameters. A total of seven "lesion states" were tested by optimizing the models free parameters to behavioural data obtained *in vivo*. Because the polarities of the individual connections were unknown, those corresponding model parameters were also exhaustively adjusted and tested against the ablation study data. A close match between the model and behavioural data was obtained.

656.10

A MULTICOMPARTMENTAL MODEL OF HEART INTERNEURONS OF THE LEECH, J.M. Crumb, R.L. Calabrese *, Department of Biology, Emory University, Atlanta, Georgia 30322

In previous studies, the laboratory has used a single-compartment model to reproduce the oscillatory behavior of heart interneurons. The model used soma-based single electrode voltage clamp data on ionic currents and a presynaptic Ca^{2+} synaptic transfer function.

We are interested in Ca^{2+} current distribution throughout the cell. To achieve this, we injected two neurons with Lucifer yellow and mapped their dimensions in unfixed ganglion whole mounts.

Using these maps, we constructed a 60-compartment model. To account for the observed variance in time constant and input resistance, we assumed that microelectrode penetration injury decreased the specific membrane resistance of the soma. Varying the membrane resistance of the soma compartment reproduced the observed distribution in passive properties. We then chose a passive model that best approximates the typical physiological behavior.

Using this passive cell model, we plan to determine the calcium current distribution and amplitude in different compartments of the cell. We will construct a series of models in which the calcium current will vary from evenly distributed to isolated only in the tips of the dendrites. In each model, the calcium currents measured in the soma compartment through a cell-body voltage clamp will approximate voltage clamp data. We will then elicit calcium plateau potentials in each model, and determine which current distribution best fits the electrophysiological data.

At the end of these studies, we should have a model that reproduces the calcium current activity in the cell, and be able to determine the possible magnitude of errors introduced by cell-body recordings.

656.11

DYNAMICAL CHANGES OF FUNCTIONAL CONNECTIVITY IN CULTURED NETWORKS. Guenter W. Gross* and David C. Tam; Center for Network Neuroscience and Department of Biological Sciences, University of North Texas, Denton, TX 76203.

We have studied the dynamical properties of neuronal networks using dissociated embryonic mouse spinal tissue in culture because they form robust monolayer networks displaying complex spontaneous activity patterns, histiotypic responses to pharmacological perturbations, and long-term cell-electrode coupling on photoetched multielectrode surfaces (cf. Gross et al., In: Neurobionics, Bothe and Samii, eds, Elsevier, 1993).

Multichannel data from such cultures were analyzed with conditional probability statistics (Tam et al., 1987, J. Neurosci. Meth.) where interspike intervals of one reference neuron were correlated with the pre-cross intervals from other neurons in the network. The conditional coupling probability statistics revealed highly dynamic interactions within the network, suggesting that the functional connectivity between neurons was not constant. We have observed that: (1) Neurons could switch their functional connections to other neurons dynamically in time. (2) During disinhibition with bicuculline or strychnine, individual neuronal firing patterns remained relatively constant, while coupling among neurons fluctuated. (3) Such fluctuations in conditional coupling probability were swift and were often observed to develop in about one minute. (4) In addition to changes in spatial coupling, the conditional probability plots also revealed how such spatial coupling varied with the temporal burst firing of these neurons.

Supported by the Texas Advanced Technology Program, the Hillcrest Foundation of Dallas, and the Office of Naval Research (to D.C.T.).

656.12

SPIKE TRAIN SERIAL DEPENDENCE, BURST RATE, BURST REGULARITY, AND NETWORK SYNCHRONY ALL INCREASE WITH INCREASING TEMPERATURE IN CULTURED SPINAL CORD NETWORKS. B.K. Rhoades*, J.C.Weil, and G.W. Gross, Department of Biological Sciences and Center for Network Neuroscience, Univ. of North Texas, Denton, TX 76203.

Neuronal networks cultured from embryonic mouse spinal cord tissue display high levels of spontaneous activity, characterized by coordinated network bursting. We have previously shown that the temporal pattern and degree of network synchrony can be systematically influenced by changes in the ionic makeup of the culture medium as well as by pharmacological additions.

Dissociated spinal cord tissue from 14-day mouse embryos was seeded onto multimicroelectrode plates (MMEPs) and cultured for 20-60 days at 38°C, to form monolayer neuronal networks of 50-200 neurons. The MMEP is a glass plate with 64 photoetched microelectrodes in a central $1mm^2$ array, which provides a stable culture substrate for multisite extracellular recording. During each recording session culture temperature was varied between 30-44°C in 2° ± 0.1° increments.

Increasing temperature produced a reduction in the amplitude and a shortening of the repolarization period of action potential profiles. Mean, maximal, and modal spike rates increased with increasing temperature. First-order spike train serial dependence increased with temperature, especially for interspike intervals near the short end of the distribution. Burst rate, burst regularity, and network burst synchrony also increased with increases in bath temperature. Simultaneously elevating both temperature and bath potassium concentration could induce the high-amplitude periodic bursting characteristic of pharmacological network disinhibition. These responses suggest that cultured networks might serve as a useful generic model of febrile seizure onset.

Supported by the Texas Advanced Technology Program and the Hillcrest Foundation of Dallas, Texas.

656.13

Stochastic versus Deterministic Variability in Hippocampal CA1 Population Spikes. P. G. Aitken, T. Sauer and S. J. Schiff*, Dept. of Cell Biology, Duke University Medical Center, Durham, NC 27710.

Reflex variability is well described in the spinal cord monosynaptic reflex. We have studied similar input-output variability in the evoked population spikes generated in rat hippocampal CA1 pyramidal cells by Schaffer collateral stimulation. Stimuli were 100 usec square wave pulses at 0.1 hz at current intensities sufficient to produce half maximal responses. Presynaptic volley and population spike peak to peak amplitude was calculated for each unaveraged event, and voltage determined by measurement of a calibration pulse coupled to each stimulus. Both the population spike amplitude, as well as the input-output transfer function of population spike divided by presynaptic volley, were calculated. Two independent methods were used to test for deterministic structure in these time-series: a nearest neighbor approach (Phys. Rev. Lett. 70; 580-582, 1993), and a discrete adaptation of a local flow approach (Phys. Rev. Lett. 68; 427-430, 1992). For each time-series, a surrogate data set was constructed that was a randomized control data set with the same linear correlation (autocorrelation) as the original experimental series. In addition, a known deterministic chaotic time-series (Henon) was used to confirm the reliability of the 2 algorithms.

Both approaches demonstrated no evidence of determinism in either the population spike or the input-output function time- series. In both cases, the matched surrogate data sets produced results that were identical to the experimental data. In this isolated simple neuronal network, we found no suggestion of chaotic deterministic behavior.

656.14

A NEW POST-CONDITIONAL CORRELATION METHOD FOR EXTRACTING EXCITATION-INHIBITION COUPLING BETWEEN NEURONS. D. C. Tam.* Center for Network Neuroscience and Dept. of Biological Sciences, University of North Texas, Denton, TX 76203.

A new post-conditional correlation statistical method is introduced to extract excitation-inhibition coupling between neurons based on their firing intervals. Since convention cross-correlation spike train analysis methods primarily detect the probability of firing of neurons given that another neuron has fired, the statistic is built on occurrence of spikes which is a suprathreshold event. But it often fails to detect subthreshold events that may contribute to the decrease in probabilities of firing. Subthreshold inhibitory effect may suppress the firing of spikes such that there may not be sufficient statistical samples of suprathreshold events (i.e., spikes) due to the fact that cross-correlation statistic is based on the firing of neurons rather than non-firing of neurons.

A new post-conditional correlation method is developed to detect the probability of firing and non-firing of neurons based on the post-conditional cross-interval after the reference spike has fired. This statistical method is an estimation of the conditional probability of subsequent firing of a spike in a neuron based on the probability of firing of another neuron after the reference spike has occurred. By examining the lag times of post-interspike intervals and post-cross intervals, the excitatory-inhibitory effects on the firing of the reference neuron can be revealed. Inhibitory effects can be uncovered when interspike interval occurrence is limited to a time window where post-cross interval occurrence time either precedes it by a fixed amount or succeeds it. Thus, such post-conditional correlation method can detect the conditions under which the non-occurrence of spikes may occur, rather than the condition under which the occurrence of spike may occur as in conventional cross-correlation. This method is very useful in detecting inhibitory effects as well as excitatory effects of coupling between firings of neurons. (Supported by ONR N00014-93-1-0135)

656.15

IDENTIFICATION OF SPIKE PROFILES USING FUZZY-LOGIC CLASSIFIERS. G. Zouridakis*† and D. C. Tam‡. †Department of Electrical Engineering, University of Houston, Houston, TX 77204-4793, and ‡Center for Network Neuroscience, Department of Biological Sciences, University of North Texas, Denton, TX 76203.

A new procedure for extracting transient signals embedded in continuous waveforms has been developed. Specifically, we formulated a technique to extract multiple spike trains from a single channel of data obtained from biological neurons. The same method can also be used in the study of other electrophysiological signals, such as the EEG and evoked potentials.

The procedure consists of two steps: spike-template creation and individual spike classification. Initially, fuzzy-logic techniques are employed to identify the number of neurons contributing to the extracellularly recorded signal and to create spike templates. In real-time processing of spike patterns, class templates are constructed using a small number of representative spikes which are often contaminated by the presence of noise or other simultaneous events, such as overlapping spikes. Fuzzy classifiers are well suited to deal with such incomplete and/or ambiguous information, because class templates are constructed using all patterns in the data set. In fact, each spike pattern belongs to all possible classes but the degree of membership is different for each class.

Finally, the templates created in the above steps are used to classify individual spikes according to their neurons of origin, utilizing a shift-invariant wavelet transformation. This particular representation has the advantage of both reducing the effect of noise present in the data and correcting the phase of individual components in a waveform.

Results from both simulated and actual recordings show that transient signals hidden in the noise-contaminated original time series can be recovered efficiently. (‡Supported by ONR N00014-93-1-0135)

657.1

THE MUSCULAR HYDROSTATIC STRUCTURE OF THE BUCCAL MASS OF *APLYSIA CALIFORNICA*. Richard F. Drushel,[1] Patrick E. Crago,[2] and Hillel J. Chiel.[1,3]* [1]Departments of Biology, [2]Biomedical Engineering, and [3]Neuroscience, Case Western Reserve University, Cleveland, Ohio 44106 U.S.A.

A *muscular hydrostatic structure* is composed of muscles and other fluid-filled spaces. The constant volume constraint of a muscular hydrostatic structure transforms forces and movements along the line of action of the muscle into forces and movements in other directions. These transformations are performed without a hard skeleton. The buccal mass of the marine slug *Aplysia californica* is a model system for the study of muscular hydrostatic structures. Histological analysis of serial cross sections of the buccal mass reveal a bilateral array of cartilaginous, fluid-filled, closed-ended tubes (termed *bolsters*), parallel to the antero-posterior axis, each array positioned lateral to each radula half. Numerous fibers of the I4 muscle originate on the radula halves and course laterally, crossing perpendicular to the bolster tubes. India ink perfusion of the vascular system shows that the I4 muscle is richly supplied with hemolymph vessels; however, there do not appear to be any direct connections between the vasculature and the bolster tubes. Compression of the closed-ended bolster tubes by I4 muscle contraction could result in both elongation of the radula/odontophore complex (by isovolumetric lengthening of the bolster tubes) and closure of the radula (by pressure against the radula halves). Indeed, since there are no direct transverse muscle connections between the radula halves, the forces involved in radula closure in *Aplysia californica* are best explained in terms of muscular hydrostatic structures. Support: HL-25830-13.

657.2

CHANGES IN BUCCAL MOTOR ACTIVITY DURING RECOVERY FROM BILATERAL CBC CRUSH IN *APLYSIA* M.L. Scott*, Y. Li, and M.D. Kirk, Div. BioL. Sci., Univ. Missouri-Columbia, Columbia, MO 65211

Aplysia consummatory feeding behavior is selectively abolished by bilateral cuts of the cerebral-buccal connectives (CBCs); however, we recently showed that consummatory feeding recovers following bilateral CBC crushes, indicating the potential usefulness of crush lesions in studies of premotor organization and regeneration in the buccal system. Here we compare behavior with buccal motor activity using *in vivo* recordings during recovery from bilateral CBC crushes. Consummatory feeding behavior was tested using bite magnitude (BM), interbite interval (IBI), and interswallow interval (ISI) (Rosen et al., 1989). Motor activity was recorded during recovery and in unlesioned controls with cuff electrodes implanted on buccal peripheral nerve n4 (BN3). Control recordings show rhythmic activity correlated 1:1 with repetitive bites and swallows. After bilateral CBC crushes, BM decreased and IBIs became long and variable. A rhythmic bite criterion was used to evaluate animals during recovery. Animals not meeting criterion showed prolonged bursts of impulses occurring without overt behavior, and occasional bursts associated with infrequent, small magnitude bites. Rhythmic n4 activity could be reliably evoked beginning around day 9, with burst period (BP) and IBI longer than controls. Animals meeting criterion during recovery showed rhythmic n4 activity with bite BP similar to controls, however, bursts were not correlated 1:1 with behavior. ISIs and swallow BPs were normal as soon as they were observed after lesions. Our results suggest that separate subcircuits of the buccal CPG control biting and swallowing, and these subcircuits have different activation pathways. We are currently examining the cellular mechanisms of behavioral recovery *in vitro*. Supported by NIH grant RO1 NS30832 to MDK and Sigma Xi Grant-in-Aid of Research to MLS.

657.3

REGENERATION OF IDENTIFIED BUCCAL MOTONEURONS FOLLOWING PERIPHERAL NERVE LESION IN *APLYSIA CALIFORNICA*. T.L. Ross and M.D. Kirk*, Div. Biol. Sci, Univ. Missouri-Columbia, Columbia, MO 65211.

To study the plasticity of buccal motoneurons (MNs) in *Aplysia*, we studied regeneration following peripheral nerve crush. Buccal nerve 4 (n4 or BN3) was unilaterally crushed, and the animals allowed to recover for 3 to 12 wks. Central and peripheral connections of selected buccal MNs were tested in a semi-intact preparation. For B15, the earliest reinnervation of the I5 muscle (ARC) was observed at 4 wks; EPSP amplitudes at this time were small, and with longer periods of recovery, neuromuscular EPSP amplitudes increased. No inappropriate innervation of target muscles was observed during MN regeneration. The proximal stumps of injured motor axons produced a myriad of fine regenerating processes, or regenerites, which could be followed through the site of injury. Between 30 and 50 regenerites have been counted for a single MN, but we found no evidence for reinnervation of the axon's distal stump. However, our results suggest that distal stumps survive beyond 10 wks and are able to produce large-amplitude synaptic responses in target muscles. In collaboration with Dr. C.K. Govind (Univ. of Toronto, Dept. Zool.), E.M. analysis was used to compare the ultrastructure of crushed nerves following regeneration with contralateral control nerves. Our results may relate to mechanisms underlying learning and memory as well as regeneration, and this system can be used to test hypotheses regarding target specificity, rates of reinnervation, and cellular responses to injury. Supported by NIH grant R01 NS30832 to MDK.

657.4

CANDIDATE COMMAND NEURONS FOR *APLYSIA* SWIMMING ARE TENTATIVELY IDENTIFIED IN CEREBRAL GANGLION. J.E. Blankenship*, P.J. Laurienti and G.N. Gamkrelidze. Marine Biomed. Inst., Univ. Tx. Med. Branch, Galveston, TX 77555.

The marine mollusc *A. brasiliana* swims by rhythmic flapping of its bilateral parapodia. Each pedal ganglion contains motoneurons and serotonergic modulator neurons (POP cells) that innervate the parapodia and fire in phase with parapodial movements, generating a swim motor program (SMP) recorded from peripheral nerves. POP cells and a CPG in each pedal ganglion are driven by cerebral input(s) that initiate and maintain the SMP. A pair of candidate "swim command cells" in the cerebral ganglion have now been identified. Using fluorescence microscopy of fixed frozen sections, these cells were first localized in the C cluster of the cerebral ganglion by retrograde labelling with rhodamine-conjugated latex microspheres injected into pedal ganglia. One cell in each C cluster was found that, when penetrated with microelectrodes, made appropriate direct EPSPs onto POP cells and produced a weak SMP. Further characterization of these neurons will aid in understanding the neural circuitry underlying *Aplysia* locomotion. Supported by NIH, NINDS NS27314 and grant 2503-92 from the John Sealy Memorial Endowment Fund.

657.5

A PAIR OF PEDAL MOTONEURONS INITIATE THE STARTLE PHASE OF ESCAPE SWIMMING IN THE PTEROPOD MOLLUSC CLIONE LIMACINA. R.A. Satterlie* Dept. of Zoology Arizona State Univ. Tempe, AZ 85287-1501.

Clione exhibit three forms of forward locomotion, including slow, fast and escape swimming. Escape swimming includes an initial startle response, in which the wings produce one or two extremely powerful wing cycles, followed by a variable period of fast swimming. A pair of motoneurons has been identified in each pedal ganglion, which appear to be involved in the startle phase of escape behavior. Spike bursts in these cells produce wing contractions that are up to five times larger than the largest contractions recorded during fast swimming. In addition, they have high activation thresholds and receive large inhibitory inputs from wing retraction circuitry. These "startle neurons" do not affect the swim pattern generator or swim motoneurons. Likewise, the swim generating circuitry does not influence the startle neurons. Startle neurons monosynaptically activate both types of swim muscles, fast-twitch fatigable and slow-twitch fatigue-resistant, indicating that the startle response utilizes the same musculature used during slow and fast swimming.

657.6

CO-ACTIVATION OF FUNCTIONALLY ANTAGONISTIC MOTONEURONS UNDERLIES THE HIGH SPEED OF HYDRAULIC EXPANSION OF PREY CAPTURE APPENDAGES IN *CLIONE LIMACINA*. T.P.Norekian* and R.A.Satterlie. Dept.of Zoology, ASU, Tempe, AZ 85287 and Friday Harbor Laboratories, Univ.of Washington, WA 98250.

The predatory pteropod mollusc *Clione limacina* catchs its prey (pteropods of the genus *Limacina*) with specialized oral appendages called buccal cones, which become tentacle-like and grasp the *Limacina* shell. The eversion and elongation of buccal cones is a hydraulic phenomenon and is accomplished by squeezing hemocoelic fluid into the central cavities of the cones. This reaction is remarkably rapid and occurs within 50-70 msec. Two groups of motoneurons have been identified in the cerebral ganglia which underlie functionally opposite movements of buccal cones: extrusion and retraction. The high speed of buccal cone extrusion is presumably achieved through initial co-activation of antagonistic neurons which produces high pressure in the head hemocoel prior to buccal cone extrusion. Sudden inhibition of retractor motoneurons results in a very rapid and powerful inflation of buccal cones. Interneurons which evoke such co-activation are identified in cerebral ganglia.

657.7

INTERJOINT COORDINATION AND MUSCLE ACTIVITY IN LEGS OF DIGGING SAND CRABS. Z. Faulkes* and D. H. Paul. Dept. Biol., U. Victoria, Victoria, B.C., V8W 2Y2. E-mail: "zenf@uvvm.uvic.ca"

Sand crabs use their thoracic legs for digging into the sand. We analyzed digging movements of *Blepharipoda occidentalis* (Albuneidae) by videotaping animals and simultaneously recording electromyograms (EMGs) from their leg muscles. In some cases, EMGs do not completely predict the timing of the final joint movements. E.g., the opener and stretcher muscles move the dactyl and propus, respectively, and their EMGs are exactly synchronous, due to a shared excitatory motor neuron. Although the movements of both joints are temporally encased by the EMGs, the stretcher-generated movement *always* precedes the opener-generated movement, with *no* significant overlap of the two.

Serially homologous legs differ in their interjoint coordination: e.g., the sequences of joint movements in leg 4 differ from those of legs 2 and 3 (the most active digging legs), which are similar to each other. Similarly, muscle activity varies in serial homologues: e.g., leg 1's closer EMG bursts are significantly shorter than the other legs'. These specializations change the movements' final form: e.g., the tip of leg 4, when seen from the side, moves in the opposite direction from 2 and 3 (e.g., when leg 4 moves clockwise, 2 and 3 move counterclockwise).

A related species, *Emerita analoga* (Hippidae), digs much like *Blepharipoda*; the phasing of legs 2 and 3 is the same in both. There is, however, at least one marked difference between them: *Blepharipoda* switches gait during digging (from moving its contralateral legs alternately to synchronously) but *Emerita* does not.

Research supported by NSERC and the Animal Behavior Society.

657.8

REGULATION OF SEGMENTAL SWIM-INITIATING INTERNEURONS BY A PAIR OF IDENTIFIED INTERNEURONS IN THE LEECH HEAD GANGLION. P.D. Brodfuehrer,* A. Burns and M. Berg. Dept. of Biology, Bryn Mawr College, Bryn Mawr, PA 19010.

The motor pattern evoked in a medicinal leech by the same mechanosensory stimulus varies from trial to trial. A large part of this variability stems from neural signals emanating from the head ganglion. With respect to initiation of swimming activity, it is clear that strong excitation of segmental-swim initiating interneurons (cells 204) is necessary. Thus one way that descending signals could bias the isolated nerve cord towards generating swimming in response to a given mechanosensory stimulus is by influencing the level of excitation in cells 204.

In the head ganglion of the leech we have found a pair of interneurons (cell Tr4) that can control the level of excitation in cells 204. Cells Tr4 are located in R2 on the dorsal surface of the subesophageal ganglion and have axons that extend at least until segmental ganglion 14. Brief intracellular depolarization (approx. 1 s) of a single cell Tr4 can, in some trials, trigger swimming activity. More striking, however, is the positive correlation between cell Tr4 spike frequency and cell 204 spike frequency. In fact, the level of excitation in cell 204 can be almost completely accounted for by Tr4's spike activity level. Each cell Tr4 spike matches one-for-one with all EPSPs in cell 204 and hyperpolarizing cell Tr4 eliminates all spiking activity and EPSPs in cell 204. Thus by modulating the spike frequency in cell Tr4 the general level of excitation in the swim generating system will simultaneously be affected and should directly influence the motor response produced in the leech by a given mechanosensory stimulus.

657.9

NON-NMDA ANTAGONISTS AFFECT THE ABILITY OF TRIGGER NEURONS TO EXCITE GATING NEURONS IN THE SWIM MOTOR CIRCUITRY OF THE MEDICINAL LEECH. M.S. Thorogood* and P.D. Brodfuehrer, Dept. of Biology, Bryn Mawr College, Bryn Mawr, PA 19010

Using antagonists to glutamate, we have investigated the role of the excitatory neurotransmitter in the swim motor program of *Hirudo medicinalis*. In the medicinal leech, previous studies have shown that pressure ejection of L-glutamate onto the unpaired segmental gating neurons (cells 204) led to (1) prolonged excitation of cell 204 and (2) swimming of the intact, isolated ventral nerve cord. Stimulation of the paired subesophageal trigger neuron (Tr1) almost always elicits sustained depolarization of cell 204, often followed by swimming. In the presence of non-NMDA blockers [i.e., Kynurenic acid, DNQX, GAMS, and L(+)-AP-3], Tr1 stimulation no longer produces sustained excitation of cell 204, and Tr1-induced swim episodes never occur. However these blockers do not eliminate the direct connection between Tr1 and cell 204, suggesting that Tr1 acts via an indirect, as well as a direct, pathway to depolarize cell 204. Further support of this hypothesis stems from previous immunocytochemical studies which revealed five pairs of glutamate-like immunoreactive neurons, none of which co-localized with Tr1. Additional experiments are currently underway to identify the putative glutaminergic cell(s), important in the activation of cell 204 and possibly swimming.

657.10

COMPARATIVE STUDY OF THE SENSORY SYSTEMS BETWEEN CAVE AND SURFACE AMPHIPODS. B. Hoyte, A. Nadel and I. Chow*. Dept. of Biology, The American Univ., Washington, DC 20016.

The antennae of the cave amphipod *Gammarus* are significantly longer and their eyes are notably smaller than their surface counterparts (Jones and Culver, 1988, Evolution 43:688-693; Fong, 1989, Am. Midl. Natur. 121:361-378). Whether these external differences are also expressed in the central nervous system of these amphipods was investigated using semi-serial sections of the supraesophageal ganglion and tracing the sensory inputs into the ganglion. The diameter of the optic ganglion was found to be significantly larger and the diameter of the olfactory lobe was smaller in the spring populations than in the cave animals. Tracing of the antennae sensory and the optic neurons with DiI and HRP will show if there is a replacement of the smaller visual system by the larger olfactory system, as a result of adaptation.

(supported in part by a TAU Senate Research Award to I.C.)

657.11

COMPARATIVE ANATOMY OF THE BASALAR MOTOR SYSTEM IN THE BLOWFLY, *CALLIPHORA*. A. Fayyazuddin*[1], M. Hummon[2], & M. Dickinson[1]. [1]Dept. of Organismal Biology. & Anatomy, Univ. of Chicago, Chicago, IL 60637; [2]College of Osteopathic Medicine, Ohio Univ., Athens, OH 45701.

Unlike the large fibrillar muscles that power flight, the 17 pairs of tubular muscles responsible for turning behaviors in flies are under direct neural control. Because of their relatively large size and firing patterns during flight, we have focused on the three steering muscles that insert upon the Basalar process, the B1, B2, and B3. As a complement to ongoing physiological and biomechanical studies, we present here a basic anatomical description of motor neuron morphology and muscle ultrastructure.

By using multiple fluorescently labeled dextran probes, we have been able to compare the central dendritic morphology of the basalar motorneurons in the same preparations. Two the motor neurons, the B1 and B2, posses very similar branching patterns and presumably share many inputs, including a strong monosynaptic input from the haltere campaniform sensory neurons. In contrast, the B3 cell shows little common overlap with the B1 and B2. Peripherally, all three motor neurons branch extensively within their muscles and virtually cover the muscle fibers with synaptic varicosities. Despite this elaborate multiterminal pattern of innervation, the muscle fibers do fire overshooting action potentials.

Analysis of muscle ultrastructure has also revealed both differences and similarities within the Basalar motor system. All the muscles posses an extensive network of SR expected of direct flight muscles. Sarcomere length is shorter in the B1 than the B2, as is the distance between concentric rings of mitochondria in cross section. These structural differences may reflect the varied functional specializations of the Basalar muscles in flight, such as the B1 muscle's ability to fire continuously at wing beat frequency, and the B2's potent effect on wing stroke amplitude.

657.12

ACTIVITY OF THE WING HINGE STRETCH RECEPTOR IN IMMATURE AND MATURE ADULT LOCUSTS. J.R. Gray & R.M. Robertson*, Dept. Biology, Queen's University, Kingston, Ontario, Canada, K7L-3N6.

The wingbeat frequency of the locust, *Locusta migratoria*, increases from 13 Hz at 1-2 days after imaginal ecdysis to approximately 23 Hz 14 days later. Comparison of intact and deafferented immature and mature locusts suggests that proprioceptive input is involved in flight maturation. We examined changes in the conduction velocity of afferent signals from the forewing stretch receptor and stretch receptor sensitivity to fixed and rhythmical wing elevation. The conduction velocity increased from 2.51 m/s in immature animals to 3.32 m/s in mature locusts. Fixed elevation of the forewing resulted in an initial increase in the firing frequency of the stretch receptor followed by a decrease to a constant, adapted frequency. Initial frequencies increased proportionally with wing elevation in immature and mature animals, however the mature frequencies were approximately three times higher. The response of the mature stretch receptor to rhythmic wing movements was characterized by bursts of four spikes precisely timed during maximum elevation. In immature animals there was only a single spike and in day 7 animals there were three spikes per burst with a lower intraburst frequency than that of a mature locust. We propose that the described changes in stretch receptor input during maturation may increase the output frequency of the central flight motor.

657.13

COMPARATIVE ANALYSIS OF FLIGHT INITIATION IN *DROSOPHILA MELANOGASTER* INDUCED BY VISUAL AND OLFACTORY STIMULI. J.R. Trimarchi* and A.M. Schneiderman, Sec. of Neurobiology and Behavior, Cornell Univ., Ithaca, NY, 14853.

We have identified two distinct types of flight initiation: visually elicited (escape) and non-visually elicited ("voluntary"). Although these behaviors differ in the movements of the wings prior to takeoff, the jumping phases of these behaviors are remarkably similar. The jumping phase of visually elicited flight initiation is coordinated by a pair of large interneurons, the giant fiber neuron. Through their connections with other interneurons and motor neurons, the giant fiber neuron sequentially activate escape response muscles that coordinate the stereotyped escape behavior. By monitoring activity in three escape response muscles— a dorsal longitudinal muscle (DLMa), a dorsal ventral muscle (DVMIc) and the tergotrochanteral muscle (TTM)— we have confirmed that the pattern of muscle activation resulting from giant fiber neuron stimulation is identical to the pattern observed during visually elicited flight initiation. We analyzed the pattern of muscle activation during non-visually elicited flight initiation and found that although the sequence of muscles activated is identical to those activated during visually elicited flight initiation, the timing of specific muscle activation differs. In addition, we have found that the pattern of muscle activation elicited by a noxious olfactory stimulus (10% benzaldehyde) is identical to the pattern observed during non-visually elicited flight initiation. We are presently investigating the neural substrate coordinating muscle activation during non-visually elicited and olfactory-induced flight initiation.

657.14

MODULATION OF THE MECHANICAL OUTPUT OF A STEERING MUSCLE FROM THE BLOWFLY *CALLIPHORA* UNDER CONDITIONS MIMICKING FLIGHT. M.S. Tu and M.H. Dickinson*. Department of Organismal Biology and Anatomy, University of Chicago, Chicago IL 60637.

Of the 17 direct synchronous steering muscles in flies, the First Basalar (B1) stands out due to its ability to fire each and every wing beat at frequencies exceeding 200 Hz. Moreover, changes in the phase of B1 firing correlate with changes in the phase of wing pronation during turning maneuvers. Because of the potentially large aerodynamic forces generated during wing pronation, rapid modulation of the B1 output by changes in stimulus phase may be crucial for flight control.

We found that changing the phase of muscle activation significantly modulates the B1 output under conditions relevant to its function in flight. Because the B1 is mechanically coupled to the wing hinge and is subjected to cyclic length changes during each wing stroke, we measured muscle work output during forced length oscillations and phasic stimulation. At wing beat frequency (150 Hz), the B1 work output was negative. The mean energy dissipation increased monotonically with strain amplitude from 1.6 JKg-1cycle-1 at an amplitude of 1% resting length to 36 JKg-1cycle-1 at 7% resting length. Changes in stimulus phase at physiological strain amplitudes resulted in modulation of energy dissipation of ±34%, and modulation in dynamic stiffness of ± 22%.

Negative work output at wing beat frequency may reflect underlying activation kinetics of the B1 necessary for dual functions during flight. Based on its isometric twitch duration of 19ms, the optimum operating frequency for positive work generation should be 26 Hz, and indeed the B1 can generate positive work at 50 Hz or less. During flight, the B1 muscle may function as an elastic element which can be fine tuned by the nervous system. Our observations indicate that the mean muscle tension has a tonic effect on wing kinematics, while changes in neural input can significantly alter the oscillatory component of the B1 output.

657.15

FMRFamide-RELATED PEPTIDES ASSOCIATED WITH THE VENTRAL NERVE CORD AND LEG MUSCLES OF THE COCKROACH (*PERIPLANETA AMERICANA* (L.)). A.J. Elia* and I. Orchard, Dept. of Zoology, University of Toronto, Toronto, Ont., Canada M5S 1A1.

The interphyletic existence of FMRFamide-related peptides (FaRPs) is now well established. Less well established are the physiological roles these peptides play in the normal functioning of an animal or their effects on a known target tissue. We are studying the innervation and putative physiological roles of FaRPs on a known target tissue in the cockroach *Periplaneta americana* (L.). Using immuno-histochemistry we show the innervation pattern of FaRP-containing axons to muscles of the metathoracic legs. Of the peripheral nerve roots, only nerve 5 of each thoracic ganglion has FaRP-containing axon profiles which appear to project from the ganglion. The effects on muscle contraction of FaRPs isolated from nerve 5 have also been investigated. Using immunohistochemical, RIA, and HPLC techniques we outline the distribution of FaRP-containing cells associated with the nervous system.

657.16

STROLLING ON THE CEILING: EFFECTS OF LOAD INVERSION IN COCKROACH WALKING. G. S. Larsen, S. F. Frazier and S. N. Zill*. Dept. Anat., Marshall Univ. Sch. Med., Huntington, WV 25755

In order to maintain dynamic postural stability, animals must generate movements and muscle contractions to adapt walking patterns to variations in load. We have examined how cockroaches resolve this problem in an extreme case, when they walk upon an inverted surface. Animals were videotaped while myograms were recorded in the tibial extensor and flexor muscles of the middle or hindlegs. In upright walking, tibial muscles showed stereotyped discharges: the flexor fired during swing and the extensor in stance, although co-contractions occurred early in stance at slow walking speeds. In inverted walking, leg movements and patterns of muscle activities were adaptively altered. Flexor bursts were prolonged and served both to move the leg in swing and support the body early in stance by pulling the animal toward the substrate. Extensor firing occurred only late in stance to propel the animal forward. These findings are compatible with a model in which stance is divided into an early support and subsequent propulsion phase. Muscle activities are apparently adapted in the support phase to variations in leg loading. We are currently examining the types of leg sense organs that can contribute to these adaptive modifications.

Supported by ONR grant N00014-93-1-0088 and a grant from the Whitehall Foundation.

657.17

ANALYSIS OF LEG MOVEMENTS EVOKED BY INTRACELLULAR STIMULATION OF NEURONS IN THE COCKROACH. J. T. Watson* and R. E. Ritzmann. Department of Biology, Case Western Reserve University, Cleveland, OH 44106.

A complete understanding of motor control circuitry requires detailed analysis of the movements produced by the circuitry as well as the connections between individual neurons. To implement biological principles into the design of legged robots, we are combining high speed video motion analysis of leg movements in the cockroach with electrophysiological techniques such as intracellular stimulation/recording from thoracic neurons and EMG recording from leg muscles. By incorporating motion analysis into our recording paradigm, we can derive the transfer functions from electrical activity to movements. We are currently using a restrained preparation to quantify movements by unloaded legs evoked by intracellular stimulation of individual motor neurons (MNs) and local interneurons (LIs).

We have observed slow MN activity which produced no movement of the leg, as well as activity which produced a slow movement to a new, maintained position. Fast and intermediate MNs produced transitory, highly damped movements with a consistent return point. Our results suggest that slow MN activity may interact with resting tonus to set limb positions, and to constrain and dampen movements. Stimulation of single MNs in unloaded legs consistently evoked single and multi-joint reflexes, showing a prominent role for proprioceptors in modulating MN activity.

We have also observed LIs which produce coordinated movements in multiple joints. Spiking LIs were found in which each action potential evoked a twitch in two muscles in separate leg segments. We plan to explore further the roles of fast and slow MNs, LIs and reflexes in the loaded leg and the walking animal. ONR grant N00014-90-J-1545 and NIH grant NS 17411.

657.18

A BIOMECHANICAL MODEL OF THE COCKROACH LEG. W.J. Marx, R.D. Beer*, G.M. Nelson, R.D. Quinn, G.A. Crocker. Depts. of Biomedical Eng., Computer Eng. and Science, and Mechanical and Aerospace Eng., Case Western Reserve University, Cleveland, OH 44106.

We are in the process of creating a computer model of walking in the American cockroach. An important step of this research program is the construction of a realistic model of the insect's periphery. This model must ultimately include some representation of the insect body and realistic models of the legs and associated musculature and sensory structures. To date, we have formulated a dynamic model of a single leg with five degrees of freedom. The Thorax-Coxa joint is modeled with three degrees of freedom, while the Coxa-Femur and Femur-Tibia joints are modeled with one degree of freedom each. By modifying the basic geometric and kinetic parameters of this model, it can be applied to all three pairs of legs.

Our initial studies with this model are focusing on the Femur-Tibia joint of the metathoracic leg. This joint provides one of the simpler neuromuscular structures of the leg and yet is very important in both walking and turning movements. Eventually neurobiological data on neural control (see poster by Watson and Ritzmann) will be incorporated into this peripheral model. The model will be integrated into an existing graphical simulation environment which allows the experimenter to observe the behavior of the model and manipulate it interactively.

We hope to use this model to address the following properties: (1) The synthesis of data on individual motor neurons and interneurons into an understanding of total leg movement; (2) The relative roles of fast, slow and intermediate motor neurons in generating various leg movements; (3) The role of complex leg reflexes in smooth and accurate movement.

Supported by ONR grant N00014-90-J-1545.

657.19

CRAYFISH ABDOMINAL POSITIONING INTERNEURONS DRIVE MOTOR ACTIVITY IN THE TAIL FAN J.A. Burdohan and J.L. Larimer*. Dept. of Zoology, University of Texas, Austin, TX 78712.

The role of interneurons producing abdominal positioning behaviors (flexion or extension), in crayfish (*Procambarus clarkii*), has been reviewed recently (Larimer TINs 11:506, 1988). Our goal in this study was to determine whether abdominal positioning interneurons (APIs) are capable of coordinating both abdominal positioning movements and those of the tail fan. Intracellular recording and dye injection techniques were used to locate and identify interneurons that originated in abdominal ganglia, and were able, when stimulated, to drive motor activity in anterior abdominal and/or uropod motor roots.

Over 60 interneurons have been examined thus far. Two general groups of neurons can be defined physiologically: Those that activate motor neurons only within anterior abdominal segments, and those that drive motor activity in the uropods as well. Among the latter class are interneurons that drive postural activity both anteriorly and posteriorly with respect to the recording site, and those with output biased in only one direction. There are APIs that increase (and/or recruit additional) motor activity in both main uropod motor roots, those that drive activity in only one of the uropod motor roots, and those that excite motor neurons in one uropod root while inhibiting activity in the other root.

Dye filling revealed several classes of neurons, some of which resemble APIs described previously. Interneurons of the abdominal positioning system are therefore concerned not only with the control of the abdominal segments but the uropods as well. Finally, we have shown in previous studies that abdominal positioning interneurons affect the swimmerets as well as the abdominal segments (Murchison and Larimer J. Comp. Physiol. 170:739, 1992). We are now examining the effects these APIs have on the swimmeret and thoracic leg motor systems. (Supported by NIH NS05423 to JLL).

657.20

COMPUTATIONAL MODELING OF THE MUSCULOSKELETAL SYSTEM OF THE LOBSTER GASTRIC MILL. K. Doya*, M. E.T. Boyle, M. Beauchamp, and A. I. Selverston. Dept. of Biology, U. C. San Diego, La Jolla, CA 92093-0332.

The lobster stomatogastric ganglion (STG) is one of the best-studied neural systems at the circuit, cellular, synaptic, and molecular levels. However, little is known about the relationship between oscillation patterns recorded from *in vitro* preparations and the actual digestive motion patterns of live animals. In order to fill the gap between neural activity and physical movements and to elucidate the role of STG network as the controller circuit, we built a computer model of the musculoskeletal system of the gastric mill.

The lobster gastric mill is a set of ossicles (teeth) that grind food in the foregut. Semi-intact animals were used to video record the movement of the ossicles and the muscles both from the dorsal side with the shell removed and from inside the stomach using an endoscope. Some of the muscles were dissected out and then stimulus-tension characteristics were measured.

The model consisted of five ossicles, six muscles, and associated connective tissue. The muscles were modeled based on A. V. Hill's formulation. The mechanical system had 10 degrees of freedom and a quasi-static approximation was used because the movement of the teeth were very slow (0.1~0.2 Hz). The output pattern of five motoneurons from the *in vitro* preparations were used as the input to the muscles. The model reproduced movement patterns that are qualitatively similar to the actual motion of the gastric mill observed by the endoscope. Supported by ONR N00014-91-J-1720 and NIH RO109322.

657.21

MIXED CHEMICAL AND ELECTRICAL SYNAPTIC INPUTS TO THE LATERAL GIANT INTERNEURON OF CRAYFISH. S.-R. Yeh, C.A. Opdyke and D.H. Edwards* Dept. of Biology, Georgia State University, Atlanta, GA 30302-4010

The crayfish responds to a sharp tap on the abdomen with a tailflip triggered by the Lateral Giant (LG) interneuron. The tap excites mechanosensory receptors on the abdominal surface that excite LG through monosynaptic connections. These synapses were identified as electrical because their transmission delay is near zero and their EPSPs follow reliably at stimulus rates up to 400 Hz (Zucker, 1972). More recently, the EPSPs have been shown to vary with post-synaptic membrane potential in a manner consistent with rectifying electrical synapses (Edwards, *et al.*, 1991). We report that the afferents can be dye-coupled to LG, which indicates that the synapses contain gap junctions that should pass electrical current as well as dye molecules. We also report that the LG EPSP can be partially and reversibly blocked with bath-applied nicotinic cholinergic blockers (mecamylamine, hexamethonium), consistent with the presence of chemical synapses between the afferents and LG. We do not yet know whether the same afferent can mediate both forms of transmission. The cholinergic blockers have their greatest effect on just-subthreshold EPSPs, and little effect on much smaller EPSPs. The low-threshold afferents may excite LG only through electrical synapses, whereas the high-threshold afferents make cholinergic and possibly electrical synapses with LG. Dye coupling and synaptic blockage occur in 1-2 cm juvenile crayfish and in 8-12 cm adults, showing that both forms of synaptic input to LG are present in early postembryonic development. Supported by NIH research grant NS26457.

657.22

POSTEMBRYONIC REORGANIZATION OF A REFLEX CIRCUIT IN *MANDUCA SEXTA*. J.L. Casagrand* and R.B. Levine. Div. of Neurobiol., Univ. of AZ, Tucson, AZ 85721.

Manduca pupae possess three paired gin traps that develop during the last 4-5 days of the larval stage (wandering). Stimulation of the gin trap sensory hairs in pupae results in a contraction of the intersegmental (ISM) muscles in the ipsilateral half of the next anterior segment. Although the sensory neurons are retained from the larval stage, larvae do not possess gin traps and stimulation of the sensory hairs evokes either no response, or a slow bending of the animal towards the stimulated side. As wandering stage animals approach pupation, they become more responsive to sensory hair stimulation, and the evoked behaviors more resemble those in pupae. Immediately after pupal ecdysis, there is a dramatic increase in the reliability of the reflex and less habituation of the behavior.

The activity recorded intracellularly in ISM motor neurons evoked by stimulation of the sensory nerve is quite different in larvae and pupae. Pupal responses are shorter in duration and exhibit a triphasic pattern of activity not seen in larvae. We have found that the responses recorded in ISM motor neurons in isolated nerve cords changes gradually during the wandering stage. Motor responses on the first day of wandering more closely resemble those from larvae, whereas activity in ISM motor neurons on the day before ecdysis are often indistinguishable from pupae. Thus, the circuit for the pupal behavior appears to develop gradually during the wandering period, and an abrupt increase in reliability of the reflex coincides with ecdysis.
Supported by NIH fellowship NS 09249 to JLC & NSF BNS 11174 to RBL.

657.23

SEGMENTAL VARIATION IN MECHANOSENSORY REFLEX CIRCUITS IN *MANDUCA SEXTA*. W.C. Lemon* and R.B. Levine. Div. of Neurobiol., Univ. of Arizona, Tucson, AZ 85721.

Stimulation of mechanosensory hairs on any abdominal segment of larval *Manduca sexta* produces a prolonged bending reflex involving the whole abdomen. When they later become part of pupal gin traps, stimulation of sensory neurons in segments 5-7 evokes a rapid bend of the abdomen only in the gin trap segments. In this study, we asked the extent to which motor activity in all segments was influenced by stimulation of sensory neurons in one segment, and how the motor patterns differed among the segments. Electrical stimulation of the sensory nerve in isolated nerve cords and mechanical stimulation of the sensory hairs in semi-intact preparations were used to evoke reflex responses that were recorded intracellularly and extracellularly from motor neurons of fifth instar larvae and day 0 pupae. Although the motor responses are quite different in larvae and pupae, stimulation of one segment evokes motor responses in many segments in both stages despite the segmentally restricted movement observed in pupae. Furthermore, stimulation of sensory nerves in all abdominal segments, including pupal segments that do not contain gin traps, evokes motor responses. The motor patterns differ, however, according to the segment stimulated, with anterior segments causing weaker, delayed responses. These findings indicate that developmental changes in mechanosensory reflex circuits occur throughout the abdominal nerve cord, although the expression of the pupal behavior is limited to the three articulated segments.
Supported by NIH fellowship NS 09008 and NSF BNS 11174.

657.24

DESCENDING THORACIC INPUTS TO AN IDENTIFIED MOTONEURON REGULATE ITS RHYTHMIC ACTIVITY DURING ECDYSIS BEHAVIOR AND ITS SUBSEQUENT DEVELOPMENTAL DEATH. A.W. DeLorme*[1], K.A. Klukas[1], S.E. Fahrbach[2], and K.A. Mesce[1]. [1]Dept. of Entomology and Graduate Program in Neuroscience, University of Minnesota, St. Paul, MN 55108 and [2]Dept. of Entomology and Neuroscience Program, University of Illinois at Urbana-Champaign, Urbana, IL 61801.

In the moth *Manduca sexta*, abdominal motoneuron 12 (MN-12) undergoes developmental cell death within four days of adult ecdysis. Ecdysis is the shedding of the old cuticle. If anterior neuronal inputs to MN-12 are removed, the MN-12 neuronal soma is spared from programmed cell death. Until now, the sparing effect on the cell's overall morphology and electrophysiology was not known. We provide quantitative evidence of a robust sparing effect. Electrophysiological measurements of spike height, spike duration at half amplitude, resting membrane potential, and input resistance show no significant differences between normal pharate adult MN-12 cells and spared MN-12 cells. Comparisons of the major arborization patterns show no difference between spared and pharate adult MN-12 cells. Currently, we are examining the changes in MN-12 cells as they undergo the normal progression of programmed cell death. Preliminary evidence suggests that MN-12 cells function normally 2 days after ecdysis, when they can no longer be spared and show initial histological evidence of degeneration. We have also developed an *in situ* preparation in which we can record intracellulary from MN-12 as the animal undergoes adult ecdysis. Our data show that MN-12 cells undergo massive depolarizations that correlate with rhythmic thoracic wing shrugging, an adult specific component of ecdysis behavior. This indicates that MN-12 is involved in adult ecdysis behavior and receives descending neuronal inputs from the thorax during ecdysis. We are attempting to determine what role these anterior, descending, neuronal inputs may have in the developmental death of the MN-12 neurons.

657.25

MULTISEGMENTAL ORGANIZATION OF THE PUPAL GIN TRAP CLOSURE REFLEX IN THE MOTH, *MANDUCA SEXTA*. B. Waldrop* Department of Zoology, University of Oklahoma, Norman, OK 73019

Movement of tactile hairs in a pupal gin trap triggers a rapid and specific closure. The reflex requires at least three segments: sensory axons from a gin trap in the fifth abdominal segment (A5) ascend ipsilaterally in the CNS to A4, and the motor neurons are in A3. In an isolated nerve cord, the motor response consists of a stereotyped pattern of excitation and inhibition of ipsilateral and contralateral motor neurons. This response pattern corresponds to closing and reopening a stimulated gin trap.

Isolation of ganglia A3-A5 allows production of the normal motor pattern. After cutting the right A4-A5 hemiconnective, normal motor responses can be elicited by stimulating the (uncut) left A5 sensory axons. Stimulation of (cut) right A5 sensory axons produces ipsilateral excitation, but not the full closure response. The sensory axons coming into right A5 must reach ascending neurons on the left side, but they do not seem to have access to the normal patterning circuitry. Stimulation of the rostral end of the cut hemiconnective can elicit a normally patterned response. Cutting the entire A4-A5 connective eliminates all physical access of the A5 sensory axons to the motor neurons. Stimulation of sensory axons in A3, however, can elicit patterned motor responses in the A3 motor neurons, although the patterns are generally not typical of the closure reflex. Isolation of ganglion A3 still permits some ipsilateral-contralateral patterning by stimulation of A3 sensory axons, but again, these patterns are not typical closure reflex responses. The results are consistent with a patterning circuit located in A3 with the motor neurons' dendrites, but accessed differently depending on the pathway used.

657.26

CRAWLING IN LEECHES: INVOLVEMENT OF NEURONS IN MIDBODY GANGLIA AND THE TAIL BRAIN. A.P. Baader* and W.B. Kristan, Jr., Dept. of Biology 0322, University of California, San Diego, La Jolla, CA92093-0322.

In addition to swimming, leeches locomote by crawling, a complex rhythmic behavior comprising alternating extensions and contractions of the whole body. Transsection experiments have shown that the nervous system in both the head and the tail ends of the animal are sufficient to produce all the behavioral subcomponents of crawling. Experiments with tethered crawling leeches, have uncovered interneurons and motoneurons in the tail brain that are modulated at various phases of crawling steps. The morphology of these neurons is unlike any seen in the midbody ganglia. The activation patterns of some neurons in the tail brain indicate very fast-conducting pathways to the head brain.

Electrical stimulation of mechanosensory cells in midbody ganglia increase the probability of the initiation of crawling steps. Some but not all of these sensory units also receive rhythmic postsynaptic re-afferent drive during the performance of crawling steps. Future studies on the tail ganglion will determine its role in the initiation and coordination of crawling behavior. Supported by an NIMH research grant (MH43396 to W.B.K.), and a DFG fellowship (A.P.B.).

HUMAN COGNITION: VISION, METHODS

658.1

INTERACTION OF MULTIPLE CUES IN 3D SHAPE DISCRIMINATION. Scott McDaniel and William H. Merigan*, Center for Visual Science, University of Rochester, Rochester, N.Y. 14642.

These experiments attempted to discover how observers base their discrimination on more than one object feature during an object discrimination task. We chose two feature dimensions along which objects may vary (curvature of the long axis of symmetry and shape of the object cross section), and constructed a series of photorealistically rendered objects which differed along one or both dimensions.

Subjects discriminated among objects varying only in curvature, only in cross sectional shape, or in both features together, using a same-different task. There were five levels of curvature and five levels of shape; separated by one just noticeable difference (jnd) along the respective dimension. Subjects' thresholds for discrimination of objects varying in both shape and curvature was close to their thresholds for discriminating either shape or curvature alone. The effect of uncertainty was measured by mixing trials in which object differed in either curvature or cross sectional shape. Unlike previous experiments in which uncertainty caused a small elevation of both thresholds, this manipulation devastated curvature discrimination, but had little effect on shape discrimination. This result suggest that when faced with difficult discriminations, subjects may monitor multiple dimensions despite decreased sensitivity for both, or monitor only a single dimension, thus losing sensitivity only on the dimension not monitored.

Supported by NIH Grants EYO8898 and ES01247.

658.2

PSYCHOPHYSICAL DEACTIVATION OF THE MAGNOCELLULAR CHANNEL WITH PERCEPTION AND RECOGNITION OF TWO-COLOR PICTURES OF FACES. H.R. Bliem (SPON: European Neuroscience Association). Department of Psychology, University of Innsbruck, Innrain 52, A-6020 Innsbruck, Austria.

Fast and reliable recognition of faces depends mainly on the processing of configurational information reflecting relational properties of eyes, nose and mouth within a face. The well known information processing properties of the magno-system (Livingstone and Hubel, *J. Neurosci.* 7:3416, 1987) include linking properties probably underlying the processing of configurational information of faces. It is, therefore, predicted that face perception and recognition should fail at isoluminant stimuli conditions. To test this hypothesis five two-tone picture categories were derived from multi-tone original b/w pictures of faces displaying only internal facial features: two-color pictures and two-tone b/w pictures at high (90%) and low (9%) luminance contrast, and two-color pictures at isoluminance.

The first experiment demonstrates that 35% of all subjects without any experience with two-color faces, who were shown a two-color face at isoluminance for 10 sec, did not perceive a face or even a face-like structure. There was no difficulty in perceiving a face with the other four picture categories. In the second experiment subjects had to recognize faces in the above described five different picture categories. In the inspection phase faces had been presented as multi-tone original b/w pictures. Error score was significantly ($p<0.001$) higher with isoluminant face stimuli compared to all other stimuli conditions.

These results strongly support the view that the color-blind and luminance contrast-sensitive magno-channel is important for the processing of configurational information of faces and innervates face specific neural mechanisms in the temporal lobe. This work is complemented by previous experimental investigations on movement-specific activation of the magno-channel with face recognition (Bliem, *Perception 19*:405, 1990).

658.3

ELECTROPHYSIOLOGICAL CORRELATES OF THE ADAPTATION TO LEFT-RIGHT REVERSED VISION. Y. Sugita*, K. Mimura and T. Ohta. Lab. for Neural Systems, Toyohashi Univ. of Technology, Toyohashi, 441 Japan.

The reversal, inversion, or displacement of the retinal image leads to extreme disruption of visually guided behavior. But, after an extended period of visual transformation, the seen would appear stable and the subjective visual normalcy is gradually restored. Adaptation to such optical distortions appeared to result in a change in visual perception. It has been claimed, however, that the adaptation consists mainly of learning specific motor response, adjusting the state of the relevant sensorimotor control system, or a proprioceptive change in the felt position of parts of the body. We report here an electrophysiological correlate of the adaptation recorded from the human visual cortex. Light flash presented on the left or right side of the fixation elicited visually evoked potentials (VEPs) of different waveforms in the two hemispheres. Wearing the left-right reversing spectacles resulted in the change in the distribution of the VEP components over the occipital region. However, after prolonged exposure to the spectacles, the VEP distribution changed again and even became similar to those measured at prewearing period, indicating that the adaptation takes place even in the relatively early stage of the visual information processing.

658.4

VISUAL RECALL WITH EYES CLOSED AND COVERED ACTIVATES EARLY VISUAL CORTICES. H. Damasio*, T.J. Grabowski, A. Damasio, D. Tranel, L. Boles-Ponto, G. L. Watkins, R. D. Hichwa. Depts. of Neurology & Radiology, Univ. of Iowa College of Medicine, Iowa City, IA 52242.

We are all able to generate internally images of entities with which we are familiar in the absence of those entities. Varied visual properties (e.g. shape, movement, color) can be conjured up coherently and attended in consciousness. In order to investigate the pattern of neural activity that correlates with visual recall, we investigated the activity in early visual cortices in 5 normal subjects tested under 3 conditions [each of which was performed twice] using [^{15}O]-$H_2$0 PET. In condition #1 the subjects were instructed to listen intently to a selection of music. In condition #2 they created mental images of specific places and persons familiar to them, and inspected them in detail. New images were suggested by an audioverbal cue, every 10 sec. In both of these conditions, the eyes were closed and covered. In condition #3, subjects viewed passively a series of nonrepresentational black and white slides, presented at a rate of one every 5 sec. Data were analyzed with two approaches using (a) PET-Brainvox, an anatomically based method, with regions of interest defined *a priori*, and (b) change distribution analysis [CDA] (adapted from Fox et al, 1988).

During visualization, both methods demonstrated increases in cerebral blood flow in a region containing V_1 and V_2. CDA identified a local maximum with a Z-score 3.1, and stereotactic coordinates which corresponded to calcarine cortex in all subjects. Analysis of activity confined to the region of V_1/V_2, defined *a priori* on coregistered MR images using PET-Brainvox, suggested that the increase in cerebral blood flow was distributed throughout the posterior half of the calcarine region.

These findings are consistent with the hypothesis that the neural correlate for explicit mental visual representation is a set of transient activity patterns in early visual cortices, in conformity with the convergence zone framework.

658.5

A TECHNIQUE FOR THE NEUROANATOMICAL ANALYSIS OF FUNCTIONAL BRAIN IMAGES. T.J. Grabowski*, H. Damasio, R. Frank, L.L. Boles Ponto, G.L. Watkins, R.D. Hichwa, M. Rizzo, M. Nawrot. Depts. of Neurology, Radiology and the Image Analysis Facility, University of Iowa, Iowa City, IA 52242.

We have developed a technique for the neuroanatomical interpretation of functional brain images (PET-Brainvox). This interactive brain rendering software clarifies individual structural-functional anatomical relationships in coregistered MRI and PET datasets. *A priori* registration depends on a pair of spectacles with paramagnetic fiducial markers. These markers are fixed with respect to the PET slice planes using the gantry lasers at the time of PET acquisition. PET-Brainvox resamples the MR volume in the PET planes.

The reliability of the positioning of the glasses across serial scans in 4 normal subjects was assessed with the *post hoc* registration method of Woods, *et al*, 1993. We obtained root mean square errors of $<1.5°$ of rotation around all axes and translational errors of <2mm in all axes. Brain phantom studies demonstrated an average 3D mapping error of 2.6mm. In human subjects, average in-plane (x- and y-axis) translational errors of 2.3 and 2.8 mm, and in plane rotational errors of 1.0° have been encountered. Analytical techniques sensitive to the residual misregistration (e.g. pixel-by-pixel subtraction) may require a *post hoc* mathematical registration step, but our approach provides the requisite starting point, permitting automated fitting.

This method offers the following advantages: 1) the interrogation of the functional image with regions of interest based *a priori* on the MR images. This approach was validated for retinotopic mapping with $[^{15}O]$-H_2O PET (after Fox, *et al*, 1987); 2) the modelling of PET gantry movement permits the advance planning of PET slice position; 3) construction of a stereotactic coordinate system (Talairach space) directly from brain landmarks, and 4) the assessment of anatomical structures corresponding to specific stereotactic coordinates.

658.6

NETWORK ANALYSIS OF OBJECT AND SPATIAL CORTICAL VISUAL PATHWAYS MAPPED WITH PET. A.R. McIntosh*, C.L. Grady, L.G. Ungerleider[1], J.V. Haxby, S.I. Rapoport and B. Horwitz Lab. of Neurosciences, National Instituite on Aging, [1]Lab. of Neuropsychology, National Institute of Mental Health, NIH, Bethesda, MD 20892 USA

Brain imaging with positron emission tomography (PET) can provide information about the functional interactions within entire neural systems, and therefore a network analysis is necessary to quantify these complex interactions. A network analysis was performed on data from a PET study of regional cerebral blood flow (rCBF) during performance of a face matching (object vision) and dot-location matching (spatial vision) task in 17 young adult right-handed males. Brain areas for the network were selected from comparisons of regional means and covariances between tasks. An anatomical network connecting selected areas represented two parallel pathways: a ventral pathway involving occipital and temporal areas extending into frontal area 46 and a dorsal pathway involving occipital and parietal areas also extending into area 46. The anatomical network and interregional correlations were analyzed using structural equation modeling to quantify the functional influence of each directional path. This created a functional network for each task. The functional network for the right hemisphere showed that in the object vision task, dominant interactions were in the ventral pathway while in the spatial vision task, interactions in the dorsal pathway were stronger. The network for the spatial vision task also had a strong feedback path from area 46 to occipital cortex, an effect which was absent in the object vision task. Functional networks for the left hemisphere did not differ between tasks. Networks for the interhemispheric interactions showed strong contralateral effects from the right hemisphere on homologous left hemisphere areas suggesting that intrahemispheric interactions were greater in the right hemisphere in both tasks, and that these influences were transmitted callosally to the left hemisphere.

658.7

COMPONENTS OF THE VISUAL EVOKED POTENTIAL IDENTIFIED BY TOPOGRAPHIC MAPPING: EVIDENCE FOR MULTIPLE VISUAL STREAMS IN HUMANS V.P. Clark*, F. Silu, N. Herold, T.C. Rubin, and S.A. Hillyard, Dept. of Neurosciences, U. C. San Diego, La Jolla, CA 92093-0608.

Multi-channel visual evoked potentials (VEPs) were recorded in 24 normal human subjects in response to a small circular checkerboard stimulus (radius 1.15°, check size 35', luminance 70cd/m²) that was flashed over a range of iso-eccentric visual field positions. Temporally and spatially overlapping components were distinguished and characterized by differences in retinotopic sensitivity and source location, as evidenced by changes in amplitude and topography of scalp voltage and current density. The Brain Electric Source Analysis (BESA) source localization algorithm, and magnetic resonance images (MRIs) of cortical topography, were also employed to help determine the identities of VEP generators. Using these methods, the C1 and P1 components have been localized to striate and lateral extrastriate cortex, respectively.[1]

The N1 complex of components (90-180 msec) was found to originate from several distinct generators. The N100op was present in occipito-parietal scalp locations, and varied little in amplitude across visual field locations. Immediately afterwards, the N150op component increased in negative amplitude (by a sigmoid function) and changed somewhat in topography with decreasing stimulus elevation. In contrast, the contralateral N160ot and ipsilateral N170ot were largest at occipito-temporal scalp sites, and showed almost no change in voltage or current density amplitude with changes in stimulus elevation. The topography and retinotopic properties of the N150op and N160/N170ot components displayed characteristics of the dorsal and ventral visual pathways, respectively, which have been described in lower primates. The N150f was maximal at frontal scalp sites and also showed little change in amplitude across visual field locations. An additional sink in the right temporal lobe (the N140t) was observed for both left and right field stimuli. No symmetric left hemisphere sink was observed. This sink did not change its topography systematically as a function of stimulus position. Further research is being performed to determine the sensitivity of these components to manipulations of selective attention.

[1] Clark et al., Abs. Soc. Neurosci. 17, 656: 1991.

658.8

VISUAL EVENT-RELATED POTENTIALS TO COLOR, LOCATION, AND ORIENTATION DISCRIMINATION. B.F. O'Donnell*, J.M. Swearer, & L. Smith. Psychiatry, Brockton VAMC & HMS; Neurology, U Mass Medical School. Neurophysiological studies suggest that the anatomical substrates of color, orientation, and location discrimination differ in primates, but these differences have been less well documented in humans. We measured scalp recorded event-related potentials (ERPs) during discrimination tasks from 28 electrode sites using a nose reference from 12 subjects. ERPs were collected both to low-probability ($p=.16$), target stimuli and high-probability, non-target stimuli in order to generate the cognitively modulated N2 and P3 components as well as the earlier N1 component (at 170 ms). 400 trials were presented for each condition with a 1400 ms ISI and a stimulus duration of 200 ms. EEG was sampled with a bandpass of 0.15 to 40 Hz. N1 topography was sensitive to the visual field of stimulation, with contralateral activation to lateralized stimuli across the entire hemisphere, and symmetric activation primarily over the occipital region to central stimuli ($p < .01$). N2 topography varied as a function of discrimination task (color, position, and orientation, $p < .03$). P3 did not vary as a function of spatial location or discrimination task. These data suggest that the N2 component may reflect activation of neuronal populations supporting a task-specific perceptual representation, while the P3 component reflects a cognitive process which is independent of a specific representation.

658.9

A COMPUTERIZED SYSTEM FOR DETERMINING PIXEL-BY-PIXEL CORRELATIONS OF FUNCTIONAL ACTIVITY MEASURED BY POSITRON EMISSION TOMOGRAPHY (PET) B. Horwitz*, J. Maisog, P. Kirschner, M. Mentis, K. Friston† and A. R. McIntosh, Lab. Neurosci., NIA, NIH, Bethesda, MD 20892, and †The Neurosciences Institute, New York, NY 10021.

Pixel-based approaches to analyzing functional neuroimaging data (such as PET measurements of regional cerebral blood flow - rCBF) have been used to study the functional organization of the human brain. One widely used method is Statistical Parametric Mapping (Friston et al. 1989,1990,1991). There have also been many studies evaluating the integration and connectivity of distributed areas using interregional correlations in PET functional data (e.g. Horwitz et al. 1984,1992). We have developed a system for performing such correlational analysis on a pixel-by-pixel basis. The system, which is compatible with SPM programs, starts with PET data that have been stereotactically normalized to the Talairach space (Friston et al. 1990) and smoothed, which results in each pixel representing the regional activity of its local neighborhood. Correlations, calculated on values normalized to global activity, can be performed on data from each run of an rCBF study separately, or on a weighted averaging of data across runs. Options for correlation analysis include: (1) correlations between one pixel and every other pixel, and (2) mutual correlations within a list of pixels. Examples of each will be provided using data obtained during a study of visual processing (Haxby et al. 1991).

658.10

SPATIAL IMAGING AND TEMPORAL DYNAMICS OF VISUAL BRAIN-ACTIVATION THROUGH INTEGRATION OF ELECTROPHYSIOLOGICAL (ERP) AND HEMODYNAMIC (fMRI) MEASURES. G.V. Simpson[1*], J.W. Belliveau[2], J.J. Foxe[1], J.R. Baker[2], and H.G. Vaughan,Jr.[1],[1]Depts. Neurology & Neuroscience, Albert Einstein College of Medicine, Bronx NY 10461 and [2]MGH-NMR Ctr., Dept. Radiology, Harvard Medical School, Boston MA 02114

Use of fMRI activation maps to improve the spatial estimates of ERP dynamic source activity may yield unprecedented spatial and temporal characterization of the dynamic networks underlying perceptual and cognitive operations. Sources of brain activation to identical visual stimuli were obtained for both physiological measures (event related brain potential source analyses and functional magnetic resonance imaging). Retinotopic stimulation was used in order to examine the spatial convergence of the two methodologies with reasonably well defined cortical regions. Checkerboard wedges (50 deg annuli) were presented at central (1-4 deg.) and peripheral (4-10 deg.) locations in each quadrant (30 ms duration, 365 ms ISI). ERPs were recorded from 64 channels (Neuroscan system) and sources were estimated with a spatiotemporal dipole source analysis system (Brain Electromagnetic Source Analysis-BESA). fMRI cortical activation was measured (1.5 Tesla GE Signa modified by Advanced NMR) using an oxygen-sensitive gradient echo imaging sequence (TR=500 ms, TE=50 ms) and a T1-sensitive (blood-flow) spin echo inversion recovery sequence. The expected retinotopic pattern of activation was found for both hemodynamic and electrophysiological measures, i.e., left vs right and inferior vs superior visual field stimulation resulted in right vs left and superior vs inferior calcarine activation, and more peripheral visual field stimulation (1-4 vs 4-10 degrees eccentricity) resulted in more anterior calcarine activation. There was good convergence of both cortical activation measures, reinforcing the electrophysiological location estimates and providing temporal activation characteristics (in milliseconds).

658.11

INTEGRATION OF P300 EVOKED POTENTIALS WITH MAGNETIC RESONANCE IMAGES (MRI) TO IDENTIFY DIPOLE SOURCES IN HUMAN BRAIN.
S.E. Lukas*[1], M. Sholar[1], D.L. Stredney[2], M.W. Torello[2], S. May[2], and F. Scheepers[2]. [1]Alcohol and Drug Abuse Research Center, McLean Hospital/Harvard Medical School, [2]Ohio State University Advanced Computer Center for the Arts and Design.

Most procedures for identifying the source of brain electrical activity rely on mathematical models that assume that the head and brain are a perfect sphere making the precise location of a calculated dipole difficult to determine. The present study was conducted to match the calculated source of the P300 evoked potential with MR images. Adult male subjects provided informed consent and were prepared with 21 scalp EEG electrodes. P300 waveforms were generated using an auditory "oddball" paradigm and estimates of the equivalent dipole were made using a 1-source inverse procedure (Bio-Logic). EEG electrodes were then removed and vitamin E capsules were glued to the electrode sites with collodion to provide a common set of reference points between modalities. MRI images were generated (G.E. Sigma 1.5 Tesla Whole Body Imager) and volumetric representations were built from contiguous MR slices. These consisted of three-dimensional arrays of voxels containing intensity values. Voxel coordinates were converted to millimeter-unit coordinates by a simple scaling transformation. Dipole data provided an estimate of the location, direction and magnitude of the P300 source. A common reference frame was developed using the voxel locations most representative of the nasion/inion and preaurical points (FPz, Oz, T3 and T4). An orthonormal reference frame based midway between the closest approach of FPz/Oz and T3/T4 was constructed. The Y-axis (superior/inferior) of the reference frame is perpendicular to both FPz/Oz and T3/T4, and the X and Z axes are approximately parallel to FPz/Oz and T3/T4, respectively. Finally, the required transformation was derived by taking the inverse of the transformation that takes the voxel reference frame to the dipole reference frame. All calculations and resultant visualizations were performed on a Silicon Graphics CRIMSON VGXT or a Sun IPX. These images provided direct visualization of both electrical and anatomical information and suggest that the hippocampus is the most likely source of the P300.
(Supported by NIDA Grants DA 00115 and 03994).

658.12

STRATEGIES FOR SOURCE SPACE LIMITATION IN NEUROMAGNETIC INVERSE PROCEDURES
P.S. Lewis, J.S. George*, H.A. Schlitt, & C.C. Wood
Los Alamos National Laboratory, MS: M-715 Los Alamos, NM 87545

The use of magnetic recordings for localization of neural activity requires the solution of an ill-posed inverse problem: i.e. the determination of the location, spatial configuration, strength and timecourse of the currents that give rise to a particular observed field distribution. Inverse procedures that produce distributed estimates of neuronal currents have received increasing attention because they do not require *a priori* estimates of model order and parameter values, they are suitable for automated analyses, and they allow localization of sources that are not point-like. To combat the parameter explosion and resulting underdetermination associated with distributed reconstructions, we have explored both algorithmic and knowledge-based strategies to limit the effective reconstruction space: variable resolution hierarchical models; anatomical constraints on the reconstruction volume; iterative procedures that effectively eliminate putative source locations that are not relevant to the specific reconstruction, such as the FOCUSS algorithm described previously; and methods that consider an evolving subspace of the reconstruction volume. We have recently achieved encouraging results by explicitly limiting the reconstruction space at each step so that the inverse problem is always well conditioned. Using a genetic algorithm, we specify a number of distinct subsets of the full reconstruction space using strings consisting of ones and zeros. At each step a metric is evaluated for the best linear reconstruction over the subspace specified by each string. Depending on this measure of "fitness", the string is either retained or removed from the evolving population of strings. New strings are produced by piecewise recombination of older strings. Under some conditions new "genetic material" is introduced into the population by stochastic processes. Genetic algorithms appear to hold particular promise for the neuromagnetic inverse problem because once a suitable subspace configuration has evolved to account for some domain within the target solution, it will tend to be preserved, and combined with other partial solutions.

658.13

SOURCE LOCALIZATION AND INTERBRAIN COMPARISONS USING CORTICAL SURFACE RECONSTRUCTIONS.
A.M. Dale* and M.I. Sereno, Cognitive Science Department 0515, University of California San Diego, La Jolla, CA 92093.

The cortical surfaces from 10 human hemispheres have been completely reconstructed from MRI images and computationally unfolded (Sereno & Dale, Neurosci. Abstr., 1992). Subjects' heads were stabilized through the use of a dental impression bite bar to prevent image blurring. The unfolded representations greatly facilitate comparisons of cortical folding patterns among individual brains, and between the two hemispheres of the same brain. Although the overall layout of the major sulci (e.g., lateral, central, intraparietal, lunate, parieto-occipital, calcarine, lateral occipital, superior temporal) is relatively constant across individuals, the locations and angles of the major sulci as well as the pattern of minor sulci vary considerably. The topology of the sulcal connections also varies to a surprising degree.

These differences in cortical folding lead to substantial variability in the spatial pattern of scalp recorded electrical potentials (EEG) and magnetic fields (MEG). By combining event-related EEG and MEG information with the cortical surface reconstruction for an individual subject, it is possible to obtain estimates of the evoked cortical electrical activity with temporal resolution on the order of milliseconds, and spatial resolution comparable to PET (See Dale & Sereno, Neurosci. Abstr., 1991 and 1992). This technique has been used successfully to localize multiple cortical sources with overlapping time courses evoked by auditory and somatosensory stimulation. The instantaneous estimates of cortical activity are displayed on a 3-D rendering of the unfolded cortical surface. The spatio-temporal pattern of cortical activity can be observed by animating the instantaneous solutions for an entire epoch.

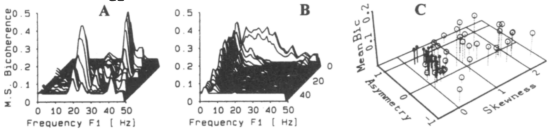
Supported by UCSD McDonnell-Pew Cognitive Neuroscience Center, the Norwegian Research Council, and Biomagnetic Technologies, Inc.

HUMAN COGNITION: ELECTROPHYSIOLOGY

659.1

NONLINEAR PROPERTIES OF EEG: BISPECTRUM IN HUMAN AND ANIMAL BRAINS.
J.Z. Achimowicz* and T.H. Bullock. Neurobiol. Unit, Scripps Inst. Oceanog. & Dept. of Neurosci., U.C.S.D., La Jolla, CA 92093-0201.

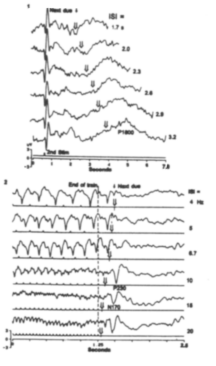
Spatio-temporal features of EEG were analyzed with **higher order spectra**. Bispectrum and its derivatives: **skewness, asymmetry and bicoherence** which shows nonlinear interactions *(e.g. quadratic phase coupling)* between different frequencies in the same or between different channels were used. Auto- and cross-bicoherence estimates for 17s segments of human EEG, from 16 scalp or subdural electrodes were plotted in different states. The traditional (linear) model of independent oscillators should be modified as phase couplings are common between nonharmonics as well as harmonics. Most common (Fig. A, awake, scalp) are wideband mountains indicating significant coupling of many frequencies up to 100 Hz, probably due to *events with sharp corners,* recurrent or transient. Narrow peaks also occur, possibly reflecting *amplitude or frequency modulation* plus extra energy at modulating frequency. Bicoherence signatures change with subject state and sensory stimulation. In slow wave sleep the fraction of nonlinearily coupled energy is usually highest <4 Hz; in the awake state the contribution from the *gamma band* is more often significant. During seizures both low and high bands are coupled (Fig. B). The dynamical pattern can differ among brain regions. Fig. C shows right lateral-frontal (●) and temporal (o) areas in the awake human. Bispectra of turtle dorsal cortex suggest that nonlinear interactions are weaker than in humans.



659.2

TWO TYPES OF EVENT RELATED POTENTIALS TO OMISSION OF STIMULI IN HUMANS.
T.H. Bullock*[1], S. Karamürsel[2] and J.Z. Achimowicz[1]
[1]Neurobiol. Unit, Scripps Inst. Oceanog. & Dept. Neurosci., UCSD, La Jolla, CA 92093, & [2]Dept. Physiol., Medical Faculty, Univ. Istanbul, 34390 Istanbul, Turkey.

Short term temporal memory was studied parametrically via the scalp-recorded SSR (Steady State Response) and the OSP (Omitted Stimulus Potential) over a wide range of stimulus (flash) rates (0.3 to 40 Hz) in 18 healthy subjects. In a high (>6 Hz) and a low frequency (<1 Hz) conditioning stimulus range the OSP has different shapes and latencies after the due-time of the first omitted stimulus (DT). The OSP is essentially absent from 2-5 Hz. Both types of OSP have nearly constant latencies after the DT. The "slow" (low frequency range) OSP (Fig. 1), seen clearly at centro-fronto-parietal regions, is a large, slow, positive wave, with a peak latency usually 500-1100 ms after the DT; it requires special attention such as counting omissions; it can be crossmodal (a click after a flash train can act like a flash); it can appear after a train as short as two stimuli; it almost totally disappears with jittered interstimulus intervals (ISI). The "fast" OSP (Fig. 2), mainly occipital, has a shorter latency after the DT (N120-P170), does not need close attention or counting, but good fixation and requires >3 or 4 flashes, is not crossmodal or much affected by jittered ISIs.



659.3

NON-LINEAR COMPLEXITY OF NEURAL ACTIVITY. N. Birbaumer*, W. Lutzenberger, H. Flor and T. Elbert. Instit. Med. Psychology and Neurobiology, Univ. Tübingen, D-7400 Tübingen, Germany.

A series of experiments with adult male and female human subjects of different levels of intellectual capacity (IQs) using non-linear analysis ("deterministic chaos") of the EEG is reported. The hypothesis was tested that the complexity of the human EEG reflects the competition of neuronal cell-assemblies. The competition of cell assemblies was operationalized by the use of cognitive tasks of increasing cognitive complexity. During processing of the different tasks the EEG from 16 electrodes was analyzed by using a.) conventional power spectra and b.) a dimensional complexity algorithm as used in deterministic chaos analysis. Simple sensory tasks (tactile, visual, acoustic perception of objects) were compared with complex ones (imagery of the same objects and complex scenes) and various resting control conditions (e.g. eyes closed vs. eyes open).

Subjects with higher mental abilities (IQ) show an increased dimensional complexity of the EEG compared to resting conditions. The execution of complex cognitive tasks with a high degree of intermodal information exchange (e.g. imagery) have higher complexity of the EEG than simple sensory tasks. This is in agreement with the assumption of focused attention in highly emotionally involved Ss and thus reduced cell assembly competition. Conventional power spectra were unable to discriminate the different tasks and groups.

Supported by the DFG (Deutsche Forschungsgemeinschaft SFB 307/B1)

659.4

SPATIOTEMPORAL DYNAMICS OF PHASE TRANSITIONS IN THE HUMAN BRAIN. G.V. Wallenstein, S.L. Bressler*, A. Fuchs, J.A.S. Kelso. Program in Complex Systems and Brain Sciences, Center for Complex Systems, Florida Atlantic University, Boca Raton, FL 33431 USA.

Human brain electric potentials show abrupt state transitions that coincide with changes in sensorimotor coordination. Whole-head EEGs (61 electrode sites) were recorded (128 Hz, passband = 0.3 to 30 Hz) while a subject produced right index finger flexions in syncopation with auditory stimuli (1.1 KHz tone pulses, 80 ms duration). Stimuli were presented at a rate that increased after every ten stimuli in steps of 0.25 Hz from 1.0 Hz to 3.0 Hz. It is known (Kelso, DelColle & Schöner, 1990) that at a critical stimulus rate a spontaneous change occurs from the syncopated relation between stimulus and finger movement to one of synchronisation. Spatiotemporal analysis of the electric field patterns revealed: a) a widespread dominant EEG power spectral peak at the frequency of coordination; b) a 60 degree phase lag of the EEG relative to the stimulus during syncopated behavior at central, antero-central and left frontal sites, c) a transition at those sites from EEG phase lag to phase lead coincident with behavioral switching from syncopation to synchronization (at 1.75 Hz); d) no such change in a control condition in which finger movement was in synchrony with the stimulus throughout the trial.

Research supported by NIMH Grant MH42900, BRS Grant RR07258 and ONR Contract N00014-88-J-119.

659.5

P300 TOPOGRAPHY IS ALTERED BY STIMULUS MODALITY BUT NOT BY TASK DIFFICULTY. S.F. Faux* and S. Law. Dept. of Psych., Drake Univ., Des Moines, IA 50311

P300 event-related potentials were recorded from 20 college-aged students while performing visual discriminations of digits (0 vs. 1 through 9) and auditory discriminations of digitized syllables (ba vs. ga, za, etc.). Stimuli were presented singly; targets occurred with 0.15 probability. Age-matched subjects were assigned to one of two levels of stimulus discriminability ("undegraded" [U] N = 10; "degraded" [D] N = 10). Both groups performed identical visual/auditory tasks, except that in the D condition the stimuli were degraded by altering visual picture elements or superimposing white noise. The signal detection measure of task difficulty (A') was statistically equal across stimulus modalities, but differed by task difficulty (U: A'= 0.98; D: A'= 0.93, $F(1, 16) = 11.18$, $p < 0.001$). Mean P300 peak component latencies (Pz site) differed only by task difficulty (U = 430 msec; D = 471 msec; $F(1, 16) = 4.78$, $p < 0.05$). Normalized P300 peak amplitudes (Fz, Cz, Pz sites) showed statistically different topographic profiles by modality ($F(2, 17) = 18.11$, $p < 0.001$), but not by task difficulty. Results suggest that P300 neural sources are not identical by stimulus modality.

659.6

INFLUENCE OF TASK DIFFICULTY AND EXPECTEDNESS ON THE P300 ELICITED BY A WARNING STIMULUS. C. Busch[1]*, G. Wilson[2] and P. Ullsperger[3]. 1) LTSI Inc., Dayton. OH 45431-7258 2) Armstrong Laboratory, AF, WPAFB, OH 45433-7022 3)Bundesanstalt fur Arbeitmedizin, Berlin, Germany.

The topographical distributions of scalp field potentials were evaluated during explicit tests of Helson's Adaptation-Level (AL) Theory (1964). This model posits the intensity of response to be a function of the mental distance from a prevailing level of adaptation with respect to the task-relevant internal dimension and has recently been adapted by those interested in evaluating the P300 component of the ERP. According to AL theory, the greater the stimulus information from the reference point, the greater the P300 amplitude expected.

In the present studies, we manipulated set size (difficulty), probability and randomness (expectedness) of number sequences presented for memorization on a CRT. Each task stimulus was preceded by a single digit warning stimulus (1 to 5) announcing the difficulty of the subsequent memory set (varied from 1 to 8 digits). Eleven subjects participated in three separate studies manipulating probability (10%-60%) and randomness (equiprobable random or blocked). Scalp EEG were recorded from 21 sites distributed according to the International 10-20 system and stored on disk for off-line analysis.

Evaluation of topographical maps indicated that between 250-650 ms post-stimulus onset, sites Cz, P3, Pz and P4 differed more than 1 sd from the calculated average across the scalp and these sites were selected for further analysis. Results indicated that, as predicted by AL theory, the P300 area increased as a function of distance from the level of adaptation (ie the geometric mean of stimulus occurrence) when the level of difficulty varied randomly and further suggested an interactive contribution of expectedness (adaptation) and preparedness (difficulty) in a non random paradigm.

659.7

WITH NETWORK MODELING, TOPOGRAPHIC EEG PREDICTS MEMORY CAPACITY AND RT IN CONTINUOUS PERFORMANCE TASKS. J.R. Moeller, B. Luber, E. Rubin* & H.A. Sackeim Dept. of Biological Psychiatry, NY State Psychiatric Institute, New York, NY 10032

In neuroimaging studies of cognitive activation, images from different task conditions are subtracted to isolate local changes in physiological activity that are associated with specific cognitive operations. However, given the multiple interconnections among brain regions, it is doubtful whether functional changes that occur at local sites can be limited by experimental design to one particular type. To address this critical measurement issue, we have used linear covariance modeling to capture the multidimensional nature of functional activity at individual brain locations. We have in applied PCA techniques to topographic EEG data to extract both "networks of regional physiological activity" and between-condition changes in "network load." Regional networks are interpreted as physiological manifestations of individual cognitive operations; and, between-condition alterations in network load, as changes in the magnitude of information processing transacted by a cognitive operation. In the analysis of group EEG data, our capacity to segregate not only a subject-dependent global factor from subject differences in network load, but also subject load differences from subject variation in structural and functional anatomy, is a critical new component of network analysis. Topographic EEG was collected from 15 male subjects who performed visual recognition tasks consisting of either novel figure or word stimuli. Two levels of memory load were used: a set size of one, and a set size that was titrated so that every subject performed at an accuracy level of 65% correct. Subject differences in network load, but not in activity at individual electrode sites, were significantly correlated with subject differences in performance. Different networks predicted: RT(set size one) with $R^2 > 76\%$ for words and figures; RT(titrated set size) with $R^2 > 80\%$; and, titrated set size with $R^2 > 62\%$. The network topographies with performance correlates had reasonable interpretations in terms of the current neurobiological information about declarative and implicit memory systems. The same modeling principles are applicable to 3D metabolic imaging.

659.8

ELECTROPHYSIOLOGICAL DIFFERENCES ASSOCIATED WITH THE DETECTION OF SIMPLE FEATURES AND FEATURE CONJUNCTIONS. R. Soria*, R. Srebro. Vision Research Lab., Univ. of TX Southwestern Med. Ctr., Dallas, TX 75235.

Evoked potential (EP) scalp topography was studied during visual search tasks which involved either parallel or serial processing. The parallel processing tasks required the detection of a simple feature either color or orientation. The serial processing task involved the detection of the feature conjunction color and orientation. Identical stimuli were used for both parallel tasks and a similar stimulus for the serial task. The subjects made a button press response. Both stimulus-locked and response-locked averages were calculated across 31 elecrodes equally spaced over the posterior half of the scalp. The global field power (GFP) was used as a reference free index pointing to EP scalp fields of interest. A chi-square test was used to test for significant differences in shape of scalp fields. Results (n=4) revealed no significant differences in scalp fields for the two parallel processing tasks. However, significantly different scalp fields between both parallel processing tasks and the serial processing task were identified for the epoch surrounding the button press (peri-decisional epoch), and for ca. 200ms after stimulus presentation. This suggests that different neural populations subserve the decision making process during parallel and serial tasks. Differences found at ca. 200 ms suggest that serial processing tasks require visuo-spatial processing for the conjoining of features.

659.9

NORADRENERGIC MODULATION OF AUDITORY AND VISUAL P300 IN PARIETAL-TEMPORAL CORTEX. J.A. Pineda*, K. Shafer, M. Belmonte. Depts of Cognitive Science and Neuroscience, University of California, San Diego, La Jolla, CA 92093

Human clinical studies have suggested that the parietal-temporal junction may be an important source of cortical P300 activity. This area is also characterized by one of the densest noradrenergic projections from the locus coeruleus. Our previous studies have argued that this widely distributed system plays a critical role in the genesis and modulation of P300-like components. In this study, we examined the relationship between noradrenergic activity in the parietal-temporal junction and auditory and visual P300-like activity.

Event-related potentials (ERPs) were recorded from chronically implanted epidural electrodes in squirrel monkeys (Saimiri sciureus). Recordings occurred before and after microinjections of L657,743, an adrenergic alpha-2 antagonist, or clonidine, an alpha-2 agonist, into the left parietal-temporal area. An 80-10-10 oddball paradigm was used consisting of stimuli presented every 2 secs. One tone was presented on 80% of the trials, a higher pitched tone on 10% of the trials, and a small, yellow rectangle on the remaining 10% of the trials. Baseline ERPs showed large, widely distributed P300-like responses to both infrequent stimuli. The amplitude of the auditory but not the visual P300-like component was significantly reduced over both hemispheres following L657,743 injections. In contrast, clonidine enhanced the magnitude of these potentials relative to baseline ERPs. These data suggest that the parietal-temporal junction is a source of auditory P300-like potentials, that noradrenergic activity in this region is critical for their genesis, and that modulation of P300 sources in the left hemisphere affects potentials recorded over the right hemisphere.

659.10

HUMAN AND MONKEY N400-LIKE RESPONSES TO FACES IN A PRIMING PARADIGM. C. Nava*, J.A. Pineda, G. Stickney. Dept of Cognitive Science 0515, University of California, San Diego, La Jolla, CA 92093

Face recognition is important for social communication in humans and non-human primates. Recent human event-related potential (ERP) studies have investigated whether the recognition of semantically associated pairs of faces elicits components similar to the linguistic N400. It has been found that incompatible faces produce these components presumably because memory representations must be activated for matching to occur, and only incompatible matches elicit N400-like responses. The focus of this study was to compare human and monkey N400-like responses related to the perception and recognition of faces.

ERPs were recorded from scalp electrodes in humans and from epidural electrodes in squirrel (Saimiri sciureus) and macaque (Macaca fascicularis) monkeys. Paradigms consisted of slides of real faces presented upright in match or mismatch pairs. Humans and squirrel monkeys were shown species match (human-human; monkey-monkey) or mismatch (monkey-human; human-monkey) pairs. All faces were familiar to human subjects but only the human faces were familiar to the squirrel monkeys. In a second paradigm, macaque monkeys were exposed to familiar (roommates) and unfamiliar faces (different monkey species) in match or mismatch pairs. Human ERPs exhibited larger N400s to mismatch trials for both monkey and human faces. Squirrel monkeys showed larger negativities to the unfamiliar mismatch monkey faces. In macaque monkeys, larger potentials occurred to the familiar match compared to the mismatch trials. These data indicate that human and non-human primates display N400-like negativities to faces. However, the conditions that elicit these components are different, suggesting that although analogous mechanisms exist, there are different overlapping processes involved.

659.11

APPEARANCE OF PARIETO-FRONTAL MIDLINE (PFm) THETA ACTIVITY RELATED TO PERFORMANCE IN HYPERACTIVE AND NORMAL CHILDREN S.J.Laukka*, P. Silven, J.Lindqvist, T.Järvilehto. Laboratory of Developmental Neuropsychology, University of Oulu, Linnanmaa P.O. BOX 222, SF-90571, Oulu, Finland.

Theta-activity (PFm theta,4-7Hz) was investigated during goal-directed behavior. In a simulated traffic situation subject had to find the right way for driving a'car' through a set of roads. Two interdependent decisions had to be made at two crossroads. Feedback about quality of performance was given. EEG was recorded from Fz and Pz. Theta-activity during seven consecutive phases (1610 ms) of behavior was analysed.

Correct driving at the first crossroad was associated with significantly more theta in the frontal area in the hyperactive children than in normal ones. On the other hand, for the normal children during wrong driving the first traffic sign was associated with significantly more theta in the parietal area than for the hyperactive children.

The results indicate a maturational lag in the hyperactive children.

659.12

REGIONAL RESPONSE CHARACTERISTICS OF AUDITORY P3 IN YOUNG ALCOHOLICS H.L. Cohen*, W. Wang, B. Porjesz and H. Begleiter
Neurodynamics Lab, Hlth Sci. Ctr, Bklyn, Bklyn, N.Y. 11203

An auditory oddball paradigm was used to assess the P3 component of the event related potential (ERP) in a group of medication - free, chronic alcoholics ($N = 25, \bar{X} = 29.3$) and an age - matched control ($N = 25, \bar{X} = 27.2$) group. Each subject received a binaurally presented series of high (1600 Hz) and low (600 Hz) frequency tones. Either tone could be designated as the rare tone (12.5% probability) depending on whether the subject number was odd or even. When the subject detected the rare tone, he made a button press as quickly as possible that recorded his reaction time (RT). Scalp recordings using the entire 10/20 System, as well as interpolated placements, were made from 31 electrodes. For statistical analyses, five electrode groups were created: frontal (F), central (C), parietal (P), occipital (O) and temporal (T). MANOVA were used to compare group differences in P3 amplitudes and latencies in each region. The results indicated that control amplitudes were significantly greater in C, P, O and T regions and control latencies were significantly longer in C and P regions. Regional response differences between the groups were also compared with measures of surface energy density (SED; Wang et al.,in press). In the control group, surface energy components in both temporal regions and in the right parietotemporooccipital region, were greater than those in the alcoholics. Our results demonstrate that young, abstinent alcoholics manifest regional deficits in auditory P3 morphology. These findings are further supported by measures of surface energy density.

Supported by Grants AA-05524 and AA-02686 from NIAAA to HB.

659.13

LONGITUDINAL EVENT-RELATED POTENTIAL MEASURES INDEX STAGES OF HIV-1 INFECTION.
M.M.Schroeder*,L.Handelsman[1],W.Ritter.Albert Einstein College of Medicine, [1]Mt.Sinai College of Medicine.

HIV-1 infection of the brain causes delays in auditory event-related potentials (ERPs). The purpose of this study was to investigate the stability of these group differences over a short time period suitable for monitoring the progression of cognitive impairment, and/or effects of anti-viral treatments.

We recorded auditory ERPs at two time points, 3 months apart. Subjects were 17 former parenteral drug users (PDUs): seronegative (6); seropositive, stage II (7); and seropositive, stage IV (4). Also 8 non-PDUs were normal controls. The stimuli were presented in an auditory oddball paradigm: there were five response conditions: Go/No-go, Count, Simple Response, Simple Count, and Ignore. ERPs were recorded from Fz, Cz, and Pz. Peak latencies and change scores of P1, N1, N2, and P3 were submitted to repeated measures ANOVAs.

N1 was delayed for the stage IV subjects compared to the other three groups (p=.02). P1 exhibited a similar, non-significant trend. N2 was delayed for both seropositive groups (p.=.008) compared to both seronegative groups. P3 showed a similar non-significant trend. Change scores for P1 and N1 increased more for the stage IV group compared to the other three groups. Change scores for N2 and P3 indicated practice effects for seronegatives in the second recording which augmented group differences.

Group differences remained stable over two recordings and a trend for progression in latency was seen in the change scores for both seropositive groups.

659.14

P300 AND SIMULATED WEIGHTLESSNESS. K.L. Murray, S.J. Benshoff and D.L. Molfese*. Southern Illinois University at Carbondale, IL 62901.

Weightlessness has effects on both the physiological and cognitive functioning of humans. The Head-Down-Tilt (HDT) model has been used to simulate weightlessness on Earth. This electrophysiological (ERP) study assessed the effects of HDT on P300 amplitude and latency. Peak analyses were performed and then input to separate ANOVAs for the amplitude and latency data. The amplitude data showed main effects for Position (HDT vs. control) and for Electrode (frontal, temporal, and parietal). A Position X Electrode interaction was also noted. The latency data ANOVA showed a main effect for Hemisphere (left vs. right). These data, particularly the amplitude data, indicate that there are attentional deficits (as indexed by an attenuated P300) associated with HDT. The implications for astronauts engaged in space exploration are significant.

660.1

COMPUTATIONAL CONSTRAINTS THAT MAY INFORM HIPPOCAMPAL & HIPPOCAMPO-CORTICAL CONNECTIONS. A. Treves*[1] and E.T. Rolls[2]. [1]SISSA, Biophysics, via Beirut 2, 34013 Trieste, Italy and [2]Dept. of Exp. Psychology, Univ. of Oxford, Oxford OX1 3UD, UK.

A candidate theory regards the hippocampus as an intermediate-term buffer store for certain types of information, and its CA3 region as an autoassociative memory network where a highly compressed neuronal representation of the data is generated, is immediately stored on recurrent collateral connections, and is later retrievable and available for directing longer-term storage in neocortex. We have previously shown (Treves and Rolls, 1992, *Hippocampus* 2, 189) how this theory constrains the organization of CA3 connections in a way consistent with observation. Here we report that the theory, coupled with the principle of maximal informational efficiency, implies further constraints on CA1 organization as well as on return projections to neocortex. First, an analytical estimate of the information content of the firing pattern induced in CA1 by retrieval of a given CA3 representation indicates the need for a) Hebbian plasticity of the Schaffer collaterals and b) nearly equal numbers of such inputs for each CA1 pyramidal cell. Second, an analytical estimate of the number of firing patterns that can be recalled in neocortex via hippocampo-cortical bacprojections indicates the need for c) Hebbian plasticity of the backprojections, d) gradual divergence through several stages of the backprojecting system and e) extensive numbers of backprojecting inputs of hippocampal origin for each neocortical pyramidal cell.

Supported by the HFSP, the MRC of the UK and the INFM.

660.2

UNIQUE CONTRIBUTION OF HIPPOCAMPAL FIELD CA3 TO INTEGRATION OF DISPARATE CUES OVER TIME. M. Taketani, R. Granger*, J. Ambros-Ingerson, R. Myers, G. Lynch. CNLM, U. Calif., Irvine, CA 92717.

Hippocampal field CA3 is a unique exception to the extremely sparse connectivity characterizing most telencephalic networks: CA3 exhibits relatively dense recurrent associational fibers, enabling dynamical activity over time, and suggesting its possible role as a reverberating short-term memory system.

Anatomically and physiologically rich simulations of field CA3 have shown that brief afferent activation can generate recurrent activity lasting for 100s of milliseconds, with some specificity to the initial input. These findings led to the suggestion that field CA3 might function as a kind of "holding memory," serving to bridge the temporal gap between transient neural signals over behavioral time (Taketani et al., Soc.Neurosci.Abs., 506.6, 1992). To serve as a holding memory, however, recurring activity patterns would have to contain patterns repeated regularly over time, so that two cues separated by time could become integrated.

Physiological rules for the induction and expression of LTP in response to time-varying afferent signalling (Larson & Lynch, Brain Res., 489:49, 1989) were incorporated into simulations of CA3. Repeated afferent stimulation at the theta rhythm leads to the potentiation of a small number of synapses in the model, strengthening a sequential chain of target cells. Subsequent brief (10 msec) stimulation with a learned input pattern sets up a sequence of recurring responses in the model, specific to the input, and repeating regularly with every theta wave.

Learning of these recurring patterns by CA3's primary target projection structure, CA1, enables field CA1 to recognize the sequence of two cortical (perforant path) afferents A and B even when they are separated by many seconds. The coordinated activity of fields CA3 and CA1 can therefore recognize the constituents of a coherent scene even when viewed from different vantage points, and at different rates of scanning. The specific hypothesis is therefore forwarded that this coordinated functional capability of fields CA3 and CA1, arising from their unique anatomical designs and the physiological operation of their LTP induction and expression rules, is what the hippocampus uniquely contributes to the processing of spatial information, as well as to non-spatial tasks requiring the integration over time of disparate sensory cues. (Supported by ONR N00014-89-J-1255 & N00014-92-J-1625).

660.3

SENSORY GATING IN SCHIZOPHRENIA: A COMPUTER MODEL OF THE CA3 HIPPOCAMPAL NEURAL NETWORK. K.A. Flach[1]*, P. Bickford[2], L. Adler[2], G.A. Gerhardt[2], R. Freedman[2], R. MacGregor[1]. [1]Dept. Of Aerospace Engineering, University of Colorado, Boulder, 80309, [2]Dept. Of Psychiatry, University of Colorado Health Sciences Center, Denver, 80262

Sensory gating deficits in schizophrenic patients have been demonstrated with an auditory evoked potential (EP) conditioning-testing paradigm. In this paradigm, the amplitude of the P50 EP is greatly decreased in response to the second of two paired auditory click stimuli in normal control subjects, but not in schizophrenic patients. Animal studies in rats, which also show a decreased response to the second click, have suggested that this gating occurs in the CA3 region of the hippocampus. Gating in the rat is lost if nicotinic binding sites are blocked. In schizophrenic patients, this gating is briefly enhanced by nicotine. A computer model of the CA3 region, using techniques discussed in R. MacGregor, Theoretical Mechanics of Biological Neural Networks, Academic Press, 1993, is used to study this phenomena. A scaled down model of the CA3 region is simulated with a 660 cell network which is defined in terms of twelve synaptic junctions: 4 internal and 8 external. Two cell populations are modeled, pyramidal cells and GABAergic cells, each by a single compartment circuit model. Using this model, the amplitude of the response to the second tone is decreased, in agreement with the data from normal controls. By modeling the blockade of nicotinic input to the CA3 system, gating is lost, as has been found in the animal research. The schizophrenia model hypothesizes that there is an increase in excitability of CA3 neurons due to the decreased nicotinic input from the septum. By doing this, the system showed a slight decrease in response to the first of the two paired stimuli, and a marked increase in response to the second, consistent with the findings of impaired auditory gating in schizophrenic patients. Thus, auditory sensory gating deficits can be successfully modelcd using a biological neural network approach. Supported by NIMH: SP50 MH44212-06.

660.4

MEMORY CAPACITY OF REALISTIC HETEROASSOCIATIVE NETWORKS. *E. P. Cook* & D. Johnston*, Division of Neuroscience, Baylor College of Medicine, Houston, TX 77030

We have examined the hypothesis that realistic levels of synaptic potentiation can be used as a mechanism for selective associative memory storage and recall in a biological neural network. We compared the memory capacity of heteroassociative feedforward networks in which the individual elements varied in their biophysical realism. Networks constructed from three types of neuron models, each with an increasingly realistic complement of membrane channels, were compared to the ideal network constructed from binary threshold elements.

Our three realistic models were reconstructed hippocampal pyramidal neurons with the following different channel distributions and types: 1) passive dendrites with H&H-like Na^+ and K^+ channels in the soma, 2) passive dendrites with a biophysically realistic soma, and 3) a complete biophysically realistic model that included active dendrites (M. Migliore et al. Soc. Neurosci. Abstr. (1993)). Individual synapses, uniformly distributed in the dendrites, were randomly potentiated by changing peak synaptic conductance from 250pS to 500pS. The effects of recurrent and feedforward inhibition were also examined. NEURON by Michael Hines was used in all computer simulations.

Using the artificial binary threshold neuron, it can be shown that the memory performance of the feedforward heteroassociative network is dependent on the model's ability to detect small changes in synaptic inputs. Only the two most realistic models (2 and 3) showed enough sensitivity to small changes in synaptic strength to be effective in our heteroassociative memory network. This sensitivity was expressed as a nonlinear increase in the latency of the first action potential after a small reduction in synaptic input. In model 3, this effect on latency was influenced by voltage and Ca^{2+}-dependent channels in the dendrites. When this model was used in a realistic heteroassociative memory network that included recurrent inhibition, the memory performance approached that of the ideal artificial model. (Keck Foundation and MH48432).

660.5

MODULAR ORGANIZATION OF CORTICAL NETWORKS OPTIMIZES INFORMATION PROCESSING CAPACITY. J. Ambati* and R.K.S. Wong. Neural & Behavioral Science Program and Dept. of Pharmacology, SUNY Health Science Center, Brooklyn, NY 11203.

Information is likely processed in cortical networks through coordinated spatiotemporal neuronal activity. Synchronous firing of neuronal assemblies is found in many cortical areas (*e.g.*, Miles & Wong, *J. Physiol.* 388: 611, 1987; Gray & Singer, *Proc. Natl. Acad. Sci. USA* 86: 1698, 1989). Interestingly, the number of neurons per radial column in the neocortex of most mammals is virtually identical (Rockel *et al. Brain* 103: 221, 1980). We suggest that this remarkable invariance serves to optimize the information processing capacity (IPC) of these networks. A model is developed to demonstrate that IPC of a network is optimized by partitioning it into neuronal ensembles, each of which has an identical number of neurons, independent of the total network size. This model predicts that the fundamental unit of representation in the CA3 region of hippocampus should contain about 2,500 neurons, which is in accord with experimental evidence (Thompson & Best, *J. Neurosci.* 9: 2382, 1989; Muller, *et al. Hippocampus* 1: 243, 1991) and simulation data (Traub & Miles, *Neuronal Networks of the Hippocampus*, 1991).

660.6

MODELING OF SPATIAL VS BEHAVIORAL FIRING OF HIPPOCAMPAL COMPLEX SPIKE CELLS.
R.E. Hampson*, T. Bunn, D.R. Byrd, S.A. Deadwyler. Bowman Gray School of Medicine, Wake Forest Univ., Winston-Salem, NC 27157.

A neural network model of hippocampal complex spike cells has been developed to simulate both spatial and task-specific correlates of neural activity for the same neurons. The model is based on a three-layer forward-only counter-propagation network (Hecht-Nielson, 1987) which has been designed to incorporate position, movement trajectory, and behavioral significance of locations within the modeled environment. Inputs to the first layer consisted of position sequences designed to simulate the movement trajectories exhibited by freely-moving rats (Deadwyler *et al.* 1989). Connections to the hidden and output layers "self-organized" in response to trajectory inputs such that firing probabilities of individual simulated neurons corresponded to adjacent positions along the trajectories. Units of the output layer showed strictly spatial firing correlates similar to those of hippocampal place cells. Task-contingent behavioral constraints were incorporated into the model via additional inputs at the hidden and output layers as defined in the design of a counter-propagation network. These additional inputs provided modulation of the trajectory-based place fields similar to the effects of rotating environmental cues as well as alteration of the behavioral relevance associated with particular locations.

Simulated neural activity derived from the model is compared with recordings of CA1 and CA3 neurons to test validity of the model. Patterns of activity between the simulated neurons (i.e. connections and weights) are examined to determine what aspects of hippocampal functional connectivity may be used to differentiate spatial and cognitive correlates of hippocampal neural activity.
[Supported by NIDA grants DA03502, DA00119 and AFOSR grant 90-0092 to S.A.D.]

660.7

A NEURAL NETWORK FOR STABLY SELF-ORGANIZING ARBITRARY RECOGNITION CODES. J. W. L. Merrill*. Center for Complex Systems, Florida Atlantic University, Boca Raton, FL 33431.

Recognition of items similar to those which have been encountered before is essential for behaving organisms. This recognition can be mediated by a self-organized abstract code, which might be either a *maximally compressed* code, in which individual codewords provide mutually exclusive information about a percept, or a *distributed* code, in which individual codewords provide complementary information about a percept. A major goal of neural network research has been the construction of neural architectures capable of stably self-organizing such codes in a non-stationary environment. Neural architectures for the self-organization of maximally compressed codes have been previously described by Carpenter and Grossberg and their colleagues. Here, a neural architecture for the stable self-organization of arbitrary recognition codes in a non-stationary environment is presented.

Self-organization of a distributed code is complicated by the possibility of interactions among the various codewords that represent a given item. Activity in one codeword could distort the class of patterns recognized by a different codeword, leading to instability in the representation of input patterns. The spectrum of instabilities arising in this fashion is shown to be completely characterizable in terms of two particular examples, the hybrid learning problem and the spurious generalization problem. It is shown that the hybrid learning problem can be avoided if the number of codewords being changed at any given instant never exceeds one, even if the number of codewords altered during a single learning bout is large. A neural mechanism for enforcing this requirement through serialization is proposed and discussed. This mechanism requires that at least one signaling pathway has a time constant much slower than that of other important pathways. Analysis of the spurious generalization problem shows that it arises in distributed coding systems because a collection of codewords could represent an input pattern well, even when some element of that collection matches the pattern very poorly. Resolution of this problem is shown to depend upon distinguishing between the match provided by a broad collection of codewords and the match provided by each individual element of that collection. It is shown that the serialization mechanism proposed for the solution of the hybrid learning problem provides exactly the extra degree of freedom necessary to make this distinction.

660.8

A CORTICAL NEURAL NETWORK MODEL OF TEMPORAL INFORMATION PROCESSING. D. Buonomano*, M. Merzenich. Keck Center, UCSF, San Francisco, CA 94143-0732.

The majority of neural network models have focused on processing spatial information, i. e., the relevant information is carried in the spatial pattern of active input channels. Biological information processing relies on utilizing spatial and temporal information. For example in speech recognition, information is encoded not only by which channels are active, but by the temporal relationships within and between activated channels. Our hypothesis was that known properties such as slow inhibitory currents and paired-pulse facilitation (PPF) would permit extraction of temporal information by altering the dynamics of the network in a state-dependent fashion. As an initial step toward understanding temporal information processing, we focused on a purely temporal task: frequency discrimination. We modeled a cortical circuit composed of modified integrate-and-fire elements with a slow inhibitory current (t=50ms), and PPF (peak=80ms). The architecture of the network was based on thalamo-cortical -> L-IV -> L-III connectivity with feed-forward excitation, and feed-forward and feed-back inhibition. The network was initially trained to discriminate between pulses at 5 frequencies (20-60 Hz). Thus, there were 5 output units which received input from all LIII excitatory units (LIII-E). The only plasticity in the network was between the LIII-E units and the output units (all time constants were nonvarying). The plasticity obeyed a supervised learning rule; during presentation of a given input frequency, active connections to the corresponding output unit were increased while active connections to other output units were decreased. Our results show that after training the output units not only respond preferentially to their trained frequency, but generalized as demonstrated by exhibiting a tuning curve that peaked at approximately their trained frequency. In the absence of noise, the first pulse will always produce the same activity pattern, but depending on when the second pulse arrived a different spatio-temporal activity pattern was generated that could be used to encode temporal information. These results suggest that time-dependent properties such as slow inhibitory currents and PPF generate enough state-dependency to alter the dynamics of the network in a time-dependent manner. Supported by NIH grants NS-10414 and NS-07067, the Coleman fund and HRI.

660.9

A STOCHASTIC MODEL OF CEREBELLAR GRANULE→PURKINJE SYNAPSES M. D. Mauk* & G. T. Kenyon. Univ. of Texas Medical School, Houston, TX 77225.

Empirical studies suggest that granule cell to Purkinje cell (Gr→PC) synapses in the cerebellum decrease in strength when active during a climbing fiber (CF) input, and increase in strength when active in the absence of a CF input (i.e. generalized Long-Term Depression or LTD). LTD involvement in Pavlovian eyelid conditioning is suggested by observations that activating CFs is necessary and sufficient for the reinforcing properties of the US, yet this idea is complicated by the low but regular spontaneous activity displayed by CFs. Specifically, how can US-evoked CF activity convey significant information against this background of spontaneous activity? Since this spontaneous activity gives rise to probabilistic pairings between Gr→PC synaptic activity and CF inputs (US-evoked and spontaneous), we propose that generalized LTD is a stochastic process. We investigate this idea using a formal, stochastic description starting with the three equations shown below. Equation 1 is the expected change at the ith Gr→PC synapse predicted by generalized LTD stated in terms of the probability of synapse activity, probability of CF input, and constants for the unit increases and decreases in strength, equation 2 is the probability of PC activity stated in terms of the sum of its Gr inputs, and equation 3 is the probability of CF activity in terms of its intrinsic and US-evoked excitatory drives and modulation via cerebellar output. These equations yield several predictions: *i*) in the absence of USs, CF activity is regulated so that the expected net change in Gr→PC synaptic strength is zero, *ii*) average synaptic input to PCs is maintained constant, *iii*) during an idealized CS-US trial the strength of Gr→PC synapses either remains constant or decreases thus, after the trial, some synapses must increase in strength to renormalize average synaptic input, and *iv*) the expected change in synapse strength for a CS-US trial and subsequent renormalization is described by equation 4. This equation predicts that the change in the strength of a given synapse for a CS-US trial is proportional to the extent to which the synapse encodes more information about the CS than the population of synapses.

1) $\Delta w_i = p_i((1-P_{CF})\delta_n - P_{CF}\delta_p)$ 2) $P_{PC} = \sum w_i p_i$ 3) $P_{CF} = (E_I + E_{US} + P_{PC})$

4) $\Delta w_i = \delta_p((p_i \sum p_i p_i^{cs} / \sum p_i^2) - p_i^{cs})$

660.10

NEURAL NETWORK BACKPROPAGATION IS ANALOGOUS TO RETROSYNAPTIC CHEMICAL TRANSMISSION. Daniel Gardner* Dept. of Physiology, Cornell Univ. Med. Coll., New York, NY 10021.

Neural network models use variable-strength synapses to interconnect layers of simplified neuron-like elements. By using learning rules to adjust synaptic weights so as to reduce error, such networks evolve successful algorithms for information processing. However, back-propagation and similar learning rules for error minimization require retrosynaptic transmission of error signals. Is this property sufficiently non-neuromorphic to make such nets uninteresting to neurobiologists?

Toward the development of neuromorphic network models, I analyze backpropagation neurobiologically to suggest a hypothetical yet plausible correspondence to retrosynaptic chemical transmission, recently implicated in LTP and other synaptic plasticity (Gardner, D. *The Neurobiology of Neural Networks*. Cambridge, MA: MIT Press, 1993).

In these nets, convergent and divergent presynaptic and postsynaptic cells with activity y are linked by synapses of weight $w_{post,pre}$. Synaptic weight optimization utilizes error δ_{post} backpropagated from each post-synaptic cell and used two ways. Presynaptic error depends on δ_{post} and synaptic weight $w_{post,pre}$: $\delta_{pre} \propto \sum_{post} \delta_{post} w_{post,pre}$. The same δ_{post} scaled by presynaptic activity y_{pre} alters $w_{post,pre}$: $\Delta w_{post,pre} \propto \delta_{post} y_{pre}$.

Via two presynaptic receptors, a retrosynaptic transmitter signalling postsynaptic error δ_{post} could implement this dual role—contributing to presynaptic error by activation of a presynaptic receptor encoding synaptic strength, perhaps uniformly distributed at terminals, and changing synaptic weight via another presynaptic receptor modulated by depolarization or increased Ca^{2+}, thereby combining error and presynaptic activity.

Supported by NIH: NS-11555 and ONR: N00014-90-J-1460.

660.11

FORAGING IN AN UNCERTAIN WORLD USING PREDICTIVE HEBBIAN LEARNING. P.R. Montague*[1,2], P. Dayan[1], T.J. Sejnowski.[1] Computational Neurobiology Lab, The Salk Institute[1], La Jolla, CA 92075, and Division of Neuroscience, Baylor College of Medicine[2] Houston, TX 77497.

There is evidence in both vertebrates and invertebrates that diffuse systems may report prediction errors to their target structures through appropriate modulation of firing rates in response to events in the world that predict future reward. We present a neural model which utilizes diffuse modulatory systems to implement a predictive version of a Hebbian rule and embed this rule in a simple neural architecture. The predictions in such a model represent the expected amount of reinforcement. We demonstrate the connection between this model and an adaptive technique called the method of temporal differences and we propose a specific hypothesis for how such predictions are made in a real brain. Moreover, we challenge this neural model with a behavioral task involving foraging for food. When required to forage in a stochastic environment, the model exhibits the foraging strategies seen in the behavior of bees and a number of other animals and provides insight into how the stochasticity of the world drives this behavior. This predictive model suggests a unified way in which neuromodulatory influences are used both to bias cell activity and to control synaptic plasticity - two roles previously suggested for diffuse ascending systems. Taken together, these results suggest that predictive Hebbian rules for synaptic plasticity offer a simple yet powerful framework for learning and provide a specific and testable role for diffuse neuromodulatory systems.

660.12

DISSIPATIVE STRUCTURES: A UNIFIED HIERARCHICAL APPROACH TO BRAIN FUNCTION. Wayne Hoss*, Department of Medicinal and Biological Chemistry and Center for Drug Design and Development, University of Toledo, Toledo, OH 43606

The thermodynamics of irreversible processes provides a unified framework for a consideration of the nervous system in terms of a hierarchical set of components. At each level, properties can be described in terms of steady states and dissipative structures that impose order in the face of continuous entropy production. This occurs because these phenomena are coupled to other irreversible processes, e.g., diffusion and chemical reaction, that maintain sufficient overall rate of entropy production. Familiar examples of steady states include resting membrane potential and resting neuronal discharge rate. Action potentials and neuronal bursting activity are the corresponding dissipative structures, which are ordered states beyond an instability (e.g., neuronal threshold) and share a common requirement for at least one positive feedback or feedforward step in their mechanisms. Expressions for the rate of entropy production for these and other steady states and dissipative structures together with their hierarchical linkages are presented. Long-term potentiation and calcium-induced calcium release are additional examples of dissipative structures, having received recent attention at the biochemical level. The central hypotheses of this line of reasoning are that dissipative structures occur naturally and pathologically within the CNS at many levels, that they are linked in a hierarchical fashion and that they are important for higher brain functions, including perception, cognition, emotion, learning and memory. It is suggested that research directed towards the detection, formation, maintenance and relaxation of dissipative structures is fertile ground for developing new tools for understanding brain function and novel diagnostic and therapeutic approaches to neurological diseases.

660.13

THE GEOMETRY OF WIN-SHIFT AND WIN-STAY BEHAVIOR.
A.G. Gittis* and J. Falleroni, Psychology Department, Westminster College, New Wilmington, PA 16172.

As a task thought sensitive to both limbic and neocortical working memory circuits, win-shift and win-stay have been conceptualized as a rodent analog of the primate match and non-match to sample memory procedures. As with primates, rodents find tasks with a shift (non-matching) contingency much easier. This study investigates whether the ease in shifting (non-matching) is due to the geometric arrangement of the arm choices.

Long-Evans hooded rats were tested in an apparatus in which a start alley led into arena in an X configuration. A task trial consisted of a forced and free component. On the forced component access to only one arm was possible. On the free component animal's had a choice between two arms, the one open on the forced component and one of the remaining alternative arms. Animals were assigned to groups in which there was a shift or stay contingency. On each of 5 days an animal received 24 test trials in which the various permutations of arm pairs were investigated.

It was found that maximal shifting behavior occurs when the midline is crossed. Little shifting behavior occurs when two choices were to one side of midline. Staying was not induced by any geometric configuration but stay animals exhibited preferences for turns which tended to "circle" them back to the point of origin. It is concluded that alternation behavior is not so much a "working memory" phenomena but a pattern co-determined by the geometry of choices and the task contingencies.

660.14

WITHIN SESSION VARIATION IN BEHAVIOR: AN UNTAPPED SOURCE OF DATA AND POTENTIAL CONFOUNDS FOR BEHAVIORAL PHARMACOLOGY. J. M. Roll*, F. K. McSweeney, R. E. See, B. P. Kelly and W. M. Meil. Dept. of Psychology, Washington State Univ., Pullman, WA 99164-4820.

Inherent in both the methodology and theory of operant psychology is the assumption that rate of responding is constant within an experimental session. The incorporation of operant methodology by behavioral pharmacologists has resulted in the constant rate assumption becoming central to behavioral pharmacology as well. Recent research (McSweeney, 1992; McSweeney & Hinson, 1992; McSweeney & Roll, 1993) has unequivocally demonstrated that rate of responding varies in a robust, systematic fashion within experimental sessions. This constitutes a major source of uncontrolled variation in studies that utilize operant methodology, introducing a potentially major confound. However, systematic changes in rate of responding within an experimental session also provide a potentially rich source of untapped dependent measures. This paper outlines and discusses the potential confounds introduced by recognizing within session changes in response rate. It also presents preliminary data demonstrating the effects of several drugs (cocaine, ethanol, and caffeine) on within session patterns of responding and shows how interpretation of these results can further our understanding of within session patterns of responding.

660.15

MICRO AND MACRO-STRUCTURE OF LEARNING IN THE ACTIVE AVOIDANCE. A. Giuliani, O. Ghirardi, A.M. Mancini, M.T. Ramacci, A. Carta* and A. Colosimo [1]. Inst. for Res. on Senescence Sigma Tau, 00040 Pomezia, Italy; [1] Dept. Biochem. Sci., "La Sapienza" Univ. of Rome, Italy

The dynamics of learning in the Active Avoidance (shuttle-box test) was analyzed at the level of the trials as well as at the level of daily sessions formed by many trials.

The two temporal scales (small scale of the trials and large scale of the sessions) highlighted very different features with no linear relation between them. By means of a renormalization group approach we investigated the intermediate scales which resulted to be coherent with the small scale but unrelated with the large scale.

This result is dynamically explained by the enormous difference between the rates of learning at the two extreme scales (4 orders of magnitude) and points out to the existence of two distinct processes of learning occurring at different temporal scales. The learning occurring at the session level (large scale) is by far more efficient of the small scale one. Nevertheless the small scale learning could be responsible for the presentation of the stimulus to be analyzed at the large scale as well as for the extinction processes.

MOTIVATION AND EMOTION III

661.1

FOS IMMUNOREACTIVITY INCREASES IN THE MEDIAL NUCLEUS OF THE AMYGDALA AFTER PUP EXPOSURE IN PRAIRIE VOLE MALES B. Kirkpatrick*, T.R. Insel. Maryland Psychiatric Research Center, UMAB, Baltimore, MD 21228 and Lab. of Neurophysiology, NIMH, Poolesville, MD 20837.

Although there is an extensive literature on the neural substrates of maternal behavior, there have been few neurobiological studies of paternal care. The prairie vole is a biparental, monogamous rodent in which both juvenile and adult males approach and huddle over pups. We previously reported that axon-sparing lesions of the medial nucleus of the amygdala of male prairie voles reduce paternal behavior, as measured by pup contact. This reduction in paternal behavior appears both anatomically specific (lesions of the basolateral nucleus are ineffective) and behaviorally selective (large medial nucleus lesions do not affect a number of non-social behavioral measures and small lesions reduce paternal behavior while sparing other forms of social interaction). In the current study, prairie vole males exposed to 3-5 day old vole pups for the first time showed extensive contact throughout the 3 hour test period. Compared to males exposed to a novel, non-social stimulus, pup-exposed males showed increased staining for *fos* peptide in the medial nucleus of the amygdala as well as areas with established anatomical connections to it: medial preoptic area, bed nucleus of the stria terminalis, midline thalamic nuclei, and lateral septum. Other areas also showed increased *fos* expression. These results support the involvement of the medial nucleus of the amygdala in paternal behavior and suggest that several limbic regions may be activated by exposure to conspecific pups.

661.2

SPINAL PATHWAY MEDIATING SUCKLING-INDUCED NURSING BEHAVIOR AND NEUROENDOCRINE REFLEXES. J. M. Stern*, Y.-L. Yu, and D. C. Crockett. Dept. of Psychology, Rutgers Univ., and Dept. of Neurosci. & Cell Biol., UMDNJ, Piscataway, NJ 08854.

Maternal behavior (MB) in rats consists of active behaviors (retrieval, licking of pups) and quiescent nursing (typically, upright crouch with rigid splayed legs). Because the latter requires suckling, we assessed whether the lateral columns (LC), the spinal pathway known to mediate the milk-ejection reflex, also mediate the upright crouching posture. Bilateral lesions of the lateral (Lx, n=8) or dorsal (Dx, n=6) columns at C5 were made on day 5-8 postpartum; controls (C, n=8) were subjected to a sham-procedure. Ventral trunk cutaneous sensitivity was not impaired in any rat. All aspects of MB and lactation were present in C and Dx dams soon after treatment. Among Lx dams, lactation ceased in all, and 6 showed good ambulatory and ingestive recovery; of these, retrieval and licking recovered in 4 and 6 rats (in 2-5 days) and all were quiescent for long periods in response to suckling by foster pups, but their nursing posture was impaired (i.e., prone or hunched over). Thus, the LC mediate both suckling-induced neuroendocrine and postural maternal reflexes. (Supported by MH40459 to JMS).

661.3

ADULT ACTIVITY AND CORPUS CALLOSUM SIZE IN THE RAT: EFFECTS OF SEX AND NEONATAL HORMONE TREATMENT.
C.M. Mack* and V.H. Denenberg, Biobehavioral Sciences Graduate Degree Program, The University of Connecticut, Storrs, CT 06269.

The presence of sex differences in both nonreproductive behaviors and corpus callosum (CC) size in the rat suggest a possible relationship between these variables. As a preliminary test of this hypothesis, multiple measures of activity using the Digiscan apparatus were obtained in adulthood for males, females, testosterone propionate (TP) treated females (Day 4), and ovariectomized (Ovx) females (Day 12). Callosal parameters, based on prior factor analysis of the rat CC, were assessed following testing. As previously reported, males had a larger CC than females, and both TP treatment and Ovx enlarged the female CC. Behavioral analyses showed females to be more active than males for horizontal movements. TP treatment reduced activity levels for both horizontal and vertical measures, whereas Ovx had no effect on activity measures. Correlations between activity and CC parameters revealed significant relationships in females between CC size and all activity measures. No relationship was found in males, TP treated females, or Ovx females. These findings indicate that 1) the enlarging effects of Ovx and TP on the CC are dissociable at the behavioral level, and 2) a relationship between CC size and activity is present in females, possibly due to the presence of estrogen in this group.

661.4

GENDER DIFFERENCES IN CROWING AND AGGRESSION IN TESTOSTERONE-TREATED CHICKS.
L. Normansell* and J. Swope, Dept. of Psychology, Muskingum College, New Concord, OH 43762.

Testosterone (T) administration in young chicks induces an adult-like morphology and a number of adult-like behaviors including crowing and aggressive pecking, and a decrease in the distress vocalization (DV) which normally accompanies social separation. The present series of studies investigated some of these T-induced emotional effects, with an analysis of gender differences. Effects of drugs known to modulate vocalizations were also assessed.

When tested in groups of four, control males were three times more aggressive than females. T-treatment raised that level of aggression in females up to the level of males. T-treated males crowed four times more often than treated females, while no control chicks of either gender ever crowed. Scopolamine (1mg/kg) decreased crowing slightly, but had no effect on pecking. When chicks were tested individually, T-treatment decreased the number of DVs emitted, with males more affected than females. Scopolamine increased the number of DVs emitted in all test groups. Morphine (1mg/kg) had no effect on either pecking or crowing, but reduced the number of DVs in males and females in both treatment conditions. While naloxone (1mg/kg) had no effects on DVs, it blocked the morphine-induced suppression of vocalization.

Males were also tested by being paired with a like-treated weight-matched chick from their same small flock or from a different flock. Chicks crowed over eight times more often and pecked each other fives times more often when they were paired with a stranger than with a fellow from their own flock.

661.5

AGGRESSION AND SOCIAL BEHAVIOR AFTER FOUR DIFFERENT MEDIAL HYPOTHALAMIC LESIONS.
M.E. Oakes* and G.D. Coover, Department of Psychology, Northern Illinois University, DeKalb, IL 60115.

Lesions of the medial hypothalamus appear to increase defensive behavior, particularly in response to handling or attack. However, both decreases and increases have been reported in offense and social aggression. Olivier (1977, Aggressive Behavior 3:47-56) found evidence for increased offense after lesions of the caudal as opposed to rostral ventromedial hypothalamus (VMH).

The present study examined the effects of bilateral electrolytic lesions of either the rostral VMH (rVMH), medial VMH (mVMH), posterior VMH (pVMH) or the dorsal medial hypothalamus (DMH). Behavioral testing involved placing an intruder (stimulus rat) into the plastic home cage (with wood chip bedding) of an experimental rat for 5 min on two separate occassions.

All four types of lesion reduced the time spent plowing and kicking wood chips compared to controls ($p < .05$, Newman-Keuls). Rats with lesions of the rVMH and mVMH spent more time immobile than controls (p's $< .005$, t-tests). The rVMH group also showed more defensive kicks plus lateral postures than the control group ($p < .005$, t-test). Fights were more likely to occur in tests of rVMH or mVMH rats than of control, pVMH or DMH rats ($p < .05$, Newman-Keuls). These data indicate increased defense after lesions rostrally in the VMH. Rats with DMH and pVMH lesions showed more on-top contact with the intruder than the controls (p's $< .05$, t-tests), with frequencies, respectively, of 16.5 ± 7.1 (Mean $\pm$ SE), 20.9 ± 7.5 and 4.0 ± 1.3. For the rVMH and mVMH groups, the values were 4.0 ± 1.2 and 7.1 ± 3.8. This behavior has been called aggressive grooming and suggests initiation or solicitation of social interaction. Caudal or dorsal medial hypothalamic lesions increase what appears to be an aspect of social aggression.

661.6

EFFECTS OF ROUGH-AND-TUMBLE PLAY ON C-FOS EXPRESSION IN THE JUVENILE RAT BRAIN.
S.M. Siviy*, S. Huguenin, L.A. Kerrigan, S.J. Kuhlman, S.W. James and K. Hiraizumi, Departments of Psychology and Biology, Gettysburg College, Gettysburg, PA 17325.

Proto-oncogenes, such as *c-fos* and *c-jun*, have been used extensively during recent years as markers of metabolic activity in the brain and to map areas of the brain which are active during discrete behaviors. In the rat, rough-and-tumble play occurs during discrete bouts and, thus, may be expected to activate *c-fos*. At 21 days of age, rats were housed individually and given a 5 minute daily opportunity to engage in rough-and-tumble play from post-natal days 22 to 34 (P22-P34). On P35, half of the rats were deeply anesthetized and perfused intracardially 90 minutes after a 5 minute opportunity to play, while the other half had no opportunity to play prior to being anesthetized. Brains were carefully removed and 70 µm sections of selected brain areas were processed immunohistochemically for the presence of c-Fos using a primary antibody which recognizes both Fos and Fos-related proteins. In both groups of rats (no-play and play), very little immunoreactivity was detected in subcortical areas, including thalamus, hippocampus and amygdala. While Fos-like immunoreactivity was detected in the parietal cortex of rats from both the no-play and play groups, there was significantly more labelling in those rats allowed to play ($p < 0.02$). These data suggest that play behavior may selectively activate *c-fos* expression in cortex. Studies are currently in progress to quantify *c-fos* mRNA as an additional measure of *c-fos* activation in the playing rat.

661.7

CONCURRENT EXPLORATION OF A COMPLEX FIELD BY RATS WITH SHAM AND FORNIX LESIONS.
T.L. Steele* & S.M. Olson, Dept. of Psychology, Univ. of Wis. Oshkosh, Oshkosh, WI 54901.

Atypical exploration by rats with hippocampal or fornix lesions has been attributed to stereotypy, hyperactivity, and memory deficits (1). Since exploration is a product of the environment, this study sought to determine which abnormalities still occur when lesioned rats explore complex familiar and novel environments with an intact conspecific.

Sham- (S) and fornix-lesioned (F) female hooded rats were housed in pairs for 22 days. Rats had continuous access to a "familiar" field containing 32 semi-enclosed compartments and several permanently located objects. Over the last 10 days rats were given concurrent 30 min/day free access to a similar "novel" field in which objects were replaced daily by new objects located in different compartments.

Investigation of novel compartments, especially those containing objects, was extensive by all rats. Habituation to certain characteristics was evident. Although all rats investigated the majority of novel compartments, visits by F rats were generally brief but numerous. F rats were also more active and shuttled between the fields frequently, exploring familiar compartments more extensively than S rats. A social influence on exploration was also noted.

Field complexity and presence of a conspecific likely contributed to the apparent normal exploration of the novel environment by F rats. However, brief contacts in the novel field coupled with repeated investigation of the familiar field suggests cognitive and motor deficits may have affected exploration. The effect of fornix lesions thus appears to be strongly, but not exclusively, influenced by environmental factors.

(1) Steele, T.L. & Devenport, L.D. (1986). Neurosci. Abs., 12, 743.

661.8

HUMAN CHORIONIC GONADOTROPIN AFFECTS OPEN FIELD BEHAVIOR, NEOPHOBIA AND MEMORY TEST PERFORMANCE IN RATS
H. Lukacs*, E. S. Hiatt, Ch. V. Rao, Dept. OB/GYN, Univ.of Louisville, Louisville KY 40292

Our laboratory has reported the presence of luteinizing hormone(LH)/human chorionic gonadotropin (hCG) receptors in several rat brain areas with highest density in hippocampus. ^{125}I-hCG injected in peripheral circulation can cross blood brain barrier. We conducted a series of studies to ask whether hCG affects behavior patterns associated with hippocampus. Female rats (n=10) were injected with 50 IU hCG (ip) or sal at 9AM on proestrus and monitored for 4 days. hCG injection decreased locomotion/exploratory behavior in open field: distance rats travelled and frequency of rearing ($p<0.05$). However, time to enter open field (reaction to novel stimulus) was unchanged by hCG. To investigate whether reduced activity of hCG-treated rats within open field might be due to increased anxiety of a novel stimulus, rats (n=7) were tested for taste neophobia. hCG-treated rats greatly preferred a novel food over rat chow ($p<0.01$), suggesting not only lack of neophobia, but increased interest. Working memory tested in a T-maze showed no effect on the correctness of choices, however, the hCG-treated rats (n=8) exhibited fewer head turns ($p<0.05$). In summary, hCG administration affects certain behavioral parameters primarily regulated by the hippocampus, suggesting that hippocampal LH/hCG receptors may be functionally coupled to regulation of rat activity, neophobia and other associated behaviors. Food preference and decreased activity have parallels in women during the first trimester of pregnancy when serum hCG is highest.

661.9

UNCONDITIONAL APPROACH PREFERENCES IN THE QUAIL: EFFECTS OF THALAMIC AND MIDBRAIN LESIONS. A. Csillag*, P. Kabai and J.K. Kovach. 1st Dept. of Anatomy, Semmelweis Univ. Med., Budapest, Hungary and The Menninger Clinic, Res. Dept., Topeka, KS, U.S.A.

Selected genetic lines of Japanese quail chicks exhibit unconditional preferences for blue and red stimulus in approaching flickering light (Kovach JK, 1980, Science 207: 549). The initial manifestation of preferences is spared by bilateral hemispherectomy (Kabai P, Kovach JK, 1993, Physiol. Behav. 53:). Newly hatched quail chicks were exposed to stereotaxic radio-frequency lesions in thalamic areas or sub-total ablation of optic tecta. The birds' approach response and color preference were tested before and 24 hours after surgery in a mass screening apparatus. Lesions to the n. rotundus, opticus princip. thalami (OPT) and geniculatus lateralis p. ventralis did not attenuate the approach behavior in BL and RL chicks, nor the approach preference in BL birds. In RL birds, lesions to these nuclei had a slight effect on red preference. Lesions to the nuclei dorsomed. ant., post.; and dorsointermed. post. attenuated both the preference and the elicitability of approach response in RL but not in BL chicks. Lesions of the nuclei of ansa lenticularis, subrotundus, ovoidalis, reticularis thalami and occipitomesencephalic and tectothalamic tracts attenuated the approach response in both RL and BL birds and had greater effect on red than on blue preference. Conversely, only blue preference was impaired by tectal ablation.

It is suggested that the deep thalamic lesions affect primarily the motor component of approach response, whereas the dorsomedial (limbic) thalamic region is likely to form a link between the visual information and the initiation of (red-biased) approach response. Thalamic and tectal centres may play different roles in the mediation of red and blue preferences.

A.C. is holder of a Fogarty-NIH CEESPN Fellowship.

661.10

MORPHINE FAILS TO PRODUCE DISCRIMINATIVE EFFECTS IN THE VENTRAL TEGMENTAL AREA (VTA). T.V. Jaeger* and D. van der Kooy, Dept. of Anatomy, Univ. of Toronto, Toronto, Canada M5S 1A8.

Previous studies have implicated ascending visceral and gustatory pathways (parabrachial nucleus), but not dopamine terminal fields (nucleus accumbens) as a possible substrate for opiate discriminative effects. These discriminative substrates are distinct from the medial forebrain bundle pathways mediating the rewarding effects of opiates. To extend these findings, we investigated the discriminative actions of morphine in the VTA, an area known to mediate morphine's rewarding actions. Rats were injected with morphine (5 mg/kg, i.p.) 15 min prior to the presentation of a 0.1% saccharin solution. Lithium chloride (130 mg/kg) was injected immediately after 20 min of exposure to the flavour. On alternate days, an injection of 0.9% saline both preceded and followed the presentation of saccharin. Animals learned to consume significantly less saccharin after morphine than after saline injections. Unilateral guide cannulae were then implanted into the ventral tegmental area and generalization to central routes of administration was evaluated following the microinjection of 2.5, 5, 10 and 20 µg of morphine. At doses as high as 10 µg, morphine failed to produce responses reliably different from the saline training condition. Decreases in saccharin consumption were observed only at the 20 µg dose of morphine. However, control data showed that these effects could not be attributed to the cueing properties of morphine. Rather, morphine produced unconditioned decreases in saccharin consumption. The absence of discriminative effects of morphine in areas known to mediate it's motivational effects implies the presence of a distinct neural pathway for the processing of the perceptual effects of morphine. To confirm this hypothesis, we are using a taste conditioning procedure to assess the motivational properties of morphine in the parabrachial nucleus.

661.11

PATTERNED DOPAMINERGIC ACTIVITY UNDERLIES THE MOTIVATIONAL EFFECTS OF MORPHINE WITHDRAWAL, K. Nader* & D. van der Kooy, Dept. of Anatomy, Univ. of Toronto, Toronto, ON, M5S 1A8.

Animals were made dependent with 3 weeks of 3 injections a day of 20 mg/kg/inj. morphine (Development). Animals were then exposed to one conditioning environment over 4 trials, each trial 18 hr after abstinence from morphine (Training). Neuroleptic pretreatment (*cis*-flupentixol 0.8 mg/kg) prior to conditioning in the training phase blocked the normal conditioned avoidance of the withdrawal paired side. In order to test if dopamine is necessary for the development of dependence, and in turn withdrawal, animals were pretreated with the same neuroleptic prior to each morphine injection during the development phase and then conditioned with morphine abstinence in the absence of neuroleptics. At testing neuroleptic pretreated animals demonstrated conditioned avoidance of the withdrawal paired side equivalent to that of animals injected with saline prior to each morphine injection. This suggests that dopamine's role is limited to the expression, rather than development, of withdrawal's motivational effects.

To further characterize the nature of the dopaminergic withdrawal signal, dependent animals were again conditioned to avoid an environment paired with morphine withdrawal. Prior to conditioning, animals were pretreated with a 1.0 mg/kg dose of either amphetamine (AMPH) or apomorphine (APO). If the aversive effects of withdrawal are mediated by an overall decrease in post-synaptic dopamine receptor activation, then we predict that both AMPH and APO pretreatment should block the acquisition of a place aversion to the withdrawal paired side. On the other hand, if withdrawal is mediated by a specific pattern of activation of post-synaptic dopamine receptors, then APO pretreatment should block, but AMPH (which potentiates dopamine release) should intensify the aversive motivational effects of withdrawal. AMPH pretreated animals demonstrated larger aversions to the withdrawal paired side compared to controls, whereas APO treated animals showed no significant aversion to the withdrawal paired side. This suggests that the aversive motivational effects of withdrawal are mediated by a patterned activation of post-synaptic dopamine receptors.

661.12

HALOPERIDOL & PENTOBARBITAL PRODUCE DIFFERENT PATTERNS OF ELEVATIONS IN OPERANT RESPONSE FORCE & DURATION IN RATS E. O'S. Hammond*, M. A. Raven, & A. Ettenberg Behavioral Pharmacology Laboratory, Dept. of Psychology, Univ. of California, Santa Barbara, CA 93106

Although haloperidol (HAL) and pentobarbital (PEN) produce comparable deficits in rats' spontaneous locomotor activity, it was of interest to determine whether the biophysical response capacity of treated subjects would differ in an operant task. Hungry rats were trained to press a force-sensing operandum for 2 sec access to sweetened milk. Responses were analyzed for temporal and force characteristics during vehicle or drug trials. While both drugs produced elevations in the mean peak force emitted during responding, within-session patterns differed. Peak force exerted by HAL animals increased over the session whereas peak force of PEN animals remained stable. Similarly, while both drugs produced response duration increases, HAL effects increased over the session while PEN durations decreased. These results demonstrate the utility of force-time data in identifying differences between classes of motor-impairing drugs.

661.13

CENTRAL CHOLINERGIC MECHANISM OF THE REACTIVE CARDIAC EFFECTS OF FG 7142. K.S. Quigley*, S.L. Hart, M.F. Sarter, & G.G. Berntson, Department of Psychology and Neuroscience Program, Ohio State Univ., Columbus, OH 43210.

We have previously demonstrated an enhancement of the cardioacceleratory response to a moderate intensity auditory stimulus following administration of the benzodiazepine inverse agonist, FG 7142 (FG), a putative anxiogenic agent. In the present study, rats were given light/shock (CS/US) pairings (conditioned, CND) or random presentations of the CS and US (pseudoconditioned, PSD). CND subjects showed an enhanced cardioacceleratory response to the CS relative to PSD. In contrast, PSD subjects, like subjects administered FG showed an augmented response to an irrelevant probe tone relative to CND.

Possible mechanisms for the augmented cardioacceleratory response after FG were examined. Intracerebroventricular administration of the cholinergic agonist, carbachol mimicked the cardioacceleratory enhancement effect of FG. Moreover, this effect was blocked by central atropine. In addition, central atropine normalized the FG-enhanced cardiac response. In sum, these data are consistent with the hypothesis that unpredictable shock produces a cardioacceleratory enhancement like that observed after FG. Additional data indicate that FG may evoke a sympathetic mechanism having a central cholinergic basis.

662.1

WHOLE-CELL PATCH-CLAMP ANALYSIS OF NEURONAL EXCITABILITY IN THE SUPRACHIASMATIC NUCLEUS (SCN). Y. Bouskila[*1,2] and F. E. Dudek[2]. [1]Interdept. Neurosci. Grad. Prog., UCLA Sch. of Med., Los Angeles, CA 90024 and [2]Dept. of Anatomy & Neurobiol., Colorado State Univ., Fort Collins, CO 80523.

The neurons of the SCN exhibit a circadian rhythm in firing rate which may result from modulation of their excitability. To examine neuronal excitability and membrane properties, coronal hypothalamic slices (400 μm) were prepared and neurons were recorded in the whole-cell patch-clamp mode. Spike trains were elicited by graded current pulses (producing 1.6-2.4 mV steps for 200 ms) near spike threshold. Most neurons (n=6) exhibited a high accommodation constant (2.32 ± 0.46, defined as the ratio of last interval to first in a spike train) and had profound spike broadening and spike amplitude reduction (except for one cell). A few neurons (n=2) showed almost no spike accommodation (accommodation constant of 1.28 ± 0.06, $p<0.003$) at all firing rates. The accommodating neurons differed from the non-accommodating neurons by their lower whole-cell capacitance and input resistance. It is unlikely that these differences are due to recording quality since the two cell groups were not significantly different in first spike amplitude, half-peak duration and threshold, resting membrane potential or slope of current-frequency (I-F) relation. Spike accomodation may be important in modulating neuronal excitability and it should now be possible to identify the underlying currents. Supported by AFOSR.

662.2

AMINO-ACIDS-MEDIATED INHIBITION AND EXCITATION OF CHARACTERIZED RAT HYPOTHALAMIC PARAVENTRICULAR NEURONS FOLLOWING SUPRACHIASMATIC NUCLEUS STIMULATION. M.L.H.J. Hermes* and L.P.Renaud
Neuroscience Unit, Loeb Research Institute, Ottawa Civic Hospital, Ottawa, Canada K1Y 4E9.

The suprachiasmatic nucleus (SCN) generates circadian rhythms in endocrine and autonomic processes. Electrophysiological data obtained *in vivo* show that the SCN influences the excitability of neurosecretory and brainstem-projecting neurons in the hypothalamic paraventricular nucleus (PVN). The transmitter candidates that mediate the responses are unknown. *In vitro* intracellular recordings were made in coronal slices of the rat hypothalamus to characterize the synaptic responses of PVN neurons to SCN stimulation. In PVN neurons that displayed linear V-I relationships with evidence of Ia but a lack of low-threshold potentials (i.e. putative magnocellular neurons), electrical stimulation of the SCN (≤ 75μA, 1Hz) evoked short latency (8-10ms) IPSPs with a reversal potential between -63 and -75 mV that could be blocked by bath application of 20 μM bicuculline. In the majority of neurons in the PVN that had linear or inwardly rectifying V-I relationships with no Ia but with small low-threshold depolarizations (generating at most one action potential: putative parvocellular neurons), short latency (7-10 ms) EPSPs were observed following SCN stimulation which could reversibly be attenuated or blocked by 0.5-1 mM bath-applied kynurenic acid.

The data suggest that the amino acids GABA and glutamate are primary transmitters involved in the relay of SCN information to the PVN (Supported by the International Human Frontier Science Program Organization and the Canadian Heart & Stroke Foundation).

662.3

EVIDENCE FOR EXPRESSION OF PLASTICITY IN THE BRAIN OF THE ADULT SIBERIAN HAMSTER: PHOTOPERIODIC CHANGES IN NCAM. W. Lee, J.D. Glass, M. Watanabe and J.M. Walro*[1]. Dept. Biol. Sci. Kent St. Univ. Kent, OH 44242. [1]Dept. Anat. Northeastern Ohio Univ. Col. Med. Rootstown, OH 44272.

The polysialylated form of the neural cell adhesion molecule (PSA-NCAM) is thought to facilitate plasticity in the adult brain. The aim of the present study was to examine the distribution of PSA-NCAM and NCAM polypeptide in a species that exhibits photoperiod-induced reorganizations in neuroendocrine functions. In hamsters maintained under long day photoperiod (LD 16:8), immunostaining for both moieties was strongest in the supraoptic, suprachiasmatic, ventromedial (VM) and arcuate (ARC) nuclei. Immunostained tanycyte-like processes radiating from the 3rd ventricle were particularly noticeable in the median eminence (ME) as well as the VM and ARC nuclei. In gonadally-regressed animals held under short day photoperiod (LD 8:16) for 8 wks, there was a striking decrease in NCAM staining of the tanycyte-like processes in the VM and ARC nuclei. The resulting increase in the ratio of PSA-NCAM to NCAM in these structures could favor the occurrence of morphological rearrangements associated with short photoperiod. Supported by AFOSR F49620-93-0086 (JDG).

662.4

POLYSIALYLATED NCAM IN THE SCN: EVIDENCE FOR PLASTICITY IN THE ADULT CIRCADIAN PACEMAKER. J.D. Glass, W. Lee, R.V. Dorman* and M. Watanabe[1]. Department of Biol. Sci., Kent St. Univ. Kent, OH 44242. [1]Div. Pediatric Cardiol., Dept. Pediatrics RB&C, Case Western Reserve Univ., Sch. Med. Cleveland, OH 44106.

In the adult brain the highly polysialylated form of neural cell adhesion molecule, PSA-NCAM, is associated with regions that exhibit neuronal plasticity. Immunochemical procedures were used to study PSA-NCAM and its NCAM polypeptide in the SCN of the Siberian hamster. Immunoreactive polysialic acid (PSA) (visualized using a monoclonal antibody against the PSA of NCAM) was evident throughout the SCN, but was most intense in the ventrolateral region. Immunoreactivity was present in the neuropil, which delineated many cellular aggregations. Preincubating sections in endoneuraminidase (endo-N) abolished staining. Staining for NCAM also was limited to the neuropil, but was more diffuse than PSA staining. The ventral SCN had the most labeling for NCAM. Immunoblots showed the PSA-NCAM as a broad band between 150-300 KDa. Immunoreactive NCAM polypeptides appeared as major bands at 120, 140, and 180 Kda. Removal of PSA from PSA-NCAM with endo-N markedly increased the intensity of the 180 and 140 Kda bands implicating these NCAM isoforms as PSA carriers. These results are evidence that cellular elements of the SCN have the capacity to undergo physiologically-regulated morphological adjustments. Supported by AFOSR F49620-93-0086 (JDG).

662.5

A DISTINCT SUBNUCLEUS OF CALBINDIN-LI IMMUNOREACTIVE CELLS IN THE HAMSTER SUPRACHIASMATIC NUCLEI (SCN). H.R. Besmer*, R. Khan, J. LeSauter, J.M. Nuñez, and R. Silver. Barnard College of Columbia University. New York, N.Y. 10027.

The SCN of mammals are known to serve as a biological clock regulating circadian rhythms. At least 25 neuroactive substances or synthetic enzymes have been found in the SCN (*Card and Moore, 82;84*). Identification of pacemaker cells of the nuclei however, has not been achieved, and there is substantial interest in further characterization of SCN neuron types. In this study, we report the distribution of a calcium-binding protein, calbindin-D28K (CaBP), in the SCN of hamsters. Though the physiological functions of calbindin are not fully understood, it is thought to play a role in buffering calcium ions. Calcium is involved in transmembrane signaling and the intracellular transmission of signals.

In coronal sections through the hamster SCN, calbindin-like immunoreactivity (CaBP-ir) occurs in a very discrete, tightly packed subregion, positioned near the center of the nucleus. No CaBP-ir cells or fibers are seen at any peripheral borders of the SCN (delineated with antisera to VIP and VP), although CaBP-ir is abundant in the rest of the hypothalamus. Analysis of alternate sections indicates that CaBP-ir cells overlap in their distribution with substance P (SP)-like ir cells. Light induced cfos-ir nuclei occur in this region as well but are more widely distributed than the distinct subnucleus characterizing CaBP- and SP-like ir. In contrast, parvalbumin, another calcium-binding protein, is noticeably absent from all hypothalamic nuclei. It is interesting that the rat does not have a subnucleus characterized by either SP- (*Morin et al., 92*) or CaBP- (*Celio, 90*) containing cells. The functional significance of this species difference, and the function of this distinct subnucleus in the hamster remains to be assessed. Supported by NIH grant NS-24292.

662.6

IMMUNOREACTIVITY OF HAMSTER INTERGENICULATE LEAFLET (IGL) CELLS PROJECTING TO SUPRACHIASMATIC NUCLEUS AND DORSOMEDIAL THALAMUS. E. Meyer*, J. Blanchard and L.P. Morin. Dept. Psychiatry, Health Science Center, SUNY at Stony Brook, NY 11794.

The hamster IGL is a 2 mm long lamination between the dorsal and ventral (VLG) lateral geniculate nuclei. It contains NPY-IR cells many of which project to the SCN in both rat (Card & Moore, JCN '89) and hamster (Morin et al., Vis. Neurosci., '92). The hamster also has a probable ENK-IR path to the SCN not evident in rat. We have compared the geniculate distributions of hamster NPY-IR and ENK-IR cells projecting to the SCN, contralateral IGL and dorsomedial thalamic nuclei.

Fluoro-Gold, placed in the SCN, IGL or dorsomedial thalamus, was used for retrograde tracing in conjunction with immunohistochemical techniques (Vis. Neurosci., '92). Colchicine (100 μg/each lateral ventricle) was administered 24 hr before perfusion. NPY-IR cells are found throughout the IGL, as previously reported. ENK-IR cells are also abundant in the IGL. At mid-levels of the IGL, both NPY-IR and ENK-IR cells are found ventral to the leaflet proper. In the VLG, NPY-IR cells tend to be more medial than the ENK-IR cells. Both ENK- and NPY-IR cell types project to the SCN. Unlike the rat, ENK-IR cells of the hamster IGL do not project to the contralateral IGL. IGL cells also project to the dorsomedial thalamus and some of these are NPY-IR. We are presently determining whether any of these are ENK-IR and whether the NPY- and ENK-IR are found in identical cells. Supported by NIH grant NS22168.

662.7

THE SUPRACHIASMATIC NUCLEUS AND INTERGENICULATE LEAFLET OF THE OCTODON DEGUS, A DIURNAL RODENT. [1]L. Smale*, [1]N. Goel and [2]T. Lee. Depts. Psychology, [1]Michigan State University, East Lansing, MI, 48824, and [2]University of Michigan, Ann Arbor, MI, 48104

Little is known about the functional neuroanatomy of circadian rhythms in diurnal mammals. One reason has been the absence of a suitable diurnal model. The degu is a caviamorph rodent that breeds well in captivity and exhibits diurnal wheel-running and temperature rhythms (Labyak and Lee, unpub. data). To provide the groundwork for future experimental studies, we examined retinal projections to, and the distribution of peptides in the SCN and IGL of 6 adult degus. Two animals received an intraocular injection of cholera toxin 2 days prior to sacrifice. Brains were processed for immunohistochemical identification of various antigens. The degu SCN contained VIP and VP immunoreactive (IR) cell bodies, a relatively small plexus of NPY-IR fibers, and a few sparse M-enk-IR fibers; a dense plexus of SP-IR fibers completely surrounded but did not enter the SCN. Retinal fibers projected evenly to ipsi- and contralateral SCN. The IGL contained some NPY-IR cell bodies, and M-enk-IR and SP-IR fibers. Retinal fibers projected to both IGL. In summary, many basic features of the SCN and IGL are fundamentally the same in the diurnal degu as in nocturnal rodents.

662.8

NEURAL-GLIAL INTERACTIONS: DIFFERENCES BETWEEN THE ANTERIOR HYPOTHALAMUS AND THE VENTROLATERAL SUPRACHIASMATIC NUCLEUS OF THE RAT. A.S. Elliott* S.M. Krauchunas and A.A. Nunez. Dept. of Psych., Neuroscience Program, Michigan State University, East Lansing, MI 48824.

Previous publications have suggested that neural-glia interactions in the suprachiasmatic nucleus (SCN) are different from other areas of the hypothalamus. For example, glial fibrillary acid protein staining is more intense within the SCN than in adjacent neural tissue (*Neuroscience Letters* **99**: 55; *Cell and Tissue Research* **242**: 9). We measured the amount of somal membrane in apposition to glia (astrocytes and astrocytic processes), neural membrane (synapses, adjacent somas, unmylinated axons and dendrites), and other components of the neuropil (oligodendrocytes, myelinated axons) from electron micrographs of the ventral lateral SCN (vlSCN) and the anterior hypothalamus (AH). The data were collected from animals entrained to a 12:12 LD cycle and sampled at 4 different time points across the circadian cycle. The initial data show that there are more glial-somal contacts in the vlSCN than in the AH, and that there are more neural-somal contacts in the AH than in the vlSCN . Circadian variations in other aspects of cell morphology are currently being investigated. Supported by grant IBN 9209437 from NSF.

662.9

THE POSTNATAL DEVELOPMENT OF RETINAL PROJECTIONS TO THE RAT SUPRACHIASMATIC NUCLEUS. D. M. Murakami*, I.-H. Tang, A. Kaplan, C. Kim, and C. A. Fuller. Section of Animal Physiology, University of California, Davis, California 95616-8519.

The suprachiasmatic nucleus (SCN) has been suggested to be the critical neural pacemaker for circadian rhythms, and the retinohypothalamic projection (RHT) mediates the effect of light on circadian rhythms. This study examined the development of the RHT to the SCN. Rat pups from birth (P0) to postnatal day 10 (P10) were sacrificed and perfused with fixative and post fixed for at least 1 week. The cornea and lens were reflected away and DiI crystals placed onto the retina. The cornea and lens were replaced, the eye sealed, and the pup placed back into fixative for a minimum of 3 months for anterograde transport. Rat brains were removed, sectioned on a microtome, and examined under a fluorescence microscope. From P0 to P2 the retinal projections through the nerve and optic chiasm were relatively light and did not appear to innervate the SCN. However, by P3 the optic nerve and chiasm exhibited dense retinal axons and the first retinal input to the SCN was revealed. Individual axon terminals were revealed that exhibited very simple arborizations primarily from the ipsilateral eye. These projections continued to become more dense for the next 7 days.

662.10

THE RAT SUPRACHIASMATIC NUCLEUS: POSSIBLE ROSTRAL-CAUDAL ORGANIZATION AND ASSOCIATED NEURONS. I-H Tang, D.M. Murakami, C.A. Fuller*. Section of Animal Physiology, University of California, Davis, California 95616-8519.

This study examined the rostral-caudal organization of the rat suprachiasmatic nucleus (SCN) by investigating the cell types and their axo-dendritic distributions. Male Wistar rats were perfused with oxygenated Krebs-Ringer's solution, the brains removed and SCN exposed by vibratome with cold oxygenated Ringer's solution in the cutting chamber. Horseradish Peroxidase (HRP) crystals were injected into specific portions of the nucleus. The brains were incubated in Ringer's at room temperature for 3-4 hrs. Fixed brains were sectioned horizontally as well as coronally on a freezing microtome at 50μ. Several distinct neuron types were identified, e.g., bipolar, pyramidal and radial shaped cells. Bipolar neurons have shorter axons and dendrites than the radial and pyramidal neurons. More bipolar cells were located in the ventrolateral portion of the SCN, while pyramidal cells were more numerous in the dorsomedial area. Bipolar cells in the ventrolateral SCN primarily project to dorsomedial SCN. Horizontal sections of ventrolateral injections showed pyramidal and radial cells projecting to the contralateral SCN and to ipsilateral hypothalamus. Dorsomedial injections showed more fibers oriented rostral-caudal in horizontal sections. The caudal SCN exhibited projections toward the retrochiasmatic area. These observations suggest that the SCN exhibits distinct functional subdivisions, interconnections and efferent patterns of projection.

662.11

CLASSIFICATION AND DISTRIBUTION OF RETINAL GANGLION CELLS THAT PROJECT TO THE SUPRACHIASMATIC NUCLEUS (SCN) OF THE RAT J.C. Speh*, J.P. Card and R. Y. Moore Depts. of Psychiatry, Neurology, Behavioral Neuroscience and the Center for Neuroscience, University of Pittsburgh, PA 15261

Recent studies have identified an alphaherpes virus,the Bartha strain of the pseudorabies virus,as a quite specific marker of the retinal projections to nuclei of the circadian timing system (Card et al.,1991). Virus injected into the eye is transported to the retinorecipient fields in the hypothalamus, particularly the SCN, and the intergeniculate leaflet and to accessory optic nuclei but not to other retinal recipient areas. Once the virus reaches the central nervous system it travels transynaptically in a retrograde direction to the next order of neurons. We have analyzed the temporal parameters of viral retrograde transport and have determined the time when the contralateral eye would have maximal retrograde labeling from the SCN. Utilizing the Bartha virus injected into the eye and the retrograde tracer Fluorogold (FG), injected into the SCN, we have identified a subset of retinal ganglion cells that projects to the SCN. The distribution of these viral infected and FG + cells is predominantly in the peripheral temporal retina. The labeled cells range 10-18μm in diameter and the majority of labeled ganglion cells is a homogeneous population with a mean diamter of 12μm. These ganglion cells have very few extremely thin dendrites. Injections of FG directly into the chiasm retrogradely labels a full range of retinal ganglion cell types. The cells that project to the SCN are a subset of small ganglion cells that corresponds to a component of the gamma or W cell classification.

662.12

A PUTATIVE RETINOHYPOTHALAMIC TRACT (RHT) IN THE HUMAN DEMONSTRATED BY SUBSTANCE P (SP) IMMUNOREACTIVITY. R.Y. Moore,* and J.C. Speh, Center for Neuroscience and Departments of Psychiatry, Neurology and Neuroscience, University of Pittsburgh, Pittsburgh, PA 15261.

The RHT is now recognized to arise from two sets of retinal ganglion cells. One set projects to the suprachiasmatic nucleus (SCN) and intergeniculate leaflet (IGL) and probably uses glutamate as a transmitter. The second set, originally described by Takatsuji et al (1991), projects to the SCN and the olivary pretectal nucleus and is SP-containing (SP+). In the rat, SP+ fibers form a dense plexus in the ventrolateral SCN and this plexus is lost after optic nerve section (Takatsuji et al, 1991). In the present study, we analyzed serial coronal sections through the SCN prepared with antisera to SP, vasopressin (VP) and vasoactive intestinal polypeptide (VIP) from 6 human brains obtained at routine postmortem examination from individuals of both sexes with ages from 22 to 75 at the time of death.

The SCN in non-human primates and other mammals (Moore, 1992) is characterized by a population of VIP+ neurons that overlap the distribution of the RHT and is surrounded by a population of VP+ neurons. In the human, VIP+ neurons are present in the ventral part of the SCN surrounded by VP+ neurons. The area occupied by the VIP+ neurons contains a dense plexus of SP+ axons which is separate from plexuses in the periventricular and anterior hypothalamic areas. If the human is homologous to other mammals in this respect, this plexus should represent the portion of RHT projections that is SP+. Supported by a grant from the AFOSR.

662.13

A COMPARATIVE STUDY OF VASOACTIVE INTESTINAL POLYPEPTIDE (VIP) IMMUNOHISTOCHEMISTRY IN RAT, MONKEY AND HUMAN HYPOTHALAMUS. R.P. Weis*, M.M. Moga, N.M. Suhan, J.C. Speh and R.Y. Moore, Depts. of Psychiatry, Neurology, Behavioral Neuroscience and the Center for Neuroscience, Univ. of Pittsburgh, PA 15261.

Vasoactive intestinal polypeptide (VIP) is a putative neurotransmitter of circadian pacemaker neurons in the suprachiasmatic nucleus (SCN). In the rat the hypothalamic distribution of VIP-immunoreactive (-ir) fibers closely corresponds to the pattern of SCN efferents as demonstrated by anterograde tracing studies. To determine the possible course of SCN efferents in the monkey and human, we compared the distributions of VIP-ir fibers in the rat, monkey and human hypothalamus. In all three species, a dense cluster of VIP-ir fibers was found in the subparaventricular zone with fibers also present in the preoptic/chiasmatic region, the paraventricular nucleus, the dorsomedial nucleus and the tuberomammillary nucleus. In monkey and human but not in rat, we observed a second dense cluster of VIP-ir fibers along the dorsomedial border of the VMH as well as additional fibers in the median eminence, the arcuate nucleus and the lateral tuberal nucleus. A VIP-ir fiber plexus was also found in the monkey paraventricular nucleus of the thalamus. In general, the VIP-ir fiber distribution in the monkey and human is more extensive than in the rat suggesting more direct circadian control of hypothalamic function. Supported by NIH grant NS-16304.

662.14

EFFERENT PROJECTIONS OF THE PARAVENTRICULAR NUCLEUS OF THE THALAMUS IN THE RAT. M.M. Moga*, R.P. Weis, and R.Y. Moore, Depts. of Psychiatry, Neurology, Behavioral Neuroscience and the Center for Neuroscience, Univ. of Pittsburgh, PA 15261.

The paraventricular nucleus of the thalamus (PVT) receives input from all major components of the circadian timing system, including the suprachiasmatic nucleus (SCN), the subparaventricular zone, the intergeniculate leaflet and the retina. Retrograde studies have demonstrated that the PVT projects to the amygdala and hippocampus (Su and Bentivoglio, '90) and to the vicinity of the SCN (Pickard, '82) but its terminal distribution within these areas is unknown. To better understand the role of this nucleus in circadian timing, we iontophoresed the anterograde tracer phaseolus vulgaris leucoagglutinin (PHA-L) into the PVT to map the distribution of its efferent projections. Major terminal fields of the PVT include: the suprachiasmatic nucleus, the dorsomedial and ventromedial hypothalamic nuclei, the lateral septum, the central and basomedial amygdaloid nuclei, the bed nucleus of the stria terminalis, the olfactory tubercle, and the insular and entorhinal cortices. The dense axonal labeling within the SCN was evenly distributed across the dorsomedial and ventrolateral subdivisions; this terminal field extended caudally into the subparaventricular zone and the retrochiasmatic area. These anatomical findings suggest that the PVT may: 1) provide feedback to the SCN, 2) relay circadian information to limbic areas, and/or 3) serve as an entraining pathway for some as yet unspecified stimulus. Supported by NIH grant NS-16304.

662.15

STIMULATION OF LOCUS COERULEUS (LC) PHASE-SHIFTS THE CIRCADIAN ACTIVITY RHYTHM IN HAMSTERS. P. D. Penev*, P. C. Zee and F. W. Turek, Dept. of Neurobiology and Physiology, Northwestern University, Evanston, IL 60208.

Non-photic stimuli that phase-shift hamster circadian rhythms are associated with increased activity and/or arousal. The LC neurons are particularly responsive to similar novel or unexpected environmental events suggesting a possible involvement of the LC in the mechanisms of non-photic phase-shifting. Nine male golden hamsters (b.w. 120-140g) were kept in constant dim light (50-100 lux) and their circadian rhythms of wheel-running activity were monitored with an on-line computer. The animals were chronically implanted with bipolar electrodes (SNEX-100S) aimed at the right LC. Seven to ten days post surgery the freely-moving animals received electrical (60-120 uA, 20 Hz, 0.5 msec pulses) or sham (no current) stimulation trials for 1 h between CT 8 and CT 9. Six of the animals received both sham and electrical stimulation with at least 7 days between trials. LC stimulations induced prolonged exploratory activity, anxiety-like behavior and considerable phase-advances in all trials (range 35-90 min). Sham stimulations were associated with short initial arousal and small or absent phase-shifts (range 0-25 min). The phase-advances following LC stimulation (58 $\pm$ 7 min) were significantly larger than the phase-shifts (14 $\pm$ 4 min) associated with sham trials (ANOVA; $p < 0.001$). Our data suggest that LC stimulation mimicks the effects of arousal and/or activity inducing stimuli upon the circadian system in hamsters suggesting a possible role for LC in the mechanisms of non-photic phase-shifting.

662.16

A 3-DIMENSIONAL MAP OF THE AFFERENT NEURONAL NETWORK OF THE LONG-TERM REM SLEEP ENHANCEMENT SITE IN THE PONTINE BRAINSTEM. O.K. Hsu, S. Datta, J. Quattrochi*, J. Hobson, *Laboratory of Neurophysiology, Harvard Medical School, Boston, MA 02115.*

Microinjection of carbachol into the feline caudolateral peribrachial nucleus (C-PBL), produces a short-latency increase in state-independent ipsilateral PGO waves for 72 hrs followed by a long-term REM sleep enhancement (LTRE) lasting 7-10 days (NeuroReport **2**: 619-622, 1991). Using retrogradely transported fluorescent nanospheres, ChAT/AMCA immunohistochemistry, and 3-D computer-assisted imaging, afferent inputs to this pharmacologically active site were mapped and quantified.

We found that the pedunculopontine tegmentum (PPT, Ch5) contributes the predominant input to the LTRE site: 51.8% of retrogradely labeled ChAT+ neurons, and 22.0% of non-ChAT retrogradely labeled neurons. The PBL contributes significantly less input: 23.1% of ChAT+ projection neurons, and 10.8% of non-ChAT projection neurons. The dorsolateral tegmentum (DLT, Ch6) contributes 20.8% of ChAT+ projection neurons, and only 1.7% of non-ChAT retrogradely labeled projections. Thus, within Ch5 & Ch6 we interpret these data to indicate that the PPT exerts the predominant cholinergic and non-cholinergic influence on the LTRE site compared to significantly less influence by labeled cells found within the DLT.

Spatial analysis of the topographic distribution of ChAT+ projection neurons revealed that the ipsilateral PPT, PBL, and DLT form a contiguous and continuous afferent network, suggesting that these cholinergic projection subpopulations converge upon the LTRE site as a single integrated unit. This unified network organization of ChAT+ projection neurons identifies a unique structural substrate within the context of a functional map of afferent connectivity which may help the understanding of the pharmacological activation of prolonged REM sleep state behavior. *(Supported in part by NIH grant MH13923)*

662.17

COMPARISON OF CIRCADIAN RHYTHMS RESTORED BY TRANSPLANTATION OF ONE OR TWO SUPRACHIASMATIC NUCLEI. F.C. Davis* and N. Viswanathan, Dept. of Biology, Northeastern Univ., Boston, MA 02115.

In rodents, transplantation of the suprachiasmatic region of the fetal hypothalamus to the brains of adult hosts restores circadian rhythms disrupted by the bilateral ablation of the host's own suprachiasmatic nucleus (SCN). In the present study, transplantation was used to test three hypotheses about the role of the SCN in circadian rhythm regulation: 1) Each bilateral representation of the SCN is a competent circadian pacemaker, 2) A single SCN is sufficient to regulate a bimodal activity pattern, and 3) The period of circadian rhythms is determined in part by the size of the SCN. Adult, SCN-lesioned hamsters (*Mesocricetus auratus*) received grafts with either one or both SCN from a single fetus. Eleven pairs of hamsters each received SCN from a single fetus and 12 hamsters each received both SCN from a single fetus. Wheel-running activity was continuously recorded in dim constant light for several weeks before and after transplantation. In four pairs that received SCN from a single fetus, both hosts showed robust restored rhythms indicating that each bilateral representation of the SCN is capable of independently regulating overt rhythms. The restored rhythms of two hosts that received a single SCN showed symmetrical bimodal patterns of activity, indicating that this pattern does not require both bilateral representations of the SCN. The average freerunning period of hosts that received a single SCN (23.98 hrs. $\pm$0.045[SE], n=13) was significantly shorter than that of hosts that received two SCN (24.23 hrs. $\pm$0.076, n=7). Although the actual sizes of the grafts have yet to be determined, these results suggest that the total amount of SCN tissue within a graft is one determinant of freerunning period. This result is consistent with the hypothesis that an age-related shortening of period is due to a loss of cells within the SCN during aging. Supported by PHS grant P01 AG09975.

662.18

WHEN DOES HOST RESET GRAFTED SCN? INFLUENCE OF IMPLANT SITE, TISSUE TYPE AND PINEAL SECRETION. J. Servière[1], G. Gendrot[1], J. le Sauter[2] and R. Silver[2]*. [1]INRA Jouy en Josas, France; [2]Barnard Coll. of Columbia Univ., New York, N.Y.10027.

In mammals, the suprachiasmatic nuclei (SCN) serve as the dominant pacemaker controlling circadian rhythms. The SCN have daily fluctuations in energy metabolism with higher glucose utilization during the day. Using ^{14}C-2-deoxyglucose (2-DG) uptake as an index of phase we previously showed that in intact hamsters bearing SCN grafts in the 3rd ventricle (3V), the phase of the native and grafted SCN become synchronized to each other and have the phase of the host clock. In this study we explored the effect of placing 1) SCN grafts in the 3V of pinealectomized hamsters, and in intact animals, 2) cortical grafts in the 3V and 3) SCN grafts in the lateral ventricle (LV). To this end, adult males (to serve as hosts) and pregnant females (source of donor tissue) were housed in opposite LD cycles. 2DG was injected either subjective day (CT05) or subjective night (CT14). The results show that the circadian rhythm of the host resets the phase of the transplanted SCN in pinealectomized, as in intacts hosts. Cortical grafts in 3V do not have circadian rhythm of glucose utilization. SCn grafts in the LV tend to fluctuate in 2DG uptake in parallel with native SCN, but the variation is not significant. We conclude that in the hamster the coupling signal from the SCN acts specifically on pacemaker cells, does not require pineal melatonin, and cannot reach pacemaker targets located at far outside the 3V. Whether the coupling signal from the host SCN is direct (via locally diffusible signals or neural efferents to the graft) is not clear, though indirect cues, such as hormonal, thermal or metabolic factors are unlikely. Supported by grants from INRA (JS), AFOSR (RS) and NATO (JS & RS).

662.19

2-MERCAPTOETHANOL AND SUCCINYLATED CONCANAVALIN A INCREASE THE NUMBER OF NEUROPEPTIDERGIC CELLS IN PRIMARY CULTURES OF THE SUPRACHIASMATIC NUCLEUS.
K.A. Zimmert, R.J. Grill, S.K. Pixley, H.T. Jansen*, and M.N. Lehman. Dept. Anat. & Cell Biol., Univ. Cincinnati, Cincinnati, OH 45267; † Div. Infect. Dis., Children's Hos. Med. Ctr., Cincinnati, OH 45229.

Transplants of the fetal hamster hypothalamic suprachiasmatic nucleus (SCN), the site of a circadian pacemaker, restore rhythmic behavior to SCN-lesioned hamsters (Lehman et al., J. Neurosci. 7:1626). The presence of peptidergic cells characteristic of the SCN is correlated with the restoration of rhythmic behavior (Silver et al., Brain Res. 525:45). We explored the use of 2-mercaptoethanol (2-ME) and succinylated concanavalin A (SCA) as additives to our standard medium for culturing SCN cells prior to use in grafting studies. Fetal (E13) anterior hypothalamic cells were dissociated by a combination of gentle trituration and enzymatic treatment. Cells were rinsed and plated at 5 x 10^5 cells/ml/16mm well on either poly-L-lysine coated coverslips or on an astroglial bed layer. At the time of plating cultures were seeded in either control standard medium (DMEM), DMEM with 25μM 2-ME, or 250μg/ml SCA. Cultures were exposed to SCA media for 48 hrs. 2-ME cultures were maintained in 2-ME media. At ten-twelve days following seeding cultures were fixed with 4% paraformaldehyde and immunostained for a variety of neuronal and glial markers. Both 2-ME and SCA cultures showed a 3-fold increase in the number of neurons immunreactive for vasoactive intestinal polypeptide. Because they lead to an increase in the number of peptidergic cells in dissociated SCN cultures, 2-ME and SCA may also be useful as additives to the medium used for grafting studies in which dissociated and cultured SCN cells are tested for their ability to restore rhythmicity.
[Supported by NIH RO1 NS28175 to M.N.L.]

662.20

IDENTIFICATION OF P34 AND CYCLIN HOMOLOGS IN THE OCULAR CIRCADIAN PACEMAKER OF BULLA GOULDIANA,
Michael H. Roberts* and Nancy K. Leader, Dept. of Biology, Clarkson University, Potsdam, NY, 13699-5806

The eye of the marine snail, Bulla gouldiana contains a circadian pacemaker. The circadian rhythm is expressed as a change in optic nerve impulse frequency driven by an alteration in potassium conductance (Michel et al. 1993). Two key modulators of potassium conductance are intracellular calcium concentration and channel phosphorylation. Thus, a rhythmic alteration in kinase activity may be a component of the circadian clock's output pathway. Our central hypothesis is that members of two protein families, some of whom regulate the eukaryotic cell division cycle through rhythmic kinase activity, are involved in circadian rhythm generation.

Using Western blotting and immunohistochemical techniques, we have identified several proteins related to the cell division kinase, p34cdc2, in the B. gouldiana eye and brain. Interestingly, one of these proteins (p40) is specific to the ocular pacemaker. We have also identified a 66kDa homolog (p66) of the regulatory phosphoprotein cyclin. Furthermore, the level of p66 is affected by treatments (100uM genistein) that have been previously shown to shift the phase of the ocular rhythm.

Our current work is aimed at identifying circadian changes in the content, phosphorylation state, and association of these proteins. Supported by NS26272.

662.21

WHOLE CELL CURRENTS IN CULTURED CIRCADIAN PACEMAKER NEURONS OF *BULLA*.
S. Michel*, K. Manivannan, J.J. Zaritsky and G.D. Block. NSF Center for Biological Timing, Dept. of Biology, Univ. of Virginia, Charlottesville, VA 22903.

Basal retinal neurons (BRNs) from the eyes of the marine snail *Bulla gouldiana* continue to express a circadian rhythm in membrane conductance for at least two cycles in primary cell culture (Michel et al. 1993, Science 259: 239).

Cultured BRNs generate action potentials that have Na^+ and Ca^{2+} components. We used the perforated patch-clamp technique to characterize the ionic currents present in these dispersed pacemaker neurons. Three distinct K^+ currents were evoked by depolarizing voltage steps. A calcium-dependent K^+ current, blocked by quinine (100 μM) and 9-aminoacridine (400 μM), an inactivating (A-like) K^+ current blocked by 4-aminopyridine (400 μM), and a delayed rectifier current blocked by tetraethylammonium (10 mM). The total normalized current amplitude for voltage steps from -80 mV to +50 mV of all perforated patch recordings before projected dawn (n=31, Zeitgeber Time (ZT) 19 to ZT 22) is significantly higher ($p<0.01$) than after projected dawn (n=22, ZT 2 to ZT 4), confirming the previous results with intracellular recordings. We are now measuring the separate outward currents at different circadian times to identify the specific conductance(s) controlled by the pacemaker and ultimately investigate its type of modulation. *Supported by NS15264 to GDB. SM, KM and JJZ were supported by the NSF Center for Biological Timing.*

662.22

SHIFTING PHASE ON ELECTRORETINOGRAM CIRCADIAN RHYTHM INDUCED BY LONG DARK STIMULUS IN CRAYFISH Procambarus. V.
Inclán-Rubio* and A. Borgonio-Aguilar. Departamento de Fisiología, Facultad de Medicina, Universidad Nacional Autónoma de México, México, D.F.

Due to their ability to be synchronized, circadian rhythms couple well to environmental signals. It is known that electroretinographic response (ERG) in the compound eye of crayfish, presents circadian changes with a predominantly nocturnal activity. This rhythm is capable of displaying phase shifts when short white or monochromatic light signals are applied on the eye photoreceptors. In these cases, conspicuous differences in the phase response curve (PRC) can be observed in basis a wavelength used. The objective of this work is to analyze the changes upon ERG light-adapted rhythm, when we applied a long term-dark stimulus (6h), during different circadian time (CT), so as to explore the complete 24h. cycle. We worked with adult crayfish Procambarus clarkii, in constant conditions of light (30 lx) and temperature (19°C). The ERG was obtained by means of a metal microelectrode (1 a 5 mv) and continuously recording at least 12 days. The results show one bimodal PRC, with advances if the stimulus was applied at 18:00-24:00 CT; delays at 24:00-06:00 CT, and any change at 12:00-18:00 CT. The transitory advances have a 12-48 hours duration, meanwhile delays occur a day after stimulus application. Studies with retinal shielding pigments pointed out the possibility of a neural mechanism as the responsible of these responses.

662.23

EFFECTS OF THE LIGHT QUALITY ON THE PHASE RESPONSE CURVE(PRC) OF THE ERG CIRCADIAN RHYTHM IN JUVENILE INSTARS CRAYFISH *Procambarus clarkii*.
M.L. Fanjul-Moles*, M. Miranda-Anaya and A.Bernal-Moreno. Depto. Biología, Fac. Ciencias, U.N.A.M. Ap.Pos. 70-371, 04510, México, D.F.

In previous investigations we proposed, during the ontogeny of crayfish, the existence of two independent systems involved in the synchronization of the electroretinographic (ERG) circadian rhythm (a short and a long wave system). The aim of this work was to test this hypothesis by studying the phase shifts evoked with two monochromatic lights of the same intensity in the ERG circadian rhythm of juvenile instars. A group of 72 animals showing an overt circadian rhythm were individually housed under constant temperature and darkness. The eye's electrical response to white test flashes (ERG) delivered every 15 min was recorded by the usual technique. Each crayfish was left in free running conditions during at least four days; on the fifth day, and at different circadian time, a 30 min. pulse of monochromatic light (blue or red) was applied. Its effect on the phase of the ERG oscillation was determined on the fourth day after. PRCs for each monochromatic light were constructed. The results showed the blue pulses elicited predominantly delays shifting phase during the subjective day and subjective night. The red pulses evoked advances shifting phase in the subjective night and delays in the subjective day. The magnitude of the changes depends both on the wave length and the circadian time. These results seem to support the hypothesis proposed. Supported by CONACyT Ref91866PFPN/92/92, and PADEP, FC-9212.

662.24

PINEAL PHOTORECEPTOR CELLS IN THE ANOLE LIZARD RHYTHMICALLY SECRETE MELATONIN.
W.X. Tang and G.E. Pickard*. Department of Psychiatry, University of Pennsylvania, Philadelphia, PA 19104-6141.

The pineal gland of the lizard (Anolis carolinensis) rhythmically secretes melatonin both in vivo and in vitro. Using a modified reverse hemolytic plaque assay for the detection of melatonin secretion from dispersed pineal cells in culture, we recently demonstrated that individual pineal cells function as circadian oscillators and rhythmically secrete melatonin (Pickard and Tang, Soc. for Neurosci. 18:879 '92).

To determine the organization of the anole pineal, EM analysis was conducted. This analysis revealed three principle cell types: sensory, support and ganglion. The most common cell is the photoreceptor, the outer segment of which is poorly developed and variable in both size and shape. The finger-shaped cell has a basal nucleus and an expanded apical region, the inner segment. Photoreceptors are found most distally in the layered pineal gland. At the light microscopic level, the photoreceptor stratum is positive for S-antigen immunoreactivity.

To ascertain whether pineal photoreceptors secrete melatonin in vitro, melatonin secreting pineal cells (as identified using the reverse hemolytic plaque assay) were tested for S-antigen immunoreactivity. All plaque producing pineal cells were S-antigen positive. Some S-antigen positive cells did not form plaques. The data suggest that pineal photoreceptors rhythmically secrete melatonin. Supported by NIH grant RR 08052.

662.25

AN ENDOGENOUS LOCOMOTOR CIRCADIAN RHYTHM IN *LYMNAEA STAGNALIS*. J. A. Tobin and T. J. Mueller*. Department of Biology, Harvey Mudd College, Claremont, CA 91711.

Endogenous circadian rhythms have been demonstrated in several marine and terrestrial gastropods (*Aplysia, Bursatella, Bulla, Navanax, Helix, Limax, Arion*). However, a literature search indicates that no endogenous circadian rhythms have been demonstrated or disproved in any freshwater gastropod, or in any gastropod belonging to the order Basommatophora. As a freshwater pulmonate basommatophore, the Minnesota pond snail *Lymnaea stagnalis* is a member of both these categories.

We measured average speed of locomotion of *Lymnaea* as a function of time both during normal light-dark cycling, and in constant darkness. We used infrared illumination and an infrared-sensitive CCD TV camera to image the snails crawling on the side of a glass aquarium. The images were digitized and processed to calculate crawling rate variations over time. In multi-day, replicate experiments for both light-dark and dark-dark conditions, average crawling rate showed significant peaks and troughs in each 24 hour period. Fourier analysis of these data indicated a dominant period of approximately 24 hours. Ecological implications for circadian rhythms in these snails will be discussed.

NEUROETHOLOGY: BEHAVIORAL STRATEGIES

663.1

THE EFFECT OF HYPOGLOSSAL AND GLOSSOPHARYNGEAL NERVE TRANSECTION ON THE PREY CATCHING BEHAVIOR OF FROGS (RANA PIPIENS). A. Weerasuriya* and C. Baker. School of Medicine, Mercer University, Macon, GA 31207.

Frogs strike at prey with their tongues and retrieve them into the mouth in a rapid movement requiring the coordination of at least 30 muscles within a period of less than 200 msec. It has been postulated that a motor pattern generator interposed between the output elements of the sensory analyzers and the motoneuron pools is responsible for the elaboration of the spatio-temporal sequence of neuronal activation necessary for successful prey capture. In *Bufo bufo*, bilateral transection of the hypoglossal nerves causes the toad to lunge at prey without any accompanying jaw movements (Weerasuriya, 1989). We extended these studies in frogs and examined the interactions among these muscles with a more extensive series of nerve transections coupled with high speed video recordings. In frogs, anesthetized with MS-222, either one or both hypoglossal nerves or both glossopharyngeal nerves were transected and ligated. The ability of these frogs to capture live mealworms, and manipulate mealworms and other objects placed in their mouth was observed and recorded for two months after the transections. After two months the transections were verified by postmortem dissection. Following bilateral glossopharyngeal transection, the frogs were able to capture mealworms successfully. A unilateral hypoglossal transection did not preclude the frogs from capturing mealworms, but the protruded tongue deviated to the unlesioned side. A bilateral transection precluded jaw movement during the lunge, but these frogs moved their jaws to swallow mealworms placed in their mouths and to reject unpalatable objects. Even after two months the mouth did not open during a lunge.

663.2

SENSORY MODALITIES THAT INFLUENCE MOTOR PATTERN CHOICE IN THE LEOPARD FROG, Rana pipiens. C.W. Anderson* and T.A. Brand. Dept. of Biology, Northern Arizona University, Flagstaff, AZ 86011.

Previous researchers have shown that multiple motor patterns can be elicited from a given stimulus. In this study, we were interested in the stimulus parameters that elicit particular feeding behavior motor patterns. If the frog can produce differing motor outputs from the same sensory input, how is the behavioral output decided?

In previous studies, we have shown that the leopard frog, Rana pipiens, exhibits distinctly different feeding behaviors in response to different prey types. When feeding on waxworms (approximately 1.5-2.0 cm), R. pipiens captures prey using tongue protraction. When offered earthworms (approximately 4.5-6.0 cm), it captures prey using jaw prehension. In this study, we have tried to elucidate the sensory modalities that determine the feeding motor pattern choice in Rana pipiens. Rana was offered 1.5, 3.0, and 4.5 cm earthworm pieces. Motor patterns elicited by the 1.5 cm piece of earthworm were very similar to the behavior observed when feeding on similar sized waxworms. Rana captured the 4.5 cm earthworm piece using jaw prehension, as when feeding on whole earthworms. The 3.0 cm piece of earthworm elicited both the tongue protraction and the jaw prehension feeding behaviors at approximately equal frequencies. This appears to represent a random choice between two discrete motor patterns. Additional experiments are in progress to determine the contribution of olfaction to feeding behavior. These studies suggest that a visual analysis of prey size is used to choose between alternative feeding motor patterns.

Supported by NSF grants IBN 8909937 and IBN 9211310 to K. Nishikawa.

663.3

THE FREE-WILL PROBLEM: MOTOR CHOICE AND INTRINSIC VARIABILITY IN FROG AND LEECH. P. Grobstein*, P. Brodfuehrer, and J. Oristaglio, Dept. of Biology, Bryn Mawr College, Bryn Mawr, PA 19010.

The Harvard Law of Animal Behavior has it that "Under carefully controlled circumstances, an animal will behave as it damned well pleases." Many investigators presume this law to reflect a failure of experimenters to adequately control surrounding experimental conditions, and so discount observed variability. We have undertaken studies from a different perspective, treating variability as a significant phenomenon in its own right rather than an experimental inconvenience.

The movements made by a given frog for a prey item at a given location vary from trial to trial. The variability does not reflect absolute limits in the precision of neural processing: reduced variability is observed under particular circumstances (increased complexity in the visual scene). The variability is not reduced by further confining the moving prey, nor increased by increasing variation in frog initial posture. The findings suggest that the variability originates in the nervous system, rather than in afferent pathways, and reflects a controllable element significant in understanding input/output relations. Variability may reduce predator predictability, and support the creation of novel motor sequences to solve novel problems.

Leeches sometimes but not always respond to cutaneous stimulation by swimming. In an isolated leech nervous system, peripheral nerve stimulation at an appropriate constant voltage triggers a swimming motor pattern on some trials but not others. Intrinsic nervous system variability, hypothesized for the frog, exists in the leech. The unpredictability of the isolated leech nervous system can be reduced by removal of the head ganglion. This suggests that intrinsic variability in leech, as in frog, is not a reflection of inevitable limits in the precision of neural processing, and that it may be possible to localize distinct variability enhancing elements.

We conclude that intrinsic variability is an important and characterizable element of nervous system function, of substantial significance for understanding input/output relationships in behavior. Organisms endowed with a probabilistic variability, as well with a capacity to monitor potential outputs and to control which will be expressed in behavior may be those that exhibit "free will."

663.4

A SMALL BENTHIC ROBOT FOR STUDIES IN MARINE ANIMAL CHEMOSENSING, T.R. Consi*, C.A. Goudey and J. Atema†, MIT Sea Grant College Program, AUV Laboratory, Cambridge, MA 02139, †Boston University Marine Program, Woods Hole, MA 02543

A great variety of marine animals navigate using chemical signals to locate food sources, mates and spawning grounds. The sensory systems and behaviors exhibited by these animals are well adapted for robust operations in turbulent and chemically complex environments. The algorithms used by an animal to rapidly extract navigational information from a discontinuous and chaotic chemical signal are presently unknown and are the topic of current research. We are developing a small benthic robot which will be used as an algorithm test bed for ideas in biological chemosensing behavior. The robot is designed to mimic the basic features of a lobster relevant to chemical sensing and associated behaviors. The lobster was chosen as a model system because its chemosensing capabilities are sophisticated and have been well studied.

Our robot is a small wheeled vehicle with the size, speed and maneuverability of an adult lobster. It is driven by two DC gear motors and is battery powered. A pair of conductivity electrodes serve as the robot's "antennae", the analogs to the chemical sensors of the lobster antennules. Vehicle control is provided by an MC68332-based microcontroller system.

The basic experimental paradigm is to set up a plume of saltwater in a freshwater flow-through flume and program the vehicle to follow the plume to its source. A saltwater plume was chosen as a reasonable approximation to an underwater chemical plume. Plume following algorithms which have been proposed for the lobster will be programmed into the vehicle and the experiments will determine the effectiveness of the various algorithms. The flow-through flume and measuring apparatus are the same as are used in studies of lobster chemosensing behavior, thus making the data from the vehicle directly comparable to that of the animal. We will present the design details of this vehicle along with initial experimental results.

663.5

SPATIAL BEHAVIOR IN THE BRAZILIAN SHORT-TAILED OPOSSUM, *MONODELPHIS DOMESTICA*: COMPARISON WITH *RATTUS NORVEGICUS* IN MORRIS WATER TASK AND RADIAL ARM MAZE. D. P. Kimble* and I. Q. Whishaw, Institute of Neuroscience, University of Oregon, Eugene, OR 97403 and Department of Psychology, University of Lethbridge, Lethbridge, AL T1K 3M4.
Six opossums (three male, three female) and an equal number of male and female rats were tested in a Morris water task with both hidden and visible platforms. Opossums were inferior on both tasks, but did show significant learning in the visible (but not hidden) platform task. Opossums tended to swim around the outer edges of the pool when the platform was hidden, rarely venturing into the middle, while rats displayed a more efficient search strategy. Opossums and rats showed distinctly different defecation patterns in the water task. Both rats and opossums were naive swimmers prior to the experiment. All animals were then tested in an 8-arm radial maze, in which they were given 14 trials with four arms baited and an additional 14 trials with the other four arms baited. Both opossums and rats reduced errors across the two tasks, with rats showing superior performance. Differences in search strategies were found, with rats showing a systematic "arm by arm" strategy on a high percentage of the trials, while opossums displayed arm by arm strategy on far fewer trials. Opossums, on the other hand, showed higher levels of a "cross maze" strategy, but overall lower levels of any discernible search strategy. Opossums were inferior to rats in both spatial situations. Differences between placental mammals and marsupials in brain morphology and evolutionary paths are considered.

663.6

MODULATING EFFECTS OF VISION ON JUVENILE PLAY BEHAVIOUR IN RATS. Mario M. Mckenna, Vivien C. Pellis*, Glen Prusky and Sergio M. Pellis, Dept. Psychology, Univ. Lethbridge, Lethbridge, AB T1K 3M4.

Play fighting behaviour in juvenile rats consists of two elements: playful attack and playful defense. Playful attack consists of snout to nape contact in which one animal gently nuzzles the nape area of its partner. Playful defense involves strategies in which the animal avoids snout to nape contact via removal of the nape area from attack (evasion) or blocking contact to the nape (rotation to a supine position). While studies have shown that vision is not necessary for normal frequencies of play fighting, previous studies have not explored the effects of vision on the components of play fighting. Specifically, is vision necessary for the organization of playful attack and defense? Eight juvenile female rats were enucleated at birth and their juvenile play behaviour compared to that of eight control females. High speed video analysis revealed that vision can be used to orient playful attacks toward the nape of the partner. Enucleated females appeared to arbitrarily attack the body of the partner and when contact was attained, relied on tactile input to direct their attack to the nape of the defender. In contrast to this, control females consistently contacted that nape area of its partner when attempting to initiate playful contact. Similarly, for defense against nape attack, enucleated rats responded when the attacker was closer to the nape. The results of this study suggest that while vision is not necessary for play to occur, it has a role in modulating playful attack and defense.

663.7

DOMINANCE STATUS, BEHAVIOR IN THE ELEVATED PLUS MAZE AND ULTRASONIC VOCALIZATIONS IN TRIAD-HOUSED RATS. L.A. Pohorecky*, S.A. Larson, and D. Benjamin. Center of Alcohol Studies, Rutgers University, Piscataway, NJ 08855.

Group housing of rodents results in the rapid establishment of dominance status. While dominance status has well known consequences on aggressive and sexual behaviors, effects on other behaviors are less known. We report on the effects of social status on body weight, and on two measures of anxiety: the elevated plus maze test (EPM) and ultrasonic vocalizations elicited by air-puffs (USV). Two sets (consisting of 10 and of 15 colonies) of male Long-Evans hooded rat triads, matched for initial body weight, were maintained and tested over a period of 38-40 days. Weight gain differences between the dominant (D), subdominant (SD) and subordinate (SO) rats developed rapidly, were significant and persistent throughout the experimental period. D rats had the greatest weight gain, while it was least in the SO rats (overall ANOVA $F=7.566$, $p=0.001$). In the EPM, an established test of anxiety, there was a rank related gradation in anxiety (overall contrast analysis of D vs SO $p=0.033$): D rats exhibited least anxiety (more open arm entries and more time in open arms), SO rats exhibited the most anxiety. Startling air puffs resulted in more USVs in SD rats in the first (contrast analysis of SD vs SO $p=0.034$), but did not reach significance, in the second experiment. Nevertheless the number of air puffs needed to elicit USV in the second experiment were greater for the SD rats. These preliminary findings indicate that there are significant rank-related differences in anxiety levels in group housed male rats which suggest differences in underlying neurochemical mechanisms subserving behavior in the EPM and the USV tests. (Supported by the Smithers Foundation and NIAAA grant AA05306).

663.8

CORTICAL 5-HT$_2$ RECEPTOR BINDING IS INVERSELY RELATED TO DOMINANCE STATUS IN TRIAD-HOUSED RATS. D. Benjamin*, E.I. Saiff, K.R. Goldstein, S.A. Larson, A. de Adrianza, and L.A. Pohorecky. Center of Alcohol Studies, Rutgers University, Piscataway, NJ, 08855.

Male rats housed in triads rapidly form a dominance hierarchy characterized by aggression and initial weight loss in subdominant (SD) and subordinate (SO) rats as compared with the dominant (D). A behavioral profile indicative of anxiety was observed in the nondominant rats (see Pohorecky *et al.*, this volume). To characterize the neurochemical basis for the significant behavioral changes induced by triad housing, 5-HT$_2$ and 5-HT$_{1A}$ receptor binding was assessed autoradiographically in four colonies of triad-housed rats previously classified by status and behavior. Subjects were sacrificed after 38 days of colony housing, and brains were sectioned in the coronal plane for autoradiographic analysis of binding. In the present sample, no significant differences were detected in 5-HT$_{1A}$ receptor binding as assessed using [^{3}H]-8-OH-DPAT. 5-HT$_2$ receptor binding was assessed using [^{3}H]-mesulergine (2 nM); binding to frontal and prefrontal cortex layer 4 differed significantly within colonies, with less labelling in D rats and the most in SO rats ($p = 0.011$). This effect was confined to frontal and prefrontal cortex, with no dominance-related differences in the claustrum or more posterior cortex. Interestingly, cortical 5-HT2 receptors have previously been shown to be associated with anxiety. These preliminary results indicate that the frontal cortical 5-HT$_2$ receptor system is affected by dominance status, which may underlie some of the observed behavioral effects. Experiments are in progress to evaluate the involvement of other receptor subtypes. (Supported by the Smithers Foundation and NIAAA grant AA05306).

663.9

ON THE USE OF VISUAL MOTION PERCEPTION TO CONTROL LOCOMOTION: SYNTHESIS OF A BIOLOGICALLY-INSPIRED VISUALLY-GUIDED CREATURE USING PARALLEL ANALOG PROCESSING. N. Franceschini*, J.M. Pichon, C. Blanes, C.N.R.S. Lab. Neurobiology, 31, Ch. J. Aiguier, 13009 Marseille (France).

In trying to understand how photoreceptor cells can be linked to muscle cells to achieve smart visually guided behavior we used biologically-inspired synthetic neural modeling to design, simulate and build a completely autonomous creature which can move about in complex environments using exclusively *visual motion perception*. This project was derived from our optical and electrophysiological analyses on *directionally selective motion sensitive neurons*, the working principle of which was first transcribed into analog optoelectonic circuits.

A battery of such smallfield neurons analyse the panoramic visual world in the azimuthal plane. The layout of obstacles in the environment is discovered step-by-step by the creature whose sensory map is transformed into a motor map that controls steering. The creature can negotiate an obstacle course to a target at a relatively high speed (50 cm/s).

We have proposed one solution to the problem of how a retina can be interfaced to a motor system for the sake of survival in cluttered environments and how multisensory fusion can be achieved by purely analog, brainlike networks. The creature we have built shows specific features common to advanced animals, such as nonuniform retinal sampling, saccadic suppression and corollary discharges. It requires no digital computer on-board and no long-term memory.

664.1

INTRAVENTRICULAR DPDPE ADMINISTRATION FOLLOWING ACUTE STRESSOR APPLICATION: DIFFERENTIAL INFLUENCE ON ACTIVITY, HEAD DIPPING AND EXPLORATION. Paul Mendella[1], Jill Irwin[2]* and R.M.Zacharko[1], [1]Unit for Behavioral Medicine and Pharmacology, Life Sciences Research Centre, Carleton University, Psychology Department, Ottawa K1S 5B6 and [2]Queen's University, Psychology Department, Kingston, Ontario K7L 3N6

Uncontrollable stressors provoke behavioral disturbances in a number of paradigms. The nature of these alterations have been attributed to variations of central CA and 5-HT activity. Neuropeptides appear to modulate neurotransmitter turnover and to influence the profile of behavioral change. We investigated the effects of intraventricular administration of DPDPE, a relatively selective ∂ receptor agonist, on activity, exploratory behavior and investigatory head dipping, following acute, uncontrollable footshock in the CD-1 mouse. The stressor produced a reduction of activity in the immediate post-stressor interval. Low dose administration of the neuropeptide (1 and 2.5 ug) provoked an enhancement of activity which exceeded baseline performance for at least 30 min post-drug administration. However, 1 ug of DPDPE appeared to have a greater effect on locomotor activity than the higher dose of the neuropeptide in non-shocked animals. Long term alterations attributable to either the stressor or the neuropeptide appeared (or persisted) after 1 week. In contrast to activity, a dose dependent reduction in exploration was evident both immediately and 24 hr after stressor exposure. The influence of 1 and 2.5 ug doses of DPDPE was negligible in stressed animals but produced behavioral alterations in non-shocked mice at more protracted intervals. Uncontrollable footshock reduced investigatory head dipping which was unaffected by drug administration at either dose. Taken together, these data suggest that the immediate and long term behavioral consequences of ∂ receptor activation are dose dependent and vary as a function of stressor imposition. Such alterations conceivably underlie sensitization effects at protracted intervals. Supported by Gustavus and Louis Pfeiffer Research Foundation and NSERC of Canada

664.2

SOCIAL STRESS MODULATES MORPHINE SENSITIVITY IN MALE AND FEMALE ROMAN RATS M. Haney*, N. Castanon, M. Le Moal and P. Mormède. INSERM U 259, Université de Bordeaux II, Bordeaux FRANCE

Roman low avoidance (RLA) rats freeze, while Roman high avoidance (RHA) rats remain motorically active in response to mild stress. What is the long-term effect of an aggressive attack on morphine-induced analgesia and locomotor activity in rats with genetically distinct responses to stress? Male and female, RHA and RLA rats (n=64) were either exposed to 2 brief attack encounters (2 min) from an aggressive, same-sex conspecific or were left undisturbed. Attack encounters were separated by a 30 min. interval, during which rats were threatened but protected from attack by wire mesh. Morphine was cumulatively administered (3, 6, 10 mg/kg IP) 1 week later. Injections occurred every 30 min, following tailflick measurement; locomotion was measured between injections (30 min) in an activity cage. There were no strain differences in defensive behavior, with both RHA and RLA rats actively attempting to avoid attack. The experience of attack greatly reduced sensitivity to morphine-induced analgesia 1 wk later, while having no effect on morphine's locomotor properties. Strains did not differ in sensitivity to morphine-induced analgesia. Yet only the RHA line showed an excitatory locomotor response to morphine, while activity in RLA rats was unchanged. Therefore: (1) RHA rats are more motorically active in response to morphine, as they are to cocaine or mild stress, and (2) Analgesia is a more sensitive measure of the long-term consequences of social stress on the opioid system than locomotor activity.

664.3

FREQUENCY OF EXPOSURE BUT NOT DIET AFFECTS ANALGESIA PRODUCED BY INTERMITTENT COLD WATER SWIMS. R.B. Kanarek*, M. Ryu, R. Olivardia, E. Chang and R. Marks-Kaufman. Dept. of Psychology, Tufts Univ., Medford, MA 02155.

Previous work using a tail flick apparatus demonstrated that chronic sucrose intake in rats decreased baseline pain sensitivity, but enhanced morphine-induced analgesia. To examine the generality of this finding, the effect of sucrose intake on analgesia resulting from intermittent cold water swims was studied in adult male Sprague-Dawley rats. Using a tail flick apparatus, analgesic responsiveness [% maximal possible response (%MPE)] was measured 30, 60 and 90 mins after 9 or 18, 10 secs swims in 2° C water separated by 10 secs rest periods in rats given ad lib access to a 32% sucrose solution, Purina chow and water (n=20), or only chow and water (n=20). Rats given the sucrose solution consumed more calories per day and gained more weight than rats not eating the sugar. Sucrose availability had no effect on analgesic responsiveness following cold water swims. However, 60 mins after swimming, rats given 9 swims had significantly ($p < 0.05$) greater %MPE than rats given 18 swims. To determine if opioid mechanisms were involved in analgesia resulting from intermittent cold water swims, rats were injected (sc) with 10 mg/kg naltrexone. Naltrexone significantly ($p < 0.05$) reduced %MPE 30 and 90 minutes after swimming in rats given 18 swims, but had no effect on %MPE in rats given 9 swims. The present results indicated that diet does not have equivalent effects on morphine and stress-induced analgesia. These data suggest that the number of experiences an animal has with a stressful stimulus is important in determining the analgesic potency of the stimulus.

664.4

THE EFFECTS OF 6-OHDA LESIONS OF THE MEDIAL PREFRONTAL CORTEX ON ELEVATIONS IN CORE TEMPERATURE INDUCED BY RESTRAINT STRESS IN THE RAT. D. Funk* and J. Stewart. Center for Studies in Behavioral Neurobiology, Dept. Psychology, Concordia University, Montreal, Canada, H3G 1M8.

The dopamine (DA)-containing projections to the medial prefrontal cortex (MFC) are activated in response to stress. The functional role of MFC DA release in the stress-induced activation of the autonomic nervous system (ANS), however remains undefined. This study examined the effects of DA depletion of the MFC with 6-hydroxydopamine (6-OHDA) on a response mediated by ANS activation, the increase in core temperature induced by physical restraint.

Male rats pretreated with desipramine or its vehicle received infusions of either 6-OHDA (4µg in 1µl ascorbic acid vehicle/site) or vehicle bilaterally into the MFC. The 3 groups consisted of rats with DAergic lesions (L-DA), DA and norepinephrine (NE) lesions (L-DA/NE) or sham lesions (VEH). Three weeks after surgery, temperature transmitters were implanted i.p. Four days later temperature samples were taken every 10 min throughout 4 contiguous 1-h periods: a 1-h baseline, 1 h of restraint, and a 2-h post-restraint period. Restraint for 1 h elevated core temperature significantly in all groups. Group L-DA failed to attain as high a level of core temperature as the other groups during restraint. These results suggest that the DAergic projection to the MFC plays a role in stress responses mediated by the ANS. Studies are in progress to assess the effects of MFC lesions on the activation of the cardiovascular and hypothalamo-pituitary-adrenal systems by stress.

664.5

LOCOMOTOR RESPONSE TO NOVELTY CORRELATES WITH AMPHETAMINE- AND STRESS-INDUCED ACTIVITY IN RATS: RELATION TO HIPPOCAMPAL NOREPINEPHRINE EFFLUX. L.A.Rosario* and E.D. Abercrombie. Center for Molecular and Behavioral Neuroscience, Rutgers University, Newark, NJ 07102.

Previous studies demonstrate that the locomotor activity (LA) response of rats placed in a novel environment is predictive of the LA response to systemic amphetamine (AMPH) administration, suggesting common mechanism(s) of behavioral reactivity in these two conditions. In such studies, rats are designated as high responders (HR) or as low responders (LR) according to LA counts obtained during 60 min exposure to a novel environment. Since the norepinephrine- (NE) containing neurons of the locus coeruleus (LC) have been implicated in brain mechanisms of arousal and stress, we are interested in whether differences in forebrain NE release might exist in HR and LR rats. We compared the LA response to 30 min tail-pinch stress and AMPH administration (1.5 mg/kg, i.p.) in HR and LR rats. Extracellular NE level in hippocampus also was monitored during these manipulations using *in vivo* microdialysis. Rats were pre-screened for assignment to HR (n=5) or LR (n=4) groups on the day before dialysis probe implantation. All animals were run blind as to group assignment. Baseline LA counts and extracellular NE levels were not significantly different in the HR versus LR groups. In response to the tail-pinch stress, LA counts were significantly greater in HR rats compared to LR rats. Preliminary neurochemical results show that the stressor produced a 65% increase in extracellular NE of the HR group whereas NE levels increased 40% in the LR group. Similar to the results obtained with stress, AMPH-stimulated LA activity was significantly greater in HR rats than in LR rats. Hippocampal NE efflux in response to AMPH was increased by a maximum of 563% in the HR group and 384% in the LR group. These data support the hypothesis that individual differences in behavioral reactivity and behavioral activation produced by stress or AMPH may share common neural substrate(s). Further, the preliminary neurochemical data suggest that evoked NE release may contribute at least in part to such behavioral differences. [Supported in part by USPHS Grant MH43947]

664.6

MODERATE STRESS ENHANCES ACQUISITION OF A SPATIAL MEMORY TASK. C.Martinez, M.Villegas, V.Luine* and B.S.McEwen, Dept. Psychology, Hunter College and Rockefeller University, N.Y., N.Y. 10021

Chronic restraint stress, 21 days, causes impaired acquisition of a spatial memory task in rats (Villegas, et al, Soc. Neurosci. Abs., 1993). We examined the effect of fewer days of restraint stress on acqusition of a spatial memory task. Rats (n=10-12) were placed in plexiglass restrainers for 6h/day for 7 or 13 consecutive days. Controls (n=11-12) were weighed only. After the last restraint, rats were food deprived, then received 9 shaping trials on the eight arm radial maze (days 3-7 post stress), 4 regular trials (days 10-13) and 5 trials with delays after the 4th choice (days 14-15). Daily restraint for 7 days did not affect performance, but rats stressed for 13 days performed better than controls: number correct in the first 8 choices was 7.32 ± 0.11 vs. 6.8 ± 0.11 ($F1,19 = 13.1$, $P<.002$). It also took stressed rats fewer total choices to finish the maze than controls (9.6 ± 0.34 vs. 10.59 ± 0.40, $F1,19 = 5.35$, $P<.03$). Serum corticosterone was measured after 2 and 6 h. of restraint on the 3rd, 8th and 13th day of stress. Performance of the stressed rats correlated with corticosterone levels at 13 days ($r=-.63$, $P<.05$); rats with higher levels took fewer choices to finish. Enhanced performance was not permanent since trials with delays, conducted immediately following the regular trials, showed no differences between the groups. These results, taken together with Villegas et al, 1993, suggest that a short duration of stress does not affect acquisition of the 8-arm maze, while a moderate duration may serve an adaptive function, and longer durations of stress are maladaptive for learning/memory. (GM08176 & GN41256)

664.7

CHLORDIAZEPOXIDE IN THE DORSAL RAPHE NUCLEUS ELIMINATES THE EFFECTS OF INESCAPABLE SHOCK ON SHUTTLEBOX ESCAPE LEARNING AND FEAR CONDITIONING. R.E. Grahn*, B.A. Kalman, L.R. Watkins, and S.F. Maier. Behavioral Neuroscience Program, Department of Psychology, University of Colorado, Boulder, CO 80309.

Increases in the excitability of the dorsal raphe nucleus (DRN) have been argued to be involved in the mediation of the behavioral changes produced by inescapable shock (IS). DRN activity is reduced by the systemic administration of benzodiazepines (BZs) and this BZ treatment before IS eliminates the escape learning deficits produced by prior exposure to IS. However, BZs given before behavioral testing fail to alter the effects of IS. It is possible that BZ actions at other brain sites resulting from systemic administration mask effects of BZ activity at the DRN. In the present experiment, rats were exposed to IS or control treatment and tested for fear conditioning and shuttlebox escape 24 hr later. Chlordiazepoxide (CDP) (30ng) was microinjected into the region of the DRN either before IS or before testing 24 hr after IS. CDP restricted to the DRN did block both the exaggerated fear conditioning and escape learning deficits produced by IS, whether administered before IS or before testing. CDP (3ug) administered intracerebroventricularly (ICV) blocked fear conditioning whether given before IS or testing, but blocked the escape deficit only when administered before IS. These findings indicate that administration of BZ isolated to the region of the DRN blocks the effects of IS, whereas this treatment administered in a nonspecific manner (either peripherally or centrally) is only effective if that administration occurs before IS.

664.8

CRH BLOCKADE REDUCES NALOXONE-REVERSIBLE, BUT NOT NALOXONE NON- REVERSIBLE STRESS-INDUCED ANALGESIA.

M.Bianchi, P.Sacerdote, A.E. Panerai * Dept. Pharmacology, University of Milan, 20129 Milan, Italy.

Depending on its temporal characteristics, inescapable foot-shock causes analgesia in rats by activating different mechanisms (1,2). Inescapable foot-shock is currently used in order to investigate the effects of stress on different biological functions (3). Since corticotropin-releasing hormone (CRH) is considered the main mediator of stress response, we evaluated the effect of the CRH antagonist alpha-Helical CRH 9-41 on the analgesia obtained by the exposure of rats (8 in each experimental group) to intermittent (naloxone-reversible) or continuos (naloxone non-reversible) electric foot-shock (2.5 mA, 60Hz). The intracerebroventricular administration of the CRH antagonist (20 µg/10 µl) significantly reduced the increase of tail-flick latencies induced by the intermittent foot-shock. On the contrary, the CRH antagonist did not modified the analgesic effect of continuos foot-shock. Our results confirm that stress-induced analgesia can be obtained by the activation of different endogenous systems and suggest that intermittent foot-shock represents the more suitable model for the stress studies.

1. J.W. Lewis, J.T. Cannon. J. C. Liebeskind, Science 208: 623-625, 1980
2. P. Sacerdote et al. Brain Res. 359: 34-38, 1985
3. S. Rivest and C. Rivier, Endocrinology 129: 2049-2057, 1991

664.9

BEHAVIORAL SENSITIZATION FOLLOWING ACUTE STRESSOR APPLICATION: INFLUENCE OF EXPERIENTIAL FACTORS. G. MacNeil* and R.M. Zacharko, Unit for Behavioral Medicine and Pharmacology, Life Sciences Research Centre, Carleton University, Psychology Department, Ottawa, Ontario,CANADA K1S 5B6

Exposure to aversive life events in infrahuman subjects has been associated with the induction of behavioral disturbances which may be reminiscent of symptoms associated with major affective and post-traumatic stress disorder (PTSD). The present investigation determined whether prior experience with major laboratory stressors sensitizes an organism to the subsequent effects of acute, uncontrollable footshock. In the absence of a stressor, general levels of activity ordinarily decline and stabilize as a function of time. In contrast, levels of exploration remain relatively stable. Application of an acute stressor (mild footshock) in CD-1 mice produced marginal alterations of locomotor activity in the immediate post-stressor interval, while profound perturbations appeared with exploratory behavior. Such stressor induced behavioral variations were transient. Interestingly, however, among animals previously exposed to the stress of a surgical intervention (applied two weeks earlier), uncontrollable footshock exacerbated the reduction in both exploration and activity ordinarily evident in animals subjected to the stressor alone. In addition, experience with a qualitatively different laboratory stressor (unpredictable fire alarm) produced effects on exploration that were reminiscent of those seen in animals sensitized to footshock by surgery. Taken together, these data suggest that sensitization (and cross sensitization) may occur more readily for some aspects of behavior (activity cf. exploration) and that the time course of such behavioral change may be fundamentally different. The implications of these data for psychological disturbances are discussed. Supported by The Gustavus and Louis Pfeiffer Research Foundation and NSERC of Canada.

664.10

BEHAVIORAL DIFFERENCES OF DOMINANT AND SUBORDINATE RATS IN A VISIBLE BURROW SYSTEM. Errol B. Yudko*, Chris Bjornson, and Robert J. Blanchard, Dept. of Psychology, University of Hawaii, Honolulu, HI 96822.

Subordinate male rats of groups maintained in semi-natural visible burrowing situations (VBS) show enhanced defensive behavior, but reduced social, sexual, exploratory and aggressive activity, within the habitat. They have higher plasma corticosterone and lower plasma testosterone than dominants, and display increased voluntary alcohol consumption and higher mortality.

In the present study VBS subordinates were removed to single cages and allowed free access to food and water during 5 hour periods every other day of a two-week grouping period. Under this regimen, they increased their intake to levels higher than those of dominants or singly-housed controls but continued to lose weight, a pattern suggesting altered metabolism.

In an open field test run at intervals throughout the grouping period, dominants and subordinates initially showed reduced activity compared to controls, but the dominants gradually increased activity to control levels while subordinates continued to be inactive. Subordinates also appeared to be deficient in risk assessment behaviors measured in both a cat-odor apparatus or in an elevated plus-maze. These findings suggest that, although subordination is generally associated with increased defensiveness, it may produce specific changes in particular components of the defense pattern.

This research was supported by MH42803, AA06220, and RR08125.

664.11

CHRONIC CALORIC RESTRICTION AND "ACTIVITY-STRESS" EFFECTS IN RATS: A COMPARISON OF TWO STRESS MODELS.
J.K. Nishita* and M.D. Kawamoto. Dept. of Psychology, San Jose State Univ., San Jose, CA 95192-0120.

When housed in our novel apparatus, rats must crawl through a Plexiglass cylinder to obtain daily food and water. Because food is always located on one side of the cylinder and water is on the opposite side, rats learn to restrict their eating and drinking in order to negotiate the cylinder each day. We hypothesized that these rats will manifest behavioral and physiological changes similar to those produced by other stress-related paradigms. In the present study, physiological correlates of stress associated with the physical restriction produced by two different cylinder sizes [Small (SM) = 3.3 cm, Large (LG) = 4.2 cm, i.d.] were compared to those in the wheel-running "activity-stress" paradigm (Paré, 1974).

Both SM and LG rats showed weight loss and decreased linear growth compared to controls housed with No Cylinder (NC). Furthermore, adrenal weights and plasma corticosterone (CORT) levels were significantly different among SM, LG, and NC female rats following 100-120 days of continuous housing. Male SM, LG, and NC also showed significantly different adrenal weights, CORT, and body temperature when housed continuously for 80-, 100-, and 140-days. These measures and the incidence of gastric ulcerations were compared to rats reared using the 1-hr feeding "activity-stress" paradigm: (a) 22-hr continuous access to running wheels, (b) partial access, consisting of 22-hr continuous access to running wheels every other day, and (c) no access.

These data show similar stress effects between the two models and are important in understanding physiological correlates of "conflict" behavior and stress in the context of chronic caloric restriction.

664.12

IMMEDIATE AND LONG TERM ALTERATIONS INDUCED BY AN ACUTE STRESSOR: VARIATIONS IN ACTIVITY, EXPLORATION AND HEAD DIPPING FOLLOWING INTRAVENTRICULAR U50,488 ADMINISTRATION. R. Dickson and R.M. Zacharko*, Unit for Behavioral Medicine and Pharmacology, Life Sciences Research Centre, Carleton University, Psychology Department, Ottawa, CANADA K1S 5B6

Stressors produce behavioral alterations ordinarily attributed to changes in central NE, DA and 5-HT activity. Increasingly greater attention has been devoted to an analysis of the influence of neuropeptides to the induction, amelioration and/or exacerbation of behavioral pathology. The nature of the opioid receptor (µ, ∂, k), their distribution in the CNS and the region specific influence of various stressors affect expression of behavioral disturbance. Moreover, k receptor activation, unlike that of µ or ∂, cannot produce behavioral change in the absence of prior activation of the associated neurochemical substrate. We examined the dose and time dependent variations of U50,488 in the CD-1 mouse in several behavioral paradigms following exposure to footshock. Low dose administration of U50,488 provoked modest reductions of activity and exploration in the absence of footshock suggesting an interaction with the stress of handling. Higher doses of U50,488 had no effect immediately but augmented exploration and activity at protracted intervals, comparable to that of no drug-stress animals.When exploratory head dipping was assessed, footshock reduced performancE only in the immediate post-stressor interval. While the immediate effects of U50,488 were masked by footshock, a dose dependent differentiation was apparent at more protracted intervals. These data suggest that (a) U50,488 is ineffective in provoking immediate behavioral change in the absence of a stressor (b) the long term behavioral effects (exploration, activity) of U50,488 may mimic the effects of a stressor (c) the immediate effects of U50,488 and footshock are similar to those of footshock alone although a dose dependent behavioral differentiation appears at longer post-stressor intervals. Supported by the Gustavus and Louis Pfeiffer Research Foundation and NSERC of Canada.

664.13

STRESS-INDUCED BEHAVIORAL DEFICITS: BEHAVIORAL AND NEUROPHARMACOLOGICAL FACTORS. Y. Zhang* and E. A. Stone, Dept. Psychiatry, New York University School of Medicine, New York, NY 10016.

Stress is known to induce in rats a number of behavioral changes which resemble signs of anxiety and depression in humans. These include heightened defensiveness, anorexia, anhedonia and hypo-exploratory activity. The following experiments were undertaken to explore some of the behavioral and neurochemical factors eliciting certain of these changes. Rats were either housed singly or in pairs and were subjected to either restraint in a mesh pouch or immobilization (four limb restraint). Some rats received an injection of clonidine prior to the stress to suppress central noradrenergic activity. Exploratory activity and saccharine consumption were measured in the post-stress interval. It was found that the stressors used produced a decrease in saccharine consumption and exploratory activity. The decrease in saccharine consumption was more severe for immobilization than pouch restraint and for the single-housed as compared to the pair-housed rats. Exploratory activity was less consistently affected by these factors. The experiments on the effects of clonidine are currently in progress. It is concluded that the type of stress and the housing condition of the animal are key factors that markedly influence the severity of behavioral impairments resulting from stress. Supported in part by grants AFOSR 89-0208, MH45265 and MH08618.

664.14

DIFFERENTIAL SENSITIVITY OF THE ELEVATED PLUS-MAZE AND SOCIAL INTERACTION TESTS OF ANXIETY TO PRIOR STRESSOR CONTROLLABILITY. B.A. Kalman*, R.E. Grahn, F.X. Brennan, L.R. Watkins, and S.F. Maier. Behavioral Neuroscience Program, Department of Psychology, University of Colorado, Boulder, CO 80309.

Inescapable shock (IS), but not an equal amount of escapable shock (ES), results in a series of consequences collectively termed learned helplessness effects. These effects include poor shuttlebox escape performance, increased fear responding, increased analgesia, decreased aggression, alterations in neurotransmitter release and other behavioral and physiological changes. The goal of the present research was to address the possible role of anxiety in mediating these various effects of IS. The social interaction test of anxiety (File and Hyde, 1978) was previously used to demonstrate that IS subjects were more anxious than their ES counterparts 24 hours after shock (Short, 1991). Recent work indicates that different putative tests of "anxiety" are sensitive to changes in different underlying neurochemical systems. We therefore explored the effect of stressor controllability on the elevated plus-maze, a test that has a profile of pharmacological sensitivity different than that of social interaction. Male Sprague-Dawley rats (350-400g) were exposed to escapable shock, yoked-inescapable shock (100 1.0mA shocks, average ITI 60s) or restraint and subsequently tested on the elevated plus-maze. Anxiety was assessed by measuring the duration of open arm activity and the percentage of open arm entries 2, 24, and 48 hr after shock. The effects of manipulating subject handling, shock intensity and test area illumination were also assessed. In some instances the level of anxiety in shocked subjects was higher than in controls, but the clear difference between IS and ES subjects that was evident in the social interaction test of anxiety was not demonstrated using the elevated plus-maze. These results lend support to the idea that these tests of anxiety measure different aspects of that state.

664.15

EFFECTS OF EARLY STRESS ON THE BEHAVIORAL RESPONSE OF ADULT RATS TO AN AMPHETAMINE CHALLENGE. C.Arons[1,2], C.Cartegenes[2], W.Shoemaker[1] and P.Kehoe[2]. Dept. of Psychiatry, UCONN Health Center, Farmington CT 06030; Neuroscience Program, Trinity College, Hartford CT 06106.

In the adult rat repeated administration of psychomotor stimulants or repeated exposure to stress produces enduring enhanced responses to subsequent drug or stress challenges. We tested whether repeated exposure of neonates and pre-weanlings similarly enhances response to an amphetamine (AMPH) challenge and whether such effects endure into adulthood. Newborn rat pups were assigned to 1 of 4 treatment groups: 1) 1-hour daily isolation, postnatal day (PND) 2-9; 2) 3-minute daily cold water swim (CWS), PND21-25; 3) isolation+CWS; or 4) no stress. When the rats reached adulthood (PND70-90) they were tested for spontaneous and AMPH induced activity. Rats that had been stressed had higher stereotypy and rearing scores than non-stressed rats. Rats exposed to both isolation and CWS generally had higher scores than rats exposed to only one stress. Gender differences were also apparent. Overall, females had higher scores than males and more clearly showed the effects of stress. The data indicate that early stress results in enhanced response to challenges in adulthood.

664.16

DEVELOPMENT OF EPISODIC STRESS REACTIONS IN ADULT RATS TREATED NEONATALLY WITH AN ANTIDEPRESSANT. I.J.Goodman* L.Beeler and A.J.Azzaro. Depts. of Psychology and Behavioral Medicine & Psychiatry, West Virginia University, Morgantown, WV 26506.

Studies report paradoxical, "major depression-like" signs in adult rats after chronic, neonatal antidepressant injections. This study attempted to confirm and extend such findings, determining whether one might distinguish between such rats and those also experiencing later-life episodic stress. Rats 7-20 d old were injected daily with clomipramine HCl (CLO) (15 mg/kg, sc) with drug controls receiving saline (SAL). Open field (OFT) and swim tests (ST) were run on same rats at ages 4 and 10 mo. On 4 d prior to testing, half of CLO and SAL rats were stressed with footshock (Sh)(15 min sessions, 2 sec/min, .6 ma), other half not shocked (nS). CLO rats' body wts at 4 and 10 mo. were less than SALs'. At 4 mo., signif. OFT activity difference between CLO vs SAL rats in novel, large but not small open field; less struggle in CLO/Sh than SAL/Sh rats in ST. At 10 mo. coherent differences between CLO/nS and CLO/Sh groups emerged: novel, large OFT hyperactivity in CLO/nS and hypoactivity in CLO/Sh rats; in ST, more floating in CLO/Sh than CLO/nS rats. Results support prior findings and provide a developmental animal model for post-traumatic stress disorder.

664.17

EFFECTS OF NOCTURNAL AND DIURNAL WHEEL RUNNING ON GASTRIC EROSION FORMATION AND SURVIVAL DURING EXPOSURE TO ACTIVITY-STRESS. P.J. Geiselman,[1] N.S. Morrow,[2] and D. Novin.[2] Dept. of Psychology, Pennington Biomedical Research Center, LSU, Baton Rouge, LA, 70803, Depts. of Psychology[2] and Psychiatry,[3] UCLA, Los Angeles, CA 90024.

The effects of differential wheel availability on running activity, estrous cyclicity, gastric erosion formation and survival were examined in 14 female rats housed in Wahman activity cages. For 4 days rats had free access to the running wheel, food and water. Over the next 4 days all rats had food and water ad libitum, but one group was blocked from the running wheels during the dark period (1000-2115 h), whereas the second group was blocked from the running wheels during the light period (2130-0830). Rats were then restricted to eating for 1 h each day for 18 days. Running activity, body weight, body temperature, estrous cyclicity, food and water intake were monitored daily. Regardless of wheel availability all rats significantly increased wheel running from baseline values, developed gastric erosions and became moribund. Rats blocked from the running wheels from 1000-2115 lost their estrous cyclicity earlier in the testing period than rats blocked from 2130-0830. On the peak day of running there were no significant group differences in any of the behavioral variables. These results suggest that rats exposed to activity-stress do not increase wheel running in anticipation of the feeding hour (NIMH Fellowship 5T32MH17140, NSF Grants: BNS 87-09982 and BNS 9196142).

664.18

CHRONIC STRESS IMPAIRS SPATIAL MEMORY IN RATS. M. Villegas,* C. Martinez , V. Luine and B.S.McEwen, Hunter College and Rockefeller University, New York., N.Y. 10021.

Excess corticosterone, through repeated restraint stress or injection, causes atrophy in apical dendrites of CA3 pyramidal neurons. We examined whether chronic stress has a deleterious effect on spatial memory in young male rats. Rats (n=14) were placed in plexiglass restrainers for 6 h/day for 21 consecutive days (lights off 0700-1900 h). Controls (n=13) were weighed only. Immediately following the last restraint, rats were food deprived, and then received 9 training trials on the eight arm radial maze (days 2-8 post stress), four regular trials (days 9-11), and 6 trials with delays after the 4th choice (days 13-16). Stressed rats made fewer correct choices in the 1st eight visits (6.7±.2 vs 7.1±.1, $F_{1,25}=3.90$, $P<.05$) and also made their first mistake earlier (6.2±.3 vs 7.3±.3, $F_{1,25}=5.31$, $P<.03$) than controls. The effects on performance were not necessarily permanent since on days 13-16 trials with delays after the 4th choice showed no stress effect. Phenytoin interferes with excitatory amino acid function and blocks stress dependent atrophy of CA3 cells (Watanabe et al Hippocampus 2 431, 1992). Phenytoin (40 mg/kg) was administered s.c. to stressed (n=14) and control (n=14) rats daily before restraint. Injections and restraint were given for 21 days, and then spatial memory was assessed as in the 1st experiment. No differences between the groups were noted, strongly suggestive that phenytoin blocks stress dependent effects on spatial memory. Results suggest that stress dependent alterations in hippocampal morphology are associated with small impairments of a spatial memory task. (GM08176 & GN41256).

664.19

BEHAVIORAL INDICES OF ISOLATION STRESS AND INFLAMMATORY NOCICEPTION IN DOMESTIC FOWL. R. A. Hughes*, K. J. Sufka, and N. C. Weed. Depts. of Psychology, Iowa State University, Ames, IA 50011 and University of Mississippi, Oxford, MS 38677.

Isolation from conspecifics can elicit a variety of behavioral responses in young domestic fowl. These include, among others, increased distress calling, ventral recumbency posturing, hypoalgesia, and hyperthermia. However, systematic evaluation of whether these behaviors reflect converging indices of stress remains to be determined. In the present study, 7-day-old chicks received IPL formalin (.05%) or saline (.05 ml) and were placed in sound-attenuating chambers with or without 2 conspecifics for a 3 min. observation period. The following measures were recorded: vocalizations (VOC), footlift frequency (LFT) and duration (DUR), pecks (PKS), ventral recumbency latency (VRL), body temperature, respiration, and body weight. Principal component analyses revealed the presence of two oblique and negatively correlated (-.25) factors, one consisting of pain-related behaviors (i.e., LFT, DUR & PKS) and the other consisting of stress-related behaviors (VOC & VRL). These findings support the construct validity of these behavioral indices of pain and stress, respectively, and are consistent with the notion of stress-effects on nociception.

HORMONAL CONTROL OF REPRODUCTIVE BEHAVIOR: NEUROPEPTIDES AND TRANSMITTERS

665.1

INCREASE IN OXYTOCIN (OT) mRNA IN THE PARAVENTRICULAR NUCLEUS (PVN) AFTER LONG TERM EXPOSURE TO ESTROGEN (E_2) AND PROGESTERONE (P). R.S. Crowley, N.B. Kim, and J.A. Amico*, University of Pittsburgh School of Medicine and VA Medical Center, Pittsburgh, PA 15261.

The hypothalamic peptide OT is an important mediator of reproductive behaviors including parturition, lactation, and production of sexual and maternal responsiveness. In rats, maternal behaviors can be elicited by long term exposure of ovariectomized females to E_2 and P. In particular, work by Bridges (Endocrinol 114:930, 1984) has determined the specific requirements for dosage, duration and sequence of steroid exposure necessary to produce the earliest induction of maternal behavior. In this study, we utilized this well-established endocrine model to examine a possible role for OT in production of steroid-induced maternal responsiveness. Female rats ovariectomized 1 week prior to study were implanted with s.c. 2mm E_2 or sham silastic capsules on day 1. Three 30 mm P or sham capsules were implanted on day 3 and then removed on day 14. Rats were sacrificed 36 hours later. Plasma levels of E_2 and P closely corresponded to those reported by other authors. Blot hybridization was performed with a ^{32}P-labeled cDNA to Exon C of the OT gene using total cytoplasmic RNA extracted from microdissected PVN of steroid-treated and sham animals. Steroid-treated animals (n = 12) had a nearly four-fold increase in OT mRNA (p = .01, Student's t-test) when compared with control animals (n = 8). Long-term E_2 and P treatment is associated with increased PVN OT mRNA. This finding supports a role for OT in the production of steroid-induced maternal responsiveness.

665.2

QUANTITATIVE MICRODIALYSIS DETERMINATION OF EXTRACELLULAR STRIATAL DOPAMINE CONCENTRATIONS IN FEMALE RATS ACROSS THE ESTROUS CYCLE. L. Xiao* and J. B. Becker, Department of Psychology, Neuroscience Program, University of Michigan, Ann Arbor, MI 48104

Estrogen modulates mesostriatal dopamine (DA) activity *in vitro* and *in vivo*. Female rats in estrus exhibit greater amphetamine-induced activity, such as locomotor and stereotyped behaviors than those in diestrus or ovariectomized (OVX) females. Using quantitative microdialysis (the no net flux method) we looked to determine whether there are differences in extracellular striatal DA concentrations across the estrous cycle. Subjects were assigned to one of six groups (females in proestrus, estrus, diestrus or OVX females, and intact or castrated males). For intact females, the stage of estrous cycle was determined by daily vaginal cytology. Two weeks after bilateral guide cannulae were aimed at dorsolateral striatum, subjects underwent microdialysis. During dialysis, Ringer solutions with 0,4, or 16 nM DA were infused through dialysis probe and the gain or loss of DA was computed by subtracting the concentrations in the dialysate from that in the perfusate. DA was measured by HPLC-EC. A regression line was drawn for each animal to find basal DA concentrations (i.e., the point of no gain/loss).

Preliminary results indicate that females have significantly higher extracellular striatal DA concentrations in proestrus and in estrus than in diestrus or after OVX. We found no difference between intact and castrated males. Thus, endogenous ovarian hormones appear to modulate basal extracellular striatal DA concentratrions in female rats.

Sponsored by BNS9021966 from NSF.

665.3

INTRA- AND INTERSEXUAL COMPARISONS OF FOREBRAIN NEUROPEPTIDE LEVELS. C. A. Marler*, S. K. Boyd, and W. Wilczynski. Depts. of Zool. and Psychol., Univ. of Texas, Austin, TX, 78712 and Dept. of Biol., Univ. of Notre Dame, IN, 46556.

Reproductive and sexual behaviors are influenced by several peptide hormones, including arginine vasotocin (AVT) and its mammalian homologs. We previously showed that increasing AVT levels in cricket frogs, Acris crepitans, increases the probability that a male will call and increases the aggressiveness of his calls (Chu et al., Neurosci. Abst. 18:894, 1992). Here we examine AVT correlates of alternative male reproductive behaviors and intersexual differences in cricket frogs. Calling males had greater densities of AVT immunoreactivity than sexually active satellite (noncalling) males in the amygdala and nucleus accumbens. AVT density was lower in females than males in the amygdala but in the nucleus accumbens female levels were intermediate between calling and satellite males. Our data suggest that alternative mating strategies in males may be influenced by fast acting neuropeptides and also that intrasexual variation in peptide levels can be greater than intersexual variation. (Supported by F32 MH10204, R29 HD24653, and R01 MH 45350.)

665.4

CHANGES IN VIP AND GNRH ASSOCIATED WITH SEASONAL CYCLES IN BIRDS. C.J. Saldanha[1], P.J. Deviche[2] and R. Silver[1]. [1]Barnard Coll. of Columbia Univ., New York, NY 10027. [2]Univ. Alaska, Fairbanks, AK 99775.

Temperate zone birds, such as the dark-eyed junco (*Junco hyemalis*) used here, have dramatic seasonal reproductive cycles. Long days of spring stimulate the gonadal axis and the birds are said to be photosensitive. Indeed, LH secretion increases after one long day (*Follet et al., 92*). In the late summer, birds no longer respond to long days, their gonads regress, and they are said to be photorefractory. After several weeks of refractoriness the birds regain sensitivity.

It is well established that peripheral hormonal fluctuations result from changes in the brain rather than the pituitary or gonads. We studied central expression of VIP (stimulates prolactin secretion in birds), GnRH, and neurophysin (NP) in photorefractory birds (housed in 16:8 LD) and in photosensitive birds (housed in 8:16 LD) and transferred to long days (16:8 LD) for one day, one month, or not at all. VIP expression was similar in all photosensitive birds. However, photorefractory birds had 3-4 times as many VIP-positive neurons in the infundibulum as photosensitive birds. GnRH-positive cell number in the preoptic area was significantly higher in one-month photostimulated and significantly lower in photorefractory birds than in other groups. NP expression was similar in all groups. The data suggest that the inverse relationship between circulating levels of prolactin and LH during refractoriness may result from neural changes in VIP and GnRH respectively. Future studies will examine how photic input influences the expression of these peptides. Supported by NIMH 029380 (RS) and NSF BNS-9121258 (PD).

665.5

IMMUNOHISTOCHEMICAL INTERACTIONS BETWEEN GnRH I OR II WITH ENKEPHALIN IN TURKEY HENS. J.R. Millam, P.L. Faris*, M. Kram, P.E. Kerr, and C. Cozzari. Dept. Avian Sciences, University of California -Davis 95616, Division of Neuroscience Research, Department of Psychiatry, University of Minnesota 55455, and Institute Biologia Cellulare CNR, Roma (Italy).

The present study sought to determine the anatomical interactions between cGnRH I or II and methionine-enkephalin. A double-label immunohistochemical procedure was used to simultaneously visualize enkephalin (ENK) and one of the GnRH's in the same tissue section. This procedure involves using diaminobenzidine and alpha-napthol/pyronin B as the chromagens in two sequentially performed peroxidase-antiperoxidase reactions. Two main potential interactions were evident in the medial extreme of the lateral septum. GnRH II fibers were observed in close association with large ENK-ergic dendritic fragments. In adjacent sections, staining in the same region was suggestive of ENK-ergic terminals juxtaposed to GnRH I immunoreactive perikarya. Despite the heavy labeling of ENK processes, only scattered, extremely small enkephalin perikarya were observed in the lateral septum. ENK staining was virtually absent in the medial septal nucleus. These findings suggest that the ENK cells in the lateral septum may be small interneurons with extensive dendritic arborizations and highly branching axonal processes. Based on these findings, we hypothesis that an ENK interneuron links GnRH II axonal terminals to GnRH I soma, thus providing a potential anatomical substrate for GnRH II modulation of reproductive function. Supported by USDA (Prime 91-37203-6606).

665.6

AN EXAMINATION OF irGnRH NEURONS IN THE PREOPTIC AREA OF THE CICHLID FISH - HAPLOCHROMIS BURTONI. R.D. Fernald* and T. Bushnik Harris. Department of Psychology, Stanford University, Stanford CA.

In the African cichlid fish, Haplochromis burtoni, there are two distinct kinds of male behaviour. Territorial (T) males, comprising about 10% of the male population, have bright body coloring, mature gonads, and aggressively defend a territory; non-territorial (NT) males have cryptic coloring, smaller gonads, and behavior indistinguishable from that exhibited by juveniles and females. Only the T males are able to reproduce. It has been previously reported that the soma sizes of cells immunoreactive for gonadotropin releasing hormone (irGnRH) in the preoptic area (POA) are strongly correlated with the social status of the fish. That is, T males have large soma sizes, NT males have significantly smaller soma sizes, and males that have been forced to change their social status, from NT to T and vice versa, exhibit large and small soma sizes, respectively (Francis, Soma & Fernald, 1993). It has been proposed that this population of irGnRH cells mediates the difference/change in reproductive status between T and NT males. In order to directly address this hypothesis, the cells in the POA were double labelled to determine if this population of irGnRH cells projects to the pituitary. Crystals of DiI were applied to the damaged pituitary stalks of paraformaldehyde fixed brains taken from T and NT males. After sufficient time for retrograde diffusion of the DiI to the POA had elapsed (2 to 3 weeks), the brains were sectioned on a vibratome and immunostained for GnRH using a fluorescein label. In addition to determining whether the irGnRH POA neurons exhibited DiI labelling, the morphology of these neurons in T and NT males was examined in order to determine whether the complexity of the neuronal processes could be correlated to social status in a manner analogous to the relationship between irGnRH POA cell soma sizes and social dominance.
Supported by NIH HD 23799 to RDF and NSERC post-doctoral scholarship to TBH.

665.7

A SECOND GENE FOR GNRH: COMPLEMENTARY DNA AND EXPRESSION PATTERN FOR THE PRE-PROHORMONE IN A TELEOST FISH. SA White*, CT Bond, RC Francis, TL Kasten, RD Fernald & JP Adelman. Neuroscience Program, Stanford University and Vollum Institute, Oregon Health Sciences University.

Gonadotropin-releasing hormones (GnRHs) are a family of decapeptides whose importance for reproductive success throughout 500 million years of vertebrate evolution is evidenced by the striking conservation of its primary sequence: Five of the ten amino acids are identical from lamprey to mammal, including the cyclized amino- and amidated carboxy-termini. Significant variation occurs only in the remaining five positions as seen in the seven peptide forms that have been sequenced to date. Frequently, more than one form has been found within a single species and immunocytochemistry indicates that these forms are expressed in distinct but overlapping neuronal populations. Hypothalamic GnRH regulates the release of pituitary gonadotropins; the bioactivity of the other forms remains unclear. Enigmatically, of the two GnRHs found in chicken, the one with greatest releasing hormone potency *in vitro*, Chicken II, is not released in detectable amounts from the median eminence. Despite the existence of multiple peptides, the gene for only one form of GnRH has been identified. Thus, the evolutionary origins and putative physiological functions of the alternate members of this potent peptide family remain unknown. To address these questions, we employed a novel PCR strategy to amplify the Chicken II-like GnRH from a teleost fish brain where multiple peptide forms were known to occur from HPLC analysis. Here we report, for the first time, the existence of a second gene for GnRH. The sequence of this precursor differs dramatically from previously described GnRH prohormones, including at least two potential peptides in addition to GnRH, and indicates that the genes encoding the GnRH family of peptides arose independently. Using *in situ* hybridization we also show that this second form is expressed only within a mesencephalic neuronal population which does not project to the pituitary, raising an obvious question about its potential role(s) in reproduction. *Supported by HD23799, MH09986 and NS28504.*

665.8

GNRH, BUT NO GNRH MRNA, IN THE PREOPTIC AREA OF THE CICHLID FISH, *HAPLOCHROMIS BURTONI*. R.C. Francis*, S.A. White and R.D. Fernald. Department of Psychology, Stanford University, Stanford, CA.

The decapeptide, gonadotropin releasing hormone (GnRH), plays a central role in the regulation of reproductive function in vertebrates. Taxonomically, the most widely distributed form of GnRH is chicken GnRH-II, which has been identified in most vertebrates with the exception of some mammals. But its expression is largely confined to the mesencephalon and it has not been implicated (*in vivo*) in the regulation of gonadotropin release from the pituitary. A number of other forms of GnRH have been identified in various taxa that are expressed in neurons of placodal origin in the ventral forebrain. In teleost fishes the most abundant form is salmon GnRH (sGnRH), which has been localized in neurons of the terminal nerve (TN) and preoptic area (POA). We have shown previously that in male *Haplochromis burtoni* the POA irGnRH neurons mediate the effects of social status on gonadal development through changes in soma size. These neurons also respond plastically to changes in gonadal hormone levels. In contrast, TN irGnRH neurons do not respond to social cues and do not project directly to the pituitary. TN irGnRH neurons project diffusely and extensively throughout the brain and may serve a neuromodulatory function (Oka et al. 1992). We used *in situ* hybridization and immunocytochemistry, alone and in combination, to explore sGnRH expression in *H. burtoni*. ICC revealed abundant sGNRH in both the TN and POA neurons. However, we were surprised to find that while sGnRH mRNA was abundant in TN irGnRH neurons, it was absent in the POA. Nor was cGnRH-II mRNA present in the POA. There are two possible explanations for this puzzling result. Either the peptides found in the TN and POA, though they cannot be distinguished by our antibody, are produced by different genes; or both the POA and TN contain sGnRH, and the sGnRH found in the POA is produced in the TN.

PSYCHOTHERAPEUTIC DRUGS: ANTIPSYCHOTICS AND OTHER AGENTS

666.1

CP88,059: A NEW ANTIPSYCHOTIC WITH MIXED DOPAMINE D2 AND SEROTONIN 5HT2 ANTAGONIST ACTIVITIES. T.F. Seeger*, A.W. Schmidt, L.A. Lebel, B.K. Koe, S.H. Zorn, D.W. Schulz, H.R. Howard, and J.H. Heym. Neuroscience Dept., Pfizer Central Research, Groton, CT 06340.

CP88,059 is a new antipsychotic which binds with high affinity to D2 receptors (Ki = 4.8 nM) and 5HT2 receptors (Ki = 0.42 nM). CP88,059 is distinguished from risperidone by its high affinity for 5HT1A (Ki = 3.3 nM) and 5HT1C (Ki = 1.4 nM) receptors, both of which may benefit antipsychotic drug action. Receptor autoradiography using 3H-CP88,059 reveals a binding pattern consistent with a 5HT2 receptor ligand. Functional studies indicate that CP88,059 is a 5HT1A agonist and an antagonist at D2 and other receptors which bind it with high affinity (see also Zorn et al., this meeting). CP88,059 has a weak affinity for alpha-1 adrenoceptors (Ki = 11 nM) relative to its D2 affinity, suggesting the potential for reduced sedative and hypotensive effects when compared to other antipsychotics. Finally, CP88,059 is a blocker of norepinephrine uptake (IC50 = 26 nM), but is relatively inactive against dopamine and serotonin reuptake. Thus, CP88,059 has a biochemical profile which predicts antipsychotic efficacy with low side effect liability, and also shares some features with known anxiolytic and antidepressant agents.

666.2

CHRONIC HALOPERIDOL ALTERS SUBSTANTIA NIGRA GABA AND EXTRACELLULAR GLUTAMATE IN THE STRIATUM. B.K. Yamamoto* and M.A. Cooperman. Dept. of Psychiatry, Case Western Reserve Univ., Cleveland, OH 44106

Striatal efferent pathways are regulated by GABA, glutamate and dopamine. These pathways have been implicated in mediating the motoric side effects of chronic antipsychotic drug treatment. The present study examined the effects of chronic haloperidol (HAL) treatment on extracellular concentrations of glutamate and GABA in the striatum and substantia nigra (SN), respectively, of awake-behaving rats.

Male rats were administered either HAL (0.5 mg/kg IP) or tartaric acid vehicle (pH 6.0) for 21 days. One or 7 days after the last injection, extracellular concentrations of glutamate and GABA were simultaneously measured *in vivo* in the caudate and SN by a dual-probe microdialysis method. One day after the cessation of chronic HAL administration, striatal glutamate was markedly elevated (500%) compared to vehicle-treated rats. Basal extracellular concentrations of GABA in the SN were also increased (240% of control). One week after the termination of HAL treatment, striatal glutamate levels remained elevated (216%), albeit lower than after only one day of withdrawal. In contrast, GABA concentrations in the SN were decreased (51% of control) following 1 week of withdrawal from chronic HAL treatment. Results will also be presented with regard to the effect of HAL on GABA in the globus pallidus. These data indicate that chronic haloperidol treatment alters striatal glutamatergic and nigral GABAergic function. These corticostriatal and striatonigral alterations are also dependent on the duration of withdrawal from chronic treatment and may be related to the motor side effect liability of chronic treatment with typical antipsychotic drugs.

[Supported by the Scottish Rite Schizophrenia Research Program]

666.3

EFFECT OF REPEATED HALOPERIDOL ADMINISTRATION ON PHENCYCLIDINE (PCP) DISCRIMINATION IN RATS. J.L. Wiley*. Dept. of Pharmacology & Toxicology, Medical College of Virginia, Richmond, VA 23298.

Acute doses of haloperidol (dose range = 0.1-0.32 mg/kg) produce partial attenuation of the PCP stimulus in rats trained to discriminate PCP from vehicle (R. Browne, In: Clouet, ed., Phencyclidine: An update, pp. 134-147, Rockville, MD: NIDA, 1986). This study examined the effects of repeated administration of haloperidol (HAL) on PCP discrimination in rats. Rats were trained to discriminate PCP (2.0 mg/kg) from saline in a two-lever drug discrimination procedure. Following acquisition, rats were tested with cumulative doses of PCP before and after repeated administration of saline and of HAL (0.5 mg/kg/day). Discrimination training was suspended during the two 14-day repeated dosing regimens. The post-saline PCP dose-effect curve for %PCP-lever responding showed little change from the pre-saline curve (pre ED_{50} = 0.6 mg/kg vs. post ED_{50} = 0.4 mg/kg). After repeated administration of HAL, the ED_{50} was 1.4 mg/kg, compared to the pre-HAL ED_{50} of 0.7 mg/kg. These results are consistent with those of acute dosing studies that tested haloperidol in PCP-trained rats and suggest that repeated administration of haloperidol may disrupt PCP's discriminative stimulus effects, although most rats were still able to discriminate the higher doses of PCP. Research supported by NIDA Grants DA-01442 and DA-07027.

666.4

EFFECTS OF CHRONIC SM-9018, A CENTRALLY ACTING 5-HT2 AND D2 ANTAGONIST, ON DOPAMINE AND 5-HT RECEPTORS IN RATS. Y. Ohno*, K. Okada, K. Ikeda, K. Ishida, T. Ishibashi and M. Nakamura. Res. Lab., Sumitomo Pharmaceuticals Co., Ltd., Konohana-ku, Osaka 554, Japan.

SM-9018 is a potential atypical neuroleptic with combined 5-HT_2 and D_2 blocking activities. Receptor binding and behavioral studies were performed to compare the effects of chronic treatment with SM-9018 and with haloperidol (HAL) on dopamine and 5-HT receptors in rats. SM-9018 treatment, at a dose sufficient to block D_2 receptors (10 mg/kg/day p.o.), for 14 days did not significantly change the density (B_{max}) of striatal D_2 receptor (labeled by ^{3}H-raclopride) or the stereotyped behaviors induced by apomorphine (APO). By contrast, a typical neuroleptic HAL (3 mg/kg/day p.o., for 14 days) significantly increased the striatal D_2 receptor density by about 55% and markedly enhanced the response to APO. The incidence of SKF 38393 (D_1 agonist)-induced vacuous oral movements was also increased by HAL but not by SM-9018. The density of 5-HT_2 receptor (labeled by ^{3}H-ketanserin) in the cerebral cortex was unaffected by HAL but slightly decreased (about 20%) by SM-9018. However, 5-HT_2 receptor-mediated hyperthermia with p-chloroamphetamine (a putative 5-HT releaser) was unaltered following SM-9018 treatment. These findings suggest that SM-9018 is weaker than HAL in inducing up-regulation and supersensitivity of dopamine receptors in rats after chronic treatment and has low propensity to induce tardive dyskinesia.

666.5

EFFECT OF A NEUROLEPTIC, NEMONAPRIDE (YM-09151), ON C-FOS EXPRESSION IN THE RAT FOREBRAIN. T. Nishio, M. Narita# and K. Satoh*# Nagahama Inst. Biochem. Science, Oriental Yeast; #Shiga Univ. Medical Science, Dept. Psychiatry, Otsu, Japan;

Neuroleptics have pharmacological effect on many neurotransmitter systems in the central nervous system. However, the mechanism of action of these drugs is still unknown. In the present study, the effect of a new neuroleptic, nemonapride (YM-09151), on the expression of immediate early gene, c-fos, was investigated. After an injection of nemonapride (0.5 - 4.0 mg/kg), the distribution of Fos immunoreactive neurons was examined in the rat forebrain, and the result was compared with that obtained after an administration of haloperidol (1-2 mg/kg). Both neuroleptics induced Fos immunoreactivity in many forebrain structures, such as the striatum, anterior olfactory nucleus, olfactory tubercle, nucleus accumbens, lateral septal nucleus, medial prefrontal cortex, and the thalamic and hypothalamic paraventricular nuclei. In addition, nemonapride enhanced Fos-immunoreactivity in cells located in the island of Calleja. In contrast, haloperidol induced many Fos-immunoreactive neurons in the lateral septal nucleus and hypothalamic paraventricular nucleus. The present findings indicated that two neuroleptics with different therapautic potentials have effect on different population of cells in the rat forebrain.

666.6

SERUM HALOPERIDOL AND TOXICITY IN GERIATRIC PATIENTS J.J. Weisbard, R.C. Young*, B. Kalayam, J. Birnbaum B. Meyers, T. Kakuma. New York Hospital-Cornell Medical Center, Westchester Division, White Plains, NY 10605.

Haloperidol is widely prescribed in the geriatric population. While it can be therapeutically effective, it may produce toxicity including reduced cognitive capacity. Serum haloperidol levels differ widely among geriatric patients receiving similar doses, and optimal treatment conditions need definition. In young patients, serum muscarinic receptor binding activity has been correlated with cognitive deterioration during neuroleptic treatment. We reviewed charts of psychiatric inpatients treated with haloperidol for a primary psychotic disorder in whom serum haloperidol had been determined. They had been routinely assessed prospectively by trained nursing staff using behavioral ratings including memory impairment and disorientation. In geriatric (age $\geq$ 60 yrs) patients (n=16) higher serum haloperidol levels, but not doses (mg/kg), were associated with cognitive deterioration since admission reflected in changes in ratings of memory impairment ($r = -.56$; $p < .02$) and disorientation ($r = -.45$; $p < .08$). In patients aged <60 yrs (n=13) these associations were not observed. Further prospective assessment of haloperidol levels and clinical effects in geriatric patients is needed.

666.7

POTENT AND SELECTIVE SIGMA$_1$ LIGANDS BASED ON CARBETAPENTANE. S.N. Calderon, S. Izenwasser, B. Heller, S. Gutkind*, A.H. Newman, Drug Development Group, NIDA-ARC, Baltimore, MD 21224, *Molecular Signaling Group, LCDO, NIDR, NIH, Bethesda, MD 20892.

The structural and pharmacological diversity of compounds that interact with sigma binding sites (σ_1 and σ_2) has prompted an intensive search for selective sigma ligands with discrete biological actions that could be undeniably attributed to their interaction at these sites. Carbetapentane (CBP, 2-[2-(diethylamino)ethoxy]ethyl-1-phenyl-1-cyclopentylcarboxylate) is a drug with anticonvulsant, antitussive and neuroprotectant actions that binds to σ_1 sites. We have prepared several series of compounds based on this drug to 1) identify structural features responsible for potent and selective binding to sigma sites, 2) identify novel anticonvulsant and neuroprotectant agents, and 3) determine whether binding to sigma sites correlates with these activities. Previously described sigma ligands that structurally resemble CBP possess one or more of the following moieties that were deemed important for potent and selective binding to these sites: a 3,4-dichlorophenyl ring, an alkyl-diamino side chain, a piperidino or morpholino function and a cycloalkyl group. All of these structural moieties were incorporated into the CBP molecule. The general synthesis began with preparing the appropriately substituted cycloalkylphenyl-carboxylate and reducing or directly reacting it with prepared side chains to give the desired ethers, esters and amides. Reduction and alkylation of the amides resulted in diamino analogs. [^{3}H](+)SKF 10,047 (in the presence of cold MK 801) and [^{3}H]DTG (in the presence of cold (+)SKF 10,047) were used to radiolabel σ_1 and σ_2 binding sites respectively, in rat brain. Optimal σ_1 binding potencies resulted when the 3,4-dichlorophenyl, cyclohexyl, and morpholino or piperidino rings were simultaneously incorporated into CBP. Structure-activity relationships for this series of compounds including σ_1, σ_2, muscarinic (m1 and m2) and PCP receptors will be discussed.

666.8

OPPOSITE EFFECTS ON LCGU IN RATS BY THE PRESYNAPTIC DOPAMINE AGONIST ORG 20223 AND APOMORPHINE.

J.A.D.M. Tonnaer*, P.C. Fedder, Th. de Boer, C.L.E. Broekkamp and A.M.L. van Delft. Scientific Development Group, Organon International B.V., P.O. Box 20, 5340 BH Oss, The Netherlands.

Org 20223 is a DA agonist which exhibits moderate affinity for D2 (pKi=7.0) and 5-HT1A (pKi=6.8) binding sites, whilst lacking substantial affinity for other DA, 5-HT, α or β adrenergic, muscarinergic or histaminergic binding sites. Org 20223 reduces DOPA accumulation (1 mg/kg, i.p.), induces yawning and penile erections, and inhibits ambulation in rats, and reduces motor activity in mice (0.5-10 mg/kg, s.c.). In rats the compound does neither induce severe symptoms of DA-related stereotyped behaviour (doses up to 100 mg/kg, s.c.), nor 5-HT1A receptor mediated behaviour (doses up to 46 mg/kg, s.c.).

Upon i.v. administration in freely moving rats (0.1 and 0.3 mg/kg) Org 20223 dose-dependently decreases local cerebral glucose utilization (LCGU) in core structures of the extrapyramidal system and in several thalamic brain areas. LCGU is not or non-significantly decreased in limbic and neocortical brain structures, and is unaffected in amygdaloid, cholinergic, serotonergic or in cerebellar brain structures. The effects induced by Org 20223 on LCGU in the thalamic nuclei are opposite to those induced by the postsynaptic DA agonist apomorphine but resemble those seen with DA receptor blocking antipsychotic drugs such as haloperidol and clozapine. Thus, Org 20223 reduces LCGU in the anteroventral, laterodorsal and ventroposterior nuclei of the thalamus and tends to increase LCGU in the lateral habenula. Plasma glucose levels and plasma corticosterone levels are not affected by treatment of the animals with Org 20223.

The neurochemical and behavioural data indicate Org 20223 has the profile of a preferential DA autoreceptor agonist. The typical pattern of LCGU changes indicates that Org 20223 is a compound with potential antipsychotic properties.

666.9

EFFECTS OF SDZ ENA 713 ON AF64A-INDUCED RATS.

H.Endo*[1],T.Tajima[2],T.Goto[2],H.Ikari[2],F.Kuzuya[2] and A.Iguchi[2].
[1]Dep of Geriatrics,Chubu Hospital, Obu,Japan,474. [2]Dep of Geriatrics,Nagoya Univ.Sch. of Med.,Nagoya,Japan,466.

SDZ ENA 713 is a potent, long-lasting, orally active acetylcholine esterase inhibitor(AChE-I). This produces a greater inhibition of AChE-I activity in brain regions than in peripheral organs, because it was developed as a brain-selective drugs. This kind of drugs is expected for the treatment of Alzheimer's disease (AD). First, we studied memory and learning on AF64A-induced rats by 8 radial maze. Secondly we measured acetylcholine (ACh) levels by micro-dialysis in freely moving rats administered SDZ ENA 713 (.3mg/kg). ACh levels in rats with the drug were higher than controls. Correct responce was reduced in AF64A-induced group, this effect was reversed by SDZ ENA 713. This drug might be useful in the treatment for AD.

666.10

IN VIVO NEUROCHEMICAL EFFECTS OF WAY-124486: A POTENT DOPAMINE AUTORECEPTOR AGONIST. T.H. Andree*, L. Cortes-Burgos, R. Scerni, T. Wasik, T. Spangler, G. Stack, K. Marquis, C. Routledge[1] and G. Gudelsky[2]. Wyeth-Ayerst Research, CN 8000, Princeton, N.J. 08543, [1]Wyeth Research Limited, Maidenhead, UK SL60PH and [2]Departments of Psychiatry and Neuroscience, Case Western Reserve University, Cleveland, OH 44106.

WAY-124486 (S)-3-[[[3-(3-aminophenoxy)propyl] amino] methyl]- 2,3-dihydro-1,4-benzodioxin-6-ol dihydrochloride is a potent D_2 autoreceptor agonist as demonstrated in neurochemical, behavioral and electrophysiological assays (1992 Neuroscience abstracts, 158.20, 158.21 and 158.22, respectively). Since dopamine autoreceptor agonists are known to decrease dopaminergic transmission by inhibition of DA release, synthesis and impulse flow, *in vivo* microdialysis studies were performed in conscious male SD rats to confirm WAY-124486's suspected effects on extracellular concentrations of DA. WAY-124486 (1 and 5 mg/kg, sc) markedly (60-65%) reduced extracellular concentrations of DA which remained depressed during the 3 hr collection period. A slight (approx. 20%) decrease in DOPAC was also observed. Following the perfusion of Ringer's buffer containing 55 mM K^+, extracellular DA was increased 3.5 fold. This increase was inhibited by quinpirole (0.75 mg/kg, sc) and WAY-124486 (5 mg/kg, sc). These results are consistent with the DA autoreceptor actions of WAY-124486. Since D_2 agonists have also been shown to have effects on the neuroendocrine axis to inhibit prolactin secretion (Caron et al., J. Biol. Chem. 253:2244, 1978) and to increase corticosterone secretion (Fuller et al., Neuroendoc. 36: 285, 1983), WAY-1244486 was likewise examined for its effects on these hormones. WAY-124486 (5 mg/kg,sc) and quinpirole(0.75 mg/kg, sc) produced marked elevations of corticosterone serum levels. Results on prolactin secretion and protection against EEDQ-induced inactivation of D_2 receptors *in vivo* will also be presented.

666.11

DISCRIMINATIVE STIMULUS PROPERTIES OF LITHIUM CHLORIDE. P. J. Winsauer*, J. F. Verrees and P. C. Mele. Behav. Sci. Dept., Armed Forces Radiobiology Res. Inst., Bethesda, MD 20889-5603.

Although much is known about the aversive properties of LiCl, little is known about the discriminative stimulus properties of this drug. For this reason, eight rats were trained to discriminate between an injection of LiCl (i.e., 56 or 75 mg/kg, i.p.) and saline in a two-lever operant chamber during a 20 min session. On training days, responding on the designated lever was reinforced under a fixed-ratio 20 (FR 20) schedule of food presentation, whereas responding on the other lever had no programmed consequences. Generalization testing with LiCl (10-100 mg/kg) was conducted after each subject reached a criterion of nine of ten sessions where 95% of overall responding occurred on the designated lever, and fewer than twenty responses were made on the other lever before presentation of the first reinforcer. On test days, responses on both levers were reinforced under an FR 20 schedule. In general, all subjects reliably discriminated between saline and the training doses of LiCl. Substituting both lower and higher doses produced graded decreases in responding on the LiCl-appropriate lever while only higher doses decreased overall response rate. Following generalization tests, subjects were divided into two groups and varying doses of LiCl were given in combination with i.p. injections of either ondansetron (0.32 and 1 mg/kg) or dexamethasone (1 and 3.2 mg/kg). At doses that had little or no effect alone, neither ondansetron nor dexamethasone pretreatment blocked the discriminative stimulus properties of LiCl. This research shows that LiCl can act as a highly discriminable stimulus in an operant drug-discrimination paradigm and suggests that the stimulus properties of LiCl do not derive from direct activation of serotonin type-3 receptors or release of adrenocorticotropic hormone.

666.12

EFFECTS OF THE SELECTIVE 5-HT_2 ANTAGONIST MDL 100,907 ON FENFLURAMINE-, MDMA-, OR DOI-INDUCED DISRUPTION OF AUDITORY OR VISUAL PREPULSE INHIBITION IN RATS. R.A. Padich*, T.C. McCloskey, J.M. Hitchcock, and J.H. Kehne. Marion Merrell Dow Research Institute, 2110 E. Galbraith Rd., Cincinnati OH 45215.

Prepulse inhibition is a model of sensorimotor gating useful for preclinical and clinical studies exploring the neurochemical basis of schizophrenia and for identifying new antipsychotic agents. The present study investigated the role of 5-HT_2 receptors in the modulation of prepulse inhibition. Agents which selectively (fenfluramine) or non-selectively (3,4-methylenedioxymethamphetamine, MDMA) release serotonin consistently reduced both auditory and visual prepulse inhibition in rats. The reduction of auditory prepulse inhibition was prevented by the selective 5-HT_2 antagonist MDL 100,907, but not by the dopamine antagonist haloperidol. The disruption of visual prepulse inhibition was not affected by either MDL 100,907 or haloperidol. The directly-acting 5-HT_2 agonist 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane HCl (DOI) selectively reduced auditory prepulse inhibition, and this effect was prevented by MDL 100,907, but not by haloperidol. These data support the conclusion 5-HT_2 receptor stimulation specifically disrupts auditory prepulse inhibition in rats. Although 5-HT release potently disrupts visual as well as auditory prepulse inhibition, the identification of the receptor(s) mediating the effect on visual prepulse inhibition requires further investigation. Finally, dopaminergic receptors are not required for the expression of the effects of DOI or the releasing agents on prepulse inhibition. These results support an independent role of 5-HT in prepulse inhibition.

666.13

EFFECTS OF THE SELECTIVE 5-HT_2 ANTAGONIST MDL 100,907 ON STIMULANT- ENHANCED LOCOMOTION IN RATS. J.H. Kehne* and H.J. Ketteler. Marion Merrell Dow Research Institute, 2110 E. Galbraith Rd., Cincinnati, OH 45215.

Previous studies suggested that the potent and selective 5-HT_2 antagonist MDL 100,907 has the profile of a potential antipsychotic with minimal extrapyramidal side effect liability (Sorensen et al., *Soc. Neurosci. Abstr., 18:* 1381, 1992). The present study further extended these findings using two models of stimulant-enhanced locomotion in rats.

MDL 100,907, like haloperidol and clozapine, significantly reduced d-amphetamine-stimulated locomotion in rats. In marked contrast, the 5-HT_2/$_{1C}$ antagonist ritanserin did not alter d-amphetamine-stimulated locomotion in doses of up to 8 mg/kg. These results suggest that MDL 100,907, but not ritanserin, would have potential antipsychotic activity. The difference in activity between MDL 100,907 and ritanserin may result from the selectivity of MDL 100,907 for the 5-HT_2 receptor.

Both MDL 100,907 and haloperidol attenuated the stimulation of locomotor activity produced by the 5-HT/DA releaser, 3,4-methylenedioxymethamphetamine (MDMA; Kehne et al., *Soc. Neuroci. Abst., 18*:352, 1992). Interestingly, haloperidol primarily affected the early (0-30 min) component of the MDMA effect, whereas MDL 100,907, and to a lesser extent, ritanserin, primarily affected the late (30-60 min) component. In the present study, clozapine also primarily attenuated the late component of MDMA-enhanced stimulation. Thus, the pattern of activity of MDL 100,907 in the MDMA-stimulated locomotion test more closely resembles that of clozapine than haloperidol.

These data further support the conclusion that MDL 100,907 is a potential antipsychotic agent with a unique behavioral profile.

666.14

METABOLISM OF PSYCHOACTIVE DRUGS BY HUMAN BRAIN MICROSOMES-IMPLICATION IN PHARMACOTHERAPY.

V.Ravindranath[1],S.Bhamre[1],S.V.Bhagwat[1],S.K.Shankar[2] & S.H.Koslow*[1] Depts. of Neurochemistry[1] & Neuropathology[2], National Institute of Mental Health & Neuro Science, Bangalore-560 029,India & *Div. of Neuroscience & Behavioral Science,NIMH,Rockville, MD-20857, USA.

Microsomal flavin-containing monooxygenase (FMO) is involved in the metabolism of nitrogen and sulfur containing xenobiotics including drugs.A number of psychoactive drugs are metabolized by hepatic FMO.We examined the presence of FMO in human brain regions obtained at autopsy using model substrates and the antidepressants,imipramine (IMI) and fluoxetine (FLUO). Microsomes from human brain metabolized IMI and FLUO efficiently.The human brain FMO demonstrated high affinity for IMI and FLUO (Km=22 & 16 μm) as compared to the model substrate N,N-dimethylaniline (Km=2.2 mM).Immunocytochemical studies using the antibody to the pulmonary FMO revealed the preferential localization of FMO in neuronal soma and co-localization of FMO and cytochrome P-450 in human brain.FMO mediated metabolism of antidepressants varied between individuals and this may explain the variable drug response typically seen in a diverse patient population.

666.15

PHENCYCLIDINE (PCP) AND (+)-AMPHETAMINE (DEX)-INDUCED STIMULATION - A PHARMACOLOGICAL STUDY. D. M. Jackson*, C. E. Johansson, A. Bengtsson and M Lindgren Department of Behavioural Pharmacology, Preclinical CNS R & D, Astra Arcus AB, Södertälje 151 54, Sweden.

Both DEX and PCP are especially interesting drugs because they can produce in man symptoms that resemble aspects of schizophrenia (SCHIZ). DEX produces symptoms that resemble some of the positive symptoms of SCHIZ, while PCP can causes symptoms that may better reflect the negative symptomatology of SCHIZ. In the present study, we have examined the effects of DEX and PCP on locomotion in rats. Both DEX and PCP caused dose dependent increases in locomotion in rats as measured in computerised activity cages. An analysis indicated that both drugs caused dose dependent increases in total activity, forward locomotion and peripheral activity While DEX dose dependently but non-significantly increased rearing, PCP significantly decreased the incidence of this behaviour. This is in agreement with earlier studies. A variety of drugs were tested for the ability to block PCP and DEX-induced excitation. The selective dopamine (DA) D1 antagonists, SCH23390 and NNC-01-0112, were 7 to 8 times more potent in blocking the DEX-induced increase in total activity than in blocking PCP-effect. In contrast, the DA D2 receptor antagonists raclopride and remoxipride, as well as haloperidol, were only 1 to 2 times more potent against DEX-induced stimulation. Non-selective DA D2 antagonists, as well as sertindole, were also tested. Chlorpromazine resembled the DA D1 antagonists in its pattern of blockade. Buspirone and sertindole were more potent in blocking PCP than DEX-induced stimulation. The selective α1 antagonist prazosin partially blocked, while the 5HT1A agonist 8-OH-DPAT potentiated, both DEX and PCP-induced stimulation. The selective 5HT2 antagonist ritanserin was inactive. The effect of the various test agents on locomotion was also tested. The data indicate that there are clear qualitative, quantitative and pharmacological differences between DEX and PCP-induced locomotor excitation. This difference could be of interest in the development of new antipsychotic agents.

GENETIC MODELS OF NERVOUS SYSTEM DISORDERS II

667.1

OVER-EXPRESSION OF TGF-ß1 IN ASTROCYTES OF TRANSGENIC MICE RESULTS IN HYDROCEPHALUS. A. Messing*, S.-J. Kim, E.J. Galbreath, and M. Brenner. School of Veterinary Medicine, Univ. Wisconsin-Madison, Madison, WI 53706; Divison of Cancer Etiology, NCI, and Stroke Branch, NINDS, NIH, Bethesda, MD 20892.

Transforming growth factor ß1 (TGF-ß1) has been proposed to play a number of roles in both the development of the CNS and its response to injury. Astrocytes produce TGF-ß1, and also express receptors for this regulator. To more critically examine the roles of TGF-ß1 in the CNS, we created transgenic mice that constitutively over-express TGF-ß1 in astrocytes by using a construct in which the TGF-ß1 coding region is under the control of the promoter of the human glial fibrillary acidic protein gene. Nineteen founder animals were produced, of which 11 developed severe hydrocephalus and died between birth and 3 weeks of age. Pathologic examination of multiple organs showed no significant abnormalities outside of the brain. Preliminary immunohistochemical studies indicate that the hydrocephalic phenotype correlated with elevated expression of TGF-ß1 in the brain. These results show that the developing CNS is highly sensitive to TGF-ß1, and suggest a role for aberrant expression of TGF-ß1 in the pathogenesis of developmental disease of the CNS.

667.2

DISTURBANCES IN THE BIOGENIC AMINE RATIOS IN THE BEHAVIORAL MUTANT, JERKY, OF XENOPUS LAEVIS. R. Tompkins*, T. Nguyen, A. Crago, D.C. Reinschmidt. Cell and Molecular Biology Dept., Tulane Univ. New Orleans, LA 70118

Tadpoles homozygous for the recessive mutant jerky begin displaying abnormal swimming behavior grading to paralysis beginning at stage 48 and ending at metamorphosis. The degree of expression of the syndrome is influenced by the amount and kind of food, photoperiod and drug treatment. The mutant also causes a lethal maternal effect on brain development and function. Measurements of the neurotransmitters norepinephrine, epinephrine, and serotonin, along with certain of their metabolites using HPLC indicate disturbed biogenic amine ratios in mutants, depending on their physiological state. In tadpole, affected animals kept in constant conditions always show a higher serotonin to dopamine ratio than normal. Under certain conditions, this is because of an elevated serotonin level; in other cirumstances, it is because of depressed levels of dopamine. The biogenic amines of embryos showing the maternal effect are normal. Therefore, differences in neurotransmitter levels are not necessarily causally related to the maternal or zygotic effects of this mutant.

667.3

TRIPHASIC RELAPSE IN DYSTONIC HAMSTER DURING PREGNANCY AS A RIGOROUS NEUROCHEMICAL MODEL FOR MOVEMENT DISORDER. A.E. Khalifa and W.B. Iturrian*. Dept. of Pharmacology and Toxicology, Univ. of Georgia, Athens, GA 30602-2356

A genetic paroxysmal dystonia occurs transiently in weanling dt sz (UGA 700) hamster with virtual complete remission by age 60 days (Movement Disorder 4:219, 1989). We now report a triphasic relapse associated with pregnancy, parturition and nursing. Over 60% have the distinctive tonic torsion and spasms of dystonia during late pregnancy and almost all being maximally susceptible on parturition day. On the third day post-partum few show dystonia even when elicited by anxiogenic doses of pentylenetetrazol. 100% of nursing mothers show full paralytic attacks on the 7th day of lactation while non-nursing individuals are resistant.

We suggested the dt sz hamster as a model of BDZ receptor deficits (Soc. Neurosci. Abst. 10:1065, 1984) and interpret current results to implicate hormonal modulation of the BDZ ligand/receptor complex. We think pregnancy produces an inverse agonist BDZ ligand, stress of parturition induces a transient antidystonic/anticonvulsant protein and that lactogenic hormone(s) exacerbate the influence of inverse agonist. Regardless of hypothesis this pregnant and nursing model of paroxysmal dystonia is an exciting opportunity to understand the underlying neuropathology of movement disorders and leads to a rigorous method of testing pharmacological and molecular theory with in-vivo response.

667.4

Analysis of Astrocyte Phenotype and Proliferation in S100β Transgenic Mice. Dana C Hilt[1], Suzanne Buck[1], Hao Jiang[1], George Dmytrenko[1*], Paul Yarowsky[3], and Roger Reeves[2]. [1] Dept. of Neurology and [3] Dept. of Pharmacology, University of Maryland School of Medicine, 22 S. Greene St., Baltimore, Md. 21201, [2] Dept. of Physiology, The Johns Hopkins Hospital School of Medicine, Wolfe St., Baltimore, Md. 21205.

S100β is a small Ca^{2+} -binding protein synthesized and secreted predominantly by glial cells in the nervous system. In vitro, S100β disulfide bonded dimers have been shown to induce alterations in astrocyte phenotype and proliferation. The brains of patients with Alzheimer disease (AD) have greatly elevated levels of S100β as well as reactive astrogliosis and abnormal neurite proliferation. In order to determine if S100β functions in vivo to control astrocyte phenotype and proliferation two S100β transgenic (tg) mouse lines have been constructed utilizing a full length mouse S100β gene. Focal S100β -dependent astrogliosis (hippocampus, cerebellum, and olfactory bulb) are found in the tg mouse brains when compared to control animals. These same regions have abnormal neurite proliferation. Astrocyte changes seen in the S100β tg mouse brains include increased astrocyte size, GFAP immunoreactivity, and complexity of glial processes. However, there is no increase in the number of astrocytes in the hippocampus or Bergmann glia in the cerebellum. These results suggest that elevated S100β expression in vivo leads to a reactive glial phenotype but that S100β does not function as a glial mitogen in the intact nervous system.

667.5

DEVELOPMENTAL ABNORMALITIES IN PROTEIN EXPRESSION IN THE LURCHER MUTANT MOUSE. L.M. Eisenman*, G.B. Grunwald and C.M. Steger. Dept. of Anatomy and Devel. Biology, Thomas Jefferson University, Philadelphia, PA.

The phenotype of the mutant mouse lurcher is characterized by the postnatal death of all cerebellar Purkinje cells (PC). This cell death commences on postnatal day 9-10 and all PC are gone by postnatal day 60. We have examined the organization of the olivocerebellar projection in the adult and during early postnatal development when there are significant interactions between the olivocerebellar afferent and the PC target. The analysis in lurcher clearly demonstrates defective development of this projection beginning on postnatal day 9. Olivocerebellar fibers in lurcher do not translocate from the PC somas, their initial targets, onto the developing PC dendrites as occurs in wildtype animals. Recent biochemical studies have been directed at determining the molecular mechanisms of this specific olivocerebellar afferent-Purkinje cell target defect in lurcher. One dimensional SDS-PAGE analyses were conducted on cerebellar homogenates of postnatal day 11, 15, 19 and adult lurcher and wildtype littermates. The results of this initial protein characterization demonstrate clearly that there are two high molecular weight proteins expressed in the wildtype which are absent from the lurcher at all developmental ages studied. Further analyses will be designed to more fully characterize these deficient proteins and to determine if these differences are related to the abnormal olivocerebellar development. Supported by NIH grant NS22093.

667.6

CHANGES IN THE LEVEL OF PROTEIN KINASE C (PKC) IN PC12 CELLS TRANSFECTED WITH THE A4-C-TERMINAL PORTION OF AMYLOID PRECURSOR PROTEIN. R.E. Majocha*, J. Newton and C.A. Marotta. Dept. Psychiatry and Human Behavior, Miriam Hosp. and Brown University, Providence, R.I. 02906.

In order to understand the affects of β/A4 amyloid on cell function we have previously examined changes in cell morphology, production of neuronal-specific proteins and response to trophic factors which are all changed in transfectants overexpressing amyloid relative to untransfected or mock-transfected PC12 cells. In the present study, we examined the levels of PKC, an enzyme which is involved in signal transduction, as a possible mechanism by which amyloid exerts its effects in these cells. Using a monoclonal antibody to PKC, we found that there is a immunohistologically significant decrease in the level of PKC as determined by optical density using an image analyzer. Changes in transduction pathways have the potential to disrupt the normal relationship of a cell with its environment.

667.7

A GENETIC FACTOR UNDERLIES THE BEHAVIORAL RESPONSE OF INBRED MICE TO THE PORSOLT TEST AND THE RESTRAINT STRESS TEST. B.J.Tarricone*, J.N.Hingtgen and J.I.Nurnberger,Jr. Institute of Psychiatric Research, Department of Psychiatry and Program in Medical Neurobiology, Indiana University School of Medicine, Indianapolis, IN 46202-4887.

To determine if mice exhibit different vulnerabilities to animal models of depression, the effect of the Porsolt swim test and the restraint stress test on behavior was examined in C57BL/6J, DBA/2J, AKR/J, and A/J inbred mice. Only the C57BL/6J mice exhibited "despair" during the Porsolt swim test ($p < 0.05$), as well as a significant decrease in open field crossings after the Porsolt test ($p < 0.05$) and the restraint stress test ($p < 0.05$). Swiss Webster mice were found to have an increase in the rate of high affinity choline uptake in the frontal cortex after the restraint test ($p < 0.05$). To identify those genes associated with the behavioral differences between C57 and DBA mice, the response of the BXD recombinant inbred mice to these models was examined. During the swim test, BXD 1, 5, 11, 12, 13, 14, 15, 16, 18, 22, and 29 floated significantly more ($p < 0.05$). After the Porsolt swim test, a decrease in open field crossings was exhibited by BXD 5, 12, 14, 29, and 31 ($p < 0.05$). Following restraint, BXD 1, 5, and 6, exhibited a decrease in open field crossings ($p < 0.05$). The identification of quantitative trait loci using behavioral and genetic strain distribution patterns is in progress. Because significant homology exists between mouse and human chromosomes, this may be a preliminary step in identifying those genes associated with affective disorders.(Supported in part by a grant from Indiana Department of Mental Health.)

667.8

CHROMOSOMAL MAPPING OF GENES WHICH ALTER THE TIMING OF DISEASE IN THE mnd MOUSE. J. Plummer*, A. Messer, M.C. MacMillen and W.N. Frankel. Wadsworth Ctr. for Labs. and Research, N.Y. State Dept. of Health and Dept. of Biomed. Sci., SUNY, P.O. Box 509, Albany, NY 12201-0509; and The Jackson Laboratory, Bar Harbor, ME.

The mouse mutant motor neuron degeneration (mnd) displays an adult-onset progressive degeneration of upper and lower motoneurons, with mild symptoms recognizable at 6 months, leading to spastic paralysis and premature death at 10 - 12 months on the C57Bl/6 (B6) background. When mnd was outcrossed to the AKR background, 35 - 39% of the mnd/mnd F2 progeny of the outcross/intercross show early onset (mild symptoms by 4.5 - 5 months, moderate symptoms by 6 months, and death by 7 months.) This accelerated timing effect seems to be strain-specific, and unlinked to the mnd gene itself. DNA from a series of 49 outcross/intercross F2 mice showing early-onset mnd was screened for endogenous polymorphic proviral fragments, with strain-specific dinucleotide repeat fragments (generated by PCR) used to assay chromosomal regions not excluded in the first assay. Data were analyzed using a chi square test.

The strongest deviation from the expected ratio of AKR vs B6 alleles occurs with markers on proximal half of Chr. 1. Additional loci on Chr's 5, 10 and 17 have also been marked for further study. Our current working hypothesis is that the timing effect is due to 2 or 3 unlinked dominant genes with incomplete penetrance at any single locus. One of these genes is on Chr. 1.

An additional series of F2 intercross and F3 backcross mice is currently aging, and will provide at least 100 additional meioses to analyze. These will also create an early-onset mnd strain in which to follow symptoms and pathology, while mapping and identifying the genes responsible. The mechanism of interaction of these modifying genes with the primary mnd gene may offer new therapeutic avenues.

667.9

Expression of the NMDA receptor in a late-onset inherited motor neuron disease in mice (Mnd).

M. Didier*, S. Bursztajn, A. Cataldo, R. Nixon and S.A. Berman. Lab. for Mol. Neurosci. Harvard Med. School/McLean Hospital, 115 Mill St. Belmont MA 02178

Motor neuron degeneration (Mnd) is a genetic neurodegenerative disease of mice displaying many characteristics of human amyotrophic lateral sclerosis (ALS). Several studies have reported a decrease in presynaptic glutamatergic mechanisms in ALS. However, a recent report suggests that such a decline of glutamate uptake would be only a consequence of the motor neuron disease in the Mnd mouse. In this study, we investigated the expression of the NMDA receptors at the postsynaptic level during the development of the Mnd disease. NMDA-sensitive L-[^{3}H] glutamate binding experiments showed a progressive increase in NMDA binding sites that correlated with the development of behavioral symptoms in Mnd mice. At 5-6 months, a 2 fold increase over controls was detected only in the lumbosacral level- the first affected region of the spinal cord. By 8-10 months, mutants displayed 2-3 fold increases in NMDA binding in both lower and upper cord. Interestingly, no differences in benzodiazepine or non-NMDA glutamate binding were found in either presymptomatic or affected Mnd mice. Autoradiography showed NMDA receptor localization mainly in the substantia gelatinosa of normal mice. In 8 month symptomatic animals, a dramatic increase of NMDA-sensitive glutamate binding was observed in the ventral horn of the lumbar spinal cord and appears to be associated with a higher expression of NMDA receptor in motor neuron during the Mnd disease. Preliminary results support an elevation in the NMDAR1 mRNA level in spinal cord motor neurons of 3 month-old Mnd mice before any behavioral modifications. These resuls suggest that alterations in NMDA receptor expression could be an early marker of the neuronal degeneration in the Mnd mouse. M.D was supported by a postdoctoral fellowship from the Human Frontier Science Organization.

667.10

IMMUNOHISTOCHEMICAL DIFFERENCES IN MESOLIMBIC DOPAMINERGIC NEURONS IN FISCHER AND LEWIS RATS. Herbert W. Harris * & Eric J. Nestler. Dept. of Psychiatry, Yale Univ., New Haven, CT 06519.

Inbred Lewis (LEW) and Fischer (F344) rat strains have served as models of mechanisms of addiction. LEW rats show greater inherent preference for opiates, cocaine, cannabinoids and alcohol than do F344 rats (see Nestler, 1992 *J. Neurosci. 12, 2439*). In comparison to F344 rats, LEW rats also have higher levels of tyrosine hydroxylase (TH) and lower levels of neurofilament proteins in the ventral tegmental area (VTA); and higher levels of the cAMP pathway and lower levels of TH in the nucleus accumbens (NAc). To investigate structural correlates of these biochemical changes, immunohistochemical studies of VTA dopaminergic neurons in these strains were undertaken. Results show that the density of TH-positive neurons in the VTA in LEW rats is about 50% of that found in F344 rats ($n=3$, $p < 0.001$). In contrast, examination of substantia nigra in the same sections revealed no differences in the density of TH-positive cells between these strains. The finding of 50% fewer TH-positive neurons in the VTA of LEW rats, with our earlier results showing 45% higher levels of TH by immunoblotting, would suggest much higher levels of TH per VTA neuron in this strain. However, no obvious strain difference in the intensity of TH staining could be detected by immunohistochemistry. This observation may reflect differences in sensitivity between immunoblotting and immunohistochemical staining or differences in the subcellular pools of TH detected by these methods. These anatomical findings shed new light on the functional differences between LEW and F344 rats that may be associated with drug preference. The observation of lower numbers of TH-positive VTA neurons in the LEW strain is consistent with a model of addiction in which dopaminergic VTA neurons play a central role in the drug-preferring state.

667.11

LOCALIZATION OF P70-LIKE IMMUNOREACTIVITY IN GERBIL BRAIN. A. Seto-Ohshima[1*], M. Onozuka[2], S. Imai[3] and S. Ozono[4]. [1]Institute for Developmental Research, Aichi Prefectural Colony, Kasugai 480-03, [2]Gifu Univ. Sch. of Med., Gifu 500, [3]Kitasato Institute, Tokyo 108, [4]Kanagawa Den. Col., Yokosuka 238, Japan.

Recently, we found a specific 70-kDa protein (P70) in a cobalt-induced cortical epileptic focus of rat that is capable of inducing epileptiform seizure activities (1), suggesting its possible linkage to epileptogenesis. In the present study, in order to more precisely evaluate the involvement of P70 in the epileptogenesis, we immunohistochemically examined the localization of this protein in the brains of Mongolian gerbils (registered as a seizure-prone strain, MGS/Idr). The animals were anesthetized, perfused intracardially and then the brain slices were prepared, followed by analysis of the localization of P70 by immunohistochemistry with an antibody to this protein. When the cerebral motor cortex was applied with cobalt, immunoreactivity to P70 was found mainly in the epileptic focus region. In cobalt-untreated animals, the significant immunoreactivity was detected only within the cell nuclei in several brain regions including substantia nigra. A similar observation was also obtained in cobalt-untreated young gerbils without any behavioral seizure activity. The cytoplasma of most of these immunoreactive cells was positive to neuron-specific enolase. Thus, P70 may be related to the inherent instability of the neuronal system of this epileptogenic animal.

1. Onozuka, M. *et al.*, Exp. Neurol., 111: 190-197 (1991)

667.12

DEVELOPMENTAL/BEHAVIORAL STUDIES IN SHAKER MUTANT RATS WITH HEREDITARY CEREBELLAR ATAXIA. L. Wolf, J. Cribb, M. LaRegina, R. Clark*, A. Bastian and D. Tolbert. Dept. Comp. Med. & Prog. Phy. Ther., Washington Univ. and Dept. Anat. & Neurobiol. St. Louis Univ., St. Louis MO.

We are characterizing a rat mutant (Shaker) which displays hereditary cerebellar ataxia coincident with degeneration of Purkinje cells and inferior olivary neurons. Ataxic Shaker rats are characterized as mild or strong depending upon the absence or presence of tremor. We report our analysis of somatic development and locomotor behaviors and the temporal onset of clinical signs in mild and strong Shakers. Somatic development was similar in Shaker and controls from birth to 6 months of age. No differences were seen in the temporal development of surface and mid-air righting reflexes in Shaker and control rats. The development of postural/placing reactions was delayed in Shaker rats. Analysis of gait revealed a longer stride length and width in 3 month old mild and strong Shakers consistent with clinical ataxia. Preliminary data from Peak Performance 2-D motion analysis suggests the average gait cycle is shorter and time spent in stance longer in Shaker rats compared to controls. Whole body tremor in strong Shakers becomes apparent as early as 6 PNW. Mild Shakers never develop tremor. These behavioral observations correlate with the magnitude and spatial pattern of Purkinje cell degeneration. (Supported by NIH grants RR07013 and NS20227)

667.13

QUANTITATIVE ANALYSIS OF GRANULE CELL AND PURKINJE CELL POPULATIONS IN SHAKER MUTANT RATS WITH HEREDITARY CEREBELLAR ATAXIA. M. LaRegina, M. Ewald, J. Haring, R. Marks, K. Smith* and D. Tolbert. Dept. Comp. Med. Washington Univ. and Depts. Anat. &. Neurobiol. and Surg. St. Louis Univ. St. Louis, MO.

In preliminary characterization of Shaker rat mutants, morphometric analysis of the cerebellum revealed degeneration of many Purkinje cells (PCs) and reduction in the area of the gray matter especially in the molecular layer. Differences in severity of clinical signs between mild (no tremor) and strong (tremor) ataxic Shakers appears correlated to the overall numerical and spatial degeneration of PCs. In this study, we report the abnormal morphology of surviving PCs, the linear spatial interrelationships of surviving PCs in mild Shakers (partial PC degeneration), and granule cell density in strong Shakers (severe PC degeneration). PCs were studied using calbindin immunostaining, granule cell density was determined from cresyl violet stained sections. The somata and dendrites of Shaker PCs appeared normal. Three abnormalities of PC axons were observed: retraction from the cerebellar nuclei, large spheroids or varicosities of PC axons in the granule cell layer, and recurrent collaterals with abnormal trajectory. Compared to controls, PCs in mild shakers were decreased/unit linear length, and inter-PC distance was increased. Granule cell density in strong Shakers was greater than controls (12.18 $\pm$1.05 vs 10.28 $\pm$0.38/1000um^2). This above normal density of granule cells in strong Shaker rats was attributed to the overall shrinkage of the cerebellar gray matter. (Supported by NIH grants RR07013 and NS20227).

667.14

SPATIAL DISTRIBUTION OF PURKINJE NEURONS IN SHAKER MUTANT RATS WITH HEREDITARY CEREBELLAR ATAXIA. D.L.Tolbert*, M. Ewald, J. Gutting, and M. LaRegina. Dept. Anat. & Neurobiol., St. Louis Univ. and Div. Comp. Med., Washington Univ., St. Louis, MO 63104.

Shaker rat mutants display characteristic clinical signs: mild Shakers are ataxic, strong Shakers are ataxic and have tremor. The onset of these clinical signs occurs coincident with cerebellar Purkinje cell (PC) degeneration. To quantitatively analyze the spatial pattern of PC degeneration, calbindin immunostaining was used to identify surviving PCs in 3, 6 and 18 month old strong Shakers. The spatial distribution of immunoreactive PCs was plotted (Bioquant) in unfolded 2-D reconstructions of the cerebellar cortex. In 3 month old strong Shakers, many PCs in the vermis and paravermis of lobules I-V had degenerated. In the other lobules significant PC loss occurred in anterior lobule VI, posterior lobule IV, lobule VIII and anterior IX. In other parts of these lobules and in lobule X, PC density appeared relatively normal. In 6 month old strong Shakers almost all PCs in the anterior lobe had degenerated. In anterior lobule VI, posterior lobule VII, lobule VIII and anterior IX there was further degeneration of PCs in the vermis and hemispheres. At 18 months the majority of surviving PCs were localized to the vermis: posteriorly in lobule VI, anteriorly in lobule VII, posteriorly in lobule IX, and in lobule X. In 3 and 6 month old Shakers many of the surviving PCs in the posterior lobe were aligned sagittally. (Supported by NIH grants RR07013 and NS20227)

667.15

ENDOGENOUS SEROTONIN RELEASE FROM THE DOPAMINE DEFICIENT STRIATUM OF THE WEAVER MUTANT MOUSE. E.H. Stotz*, B. Ghetti and J.R. Simon. Indiana Univ. Sch. of Med., Indianapolis, IN 46202

The striatum (STR) of the weaver mutant mouse has major deficits in dopamine (DA) content, tyrosine hydroxylase activity and DA uptake. Striatal serotonin (5-HT) is also altered in weaver mice compared to wild-type as seen by increased 5-HT levels in weaver STR with the dorsal aspect being the primary site of the increase. 5-HT uptake is also elevated in the dorsal portion of the weaver STR. The functional relevance of the 5-HT increase was investigated by studying endogenous 5-HT release. Endogenous 5-HT release was measured in a superfusion system at a flow rate of 0.25 ml/min. Basal fractions were collected prior to a 5 minute exposure to either high K^+ or fenfluramine. Aliquots of the superfusate fractions were analyzed for 5-HT by HPLC-EC. The effects of stimulation on 5-HT release were calculated as evoked release, which is the amount of 5-HT released above basal release. Data are presented on a fractional basis. Basal 5-HT release is decreased by 50% in the weaver STR compared to wild-type in spite of the weaver STR having higher 5-HT levels. The evoked release elicited by high K^+ was lower in the weaver STR than in the wild-type STR. The evoked release elicited by fenfluramine was greater in the weaver STR than in the wild-type STR. Conclusions drawn from the evoked release stimulated by high K^+ and fenfluramine are not consistent in as much as high K^+ release data suggest the increased 5-HT content is in a nonreleasable pool while the fenfluramine data suggest that the 5-HT is in a releasable pool. Since high K^+ is a nonselective releaser, it is possible that a second neurotransmitter is released during high K^+ stimulation and it could affect 5-HT release. This neurotransmitter would be less likely to be released during fenfluramine stimulation as the drug is more selective for 5-HT. Another explanation of the decreased 5-HT release seen in the weaver STR is that the 5-HT system is a primary site of action for the weaver mutation and the decreased release is a direct result of the mutation. (R01 NS14426 and P01 NS27613)

667.16

A GENETIC ANALYSIS OF NEOCORTICAL ECTOPIAS IN NEW ZEALAND BLACK MICE. G.F. Sherman*, L.V. Stone, A.M. Galaburda, V.H. Denenberg, and D.R. Beier. Department of Neurology, Beth Israel Hospital, and Harvard Medical School, Boston, MA 02215; Biobehavioral Sciences Graduate Degree Program, University of Connecticut, Storrs, CT 06269; and Genetics Division, Brigham and Women's Hospital, and Harvard Medical School, Boston, MA 02115.

Approximately 30-40% of New Zealand Black mice (NZB/BINJ) develop prenatal ectopic collections of neurons in layer I of the neocortex (Sherman et al., *Acta Neuropathol.*, 74:239-242, 1985). These ectopias are similar in appearance to those seen in the brains of dyslexic individuals (Galaburda et al., *Ann. Neurol.*, 18:222-233, 1985). In earlier studies we hypothesized that the cortical malformations in NZB mice resulted from intrauterine influences arising in the affected offspring's mother. However, embryo transfer experiments with mice, in which the influence of the intrauterine environment was examined, failed to show a significant role for this mechanism (Denenberg et al., *Brain Res.*, 563:114-122, 1991). Furthermore, both the incidence of ectopias in the F_1 crosses from NZB and normal control strains and the incidence in the recombinant inbred strains NXSM (NZB x SM; provided by E. Eicher) suggested that the predisposition to cerebral ectopias in NZB mice is heritable. The present study was a mendelian breeding experiment designed to test the hypothesis that a single recessive gene is involved in the development of ectopias.

NZB and control DBA/2 mice were bred, and the F_1 offspring were backcrossed to DBA or NZB resulting in eight reciprocal combinations of breeding pairs. All brains were processed for celloidin embedding and serially sectioned at 30 µm. Every fifth section was stained with cresyl violet and examined for the presence of ectopias.

Thirty-eight percent of the NZB progenitor strain had ectopias, whereas the DBA and the F_1 offspring had an incidence of 1-2%. The incidence of ectopias in the DBA backcross (1.5%) was not different from the DBA progenitors, whereas the incidence in the NZB backcross (14.2%) was half that seen in the NZB progenitors (χ^2=19.71, df=1, p<0.01). The NZB backcross did not differ from the expected distribution based on the hypothesis that a recessive gene was involved (χ^2=1.99, df=1, n.s.). The results support the hypothesis that the predisposition to cerebral cortical ectopias in NZB mice is a recessively inherited trait with incomplete penetrance. Supported in part by NIH grant HD 20806.

667.17

BEHAVIORAL AND IMMUNOLOGICAL ASSOCIATIONS OF CORPUS CALLOSUM ABNORMALITIES IN NZB-RELATED MICE L.M. Schrott*+, G.F. Sherman#, S. Ansar Ahmed^, N.S. Waters†, R. Wimer°, C. Wimer°, G.D. Rosen#, A.M. Galaburda#, and V.H. Denenberg†. †Biobeh. Sci. Grad. Deg. Prog., Univ. of CT, Storrs CT 06269; #Beth Israel Hosp., Harvard Med. School, Boston MA 02215; ^VA-MD Reg. Col. Vet. Med. VPI, Blacksburg VA 24061; °Beckman Res. Inst., Duarte CA, 91010; +Univ. of CO Health Sci. Cen., Denver CO 80262

NZB mice have been used to explore relationships among learning disorders, brain anomalies, and autoimmunity. Prior studies related learning deficits to brain anomalies (cortical ectopias and hippocampal anomalies) or degree of autoimmunity. NZBs also display a low incidence of corpus callosum agenesis (CC Agen). To assess the influence of CC Agen on behavior and autoimmune parameters, recombinant lines with NZB as one progenitor were examined. NXSM (n=179) and NXRF (n=112) mice were behaviorally tested. Brains were examined for CC Agen and sera samples were examined for autoantibodies to dsDNA and cardiolipin.

CC agenesis was found in 3/15 NXSM and 1/4 NXRF lines. Comparisons were made among mice with CC Agen, mice from the same lines with Intact CC and mice from lines with no neural anomalies (Controls). CC Agen mice did not learn a spatial water escape task, while Intact CC and Control mice did. In NXSMs Intact CC mice had better performance than Control mice. NXRFs had a similar pattern on the last 2 trials. In the spatial Morris maze task, CC Agen mice traveled a greater distance and spent more time in the quadrant opposite the escape platform. Intact CC NXRF mice traveled a greater distance than Controls. CC Agen was also associated with high levels of antibodies to dsDNA in NXSMs.

CC Agen led to poor spatial learning, implying that inter-hemispheric transfer of information facilitates this learning. These mice had high levels of antibodies to DNA suggesting an altered neural-immune axis. Supported in part by NIH grant HD 20806 and RR 01183. We thank Dr. E. Eicher for generous donation of mice.

667.18

SEVERE GAUCHER DISEASE IN NEONATAL MICE AND HUMANS. E. Sidransky, and E. I. Ginns*. Section on Molecular Neurogenetics, Clinical Neuroscience Branch, NIMH, NIH, Bethesda, MD 20892.

A group of infants with Gaucher disease is described with a devastating course similar to that of transgenic Gaucher mice created by targeted disruption of the mouse glucocerebrosidase gene. These mice have lipid storage in macrophages of liver, spleen, bone marrow and brain. They are cyanotic, lethargic, have an unusual skin appearance at birth and die within 24 hours of life. The severely affected humans also die in the neonatal period and many have associated congenital ichthyosis. Both the affected mice and ichthyotic Gaucher patients have a similar histologic appearance with a thickened overlying keratin layer. Thus, specific clinical phenotypic manifestations of the transgenic Gaucher mouse have contributed to the recognition of a new human phenotype of Gaucher disease. The anticipated generation of other, less severe mouse phenotypes, should similarly provide insight into other aspects of the human disease.

667.19

THE SPONTANEOUS AGE-RELATED INCREASE IN THE NUMBER OF REVERTANT VASOPRESSIN CELLS IN THE HOMOZYGOUS BRATTLEBORO RAT DECLINES AFTER 100 WEEKS OF LIFE. F.W. van Leeuwen* and M.A.F. Sonnemans. Neth. Inst. Brain Res., 1105 AZ Amsterdam, The Netherlands.

The homozygous Brattleboro rat suffers from hypothalamic diabetes insipidus caused by a single base deletion in exon B as a result of which the frame-shifted mutant vasopressin (VP) precursor cannot be translocated over the membranes of the endoplasmic reticulum. Therefore this mutant precursor does not reach the Golgi apparatus and cannot be processed enzymatically.

We found a remarkable age-dependent increase (until 100 weeks of life) in the number of cells displaying the wild type VP phenotype. Through an unknown mechanism a low number of cells become phenotypically heterozygous. The wild-type precursor can again be translocated in the endoplasmic reticulum and is axonally transported towards the neural lobe (van Leeuwen et al., PNAS USA, 86, 6417, 1989).

Using a new antiserum directed against the C-terminus of VP-neurophysin (# THR, obtained through A.G. Robinson, Univ. Pittsburgh) we could label these solitary neurons. The immunoreactivity obtained with THR indicates that the sequence alteration leading to the phenotypic repair occurs in a region upstream of the C-terminus of neurophysin (Evans et al., Soc. Neurosci. 18, 1334, 1992).

In addition, the increase in solitary cell number declines after 100 weeks of life in females. The number of cell profiles was assessed in female rats with an age of 92, 100, 110 and 120 weeks. Their mean number was respectively 119, 120, 97 and 110. This suggests that the mechanism leading to phenotypic repair of the wild-type can change after 100 weeks of life. Alternatively there is no change, but the increase is compensated by cell death.

667.20

MAGNETIC RESONANCE IMAGING OF CEREBRAL ANOMALIES IN SUBJECTS WITH GENERALIZED RESISTANCE TO THYROID HORMONE. C. M. Leonard*, S. An, N. Sorensen, L.DeBusc, E. Wiggs, P. Martinez, B. Vitiello, B. D. Weintraub, and P. Hauser. Dept. Neuroscience, Univ. Fl. Gainesville 30610, NIDDK, and NIMH, Bethesda, MD 20892.

Individuals with generalized resistance to thyroid hormone due to a mutation in the human thyroid receptor beta gene on chromosome 3 have an increased incidence of attention deficit hyperactivity disorder and language deficits. We have used magnetic resonance imaging (MRI) to determine whether mutations in this gene are associated with an increased incidence of the cerebral anomalies that have been described in other inherited language disorders. Subjects [20 male (AM), 22 female (AF)] with the gene and unaffected first degree relatives [18 male (UM), 16 female (UF)] underwent MRI brain scans that provided contiguous 2 mm thick sagittal images. Films of images taken at standard sagittal positions were analyzed by an investigator blind to subject characteristics. Affected male subjects had a significantly higher incidence of duplicated Heschl's gyri (primary auditory cortex) combined with anomalous gyri in the *left* parietal association cortex (AM: 40%; AF: 5%; UM: 6%; UF: 0%). There were no differences in the incidence of right sided anomalies. These findings suggest that an inability to utilize thyroid hormone early in development has a greater impact on the left hemisphere in male than female brains, providing indirect support for the "Geschwind hypothesis." This mutation provides a potential model system for the study of interacting genetic and environmental effects on human neural and cognitive development.

667.21

GENDER DIFFERENCES IN THE BEHAVIOR OF AN ANIMAL MODEL OF ATTENTION DEFICIT HYPERACTIVITY DISORDER. D.F. Berger and T. Sagvolden. Department of Neurophysiology, University of Oslo, Norway. *(SPON: European Brain and Behaviour Society).*

There is general agreement among investigators that the prevalence of Attention Deficit Hyperactivity Disorder (ADHD) is greater in male than in female children. The goal of the present experiment was determine if a gender difference could also be observed in the behavior of the spontaneously hypertensive rat (SHR), an animal model of the disorder. The operant behaviors of 32 approximately 90-day old male versus female SHR, and their normotensive progenitor control strain, the Wistar-Kyoto rat (WKY), were compared (n = 8). All were trained to leverpress on a multiple 120-s fixed interval (FI), 5-min extinction (EXT) schedule, for water. Gender differences were observed with the SHRs, but not the WKYs. Males SHRs responded considerably more during EXT than female SHRs; and both WKY groups, which did not differ. Male SHRs also had relatively more short interresponse times than the other groups. These findings increase the generality of the animal model, and suggest that the gender difference in the prevalence of ADHD may have a physiological, rather than social etiology.

668.1

FAST AND SLOW Na CHANNEL INACTIVATION AND THE ACTION OF PHENYTOIN IN RAT HIPPOCAMPAL NEURONS. Chung-Chin Kuo and Bruce P. Bean*. Dept. of Neurobiology, Harvard Medical School, Boston MA 02115

Previous experiments have shown that the anticonvulsant phenytoin (DPH) interacts with Na channels. In rat hippocampal neurons, DPH up to 100 μM had little effect on Na current elicited by short (~20 ms) depolarizations from -100 mV, suggesting very weak binding to channels in the resting state (apparent Kd > 1 mM). Potent block (Kd ~10 μM) could be observed only with protocols containing long-lasting (~20 s) depolarizations, suggesting either slow binding of DPH to the fast inactivated state (which is reached within msecs) or selective binding of DPH to the slow inactivated state (reached ~1000 times slower). Binding to the fast inactivated state seems to occur, since at potentials where there was almost only fast inactivation (< -50 mV), DPH still potently affected recovery from inactivation. Nevertheless, other experiments showed that DPH could also bind to the slow inactivated state, with a Kd similar to or slightly smaller than ~10 μM. The findings explain why Na channels should be strongly inhibited by DPH in the therapeutic range (~10 μM) during a long paroxysmal depolarization shift but would be almost unaffected during normal action potentials.

668.2

ADDITIVE CALCIUMANTAGONISTIC EFFECTS OF CARBAMAZEPINE AND BUSPIRONE AS MECHANISM OF ACTION IN EPILEPSIES AND AFFECTIVE DISORDERS. J.Walden[1,2]*, J.v.Wegerer[1], D.Bingmann[3]. 1=Psychiatr. Clini,Freiburg,2=Tropon Köln,3=Inst. Physiol. Essen,FRG

Carbamazepine (CBZ) in addition to its antiepileptic properties has been shown to be effective in the treatment of affective and schizoaffective disorders. In previous investigations calcium antagonistic actions of CBZ were described as a common mechanism in the efficacy in both pathophysiological states (Walden et al., Pflügers Arch. 420,R35, 1992). The aim of the present study was to compare effects of CBZ with those of the calcium antagonist verapamil (VERA) and the $5HT_{1A}$-agonist buspirone (BU). In hippocampal slices of guinea pigs (CA1 and CA3)threshold concentrations of 10 μmol/l CBZ, 5 μmol/l BU and 10 μmol/l VERA had no effect on the frequency of extracellular field potentials (EFP). Combinations of these concentrations resulted in reduction of EFP frequencies. The relative decrease was 0.66 (range 0.44-0.82) for 5 μmol/l Bu + 10 μmol/l CBZ; 0.33 (range 0.1-0.53) for 5 μmol/l BU + 10 μmol/l VERA and 0.39 (range 0.28-0.55) for 10 μmol/l CBZ + 10 μmol/l VERA. The results indicate that the VERA induced reduction of EFP is intensified by CBZ and BU.

668.3

CHRONIC ADMINISTRATION OF SODIUM VALPROIC ACID RETARDS PUBERTAL MATURATION IN INBRED DBA/2J MICE. P.J. Snyder* and L.L. Badura. Psychiatry Research & Neurology, Long Island Jewish Med. Ctr., and Behavioral Neuroscience, Univ. of Connecticut, Storrs, CT, 06269.

Sodium valproic acid (VPA), a widely prescribed anticonvulsant, appears to act on GABAergic systems although its precise mechanisms of action remain controversial. Because VPA has been reported to occasionally delay pubertal maturation in children, the current study sought to establish a valid animal model with which to further investigate the neuroendocrinological sequelae of VPA administration.

Male DBA/2J mice, raised form birth under a 12L:12D photoperiod, were weaned at 2 weeks and administered either VPA (17-20 mg/kg/day) or control solution via drinking water. Animals were weighed, blood sampled via retro-orbital puncture, and sacrificed via anesthetic overdose at 4, 6, or 8 weeks of age. Testes were removed, weighed, and fixed in 10% formalin for later histological analyses. In addition, the lengths (mm) of the left humerus bone from each animal were obtained as an index of skeletal growth.

The testes weights of the animals receiving VPA were significantly smaller than the control animals at both 6 and 8 weeks of age (148 ± 9 vs. 175 ± 5, and 179 ± 4 vs. 200 ± 5 mg respectively), and a strong trend in the predicted direction was found for the animals sacrificed at 4 weeks. No between-group differences were found for humerus length or body weight at any sampling age. These data indicate that chronic administration of VPA delays reproductive (but not skeletal) maturation in mice. Further work will center on: 1) the investigation of an hypothesized attenuation of reproductive function via histological analyses of gonadal tissue; and 2) RIA analyses of blood serum for determination of anterior pituitary and gonadal steroid content.

668.4

NEUROCHEMICAL CHANGES AND SEIZURE SEVERITY IN MALE GERBILS AFTER CHRONIC MELATONIN ADMINISTRATION. TH Champney* and JC Champney. Dept Human Anat, Coll Med, Texas A&M Univ, College Station, TX 77843-1114.

Recently, melatonin's anticonvulsant action has been demonstrated in gerbils (*Neuroreport* **3**:1152-1154, 1992). The present studies explored changes in γ-aminobutyric acid (GABA) and amine content from selected brain regions of animals tested for melatonin's anticonvulsant activity. Male Mongolian gerbils (*Meriones unguiculatus*) received daily afternoon injections of melatonin (MEL, 25 μg/inj, sc), biweekly MEL implants (1 mg/imp, sc) or no treatment for 12 weeks. One half of each of these groups was exposed to long photoperiods (LP, 14L:10D) while the other half was exposed to short photoperiods (SP, 10L:14D). At the end of the treatment period, the gerbils were challenged with pentylenetetrazol (60 mg/kg, sc) to produce convulsions, were monitored for convulsive behavior and 3 minutes prior to death received 3-mercaptopropionic acid (100 mg/kg, ip) to prevent GABA degradation. The gerbils were anesthetized with metofane, killed by decapitation and cortex, hippocampus and hypothalamus collected. These regions were assayed for GABA, dopamine, serotonin (5HT) and 5-hydroxyindoleacetic acid (5-HIAA) content (*J Chromat Biomed Appl* **579**:334-339, 1992). MEL injected gerbils were able to survive and respond to seizures better than control animals. Total convulsion scores were reduced in LP-MEL injected gerbils (2.47 ± 0.90 vs 7.67 ± 1.83 in LP-CONTROLS, $p < 0.05$). No MEL injected gerbils died during seizure-induction (0/31 compared to 5/33 in control animals). MEL implants were ineffective in preventing seizures (4/30 died). Hippocampal GABA levels were depressed in all SP exposed gerbils and in LP-MEL injected animals ($p < 0.05$ vs LP-CONTROLS). Dopamine content was unchanged in all brain regions examined. Hippocampal 5HT was increased in LP-MEL injected gerbils and hypothalamic 5HT was increased in SP-MEL injected gerbils ($p < 0.05$ vs LP-CONTROLS). Hippocampal and hypothalamic 5-HIAA levels were increased in SP-MEL injected gerbils and hypothalamic 5-HIAA was increased in LP-MEL injected animals ($p < 0.05$ vs LP-CONTROLS). These results suggest that MEL's anticonvulsant activity does not correlate with changes in neurochemicals in the regions studied.

668.5

ADCI, A NOVEL ANTICONVULSANT, DOES NOT IMPAIR AVOIDANCE OR SPATIAL LEARNING IN THE RAT. L. Rajachandran, A.D. Kastello, J.V. Cassella, A. Thurkauf, L. Marshall, A.J. Hutchison and J.F. Tallman*. Neurogen Corporation, Branford, CT 06405.

Seizure disorders are prevalent CNS disorders. A notable side effect of many anticonvulsant compounds is a disruption of learning and memory processes. This study assessed the effects of enantiomers of the novel broad spectrum anticonvulsant ADCI (30,100 mg/kg,PO), originally synthesized as a hybrid of Carbamazepine and MK801 (Rogawski et al., J. Pharmacol. Exp. Ther., 259, 1990), in a step down passive avoidance procedure and a Morris water maze task in adult male Sprague Dawley rats. (+) ADCI competitively antagonizes the high affinity binding of MK801 to rat brain cortical membranes with an IC_{50} of 15μM. In contrast, (-) ADCI has an IC_{50} of 300μM while Carbamazepine's IC_{50} is >300μM. ADCI, Carbamazepine (10,35 mg/kg,PO) and the noncompetitive NMDA antagonist MK 801 (0.25-1.0 mg/kg,PO) as well as Phenobarbital (30,60 mg/kg,PO) were administered 30 min prior to acquisition training in both of these paradigms. At the doses tested (3x and 10x the ED_{50} in maximal electroshock test for anticonvulsant activity), Carbamazepine did not produce deficits in passive avoidance. In contrast MK801 and Phenobarbital produced marked deficits in the avoidance and spatial learning task. Neither enantiomer of ADCI produced significant deficits in either task. Therefore, ADCI lacks amnestic effects in these rodent models of learning and memory and thus may offer distinct advantages over the existing pharmacological therapies for the treatment of seizure disorders.

668.6

FOREBRAIN AREAS SENSITIVE TO THE CONVULSANT EFFECTS OF THE ANTICHOLINESTERASE AGENT VX. J.H. McDonough*, T.-M. Shih and N. Adams. U.S. Army Med. Resch. Inst. Chem. Def., APG, MD 21010-5425

This study mapped brain sites sensitive to the convulsant effects of VX to identify areas responsible for initiation of nerve agent seizures. Rats previously implanted with cannula and recording electrodes were infused with VX (0.47-1.88 ug; 0.94 mg/ml; 0.25 ul/min); EEG and behavior were monitored for 4 h after infusion. Varying levels of sensitivity were found among limbic structures. The basolat. amygdala, amygdala-cortex transition zone, the piriform cortex, and ant. accumbens were most sensitive; other piriform sites, post. accumbens and entorhinal cortex were moderately sensitive; dorsal hippocampus, cent. and ant. amygdala were least sensitive. Preseizure behaviors were site-dependent. The mildest seizures consisted of episodic spike trains accompanied by class I-III kindled seizure behaviors. In the extreme form, spike trains merged to become continuous and class IV-VII behaviors occurred. Of the 20 sites studied, only limbic forebrain areas were sensitive to the convulsant effects of VX. Since there are multiple areas with slightly different levels of sensitivity, no single area may be critical to the initiation of seizures after systemic intoxication with nerve agents.

668.7

ISOBOLOGRAPHIC ASSESSMENT OF ANTICONVULSANT AND NEUROTOXIC INTERACTION BETWEEN DIAZEPAM AND NEW BENZODIAZEPINE RECEPTOR LIGANDS: INDICATIONS FOR SUBTYPE SPECIFICITY

C. Rundfeldt[1]*, C.D. Torchin[2], J.M. Kapetanovic[2], C. Gennings[3], and H.J. Kupferberg[2]
[1]School of Vet. Med., Dept. Pharmacol., Toxicol. & Pharmacy, Bünteweg 17, 3000 Hannover 71, FRG. [2]Epilepsy Branch, NINDS, NIH, Bethesda, MD 20892. [3]Med. Coll. Virginia, Dept. Biostatistics, Richmond, VA 23298-0032.

Benzodiazepine (BZ) receptor ligands are potent anxiolytic, anticonvulsant, sedative, and hypnotic drugs; partial BZ agonists are limited in their maximum effect. Recent work revealed that multiple isoforms of the GABA-BZ,receptor complex exist. The aim of this study was to investigate if isobolographic analysis (IA) of drug-drug interaction is a tool to get information on the pharmacological profile of partial BZ ligands. The IA was based on the estimation of a three-dimensional response surface using the logistic regression. We tested 4 new partial BZ ligands for interaction with diazepam (DZ) using the s.c. pentylenetetrazol (PTZ) test for anticonvulsant effect and the horizontal screen test for motor impairment. For both tests each of the drugs, DZ, Ro 17-1812 (1), Ro 18-5607 (2), Ro 40-4214 (3) and Ro 42-5787 (4), was administered i.p. alone and in fixed combinations with DZ in a range of doses to groups of 7-16 mice. The dose-response curves obtained with the PTZ test indicated median effective doses of 0.19 mg/kg for DZ, 0.15 mg/kg for 1, 0.06 mg/kg for 2, 0.33 mg/kg for 3 and 0.23 mg/kg for 4. Co-administration of DZ with the Ro-compounds showed that all 4 drugs shifted the dose-response curve of DZ to the left. The IA indicated for all 4 drugs no significant deviation from additivity. DZ induced motor impairment with a TD_{50} of 1.96 mg/kg. The TD_{50} of 1 and 2 was 20 and 35.8 mg/kg, respectively; 3 and 4 did not evoke motor impairment up to 200 mg/kg. The IA revealed a subadditive or antagonistic interaction for motor impairment with 1, 2, and 3; the data obtained with 4 were not sufficient for the IA, but the effect of diazepam was antagonized. The results indicate that the IA is a suitable tool to get information on the pharmacological profile of partial BZ agonists. Further work is needed to elucidate, if the difference between the two tests used can be explained only by differences in receptor reserve or if the results indicate subtype specific effects of the new BZ receptor ligands.

668.8

SINGLE UNIT RECORDINGS IN THE SUBSTANTIA NIGRA OF RATS: INFLUENCE OF THE ANESTHESIA ON SPONTANEOUS ACTIVITY OF GABAERGIC NEURONS AND ON THE EFFECT OF THE ANTICONVULSANT DRUG VALPROATE.

A. Rohlfs, C. Rundfeldt and W. Löscher*, Dept. of Pharmacol., Toxicol. & Pharmacy, School of Veterinary Medicine, Bünteweg 17, D-3000 Hannover 71, FRG.

The substantia nigra pars reticulata (SNR) is in current discussion to play a critical role in the propagation of epileptic seizures. Valproic acid (VPA) reduces SNR output by decreasing the spontaneous activity of GABAergic SNR neurons. We wanted to test if this effect is influenced by the anaesthesia used to measure SNR neuron activity. Three different forms of anaesthesia were compared: 1) Chloral hydrate (CH), 360 mg/kg i.p., followed by i.v. infusion of 140-160 mg/kg/h CH. 2) Fentanyl, 50 µg/kg, combined with methohexital, 60 mg/kg i.p., for induction, followed by an i.p. bolus of methohexital 20 mg/kg after 20 min. After preparation, the rats were relaxed with an i.p. bolus of 20 mg/kg gallamine; analgesia and relaxation were maintained with a co-infusion of fentanyl (100 µg/kg/h) and gallamine (20 mg/kg/h). 3) Ketamine, 200 mg/kg i.p., followed by i.v. infusion of 30 mg/kg/h ketamine. For the experiments, female Wistar rats were ventilated and carbon dioxide in exspiratory air, heart rate, blood pressure, and rectal temperature were continuously monitored. The spontaneous firing rate of one identified GABAergic neuron in the SNR was monitored over 10 minutes after stabilization and for further 40-50 min after i.v. application of 100 mg/kg VPA. The mean (x±SD) basic firing rate of SNR neurons was 19.9±7.1 Hz under CH anaesthesia. The maximum depression after 100 mg/kg VPA was 14%. VPA deepened the anesthesia; to maintain a stable state, the infusion rate of CH had to be reduced to 70 mg/kg/h. Under fentanyl analgesia, the basic firing rate of 23.5±6.5 Hz was significantly higher than under CH anaesthesia. The maximum depression by VPA of 49% was significantly higher than under CH anaesthesia; the infusion of fentanyl had to be reduced to 75 µg/kg/h. Under ketamine anaesthesia, the basic firing rate of 22.7±10.7 Hz was not significant different from the rate under CH anaesthesia. The maximum depression by VPA of 36% was significantly higher than under CH anaesthesia.

The results indicate that the anaesthesia can influence the results obtained from single unit recordings in rats in the SNR. Both the basic activity and the effect of the drug differed depending on the anaesthesia used.

668.9

THE ANTICONVULSANT LAMOTRIGINE BLOCKS SODIUM CURRENTS FROM CLONED ALPHA-SUBUNITS OF RAT BRAIN Na^+ CHANNELS IN A VOLTAGE-DEPENDENT MANNER BUT GABAPENTIN DOES NOT.

C. P. Taylor*, Dept. Pharmacology, Parke-Davis Pharm. Res., Div. of Warner-Lambert Co., Ann Arbor, MI 48105.

Lamotrigine (6-(2,3-dichlorophenyl)-1,2,4-triazine-3,5-diamine) and gabapentin (1-(aminomethyl)cyclohexaneacetic acid) each prevent seizures in animal models and clinical trials. This study compares their action on rat brain type IIA sodium channel α subunits stably expressed in Chinese hamster ovary cells (West et al., *Neuron* 8:59-70, 1992). Sodium currents were recorded by whole-cell voltage clamp and drugs were pressure-applied from blunt micropipettes or added to the bathing medium.

Within 2 min, lamotrigine (5-50 µM) increased steady-state inactivation of sodium currents and at high concentrations (50 µM) slowed the time course of recovery from inactivation and reduced peak currents. Lamotrigine (5 µM) decreased peak current progressively for 50 sec when holding potential was stepped from -120 to -60 mV and current was restored by holding at -120 mV for 25 sec, whether or not depolarizing pulses were applied. Blockade of peak current by 50 µM lamotrigine was increased even by short (1 msec) prepulses to 0 mV. Voltage-dependence of activation and rate of activation or inactivation were not consistently changed.

Gabapentin (200 µM) was applied by blunt pipettes, by bath application for 24 hr, or by the recording micropipette but sodium currents were unchanged.

These results show that lamotrigine modulates voltage-dependent sodium channels in much the same manner as lidocaine or phenytoin (Ragsdale, et al., *Mol. Pharmacol.* 40:756-765, 1991), while gabapentin does not.

668.10

Effects of Antiepileptic Drugs on Seizure Activity in Cultured Rat Hippocampal Neurons.

Flora W. Jow* and Stephen S. Lin Departments of CVMD and CNS, Wyeth-Ayerst Research, CN 8000 Princeton, NJ 08543.

The type of medication used for treating epilepsy depends on the kind of seizure. We investigated the effects of seven different anticonvulsants on epileptic seizure activity in cultured rat hippocampal neurons (initially described by Furshpan and Potter, Neuron, 1989). Phenytoin, one of the most effective drugs against partial and generalized tonic-clonic seizures, reduces the frequency of paroxysmal depolarization shifts (PDSs) in these cells at 10 µg/ml, and completely blocks seizure activity at 20 µg/ml. Phenobarbital, the drug of choice for febrile seizures, reduces the frequency of seizure activity at 20 µg/ml and blocks all activity at 40 µg/ml. Drugs that are effective against absence seizures, such as ethosuximide and valproate, also reduce the frequency of PDSs at 50-100 µg/ml. The benzodiazepine, diazepam, inhibits seizure activity at 1 µg/ml. However, nitrazepam, is ineffective at 1-10 µg/ml. Carbamazepine also has no effect at 1-20 µg/ml.

668.11

ANTICONVULSANT DRUGS INHIBIT NMDA STIMULATED [^{3}H]-NOREPINEPHRINE EFFLUX IN RAT BRAIN CORTEX AND HIPPOCAMPUS.

Gladys Yi-Ping Lee*, Timothy J. Teyler and Laurie M. Brown, Departments of Pharmacology and Neurobiology, Northeastern Ohio Universities College of Medicine, Rootstown, Ohio 44272-0095.

Some investigators have observed inhibitory effects of anticonvulsant agents on NMDA mediated processes (Sethy and Sage, Neuropharmacol. 31 (1992) 111-114; Cotariu et al., Proc. Neurobiol. 34 (1990) 343-354). The anticonvulsant drugs phenobarbital, phenytoin, and valproic acid were used to assess their interaction with NMDA mediated [^{3}H]Norepinephrine ([^{3}H]NE) efflux.

Brain slices (350 µm) from the cortex and hippocampus of adult Long-Evans hooded rats were used. The slices were washed and incubated in 37°C oxygenated Krebs-Ringers bicarbonate buffer and allowed to accumulate [^{3}H]NE. After uptake of the label, the slices were washed again and aliquots placed into baskets which were transferred through a series of vials containing 100 µM NMDA and varying concentrations of the anticonvulsant drugs. Phenobarbital and valproic acid were used in concentrations of 0.001, 0.01, 0.1, and 1 mg/ml, and phenytoin was used in concentrations of 0.0001, 0.001, 0.01, and 0.1 mg/ml. The human therapeutic serum concentrations of phenobarbital, phenytoin, and valproic acid are 0.035, 0.02, and 0.1 mg/ml respectively.

Phenobarbital at the concentration of 1 mg/ml caused a 52 and 48% inhibition of NMDA stimulated [^{3}H]NE efflux in the cortex and hippocampus, respectively. Phenytoin at 0.1 mg/ml caused a 39 and 17% inhibition of [^{3}H]NE efflux in the cortex and hippocampus, respectively. Valproic acid had a tendency to inhibit [^{3}H]NE efflux at the 1 mg/ml concentration in cortex. These results indicated that some anticonvulsants may inhibit NMDA mediated processes in the therapeutic range.

668.12

ANTICONVULSANT EFFECTS OF NS004 AGAINST PENTYLENETETRAZOLE-INDUCED SEIZURES

A. Ortiz*, S. Ward, and N. Meanwell.
Neuropharmacology and Division of Chemistry, Bristol-Myers Squibb, Wallingford, CT 06492-7660.

The selective opening of K^+ channels has been described as a possible therapeutic strategy for the treatment of seizure disorders. Recent work suggests that NS004 [1-(5-chloro-2-hydroxyphenyl)-5-trifluoro-methyl-1,3-dihydro-2*H*-benzimidazol-2-one] behaves as a maxi-K potassium channel opener: NS004 activates maxi-K channels in cerebellar granule cells and smooth muscle cells in culture (Olesen & Wätjen, Eur. Patent 477,819). We have studied the anti-convulsive activity of NS004 in the iv. pentylenetetrazole (PTZ)-induced seizure model. Fasted $CD2F_1$ mice were pretreated with vehicle or NS004 at 1, 30, or 60 mg/kg, ip. After 30 min, 10 mg/ml of PTZ was infused iv. at 0.20 ml/min. The freely moving animal was observed for five minutes and the amount of PTZ (mg/kg) required to produce the following seizure components was calculated: the "first twitch", the "clonic seizure", the "tonic seizure", and PTZ-induced lethality. The thresholds for the "tonic seizure" and the "clonic seizure" were significantly increased by 30 and 60 mg/kg of NS004. Moreover, NS004 at 60 mg/kg increased the threshold of PTZ-induced lethality. Results from this experiment demonstrate the anticonvulsive activity of NS004 and suggest the potential of maxi-K channel openers as candidates in the treatment of seizure disorders.

668.13

CHARACTERIZATION OF FELBAMATE'S ACTIONS ON NMDA AND GABA RECEPTORS IN CULTURED RAT HIPPOCAMPAL NEURONS. Jong M. Rho*, Sean D. Donevan, and Michael A. Rogawski, Neuronal Excitability Section, Epilepsy Research Branch, NINDS, NIH, Bethesda, MD 20892.

Felbamate (FBM, 2-phenyl-1,3-propanediol dicarbamate) is a promising new antiepileptic and neuroprotective agent that may interact with the glycine site on NMDA receptors (McCabe et al., *J. Pharmacol. Exp. Ther.* 264:1248–1252, 1993) and is generally believed not to modulate $GABA_A$ receptors (Ticku et al., *Epilepsia* 32:389-391, 1991). In the present study, we characterized the functional effects of FBM on NMDA and $GABA_A$ receptors using whole-cell voltage clamp recordings in cultured rat hippocampal neurons. FBM caused a concentration-dependent (1 mM, 20 ± 2%, $n=6$; 3 mM, 49 ± 4%, $n=6$), voltage-independent block of responses evoked by 10 μM NMDA in the presence of 10 μM glycine. The blocking action of FBM could not be overcome by increasing the concentrations of either NMDA or glycine, indicating that the block may, at least in part, occur at a site distinct from the NMDA or glycine recognition sites. In the same range of concentrations that blocked NMDA responses, FBM produced a marked potentiation of Cl^- currents activated by 3 μM GABA (1 mM, 141 ± 6%, $n=3$; 3 mM, 437 ± 24%, $n=3$). The effect of FBM on GABA responses was unaffected by 10 μM flumazenil (Ro 15-1788), indicating that FBM is not a benzodiazepine site agonist. In the presence of supramaximal pentobarbital (3 mM), FBM produced no further potentiation of GABA responses, suggesting that FBM may be a barbiturate-like modulator. Thus, FBM appears to both inhibit NMDA responses and potentiate GABA responses. This novel combination of actions may account for FBM's unique clinical profile.

668.14

Gamma-vinyl GABA (GVG) reverses activity-dependent disinhibition in the rat hippocampus *in vitro*. M. Jackson, B. Esplin and R. Čapek*. Department of Pharmacology and Therapeutics, McGill University, Montreal, Quebec H3G 1Y6.

GVG is an irreversible inhibitor of GABA transaminase (GABA-T) which increases brain GABA levels and is an anticonvulsant in both animals and humans. Although it is generally believed that GVG prevents seizures by enhancing GABA-mediated inhibition, direct evidence in support of this is unconvincing. The goal of this study was therefore to determine whether an increase in GABA-mediated inhibition could be demonstrated using the antidromic-orthodromic stimulation test during conventional field potential recordings in hippocampal slices obtained from GVG-treated rats.

Slices were obtained from male Sprague-Dawley rats 24 hours after a single i.p. injection of either an anticonvulsant dose of GVG (1500mg/kg) or saline. Population spikes (PS) were recorded from the CA1 region following orthodromic stimulation delivered to the stratum radiatum. They were inhibited by conditioning antidromic stimulation delivered to the alveus at various interpulse intervals (IPI). The conditioning antidromic stimulation consisted of either a single pulse or a train of 20 pulses delivered at 5 or 100 Hz.

In slices taken from saline-treated animals the inhibition produced by stimulation of the alveus at 5 Hz was less than the inhibition following a single conditioning stimulus. In contrast to this, GVG pretreatment caused the inhibition at 5 Hz to be greater than that produced by a single pulse. GVG pretreatment did not affect the inhibition produced by a single pulse or by a train of pulses at 100 Hz.

These results suggest that GVG may suppress seizures by preventing activity-dependent disinhibition. (Supported by MRC of Canada and FCAR)

668.15

LACK OF ANTICONVULSANT TOLERANCE FOLLOWING CHRONIC (21 DAY) ADMINISTRATION OF THE GABA UPTAKE INHIBITOR TIAGABINE. P.D. Suzdak*. Novo Nordisk A/S, Pharmaceuticals Division, Novo Nordisk Park, DK-2760 Måløv, Denmark.

Tiagabine (NNC-05-0328) is a potent and selective GABA uptake inhibitor currently in Phase II/III clinical trials for epilepsy. The present investigation focused on determining if tolerance developed to the anticonvulsant effects of tiagabine following chronic (21 day) administration. NMRI mice received either vehicle, or tiagabine (at either 15 or 30 mg/kg) p.o. twice daily for 21 days. Subsequent behavioral testing was conducted 24 hours following the termination of chronic treatment. There was no tolerance to the anticonvulsant effect of acutely administered tiagabine (ED_{50} for inhibiting DMCM-induced seizures were 1.7, 1.9 and 2.0 mg/kg i.p., respectively). However, there was a significant decrease in the ability of acutely administered tiagabine to impair rotarod performance (ED_{50} of 6, 12 and 19 mg/kg i.p., respectively), inhibit traction (10, 23 and 32 mg/kg i.p., respectively) and to inhibit spontaneous locomotor activity (10, 19 and 29 mg/kg i.p., respectively) following chronic tiagabine administration. The lack of anticonvulsant tolerance following chronic administration of tiagabine further suggests that inhibition of GABA uptake is a highly promising, and novel, mechanism for future antiepileptic agents.

668.16

EFFECTS OF N^6-CYCLOPENTYLADENOSINE AND 8-CYCLOPENTYL-1,3- DIPROPYLXANTHINE ON N-METHYL-D-ASPARTATE - INDUCED SEIZURES IN MICE. Dag von Lubitz, Jan A. Paul, Kenneth A. Jacobson*, NIDDK, National Institutes of Health, Bethesda, MD 20892.

Adenosine agonists prevent convulsions in several chemical and electrical seizure models and protect against excitotoxic neurodegeneration involving NMDA receptors. However, the effect of receptor specific adenosinergics on NMDA evoked seizures is poorly known. Therefore, the effect of acute and chronic administration of a selective adenosine A1 receptor agonist N^6-cyclopentyladenosine (CPA) and antagonist 8-cyclopentyl-1,3-dipropylxanthine (CPX) on NMDA-evoked seizures was studied in C57BL/6 mice (20/group). In acute experiments, animals were injected i.p. either with CPA (0.5, 1, 2 mg/kg) or CPX (1, 2 mg/kg) 15 min prior to administration of NMDA (30, 60, 125 mg/kg). In chronic studies, mice were given either CPA (1 mg/kg) or CPX (1 mg/kg) for 9 days. One day later they were challenged with a single i.p. injection of 60 mg/kg NMDA. Administration of NMDA alone resulted in a dose-dependent increase in the intensity of clonic/tonic seizures and mortality. Acute administration of CPA delayed seizure onset, eliminated tonic episodes, and reduced mortality. Acute pretreatment with CPX significantly exacerbated the effect of NMDA. Conversely, compared to NMDA controls, chronic treatment with CPA led to a significantly increased mortality, while chronic CPX caused an equally impressive protection against seizures and postictal deaths ($p<0.01$) These results indicate that A1 receptor agonists may protect against NMDA-evoked seizures and that A1 receptors may be directly involved in these actions.

668.17

VALPROATE CONCENTRATION IN THE OLFACTORY BULB OF THE PURKINJE CELL DEFICIENT MUTANT MOUSE. T. J. Hoeppner* Department of Neurological Sciences, Rush Medical College, Chicago, IL 60612.

Previously we have shown that in the rat the anticonvulsant valproate (VPA) concentrates in the olfactory bulb. To help determine the cell type in which VPA concentrates tritiated sodium valproate (300 uCi/kg, i.p.) was administered to eight month old purkinje cell deficient (PCD) mutant mice and unaffected littermates. (By this age PCD mutants lose almost all of their cerebellar purkinje cells and their mitral cells). Thirty minutes later mice were perfused with saline and formalin and the brain frozen. Thaw mounted sections were apposed to X-ray film for autoradiography. The distribution and concentration of radiolabel was similar in PCD mutants and in unaffected littermates despite the marked loss of mitral cells in the mutants. VPA concentrated in the dorsal and medial portions of the glomerular layer of the olfactory bulb. VPA was undetectable in other parts of the brain. These findings indicate that the concentration of VPA in the olfactory bulb is not in the principal cells of the bulb: the mitral cells. This suggests that VPA concentrates in the olfactory nerve terminals or in periglomerular cells.

668.18

THE EFFECTS OF ANTICONVULSANT AGENTS ON 4-AMINOPYRIDINE-INDUCED EPILEPTIFORM ACTIVITY IN RAT HIPPOCAMPUS *IN VITRO*. W.D. Yonekawa*, I.M. Kapetanovic and H.J. Kupferberg, Epilepsy Branch, NINDS, NIH, Bethesda, MD 20892.

The Antiepileptic Drug Development (ADD) Program is attempting to identify novel anticonvulsants by their effects on various parameters of a stimulated hippocampal slice. We examined the effects of known anticonvulsant drugs, including phenytoin (PHT) and carbamazepine (CBZ), and a potent experimental compound, ADD 172014 (D20443 Asta Medica AG, Germany), on epileptiform activity evoked by the K^+ channel inhibitor, 4-aminopyridine (4-AP). Evoked extracellular population spikes (PS) and spontaneous activity with or without 4-AP and/or test compound addition were recorded from areas CA1 and CA3. The results indicate that EPSP duration is very sensitive to 4-AP perturbation, increasing approximately 2.6x in both CA1 and CA3 compared to control. PHT, CBZ, and ADD 172014 affected this increase by 1.1x, .8x, and .6x in CA1 and .8x, .9x and .7x in CA3. The PS amplitude increased with 4-AP by 1.6x in CA1 and 1.1x in CA3 and, while PHT and CBZ had little effect on these increases (1x-1.1x), ADD 172014 increased the amplitude by 1.4x. ADD 172014 also abolished spontaneous bursting, while both PHT and CBZ merely reduced the frequency and amplitude by .8x. These data indicate that ADD 172014 may act differently than PHT or CBZ and that this in vitro model can be useful in differentiating mechanisms of action.

668.19

ANTICONVULSANT EFFECTS OF MAGNESIUM SULFATE IN HIPPOCAMPAL KINDLED RATS. C.A. Janusz*, S.M. Irtenkauf, R.F. Berman and D.B. Cotton. Dept. OB/GYN, Wayne State Univ., Detroit, MI 48201.

The purpose of the present study was to determine whether magnesium sulfate has anticonvulsant actions in the hippocampal-kindled rat model of epilepsy. Fully kindled rats received acute intraperitoneal injections of magnesium sulfate (270 mg/kg), phenytoin (20 mg/kg) or saline in random order. Seizure duration, behavioral seizure stage and duration of postictal EEG depression were examined at 15, 30 and 60 minutes post-injection. A second experiment was initiated to examine the effect of chronic (2 hour) i.p. injections of magnesium sulfate on fully kindled seizures. There was a significant decrease in seizure duration ($p<0.01$) and behavioral seizure stage ($p<0.01$) with magnesium sulfate compared to saline. Phenytoin had no statistically significant effects on hippocampal kindled seizures. Chronic magnesium sulfate treatment significantly reduced behavioral seizure stage at 2, 24 and 48 hours ($p<0.05$). There was a significant time by treatment effect for magnesium sulfate on postictal EEG depression ($p<0.01$). We conclude that magnesium sulfate has anticonvulsant effects in this model of hippocampal seizures.

668.20

THE ANTICONVULSANT EFFECT OF IMIDAZENIL ON ELECTRICAL AND PENTYLENTETRAZOLE (PTZ)-INDUCED KINDLING. G. Bregola@, D. Ferrari@, M. Simonato@, A. Zanotti, P. Giusti, and E. Fadda*. @ Dept of Pharmacology, University of Ferrara , Dept. of Pharmacology, University of Padova, Italy.

The anticonvulsant effect of Imidazenil, a new imidazobenzodiazepine carboxamide, was studied in Sprague-Dawley rats underwent to PTZ or electrical kindling. The former was produced injecting i.p. a subconvulsant dose of PTZ (217 μmol/kg) every second day until tonic-clonic convulsion were evoked. Electrical kindling was obtained stimulating the right amygdala once daily with a single 1 sec train of 60 Hz bipolar pulse.

One week after reaching the criteria for kindling, animals were orally treated with the reference drug Diazepam or with Imidazenil. After 30 min, they were challenged with the appropiate stimulation. ED_{50} (95% C.L.) of Diazepam and Imidazenil for full suppression of convulsion were 19 (8.3-37) and 0.75 (0.21-2.6) μmol/kg respectively in PTZ-kindling, 18.8 (12.3-28.8) and 7.5 (4.9-11.3) μmol/kg in the electrical kindling. Furthermore, Imidazenil, but not Diazepam, appeared to increase the latency to amygdala kindling convulsion in non-responsive rats.

The present data are consistent with other reports of a potent anticonvulsant effect of Imidazenil.

DEGENERATIVE DISEASE: ALZHEIMER'S— β -AMYLOID XII

669.1

DEGRADATION OF THE BETA-AMYLOID PRECURSOR PROTEIN IN A CELL-FREE SYSTEM. S. Mistretta, J.T. Durkin, S. Murthy, R.W. Scott, B.D. Greenberg and R. Siman*. Cephalon, Inc., West Chester, PA 19380

It is now established that β/A4(1-40) is normally secreted from a variety of cells, but the proteases responsible for generating the β/A4 protein from the β-amyloid precursor proteins (APP) and the subcellular compartments involved in β/A4 protein production have not been identified. To address these issues, we report here on the use of a cell-free system for analysis of APP degradation. In human cells overexpressing APP751, the APP was tagged by metabolic labelling for 45 minutes. This permits some radiolabelled APP to enter constitutive processing pathways, as evidenced by the appearance of label in fully glycosylated full-length APP as well as in a number of COOH-terminal derivatives. Following homogenization, cell-free extracts were incubated under various conditions, and APP degradation was assessed by immunoprecipitation, Tris/Tricine SDS-PAGE, and phosphor-imaging. Both mature and immature full-length APPs were subject to processing *in vitro*. Among the COOH-terminal derivatives produced from degradation of full-length APP is a 12 kDa fragment. This derivative likely has the same NH_2-terminus as β/A4 protein and may be an intermediate in β/A4 protein formation (Durkin et al., this vol; Cai et al., Science *259*, 514 (1993)). The 12 kDa derivative is selectively immunoprecipitated by anti β(1-9), an antibody that also labels a single identically-sized fragment generated by intact cells. The method is amenable to the identification of distinct subcellular fractions, and the protease(s) within them, which generate the 12 kDa derivative and, therefore, which may be involved in β/A4 protein formation.

669.2

CLEAVAGE OF THE AMYLOIDOGENIC SITE OF APP BY CATHEPSIN D AND BRAIN EXTRACTS. US Ladror*, SW Snyder, TF Holzman, GT Wang, GA Krafft. Abbott Labs. Abbott Park, IL 60064.

A new fluorogenic substrate that mimics the N-terminal cleavage site (positions 649-656 of APP751) of Alzheimer's β-amyloid peptide was used to assay cathepsin D and proteinase activities from human and bovine brains. Ether spacers were used to distance the Edans and Dabcyl chromophores from the amino acid sequence to avoid artifacts previously observed with smaller substrates.

Proteinase from human and bovine brain extracts cleaved this substrate at pH 3.5 and 7.5, primarily at the Met-Asp bond, consistent with the most frequently reported amyloidogenic cleavage of APP. Human brain extract produced an additional cleavage at the Ala-Glu bond, as we reported previously with smaller substrates. This cleavage was absent in bovine brain extract. Purified cathepsin D also cleaved at the Met-Asp bond.

A similar substrate that incorporates the Lys-Met → Asn-Leu mutation, reported in a Swedish family with early onset Alzheimer's disease, was cleaved by cathepsin D at a rate 72-fold higher than the rate with the wild type substrate. Both cathepsin D and bovine brain activity at pH 3.5 cleaved this substrate at the amyloidogenic Leu-Asp site. At pH 3.0, bovine brain activity against the mutated substrate was 70 fold higher than the rate against wild type substrate. At pH above 5.5, bovine brain activities against both substrates were similar. These results suggest that Cathepsin D is a prime candidate for the "N-terminal amyloidogenic proteinase".

669.3

IDENTIFICATION AND CHARACTERIZATION OF A PROTEASE WHICH PROCESSES A PEPTIDE CORRESPONDING TO THE AMINO-TERMINUS OF β-AMYLOID. J. Koehn, A. Boon, L. Kent, K. Rogers, L. Fleissner, M. Ator, G. Trakshel, S. J. Ward*, and M. Miller. Pharmaceuticals Research Division, Sterling Winthrop Inc., Collegeville, PA 19426.

βA4 protein is a major component of AD plaques. It is thought to be proteolytically derived from one or more larger amyloid precursor proteins (APPs). We report here the purification and characterization of a peptidase from normal and AD brain which cleaves a peptide substrate, SEVKMDAE. Cleavage occurs on the carboxyl side of Met, mimicking the cleavage yielding the amino terminus of βA4. Using ion exchange, size exclusion, and affinity chromatography, this activity has been purified approximately 4000-fold. Class-specific protease inhibitors suggest this activity is a thiol-dependent metalloprotease. No proteolytic cleavage was observed using native full-length human APP isoforms or baculovirus expressed APP_{751}, or APP C-terminal fragments from human brain, human cell-lines, or a baculovirus expression system. Data suggest that hydrolysis of the βA4 N-terminus is regulated by factors in addition to primary peptide sequence.

669.4

CATHEPSIN S, AN ATYPICAL CYSTEINE LYSOSOMAL PROTEASE. S. Petanceska, S. Cvejic and L. Devi*. *Department of Pharmacology, New York Univ. Medical Center, New York, 10016.

The cysteine lysosomal proteases are a large family of highly conserved enzymes that are essential for intracellular protein turnover. These enzymes are strongly implicated in processes of cell growth, malignant transformation, inflammation, and in the metabolism of the amyloid precursor protein.

We have cloned and characterized a rather atypical member of this family of enzymes, cathepsin S. Unlike the other cysteine lysosomal proteases that are mostly inactive at neutral pH, cathepsin S retains around 70% of its activity at neutral pH. Also it is not expressed in detectable levels in many transformed rat embryo fibroblast cell lines of different tumorigenic potential that express large amounts of cathepsin B and cathepsin L. In a separate study, we compared the distribution of the mRNA for rat cathepsins S, B, and L in adult rat brain by *in situ* hybridization histochemistry; the distribution pattern of cathepsin S mRNA is profoundly different from the distribution of cathepsin B and L mRNA. Cathepsin S has a wide distribution throughout the grey and white matter and in cells that have microglial morphology.

Here we present the results of a comparative study of the expression of cathepsins S, B, and L during brain development and after entorhinal cortex lesions. During development, unlike cathepsin B and L, cathepsin S mRNA has a different (biphasic) temporal expression pattern in the time between E13 and P11. Also, unlike cathepsin B and L, after entorhinal cortex lesion cathepsin S is highly upregulated at the site of lesion and in the hippocampus in cells that have the morphology of activated microglia and reactive astrocytes. These data support the possibility that cathepsin S is involved in the Alzheimer's pathology.

669.5

TISSUE- AND CELL SPECIFIC EXPRESSION OF A NOVEL BRAIN SERINE PROTEASE. B. Meckelein*, K.J. Conn and C.R. Abraham. Arthritis Center, Boston Univ. Sch. of Med., Boston MA 02118

Brain proteases are believed to play a crucial role in the development of Alzheimer's disease (AD). Among those are the enzymes of the different secretory and lysosomal pathways which process the amyloid precursor protein (APP) to yield a variety of potentially amyloidogenic and non amyloidogenic fragments, as well as enzymes which are involved in inflammatory or "acute phase" processes. The identification of such proteases is important towards understanding the development of AD and may provide promising targets for a therapeutical approach.

We screened a human fetal brain cDNA library with anti cathepsinG antibodies, and isolated four crossreacting clones. They comprise a cDNA population which is derived from three differentially spliced RNA transcripts of one gene encoding a hitherto unknown serine protease.

The tissue distribution of these three differentially spliced RNAs was determined by PCR using isoform-specific primers. Examination of a variety of monkey tissues demonstrated that RNA for the novel serine protease is found in most of the tissues investigated. The tissue distribution of the differentially spliced forms, however, varies greatly. Interestingly, one of the transcripts is expressed predominantly in brain and testis. This could indicate a role for the enzyme in the processing of neuropeptides or hormones, but it is also intriguing in light of the fact that brain and testis are two of the organs with the highest levels of APP synthesis.

On brain sections, antibodies directed against peptide fragments of the novel protease stain blood vessel walls and reactive astrocytes. The same antibodies specifically precipitate a population of 28-35 kDa proteins from conditioned media of cultured astrocytes and glioblastoma cells.

The possible involvement of the novel brain serine protease in APP processing or acute phase reactions is under investigation.

669.6

BRAIN SERINE PROTEASES GENERATE AMYLOIDOGENIC FRAGMENTS. B.L. Razzaboni*#, K.J. Conn, M. Pietropaolo, and C.R. Abraham#. Arthritis Center and Biochemistry Dept.#, Boston Univ. Sch. of Med., Boston, MA 02118.

A serine protease appearing as a 26-28kDa doublet has been characterized from adult monkey brain. The protease(s) are able to cleave a synthetic substrate (P1) made according to the sequence flanking the N-terminus of ß-protein (Aß) and to degrade APP (Razzaboni et al., Brain Res. 589:207-216). Using aged monkey and aged human brains, similar serine protease(s) have been found. Further purification techniques enabled us to separate the two serine proteases from each other. Following chromatofocusing two bands were identified: a 26kDa band with a pI around 5.5 and a 28kDa band with a pI around 6.5. Both enzymes are cross-reactive with cathepsin G antibodies. The two may exist as isoforms since they both cleave P1 in the same position, APP degradation is blocked by preincubation of the enzymes with an antibody made to the N-terminus of the Aß (gift of E. Koo), and both are inhibited by an inhibitor specific for chymotrypsin-like proteases (gift of J. Oleksyszyn). Additionally, the 26kDa serine protease is inhibited by α1-antichymotrypsin (ACT) and aprotinin and the 28kDa serine protease is not.

Furthermore, we observed an increase in the specific activity for the serine protease inhibited by ACT and aprotinin from AD brains versus aged matched controls indicating that the enzyme may have a role in increased production of Aß. We are in the process of determining the relevance of these serine proteases to the in vivo degradation of APP and to the pathogenesis of AD.

669.7

CHARACTERIZATION OF AMYLOID PRECURSOR PROTEIN-ASSOCIATED SERINE ESTERASE ACTIVITY IN DIFFERENT CELL LINES. U.S. Kayyali*, and H. Potter. Dept. Neurobiology, Harvard Medical School, Boston MA 02115.

The precursor of the Alzheimer amyloid β/A4 protein, β-APP, is generated in at least three forms by alternative mRNA splicing. Two of the forms contain an extra domain with a Kunitz-type protease inhibitor (KPI) function. The secreted isoforms of the APP molecules that contain the KPI domain have been shown to be identical to the known protease inhibitor, protease nexin 2, and are therefore presumed to have as their function the inhibition of trypsin- and chymotrypsin-like proteases released during inflammation. We have obtained evidence that the 695 amino acid isoform of β-APP has an esterase enzyme activity that is capable of cleaving acetylcholine and other ester substrates such as p-nitrophenylacetate. The APP-associated esterase activity has been shown by inhibitor profile determination to be distinct from other serine esterases/proteases such as acetylcholinesterase. Site-directed mutagenesis of selected amino acid residues is being performed to test the hypothesis that the esterase active site serine is inside the beta protein itself. In addition the consequences on cellular physiology of abolishing that esterase activity in mutant cells will be characterized to ascertain the normal function of this novel esterase. We propose that the various isoforms of β-APP constitute a family of serine esterases and inhibitors whose normal function may be to control an important physiological process. The increase in acetylcholinesterase activity due to over-expression or aberrant processing of β-APP695 may also be significant to the pathology of Alzheimer's disease by reducing the level of the key neurotransmitter, acetylcholine.

669.8

SECRETASE-LIKE ENZYMES IN THE HUMAN AND RAT BRAIN. C. Schönlein[1], A. Probst[2] and G. Huber[1,*]. [1]Pharma Division, Preclinical Research, F. Hoffmann-La Roche LTD and [2]Institute of Pathology, 4002 Basel, Switzerland.

In the brain of Alzheimer's disease (AD) patients proteolytic deposition and accumulation of ßA4-amyloid in extracellular plaques is one of the pathological hallmarks of the disorder. Proteolysis of the amyloid precursor protein (APP) may occur through different cellular pathways, one of which gives rise to extracellularly secreted APP isoforms. The enzyme responsible for this cleavage, the secretase, is not yet clearly characterized. Using chromogenic peptide substrates derived from the secretase cleavage site within APP, proteolytic enzymes were investigated in the brain of Alzheimer patients and control individuals. Mean differences in enzyme activity were observed between the two groups, yet no statistical significance was reached. Further analysis of the enzymes suggest that under these experimental assay conditions secretase-like activity may be attributed to different enzymes and may be slightly altered under pathological conditions.

669.9

AMYLOID PRECURSOR PHOSHOINOSITIDE-REGULATED "α-SECRETASE" LEADS OTHER MEMBRANE PROTEASES AS THERAPEUTIC TARGET. D.M. Bowen[1], G.C. Stratmann[1], M-T. Webster[1], I.P. Chessell[1], R.C.A. Pearson*[2] & P.T. Francis[1]. [1] Inst. Neurol., London, [2] Univ. Sheffield, UK.

Gelatinase A (Miyazaki et al Nature, 362, 89, 1993) is the most recent of a myriad of membrane proteases being considered as candidates. Here "Clipsin" (Savage and Siman, Soc. Neurosci. Abst. 16, 787, 1990) has been studied as activity has only been reported towards precursor protein (APP) with enzyme from immature rat. APP from adult rat cortical membranes was partially purified (heparin-agarose), incubated (2h, pH 7.5, with EDTA) with enzyme (detergent- high ionic strength buffer extracts of purified cortical membranes of samples subject to short, 2h, and routine postmortem delay) and then analysed by Western blotting (mcab 22C11, Boehringer Mannheim; Biochem. Soc. Trans. 21, 241S, 1993). Substrate (APP, 116 kDa) was subject to slight (9%) autodegradation (product, 100 kDa). This was not increased by adult rat extract but it was enhanced to 62 ± 21% (n=6) by extract of human control temporal cortex (2h delay). This was not different (Mann Whitney U test) from Alzheimer's disease (69 ± 23%, n=9) as was also found with frontal cortex, 2 series with short and routine postmortem delays (control in parenthesis) 51 ± 14%, n=8 (58 ± 26%, n=6) and 70 ± 22%, n=8 (66 ± 27%, n=8), respectively. As product formation by this method was not different by diagnosis but was altered based on studies of phoshoinositide-regulated "α-secretase" (Francis P.T. et al, this meeting) the use of a cholinomimetic agent, in combination with a drug to block cortical pyramidal neurone hyperpolarization, is indicated for treating Alzheimer's disease. Support, in part, from Astra Arcus and Brain Research Trust.

669.10

Identification of the Cleavage Site Involved in Increased Production of Aß from a mutant ßAPP (ΔNL) Linked to Familial AD. T. T. Cheung*, J. Ghiso, X-D Cai, M. Shoji, B. Frangione, and S. G. Younkin. Case Western Reserve University, Cleveland, OH 44106; New York University. NY, NY 10012

We have previously shown that normal processing of the amyloid ß protein precursor (ßAPP) in cultured cells produces a complex set of four COOH-terminal ßAPP derivatives with molecular weights between 8.7 and 11.4 kD. We have also shown that the "Swedish" mutant ßAPP (ΔNL) linked to familial AD produces increased amounts of the 11.4 kD COOH-terminal dervative and of 4 kD amyloid ß protein (Aß). To identify the NH2 terminus of the various COOH-terminal fragments produced by ßAPP695-transfected human neuroblastoma (M17) cells, we metabolically labeled these cells with [3H]-phenylalanine and then sequenced or radiosequenced them (Aß has phenylalanines at positions 4, 19, and 20). Direct sequencing of the excised 8.7 kD derivative yielded LVFFAED at the 2 to 4 pmole level confirming that this derivative begins at Aß17. Radiosequencing of this fragment showed [3H]-Phe at cycles 3 and 4 as expected. These data confirmed that the 8.7 kD protein is a COOH-terminal ßAPP derivative beginning at Aß17, and they validated the utility of the radiosequencing methodology. Similar analysis of the 11.4 kD derivative showed a phenylalanine at position 4 indicating that this derivative begins with the sequence DAEF and thus has the entire Aß at its NH2 terminus. Analysis of the intervening ~10.9 and ~9.6 COOH-terminal derivatives, although less definitive, indicated that they begin at Aß4 and Aß10 respectively. Examination of the 11.4 kD COOH-terminal derivative and the 4 kD Aß produced from ßAPPΔNL showed that, like the corresponding derivatives from wild type ßAPP, both begin with the sequence DAEF. Thus the mutation replacing KM on the NH2 side of Aß with NL appears to accelerate a normal ßAPP cleavage thereby producing increased amounts of an Aß-bearing COOH-terminal derivative from which Aß is released.

669.11

SPECIFIC INHIBITION OF BETA AMYLOID 1-42 AGGREGATION BY BETA AMYLOID 1-40. S. W. Snyder, U. S. Ladror, G. W. Wang, G. A. Krafft,*and T. F. Holzman. Drug, Design and Delivery, Abbott Laboratories, Abbott Park, IL 60064.

One of the hallmarks of Alzheimer's disease is the presence of extracellular senile plaques composed primarily of aggregated beta amyloid (βA4). βA4 is a 40-43 residue protein proteolytically derived from the transmembrane region of amyloid precursor protein (APP). Using analytical ultracentrifugation, we have characterized the size of aggregates, as well as the distribution between monomeric and aggregated populations, formed by βA4. Using turbidity, we have investigated the kinetics of aggregation from a well-defined monomeric starting state to a fibrillar aggregated state as a function of βA4 sequence and environmental conditions. Aggregation was several orders of magnitude faster for the more hydrophobic βA4 1-42 protein than the more soluble βA4 1-40. We observed a specific, and dose dependent, kinetic inhibition of aggregation of 1-42 by 1-40. In addition, we observed a kinetic enhancement of 1-40 aggregation by pre-formed βA4 aggregates. These results reveal that the rate of βA4 aggregation is strongly dependent upon relative concentrations of its shorter and longer forms. Therefore, specificity in the carboxy-terminal cleavage of APP might play a critical role in partitioning between deposited aggregates of βA4 and cleared protein. This work was supported in part by the NIH-NIA grant AG10481-02.

βA4

DAEFRHDSGY EVHHQKLVFF AEDVGSNKGA IIGLMVGGVV IAT

669.12

NEURONAL TYPE IV COLLAGENASE DEGRADES THE AMYLOID BETA PEPTIDE (Aβ 1-40) AND SUBSTANCE P. J. R. Backstrom and Z. A. Tokes*. Department of Biochemistry and Molecular Biology, University of Southern California, Los Angeles, CA 90033.

The 100 kD type IV collagenase (matrix metalloproteinase-9, MMP-9) was immunologically detected in pyramidal neurons and plaques in the hippocampus from Alzheimer affected tissues. Therefore, experiments were designed to determine which cells produce MMP-9 mRNA, which structures bind exogenous biotinylated MMP-9, and whether the enzyme cleaves the amyloid beta protein (Aβ1-40). MMP-9 riboprobes labeled pyramidal neurons in the CA1 to CA4 fields, but not neurons in the dentate gyrus. Although biotinylated MMP-9 attached to subendothelial, perivascular structures, antibodies to MMP-9 did not detect the endogenous enzyme in this region. Thus, basement membrane constituents such as collagen may not be the primary substrate in the brain. Since the antisera labeled amyloid plaques, synthetic Aβ1-40 was incubated with purified MMP-9. The peptide was cleaved at several sites including the Phe-Phe region. This region shares homology with the tachykinins and somatostatin. Therefore, the metalloproteinase was incubated with substance P, neurokinin A, neurokinin B, kassinin, and somatostatin. The enzyme cleaved substance P but not the other peptides. The results suggest that the main function of the metalloproteinase MMP-9 may be to process peptides, and not collagen, within the central nervous system. Supported by NIA grant AG09681.

669.13

AMYLOID ß PROTEIN ($A\beta_{1-40}$) IS DEGRADED BY PROTEASES PRESENT IN AND RELEASED FROM RAT MICROGLIA. L. M. Shaffer,* K. R. Brunden, S. G. Younkin and M. L. Cohen. Gliatech, Inc., Cleveland, OH 44122 and Case Western Reserve University, Institute of Pathology, Cleveland, OH 44106.

Microglial cells are closely associated with the amyloid cores in the mature senile plaques of Alzheimer's Disease (AD) brain. Plaques contain a high concentration of fibrillar aggregates of Aß, an ~40 residue peptide. To examine processing of Aß by microglia and to determine whether the physical state of Aß alters this processing, Synthetic $A\beta_{1-40}$ (0.01-40 µg/ml) + ^{125}I-$A\beta_{1-40}$ was added to cultures of primary rat microglia and incubated for various periods of time. The peptide was either in a soluble (1 day in neutral solution at 4°C) or aggregated form (1 day in pH 5.0 NaOAc). In some cases, "labeling" medium was subsequently replaced with "chase" medium containing no peptide. Labeling medium, cell lysates and chase medium were analyzed by gamma counting and/or SDS-PAGE (16.5% tris-tricine) and autoradiography. The amount of Aß associated with the exterior surface of microglia increased linearly as peptide concentration was increased over a large range. For both soluble and aggregated Aß, (i) intracellular accumulation was maximal in one hour, and (ii) during a 24 hr chase after a 3 hr pulse, 70% of the intracellular ^{125}I-dpm were externalized in 4 hrs, and 15% over the next 20 hrs. The released ^{125}I-dpm were in a form that was virtually undetectable on gels that readily detected equivalent dpm of ^{125}I-Aß. Further investigation showed (i) substantial dose-dependent breakdown of Aß by conditioned medium alone, and (ii) most of the *intracellular* ^{125}I-dpm were undetectable on gels when compared to equivalent ^{125}I-Aß dpm. Thus Aß is degraded by proteases present in and released from rat microglia, and microglia may remove aggregated as well as soluble Aß released during normal processing of the Aß precursor protein. Several factors could account for the resistance of plaque core amyloid to microglial proteolysis, including association of the peptide with additional plaque components.

669.14

THE ALZHEIMER'S β(1-40) PEPTIDE FORMS SOLUBLE MULTIMERIC COMPLEXES IN DILUTE SOLUTION. H. LeVine III*, Department of Neuroscience Pharmacology, Parke-Davis Pharmaceutical Research Division of Warner-Lambert Co., Ann Arbor, MI 48106-1047.

Multimers of synthetic β(1-40) Alzheimer's amyloid peptide can be detected in solution at sub-microgram per ml concentrations by cross-linking with glutaraldehyde followed by borohydride reduction. SDS-Tricine SDS-PAGE suggests the formation of up to pentameric or hexameric complexes. Under amyloidogenic conditions pf pH and high peptide concentrations, sedimentable and filterable structures display the characteristic amyloid fluorescent binding of Thioflavine T. Similar cross-linked products at high and low peptide concentrations indicate that intramultimer reactions predominate over intermultimer reactions for glutaraldehyde, supporing a non-random association of β(1-40) peptides in amyloid fibril. The presence of such pre-amyloid structures at low peptide concentrations suggests that amyloid plaques could accrete additional material by cooperative rather than by monomeric growth.

669.15

CHRONIC INTRAVENTRICULAR AMYLOID INFUSION: A PARADIGM FOR INDUCTION OF AMYLOID ACCUMULATIONS IN THE RAT BRAIN. B.A. Tate*, R. Tovar, R.E. Majocha and C.A. Marotta. Dept. Psychiatry and Human Behavior, Miriam Hosp. and Brown University, Providence, RI 02906.

The production of a rodent model for the neuropathological and behavioral features of the human disorder of Alzheimer Disease would be economically and scientifically advantageous. We have used subcutaneuosly implanted osmotic pumps and indwelling intraventricular cannulae to chronically infuse synthetic amyloid peptide 1-40 into the rat brain over a period of two weeks. The vehicle we use does not contain acetonitrile. The combination of intraventricular infusion and this vehicle eliminates the nonspecific brain damage induced by other methods of amyloid administration. Immunopositive amyloid accumulations are present in the brain parachyma several weeks post infusion. The cell body of cells lining and immediately adjacent to the ventricles are immunopositive. The processes of some cells immediately adjacent to the ventricles are also immuno-positive for amyloid. Behavioral and neuropathological studies are underway.

670.1

INDUCTION OF COMPLEMENT MRNAS AND PROTEINS IN ENTORHINAL CORTEX AND HIPPOCAMPUS AFTER PERFORANT PATH TRANSECTION.
S.A. Johnson*, N.J. Laping, C.S. Young-Chan, G.M. Pasinetti and C.E. Finch. Neurogerontology Division, Andrus Center, Dept. Biol. Sci., Univ. of Southern California, Los Angeles, CA 90089-0191.

We and others have shown the presence of complement (C) system mRNAs in normal and diseased mammalian brain; the presence of C proteins in AD pathological structures; and induction of C mRNAs and proteins in AD and in response to experimental brain lesions. The entorhinal cortex (EC) perforant path lesion is a well documented acute model of EC neurodegeneration and hippocampal reactive synaptogenesis found in Alzheimer disease (AD). We are using this model to further examine the potential role of the complement system, a cytotoxic arm of the immune system, in both neurodegenerative (EC) and synaptogenic (hippocampus) mechanisms that occur simultaneously in AD.

Unilateral perforant path transection using a stereotaxically placed Scouten retractable wire knife caused detectable increases of C1qB, C4 and clusterin (SGP-2) mRNAs in ipsilateral EC within 1 day post lesion, as assayed by x-ray film exposure after in situ hybridization. Immunocytochemistry on adjacent sections shows the lesion specific presence of both C1 and C9 proteins in the ipsilateral EC; C9 detection suggests, but does not prove, C cascade activation leading to the presence of membrane attack complex, the terminal cytolytic component of the classical C pathway. These studies provide further evidence for C involvement in neurodegenerative lesions. Supported by grants from the NIA to SAJ (AG10673) and CEF (AG07909).

670.2

TWO DIMENSIONAL ELECTROPHORETIC ANALYSIS OF A68 FROM ALZHEIMER'S DISEASE BRAIN.
B. Miller*, G. Johnson, H. Ghanbari, and P. Davies. Molecular Geriatrics Inc, Lake Bluff, IL 60044

To characterize the various polypeptide components of the Alzheimer's disease (AD)-specific protein, A68, we used two dimensional (2D) gel electrophoresis and computer assisted analysis. A68 was affinity purified from an AD brain showing characteristic pathology using antibodies which show high affinity for PHF. The purified A68 was then separated by 2D gel electrophoresis and either silver stained or electroblotted to membrane. Silver staining revealed a pattern of three distinct groups of A68-related polypeptides of Mr of 60 to 68 kD and pI 5.0-6.3. Within each of the three A68 groups, 10-20 individual polypeptides were present that appeared to be related by differences in post-translational modification. Individual polypeptides were further characterized by Western blot analysis according to their reactivity with a series of monoclonal antibodies specific for identified tau eptitopes including Alz50 and Tau-1. Post-translational modifications of A68 including phosphorylation were characterized by alterations in both the 2D silver stain and Western blot pattern of A68 after enzymatic removal of the modification. Biochemical and immunochemical characterization of the individual polypeptides comprising A68 may lead to a greater understanding of the alterations and/or modificatons of this protein unique to the AD brain.

670.3

TG3: A BETTER ANTIBODY THAN ALZ-50 FOR THE VISUALIZATION OF ALZHEIMER-TYPE NEURONAL PATHOLOGY.
P. Davies, [1]H. Ghanbari*, A. Issacs, D.W. Dickson, L.A. Mattiace, M. Rosado, and I.J. Vincent. Dept. Pathology, Albert Einstein College of Medicine, Bronx, NY 10461, [1]Molecular Geriatrics, Lake Bluff, IL 60044

TG3 is a mouse IgM produced from mice immunized with Alz-50-reactive proteins purified from Alzheimer's Disease brain tissue. By immunocytochemistry, TG3 appears to be more sensitive and specific than Alz-50. Alz-50 stains small numbers of neurons and neuronal processes in the normal adult human cerebral cortex, and in the rat and human hypothalamus. TG3 does not stain these neurons in either species; to date, no neuronal staining has been found in the normal adult human or rat, although small numbers of fibers are found in sub-cortical regions of the rat brain. In AD brain tissues, TG3 intensely labels neurons, neuritic processes, neurofibrillary tangles and neurites in plaques. The antibody appears to work equally well in vibratome sections of fixed tissue (even after 20 years in formalin) in paraffin embedded tissue, and in cases with a wide range of disease severity. In cases with early AD, TG3 intensely labels neurons of the entorhinal cortex and CA1 pyramidal neurons many of the latter appearing free of tangles. These neurons are much less evident in Alz-50 stained sections.

By western blotting, TG3 reacts with a phosphorylated epitope on the same A68 or PHF-tau proteins recognized by Alz-50, but may also detect additional associated proteins. TG3 does not appear to have significant reactivity with normal adult human, rat or mouse tau, nor with any other proteins in brain homogenates.

670.4

TG1: A MARKER FOR NUCLEAR TAU WITH SPECIFICITY FOR NEURONAL NUCLEI IN ALZHEIMER'S DISEASE.
I.J.Vincent*, R.N.Katen II, A.Isaacs, L.A.Mattiace & P.Davies
Department of Pathology, Albert Einstein College of Medicine, Bronx, NY 10461.

The monoclonal antibody TG1 was derived from mice immunized with PHF preparations isolated from Alzheimer's Disease (AD) brain tissue by immunoaffinity chromatography on a column of Alz-50. On immunoblots of immunoaffinity purified PHF, TG1 reacts with only the 60 and 64 kDa components of the characteristic triplet proteins, as well as a 24 kDa protein. The antibody has some reactivity with tau from human fetal brain but none with normal adult tau. Immunocytochemical analysis of AD brain tissue reveals intense reactivity localized to nuclei in neurons of cerebral cortex, but no staining of the typical pathological features of the disease. Although the antibody does not stain normal adult brain tissue, it labels neuronal nuclei in human fetal brain and in adult rat and mouse brain tissues. In order to identify the TG1 antigen in neuronal nuclei, proteins were extracted from enriched nuclear fractions of rat brain by formic acid treatment. Immunoblot analysis of these extracts with TG1 shows a triplet in the 60-68 kDa range. These proteins are recognized by the antibodies Tau-1 and PHF-1, indicating that they are tau-like. However, TG1 does not display any reactivity with the heat stable cytoplasmic tau fraction in which Tau-1 and PHF-1 immunoreacitvities are enriched. The appearence of nuclear TG1 reactivity in AD and in fetal brain reinforces the idea of reexpression of developmental programs in AD. The widespread staining of neuronal nuclei suggest that tau-pathology in AD is more diffuse than is indicated by the more localized neurofibrillary aberrations.

670.5

INHIBITION OF PHOSPHATASES IN NEUROBLASTOMA CELLS INDUCE THE FORMATION OF ALZHEIMER-TYPE TAU PROTEINS.
P.E. Sautière, M.L. Caillet, A. Wattez and A. Delacourte*, INSERM U156, 59045 Lille, France.

Pathological Tau proteins are the basic components of Paired Helical Filaments (PHF) found in degenerating neurons during Alzheimer's disease (AD). They have a Molecular Weight (MW) of 55, 64 and 69 kDa and are abnormally hyperphosphorylated. They differ from normal Tau by their apparent higher MW, their more acidic isoelectric point and their conformation generating Alzheimer epitopes.

In order to set up an experimental model of the Alzheimer-type degenerating process, we induced the hyperphosphorylation of Tau by incubating human differentiated neuroblastoma cells (SKNSH-SY 5Y) with okadaic acid (OA), a potent inhibitor of protein phosphatases 1 and 2A. After OA treatment (0,25 µM), neurites quickly disappeared, cells rounded up and detached. In control cells, a doublet of normal Tau isoforms, with a MW of 48 and 53 kDa, was detected by Tau 1 and not detected by our specific anti-PHF serum. After OA treatment, in parallel with morphological changes, we observed the formation of Tau 55 and Tau 64 isoforms incorporating 32P, with a more acidic isoelectric point, a loss of Tau 1-immunoreactivity and an apparition of Alzheimer epitopes detected in a dose-dependant fashion with anti-PHF.

1 nM Calyculin A, another inhibitor of phosphatases 1 and 2A, also produced the formation of pathological Tau 55, 64. The formation of abnormal Tau proteins is likely related to an hyperphosphorylation of preexisting proteins because cycloheximide did not perturb their detection.

Such a model of degenerating process with a production of Alzheimer-type Tau proteins will be useful to elucidate the biochemical dysfunctions occuring during AD and to screen for neuroprotective drugs able to slow down or to stop the dementing process.

Supported by Laboratoires Glaxo and the Conseil Régional Nord Pas-de-Calais.

670.6

COLOCALIZATION OF CHOLINESTERASES WITH ABNORMALLY PHOSPHORYLATED TAU PROTEIN IN AGED AND ALZHEIMER'S BRAINS.
M.A. Morán* and P. Gómez-Ramos. Dpto. Morfología, Fac. Medicina, Universidad Autónoma, Madrid. Spain.

Both acetyl- and butyryl-cholinesterases colocalize with the ß-amyloid protein (Morán et al '93), and are ultrastructurally located over paired helical- and straight-filaments, in aged and Alzheimer brains (Gómez-Ramos et al '92). In free-floating sections from 10 Alzheimer's and 4 non-demented brains, two double protocols were performed: A) Tau immunocytochemistry followed by cholinesterase histochemistry and B) cholinesterase histochemistry followed by tau immunocytochemistry. A modified Karnovsky-Roots method for cholinesterases and the mAb 5E2 against abnormally phosphorylated tau were used. Either alkaline phosphatase with fast red or extravidin-peroxidase with naphthol allowed the distinction of the tau precipitate from the cholinesterases reaction product. In any case, after the second protocol, sections were re-mounted, using previously drawn section outlines, and re-photographed. Cholinesterases were present in a certain proportion of all the structures with neurofibrillary degeneration: neurons with either extra- or intra-cellular neurofibrillary tangles, neuropil threads and plaque-associated neurites. Colocalization was clearly demonstrated in some of these structures, whereas others were either only tau positive or only cholinesterase positive. Triple staining procedures for cholinesterases, tau and thioflavin-S, showed that some of these degenerated structures did not have neither cholinesterases nor tau precipitates. These results are discussed in relation to the evolution of the neurofibrillary degeneration. Supported by FISss nº 93/0189. Spain.

670.7

DYNAMICS OF TAU PHOSPHORYLATION AND BINDING AFFINITY FOR MICROTUBULES IN A HUMAN NEURONAL (NT_2N) CULTURED SYSTEM Sandra E Merrick*, Virginia M-Y Lee. Inst Neurol Sci and Dept of Path and Lab Med, Univ of Pennsylvania, Philadelphia, PA 19104.

Tau isolated from paired helical filaments (PHFs), the major building blocks of neurofibrillary tangles of Alzheimer's disease, has been shown to be abnormally phosphorylated and unable to bind microtubules (MTs), but enzymatic dephosphorylation of PHF tau restores the ability to bind MTs. Previous studies have shown that increased phosphorylation of tau decreases the affinity of tau for MTs. To examine the dynamics of tau phosphorylation and to identify specific phosphorylation sites on tau that are involved in the stabilization of MTs we have used various agents that may alter tau phosphorylation in postmitotic neurons (NT_2N) derived from a human teratocarcinoma cell line. When NT_2N cells, were treated with drugs (i.e. colchicine or nocodazole) that depolymerize MTs, both tau and tubulin subunits were recovered in the supernatant fractions. However, the tau that was recovered was found to be dephosphorylated as monitored by an increase in electrophoretic mobility, a decrease in ^{32}P PO_4 incorporation and a lack of immunoreactivity to specific phosphorylation dependent tau antibodies. In contrast, treatment of NT_2N cells with a calcium ionophore resulted in degradation of tau protein with no change in MT binding or sites of tau phosphorylation. Finally, treatment of NT_2N cells with okadaic acid resulted in an increase in tau phosphorylation at specific sites and eliminated the binding of tau to MTs. We conclude that tau phosphorylation/dephosphorylation in cultured neurons is dynamic and can be modulated by exogenous factors.

670.8

TOTAL UBIQUITIN IS NOT ELEVATED IN ALZHEIMER BRAIN AND FIBROBLASTS. RS Black*, ES Kelman, SG Greenberg, HT Smith, VA Fried Cornell Univ. Med. Coll. at Burke Medical Res. Inst., White Plains, NY and New York Med. Coll., Valhalla, NY

Levels of ubiquitin have been reported to be elevated in postmortem brain samples from Alzheimer's disease (AD) (Wang et al. 1991). We developed a solid phase dot blot immunoassay to measure ubiquitin in brain and cultured cell samples using both polyclonal and monoclonal antibodies, and ^{125}I-conjugated Protein A. Bovine ubiquitin was used as the standard. Ubiquitin was measured in samples of Alzheimer and control brain matched for age, sex and postmortem delay, with the longest postmortem delay in this series being 3 hours. Values for Alzheimer and control frontal cortex were 2.81 ± 0.24 and 2.76 ± 0.23 mg ubiquitin/gm protein, respectively. In contrast, an abnormally phosphorylated form of tau (assayed in parallel with the antibody PHF-1) was elevated six to 30 fold in AD frontal cortex. Cultures of fibroblasts from cases of familial and sporadic AD were examined and compared to control cultures matched for age and sex of donor, and age in culture (cPDL); no differences in ubiquitin content were found. Reported differences in ubiquitin content between AD and control brain may reflect preferential recognition of ubiquitin conformations present in AD brain, or increased postmortem stability of tangle-associated ubiquitin.

670.9

UBIQUITIN IS CONJUGATED WITH AMINO-TERMINALLY PROCESSED tau IN PAIRED HELICAL FILAMENTS. M. Morishima-Kawashima[1], M. Hasegawa[1], K. Takio[2], M. Suzuki[3], K. Titani[3], K. Murayama[1]*, and Y. Ihara[1] [1]Institute for Brain Research, University of Tokyo, Tokyo 113, [2]Institute of Physical and Chemical Research (RIKEN), Saitama 351-01, [3]Institute for Comprehensive Medical Science, Fujita Health University, Aichi 470-11, Japan.

In a subset of neurons in Alzheimer's disease (AD), paired helical filaments (PHF) appear to progressively accumulate despite their ubiquitination. To obtain an insight into the ubiquitin pathway in the brain affected with AD, we have investigated ubiquitinated PHF, which show a smear in SDS-polyacrylamide gel electrophoresis and immunoblotting. Smearing materials with both tau- and ubiquitin-immunoreactivities were purified and their digests were subjected to protein sequence and mass spectrometric analyses. The smear consisted largely of the carboxyl-terminal portion of tau and ubiquitin. Several peaks specific for the smear peptide map were found to contain a ubiquitin-ubiquitin and ubiquitin-tau conjugates. Thus the ubiquitin-targeted protein was tau in PHF and the conjugation sites were localized to the microtubule-binding region. Most ubiquitin in PHF occurred as a monoubiquitinated form and only a small proportion of ubiquitin formed multiubiquitin chains. There was a ubiquitin-negative smear in which tau is much less processed in the amino-terminal portion. This strongly suggests that the amino-terminal processing of tau in PHF precedes its ubiquitination.

670.10

THE EXTENT OF PHOSPHORYLATION OF FETAL TAU IS COMPARABLE TO THAT OF PHF-TAU FROM ALZHEIMER PAIRED HELICAL FILAMENTS A. Kenessey, D. W. Dickson*, and S. H. Yen. Department of Pathology, Albert Einstein College of Medicine, Bronx, New York, 10461

The relationship between Alzheimer's disease (AD) and expression of fetal proteins was examined by (i) determining the phosphate content of tau prepared from fetal brains (F-tau), (ii) comparing F-tau, tau from normal adult human brains (N-tau) and tau from paired helical filaments in AD brains (PHF-tau) for phosphate content, and (iii) testing the reactivity of F-tau with five antibodies known to recognize PHF-tau. The antibodies have been reported to recognize phosphate dependent epitopes at the carboxy terminal half of the tau molecule. Our data shows that on the average, F-tau contains 7 mol phosphate/mol protein, which is comparable to the phosphate content of PHF-tau, but is 3-4 times higher than that of N-tau. Immunoblotting shows that all of the tested antibodies reacted with F-tau on immunoblots, indicating that F-tau and PHF-tau are phosphorylated at similar sites. A difference between PHF-tau and F-tau is the state of phosphorylation in the Tau-1 epitope, an epitope reactive with a monoclonal anti-tau antibody, Tau-1. This epitope, which is phosphorylated in all PHF-tau, is phosphorylated only in some of the F-tau. Our results suggest that tau phosphorylation does not necessary lead to AD type cytoskeletal abnormalities, since fetal brains are free of such pathology.

670.11

TAU IMMUNOREACTIVITY IN THE HIPPOCAMPAL FORMATION OF YOUNG DOWN SYNDROME AND NON-DEMENTED ELDERLY PATIENTS. J. Leverenz*, C.B. Saper and M. Raskind. Depts. of Medicine (Neurology) and Psychiatry & Behavioral Sciences, Univ. of Washington, Seattle, WA 98195, Dept. Neurology, Beth Israel Hosp./Harvard Med Sch., Boston, MA 02115

Post-translational modification of tau protein may be an early event in the development of neurofibrillary tangles in Alzheimer's disease. Alz-50, a monoclonal antibody to the amino terminus of tau, has been noted to stain some neurons in a granular fashion in regions susceptible to neurofibrillary tangles, before tangles are present. This granular staining may represent pre-tangle alterations of tau protein. We examined the expression of the Alz-50 antigen, and two tau phosphorylation epitopes (Tau-2 and PHF-1) in the hippocampal formation of two "models" of early Alzheimer's disease: young Down syndrome patients with little or no evidence of neurofibrillary tangle formation, and elderly non-demented patients with amyloid deposition and few neurofibrillary tangles. The findings in the two groups were similar. Prior to substantial development of neurofibrillary pathology in hippocampal neurons, we saw granular staining of neurons with all three antibodies. Using double labeling and consecutive sections, Alz-50 and Tau-2 immunostaining were more extensive than PHF-1. These results support the contention that phosphorylation of tau is an early event in the development of neurofibrillary tangles. It also appears that phosphorylation of the Tau-2 epitope and exposure of the Alz-50 epitope occur earlier than the development of PHF-1 antigenicity. Further study of the development of antigenic changes in these groups may yield important clues to the instigating factors in neurofibrillary tangle formation.

670.12

CORTICAL MAPPING OF ALZHEIMER PATHOLOGY IN BRAINS OF AGED NON-DEMENTED SUBJECTS. P. Vermersch[1]*, J. Ph. David[2], B. Frigard[1], C. Fallet-Bianco[2], A. Delacourte[1]. 1) INSERM U156, 59045 Lille; 2) Department of Neuropathology, Hôpital Sainte-Anne, Paris, France.

The presence of neurofibrillary pathology of the Alzheimer type and amyloid deposits within the brains of aged non-demented subjects were investigated by immunoblotting and immunohistochemistry using antibodies directed against abnormal tau proteins 55, 64 and 69 (Tau-PHF) and βA4 respectively.

As part of a prospective study on aging, only brains from non-demented patients were included. All of them, aged from 69 to 97, had a MMS score higher than 24/30. The abnormal tau triplet, a biochemical marker of neurofibrillary degeneration (NFD) was quantified by western blot and densitometric analysis of several cortical areas including entorhinal cortex (EC), hippocampus, Brodmann areas (BA) 20, 22, 38, 9, 45 and 39, as previously described (Vermersch et al., Acta Neuropathol 1992; 85: 48-54).

The abnormal tau triplet was detected in the EC of all controls over 65 and in the hippocampus of controls aged over 80 years. In some of the oldest cases the degenerating process was also detected in the isocortex in BA38 alone or also in BA20 and BA22. These observations suggest that there is a continuum between aging and AD. Some cases and especially those with tau pathology in BA38 contained numerous senile plaques (SP) in the isocortex and were likely to be subclinical stages of AD. However, approximatively fifty per cent of controls with tau patholgy in the EC and hippocampus were almost devoid of SP in the isocortex (detection with different antibodies anti-A4 and thioflavine). We conclude that this last group is more likely to be related to normal aging than to AD. A higher vulnerability of the hippocampal region to numerous injuries during life could explain this group.

Supported by CH&U de Lille and INSERM clinical network CAR492011.

670.13

INTRAHIPPOCAMPAL COLCHICINE INJECTION ALTERS TAU IMMUNOSTAINING BUT DOES NOT MODEL ALZHEIMER'S DISEASE PATHOLOGY. J.W. Geddes*, V. Bondada and J.N. Keller. Sanders-Brown Center on Aging and Dept. Anatomy & Neurobiology, Univ. Kentucky, Lexington, KY 40536.

The microtubule disrupting agent, colchicine, has been used to model several aspects of Alzheimer's disease (AD)-related neuropathology. This study examined the effects of colchicine on microtubule-associated proteins (tau, MAP2, and MAP5) to determine if changes in their levels or distribution might be similar to those which have been suggested to precede the formation of neurofibrillary tangles in AD. Six hours following intrahippocampal colchicine injection (3.5 µg injected into two rostro-caudal locations) tau immunostaining was increased in CA1 s. radiatum and decreased in the outer molecular layers of the dentate gyrus. In addition, a downward shift in the electrophoretic mobility of some tau isoforms was observed in Western blots, indicative of dephosphorylation. Three days postinjection, there was a loss of MAP2 and MAP5 which coincided with a loss of cresyl violet staining in granule cells, CA3, subicular and entorhinal neurons. Accumulation of the MAPs in neuronal perikarya was not observed at any postinjection time points. The results demonstrate that although intrahippocampal colchicine does alter tau immunostaining, it does not model the cytoskeletal alterations that are suggested to precede or accompany the formation of neurofibrillary pathology in AD.

670.14

CALCIUM/CALMODULIN-DEPENDENT PROTEIN KINASE II IN ALZHEIMER'S DISEASE. N.A.Simonian*[1], T.Elvhage[1], A.J. Czernik[2], P.Greengard[2] and B.T.Hyman[1]. [1]Dept. of Neurology, Mass.General Hospital, Boston, MA 02114 & [2]Lab. of Molec. & Cell. Neurosci., The Rockefeller University, NY, NY 10021 USA.

Calcium/calmodulin-dependent protein kinase II(CaM K) has beeen implicated in the regulation of synaptic transmission and in the induction of long term potentiation(LTP). The hippocampal formation, a structure important in memory formation, is highly enriched in this enzyme. Transgenic mice lacking the alpha subunit of CaM K show abnormalities in some memory tasks and slices from the CA1 region of these animals exhibit deficiencies in LTP. In Alzheimer's disease(AD), memory loss is universal but the molecular basis is unknown. We examined whether changes in CaM kinase occur in AD by performing immunohistochemistry using an antibody to the alpha subunit of CaM K. The diffuse density of the immunohistochemical product was quantitated in hippocampal sections from 11 AD and 9 aged control individuals. CaM K immunoreactivity was decreased by 50% in CA1, but not in dentate gyrus or presubiculum in individuals with AD($p<.002$). In contrast to the dentate gyrus and presubiculum, CA1 is severely affected by neurofibrillary tangles and cell loss in AD. The decrease in CaM K immunoreactivity in CA1 may be due loss of neurons and/or to an alteration in function of remaining neurons A selective decrease of CaM K immunoreactivity in remaining neurons suggests that alterations in second messenger systems important in synaptic transmission may accompany these changes. Supported by a Neuroscience Training Grant NS07009-18 (NAS); NIA AG09464 (AJC,PG) and AG08487 (BTH)

670.15

DISTRIBUTION OF PROLINE-DIRECTED PROTEIN KINASES AND REGULATORY ENZYMES IN PRIMARY HIPPOCAMPAL CULTURES. C.A. Reich and J.G. Wood*. Emory Univ. Sch. of Med., Atlanta, GA 30322

Recent reports indicate that the proline-directed protein kinases, MAP kinase and cdc2 kinase, have the ability to phosphorylate tau *in vitro* in a manner that mimics many of the changes associated with Alzheimer's disease pathology, yet little is known about the distribution of these kinases and their regulatory enzymes in neurons. Although these kinases have been isolated from brain, typically considered a non-proliferating tissue; studies characterizing the *in vivo* activities of these enzymes reveal that they play a key role in the control of cell division. Therefore, these enzymes might actually be located in glia, rather than neurons, and have little opportunity to interact with tau. Using antibodies to the proline-directed protein kinases, cdc2 and MAP kinase, as well as to the potential regulatory components, MAP kinase kinase, raf-1, phosphoinositide-3 kinase, protein phosphatase 2A, cdc25, wee-1 kinase and fyn kinase, we have immunohistochemically characterized the cellular distribution of these enzymes in primary rat hippocampal cultures. Our results indicate that many components of this kinase/phosphatase system are predominantly present in neurons. The relative absence of staining for these enzymes in the abundant, actively dividing glia in these cultures is a significant observation as well. The neurons in this culture system, like their *in vivo* counterparts, are post-mitotic and terminally differentiated. Therefore, enzymes which function strictly to regulate mitosis or merely initiate differentiation are less likely to be present. The fact that these proline-directed protein kinases and their regulatory enzymes are found in neurons suggests additional physiological roles for these kinases in addition to cell cycle control. The presence of these kinases in a primary neuronal culture system provides the opportunity to study factors which may influence regulation of the protein levels and the activation state of these enzymes. AG 11123 and NS 27847.

670.16

MAP KINASE $p44^{mpk}$ INDUCES ALZHEIMER TYPE ALTERATIONS IN TAU FUNCTION AND IN PRIMARY HIPPOCAMPAL NEURONS. Q.LU*, J.P.SORIA and J.G.WOOD. Dept.of Anatomy and Cell Biology, Emory Univ. Sch. of Med., Atlanta, GA 30322

Abnormally phosphorylated tau protein is a major component of the cytoskeletal pathology of Alzheimer's disease (AD) found in the neurofibrillary tangle and neuritic plaque. Identification of the kinase responsible for this phosphorylation has been difficult. In the test tube, several proline-directed kinases, particularly mitogen activated protein (MAP) and cdc2 kinase phosphorylate tau on sites that appear to mimic the abnormally phosphorylated sites in AD. Important unanswered issues include: 1) whether this phosphorylation event occurs in the tightly regulated environment of a living cell, 2) whether this phosphorylation of tau affects its functional properties, and 3) what is the subcellular relationship of proline-directed kinases and tau. We show here that tau can be phosphorylated in cultured hippocampal neurons by the MAP kinase $p44^{mpk}$ and the effects appeared to be mimicked by microinjection of the phosphatase inhibitor okadaic acid. In addition, phosphorylation of aged non-demented human brain tau compromises its functional ability to assemble microtubules. We show further that a number of protein kinases including p34 cdc2 and MAP kinase copurify with microtubule fractions. Both p44 and p42 MAP kinases are tyrosine phosphorylated and presumably active. These studies address and raise several important issues regarding the regulation of tau phosphorylation in normal and AD brain. AG 11123 and NS 27847.

670.17

DEGRADATION OF *TAU* BY A MAJOR EXTRALYSOSOMAL PROTEINASE, THE MULTICATALYTIC PROTEINASE COMPLEX (PROTEASOME). M.E. Figueiredo-Pereira, S.Wilk* and G.V.W. Johnson[¢]. Dept. of Pharmacology, Mount Sinai Medical School of C.U.N.Y.,N.Y.,N.Y. and [¢]Dept. of Psychiatry and Behavioral Neurobiology, University of Alabama at Birmingham,Birmingham,AL.

Tau is a family of microtubule-associated proteins which plays a significant role in the structure and function of the neuronal cytoskeleton. It is a major component of PHFs of Alzheimer's disease. Although the mechanism of formation remains unknown, it can be suggested that impaired proteolysis of *tau* may be associated with its accumulation. *Tau* in PHFs is abnormally phosphorylated. It was shown that *tau* is a substrate for the proteinase calpain, and that phosphorylation decreases its susceptibility to calpain degradation. In this report we demonstrate that *tau* is rapidly degraded by another major proteinase, the multicatalytic proteinase complex (MPC). MPC is a unique high molecular mass proteinase (700 kDa) with multiple distinct proteolytic activities. It is found both in cytosol and nuclei, and is present in brain in relative high concentrations (0.12% of soluble brain protein). *Tau* was purified from fresh bovine brains and MPC from frozen bovine pituitaries. Assay mixtures (100 µl) containing 7 µg of *tau*, 2 µg of MPC and 0.05M Tris-HCl buffer, pH 8.0 were incubated at 37°C, aliquots were removed at various times, subjected to SDS-PAGE and immunoblotted with Tau2. Virtually all of intact *tau* was proteolyzed after 30-min of incubation. The rate of degradation of *tau* by MPC was approximately 30 mg of *tau* degraded/mg of MPC/hour. This is the highest described rate of degradation of an endogenous protein substrate by MPC, under physiological conditions. Malfunction or changes in the catalytic activities of MPC, and/or alterations in the phosphorylation state of *tau* could result in abnormal degradation of *tau*, and contribute to neuronal dysfunction and pathological lesions in neurodegenerative diseases. (Supported by NIH grants NS-29936 and MH-00350 (S.W.)and NS-27538 and AG-06569 (GVWJ).

670.18

CLONING AND CHARACTERIZATION OF AN ALZ50 IMMUNOREACTIVE GENE PRODUCT FROM FETAL BRAIN THAT IS NOT TAU. R. Bowser*, A. Giambrone and P. Davies. Dept. Pathology, Albert Einstein College of Medicine, Bronx, NY 10461.

By immunocytochemistry the monoclonal antibody Alz50 identifies a tau epitope that is present in the brain of individuals with Alzheimer's disease (AD) but not apparent in age-matched controls. Alz50 also recognizes a developmentally regulated antigen that appears to be present in subplate and marginal zone neurons of fetal and neonatal brain but absent by age 6. It is possible that an Alz50 immunoreactive protein(s) may function in programmed cell death and is re-expressed in AD. To identify such a protein we immunoscreened a fetal cDNA expression library with Alz50. A 1.3 kb cDNA clone was isolated and sequenced. Comparison with the GenBank database revealed no homologies to known proteins. The 1.3 kb clone, designated FAC1 (Fetal Alz50 reactive Clone 1), encodes a 41 kD open reading frame with 3 in-frame stop codons near the 3' end of the clone. Additional 5' upstream coding sequences are currently being identified by PCR cloning techniques. The FAC1 gene is expressed in multiple human tissues and is conserved across species. 9 monoclonal antibodies, FA1-9, were produced to purified recombinant FAC1 protein. Immunoblots of fetal brain tissue have determined that the full-length FAC1 protein is ~120 kD. Immunocytochemical analysis indicates that the FAC1 protein is located in the nucleus of neurons throughout the fetal brain. Decreased levels of FAC1 protein are detected in normal adult brain sections but increases in FAC1 protein are often observed in AD. Therefore the FAC1 gene encodes a novel ~120 kD Alz50 immunoreactive protein that is developmentally regulated and may be re-expressed in AD.

670.19

DISTRIBUTION OF ALZ-50- AND AMYLOID P COMPONENT-IMMUNOREACTIVE NEUROFIBRILLARY TANGLES IN ALZHEIMER'S DISEASE. T. Duong* and P.J. Acton. Biomedical Research Institute, Indiana Univ. Sch. of Med., Terre Haute, IN 47809.

Alz-50 monoclonal antibody labels the A68 protein which is abundantly expressed in neurofibrillary tangles (NFT) in Alzheimer's disease (AD). Amyloid P component (AP) is derived from the normal circulating serum AP and it has been localized by immunohistochemistry to AD brain lesions, including NFT. In this study, NFT immunoreactive to ALZ-50 and/or to AP were compared in the AD hippocampal gyrus. Paraformaldehyde-fixed AD brain samples were cut on a cryostat and free floating sections were processed by double-antigen immunohistochemistry. ALZ-50- and AP-labeled NFT formed 2 separate populations which overlapped partially. In layer II of the entorhinal cortex, NFT derived from the stellate neurons showed either strong immunoreactivity to ALZ-50 or to AP. Occasional patches of neurons displayed immunoreactivity to both antibodies. In CA1 region of the hippocampus, ALZ-50-, AP- and double-labeled NFT accounted for 60%, 24% and 16% of immunoreactive NFT respectively. In CA4 region of the hippocampus, the majority of NFT were AP-immunoreactive. ALZ-50- and double-labeled NFT were occasionaly observed. In double-labeled NFT, AP-immunoreactivity was seen in the perikaryon and ALZ-50 labeling in the proximal portion of dendrites. **The results showed that the majority of NFT is immunoreactive to either ALZ-50 or AP and that a small population of NFT is immunoreactive to both.** This small group of NFT may represent a transition phase from ALZ-50 to AP labeling in NFT, suggesting changing patterns of immunoreactivity in NFT. This work was supported in part by a Turken Scholarship from the ADRDA..

670.20

MODULATION OF THE IN SITU PHOSPHORYLATION OF TAU IN RAT BRAIN CEREBRAL CORTICAL SLICES. L.M. Fleming* and G.V.W. Johnson. Dept. of Psychiatry, Univ. of Alabama at Birmingham, Birmingham, AL 35294.

Tau is a neuronal microtubule-associated phosphoprotein which plays a role in the normal development and maintenance of the axon. In Alzheimer's disease brain, inappropriately phosphorylated tau is an integral component of paired helical filaments (PHFs). It has been postulated that this aberrant phosphorylation precedes PHF formation. Although tau has been shown to be a substrate for many kinases in vitro, little is known about its in situ phosphorylation. In this study we examined the phosphorylation of tau in rat brain cerebral cortical slices and how the phosphorylation state is modulated by agents which increase intracellular cAMP or calcium concentrations.

Rat brain slices were labeled with $^{32}P_i$ prior to incubation in the presence or absence of drugs. The incubations were terminated and tau was immunoprecipitated from 250 μg of each sample. Tau was ^{32}P-phosphorylated under basal conditions in situ. Treatment of the slices with forskolin plus rolipram significantly enhanced the incorporation of ^{32}P into tau, suggesting that tau is an endogenous substrate for cAMP-dependent protein kinase. Incubation of the slices with either KCl or the excitatory amino acid, NMDA did not significantly alter tau phosphorylation. In contrast, incubation of the slices in the presence of both NMDA and KCl greatly increased incorporation of ^{32}P into tau in a calcium-dependent manner. This data suggests that depolarization of the slices is required for the NMDA-mediated calcium influx into the cell and activation of calcium-dependent protein kinases. (Supported by NIH grants NS27538, AG06569, and a grant from the Alzheimer's Association.)

670.21

TRANSGLUTAMINASE CATALYZES THE FORMATION OF HOMO-POLYMERS OF TAU AND β-AMYLOID: IMPLICATION FOR A ROLE IN ALZHEIMER'S DISEASE. G. V. W. Johnson* and S. M. Dudek. Dept. of Psychiatry, University of Alabama at Birmingham, Birmingham, AL 35294-0017

Transglutaminases are a family of calcium-activated enzymes which catalyze the formation of covalent crossbridges between certain proteins. Previous studies in brain have led to suggestions that transglutaminase may play a role in neuronal differentiation, programmed cell death, aging, and Alzheimer's disease (AD). Here we describe experiments aimed at determining if transglutaminase could contribute to the formation of two hallmarks of AD pathology: neurofibrillary tangles (NFTs) and β-amyloid (βA) aggregation.

Since paired helical filaments (PHFs), a constituent of NFTs, consist primarily of the microtubule-associated protein, tau, we tested the hypothesis that tau is a substrate of transglutaminase. Purified bovine or human tau was incubated with transglutaminase and examined on immunoblots. Crosslinking of tau was evident with the loss of monomeric tau and the formation of higher molecular weight complexes. Aggregates too large to enter the gel were also formed, since tau immunoreactivity was detected (on slot-blots) in pellets after centrifugation. These aggregates remained insoluble in SDS and β-mercaptoethanol, in guanidine-HCl and in urea, indicating that covalent crosslinking of tau was likely. Interestingly, an increase in the Alz-50 immunoreactivity of tau was also observed in the aggregates after treatment with transglutaminase. Electron microscopy revealed the polymers to be filamentous in structure.

As insoluble fibrils of β-amyloid are also abundant in AD brain, the susceptibility of the βA peptide to crosslinking by transglutaminase was studied. Like tau, βA was readily crosslinked. Distinct dimers, trimers, tetramers (up to very high multiples of the peptide) were clearly visible on immunoblots. No covalent aggregation was observed when βA was incubated under similar conditions without transglutaminase or in the presence of EGTA. Though it remains to be shown that these reactions occur *in vivo* in AD, these studies suggest a possible mechanistic link between the processes mediating NFT formation and βA aggregation. (NIH #s NS27538 and AG06569)

ISCHEMIA: ACIDOSIS

671.1

MILD ACIDOSIS DELAYS HYPOXIC SPREADING DEPRESSION AND IMPROVES NEURONAL RECOVERY IN RAT HIPPOCAMPAL SLICES. G.C. Tombaugh*, P.G. Aitken, and G.G. Somjen. Dept. of Cell Biology, Duke Univ. Med. Ctr., Durham, NC 27710.

Severe tissue acidosis has been viewed traditionally as a damaging component of cerebral hypoxia. However, a protective action of moderate acidosis (pH_O=6.5-7.0) against hypoxic neuronal injury has been described in primary cell cultures. To determine whether a similar effect occurs in mature brain tissue, we subjected adult rat hippocampal slices to a fixed period of hypoxia after ambient pH was changed slightly with HCl or NaOH. Slices were maintained in an interface chamber at 35°C. Ion-selective microelectrodes were positioned in CA1 to record evoked field potentials, extracellular voltage (V_O), pH_O, and $[Ca^{2+}]_o$. Orthodromic population spike amplitude was used as a measure of slice recovery 2hr after reoxygenation. When compared to control conditions (bath pH=7.4; pH_O=7.22), mild acidosis (pH_O=6.9-7.1) caused a reversible decrease in the population spike, an increase in the latency of hypoxic spreading depression (HSD=anoxic depolarization), and a decrease in the magnitude of the negative V_O shift; mild alkalinity (pH_O=7.53) had the opposite effect for each measurement. All slices became markedly acidotic during hypoxia; following restoration of O_2 and bath pH to 7.4, slice pH_O returned to its pre-treatment level regardless of experimental treatment or the degree of slice recovery. A reduction of pHo by as little as 0.1pH unit resulted in a dramatic improvement in post-hypoxic recovery. Because the duration of HSD has been inversely correlated to the degree of slice recovery and could have explained this protective effect, additional experiments were performed in which the duration of HSD was held constant (5'). Under these conditions, acid-treated slices exhibited a more modest though significant improvement in recovery. In neither paradigm did the recovery of alkaline-treated slices differ from controls. Both acid-treated and control slices exhibited an identical decrease in $[Ca^{2+}]_o$ during HSD that recovered with a similar time-course after reoxygenation. These results suggest that mild acidosis may limit hypoxic neuronal injury in the CNS by delaying SD onset in addition to other direct, though currently undefined mechanisms.

671.2

HYPOXIA AND ACIDOSIS ACT SYNERGISTICALLY TO CAUSE ASTROCYTE DEATH AND DYSFUNCTION K. Farrell* and R.A. Swanson. Dept. of Neurology, Univ. of California and V.A.M.C., San Francisco, CA 94121.

Cerebral ischemia may spare glia and cause selective neuronal death, or may cause infarction with pan-necrosis of all cellular elements. We used primary rat cortical astrocytes to study factors that may determine these outcomes. Acidosis was induced with PIPES- and bicarbonate- buffered media, and chemical hypoxia with the mitochondrial poisons sodium azide (25mM), 2,4 dinitrophenol (0.1mM), or antimycin A (10 g/ml). Glutamate uptake was followed with [^{14}C] GLU, and cell death was assessed by measuring LDH activity in lysates of surviving cells.

With hypoxia alone or acidosis alone (pH 6.2) glutamate uptake was maintained at 50%-70% of control rates. Combined hypoxia plus acidosis reduced uptake to <10% of control rates. Similarly, 6 hrs exposure to either pH 6.2 or hypoxia alone caused little or no cell death, whereas combined acidosis plus hypoxia caused >70% cell death. These results support acidosis as a critical variable determining type and extent of brain injury during ischemia.

671.3

EFFECT OF ANOXIA ON INTRACELLULAR pH IN ISOLATED NEWBORN AND MATURE RAT HIPPOCAMPAL CA1 NEURONS. T.R. Cummins*, D.A. Levy and G.G. Haddad. Section of Respiratory Medicine, Dept. of Pediatrics, Yale University School of Medicine, New Haven, CT 06510.

Brief periods of anoxia can lead to loss of excitability and neuronal damage. However, newborn animals are more resistant to these effects than mature animals. The mechanism(s) for this difference in sensitivities is not known, but could be related to many factors. One such factor is intracellular pH (pH_i), which can modulate a number of cellular properties, including calcium levels and excitability. Anoxia may have a different effect on pH_i in newborn animals than mature animals, and this difference may contribute to the newborn's resistance to anoxic damage. To study the effect of anoxia on pH_i, we freshly isolated pyramidal neurons from mature (20-30 days old) and newborn (4-7 days old) rat hippocampal slices using enzymatic and mechanical dissociation techniques. pH_i was measured using the pH-sensitive dye SNARF-1 and a LASER confocal microscope.

In HEPES buffered saline (34°c) bubbled with oxygen, mature and newborn isolated neurons had a resting pH_i of 7.06±0.23 (mean±SD; n=20) and 7.20±0.13 (n=22), respectively. Single neurons in slices were also studied, in which mature neurons had a resting pH_i of 7.09±0.16 (n=37) and newborn neurons had a resting pH_i of 7.29±0.17 (n=40). Anoxia was induced by perfusing with nitrogen bubbled HEPES saline containing 1mM sodium dithionate (an oxygen scavenger) for 10 minutes. pH_i in both mature and newborn isolated neurons decreased during anoxia and the mean decrease was smaller in newborn than mature neurons (newborn 0.19±0.13, n=20; mature 0.25±0.16, n=22). Compared to mature, newborn neurons tended to have a longer latency to the anoxic pH_i drop, and showed a significantly faster rate of fall in pH_i (p=0.05) and a faster rate of recovery with re-oxygenation (p<0.05).

Our data indicate that anoxia induces a different pattern of pH_i drop in newborn than in mature neurons. This differential response could be related to differences in production of acid from intermediary metabolism, rates of proton and other exchangers and intracellular buffering capacity.

671.4

THAM BUFFERS CEREBRAL ACIDOSIS DURING FOCAL ISCHEMIA. J.E. Duldner, K.R. Wagner*, M. Kleinholz, G.M. deCourten-Myers, R.E. Myers. Research Service, Veterans Affairs Medical Center; Depts. of Neurology and Pathology, Univ. Cincinnati, College Medicine, Cincinnati, Ohio 45220.

Hyperglycemia leading to markedly elevated brain lactic acid concentrations during cerebral ischemia exacerbates injury. Treatment of cerebral acidosis during focal ischemia may reduce tissue infarction.

We determined if treatment with the buffer tromethamine (THAM) during middle cerebral artery (MCA) occlusion in hyperglycemic cats (serum glucose = 20 mM) would reduce hydrogen ion accumulation in the ischemic territory. THAM (0.3 M) was infused (i.v.) at 0.9 mmol/kg/hr for the first hour post-occlusion and at 0.2 mmol/kg/hr until in situ brain freezing at 4 hr. Brain tissue lactate and high energy phosphates were determined topographically in 14 MCA territory sites by standard enzymatic-fluorometric methods. Intracellular pH (pHi) was derived from the creatine kinase equilibrium.

MCA territory sites in THAM-treated cats (N=4) had significantly (p<0.05) higher pHi's (6.63 ± 0.06) versus comparable sites in control cats (6.24 ± 0.11, N=5). THAM treatment also resulted in fewer MCA territory sites with pathologic pHi's (pHi<6.50) (30.4%, 17/56 sites versus 50.0%, 29/58 sites in controls). THAM treatment effectively reduces tissue acidosis during focal cerebral ischemia and may reduce infarction following stroke.

ISCHEMIA: CALCIUM

672.1

mRNA LEVELS OF Ca^{2+} INDEPENDENT FORMS OF PROTEIN KINASE C IN POSTISCHEMIC GERBIL BRAIN BY NORTHERN BLOT ANALYSIS. K. Kumar*, S. Savithiry, Dept. of Pathology, Michigan State University, E. Lansing, MI 48824.

To investigate the role of Ca^{2+} independent forms of protein kinase C (PKC) in ischemic neuronal injury, their mRNA expression was studied by Northern blot analysis in the gerbil model with global forebrain ischemia produced by 10 min of bilateral carotid artery occlusion. RNA was prepared from forebrains of nonischemic controls and postischemic (PI) animals following 15 min, 6 h and 24 h of recirculation (n = 3 to 4 in each group) and hybridized with synthetic oligonucleotide probes for PKC, δ, ϵ, and ζ, based on cDNA sequences in rat and labelled with ^{32}P. Increase in mRNA levels of all three forms of PKC was noted in the 6 h PI group which continued through 24 h PI. Of these, the increases in PKC δ, and ζ mRNA were statistically significant ($p < .05$). These results suggest that the mRNA expressions of PKC δ, and ζ, in particular, are temporally stimulated by ischemic injury in the brain and imply an important role of the enzyme in postischemic neuronal damage. However, since the protein itself was not examined, it cannot be said whether the increased expression translates into increased synthesis of the protein.

672.2

BIPHASIC CHANGES IN MEMBRANE PROTEIN KINASE C (PKC) ACTIVITY CORRELATE WITH CA1 NEURONAL CELL DAMAGE FOLLOWING CEREBRAL ISCHEMIA. B. Chakravarthy, H. Li*, A. Buchan and J. Durkin. Institute for Biological Sciences, National Research Council Canada, Ottawa, Ontario, Canada K1A 0R6 and Ottawa Civic Hospital, Ottawa, Ontario, Canada K1Y 4E9 from the *Canada/Fisons Fight Stroke* program..

Previous studies, using a conventional PKC assay, indicate that a rapid and sustained loss in activatible membrane PKC occurs in hippocampal neurons subjected to ischemic insult. However, the nature of the conventional assay is such that it measures 1) only PKC that is tightly associated with membranes, and b) the amount and not the activity of the enzyme present in that fraction. Our recently developed *direct PKC assay* gives an actual measure of the enzyme *activity* associated with both the tightly-associated and peripherally-bound pools of membrane PKC. This direct assay was used to explore the effects of ischemia on membrane PKC activity in CA1 neurons. Transient global ischemia was induced in male Wistar rats by a 15 min 4 vessel occlusion at 37° C followed by reperfusion for up to 72 hr. The PKC activity in the membranes from CA1 neurons was found to follow a biphasic pattern. A rapid 2-fold increase in membrane PKC activity was observed during ischemia, and this was followed by a rapid decline in activity upon reperfusion until levels 50% below that of sham-operated animals was reached 6 hr post-reperfusion. These subnormal levels of activity were maintained during the ensuring 48 hr. Mild hypothermia during ischemia (32° C) did not affect the initial increase in PKC activity but prevented enzyme activity from dropping below basal levels upon reperfusion. Hyperthermia (39.5° C), which exacerbated neuronal injury, triggered a decline in PKC activity to levels 80% below that of matched sham-treated animals. A tight correlation was found between the extent of CA1 neuronal cell damage and the levels of PKC activity 6 hr. post-ischemia.

672.3

RELATIONSHIP BETWEEN SPECTRIN-BREAKDOWN AND LDH RELEASE IN CORTICAL NEURONAL CULTURE CHALLENGED WITH GLUTAMATE. A. Posner, G.W. Campbell, K.K.W. Wang*, and D.M. Rock, Neuroscience Pharmacology, Parke-Davis Pharmaceutical Research, Division of Warner-Lambert Company, 2800 Plymouth Road, Ann Arbor, MI 48105, USA.

Excess amounts of glutamate can cause excitotoxicity in neurons by raising intracellular calcium levels. It has been suggested that calpain overactivation may play a key role in this neurodegeneration. Fetal rat cortical neurons were subjected to 500 µM glutamate for 5 min and the cells were then returned to normal medium. The time course of LDH release and spectrin breakdown were measured. After the glutamate challenge, both spectrin isoforms (α, β) (220 kDa) were found partly degraded to immunostained spectrin breakdown products (SBDP) of 150 kDa and 145 kDa, presumably originated from the two spectrin isoforms, respectively. The 150 kDa product was formed very rapidly (present after 15 min), followed by the appearance of the 145 kDa SBDP. Maximal levels of both SBDP were reached after 1 h. In contrast, LDH release continued to rise after 1 h and plateaued out at about 4 h. SBDP formation was shown to be largely dependent on extracellular calcium, suggesting the involvement of calpain. MK-801 (1 µM) effectively blocked LDH release and spectrin breakdown when added before and during the glutamate challenge, but was ineffective when added after the challenge. Calpain inhibitor I (Ac-Leu-Leu-Nle-H) at 50 µM reduced the level of 150 kDa SBDP and blocked formation of the 145 kDa SBDP at 45 min post-glutamate but did not significantly reduce LDH release.

672.4

THE INVOLVEMENT OF CALCIUM-CALMODULIN PATHWAY IN THE NEURONAL INJURY INDUCED BY HYPOXIA-HYPOGLYCEMIA IN THE ORGANOTYPIC CULTURE OF RAT HIPPOCAMPUS. C. Shin*, X. Sun and M. Colberg Departments of Pharmacology and Neurology, Mayo Clinic/Mayo Foundation, Rochester, MN 55905

We have previously reported that the NMDA receptor is involved in the process of neuronal injury produced by ischemia (Shin et al., Neurosci. Abstr., 1992). The NMDA receptor gates channel permeable to Ca^{2+} which subsequently activates many intracellular mechanisms. The present study was designed to test the hypothesis that activation of Ca^{2+}-calmodulin pathway plays a key role in the development of neuronal injury after ischemic insults.

The organotypic cultures from 6 day old pups were exposed at 10 DIV to 2-deoxyglucose substituted HBSS equilibrated with 95% N_2 plus 5% CO_2 atmosphere. Calmidazolium, which was shown to inhibit the activities of calcium calmodulin dependent kinase (CaMKII), was applied 30 min before, during and for 24 hours after the ischemia. At 24 hrs, a severity index of neuronal injury (0=none, up to 4=severe) was assigned to propidium iodide fluorescence.

With 40 min exposure to ischemic treatment, CA1 specific pattern of injury to the pyramidal neurons in the hippocampal formation was obtained. Calmidazolium reduced the injury index to 0.4 ± 0.13 (1 µM; n=13) from 1.83 ± 0.26 (vehicle control; n=30) [p < 0.05; Mann-Whitney U]. There was also dose-dependent protection at lower doses.

The results showed that calmidazolium, a specific inhibitor of CaMKII, suppresses neuronal injury induced by ischemia. These data support the hypothesis that activation of Ca^{2+}-calmodulin pathway may play a key role in the development of brain injury produced by ischemic insults.

672.5

N-METHYL-D-ASPARTATE RECEPTORS AND EXTRACELLULAR CALCIUM CHANGES DURING ISCHEMIA IN THE NEWBORN AND ADULT BRAIN IN VIVO. H. Hagberg, P Andiné*, M.Puka, J Lazarewicz, R. Gadamski. Institute of Neurobiology, University of Göteborg, 41390 Göteborg, Sweden.

The objectives were to 1. follow extracellular (ec) calcium during complete anoxia in the immature brain after blockade of N-methyl-D-aspartate (NMDA) receptors and 2. compare the calcium changes induced by NMDA in the adult and immature striatum. In the anoxia experiments, ec calcium was measured with ion-sensitive microelectrodes placed into the parietal cortex of 9-11 days old rats before and during 60 min of complete anoxia. One group was treated with MK-801 (0.3 mg/kg) and the other served as control. In the NMDA-experiments, microdialysis probes were placed into the striatum of 8-10 days old and adult rats. Calcium changes, induced by 5 mM of NMDA in the perfusion solution, were monitored with the 45-calcium washout method. In addition, 14C-sucrose was measured as a marker of ec volume. After induction of anoxia e.c. calcium increased from 1.1±0.1 mM to 1.4±0.2 mM in control and to 1.9±0.2 mM in MK-801 treated rats (NS). Thereafter, e.c. calcium decreased to 0.3 mM after 58±2.2 min in the control and after 39±7.5 min in the MK-801 group ($p<0.05$). NMDA induced a 50% decrease of 45-calcium in the 8-10 days old rats in opposite to a 30% increase followed by a <10% decrease in the adult striatum. Changes in 14C-sucrose were similar in immature and adult rats.

In conclusion, ec calcium movements during anoxia were considerably slower in neonatal than in adult rats. The NMDA receptor antagonist, MK-801, delayed the ec decrease of calcium after anoxia. NMDA induced a marked uptake of 45-calcium in 8-10 days old rats which may be important to explain the hypertoxicity of NMDA in the immature striatum.

672.6

KINETICS OF CALCIUM AND EXTRACELLULAR RADIOTRACERS IN BRAIN SLICES. F.E.Hospod, G.C.Newman, H.Qi, C.S.Patlak. Depts. of Neurology and Neurol. Surgery, SUNY at Stony Brook, NY and VAMC at Northport, NY.

In an effort to understand ion and water fluxes in the ischemic penumbra we are studying the movement of ^{45}Ca and several "extracellular" markers, along with water spaces and histology, in 500μ and 1000μ hippocampal slices.

Equilibrium measurements of ^{45}Ca indicate that total tissue calcium is linearly dependent on the extracellular calcium concentration between 1 μM and 4.5 mM when slices are incubated in standard Krebs-Ringer (K-R). 500μ hippocampal slices incubated for 4 hours in standard K-R gain up to 80% more water than that present *in vivo* with most of the increase prior to 2 hours. The "extracellular" space of slices incubated for 2 hours is 35% when measured with ^{3}H-sucrose or ^{14}C-inulin and 28% when measured with PEG-4000. Sucrose space rises to 45% and PEG space rises to 33% after 4 hours *in vitro*. Lajtha and co-workers (*Brain Res. 65:265, 1974*) have reported that incubation of parietal slices in the presence of 4% PEG-4000 or 80,000 MW dextran prevents this water gain. We have reproduced their results with PEG and also confirm that, despite preventing water gain, there is only minimal change in the "extracellular" space measured with ^{3}H-sucrose or ^{14}C-PEG when PEG is varied from 0 to 4%. Our histology reveals that the CA1 and CA3 regions respond very differently to increasing concentrations of PEG in the buffer, 4% PEG improves the CA3 histology after 4 hours but induces severe pyknosis of CA1 pyramidal neurons and dentate granular cells.

These results are not consistent with a simple two compartment model of homogeneous intracellular and extracellular water spaces. Histology suggests that there is regional heterogeneity within the slice and that PEG should be used in slices only with caution. Further studies with dextran and additional ^{45}Ca kinetics in both thin and thick slices will also be presented.

672.7

SYSTEMIC ADMINISTRATION OF A CALPAIN INHIBITOR IS NEUROPROTECTIVE IN A RAT MODEL OF FOCAL CEREBRAL ISCHEMIA. S.C. Hong*, Y. Goto, G. Lanzino, S. Soleau, N.F. Kassell, and K.S. Lee. Dept. of Neurosurgery, Univ. of Virginia, Charlottesville, VA 22908.

Excessive elevation of intracellular calcium, and uncontrolled activation of calcium-sensitive events are believed to play a central role in ischemic neuronal damage. Calcium-activated proteolysis by calpain is a candidate to participate in this form of pathology because it is activated under ischemic conditions and its activation results in the degradation of crucial cytoskeletal and regulatory proteins. The present studies examined the effects of a cell-penetrating inhibitor of calpain on the pathological outcome following transient, focal ischemia in the brain. Rats treated with the calpain inhibitor, Cbz-Val-Phe-H (MDL28170), exhibited significantly smaller volumes of infarction than saline-treated, or vehicle-treated control animals. Intravenous injections of cumulative dosages of 30 mg/kg or 60 mg/kg of Cbz-Val-Phe-H were effective in reducing infarction, while only the higher dosage reduced cerebral edema following ischemia. These results demonstrate the neuroprotective effect of a cell-penetrating calpain inhibitor when administered systemically. The findings suggest that targeting intracellular calcium-activated mechanisms, such as proteolysis, represents a potentially useful therapeutic strategy for limiting neurological damage following ischemia. (Supported by NS30671)

672.8

KETOAMIDE CALPAIN INHIBITORS PROTECT HIPPOCAMPAL SLICES FROM HYPOXIA H. Ton, A. Arai, D. Eveleth*, R. Bartus and G. Lynch, Cortex Pharmaceuticals, Inc., Irvine CA, Center for the Neurobiology of Learning and Memory, University of California, Irvine CA, and Alkermes, Inc., Cambridge MA.

Several lines of evidence suggest that the calcium-activated protease calpain might be involved in various types of pathology, in particular ischemia/hypoxia. Previously, we have shown that calpain inhibitors improved the recovery of the synaptic transmission after a brief period of hypoxia in the field CA1 of hippocampal slices. The present experiments sought to examine whether a different class of calpain inhibitors (ketoamide) shows a protective effect against hypoxia-induced acute neuronal dysfunction and also examined the effects of various classes of drugs, such as NMDA receptor antagonists (CPP, MK-801 and dl-AP5), free radical scavengers (D-mannitol, superoxide dismutase together with catalase, PBN) and a PAF receptor antagonist on this system.

In our system, the PAF antagonist was ineffective. Among the free radical scavengers, only the spin adduct PBN caused a significant improvement in the recovery of EPSPs after hypoxia. Protection was provided by the NMDA receptor antagonists CPP (20 μM) and MK-801 (50 μM), but not by dl-AP5, even at a concentration of 100 μM.

Two newly synthesized calpain inhibitors (CX269 and CX275) are more potent and selective than those reported previously (K_i = .2 μM for CX269; K_i = .37 μM for CX275 against purified human calpain). Both drugs at 20 μM showed significant effects on the recovery of synaptic transmission after hypoxia. This protection appears not to be due to a drug action on NMDA receptors. These results give further support to the idea that activation of calpain is involved in acute pathophysiology caused by hypoxia.

672.9

MECHANISMS OF INTRACELLULAR CALCIUM ALTERATIONS DURING ENERGY DEPLETION AND REPERFUSION INJURIES. M.H.Kim-Lee*, B.T.Stokes Dept. of Physiology, The Ohio State University.,Columbus, OH 43210.

Fura-2 microspectrofluorometry was used to examine the intracellular calcium ($[Ca^{2+}]_i$) alterations produced by energy depletion or reperfusion injury. The mechanisms by which local anesthetics (lidocaine) are able to alter changes induced by these insults in vitro were also investigated. The $[Ca^{2+}]_i$ increase during energy depletion was determined to be mostly from internal calcium stores. Furthermore, intracellular Na^+ played a major role in $[Ca^{2+}]_i$ release from such stores during energy depletion. Addition of lidocaine (0.5 mM) after de-energization effectively blocked $[Ca^{2+}]_i$ increases with normal perfusate calcium but failed to block after calcium removal. This may indicate that lidocaine stimulates forward activity of the surface Na/Ca exchanger. In addition, 0.5 mM lidocaine prevented $[Ca^{2+}]_i$ increases during reverse Na/Ca exchanger activity associated with reperfusion protocols. Therefore, these data support the hypothesis that local anesthetics can alter Na/Ca exchanger activity and suggest that they may be effective in a variety of ischemic and reperfusion-induced cytotoxic insults in vitro.

672.10

EARLY ANOXIA-INDUCED VESICULAR GLUTAMATE RELEASE RESULTS FROM MOBILIZATION OF CALCIUM FROM INTRACELLULAR STORES. N.Hershkowitz, K.L. Kelner* and A.N. Katchman, Georgetown University Medical Center, Dept. Neurol., Washington, DC 20007.

Glutamate release is thought to contribute to cell injury during anoxia of the brain. An understanding of mechanisms by which this release occurs may assist in the development of effective therapeutic agents. Whole-cell patch clamp recordings (K gluconate electrodes) were made in CA1 neurons of hippocampal slices in the presence of 1 uM of tetrodotoxin using an interface chamber. Anoxia was induced by switching perfusion and ambient gas from a 95%O_2/5%CO_2 to a 95%N_2/5%CO_2 mixture. Transient inward currents, representing miniature excitatory postsynaptic currents (mEPSCs), are observed under control normoxia conditions. Following the onset of anoxia a significant (paired t test; $P<0.05$), 3.17-fold increase, in the mEPSCs frequency is observed ($n=9$). The kinetics of the mEPSCs (i.e. amplitude, rise time and half decay time) did not significantly change following anoxia. In calcium-free extracellular medium, 8 cells still exhibited a significant 3.37-fold increase in the frequency of mEPSCs; in calcium-free extracellular medium plus 0.2 mM $CdCl_2$, 11 cells showed a significant 3.38-fold increase. In contrast, in cells preincubated in 20 uM of dantrolene ($n=20$) or 10 mM of caffeine ($n=11$), agents which can inhibit release or deplete calcium from intracellular stores, there was no significant anoxia-induced increase in mEPSC frequency (1.20- and 1.44-fold). Thus, a component of the early glutamate release after anoxia results from calcium mobilization from intracellular stores. The absence of changes in the kinetics of the mEPSCs following anoxia supports a presynaptic locus to the early anoxia-induced synaptic depression of the orthodromically elicited EPSC. (Supported by NINDS grant NS 14600-02).

672.11

CALCIUM CHANNEL ACTIVATION DURING SPREADING DEPRESSION IN NORMAL RATS. Osuga S, Osuga H, Hakim AM, Hogan MJ*. Institute of Neuroscience and Molecular Genetics, University of Ottawa, Ottawa, CANADA K1H 8M5.

We have used in vivo binding of the L-type voltage sensitive calcium channel (VSCC) antagonist [^{3}H]nimodipine to identify calcium channel activation during repeated spreading depression in normal rats. In cerebral ischemia we have previously demonstrated increased in vivo [^{3}H]nimodipine binding to be specific to the L-type VSCC and to be a sensitive indicator of impending injury. We undertook these studies to assess [^{3}H]nimodipine binding in a non ischemic model of cell membrane depolarization. Male 250g Sprague Dawley rats were anesthetized with Halothane and a 300 μm diameter microdialysis probe was inserted stereotactically 2 mm into the right occipital cortex. Repetitive waves of spreading depression (every 7 to 12 minutes) were induced by instilling 20 μl of 3M KCl. After 1 hour [^{3}H]nimodipine was infused intravenously (250 μCi, 130 Ci/mmole) and circulated for 15 minutes. The rats were then decapitated, brains removed, frozen and sectioned for autoradiography. [^{3}H]nimodipine binding was determined in both hemispheres and was observed to be increased by 20% in the dorsal aspect of the frontal and parietal cortex, remote from the site of KCl application, on the side of spreading depression ($p<0.025$, $n=4$). We hypothesize that increased nimodipine binding in spreading depression is the result of L-type VSCC activation. This observation may be relevant to studies on the pathopysiology of cerebral ischemia where recurrent spreading depression has been reported to result in increased ischemic injury.

Supported by Heart and Stroke Foundation of Ontario, the Medical Research Council of Canada and Miles Laboratories.

672.12

IMMUNOLOCALIZATION OF SPECTRIN DEGRADATION IN VULNERABLE REGIONS OF ISCHEMIC GERBIL BRAIN. J. M. Roberts-Lewis*, M. J. Savage, V. R. Marcy, L. R. Pinsker and R. Siman Cephalon, Inc., West Chester, PA 19380

The calcium-activated cysteine protease calpain I (μ-calpain) has been suggested to play a vital role in the mechanisms underlying ischemia-induced, calcium-mediated neuronal degeneration. Several studies have linked the pathological induction of calpain-mediated spectrin proteolysis to neuronal death. Using an antibody selective for calpain-generated spectrin breakdown products, we have examined the time course and localization of calpain activation in the gerbil brain following transient global ischemia. Immunoreactivity for spectrin breakdown products was present in the hippocampus as early as 30 minutes following a five minute occlusion of the carotid arteries. This effect was maximal in the CA1 region of the hippocampus at two days, and essentially absent by 5 days following ischemia. Intense immunoreactivity was neuron-specific, and present in both perikarya and dendrites. In contrast, spectrin degradation was also present in the cortex, striatum and thalamus at 30 minutes following a 5-minute insult, but virtually absent in these regions by two days post-ischemia. Silver impregnation histochemistry confirmed that only neurons in areas exhibiting prolonged calpain activation degenerated. Treatment of gerbils with the non-competitive NMDA antagonist, MK-801, substantially reduced staining for spectrin breakdown products in all brain regions. Together, these results suggest that ischemia triggers NMDA receptor-mediated calpain activation in several regions of the brain within at least 30 minutes following a modest ischemic insult; however, only those neurons showing sustained calpain activation are destined to die.

ISCHEMIA: DRUG TREATMENT I

673.1

ACADESINE REDUCES INDIUM LABELED PLATELET DEPOSITION IN RAT BRAIN FOLLOWING PHOTOTHROMBOSIS OF THE COMMON CAROTID ARTERY. L.P. Miller*, W.D. Dietrich, R. Prado, M.K. Dewanjee, & H. Gruber. Gensia Pharm, San Diego, CA 92121 and Univ. Miami Sch. of Med., Miami, FL. 33101.

Adenosine regulating agents (ARAs) are a class of compounds designed to enhance the beneficial effects of adenosine (Adn) in an event and site-specific manner. Acadesine (ACA)(5-amino-1-β-D-ribofuranosyl-imidazole-4-carboxamide), a prototypical ARA, has cardioprotective effects in animal models and reduces the incidence of myocardial infarction and stroke following bypass surgery. The present study was designed to determine whether the beneficial effects of ACA could be observed in a model of photothrombotic stroke. Rats were anesthetized with halothane and preloaded with indium 111-tropolone labeled platelets 30 min prior to common carotid artery (CCA) thrombosis induced by a rose bengal-mediated photochemical insult. Intravenous infusion of ACA (n=10) at 0.5,1, 2 mg/kg/min or vehicle was begun 30 min prior to CCA thrombosis and continued for an additional 15 min. Animals were then sacrificed and brains processed for autoradiographic visualization of labeled platelets. The ratio of right-to-left CCA radioactivity was not affected by ACA administration. However, ACA at 1 and 2 mg/kg/min significantly decreased ($p<0.01$) platelet deposition within the ipsilateral cerebral cortex, hippocampus and striatum. These results support a prophylactic role for ACA in reducing the deposition of platelet emboli in brain during periods of acute thrombus formation.

673.2

EFFECTS OF PERIPHERAL IL-1RA TREATMENT AFTER FOCAL CEREBRAL ISCHAEMIA IN THE RAT. J.K.Relton, D. Martin* and D. Russell. Preclinical Pharmacology, Synergen, 1885 33rd St. Boulder, CO80301.

The objective of these experiments was to determine the effect of peripherally administered recombinant human interleukin-1 receptor antagonist (rhIL-1ra) on infarct size and cerebral oedema formation after focal cerebral ischaemia in the rat. Ischaemia was induced by permanent unilateral occlusion of the left middle cerebral artery (MCAo) under isoflurane anaesthesia (2% in O_2). The histological assessment of neuronal damage was performed on tetrazolium stained 500μm coronal brain sections and tissue H_2O composition (as an index of cerebral oedema formation) determined by wet and dry weight measurements. Animals were injected (iv and sc) immediately after MCAo (0h) with either vehicle (0.25-0.5ml) or rhIL-1ra (10mg/kg, 50mg/kg or 100mg/kg) followed by further injections (sc) 4, 8, 12 and 18h after MCAo. All animals were sacrificed after 24h to assess brain damage. Treatment with IL-1ra (100mg/kg) significantly reduced infarct size by 48% compared to vehicle-treated control rats ($76.17 \pm 12.06mm^3$, $n=16$ vs $39.96 \pm 8.68mm^3$, $n=12$, $P<0.05$ Mann Whitney U-test). Lower doses of IL-1ra (50mg/kg and 10mg/kg had no significant effect on infarct size compared to controls. Cerebral oedema was significantly reduced by 36% and 33% in 100mg/kg and 50mg/kg IL-1ra-treated rats respectively (left-right hemisphere $\%H_2O$: 100mg/kg; $1.69 \pm 0.25\%$, $n=14$ vs $1.08 \pm 0.1\%$, $n=14$, $P<0.05$ one way ANOVA with Tukey's post hoc test; 50mg/kg; $2.39 \pm 0.23\%$, $n=10$ vs $1.58 \pm 0.15\%$, $n=10$, $P<0.01$ one way ANOVA with Tukey's post hoc test). A dose of 10mg/kg IL-1ra had no discernable effect on cerebral oedema 24h after MCAo. These data indicate that IL-1ra may be of considerable therapeutic benefit in the treatment of stroke.

673.3

FLUNARIZINE FAILS TO REDUCE INFARCT VOLUME IN A NOVEL MODEL OF FOCAL ISCHAEMIA IN THE MARMOSET A J Hunter*, N A Milkowski, N I Wood, S Patel, and A L Rothaul SB Pharmaceuticals, Coldharbour Road, The Pinnacles, Harlow, Essex, CM19 5AD, UK.

We have used a light sensitive dye to produce a novel, minimally invasive, model of primate focal ischaemia. Flunarizine, a T-type calcium antagonist, has been claimed to reduce photothrombotic lesions in rodents (van Reempts et al, 1987, Stroke 18: 1113-9); therefore we have tested this compound in our primate model. Common marmosets (C. jacchus) weighing 300-450 gms were anaesthetised with Saffan and placed in a stereotaxic frame. Rose bengal dye (20 $mgkg^{-1}$) was infused via the tail vein at the same time as bilateral illumination of the exposed anterior skull surface. Flunarizine (1 or 10 $mgkg^{-1}$) or 0.9% saline was given i.p. 30 minutes post ischaemia and b.i.d. for 3 days. Animals were euthanased at 7 days post surgery and serial transverse sections taken. The lesion volumes were similar in both hemispheres in all groups eg saline left side = 50.9 ± 3.7, right = 50.4 ± 4.1 mm^3. Treatment with flunarizine failed to reduce lesion size eg left side 1 $mgkg^{-1}$ = 53.2 ± 2.4; 10 $mgkg^{-1}$ = 55.6 ± 3.7 mm^3. We conclude that post ischaemia dosing of flunarizine is ineffective in this primate focal ischaemia model.

673.4

PORTECTIVE EFFECTS OF U-92032, A NOVEL T-TYPE CALCIUM CHANNEL BLOCKER, ON CEREBRAL ISCHEMIC INJURY. S.Y. Liou*, M. Takahashi, H. Takagi, M. Takashima, C. Itoh, W.B. IMM and M. Kunihara. Upjohn Pharmaceuticals Limited, Tsukuba Research Laboratories, Wadai 23, Tsukuba 300-42, Japan.

U-92032,(7-[[4-[bis(4-Fluorophenyl)methyl]-1-piperazinyl]methyl]-2-[(2-hydroxyethyl)amino]4-(1-methylethyl)-2,4,6-cycloheptatrien-1-one) proved to be a selective T-type calcium channel blocker using the whole-cell version of the pathch-clamp technique. We also investigated the anti-ischemic and anoxic effects of U-92032 in a number of models in rodents. In *in vitro* hypoxia injury, U-92032 at $3x10^{-6}$ M had protective action against functional failure of CA1 neuron in hippocampal slice after 10 min hypoxia/hypoglycemia. In KCN anoxia, U-92032 at a dose of 10 mg/kg, i.v. or 100 mg/kg, p.o. prevented lethality in mice. In stroke model in gerbils, U-92032 at 10 mg/kg, p.o. reduced stroke index induced by unilateral ligation of the carotid artery. Following transient global ischemia, U-92032 had protective action against delayed neuronal death in hippocampus and improved impairment of passive avoidance performance. In addition, unlike dihydropyrizine Ca channel blockers (nifedipine and nicardipine), U-92032 potently blocked ^{45}Ca uptake evoked by glutamate and hypoxia in the hippocampal brain slice preperation. These results suggest that the selective T-type Ca channel blocking effect of U-92032 might contribute to it's cerebroprotective efficacy in these cerebral ischemic models.

673.5

SNX-111 REDUCES NEOCORTICAL INFARCTION DESPITE INTRA- AND POST-ISCHEMIC HYPOTENSION FOLLOWING FOCAL ISCHEMIA. D. Xue*, Z.G. Huang, K. Barnes, H. Lesiuk, K.E. Smith and A.M. Buchan. Ottawa Civic Hospital, Ottawa, Ontario, Canada K1Y 4E9, from the *Canada/Fisons Fight Stroke* program.

The potent N-type calcium channel blocker, SNX-111 (NEUREX Corp.), was used to see if it would reduce cortical infarction in focal stroke. Adult male spontaneously hypertensive rats (n=18) had permanent occlusion of the right common carotid artery and transient occlusion of the right middle cerebral artery (2 hr), followed by 22 hr reperfusion. To mimic the further reduction in cerebral blood flow that may follow the hypotensive actions of SNX-111 (and to replicate the resulting ischemic insult), a second group of control rats were subjected to additional transient occlusion (4 hr) of the left carotid artery (LCCAO). Rats were given saline or SNX-111 (5 mg/kg) injected iv 1 hr *after* the onset of ischemia. Regional cerebral blood flow was monitored. The volume of cortical infarction was then quantified with frozen-sectioned brain slices.

Group (n)	Cortical Infarction (mm^3 ±SD)	
Saline (6)	211 ± 51	
Saline + LCCAO (6)	271 ± 35	*p<0.001 vs. saline
SNX-111 (6)	141 ± 49*	+ LCCAO group (ANOVA)

Despite the hypotensive effect of SNX-111, which *may* further compromise intra- and post-ischemic cerebral blood flow, a 33% reduction in mean cortical infarction was demonstrated in the treatment group compared with the saline control group. Striking protection (48%) was shown in the SNX-111-treated animals compared with control animals with cerebral blood flow depressed to comparable levels to that seen with SNX-111 treatment.

(The authors thank the NEUREX Corporation for providing SNX-111).

673.6

MARKEDLY DELAYED TREATMENT WITH SNX-111, AN N-TYPE CALCIUM CHANNEL BLOCKER, PREVENTS THE DEATH OF HIPPOCAMPAL CA1 NEURONS FOLLOWING GLOBAL ISCHEMIA. A. Buchan, H. Li, K. Barnes, K.E. Smith* and H. Lesiuk. Neuroscience, Loeb Research Institute, Ottawa Civic Hospital, Ottawa, Ontario, Canada K1Y 4E9, from the *Canada/Fisons Fight Stroke* program.

SNX-111 (NEUREX Corp.), a potent N-type calcium channel antagonist, was tested following ischemia using a 4-vessel occlusion model of severe global (forebrain) ischemia which reliably induces hippocampal CA1 necrosis. Adult male Wistar rats (n=22) were subjected to 10 min of forebrain ischemia and seven days of reperfusion. A single dose of SNX-111 (5 mg/kg) was injected iv either at 6 or at 24 hr post-ischemia. Damaged CA1 neurons of the dorsal hippocampus in surviving animals (n=22) were counted and the percentage of injured cells calculated.

Group (n)	% CA1 Injury (Mean ±SD)
Saline (9)	77 ±14
SNX-111 (13)	
delayed 6 hr (6)	36 ±28*
delayed 24 hr (7)	44 ±31*

* p < 0.001 Mann-Whitney U test with Bonferoni correction

Twenty-four hours after ischemia, CA1 cells could still be resuscitated with SNX-111. This suggests that calcium fluxes 24 hours after ischemia are critically involved in the pathogenesis (and reversibility) of selective neuronal death.

(The authors thank the NEUREX Corporation for providing SNX-111).

673.7

AUTORADIOGRAPHIC ANALYSIS OF [^{125}I]OMEGA-CONOTOXIN BINDING IN CANINE CORTEX AND HIPPOCAMPUS FOLLOWING GLOBAL CEREBRAL ISCHEMIA / REPERFUSION. D.C. Perry*, E. Sidel, R.E. Rosenthal and G. Fiskum. Depts. of Pharmacology, Biochemistry and Molecular Biology, and Emergency Medicine, George Washington University Medical Center, Washington, D.C. 20037.

Changes in Ca^{2+} flux may contribute to selective neuronal damage occurring after cerebral ischemia. We used [^{125}I]ω-conotoxin to look for changes in N-type VDCC in selected regions in ischemic brain. Canines were divided into one of five groups: non-arrested controls; complete global ischemia induced by 10 min cardiac arrest (CA); or CA followed by 30 min, 2 hr or 24 hr restoration of spontaneous circulation (ROSC). Frozen sections from parietal and temporal cortex and hippocampus were incubated with 0.5 nM [^{125}I]ω-conotoxin, ±1 μM unlabeled peptide to define specific binding. Sections were exposed to film and resultant images analyzed by computerized densitometry. No effect of cerebral ischemia on binding was seen after CA or early time points after ROSC, but at 24 hr after ROSC, binding over the outer laminae was increased by 43% in the parietal cortex. This binding increase was not apparent over inner laminae or in temporal cortex. In contrast, hippocampal binding over strata radiatum and oriens was sharply decreased after CA and even more at short times after ROSC (up to 70%), but largely recovered by 24 hr. This effect was seen in CA1 and dentate gyrus molecular layer, but not CA3. We are currently examining whether other calcium channels, both voltage- and receptor-activated, show parallel alterations post-ischemia. Supported by Sigma Tau, S.p.A. and the Souers Stroke Fund.

673.8

HEMODILUTION WITH DIASPIRIN CROSS-LINKED HEMOGLOBIN (DCLHb) REDUCES NEUROLOGICAL DAMAGE FOLLOWING REVERSIBLE BUT NOT IRREVERSIBLE CNS ISCHEMIA IN RABBITS. M.P. Bowes, J.A. Zivin*, and K.E. Burhop. Univ. California San Diego, Dept. of Neurosciences, La Jolla, CA 92093-0624 and Baxter Healthcare Corp. Blood Substitutes, Round Lake, IL, 60073.

Hemodilution using hemoglobin (Hb) solutions may reduce ischemic or hemorrhagic injury. Cell-free Hb may be intramollecularly cross-linked, enhancing O_2 offloading to tissues and increasing intravascular retention time without systemic side effects. We evaluated the ability of purified diaspirin cross-linked hemoglobin (DCLHb) to reduce neurological damage in two rabbit CNS ischemia models. Reversible spinal cord ischemia [Zivin et al., Arch. Neurol. 39: 408-412, 1982] was produced by temporary occlusion of the abdominal aorta. DCLHb was infused (1 ml/min; .1 g Hb/ml) 5 min after ischemia as either a 10 ml/kg infusion, 10 ml/kg exchange transfusion, or a 20 ml/kg infusion. Control animals received 20 ml/kg oncotically matched human serum albumin (HSA). Neurological status was evaluated 18 hr and 4 days following ischemia/reperfusion. Neurological outcome was significantly improved in the 10 ml/kg exchange transfusion and 20 ml/kg conditions, compared to HSA-treated controls. In an irreversible model of cerebral ischemia, rabbits were embolized with 50 μM plastic microspheres injected into the carotid circulation [Zivin et al., Brain Res. 435: 305-309, 1987]. DCLHb (20 ml/kg, 5 min post ischemia) did not significantly reduce neurological damage in this model. These data suggest that cross-linked hemoglobin may improve neurological outcome in reversible but not irreversible ischemia, and this is not attributable only to hemodilution or hypervolemia.

673.9

CYCLOTHIAZIDE POTENTIATES AMPA NEUROTOXICITY AND OXYGEN-GLUCOSE DEPRIVATION INJURY IN CORTICAL CULTURE. M.C. Bateman*, M.R Bagwe, K.A. Yamada, and M.P. Goldberg. Center for the Study of Nervous System Injury, Dept. of Neurology, Washington Univ. School of Med., St. Louis MO 63110.

Rapid desensitization may limit the contribution of AMPA/kainate (A/K) receptors to glutamate neurotoxicity. The benzothiadiazide compound, cyclothiazide (CYC) prevents desensitization of AMPA responses and potentiates hippocampal glutamate toxicity (Yamada et al, Soc. Neurosci. Abs. 18:757, 1992; Moudy et. al., ibid). We examined the effects of CYC on excitotoxic injury in murine neocortical cultures (15-17 days in vitro). 10-100 μM CYC markedly potentiated $[Ca^{2+}]_i$ elevation induced by AMPA or kainate application on both neurons and glia, assessed by fura-2 fluorescence ratio imaging. Furthermore, CYC (30μM) significantly increased neuronal injury during 24 hr exposure to AMPA or kainate and had no effect on NMDA toxicity. CYC-enhanced neurotoxicity of AMPA could be blocked by A/K antagonists CNQX or GYKI 52466.

Neuronal injury produced by deprivation of oxygen and glucose (45-60 min) was enhanced by addition of 10-100 μM CYC (Neurology 43:A339, 1993). In the absence of CYC, neuronal injury could be blocked by NMDA antagonists but not by A/K antagonists alone. In contrast, the enhanced neuronal loss produced by oxygen-glucose deprivation in the presence of CYC could be blocked by CNQX. This CYC-enhanced neuronal injury occurred even with the addition of MK-801. These observations suggest that AMPA receptor desensitization may limit calcium entry and alter glutamate receptor pharmacology of hypoxic-ischemic cerebral injury.

Supported by NIH NS01443 (KAY) and NS01543 (MPG) and a grant from the Brain Trauma Foundation (MPG).

673.10

EFFECTS OF NEUROTROPHIN-4/5 TREATMENT ON INFARCTION VOLUME INDUCED BY FOCAL CEREBRAL ISCHEMIA IN THE ADULT RAT. K.M. Chan*, H.R. Widmer and F. Hefti. Andrus Gerontology Center, Univ. of Southern California, Los Angeles, CA 90089-0191.

We are studying the effects of neurotrophin-4/5 (NT-4/5) treatment on a rat model of focal cerebral ischemia induced by intraluminal occlusion of the middle cerebral artery (MCA).

One day prior to MCA occlusion, a cannula was implanted into the right lateral ventricle of each young adult (2-month old) rat. A dose of 0.87μg of either NT-4/5 or a control protein (cytochrome c, cc) was administered immediately following cannula implantation. Twenty-four hours later, focal cerebral ischemia was induced by permanent ligation of the right common carotid artery and the intraluminal occlusion of the right MCA by a piece of 3-0 nylon suture thread. The same dose of NT-4/5 or cc was administered immediately following the procedure. Normal body temperature was monitored by a digital thermometer and maintained by a warming blanket. The animals were sacrificed after a survival period of twenty-four hours and 30μm thick coronal sections of the perfusion-fixed brains were stained with cresyl violet. The areas of infarction were determined by computer-aided image analysis and the volume of infarction estimated from at least eight coronal levels.

While the volumes of infarction of the cc-treated animals were relatively consistent (n=4), four out of six of the NT-4/5-treated animals showed significant reduction of the infarct volumes. We are presently performing more surgeries to substantiate this preliminary finding.

673.11

PROTECTIVE EFFECTS OF bFGF AGAINST NEURONAL DAMAGES IN *IN VITRO* AND *IN VIVO*. Y. Morita*, N. Murayama, T. Inoue, R. Ogino and T. Ohno. Lab. of Exp. Pharmacol., SUNTORY Inst. for Biomed. Res., Osaka 618, JAPAN

Neuroprotective effects of bFGF was examined in the damaged brains with temporal ischemia and in primary neurons cultured under different experimental conditions. Male Wistar rats received a surgery of 60-min unilateral middle cerebral artery occlusion (MCAO). bFGF (25 ug, i.c.v., [1ug/ul]) was administered 24 hr prior to MCAO, and the brains were treated according to a TTC protocol and measured volumetrically. Primary neurons from the neocortex, hippocampus and striatum of rat fetuses (E 18) were cultured in DMEM containing 10% serum. bFGF was applied at the concentrations of 5, 10 or 25 ng/ml 36 hr after plating. The types of injuries applied were glutamatetoxicity, hypoglycemia and hypoxia. The expression of *FLG*/FGF receptor was detected immunocytochemically in the brains and primary neurons.

Postoperative 48 hr after MCAO, brains were less damaged in the bFGF-treated rats compared to the control rats. The infarcted tissue-volume per the examined brain region in the bFGF-treated rats was 7.73% ± 1.09 (n=19) ($p<0.001$), while that of the control rats 27.74% ± 2.08 (n=15). Primary cultured neurons from 3 brain regions were protected from damages of glutamatetoxicity, hypoglycemia, and hypoxia at either 5, 10 or 25 ng/ml of bFGF. Those neurons expressed immunoreactive *FLG*/FGF receptor as early as 36 hr after plating.

It suggests that FGF receptor-mediated cellular events and gene regulation are involved in the neuroportective effects of bFGF, because such protection of bFGF are eliminated when either transcription or translation is suppressed.

673.12

THE NEUROPROTECTIVE EFFECTS OF DEXMEDETOMIDINE IN THE GERBIL HIPPOCAMPUS FOLLOWING TRANSIENT ISCHEMIA. J.Sivenius*, J. Kuhmonen and P. Riekkinen Sr. Univ. of Kuopio, Dept. of Neurology, P.O.B. 1627, SF-70211 Kuopio, Finland.

Excessive neuronal activity with an increased release of excitatory neurotransmitter glutamate is supposed to contribute to the delayed neuronal degeneration in animal models of transient cerebral ischemia. Since evidence is accumulating that agonizing of alfa-2-adrenergic receptors might depress glutamate release, the neuroprotective effects of dexmedetomidine, an alfa-2-adrenergic agonist, were investigated in a gerbil transient cerebral ischemia model, involving bilateral carotid occlusion of 5 min in ether inhalated normothermic animals. Dexmedetomidine (3 or 30 µg/kg) was administered s.c. 30 min before and 3, 12, 24 and 48 h after occlusion;or the same doses only 3, 12, 24 and 48 h after occlusion. Control animals were subjected to cerebral ischemia without medication. After one week the animals were killed and brains fixed by transcardiac perfusion. Dorsal hippocampus was sectioned coronally into 60 µm thick slices which were stained with silver. Delayed neuronal death of sector CA1 pyramidal cells was evaluated and the data was analyzed by Mann-Whitney U-test. The dose of 3 µg/kg before and after ischemic insult decreased neuronal loss ($p\leq0.05$). We conclude that dexmedetomidine, an alfa-2-adrenergic agonist, is neuroprotective in this gerbil transient cerebral ischemia model.

673.13

COCAINE EXACERBATES THE OUTCOME OF GLOBAL BRAIN ISCHEMIA. B. Lin*, W.D. Dietrich, R. Busto, S. Kraydieh, M.Y.-T. Globus, and M.D. Ginsberg. CVD Research Center, Univ. of Miami, Sch. of Med., Miami, FL, 33101.

To investigate whether acute cocaine would affect the outcome of global cerebral ischemia, fasted halothane-anesthetized rats received 10 min global forebrain ischemia by bilateral carotid artery occlusion plus hypotension (50 mm Hg). Brain temperature was maintained at 36.5-37°C. Cocaine, 2 mg/kg or 10 mg/kg was injected i.p. 20 min prior to ischemia. Control rats received water. Ischemic cell change (ICC) was assessed blindly at 3 days following the insult. Seven of 10 rats with 10 mg/kg cocaine, but no control rats, showed damage to hippocamal sector CA3 ($p=0.0015$, Fisher exact test); 5/10 cocaine rats (but no controls) showed injury to medial geniculate body ($p=0.016$), and 2 of 10 cocaine rats displayed subarachnoid hemorrhage. By contrast, injury to hippocampal CA1 sector, striatum, and neocortex were unaffected by cocaine. The 2 mg/kg cocaine dose did not accentuate ischemic injury. After i.p. cocaine, blood pressure increased 20-40 mm Hg for approx. 10 min in the 2 mg/kg series and decreased 20-40 mm Hg in the 10 mg/kg group but returned to normal prior to ischemia. Our findings suggest that high-dose cocaine adversely affects the outcome of normothermic global ischemia.

673.14

EFFECT OF ACETYL-L-CARNITINE ON THE RECOVERY OF BRAIN ENERGY METABOLISM AND LACTIC ACID LEVELS FOLLOWING TRANSIENT FOREBRAIN ISCHEMIA IN THE RAT. A ^{31}P AND ^{1}H NMR SPECTROSCOPY STUDY. T. Aureli, M.E. Di Cocco, O. Ghirardi, M. Vertechy, A. Giuliani, M.T. Ramacci, M. Calvani*, F. Conti[1]. Inst. for Res. on Senescence Sigma Tau, 00040 Pomezia, Italy; [1] Dept. Chemistry, "La Sapienza" Univ. of Rome, Italy

The four-vessel occlusion (4-VO) model of transient ischemia in the rat produces a histological damage profile similar to that observed in man after total ischemia following a cardiac arrest. This coincident pathophysiology of ischemic brain damage suggested that 4-VO model can be very useful for the evaluation of a neuroprotective efficacy of novel compounds after global ischemia. Acetyl-L-Carnitine (ALCAR) is a molecule recently shown to exert a beneficial effect on brain energy and phospholipid metabolism in both adult and aged rats. In the present study, we tested by NMR spectroscopy the effectiveness of post-ischemic administration of ALCAR on the recovery of brain high-energy phosphates (i.e., phosphocreatine, ATP) and lactic acid levels following a 20-min global ischemia in Fischer 344-adult rats. Ischemia produced a remarkable change in all the measured parameters involved in energy metabolism, especially in the levels of lactic acid and inorganic phosphate. ALCAR was demonstrated to exert a significant effect on the recovery of energy phosphate levels and of brain lactic acid content. Principal component analysis of data at 2, 24, and 48 hours after reperfusion, indicated a significant ALCAR effect on brain energy metabolism as a whole. These results indicate that when administered immediately after ischemic insult, ALCAR exerted a significantly protective effect on ischemia-induced brain damage; in addition, ALCAR enhanced brain energy metabolism during reperfusion so as to ameliorate the overall activation of cellular recovery mechanisms.

673.15

EXPERIMENTAL CEREBRAL ISCHEMIA IN MONGOLIAN GERBILS: EFFECTS OF JO 1784 ON HISTOLOGICAL PARAMETERS AND LOCOMOTOR ACTIVITY. B. Farley*[1], M .Canney[1], M. O'Neill[1], B.E. Leonard[1], C. Benicourt[2] and J-L Junien[2], Pharmacology Department[1], University College, Galway, Ireland and Institut de Recherche Jouveinal[2], 94265, Fresnes, France.

In mongolian gerbils, 5 minutes of bilateral carotid occlusion (BCO) followed by reperfusion, causes forebrain ischemia, glutamate release and permanent brain injury to the hippocampus after a 24 hours window of vulnerability. During this period, use of NMDA receptor antagonists or blockers of the NMDA-regulated calcium channels, markedly reduces the extent of brain damage evaluated histologically one week later.

The purpose of the present series of experiments was to:

1) To determine the extent of hippocampal damage in gerbils following 5 min bilateral carotid occlusion.

2) Based upon the possibility that sigma ligands might modulate the NMDA receptor, JO 1784 (25, 50, 75 and 100 mg/kg p.o.) a sigma ligand was tested for its ability to reduce brain damage in the gerbil model. MK-801 (2.5 mg/kg i.p.) used as a standard neuroprotective agent, was administered 1 hour after the occlusion and JO 1784 at 1, 24, 48 and 72 hours after occlusion. Locomotor activity was used as an index of hippocampal damage.

In the first study, results showed that, following bilateral carotid occlusion, ischemia induced degeneration was observed with the cresyl violet stain after 72 hours and was maximal 96 hours post-occlusion. The damage was marked along the entire CA1 pyramidal cell layer of the hippocampus.In the second study, JO 1784 provided some degree of neuroprotection as assessed by histological examination, but locomotor activity was unchanged. MK-801 (2.5 mg/kg i.p.) produced significant protection and attenuated the hyperactivity induced by BCO. JO 1784 may be a useful ligand for minimizing brain hippocampal injury due to ischemia.

673.16

POST-ISCHEMIC ADMINISTRATION OF ANIRACETAM AUGMENTS NEURONAL DAMAGE. A.K. Vinter, C.T. Nielsen, P. Jakobsen, L. Nordholm, H. Laursen, and A.J. Hansen*. Novo Nordisk A/S, Pharmaceuticals Division, Novo Nordisk Park, DK-2760 Måløv, Denmark, and Institute of Neuropathology, University of Copenhagen, DK-2100 Copenhagen, Denmark.

Compounds which block AMPA receptors have been repeatedly shown to ameliorate neuronal damage in the wake of cerebral ischemia. Stimulation of AMPA receptors by glutamate is coupled to a brief opening of an ion channel which gates an inward current. Prolongation of the channel opening is achieved by compounds which inhibit the rapid desensitization of the receptor. If AMPA receptor activation is the cause of neuronal damage, we would expect an aggrevation of the damage when the desensitization is inhibited. Rats were exposed to 10 min of global ischemia (neck-cuff inflation and hypotension). One group (n=24) received aniracetam (75 mg/kg) 15 and 45 min after ischemia, another (n=18) was given the vehicle. Seven days later, histological sections of the hippocampus CA1 region were evaluated for dead (eosinophilic), normal pyramidal nerve cells and for glial cells. The aniracetam-treated animals had significantly fewer normal nerve cells and more glial cells, whereas the number of eosinophilic nerve cells was insignificantly increased. We concluded that aniracetam - hence augmentation of the AMPA-mediated transmission in the immediate post-ischemic period - aggrevates neuronal damage after ischemia

673.17

CURATIVE EFFECT OF PIRIBEDIL, A D2 DOPAMINERGIC AGONIST, IN GERBIL CEREBRAL ISCHEMIA.

G. Delbarre*, B. Delbarre, C. Rochat and F. Calinon, F. de Médecine, 37032 Tours, Euthérapie, Neuilly sur seine, FRANCE.

Gerbil model of brain ischemia has been widely used to assess pharmacological protection from cerebral damage because of its incomplete circle of Willis in 40 to 60 %. 120 gerbils (60 males and 60 females), 8 to 10 months old, were subjected to left carotid occlusion during 60 minutes. They were selected according to Delbarre method (Delbarre, G., Stroke, 19: 26, 1988). Piribedil, (5 and 2.5 mg.kg-1, i.p.) was administered 30 or 60 min. after reperfusion. Neurological signs were evaluated as Stroke Index (SI) from 4h to 15 days after release of the clip. In acute curative treatment, Piribedil (5 mg.kg-1), did not modify the SI versus the placebo group, while at the dose of 2,5 mg.kg-1 i.p., 60 min. after reperfusion significantly improved the SI from 72h until 15 days after release of the clip and 30 min. significantly improved the SI from 24h to 72h and from 8 days to 10 days.

	4h	24h	48h	72h	96h	8 days	10 days	15 days
60 min.		Percentage of modifications versus control.						
2.5 mg.kg-1	15.24	32.00	36.16	38.92	40.29	47.00	46.33	46.02
	NS	NS	NS	*	*	**	*	*
5 mg.kg-1	-1.45	30.09	27.87	25.06	20.43	11.74	10.49	9.46
30 min.	NS	NS	NS	NS	NS	NS	NS	NS
2.5 mg.kg-1	16.98	44.68	44.12	46.09	43.92	55.76	56.89	34.94
	NS	*	*	*	NS	**	**	NS
5 mg.kg-1	14.23	33.78	20.90	24.60	16.65	9.23	12.12	-1.03
	NS	NS	NS	NS	NS	NS	NS	NS

Mann Whitney U test. $p \leq 0.05$ *. $p \leq 0.01$ **.

In acute curative treatment, Piribedil (2.5 mg.kg-1, i.p.) significantly protects from neurological signs in brain ischemia reperfusion.

673.18

NMDA RECEPTOR INDEPENDENT REDUCTION OF PROTEIN SYNTHESIS BY MK-801 MAY BE MEDIATED BY ALPHA2 ADRENERGIC RECEPTORS.

F. Dessi, Y. Ben-Ari, MP Junier* and C. Charriaut-Marlangue. INSERM U29, 123 Bld de Port-Royal, 75014 Paris, France.

MK-801 has been extensively utilised as a selective non competitive NMDA channel blocker. MK-801 is also a potent anti ischemic agent in several animal models and is as such frequently used to reduce the excitotoxic effects of glutamate. We now report that MK-801 (10 μM) inhibits [^{35}S]methionine incorporation into polypeptides by 45 ± 7.5 % (n=10) after one hour incubation in adult rat hippocampal slices and by 35 ± 10 % (n=8) in primary glial cell culture. This effect was NMDA-receptor independent since neither APV (50-500 μM, n=4), a competitive NMDA receptor antagonist, nor Mg^{2+} (10 mM, n=4) a NMDA channel blocker, reversed the inhibition of protein synthesis. $GABA_{A/B}$ (10 μM) or adenosine antagonist receptors also did not reduce protein synthesis. In contrast this inhibition is reversed by clonidine (1 μM), an α2-adrenergic receptor agonist. Selective (efaroxan, 10μM) and non-selective (phentolamine, 10μM) alpha2-adrenergic antagonists also induce an inhibition of protein synthesis. We suggest that MK-801 could act on adenylate cyclase cascade and modulate transcription. This inhibition of protein synthesis may also mediate some of the observed effects by MK-801 *in vivo*.

673.19

ENDOTHELIN-1 INDUCED MIDDLE CEREBRAL ARTERY OCCLUSION: PATHOLOGICAL CONSEQUENCES AND NEUROPROTECTIVE EFFECTS OF MK-801

J. Sharkey, S.P. Butcher* and J.S. Kelly. Fujisawa Institute of Neuroscience, and Department of Pharmacology, University of Edinburgh, Edinburgh, UK.

In the present study we utilise the potent vasoconstrictor properties of endothelin-1 (Et-1) in a model of middle cerebral artery (MCA) occlusion in the pentobarbital anaesthetized rat. We evaluate the reproducibility of the model and examine the neuroprotective efficacy of the potent anti-ischaemic agent, MK801.

Adult male SD rats received MK801 (5mg/kg, n=7) or saline vehicle (n=7) 30 mins prior to the microinjection of Et-1 (60pmols in 3μl) via a 31-g cannula stereotaxically positioned 0.5mm above the MCA (AP=0.2mm: L=5.9: V=8.5). Three days after treatment, rats were perfusion fixed, the brain removed and immersed in fixative prior to cryostat sectioning and histological staining. Sections at 8 predetermined levels were examined by light microscopy and the volume of infarction calculated .

Following administration of Et-1, saline pretreated rats exhibited a similar pattern of ischaemic damage to that previously reported following permanent occlusion of the rat middle cerebral artery (1). This pattern was characterised by a large volume of infarction in dorsal and lateral neocortex (98 ± 12mm^3) and in striatum (32 ± 3mm^3) ipsilateral to the insult. Power analysis predicted a group size of 7 would be required for a 50% reduction in ischaemic damage to be recorded as statistically significant at the 5% level. Pretreatment with MK801 resulted in significant reductions (by 51%; p=0.026) in cortical tissue damage but did not significantly alter either the pattern or the volume (33 ± 4mm^3; p=0.95) in the striatum.

The present studies show that perivascular microinjections of Endothelin-1 around the rat MCA results in a reproducible pattern of focal cerebral infarction similar to that produced by permanent occlusion of the artery and that the volume of infarction can be significantly reduced by the potent NMDA receptor antagonist MK801.

1. Tamura et al., (1981) J. Cereb. Blood Flow Metab 1:53-60.

673.20

NEUROPROTECTIVE EFFECT OF DEXTRORPHAN IN HYPOXIC SPINAL CORD.

V. Iyer, A. Schurr* and C. Young. Depts. Neurology and Anesthesiology, Univ. of Louisville, Louisville KY 40292.

Spinal cord damage may result from pressure, stretch or ischemia during spinal surgical procedures. Abnormalities of stimulus-evoked potential (SEP) can prompt measures to correct the cord damage while it is reversible. This study was undertaken to evaluate the neuroprotective effect of dextrorphan, a non-competitive NMDA antagonist during spinal cord hypoxia, using SEP model described by Iyer etal.[1]

Spinal evoked potentials (SEP) were recorded in vitro, in mouse spinal cord preparations which were subjected to hypoxia until the SEP became flat and continued for 30 minutes. The perfusate was artificial cerebrospinal fluid (ACSF) for the control and ACSF containing 100 μM of dextrorphan for the test group during hypoxia. 42% of the specimens recovered in the control and 83% in the dextrorphan group. The mean amplitude recovery was 43% of the pre-hypoxic value in the control and 92% in the dextrorphan group. It is concluded that dextrorphan has definite neuroprotective effect during spinal cord hypoxia.

[1]Iyer etal., Neurological Research 10:200,1988.

ISCHEMIA: DRUG TREATMENT II

674.1

7-CL-THIOKYNURENIC ACID: A GLYCINE ANTAGONIST AND FREE RADICAL SCAVENGER THAT REDUCES CA1 ISCHEMIC DAMAGE *IN VIVO*.

D.E.Pellegrini-Giampietro*, A.Cozzi and F.Moroni. Dipartimento di Farmacologia, Università di Firenze, Firenze, Italy.

Glutamate and oxygen free radicals are thought to cooperate in the pathogenesis of excitotoxic neuronal damage. In this study we examined whether 7-Cl-thio-kynurenic acid (7-Cl-thioKYNA), a potent antagonist at the glycine site of the NMDA receptor that also inhibits lipid peroxidation (*Eur. J. Pharmacol.* **218**, 145-151, 1992), protected CA1 pyramidal cells against postischemic damage. Sham-operated gerbils and gerbils subjected to 5 min of bilateral carotid occlusion were treated i.p. with either vehicle (0.5 ml) or 7-Cl-thioKYNA (100 mg/kg at 0, 30, 90, 180 and 270 min following reperfusion). Gerbils' temperature was maintained at 37°C throughout the surgery and for 30 min after reperfusion. Five days later, the extent of CA1 cell loss in brain sections was evaluated by toluidine blue staining. Transient bilateral carotid occlusion induced neurodegeneration of almost all (95±0.7%) CA1 pyramidal cells. 7-Cl-thioKYNA dramatically attenuated ischemia-induced CA1 cell loss to 5±2.6%: the protection was associated with a 4 degree reduction in the animals' temperature that developed between 90 and 180 min post-ischemia and lasted for several hrs. However, when the temperature was maintained at 37°C until 6 hrs following reperfusion, 7-Cl-thioKYNA still provided partial (54±11.8%) protection, indicating that the latter could not be simply ascribed to hypothermia alone. Our results indicate that glutamate receptor blockers possessing radical scavenger properties may be useful in the treatment of excitotoxic brain damage.

674.2

NOVEL GLYCINE ANTAGONISTS REDUCE NEURONAL DAMAGE IN GLOBAL AND FOCAL OF CEREBRAL ISCHEMIA MODELS.

J.B. Patel*, L. Ross, K. Siddiqui, L. Walczak, T. Bare, M. Asif and B. Duncan. ZENECA Pharmaceuticals Group, a business unit of ZENECA Inc., Wilmington, DE 19897.

Several reports indicate that the N-methyl-D-aspartic acid (NMDA) receptor complex plays a critical role as a mediator of cell damage following cerebral ischemia. It is also well established that glycine acts as a coagonist on the NMDA receptor. This study examined the ability of novel glycine antagonists, 7-chloro and 7,9-dichloro 2,3-dihydro-10-hydroxypyridazino[4,5-b] quinoline-1,4-dione (M241247 and M244249 respectively) to protect against neurodegeneration in global (GI) and focal (FI) models of cerebral ischemia.

GI was induced by bilateral carotid occlusion (10 min) in gerbils and hippocampal CA1 area damage was determined 4 days post ischemia. FI was produced by permanent occlusion of the left middle cerebral artery (MCA) and ligation of ipsilateral carotid artery in SHR rats. Infarct volume was determined 24 hrs later. In GI, M241247 (20-80 mg/kg IP) exhibited significant (>75%) reduction in neuronal damage score. Additionally, in FI, M241247 (33.0 mg/kg/hr IV infusion for 4 hrs) resulted in 22% reduction in infarct volume. M244249 at 10.0 mg/kg IP demonstrated reduction (>75%) in neuronal damage in GI and at 66.0 mg/kg/hr IV infusion for 4 hr it produced 23% reduction in infarct volume. These findings provide the first evidence to suggest that glycine antagonists are efficacious in both global and focal models of cerebral ischemia and, consequently, may be beneficial in the treatment of cerebrovascular stroke.

674.3

GLYCINE NMDA RECEPTOR ANTAGONISTS REDUCE FOCAL BUT NOT GLOBAL ISCHEMIC BRAIN DAMAGE IN THE RAT. DS Warner*, H Wu, P Ludwig, MM Todd, Neuroanesthesia Research Group, Department of Anesthesia, University of Iowa, Iowa City, IA 52242

Glycine is a co-agonist at the NMDA receptor complex. Interstitial glycine concentrations are elevated during ischemia and early reperfusion. Thus, effects of the glycine NMDA receptor antagonist, ACEA 1021, were evaluated in outcome models of cerebral ischemia. First, rats underwent 90 min of reversible MCA occlusion while awake (filament technique). Rats (n=9/group) received either 10 mg/kg or 30 mg/kg ACEA 1021 i.p. prior to, during, and 3 hrs after ischemia. Control rats received vehicle (DMSO) only. Mean±SEM cortical infarct volume (4d recovery) was reduced in a dose-dependent manner (Control=160±41; 10mg/kg=76±26; 30 mg/kg=40±26 mm^3, $p<0.05$). Subcortical infarct volume was not significantly different between groups. Neurologic scores correlated with infarct volume ($p<0.01$). Second, rats were prepared for 2-vessel occlusion forebrain ischemia (10 min). ACEA 1021 (10mg/kg or 30 mg/kg or vehicle) was administered i.p. (n=15/group) 60 min prior to ischemia, and repeated at 20 min and 180 min after reperfusion. Brains were graded for CA1 and striatal damage (5d recovery). No difference between groups was observed in either structure. ACEA 1021 caused sedation in the global ischemia experiment only. No difference between groups occurred for hemodynamic or respiratory values in either experiment. Antagonism of glycine at the NMDA receptor appears to provide protective properties similar to those previously observed for antagonists of glutamate at the NMDA receptor. To date, no psychotomimetic effects for this class of agents have been reported suggesting a potential role for glycine antagonism in stroke therapy.

674.4

PROTECTION FROM HYPOXIC NEURONAL INJURY WITH AMINOGLYCOSIDES, K.L. Panizzon*, P. Marangos & R.A. Wallis, Dept. of Neurology, UCLA and Sepulveda VAMC, Sepulveda, CA 91343, and Cypros Pharmaceuticals, San Diego, CA 92121.

Release of neurotransmitters during hypoxia and ischemia may play a key role in the development of excitotoxic neuronal injury. N-type calcium channels modulate this release, and are inhibited by aminoglycoside antibiotics. Using the hippocampal slice, we tested the neuroprotective effect of aminoglycosides upon recovery of CA1 PS activity following hypoxic exposure. We found that neomycin 1.0 mM provided significant protection against hypoxia yielding CA1 PS orthodromic recovery of 88 ± 5% of original amplitude and antidromic recovery of 94 ± 0.3%. This compared to CA1 PS orthodromic recovery of 0% and antidromic recovery of 15 ± 6% in paired slices exposed to hypoxia alone. The EC_{50} for neomycin was 573 and 458 μM for recovery of CA1 orthodromic and antidromic PS amplitude after hypoxic exposure. Hypoxic protection was also seen with gentamicin 2.5 mM which yielded recoveries of 60 ± 23% and 72 ± 16%, and with sisomicin 1.0 mM which produced recoveries of 76 ± 9% and 60 ± 15%. No significant protection was seen from streptomycin 10 mM. This order of aminoglycoside protection against hypoxia paralleled the affinity of these compounds for N-type calcium channel receptors. In receptor binding studies, neomycin, sisomicin, gentamicin and streptomycin inhibited [I^{125}]conotoxin binding with K_i's of 1.4 μM, 3.1 μM, 7.4 μM and 24.5 μM respectively. These results indicate that aminoglycosides can provide neuroprotection against hypoxic neuronal injury, and that inhibition of N-type calcium channels may represent a novel neuroprotective strategy.

674.5

EFFECTS OF BERAPROST Na (BPS) AND MK-801 ON CHRONIC CEREBRAL ISCHEMIA IN GERBIL.

S. UENO*, Y. MIYAUCHI, N. IZUMIMOTO, S. MATSUDA and T. ENDO. Basic Research Laboratories, Toray Industries Inc., Kamakura 248, Japan.

We previously reported that BPS, a new prostacyclin derivative, shows protective effects in various ischemic models. The present report describes the effects of BPS and MK-801 on chronic cerebral ischemia in gerbils. The unilateral carotid artery was repeatedly occluded (four times; total: 31min) at intervals of 24 h. The animals were given BPS (1 to 100 µg/kg, p.o.) or MK-801 (3 to 300 µg/kg, s.c.) after the first ischemic insult, and then twice daily for 4 weeks. Neuronal loss, acidophilic neurons, and progressive atrophy were observed with increasing time of reperfusion in the cerebral cortex of ischemic hemisphere. Cortical sections revealed no GFAP-positive astrocytes, while the hippocampal CA1 area showed neuronal loss accompanied by GFAP-positive astrocytes. In the controls, the area ratio and the cortical neurons ratio were 89.8±3.0% and 74.6±3.4%, respectively. BPS was found to inhibit atrophy and chronic, cortical neuronal loss in the ischemic hemisphere in a dose-dependent manner, while MK-801 showed no inhibitory effects at any dose tested. These results suggest that nature of neuronal degeneration differs the between cortical and hippocampal areas, and that BPS has a protective effect on ischemia-induced progressive atrophy.

674.6

DEPRENYL PROTECTS RAT HIPPOCAMPAL PYRAMIDAL CELLS FROM ISCHAEMIC INSULT. A.J. Barber(1)*, I.A. Paterson(1), D.L. Gelowitz(1) and C.L. Voll(2). (1)Neuropsychiatric Research Unit and (2)Dept. of Medicine, University of Saskatchewan, Saskatoon, SK, Canada S7N 0W0.

Adult male Wistar rats were given unilateral ischaemic lesions to the hippocampus by ligation of the left common carotid artery followed, 24 hrs later, by a 30 min exposure to carbon monoxide (1% mixture in air). The rats were injected with (-)deprenyl (0.25 mg/kg, s.c.) or saline just before the onset of carbon monoxide and then every day for 1, 7 or 14 days. Rats were killed by transcardiac perfusion of 4% formalin under deep anaesthesia, on the last day of drug treatment. The brains were removed, fixed, cryoprotected, frozen and 7µ coronal sections were cut and stained with thionin.

Morphological changes were observed in the pyramidal neurones at days 1 and 7 which were consistent with programmed cell death (intact neurones with cytoplasmic shrinkage). There was no difference in the survival of pyramidal neurones between the (-)deprenyl and saline treated groups after 1 and 7 days. After 14 days more CA1 and CA3 cells had survived in the (-)deprenyl treated animals (38% & 83% respectively) compared to those of the saline treated animals (5% & 47% respectively). This was statistically significant for CA1 cells ($f(1, 28)=13.34$, $p<0.001$).

These data demonstrate that (-)deprenyl can protect hippocampal pyramidal cells and increase survival of at least the cell bodies, following an ischaemic insult.

674.7

MK-801 IS BOTH AN NMDA AND A CALCIUM CHANNEL BLOCKER IN HYPOXIC HIPPOCAMPAL SLICES. A. Schurr, R.S. Payne, and B.M. Rigor*, Department of Anesthesiology, University of Louisville School of Medicine, Louisville, KY 40292

We compared the potency of two NMDA antagonists as protectants of neuronal tissue against NMDA-enhanced hypoxic damage. MK-801, a noncompetitive NMDA antagonist, protected rat hippocampal slices not only from NMDA-enhanced hypoxic damage, but also from damage elicited by hypoxia in the absence of NMDA. APV, a competitive NMDA antagonist, blocked the enhanced hypoxic damage in the presence of NMDA, but did not protect against hypoxic damage in the absence of NMDA. Two plausible mechanisms were tested to explain the difference between the two antagonists' effects: 1) MK-801, while antagonizing the NMDA receptor, also blocks hypoxic glutamate (Glu) release and thus, the activation of non-NMDA (kainate, AMPA) Glu receptors; 2) MK-801 blocks not only the voltage-dependent NMDA channel, but other voltage-dependent calcium channels as well. The first possibility was tested by perfusing hypoxic hippocampal slices with both NMDA and non-NMDA anatagonists at concentrations sufficient for a complete blockade of their corresponding receptors. A mixture of APV (100 µM), kynurenate (a mixed NMDA and kainate receptor blocker, 500 µM), and dihydroxyquinoxaline (a kainate/AMPA receptor blocker, 500 µM) did not produce the the degree of protection produced by 100-200 µM MK-801 in slices that were exposed to severe hypoxia (15 min). To test the second possibility, we reduced the level of magnesium in the medium perfusing the slices (0.2-0.5 mM) to lessen the ion's blockade of voltage-dependent calcium channels. Although the tissue sensitivity to hypoxia was increased due to the reduction in magnesium level, MK-801 still provided strong protection.

These preliminary results indicate that MK-801 blocks NMDA and other voltage-dependent calcium channels.

674.8

AMPHETAMINES PROMOTE ANATOMICAL PLASTICITY AND BEHAVIORAL RECOVERY FOLLOWING CORTICAL INFARCTION R.P. Stroemer*, T.A. Kent and C.E. Hulsebosch. Depts. of Anat. and Neurosci., Marine Biomed. Inst., Neurol., Univ. of Texas Med. Br., Galveston, TX 77550.

Amphetamine administration has been shown to accelerate recovery of behavioral dysfunction following cortical ablations. The mechanisms of this therapy are not understood. We hypothesize that the long term effects of amphetamines are due to increases in protein expression allowing for anatomical plasticity. Earlier work demonstrates the existence of axonal sprouting and synaptogenesis in the cortex of SHR rats following distal middle cerebral artery occlusion, (MCAO), using antibodies to synaptophysin, a synaptic vesicle protein, and GAP 43, a growth cone associated protein.

The present study demonstrates that one week following MCAO, amphetamine therapy (5mg/kg) combined with behavioral experience increases the GAP 43 expression in areas surrounding the infarction when compared to the vehicle treated MCAO group. The optical densities of GAP 43 immunoreactivity in the cortex surrounding the infarction were 3 times higher than contralateral cortex in the amphetamine treated group, compared to 2 times higher than control cortex in the vehicle treated group. The amphetamine treated group also demonstrates improved scores on foot faults in behavioral testing when compared to the vehicle group. This supports the hypothesis that amphetamine therapy when accompanied with physical experience increases levels of proteins necessary for anatomical plasticity. Supported by NS 11255, NS 01217, and Bristol Myers-Squibb.

674.9

NS004 IS NEUROPROTECTIVE IN RODENT MODELS OF GLOBAL AND FOCAL CEREBRAL ISCHEMIA. S.L. Moon*, L. Lombardo, J. Hibbard, B. Myers, D. Lindell and N. Meanwell[1]. CNS Neuro-pharmacology and [1]Central Chemistry; Bristol-Myers Squibb, Wallingford CT, 06492.

NS004 is an opener of calcium activated K_{maxi} channels. We have investigated the ability of NS004 to attenuate neuronal damage after cerebral ischemia.

In a model of transient global forebrain ischemia, gerbils were subjected to a 15-min. bilateral carotid occlusion. NS004 (10, 30, or 60 mg/kg, ip) or vehicle was administered at 1, 24, 48, and 72 hrs post-occlusion. Hippocampal histopathology was evaluated by means of a damage rating scale of Nissl-stained sections. Compared to vehicle-treated animals, NS004 at 60 mg/kg significantly reduced the hippocampal damage.

After permanent occlusion of the middle cerebral artery in the spontaneously hypertensive rat (SHR), a single dose of NS004 (0.3, 1, 5, and 10 mg/kg, ip) or vehicle was given at either 1, 2, or 3 hrs post-occlusion. At 24 hrs, the brains were perfused and incubated in 3% 2,3, 5-triphenyltetrazolium chloride. Infarcts were measured by means of computer-assisted planimetry. When given one hr after occlusion, 0.3 mg/kg did not alter infarct volume, however all other doses were effective. Only the 5 mg/kg dose was tested at the later time points. NS004 at 5 mg/kg significantly reduced infarct size when given 2 hrs post-occlusion, but not when administered at the 3 hr time point.

In another group of SHRs with mean arterial blood pressures ≥150 mm Hg, NS004 at 10 mg/kg, iv, had no effect blood pressure, but at 20 mg/kg, iv, mean arterial blood pressure decreased by 15% . There was no effect on heart rate at either dose.

674.10

DELAYED TREATMENT WITH THE AMPA ANTAGONIST NBQX, REDUCES EDEMA FOLLOWING FOCAL ISCHEMIA. Z.G. Huang*, D. Xue, K.E. Smith, H. Lesiuk and A.M. Buchan. Neuroscience, Loeb Research Institute, Ottawa Civic Hospital, Ottawa, Ontario, Canada K1Y 4E9, from the *Canada/Fisons Fight Stroke* program.

NBQX, a potent AMPA receptor antagonist, protects against neuronal injury following brain ischemia[1]. This study was conducted to determine whether NBQX attenuates brain edema in a well-characterized rodent model of transient middle cerebral artery occlusion (MCAO). Thirty male spontaneously hypertensive rats were subjected to 2 hours of reversible right MCAO. Ninety minutes following the onset of ischemia, saline or a known cytoprotective dose of NBQX (30 mg/kg) was administered ip. Subsequent doses were given at the time of reperfusion (RP) and 30 minutes following RP. Animals were kept normothermic throughout ischemia and RP, and sacrificed at 24 hours. Brain edema was calculated based on the differences between hemispheric volumes. Cortical infarct volume was also determined. The student T-test was used for statistical analysis.

Group (n)	Mean Volume ± SD (mm^3) Edema	Cortical Infarction
Saline (15)	45 ± 18	137 ± 24
NBQX (15)	29 ± 15*	108 ± 21**

* $p < 0.05$, ** $p < 0.01$

Treatment with NBQX significantly reduced both neocortical infarction and significantly attenuated the accumulation of edema even when administered 90 minutes after the onset of ischemia.

[1]Buchan et al., *NeuroReport*, 2:473-476, 1992.

674.11

DELAYED TREATMENT WITH THE AMPA RECEPTOR ANTAGONIST NBQX, PREVENTS ISCHEMIC NEURONAL INJURY. H. Li, K. Barnes, H. Lesiuk* and A.M. Buchan. Neuroscience, Loeb Research Institute, Ottawa Civic Hospital, Ottawa, Ontario, Canada K1Y 4E9, from the *Canada/Fisons Fight Stroke* program..

Delayed treatment with NBQX, an AMPA glutamate receptor antagonist, was tested in a 4 vessel occlusion model of severe forebrain ischemia which reliably induces hippocampal CA1 injury. Adult male Wistar rats (n=29) were subjected to 10 min of forebrain ischemia and seven days of reperfusion. NBQX (30 mg/kg) was injected IP at the time of reperfusion, or following a delay of 6, 12 or 24 hr post-ischemia. Damaged CA1 neurons of the hippocampus were counted and expressed as a percentage.

Group (n)	% CA1 Injury (Mean ±SD)
Saline (7)	79 ±16
NBQX	
immediate (6)	17 ±17*
delayed 6 hr (5)	27 ±32*
delayed 12 hr (6)	25 ±17*
delayed 24 hr (5)	50 ±28

*, $p < 0.01$ Mann-Whitney U test with Bonferoni correction

AMPA receptor blockade delayed by up to 12 hr prevents CA1 injury, suggesting a causal relationship between selective injury and Ca^{++} entry through this glutamate-regulated but ischemia-modified ionophore.

674.12

PHARMACOLOGICAL PROFILE AND NEUROPROTECTIVE ACTIVITIES OF RP 66055, A RILUZOLE DERIVATIVE, IN RODENTS. J.M. Stutzmann, S. Mignani, F. Debarnot, J. Rataud, O. Piot, C. Pauchet, P. Jimonet, M. Reibaud, C. Malgouris, A. Uzan*, J Pratt, J.C. Blanchard and M. Barreau. Rhône-Poulenc Rorer, CRVA, 94403, Vitry sur Seine, France.

RP 66055, (3-[2-[4-(4-fluorophenyl)-1-piperazinyl]ethyl]-2-imino-6-trifluoromethoxy-benzothiazoline), prepared from riluzole in a five-step synthesis, shows potent anticonvulsant properties against glutamate-induced convulsions (ED_{50} = 2.2 mg/kg ip in rats) and maximal electroshock (MES : ED_{50} = 2.3 mg/kg ip in mice). Furthermore, it shows a long duration of action, indeed after po administration its ED_{50}s at 20 hrs are only 5 or 9 times higher than those at 1 hour (glutamate : ED_{50}s = 20 and 3.85 mg/kg po respecti-vely ; MES : ED_{50}s = 6 and 0.66 mg/kg po respectively). RP 66055 protects mice against tonic hindpaw extension in the pentylenetetrazole test (ED_{50} = 3.1 mg/kg po), but not against generalized clonic seizures. RP 66055 is a very potent neuroprotective agent in models of hypoxia and ischaemia in rodents. It produces a dose-dependent increase in mean survival time in mice under hypobaric hypoxia (ED_{50} = 1.3 mg/kg ip). When administered at 4x8 mg/kg ip starting 30 minutes post bilateral carotid occlusion in the gerbils, RP 66055 reduces EEG spectrum shift towards the slow waves and hippocampal CA1 necrosis. In the rat from the low dose of 2 x 0.5 mg/kg iv; it significantly decreases the volume of cortical infarction and the neurological deficit induced by a focal and definitive ischaemia (the MCA model). Binding assays only reveal an affinity for voltage sensitive Na+ channels (IC_{50} = 0.4 μM).

674.13

POST-TREATMENT WITH THE NOVEL DEXTROMETHORPHAN ANALOG AHN-649 ATTENUATES ISCHEMIC DAMAGE TO CA1 NEURONS AND IMPROVES RECOVERY IN RATS. X-C. Lu*, L.Robles, J. Rose, C. Wingfield, M. DeCoster, A. Newman+ and F. Tortella. Walter Reed Army Inst. Res., Washington, DC 20307 and +NIDA Addiction Res. Ctr., Baltimore, MD 21224.

AHN-649 is a novel 3-substituted 17-methylmorphinan analog of dextromethorphan (DM) possessing *in vivo* anticonvulsant activity (Newman et al., J. Med. Chem 35, 1992) and *in vitro* neuroprotective properties (Tortella et al, In Press, 1993). The purpose of the present study was to determine the neuroprotective capacity of AHN-649 in an *in vivo* model of brain injury. Using a rat model of severe forebrain ischemia (15 min of 4-vessel occlusion followed by 72 h reperfusion) we now report *in vivo* neuroprotection and improved functional recovery with AHN-649 (20 mg/kg, s.c.) administered 1, 2 and 4 h post occlusion. Functional recovery was evaluated using treadmill running performance (TRP) assessed at 6, 24 and 72 h post injury. Animals were perfusion fixed 72 h post occlusion for histological quantification of CA1 damage. In saline-treated rats ischemia caused severe impairment of TRP which was most evident during the first 24 h but persisted throughout the 72 h course of study. Extensive and consistent loss of pyramidal CA1 neurons was measured (75 ± 9% damage). Post-treatment with AHN-649 significantly attenuated the ischemia-induced CA1 injury (21 ± 11% damage). TRP was significantly improved at 6 h, and normal by 24 h, post-injury. DM (20 mg/kg, s.c.) was less neuroprotective (44 ± 14% damage), and comparisons of TRP indicate that it may be less effective, than AHN-649 in improving functional recovery, particularly during the early (6-24 h) stages post injury. Thus, AHN-649 appears to represent a new class of neuroprotective agents.

674.14

THE EFFECTS OF CGS-19755 IN RAT FOCAL CEREBRAL ISCHEMIA PRODUCED BY TANDEM IPSILATERAL COMMON CAROTID ARTERY AND MIDDLE CEREBRAL ARTERY OCCLUSION. J.T. Simmonds, T.L. Sailer, and J.A. Moyer*. CNS Division, Wyeth-Ayerst Research, CN 8000, Princeton, NJ 08543-8000.

It has been previously reported that CGS-19755 produces a 50% decrease in ischemic damage by modified proximal middle cerebral artery (MCA) occlusion (Simon, 1990) and a 28% decrease in a modified distal MCA occlusion model of focal ischemia (Takizawa, 1991). As a means of establishing a pharmacological model for evaluating known and novel EAAA (excitatory amino acid antagonists), ligation of the right common carotid artery and occlusion of the distal MCA was performed. In the present studies, male Fischer-344 (290-310 g) rats were administered a 10 mg/kg i.v. bolus of CGS-19755 five minutes post-occlusion and were sacrificed 24 hours later. Infarct volumes were determined by area measurements on every tenth section through the lesion by image analysis. Body temperatures were taken every hour out to 4 hours post-occlusion. Hemiparesis was recorded as present if the animals exhibited forelimb flexion within the first 4 hours following occlusion. In the initial study, infarct volumes for saline and CGS-19755 treated groups were 45.7 mm^3 and 21.9 mm^3, respectively. CGS-19755 also produced a 50% decrease in infarct volume (61.3 mm^3) as compared to the saline control group (31.5 mm^3) in a second study. In both studies, the body temperatures of the CGS-19755 group at the 2, 3 and 4 hour timepoints were significantly different from the saline control. Although significant, hypothermia produced was approximately 1° or less. Forelimb flexion was significantly reduced in both studies by CGS-19755. These results provide evidence that the focal ischemia produced by right common carotid artery and MCA occlusion provides reproducible results. These studies also replicate reduction in infarct volume produced by CGS-19755 in other focal ischemia models.

674.15

NEUROPROTECTIVE EFFECT OF U69593 AND DEXTRO-METHORPHAN IN A MOUSE MODEL OF PERMANENT FOCAL CEREBRAL ISCHEMIA. I. P. Karatchounova# and G. T. Pryor. Neuroscience Department, SRI International, Menlo Park, CA 94025.

U69593, a κ-opioid agonist, and dextromethorphan (DM), a (+)-opioid with affinity at the PCP/MK-801 channel site of the NMDA receptor complex, have been reported to reduce ischemic neuronal injury following transient global ischemia and infarct volumes following focal ischemia. To confirm further these neuroprotective effects, we tested them in a mouse model of permanent middle cerebral artery occlusion (MCAO) that we were establishing for use in screening new neuroprotective compounds. The left middle cerebral artery was occluded in 37 adult male Swiss-Webster mice (28-32 g) according to Welsh et al. (J. Neurochem. 49:846-851, 1987), anesthetized with tribromo-ethanol (600 mg/kg). Rectal temperature was monitored and maintained at 37 ±1°C throughout the surgical procedure and up to 120 min after induction of ischemia.U69593 (20 mg/kg) or DM(20 mg/kg)were injected i.p. in saline 5 min, 1 hr, and 2 hr after cauterization. All mice were sacrificed after 48 hr and per-fused transcardially with a suspension of carbon black. Cortical infarct areas were digitized using a Quantex image analyzing system. The mean (±SEM) infarct area in the saline-treated mice (n=18) was 25.7 (±8.8) mm^2 compared with 17.7 (±3.4) and 12.9 (±3.7) in the mice treated with DM and U69593, respectively ($p<0.05$). Our data confirm that U69593 and DM significantly alleviate ischemic cell damage and further support a potential therapeutic use for these compounds in cerebral ischemia.

674.16

DIAZEPAM IS NEUROPROTECTIVE AND PRESERVES $GABA_A$ RECEPTORS IN STRIATUM BUT NOT IN CEREBRAL CORTEX FOLLOWING TRANSIENT GLOBAL ISCHEMIA IN THE RAT. Xiao Yu, Huiling Li and Rochelle D. Schwartz# Department of Pharmacology, Duke University School of Medicine, Durham, NC. 27710.

We studied the role of enhancing $GABA_A$ neurotransmission following a moderately severe ischemic insult. Rats were subjected to 15 min of ischemia using the 4-vessel occlusion technique. Diazepam was administered systemically 1 hr and again 2 hrs following the onset of reperfusion. Four days later the rats were sacrificed and the brains were prepared for histologic analysis, receptor autoradiographic analysis of [^{35}S]TBPS binding to the GABA-gated chloride channel, and in situ hybridization of $GABA_A$ receptor subunit mRNAs. Histological analysis of the dorsolateral striatum from 7 rats revealed that neuroprotection was complete in 5 rats, 75% protection was acheived in 1 rat and no protection was achieved in 1 rat. In receptor autoradiography studies, [^{35}S]TBPS binding was reduced by 75% in the dorsolateral striatum of occluded rats (n=6) and by 33% in the diazepam-treated rats. [^{35}S]TBPS binding in the somatosensory cortex (layers 1-4) was reduced by 25-33% in occluded rats; the diazepam treatment had no effect on cortical damage. The enhancement of GABAergic neurotransmission at a critical time following an ischemic insult suggests that loss of GABAergic neurotransmission may contribute to ischemia-induced neuronal injury.

(Supported by PHS Grant NS 28791)

674.17

α-TOCOPHEROL DEPLETION OF PERIPHERAL NERVE WORSENS DIABETIC NEUROPATHY. K. K. Nickander, J. D. Schmelzer* and P. A. Low. Neurophysiology Laboratory, Department of Neurology, Mayo Clinic, Rochester, MN 55905

In streptozotocin (STZ) induced diabetic neuropathy, endoneurial hypoxia is present and ischemia is associated with reduced endoneurial superoxide dismutase, norepinephrine, and increased endoneurial conjugated dienes. To evaluate the importance of endoneurial α-tocopherol on severity of neuropathy, we performed nerve conduction and blood-nerve barrier (BNB) studies in 4 groups of rats at 1 and 3 months of diabetes: (1) normal α-tocopherol Controls (CONT-N); (2) α-tocopherol deficient Controls (CONT-def); (3) normal α-tocopherol Diabetics (STZ-N); (4) α-tocopherol deficient Diabetics (STZ-def). Whole nerve α-tocopherol was significantly increased in STZ-N ($p<0.001$). α-Tocopherol depletion significantly induced or worsened both amplitudes and conduction velocities of both sciatic-tibial and caudal nerves. Sciatic-tibial motor nerve conduction velocity (CONT-def vs CONT-N, $p<0.001$; STZ-def vs STZ-N, $p<0.001$), compound nerve action potential (STZ-def vs STZ-N, $p<0.01$), caudal conduction velocity (STZ-def vs STZ-N, $p<0.001$), and caudal nerve action potential (CONT-def vs CONT-N, $p<0.01$; STZ-def vs STZ-N, $p<0.001$) all showed significant abnormalities. The BNB indices were not worsened by α-tocopherol depletion. We suggest that the increase in α-tocopherol is compensatory and these findings support the notion that oxidative stress may cause neuropathy and worsen experimental diabetic neuropathy.

ISCHEMIA: GLIA

675.1

INHIBITION OF ISCHEMIA-INDUCED GLUTAMATE RELEASE IN GERBILS BY AN ANION TRANSPORT INHIBITOR (L-644,711) P.J.Feustel, L.Rodriguez, R.W.Keller,Jr., A.M.Snyder-Keller+, K.Barron, A.J.Popp and H.K.Kimelberg. Albany Medical College, Albany, NY 12208; and +NY State Dept of Health, Wadsworth Ctr, Albany, NY 12201

We have previously shown that an anion transport inhibitor (L-644,711) will inhibit swelling induced release of glutamate and aspartate from primary astrocyte cultures *in vitro* (see abst. by Kimelberg et al.,this vol.; and Kimelberg et al.,*J.Neurosci.*,1990). Swelling of astrocytes is well-known to occur in ischemia, and therefore it is possible that some of the aspartate and glutamate release seen during ischemia could be coming from swollen astrocytes. Ten minute bilateral carotid occlusion of gerbils, with microdialysis probes implanted bilaterally into the dorsal hippocampus was used as the ischemia model. L-644,711 was infused at varying concentrations in one probe and the other served as the contralateral control. A relatively low dose of the drug (0.5mM) produced no basal changes in glutamate release but did inhibit the glutamate release seen during carotid occlusion by 70-80%. A decrease in glutamate levels during reperfusion was unaffected. At higher concentrations (1 or 5mM), the drug had no significant effect on glutamate or aspartate or caused increased levels of aspartate. At these concentrations of drug decreases in extracellular glutamine were observed. These data are consistent with work *in vitro* that showed that lower concentrations of L-644,711 inhibited cell swelling-induced release of glutamate from primary astrocyte cultures (Bednar et al., *Neurol.Res.*14:53-56, 1992), but at higher concentrations glutamate uptake was inhibited. Preliminary studies also indicate that in the region adjacent to the probe containing L-644,711 there was less induction of *c-fos* than on the control side.(Supported by NS 30303).

675.2

O2A PROGENITOR CELLS DO NOT TOLERATE HYPERTHERMIC AND ISCHEMIA-RELATED STRESSES AS READILY AS TYPE-1 ASTROCYTES. B.H.J. Juurlink*. Department of Anatomy & The Saskatchewan Stroke Research Centre, University of Saskatchewan, Saskatoon, SK, CANADA S7N 0W0

I have previously demonstrated that type-2 astrocytes have little ability to tolerate elevated temperature. The objective of this study was to examine the ability of O2A progenitor cells to tolerate stresses in the form of elevated temperature, hypoxia and ischemia. Experiments were performed on rat mixed glial cultures whose major populations consisted of type-1 astrocytes (T1As) and O2A progenitor cells (O2As). To examine the ability to withstand elevated temperature, cultures were subjected to 43 °C and returned to 37 °C for recovery. Although T1As could readily tolerate 6 hr of hyperthermia, the majority of O2As died during the recovery period if exposed to more than 1 hr of hyperthermia. As little as two hours of simulated ischemia resulted in the death of ~90% of the O2As during the first 24 hr of recovery whereas no death was observed amongst the T1As; however, following this period there was an increase in the rate of proliferation of the surviving O2As such that the cultures became repopulated by large numbers of O2As during days 2 and 3 following the ischemic insult. Similar results were observed following 24 hr of hypoxia in the presence of substrate. In conclusion, O2As appear to have a much lower ability to tolerate environmental perturbations than T1As. *I thank the Ralston Brothers Medical Research Foundation and the Melfort Memorial Hospital for supporting this research.*

675.3

CELLULAR LOCALIZATION OF QUINOLINATE FOLLOWING TRANSIENT FOREBRAIN ISCHEMIA. KJ Blinder[1], JR Moffett[1*], K Saito[2], K Suyama[3], MP Heyes[2] and MAA Namboodiri[1]. Dept of Biol.[1], Georgetown U., Washington, DC 20057; Sect. on Anal. Biochem.[2], Lab. of Clin. Sci., NIMH, Bethesda, MD 20892; Clinical Pharmacology[3], NIMH, Bethesda, MD 20892

Localized neurodegeneration of the hippocampus can be produced in the Mongolian gerbil (Meriones unguiculatus) by transient occlusion of both common carotid arteries. This lesion is considered a model of delayed-onset neurodegeneration in stroke. Excitotoxicity has been implicated in such ischemic brain damage. Quinolinic acid, a known NMDA agonist, is normally present in the brain in only nanomolar amounts, but is found in the brain in potentially excitotoxic concentrations in many acute inflammatory brain conditions, including ischemic brain damage. The source of the QUIN is not known. It has been hypothesized that the QUIN may be produced by activated resident or infiltrating macrophages.

We now report QUIN staining in microglia-like cells of the CA1 region of the hippocampus four days after the induction of transient forebrain ischemia in adult gerbils by 5 or 10 minutes occlusion of both common carotid arteries. Four days after the ischemic insult, the animals were sacrificed by intracardiac perfusion with a carbodiimide fixative to assure retention of soluble QUIN. Brain immunohistochemistry was done on using rabbit polyclonal antiserum against protein-conjugated QUIN, purified by extensive blocking with potential cross-reactants.

Degenerative changes were apparent in the hippocampus, in the pyramidal cell layer of CA1, which showed extensive neuronal loss. QUIN-positive cells of microglial morphology were numerous in the CA1 pyramidal cell layer, but not noted elsewhere. Sham-operated controls had no CA1 degeneration, and no QUIN-positive cells in CA1. This finding suggests that microglia may serve as a source of the potential excitotoxin, QUIN, in delayed-onset ischemic brain damage.

675.4

REGIONAL ACCUMULATION OF MACROPHAGE/MICROGLIA AFTER HYPOXIC-ISCHEMIC (HI) BRAIN INJURY IN PERINATAL RODENTS. JA Shubitowski, R Sun, FS Silverstein*. University of Michigan, Ann Arbor, MI 48109.

Macrophages (MØ) and microglia (MCG) may play important roles in CNS development, and may also contribute to neuronal injury after diverse CNS insults. To begin to study the role of MØ/MCG in perinatal CNS injury, we used 2 complementary detection methods to visualize their regional distributions after a focal HI insult in 7 day old (P7) rats. To elicit injury, P7 rats underwent right carotid ligation followed by 3h 8% O_2 exposure; rats were sacrificed 1-5 days later (n=3/group). A histochemical assay using Griffonia simplicifolia B4-isolectin enabled detection of activated MCG; MØ and vascular cells were also reactive. MØ were identified immunocytochemically using a MØ-specific monoclonal antibody, ED1 (which also labeled some MCG). In normal P7-12 brain MØ and MCG were concentrated in white matter. After HI, in the lesioned hemisphere, lectin staining of MCG peaked at 48h post-injury; reactive MCG were concentrated in cortex (C), hippocampus (HIP) (in particular CA1 and CA3), striatum (STR), septum (SEP), and habenula. MØ accumulation peaked 1-2 days post-injury, with prominent collections of immunoreactive cells in C, HIP, STR, and SEP. In brain sections in which no neuronal injury was evident, no MCG or MØ accumulation was detected. Yet, MØ and MCG did not accumulate uniformly in all regions where neuronal injury was apparent. Perinatal HI brain injury induced anatomically and temporally distinct patterns of MØ and MCG accumulation; these cells may be important mediators of progressive neuronal injury.

675.5

POTASSIUM HOMEOSTASIS IN ENERGY-DEPRIVED ASTROCYTES. W. Walz.* Dept. of Physiology, Univ. of Saskatchewan, Saskatoon S7N 0W0, Canada.

One of the earliest events of an ischemic episode is an increase of the extracellular K^+ concentration to 30-80mM, which is followed by astrocytic swelling. The pathophysiological relevance of the phenomena was investigated with the use of ion-sensitive microelectrodes in primary cultures of type-1-like cortical mouse astrocytes. If these astrocytes are exposed to saline containing 60mM K^+ their K^+ concentration increases by about 40mM accompanied by Cl^- and HCO_3^- increases as well as swelling. If the cells are exposed to the metabolic inhibitors sodiumfluoride and antimycin a they exhibit a slow depolarization that is caused by loss of intracellular K^+ ($E_M = E_K$). If such a depolarized astrocyte was exposed to 60mM K^+ the intracellular K^+ concentration increased rapidly to a level higher than the previous resting value. This increase was independent of the degree of depolarization and amount of K^+ left in the cells. Replacement of HCO_3^- and Cl^- by glucuronate and HEPES did not change these responses. The K^+ accumulation of metabolically intact cells was not inhibited by ouabain application. Thus, astrocytes possess a rapid K^+ uptake system that is energy-independent and is bound to play a role in cytotoxic swelling following metabolic interruption. A Boyle-Conway mediated mechanism involving voltage-dependent anion channels is not likely to be involved.

ISCHEMIA: HEAT SHOCK PROTEIN

676.1

Cloning of a novel hsp70 family member and its expression in brain ischemia. S.M. Massa,* F.M. Longo, S. Wang, J. Zuo and F.R. Sharp, Dept. of Neurology Univ. of California and SFVAMC, San Francisco, CA 94121

Stress proteins, including the heat shock (hsps) and glucose-regulated proteins (grps), are induced in the brain in response to ischemia, seizure, trauma and other stresses. The induction of these proteins has been hypothesized to protect the brain against subsequent more severe (supralethal) stresses. However, expression of the well characterized stress protein family members does not completely account for the observed protective effects. Since the involvement of undiscovered and uncharacterized members of these families has been hypothesized a search for such members was undertaken.

A fragment of a novel gene (hsr1, hsp70 related sequence) was generated from mRNA isolated from rat brain subjected to kainate-induced status epilepticus, using degenerate primer-PCR and screening to exclude known family members. Northern analysis revealed a single approximately 3.5 kb mRNA species in normal rat brain; slot blot analysis of mRNA derived from whole brain hemispheres subjected to 1 hr. of middle cerebral artery occlusion followed by 8 hrs of reperfusion, or kainate treatment, suggested the quantity of hsr1 message was slightly increased (1.2-1.3 fold) after injury. Database comparison of the amino acid sequence encoded by the longest open reading frame of the hsr1 fragment revealed many highly similar sequences, including the presumptive mitochondrial protein *c. elegans* hsp70f (73% identity), mitochondrial proteins *S. cerivisiae* SSC1 (64%) and *P. sativum* hsp70 (60%), and *E. coli* dnaK(56 %). Hsr1 is less similar to hsp70 (33%), hsc73 (37%) and grp78 (41%) but like grp78, lacks the nuclear localization consensus sequence found in the cytoplasmic hsp70 and hsc73. The relationship of hsr1 to mitochondrial hsps in other species suggests the possibility that hsr1 is closely related or identical to grp75 (also called mhsp70), a vertebrate mitochondrial hsp70 family member.

676.2

INDUCTION OF HEAT SHOCK PROTEIN IN CINGULATE AND RETROSPLENIAL CORTEX BY ANTI-ISCHEMIC AGENTS. R.P.Simon, J.Q.Lan, J.Chen, D.Ferriero*, F.R.Sharp, S.H.Graham. Department of Neurology, University of California, San Francisco 94143.

NMDA antagonists injure neurons in layers 2 and 3 of both cingulate and retrosplenial cortex, resulting in induction of heat shock protein (HSP70). The purpose of this study is to determine if alternative means of blocking excitotoxicity also induce HSP expression in these regions.

Five agents were studied in 74 rats: CGS19755 (10, 20, 30mg/kg) a competitive NMDA antagonist; 7-chlorothiokynurenic acid (10, 20, 30 or 50mg/kg) a glycine site antagonist; NBQX (3, 10 or 30 mg/kg) an AMPA-kainate antagonist; and the lamotrigine congeners, BW1003 (6, 20, or 50mg/kg) and BW619 (1, 10, 20, 30 or 50mg/kg), which block excitotoxicity by reducing glutamate release. MK801 (1mg/kg) (n=50) was used as a positive control in all groups. Brains were removed and sectioned for HSP70 immunocytochemistry 24 hrs after i.p. injection of drug.

CGS19755 induced HSP70 in cingulate and retrosplenial cortex at doses as low as 20mg/kg (3/12 rats expressed HSP70), but not 10mg/kg (0/8 rats). The effect was maximal at 30mg/kg (4/6 rats). 7-chlorothiokynurenic acid, NBQX, BW1003, and BW619 did not induce HSP70 at any dose tested. These results suggest that alternative approaches to ameliorating excitotoxicity may not share the neurocytopathologic toxicity of competitive and noncompetitive NMDA antagonists.

676.3

EXPRESSION OF HEME OXYGENASE, A CNS STRESS PROTEIN, IN E. COLI. B.E. Dwyer*, R.N. Nishimura, T. Yoshida. VA Medical Center, Sepulveda, CA 91343, USA.

Heme oxygenase catalyzes the rate-limiting step in the degradation of heme to biliverdin and ultimately bilirubin. Heme oxygenase is also a stress protein in many cell types including cells in the CNS. Rat heme oxygenase-1 was expressed in E. coli using the QIAexpress system (Qiagen, Inc). A DNA fragment comprising the coding region of the rat heme oxygenase gene (kindly supplied in plasmid pRHO1 by Dr. Shigeki Shibahara) was amplified by PCR and subcloned into plasmid QE-30 (Qiagen) to give pQE-30/HO-1. The coding region of rat heme oxygenase-2 gene was amplified from rat testes polyA$^+$ by PCR and subcloned into QE-30 to give pQE-30/HO-2. pQE-30/HO-1 and HO-2 were used to transform M15p[rep4] cells and the synthesis of heme oxygenase-1 and 2, induced by addition of IPTG, was verified by gel electrophoresis/autoradiography and Western blotting. Probes based on full length sequences confirm that heme oxygenase-1 is a glial heat shock protein in vitro. Neuronal induction of heme oxygenase-1 is weak.

Supported by the Research Service of the Department of Veterans' Affairs, the United Cerebral Palsy Research and Educational Foundation, Inc., and the American Heart Assn.

676.4

DEEP HYPOTHERMIA REDUCES POSTISCHEMIC HSP72 EXPRESSION IN PIGLET BRAIN. E.G. Shaver, L.N. Sutton, F.A. Welsh*, G. Mora, L.M. Gennarelli, C.R. Norwood, Div. of Neurosurgery, Univ. of Penn. Sch. of Med., Philadelphia, PA 19104.

The 72 kDa heat-shock protein, hsp72, has been induced in previous models of cerebral ischemia. Further, severe ischemia models which deplete ATP usually demonstrate hsp72 expression. The mechanism which triggers hsp72 induction is not clear. The objective of this investigation was to determine the ability of deep hypothermia (15°C) to alter the expression of hsp72 following severe cerebral ischemia.

Three-week-old piglets were placed on cardiopulmonary bypass and subjected to 60 min of cardiac arrest at 15°C or 24°C, followed by 2h of normothermic reperfusion. Regional expression of hsp72 mRNA was determined using in situ hybridization, and calculated as a percentage of total area of cortex. In the 24°C group, hsp72 was induced in a patchy pattern throughout most of the cerebral cortex (67± 22%, N=9). In the 15°C group, the percentage of hsp72 expression was significantly reduced (23± 20%, N=9). Hsp72 expression was patchy in both groups with no apparent anatomic localization.

This study demonstrates that hsp72 mRNA expression is strongly increased after global ischemia at 24°C, and the expression is significantly decreased if the temperature is lowered to 15°C. We speculate that deep hypothermia reduces cell stress below the threshhold required to trigger hsp72 expression, despite depletion of ATP.

676.5

HSP72 AND UBIQUITIN CONJUGATE EXPRESSION IN THE POSTISCHEMIC AND POSTHYPOGLYCEMIC RAT BRAIN. INFLUENCE OF HYPOTHERMIA AND NBQX. Kerstin Bergstedt* and Tadeusz Wieloch, Laboratory for Experimental Brain Research, Lund Hospital, Lund University, S-221 85 Lund, Sweden.

HSP72 expression and ubiquitin conjugate immunoreactivity (UIR) was studied in the postischemic brain. HSP72 was observed at 24 hrs following ischemia in the hippocampus and neocortex layer IV, in both saline and NBQX treated rats. Two days postischemia, HSP72 IR was evident in the CA3 region in saline treated rats, but not in the degenerated CA1 region. In NBQX and hypothermia treated rats HSP72 was seen in the CA1 but not in the CA3 region.The UIR was absent in both CA1 and CA3 region at 24 hrs following normothermic ischemia, but was less depressed in the hypothermic animals. An increase in UIR was seen at 2 and 3 days following normothermic ischemia in the CA3 region and in the CA1 region in the hypothermic animals. Blockade of the AMPA receptor stimulated recovery of UIR in the CA1 region at 2 and 3 days postischemia. A functional ubiquitin system may be essential for the neuronal survival following ischemia. A defect in ubiquitination may cause an accumulation of damaged or defective proteins and lead to sequestration of HSP's into large protein aggregates, depleting the vulnerable neurons from the protective influence of HSP's. Expression of HSP72 despite AMPA-receptor blockade suggests that the receptor blockade dampens the postischemic stress on vulnerable neurons. Following insulin-induced hypoglycemia a delayed and expression of HSP72 in surviving neurons, which may be protective. Supported by the Swedish MRC.

676.6

ENHANCEMENT OF hsp70 AND c-fos mRNA EXPRESSION FOLLOWING TRANSIENT FOCAL CEREBRAL ISCHEMIA IN TRANSGENIC MICE OVEREXPRESSING CuZn-SUPEROXIDE DISMUTASE. H. Kamii, H. Kinouchi, J. Koistinaho, F. R. Sharp, C. J. Epstein, S. F. Chen*, and P. H. Chan. Depts. of Neurology, Neurosurgery, and Pediatrics, Univ. of California at Sun Francisco and Dept. of Neurology, VA Medical Center, San Francisco, CA 94143.

A heat shock gene hsp70 and a proto-oncogen c-fos are activated by a variety of brain injuries including cerebral ischemia/reperfusion. Recently, these gene expressions are suggested to contribute to part of a survival mechanism. It is not known, however, whether oxidative stress produced by ischemia/reperfusion plays a role in these gene expressions. The purpose of the present study is to investigate the involvement of oxidative stress in these gene expressions following transient focal cerebral ischemia, using transgenic (Tg) mice overexpressing CuZn-superoxide dismutase (SOD). Male Tg mice and nontransgenic (nTg) littermates (30-35 g) were subjected to focal cerebral ischemia and reperfusion. After 10 min of middle cerebral artery occlusion, the mice were allowed variable periods of recovery up to 7 days. At each time point, the mice, both nTg and Tg (n=3 each), were decapitated and the brains were processed for in situ hybridization with a [^{35}S]-labeled hsp70 or c-fos oligonucleotide probe. After the hybridization experiments, the sections were counter-stained with hematoxylin and eosin for histological examinations. In the ischemic cortex, expression of hsp70 mRNA was observed only up to 6 h after reperfusion in nTg mice, while it was seen up to 24 h in Tg mice. In the hippocampus, nTg mice showed the expression only at 1 h post reperfusion, whereas Tg mice revealed it up to 24 h with an intensive expression in the CA1 subfield. Likewise, prolonged expression of c-fos mRNA was observed in the hippocampus of Tg mice at up to 6 h, whereas it was observed at up to 1 h in nTg mice. There was no observable infarction for up to 7 days in both nTg and Tg mice. These results suggest that oxidative stress, along with other factors, affects the expression of hsp70 and c-fos mRNA following transient focal cerebral ischemia. We speculate that enhancement of these gene expressions in Tg mice may be associated with neuronal protection against ischemia/reperfusion injury.

676.7

POST-ISCHEMIC DEPOLARIZATION THRESHOLD FOR HSP72 FOS, AND JUN INDUCTION IN RATS. I.A. Halaby*, Y. Takeda, T.S. Nowak, Jr. and W.A. Pulsinelli. Dept. of Neurology, University of Tennessee, Memphis, TN 38163.

Following short periods of ischemia, HSP72 as well as the immediate early gene products, Fos and Jun, are induced in hippocampal neurons. Brief depolarizations are known to induce c-fos but not HSP72 in a number of experimental paradigms, and the mechanisms underlying the co-induction of these mRNAs after ischemia remain to be defined. In the present study, we examined the correlation between post-ischemic depolarization and the induction of HSP72, Fos and Jun. Extracellular electrodes were stereotactically positioned in CA1 and cortex of male Wistar rats, and forebrain ischemia was achieved by 4 vessel occlusion for 1 to 5 min. Brain and body temperature were maintained within physiological limits before, during, and after ischemia. At 3 h recirculation, animals were killed and consecutive frozen sections prepared for analysis. HSP72 mRNA was detected by *in situ* hybridization with a ^{35}S labelled oligonucleotide probe and the results quantitated by densitometry. Fos and Jun proteins were detected by immunocytochemistry using polyclonal antibodies (Oncogene Science).The length of post-ischemic depolarization varied for ischemic insults of apparently equivalent duration. Depolarization time was the better predictor of HSP72 and Jun expression with an apparent threshold of 2.5 min. Preliminary results identified a threshold of 1.5 min depolarization for Fos expression. These results implicate depolarization as a critical variable affecting gene expression after ischemia, and suggest that different thresholds may exist for Fos vs. Jun and HSP72 induction.

676.8

PROTEIN SYNTHESIS AND EXPRESSION OF HSP72, FOS AND JUN AFTER TRANSIENT FOREBRAIN ISCHEMIA IN THE RAT. M. Okawa, I. Halaby, T.S. Nowak, Jr. and W. A. Pulsinelli* Dept. of Neurology, University of Tennessee, Memphis, TN 38163

Hsp72, c-fos and c-jun mRNAs are induced in brain after ischemia but their translation is impaired in vulnerable CA1 neurons in the gerbil. In this study, we investigated translational recovery and expression of proteins encoded by induced mRNAs after ischemia in the rat. Male Wistar rats were subjected to 2 min or 10 min 4-vessel occlusion. L-1-[^{14}C]leucine (25 μCi) was given i.v. at various recirculation intervals, and incorporation measured by autoradiography of frozen sections (20 μm) extracted with 5% trichloroacetic acid. Other rats were perfused with 4% paraformaldehyde, and 50 μm vibratome sections processed for Fos, Jun and hsp72 immunoreactivity. After 10 min ischemia protein synthesis recovered progressively except in CA1, where a persistent 50% deficit remained at 24 h after the insult. CA3 showed more severe inhibition during early recirculation, reaching 25% of control at 1 h vs. 40% in CA1. This early selective deficit in CA3 was not seen after 2 min ischemia and recovery was almost complete in all regions at 24 h. Fos staining in CA3 was prominent after 2 min but not after 10 min ischemia, while CA1 Fos staining was more pronounced after 10 min vs. 2 min ischemia. Jun and hsp72 were detected in CA1 24 h after both insults, while CA3 staining was prominent after 10 min but not after 2 min ischemia. Thus protein synthesis recovery limits Fos expression in CA3 neurons that survive after 10 min ischemia, while there is sufficient recovery in vulnerable neurons of CA1 to allow Fos accumulation. It appears there is no simple relationship between expression of these induced proteins and neuronal survival after ischemia.

676.9

PROTEIN SYNTHESIS THRESHOLD FOR FOS EXPRESSION FOLLOWING TRANSIENT FOCAL ISCHEMIA IN RAT BRAIN. T. Kamiya, T. S. Nowak, Jr., M. Jacewicz* and W. A. Pulsinelli, Dept. of Neurology, Univ. of Tennessee, Memphis, TN 38163

The mRNA encoding the proto-oncogene c-fos is expressed in regions of a focally ischemic hemisphere in rats in which residual CBF remains above 40-50 ml/100g/min (30-40% of control). Cerebral protein synthesis begins to fail at somewhat higher residual blood flows (55-60 ml/100g/min or 50% of control). The present study investigated the relationship between protein synthesis recovery and expression of Fos protein after transient focal ischemia. The right middle cerebral artery (MCA) and common carotid artery were reversibly occluded for 1 h (n=4) or 3 h (n=3) in spontaneously hypertensive rats (1% halothane anesthesia, mechanical ventilation). After 1 h recirculation, 25 μCi L-1-[^{14}C]leucine was infused intravenously and an additional 1 hr elapsed for radiolabeling cerebral proteins. Frozen brain sections (20 μm) were collected on glass slides, extracted with 5% trichloroacetic acid, and imaged on Kodak SB-5 film. Regional [^{14}C] incorporation into protein was determined by densitometry and expressed in the ischemic hemisphere as a percent of the average [^{14}C] incorporation in the nonischemic hemisphere. Fos was detected in adjacent brain sections using a commercial antibody (Oncogene Science). Protein synthesis was severely reduced in the MCA territory after both 1 h and 3 h of ischemia. Fos immunoreactivity was not detected in cortex with less than 30% of control [^{14}C] incorporation, was faintly detectable in cortex with 35-50% of control, and was striking in regions with above 50% of control. These results demonstrate that the lack of translational recovery may limit the expression of proteins encoded by induced mRNAs after focal ischemia.

676.10

HEAT PRETREATMENT CAN PROTECT ASTROCYTES IN CULTURE FROM OXYGEN GLUCOSE DEPRIVATION INJURY. R.G. Giffard, S.M. Amagasu, V.M.G. Bruno, J. Cao* and A.J. Giaccia. Depts. of Anesthesia and Radiation Oncology, Stanford University School of Medicine, Stanford CA 94305

Heat and other stresses are known to induce production of heat shock proteins (hsps). Prior treatment with heat has been shown to protect many cell types from subsequent heat injury and retina from subsequent laser injury. To better understand the role of hsps in such protection we characterized the heat tolerance of primary cultures of astrocytes from mouse cortex to heat, and then determined their sensitivity to oxygen glucose deprivation (OGD) with or without prior heat treatment. An insult of about 2 hr at 44°C led to loss of essentially all the astrocytes, while durations of 15-30 min produced essentially no cell loss. Injury was quantitated by release of lactate dehydrogenase. Brief heat treatment induced production of hsp70, the inducible form of the 70 kd hsp, as detected by immunohistochemical staining (Amersham RPN 1197) 18 hr after heating. Astrocytes heated at 44°C for 30 min and allowed to recover at 37°C for 16-20 hr showed a significant reduction in injury due to 10 or 12 hr of OGD compared to sham washed sister cultures subjected to OGD. These results demonstrate sublethal heat pretreatment reduces astrocyte injury due to subsequent OGD.

Supported in part by NS 01425

676.11

HSP70 HEAT SHOCK GENE IN ISCHEMIC RAT BRAIN: CLONING, SEQUENCING AND CHARACTERIZATION. P.Narasimhan*, F.L.Longo, S.Wang and F.R.Sharp. Department of Neurology, Univ. of California and SFVAMC, San Francisco, CA 94121.

Heat shock genese are induced following ischemia and other injuries in mammalian brain. Five human and six mouse inducible hsp70 genes have been characterized. In this work, a rat ischemic forebrain cDNA library was screened with a reverse transcriptase PCR product from ischemic rat brain RNA to obtain an inducible rat hsp70 gene. The 1926 nucleotides in the open-reading frame of this gene codes for a 642 amino acid protein. Rhe complete coding sequence of the rat hsp70 gene is most similar to the mouse hsp70 gene reported by Hunt and Calderwood (1990) and the human hsp70 gene characterized by Hunt and Morimoto (1985) with atleast 90% similarity at the amino acid level. The rat hsp70 gene is distinct from the constitutive rat hsc73 and grp78 sequences. Northern blotting of rat ischemic brain RNA shows the rat hsp70 gene as a 2.9kb primary transcript. The rat hsp70 gene also showed low levels of basal expression in normal rat brain in northern analysis. This is consistent with similar expression reported by Hunt and Morimoto (1985). The cloning of the rat hsp70 gene should help in designing probes to study the other rat hsp genes in the CNS and help in defining their role in brain injury.

676.12

IDENTIFICATION BY DIFFERENTIAL SCREENING OF HEAT SHOCK PROTEIN-86 INDUCED IN GERBIL HIPPOCAMPUS FOLLOWING TRANSIENT GLOBAL ISCHEMIA. R. M. Sklar, C. G. Caday, D. J. Berlove, A. Kemmou, R. H. Brown, Jr. and S. P. Finklestein*. CNS Growth Factor Research Laboratory, Department of Neurology, Massachusetts General Hospital and Harvard Medical School, Boston, MA 02114.

Transient global forebrain ischemia in gerbils selectively damages vulnerable neurons, most prominently in the CA-1 sector of the hippocampus. Immediately following global forebrain ischemia, CA-1 neurons appear normal and respond to electrical stimulation; but then go on to die within 2-4 days. Elucidation of the molecular events that lead to delayed neuronal death is key towards developing alternative therapies for stroke and related ischemic disorders.

Using a differential screening method, we selected 30 gene clones that are modulated in gerbil hippocampus following transient global ischemia. Dot blot analysis showed at least six different patterns of expression of these genes after ischemia. DNA sequencing revealed one of these genes to be homologous to heat shock protein (HSP)-86. Northern blot studies showed about 4- to 6-fold induction of HSP-86 gene in gerbil hippocampus at 4 hours to 1 day after ischemia; the mRNA levels declined to basal level at about 2-4 days after ischemia. Maximal expression of the putative HSP-86 occurred before the onset of delayed neuronal death in the hippocampus after ischemia.

The present study is consistent with the hypothesis that transient global ischemia initiates cascades of protein syntheses -- including those involved in either cell death or cell survival response pathways.

676.13

EFFECTS OF MILD HYPOTHERMIA ON EXPRESSION OF HEAT SHOCK PROTEIN-70 MRNA AND IMMUNOREACTIVITY FOLLOWING FOCAL ISCHEMIA IN RAT BRAIN. J.Chen, J.Q.Lan, M. Butman, R.P.Simon*, F.R.Sharp, and S.H.Graham, Department of Neurology, Univ. of Calif. San Francisco, CA 94143

Although hypothermia may reduce infarction after temporary focal ischemia, the degree of selective neuronal injury in the spared regions is difficult to asses using conventional histology. Therefore, we used the expression of HSP70, a sensitive marker of neuronal injury, to study the effect of hypothermia upon neuronal injury after ischemia.

Sprague-Dawley rats (n=16) underwent MCA occlusion for 2 hours followed by reperfusion. Brain temperature was maintained either at 37C^{o} or 32C^{o} during occlusion and at 37C^{o} during reperfusion. Distribution of HSP70 mRNA in the brain was investigated by in situ hybridization 8 hours after reperfusion. Expression of HSP70 protein was determined at 24hrs after reperfusion by immunocytochemistry.

In normothermic brains: HSP70 mRNA was present throughout the ipsilateral MCA territory, most intense adjacent to the infarcted regions in caudate and cortex. Neuronal HSP70 immunoreactivity was only present in the narrow rostral border zone in cortex while glial and endothelial expression was present within the infaction. In hypothermic brains: Intense HSP70 mRNA was present in caudal cortex and caudate putamen, but reduced or absent throughout rostral cortex. HSP70 immunoreactivity was widespread in neurons of layers 3 and 5 in the caudal cortex but absent in rostral cortex.

These results suggest that while mild hypothermia prevents infarction of much of cortex, many neurons in this region are injured and express HSP70 protein. The lack of expression of HSP in the rostral cortex suggests that this region is protected by mechanisms other than HSP70 expression.

676.14

C-FOS, JUN-B, AND C-JUN mRNA EXPRESSION FOLLOWING FOCAL CEREBRAL ISCHEMIA IN RATS

H. Kinouchi, F. R. Sharp, J. Koistinaho, S. M. Sagar, T. Yoshimoto, R. A. Fishman*, P. H. Chan.

Dept. of Neurosurgery, Tohoku Univ. Sendai 980, Japan, Dept. of Neurology, UCSF, CA 94143, Dept. of Neurology, VAMC, San Francisco, CA 94121

Thalamus and substantia nigra become atrophic following middle cerebral artery (MCA) occlusion by retrograde degeneration. Immediate early genes (IEG), c-fos, c-jun, and jun-B are induced by a wide variety of stimuli, including transsynaptic excitation. In the present study, we have investigated the IEG and heat shock protein (HSP) mRNA expression at 1, 4, 24 hrs. following MCA occlusion to demonstrate the neuronal network disturbance using *in situ* hybridization (ISH). The c-fos and jun-B mRNAs were co-induced rapidly outside the ischemic core, the cingulate gyrus and medial striatum at 1 hr. following MCA occlusion. At 4 hrs. of MCA occlusion, both c-fos and jun-B mRNAs were induced in both hippocampi and medial geniculate nucleus and in the ipsilateral substantia nigra and thalamus. These signals decreased after 24 hrs. of MCA occlusion. Only a slight induction of c-jun mRNA occurred in the ipsilateral cortex and striatum outside the ischemic core at 4 hrs. of MCA occlusion, whereas the signal was greater at 24 hrs. than at 4 hrs. of MCA occlusion. The ischemic core showed no hybridization signals for IEG throughout the time course. hsp70 mRNA was markedly induced in the peripheral regions of the ischemic cortex and the striatum of MCA distribution even at 24 hrs. of MCA occlusion. However, some animals showed hsp70 mRNA induction in the ipsilateral hippocampus, thalamus, substantia nigra, and medial geniculate nucleus. These data suggest that transsynaptic activation of neurons may account for the ipsilateral and contralateral expression of IEG in areas outside of the ischemic core.

Supported by NS14543, NS25372, AG08938 and Department of Veterans Affairs

677.1

FACTORS AFFECTING HIPPOCAMPAL BRAIN SLICE HISTOLOGY. G. C. Newman*, H. Qi, F. E. Hospod and Z. Bekele-Arcuri. Neurology Service, VAMC at Northport, NY and SUNY at Stony Brook, NY 11794.

Histology is useful both for assessing brain slice quality and as an assay of tissue injury in response to chemical insults. Pre-incubation at 22°C. for 45 min prior to warming to 37°C. yields slices with 90% of normal appearing neurons in CA1, CA2 and the suprapyramidal blade of dentate after 4 hours *in vitro*. Despite this, neurons in CA3 and CA4 show uniformly poor histology even after much shorter incubations. We have attempted to identify the factors which contribute to neuronal injury during slice incubation.

Hippocampal brain slices prepared from 250g male Sprague-Dawley rats were incubated at 22°C. for 45 min, warmed to 37°C. and incubated for up to 4 hours *in vitro*. Slices were fixed with Bouin's fixative, paraffin embedded, sectioned at 7μ and stained with H&E. CA1, CA2, CA3, CA4 and dentate histology was assessed at 100X with a 5 point scale by a blinded observer.

Histologic injury of CA1, CA2 and dentate occurred within 15 min of slice isolation and did not increase after 4 hours while CA3 and CA4 histology was even more severely injured after 15 min and continued to worsen throughout the incubation. Incubating slices in buffer with 0 mM Ca^{++} and 10 mM Mg^{++} for the first 45 min produced slight additional improvement in CA1 and considerable improvement in CA3 after 4 hours *in vitro* but 0 mM Ca^{++} without Mg^{++} worsened histology of CA1, CA2 and dentate. Replacing Na^{+} with choline for the first 15 min of slice incubation improved histology of all regions but continuing choline beyond 30 min caused severe histologic injury. Incubation with 400μM ascorbic acid or acutely cutting the mossy fibers at the time of slice preparation failed to improve histology. The effects of technical issues such as animal size, depth of slice submersion and fixation temperature will also be presented.

677.2

QUANTITATION OF PERIVASCULAR MACROPHAGES IN THE BRAINS OF HYPERTENSIVE OR AGED RATS. Y. Liu, D.M. Jacobowitz, G. Feuerstein, J.M. Hallenbeck* and A.-L. Sirén. Dept. of Neurology, USUHS, Bethesda, MD 20814; Lab. of Clinical Science, NIMH; Stroke Branch, NINDS, Bethesda, MD 20892; and Dept. of Pharmacology, SmithKline Beecham, King of Prussia, PA 19406.

The numbers of perivascular macrophages in the brains of hypertensive or aged rats were studied using the immunohistofluorescence technique. Perfusion-fixed frozen brain sections (16 μ) of spontaneously hypertensive rats (SHR), stroke-prone SHR (SHR-SP) and normotensive Wistar-Kyoto (WKY) rats as well as 2-year-old (2Y) and 16-week-old (16W) Sprague-Dawley (SD) rats were exposed to specific monoclonal antibodies against rat macrophages (ED2). All slides were double stained with anti-Factor VIII antibodies for the identification of endothelial cells which showed no ED2 staining. The quantity of perivascular cells per square millimeter of high power field was significantly greater in SHR-SP (8.6 ± 2.1, n=4) and SHR (6.7 ± 0.9, n=6) than in normotensive WKY (4.0 ± 0.5, n=6) ($p<0.01$). In addition, the number of perivascular macrophages was greater in 2Y-SD-rats (7.5 ± 2.7, n=9) than in 16W-SD-rats (2.9 ± 1.8, n=8, $p<0.01$). No ED2 staining was found in the parenchymal resident microglia or in the endothelial cells. The results suggest that an increased subendothelial accumulation of macrophages is associated with the stroke-risk factors, hypertension and advanced age. This could increase the tendency for the endothelium to convert from an anticoagulant to a procoagulant surface in response to proinflammatory and procoagulant mediators released from these subendothelial cells.

677.3

GLOBAL ISCHEMIA IN RATS YIELDS STRIATAL PROJECTION NEURON AND INTERNEURON LOSS RESEMBLING HUNTINGTON'S DISEASE. G. Figueredo-Cardenas*, K. Baxter, T.S. Nowak, W. Pulsinelli and A. Reiner. Dept. Anat & Neurobiol., Dept. Neurol., UT, Memphis, TN 38163.

Huntington's disease (HD) is characterized by complete sparing of striatal interneurons and profound loss of projection neurons. Among projection neurons, loss of enkephalinergic (ENK+) neurons projecting to the lateral globus pallidus and substance P-containing (SP+) neurons projecting to the substantia nigra pars reticulata (SNr) occurs more rapidly and earlier in the disease process than does loss of SP+ striatal neurons projecting to the medial globus pallidus. Since preferential sparing of striatal interneurons has also been demonstrated after transient ischemia, we examined in detail the pattern of both striatal projection and interneuron loss after global ischemia in rats. One week after 30 min bilateral carotid and vertebral artery occlusion, alternate brain sections from the animals were processed for the visualization of striatal interneurons or terminals in striatal targets using histochemical or immunohistochemical methods.

The ischemic episode produced profound loss of striatal neurons in the dorsolateral striatum, with survival of many neurons medially and caudally. We found complete sparing of diaphorase-containing, parvalbumin-containing, and choline acetyltransferase-containing interneurons even in striatal regions seemingly devoid of other neurons. We also found severe loss of ENK+ terminals in the lateral globus pallidus and SP+ terminals in the SNr, but much less loss of SP+ terminals in the medial globus pallidus (i.e.ento-peduncular nucleus) and the SN compacta. This pattern of striatal cell loss following transient ischemia resembles that in HD even more closely than previously realized, suggesting that the pathogenetic process in HD and ischemia may involve related mechanisms. Further study of the mechanisms producing ischemic striatal cell death may thus clarify the mechanisms involved in HD. HDF, NS-19620, NS-28721 (AR).

677.4

IMMEDIATE AND LONGTERM MORPHOLOGICAL CHANGES IN THE RAT HIPPOCAMPUS AFTER FOREBRAIN ISCHEMIA. M. Hsu*, F. Gallyas, A. Sik and G. Buzsáki. Ctr. for Neurosci., Rutgers University, Newark, NJ.

Ischemia-induced morphological changes in the rat hippocampus were studied 1-365d after induction of ischemia using the 4-vessel-occlusion model of Pulsinelli and Brierley (1979). Degenerative changes were assessed using the Gallyas "dark neuron" stain, microglia stain, and the Gallyas stain for degenerating terminals. In addition, short-term (1-7d) morphological changes were also studied using immunocytochemistry against heat shock protein (HSP72), the calcium binding proteins (CaBPs) parvalbumin, calbindin and calretinin, and the neuropeptides somatostatin (SS) and neuropeptide Y (NPY). The time course of degeneration of vulnerable cell populations determined using the "dark neuron" stain was similar to that previously reported, i.e. argyrophilic hilar cells were found 1d after the ischemic insult while CA1 pyramidal cells did not become argyrophilic until 3-4d postischemia. CA3 pyramidal cells and dentate granule cells were resistant. Other vulnerable cell populations included subicular neurons, CA2 pyramidal cells, and spiny interneurons in the stratum lucidum of CA3. Results from both silver-stained material and double immunolabelling of HSP and neuropeptides and CaBPs confirmed our previous results that many hilar cell groups (other than SS neurons) were susceptible. A continuous state of degeneration was evident 7-365d postischemia, as indicated by the presence of degenerating terminals, argyrophilic neurons, and reactive microglia. Reactive microglia could be detected at 1-9 mo postischemia, but only in areas where argyrophilic cells were found after ischemia, i.e. the dentate hilus, subiculum, stratum lucidum of CA3, and area CA1. Cell counts of both Nissl- and immunostained material in these areas provide further support that the argyrophilic (or "dark" neurons) degenerated after ischemia.

677.5

PERSISTENCE OF CHOLINE ACETYLTRANSFERASE- IMMUNOPOSITIVE NERVE FIBERS IN THE ISCHEMIC RAT HIPPOCAMPUS. A.B. Kopstein*, L.A. Sebring, B.K. Hartman, P.L. Faris, and W.C. Low. Depts. of Neurosurgery, Physiology, Psychiatry, and Graduate Program in Neuroscience, Univiversity of Minnesota Medical School, Minneapolis, MN 55455.

It has been demonstrated previously that intrahippocampal transplantation of fetal pyramidal neurons restores spatial navigation function in rodents with ischemic brain injury. These functional effects are thought to be mediated by graft-to-host projections to the subiculum and host-to-graft inputs from CA3 pyramidal neurons. In normal animals, these connections appear to convey precise information about the external spatial environment. Inputs of cholinergic fibers from the medial septal nucleus to the hippocampal formation (HF) have also been shown to play an important role in spatial memory function. We have used choline acetyltransferase (ChAT) immunohistochemistry to examine whether cholinergic afferent fibers persist in the HF after ischemic brain injury and thus may be available to form connections with transplanted pyramidal neurons. Unanesthetized Wistar rats were subjected to 20 minutes of global cerebral ischemia by the four-vessel occlusion technique of Pulsinelli and Brierly. Animals were subsequently processed for ChAT immunocytochemistry at 2, 4, and 8 weeks following the ischemic episode. At all of these time periods, we have found that ChAT-positive fibers persist in the stratum oriens and radiatum of the CA1 subfield of the rat HF. The presence of these cholinergic fibers within the CA1 hippocampal region long after ischemic injury provides the opportunity for these neurons to establish connections with transplanted pyramidal neurons and to incorporate the grafted cells within the neural circuitry of the host brain. [This work was supported in part by an American Heart Association Medical Student Research Fellowship Award (AHA-91005050) and PHS grant R01-NS-24464.]

677.6

LOCOMOTOR ACTIVITY IN THE ISCHEMIC GERBIL. AM Babcock[1], and P. Lomax[2,*] [1]Dept. of Psych., Montana State Univ, Bozeman MT, 59717; [2]Dept of Pharm., UCLA, Los Angeles, CA 90024.

Previous studies have shown that gerbils exhibit increased locomotor activity within 24 hrs following an ischemic insult. Since activity gradually diminishes to normal levels with repeated testing, it has been argued that this behavior represents a reversible or transient effect of ischemia . We challenged this notion by testing ischemic gerbils at a time point where increased activity is not observed with repeated testing. Ischemic (5-min bilateral carotid occlusion) and sham gerbils were tested for 14 consecutive days following reperfusion in an open-field apparatus (n=6/condition). As previously reported, ischemic gerbils exhibited a significant increase in activity (days 1 and 2) which returned to sham levels with repeated testing (days 13 and 14). A second group of ischemic and sham gerbils (n=6/condition) were tested only on days 13 and 14 following reperfusion. In contrast to those tested repeatedly, these ischemic gerbils displayed increased locomotor activity as compared to sham controls. In addition, gerbils in the repeated testing conditions were evaluated in a semi-novel testing environment on days 15 and 16 following surgery. The locomotor activity of these ischemic gerbils significantly increased in response to the semi-novel environment. These results suggest that the effects of ischemia on locomotor activity are not limited to a brief period following occlusion and may represent a permanent deficit. In addition, as previously suggested, this behavior may represent a deficit in habituation or spatial mapping rather then motor hyperactivity.

677.7

LOCOMOTOR DEFICITS IN ADULT ANIMALS FOLLOWING NEONATAL ISCHEMIC-HYPOXIA. E.M. Jansen* and W.C. Low. Depts. of Neurosurgery and Physiology, Grad. Prog. in Neuroscience, Univ. of Minnesota, Mpls., MN 55455.

Perinatal ischemic-hypoxia in humans creates neurologic injury that often manifests as motor deficits throughout development and at maturity. In these studies, we utilized an established model of neonatal ischemic-hypoxia that creates unilateral striatal, cortical and hippocampal damage (Rice, et al, Ann. Neurol., 9:131-141, 1981) to investigate locomotor deficits in the animals as adults. Seven day-old Wistar rat pups underwent unilateral carotid artery ligation and were then exposed to 2.5 hours of hypoxia (8% O_2, 92% N_2, 37°C). Following this, animals were reared normally and locomotor ability was assessed throughout development using a rotating treadmill (Rota-Rod, Ugo Basile). Normal animals tested once a week for seven weeks improved significantly over time. Animals that experienced ischemic-hypoxia as neonates were not able to remain on the treadmill as long as their same-sex littermate controls at 3, 4, 5 and 6 weeks of age ($p < 0.027$, ANOVA). There were no significant differences after 6 weeks of age, suggesting that this motor task revealed a developmental delay rather than a permanent deficit. Upon examination of whole brains after perfusion, some brains showed generalized hypertrophy in the hemisphere contralateral to the ligation. The Rota-Rod performance scores for these animals were significantly better than those of the animals without contralateral hemisphere hypertrophy over all weeks tested ($p < 0.02$, ANOVA). These data suggest that 1) Rota-Rod performance is an appropriate test of motor ability for use in this model of neonatal ischemic-hypoxia to screen for animals with severe neural injury, and 2) in this model of neonatal ischemic-hypoxia, there may be compensatory mechanisms which occur in the undamaged hemisphere of some animals that may account for a recovery of function. (Supported in part by grants from the United Cerebral Palsy Association, the American Heart Association, and PHS grant RO1-NS-24464.)

677.8

LOCOMOTOR DEFICITS IN RATS WITH A COLLAGENASE-INDUCED INTRASTRIATAL HEMORRHAGE. I. A. Chesney *, T. Kondoh and W.C. Low. Depts. of Neurosurgery and Physiology, Graduate Program in Neuroscience, University of Minnesota Medical School, Minneapolis, MN, 55455.

Previous studies have shown that injection of the metalloproteinase collagenase directly into the caudate nucleus of rats causes an intracerebral hemorrhage (Rosenberg *et al.*, Stroke 21:801-807, 1990). In the present study we have examined the functional deficits associated with a hemorrhagic lesion of the striatum. Twelve adult rats received a 2 µl infusion of bacterial collagenase (0.5 unit in saline) into the right striatum. The rotational response to apomorphine (1 mg/kg, s.c.) injection was then examined at 1, 4, 7, 21, 35 and 70 days after the surgery. A net ipsilateral rotation was noted for all days. The average rotational asymmetries on these days were: 14.57 ± 2.9, 20.33 ± 2.7, 19.99 ± 4.4, 18.95 ± 4.9, 17.03 ± 4.9 and 14.4 ± 4.7, respectively (data expressed as mean clockwise rotations/5 min ± SEM). In addition to rotation studies, the initiation of stepping movements in each forelimb was determined 8 weeks after surgery. The average number of steps initiated by the forelimb ipsilateral and contralateral to the lesion was 28.3 ± 2.1 steps/min and 13.6 ± 1.5 steps/min, respectively. This significant difference ($P < 0.05$) was stable and reproducible for three consecutive days. We conclude that collagenase-induced intrastriatal hemorrhage results in long-term locomotor deficits and therefore is a useful model for developing strategies for the restoration of neurologic function after intracerebral hemorrhage. (Supported by grants from the American Heart Association and PHS grant RO1-NS-24464.)

677.9

BEAM WALKING WITHOUT ADVERSIVE STIMULI AS A TOOL TO RECORD MOTOR RECOVERY AFTER PHOTOCHEMICAL INDUCED UNILATERAL LESION OF THE SENSORY-MOTOR CORTEX. TRAINING EFFECT CONTRA d-AMPHETAMINE TREATEMENT.

B. Bjelke*; §A. Brown; S. Sabouri; K. Fuxe

Dept. of Histology and Neurobiology, Karolinska Institutet, Stockholm, Sweden. §Dept. of Physical Medicine and Rehabilitation, Mayo Clinic, Rochester, Minnesota USA

In various stroke models d-amphetamine has been shown to improve the rate of motor recovery after brain damage. Sprague-Dawley male rats b.w. 300g housed under standardised conditions were trained in beam walking until they achieved a score of 7 on a motor deficit rating scale from 1 (unable) to 7 (normal) on two consecutive 90 sec. daily trials. The home cage was used as a goal and no aversive stimuli were used in training or testing. A unilateral sensory-motor cortex lesion was photochemically induced using cold white light and Rose Bengal as the photosensitive dye. Recovery of the locomotor deficit was recorded as early as 4h after the lesion and up to ten days after the lesion. The most rapid recovery was seen in rats that received only one 2 hour training session 24 h after the lesion. Animals treated with a single 2mg/kg dose of amphetamine 24 h after the lesion recovered less rapidly than animals with lesion alone. Morphological analysis was done using immunohistochemical markers and Nissl staining. Thus, using nonaversive stimuli and photochemically induced sensory-motor cortex lesion no enhancement of locomotor recovery was obtained from amphetamine treatment.

677.10

N-*tert*-BUTYL-α-PHENYL-NITRONE (BPN), A FREE RADICAL SCAVENGER, FACILITATES LEARNING BY ELDERLY GERBILS FOLLOWING BILATERAL CAROTID OCCLUSION (BCO) I. A. Stanley*, C.O. Bull, S. L. Moon, and A. A. Ortiz. CNS Neuropharmacology, Bristol-Myers Squibb Company, Wallingford, CT 06492

Because of an anomalous circulatory pattern in the Mongolian Gerbil, temporary bilateral occlusion of carotid arteries (BCO) can be used to produce a reliable model of transient, global cerebral ischemia. Drugs have been shown to protect sensitive brain regions, e.g., the hippocampus, from damage resulting from BCO. Since the hippocampus is involved in spatial learning, tasks that rely on spatial discrimination are used to assess behavioral dysfunction following BCO. Elevated levels of free radicals (1) have been implicated in the damage that follows transient ischemia and (2) are associated with the detrimental effects of aging on memory. Free radical scavengers attenuate BCO-induced cell death in the hippocampus of young gerbils. They also reduce the number of errors made by normal old gerbils during training in a radial arm maze.

This study investigated the effect of short-term treatment with N-*tert*-butyl-α-phenyl-nitrone (BPN), a free radical scavenger, on performance by elderly gerbils in a radial arm maze following BCO.

A total of 82 fasted old male gerbils were used; half underwent a 3-min BCO. In the remaining (shams) the carotids were exposed but not clamped. All were treated with 30 or 60 mg/kg BPN or saline 1 hr after reperfusion and every 12 hrs thereafter for 3 days. Starting 2 wks after surgery, spatial learning was assessed in an 8-arm radial maze using the number of errors made during 15 days of training. After the last trial, the brains were removed and the damage in the hippocampus was histologically analyzed.

When normal blood flow is interrupted for 3 mins in non-treated old gerbils, the rate of acquisition of spatial learning is significantly impaired when compared to sham gerbils. Gerbils treated with BPN following BCO made significantly fewer errors during acquisition of the task when compared with those who were occluded but not drug treated. Sham operated gerbils treated with BPN also tended to perform better than those given only saline.

The results of this study suggest that compounds that are free radical scavengers may be useful in the treatment of age-related memory impairment as well as in facilitating learning following stroke.

677.11

BRIEF ASPHYXIA DURING BIRTH PRODUCES DEFICITS IN ACQUISITION, BUT NOT RETENTION, OF A SPATIAL LEARNING TASK IN THE ADULT ANIMAL. A. Krishnamurthy, W. Brooks, B. Noel, A. Gratton and P. Boksa*. McGill University, Depts. of Psychiatry and of Neurology & Neurosurgery, Douglas Hospital Research Center, Montreal, Quebec, Canada, H4H 1R3.

While birth complications are thought to contribute to major deficits in some neurological disorders, a role for brief asphyxia in more subtle alterations of CNS functioning is much less recognized. Morphological studies indicate that the hippocampus, an area which plays an important role in learning and memory, is particularly vulnerable to perinatal asphyxia. The present study examined consequences of brief asphyxia at birth on spatial learning in male rats in the Morris water maze. In the model of birth asphyxia (Bjelke et al, Brain Res., 1991, 543, 1), the entire uterus was removed on the day of birth and immersed in a water bath for 5-20 min before delivery. Control animals were either born vaginally or delivered by C section. At 1 month of age, rats who had undergone 15 min of birth asphyxia showed no deficit in acquisition of place learning (i.e. time to find the hidden platform). At 4 months of age, rats who had undergone 10, 15 or 20 min of birth asphyxia showed a deficit in initial acquisition of the place navigation task compared to control groups, while the 5 min group performed similarly to controls. With further training, all animals eventually learned the task effectively. Following overtraining, there was no difference among groups on retention of the place navigation task. There was also no difference in latency to find a visible platform, indicating no deficits in sensorimotor ability or motivation. In addition, there were no group differences in locomotor activity in an open field, indicating that motor activity is undisturbed. Our results indicate that a brief period of asphyxia during birth can produce subtle deficits in spatial learning that become evident only in adulthood. (Supported by the Medical Research Council of Canada.)

677.12

BMS-183729 PREVENTS MEMORY LOSS IN THE ISCHEMIC GERBIL. MA Kapin[1*], AM Babcock[2]. [1]Bristol-Myers Squibb Co., Wallingford, CT 06492; [2]Dept. of Psych., Montana State Univ., Bozeman, MT, 59717

The present study evaluated the neuroprotective efficacy of the novel hypothermic producing agent, BMS-183729 (formally BMY-20862; 5,6-dihydro-11-methyl-11H-pyrimido[4,5-6][1,4]benzodiazepinemono-hydrochloride hydrate) using a standard step-down avoidance paradigm. In Exp. I, conscious gerbils were injected with BMS-183729 (30 mg/kg) or vehicle and changes in rectal temperature were monitored for 4 h. Drug, but not vehicle treated gerbils, exhibited a significant reduction in temperature within 10 mins. that continued for at least 240 mins. In Exp. II, the histopathological and behavioral changes following 5 mins. of carotid artery occlusion in gerbils pretreated with BMS-183729 were evaluated. As previously reported, BMS-183729 (30 mg/kg) attenuated cell death to the hippocampal CA1 sector following an ischemic insult. Step-down latencies of both sham and BMS-183729 treated gerbils 24 h following training were significantly increased, indicating successful avoidance learning. Ischemic control gerbils failed to exhibit any significant change in latency suggestive of no avoidance learning. Our findings indicate that, in addition to protecting the hippocampal CA1 sector from necrosis, BMS-183729 also prevents memory deficits typically associated with ischemia.

677.13

CHARACTERISTICS OF A NEW REVERSIBLE CEREBRAL ISCHEMIC STROKE MODEL IN THE RAT. K.H.LEE* and J.S.Han. Dept.of Neurosurgery,Sch.of Med.,Hallym Univ.,Kangdong Sacred Heart Hospital,Seoul,134-701, Korea.

We have been tried to develop a new reversible ischemic model in the rat and to characterize it. Under chloral hydrate(300 mg/kg,IP) anesthesia,the common,internal and external carotid arteries exposed. Temporary trapping was done at the exposed common carotid artery with two microclips and a 5 cm 6-0 or 7-0 nylon monofilament topped with a ball(200-300 μm in diameter) of iron powder and epoxy was inserted through a microhole at the mid-trapped area. After removal of microclips,the inserted monofilament was introduced about 2 cm to the origin of middle cerebral artery via internal carotid artery until feeling of some resistance. After 1,2,4 and 24 hours occlusion,each introduced monofilaments were withdrawn and all rats kept alive for 2 days. We have periodically checked behaviors from the time of awakening to death and measured the infarcted areas of each groups using 2 % neutral red infusion for counter-staining. All rats revealed dense contralateral forelimb weakness at awakening but none of 1-hr occlusion group(9 rats),2 of 2-hrs(9),5 of 4-hrs(6) and 10 of 24-hrs (13) noted weakness before death. There was no infarction in 1-hr occlusion while definite and wider infarct areas in others according to occlusion times($p<0.001$,$p<0.001$,$p<0.001$). We have considered this animal model as a new, simple,reproducible,confident and reversible one.

677.14

Recovery from anaesthesia improves outcome following transient but not permanent focal cerebral ischaemia in the rat.
D.A. Dawson, J. McCulloch*, D.I. Graham and I.M. Macrae, Wellcome Surgical Institute, University of Glasgow, Glasgow G61 1QH, U.K.

Adult male Sprague-Dawley rats were anaesthetised with halothane in N_2O:O_2. The left middle cerebral artery was occluded (MCAO) permanently by diathermy, or transiently by application of endothelin-1 (2.5nmoles). Physiological variables were monitored throughout. Following surgery rats were maintained under anaesthesia or allowed to recover consciousness. All rats were perfusion fixed 4 hours post-MCAO and volumes of ischaemic damage assessed using quantitative histopathology.

In the anaesthetised groups mean arterial pressure (MAP) remained relatively stable whereas in the recovery groups MAP rapidly increased following withdrawal of anaesthetic. Volumes of ischaemic damage did not differ significantly between anaesthetised and recovery groups for permanent MCAO (n=6) but were significantly reduced in the recovery group compared to the anaesthetised group for transient MCAO (n=10-12). Data is presented as mean ± S.E.M., *$P<0.05$, unpaired Student's t-test.

	MAP (mmHg)			Volume of damage (mm^3)		
	MCAO	+1h	+4h	Hemisphere	Cortex	Striatum
Permanent						
Anaesthetised	91±8	85±3	88±4	107± 6	69± 5	30±2
Recovery	89±4	114±4	123±7	131±15	95±14	28±1
Transient						
Anaesthetised	86±3	82±2	87±2	48± 8	32± 7	13±3
Recovery	84±4	121±3	132±3	23± 4*	17± 3	5±1*

We have shown that recovery from anaesthesia does not improve early outcome following permanent MCAO but is of benefit in the endothelin-1 transient occlusion model where volume of infarct is reduced by 50%. In this model augmentation of blood pressure may improve reperfusion through the previously constricted MCA. It remains to be established whether this early beneficial effect may be superceded by a later increase in free-radical associated damage.

677.15

CHANGES IN LESION VOLUME AND EDEMA OVER TIME FOLLOWING FOCAL CEREBRAL ISCHEMIA IN THE RAT.
T.L. Sailer, T.L. Graessle, J.T. Simmonds, J.T. Haskins*, and J.A. Moyer, CNS Division, Wyeth-Ayerst Research, CN 8000, Princeton, NJ 08543-8000.

Ischemic damage caused by occlusion of cerebral arteries involves a dynamic process of events. While most studies of cerebral ischemia focus on relatively early time points (<48 hours post-occlusion), evolution of the infarcted region continues for several weeks post-insult. This continuing evolution may be responsible for some of the recovery seen in human stroke patients over time. To assess changes in the nature of ischemia-induced infarcts over time, infarct volume and edema were measured in a model of focal cerebral ischemia in the rat. Tandem permanent occlusion of the right common carotid and middle cerebral arteries was performed on male Fischer-344 rats. Infarct volume and edema were measured at 1, 2, 4, 7, and 14 days post-occlusion.

Infarct volume increased to a maximum of 48.88 mm^3 at 2 days post-occlusion. Edema in the infarcted right hemisphere also peaked at 2 days at 79.59% water content. After 2 days, infarct volume and edema diminished rapidly. At 7 days infarct volume was reduced to 12.75 mm^3 and by 14 days volume had further decreased to 4.62 mm^3. Edema in the infarcted hemisphere was reduced to 79.17% by 4 days and decreased to a normal range by 7 days. These results suggest that while pharmacological assessment of neuroprotection at early time points may produce significant differences between groups, later time points should be used to assess the long term benefit of treatment.

677.16

THE EFFECT OF TIME AFTER OCCLUSION ON INJURY VOLUME IN A RABBIT MODEL OF FOCAL CEREBRAL ISCHEMIA.
J.L. Browning*, M.A. Widmayer, K.K. Hoffmann and D.S. Baskin Dept. of Neurosurgery, VAMC Houston and Baylor College of Medicine, Houston, TX 77030.

Studies by others have suggested that infarct volume changes as a function of post-occlusion survival time in rodents (Gonzales Soc. Neurosci. Abstr.18:1260,1992). Therefore, as a prelude to studies of the effects of drug treatment on survival and injury volume, the effect of time post-occlusion on injury volume was studied in rabbits with focal cerebral ischemia. Focal cerebral ischemia was induced in twenty-eight male rabbits, 2.6-3.5 kg, by occlusion of 1 mm of the middle cerebral and accessory middle cerebral arteries. At 28 hr, 7 days, and 3 weeks after occlusion, the animals were sacrificed, their brains removed, sliced coronally and stained with triphenyltetrazolium chloride (TTC). In a blinded fashion, areas of infarct (colorless), ischemic (lightly stained), and normal (fully stained) were delineated from photographic slides, and digitized to determine injury volume. No differences were found in any injury measure between animals surviving 28 hours and those surviving 7 days. However, at three weeks, infarct volume had decreased to 4.5±1.9% of the ipsilateral hemisphere as compared to 11.2±2.7% at 28 hr and 14.3±4.1% at 7 days ($p<0.07$ for 3 weeks vs 28 hr; $p<0.075$ vs 7 days). Additionally, total hemispheric volume decreased from 28.1±1.1 cm^3 at 28 hr to 25.2±0.6 cm^3 ($p<0.01$) at 7 days and 22.4±0.7 cm^3 ($p<0.01$) and 3 weeks respectively. These results suggest that hemispheric volume must be considered when comparing injury volumes in animals at differing times after occlusion. However, injury volumes in rabbits surviving one day may readily be compared to rabbits surviving 7 days. At three weeks after injury, there is a trend that falls short of statistical significance. Thus, in experiments where drug treatments increase survival after focal cerebral ischemia, survival measurements of infarct volume should preferably be made within one week.

677.17

A PHOTOTHROMBOTIC MODEL OF STROKE-IN-EVOLUTION
P. Wester*, B.D. Watson, R. Prado, W.D. Dietritch, Umeå Stroke Research Group, Univ. of Umeå, Sweden and CVD Research Center, Univ. of Miami, School of Medicine, FL 33101.

This study aimed at developing a thrombotic cortical infarction model with a large, consistent and easy observable intrafocal "area at risk". A ring-shaped pattern of cortical vascular occlusion was induced photochemically by the intravascular interaction of the photosensitizing dye, Erythrosin B (ErB), with a 514.5 nm ring beam, derived from an argon laser and incident on the intact skull surface of anesthetized Wistar rats (n=84). The ring beam was generated by focussing the argon laser beam into an optical fiber at a 12° off-axis angle of incidence. At the fiber output end, the resultant 5 mm diameter, 0.5 mm thick ring beam was located within the coronal, saggital and lambdoid sutures and the parietal bone crest. Transcardial carbon black perfusions (n=67) were performed at 1, 4 and 24 hr to study vascular patency at various irradiation powers (25-80 mW), duration's (2-5 min) and [ErB] from 8.5-34 mg/kg. To evaluate blood-brain barrier integrity, horseradish peroxidase (HRP) was administered i.v. followed by histopathological analysis of coronal and saggital brain sections.

With [ErB] at 17 mg/kg, irradiation for 2 min at 65 mW produced at 1 hr a ring-shaped cortical deficit which expanded in time, as observed at carbon black perfusion; the ring interior was spared at 4 hr but was occluded by 24 hr (n=3 in each group). Coronal sections which bisected the irradiated ring region revealed two ischemic foci (originating from the ring segments) which expanded over a 24 hr period to include the region between. A ring pattern of severe HRP extravasation surrounding an area of minimal HRP leakage was observed 15 min following irradiation (n=3). Examination of hematoxylin and eosin stained coronal sections at 4 hr revealed a toroidal shaped cortical infarct with a central core of normal appearing neuropil extending to the subcortical white matter and containing a few scattered ischemic neurons (n=3). By 24 hr the central core progressed to infarction with only superficial cortical layers exhibiting some degree of neuropil conservation, but containing extensive ischemic neurons (n=3). This model of a controllable "penumbral zone" may facilitate reliable and reproducible tests of antiischemic drugs.

677.18

BOLD MR IMAGING OF ACUTE EMBOLIC STROKE.
K. H. Taber*, S.R. Northrup. Herbert J. Frensley Center for Imaging Research, Dept. of Radiology, Baylor College of Medicine, Houston, TX 77030

BOLD (Blood Oxygenation Level Dependent) is an exciting new natural-contrast method of magnetic resonance (MR) imaging. This method utilizes the fact that as blood becomes deoxygenated the RBCs change from their normal diamagnetic state to paramagnetic. Thus, deoxygenated intravascular blood becomes a naturally-formed MR contrast agent. We have performed BOLD MR imaging 30-360 minutes after induction of embolic stroke in rabbits. MR imaging was done on a Bruker BioSpec 2.35 Tesla system. A gradient echo (350/40/30) imaging sequence, 2 mm slice thickness, 128 gradient steps, 10 cm field of view and 4 averages were used. Following completion of the imaging session the brain was removed, sliced and placed in TTC for staining. Excellent delineation of areas of signal abnormality was obtained even at the earliest imaging times, and was maintained throughout the 4-6 hour imaging session. However, in some cases the areas of abnormality was smaller on the BOLD images than on the TTC stained sections, indicating that in some cases there was trapped intravascular blood in only a portion of the affected tissue.

677.19

INFARCTION IS NOURISHED BY DELAYS.—DITHIZONE DELAYS NEURONAL DAMAGE.— K. Shiraishi,* MD, S. Kobayashi, MD, S. Nakazawa, MD, Dept. of Neurosurgery, Nippon Medical School, Bunkyo-ku, Tokyo, 113 Japan

We reported that zinc enhances kainate neurotoxicity in the rat hippocampus. Pre-treated zinc induces selective neuronal damage in the CA 3–4 of the hippocampus with an intravenous injection of kainate (a dose is 0.5 mg/kg, BW). On the contrary, zinc depletion might reduce neuronal damage caused by excitatory amino acids. We used dithizone (DZ) as a chelating agent for zinc in the ischemic models in the rat. [METHODS] (1) DZ disolved in 0.2% ammonia and was injected to rat (100 mg/kg) intraperitoneally (Otsuka, N. Histochemie, 1966). (2) 30 minutes after the DZ i.p. injections, left middle cerebral artery (MCA) was occluded by cauterization at the proximal site (Shiraishi & Simon, J. Neurosci. Methods, 1989). 24 (group 1) and 72 (group 2) hours later, rats (each group had five) were sacrificed under general anesthesia and H. & E sections were made. (3) 30 minutes after the DZ i.p. injection, forebrain ischemia was induced by bilateral temporary occlusion of the carotid artery and systemic hypotension; mean blood pressure was maintained 60 mmHg for 10 minutes. 5 (group 3) and 7 (group 4) days later, rats (each had five) were also sacrificed and examined the pathological sections at the hippocampus. [RESULTS] In MCA occlusion model, infarction size was measured at the slice 5mm from the frontal pole; group 1 was 12.5±3.1% and group 2 was 28.8±3.6% (%hemisphere). And group 3 and 4 did not have any apparent neuronal damage at the hippocampus. [REMARKS] In global ischemia models, delayed neuronal damage is well known. But, focal ischemic models also have same kinds of issues in cortical infarction. In the proximal MCA occlusion model, the difference of cortical infarction size between 24 and 72 hours after is 3.1±1.1% (unpublished data). In this experiment, zinc depletion could enlarge the time lag of cortical infarction and also could cancel delayed nelayed neuronal damage of the hippocampus, which might be due to the decrease of non-NMDA neurotoxicity.

ISCHEMIA: MOLECULAR BIOLOGY/IMMUNOCYTOCHEMISTRY

678.1

Comparison of the Effects of Global Ischemia on Hippocampal Morphology and Glutamate Receptor Binding Sites. *...by John Steck, Kheir Zuobi, Art Day, Joanna Peris, Judson Chandler* and Fulton T. Crews . Dept. of Pharmacology and Neurological Surgery, University of Florida Brain Institute, Gainesville, Fla. 32610*

Histological damage and autoradiographic distribution of glutamate receptor binding sites were compared following 20 min of global ischemia. Global ischemia was produced in Wistar rats through the four vessel occlusion model as described by Pulsinelli. Sham operated controls and ischemic animals were sacrificed 3 and 7 days following surgery. After both 3 and 7 days post-ischemia, hippocampal CA1 consistently showed severe neuronal loss whereas dentate gyrus (DG) showed no neuronal damage. CA3 showed variable damage at both time points and in CA4 the majority of hippocampi showed signs of ischemic injury characterized by eosinophilic cytoplasm and pyknotic nuclei after 3 days whereas 7 days post-ischemia only a few CA4 regions showed damage. In sham operated controls [^{3}H]-AMPA sites were highest in dentate gyrus with similar amounts in CA1, CA3 and CA4. Ischemia reduced [^{3}H]-AMPA sites in all hippocampal regions by approximately 30-40% in all hippocampal regions at both 3 and 7 days post-ischemia. [^{3}H] kainate sites were lowest in CA1 and greatest in CA3 with intermediate levels in CA4 and DG. Ischemia produced progressive decreases in CA1 on 3 and 7 days post-ischemia, small changes in CA3 and CA4, and no change in DG. NMDA glutamate receptors were studied using [^{3}H]-MK801, [^{3}H]-CGS14859, and [^{3}H]-glutamate-NMDA specific binding. All 3 NMDA ligands had the greatest number of sites in CA1 and DG, which were comparable. MK-801 and CGS binding were decreased approximately 30-40% in both CA1 and DG with smaller decreases in CA3 and CA4 at both 3 and 7 days. NMDA specific glutamate binding showed little or no change at 3 days post ischemia in CA1, CA3 and DG whereas after 7 days all regions showed a 25-35% decrease in binding. These findings suggest that NMDA receptor sites may change differentially following global ischemia and that changes in glutamate receptor sites do not correspond with histological changes. (Funded by AA06069).

678.2

THE RELATIONSHIP BETWEEN GLUTAMATE EXPOSURE, ^{3}H-NIMODIPINE BINDING AND CBF IN RAT FOCAL ISCHEMIA.
H.Osuga, A.M.Hakim*. Neuroscience institute, University of Ottawa, Ottawa, Canada, K1H 8L6

The increase in extracellular glutamate concentration and intracellular calcium after ischemia are presumed to be major contributors to cell death. The purpose of this study is to define the relationship between the increase in extracelluar glutamate, binding of ^{3}H-nimodipine, a ligand of L-type voltage sensitive calcium channel (VSCC)), and CBF in rat focal ischemia.

Materials and Methods: Eight rats weighing 275-300g were anesthetized with halothane. Microdialysis probes (OD:0.3mm, membrane length 2mm) and adjacent platinum wires for CBF measurements by means of H_2 clearance were placed in 6 cortical regions and perfused with Krebs-Ringer-Bicarbonate solution at 2µl/min. Dialysate was collected at 10min intervals and glutamate concentration measured using HPLC. Two hundred µCi of ^{3}H-nimodipine were injected and ischemia was produced by inserting 4-0 nylon thread through common carotid artery toward anterior-middle cerebral artery bifurcation. CBF was measured frequently and was stable over the experimental duration.Animals were sacrificed by decapitation 30 min after occlusion and autoradiography was performed.

Conclusion: Regional CBF varied from 8 to 110% of preocclusion values. Glutamate concentration increased 6.9 fold from the base line after ischemia in parietal cortex but the course of extracelluar glutamate was variable. The glutamate exposure index (GEI: Integrated glutamate concentration in dialysate over duration of ischemia) correlated with CBF below 30 % of control hemisphere ($p<0.00003$). ^{3}H-nimodipine binding increases with severity of ischemia and is related to GEI.

678.3

MEF2C Immunoreactivity in Gerbil Brain Following Global Cerebral Ischemia. E.K. Speliotes*, N.W. Kowall, B.F.Shanti, S.P. Finklestein, D. Leifer. Massachusetts General Hospital, Children's Hospital, Boston University Medical School, and Harvard Medical School , Boston, MA, & Veteran's Administration Medical Center, Bedford, MA.

MEF2C (myocyte-specific enhancer binding factor 2C) is a transcription factor that is expressed in muscle and brain, and may play a role in the terminal differentiation of neurons during development (Leifer et. al., this meeting). To examine the role of this factor in adult mammalian brain, we looked at MEF2C immunoreactivity (MEF2C-IR) in gerbils following global cerebral ischemia which results in the preferential death of hippocampal CA1 neurons within 2-4 days. Animals were sacrificed at 4hrs, 1,3,7,14,and 30 days after transient (10 min) bilateral carotid occlusion, and fixed brains were stained with antisera to MEF2C. MEF2C-IR was noted in the nuclei of many subpopulations of neurons and in microglia in normal brain, as well as in activated microglia in the CA1 region following cerebral ischemia. MEF2C-labeled neurons in the CA1 region, unlike CA1 pyramidal neurons which were not MEF2C-immunoreactive, survived the ischemic insult. A subpopulation of these surviving MEF2C immunoreactive cells stained with antibody against parvalbumin, a calcium binding protein found in a subset of GABAergic inhibitory interneurons. These findings suggest that MEF2C may play a role in the functioning of some normal subpopulations of neurons and microglia as well as in the functioning of activated microglia. Moreover, MEF2C may contribute to helping some types of cells survive ischemic insult.

678.4

REGIONAL ACCUMULATION OF AMYLOID PROTEIN PRECURSOR FOLLOWING A BILATERAL CAROTID OCCLUSION IN THE GERBIL. J.A.Oostveen* and E.D.Hall, CNS Diseases Research, The Upjohn Company, Kalamazoo, MI 49001.

Amyloid deposits comprise the major pathological characteristic of Alzheimer's disease. The primary component of these deposits is a peptide known as the β-amyloid protein which is derived from a larger precursor, the amyloid protein precursor (APP). The physiological function of APP is unclear, but evidence is accumulating that APP plays a role in the cerebral acute phase response to injury. Increased APP expression has been demonstrated in experimental and clinical cerebral trauma and, to a lesser degree, ischemic injury. We report the distribution of APP immunoreactivity in a model of transient global cerebral ischemia in the gerbil.

Male Mongolian gerbils (50-65 g) were exposed to a 10 min. bilateral carotid occlusion. Following 24 hrs of reperfusion, the animals were perfused intracardially and the brains frozen for free floating immunocytochemistry. Frozen coronal sections were processed and immunoreactivity to APP was visualized using clone 22C11 mouse monoclonal anti-APP, which recognized the mature and carboxytruncated forms of APP (Boehringer Mannheim).

APP immunoreactivity was found in the CA3 region of the hippocampus, the dentate gyrus, as well as layers III, V, and VI of the parietal cortex.

Following a cerebral insult, APP immunoreactivity increases and may provide a useful marker of neuronal injury. Elucidation of this protein's role in the neuronal stress response may have therapeutic implications.

678.5

EFFECT OF ISCHEMIA ON THE AMYLOID PRECURSOR PROTEIN. N. Komori*, T. Saitoh, B.-L. Chen, J.-M. Roch, and J. Zivin. Dept. of Neurosci, Univ. of California, San Diego, CA 92093.

Amyloid precursor protein (APP) and its trophic sequences (Roch et al., 1993 Neurosci. Abst.) have been found to promote neurite extension in vitro, to increase synaptic density in rat brain, and to alleviate ischemia-induced damage in a rabbit spinal cord ischemia model (RSCIM). Thus, one of the physiological functions of APP appears to be assisting cellular function and survival. Using RSCIM, we have studied APP mRNA and protein levels. The concentration of APP mRNA increased in the lumbar region after 15 min ischemia and/or 1 hr reperfusion, and then went down to near control. However, elevation of APP mRNA was not seen at 60 min ischemia. APP protein level was also examined by Western blot. We detected three distinct APP bands in the particulate fractions. The concentration of the highest molecular weight APP band decreased after 60 min ischemia and disappeared by the end of 18 hr of reperfusion. The ratio of the middle and the lowest APP bands changed after 15 as well as 60 min ischemia and also reperfusion periods following 60 min ischemia suggesting a differential effect of ischemia/reperfusion on different APP isoforms. The differential expression and/or disappearance of APP isoforms may be of significance in protecting neurons from ischemia-induced death.

678.6

TUMOR NECROSIS FACTOR α mRNA AND PEPTIDE ARE MARKEDLY EXPRESSED IN NEURONS PRESENT IN FOCAL CEREBRAL STROKE. G. Feuerstein*, R. Clark, T. Liu, R. White, F. Barone. Dept. of Cardiovascular Pharmacology, SmithKline Beecham Pharmaceuticals, King of Prussia, PA 19406

Ischemic stroke elicits inflammatory reactions marked by leukocyte adherence to microvessels and transendothelial migration. Tumor Necrosis Factor α (TNF_α) is a cytokine known to initiate inflammation. In order to prove that TNF_α is produced in ischemic cortex, molecular biology techniques and immunocytochemistry were employed to identify TNF_α mRNA and peptide in the cortex of rats (300-350 gm) subjected to permanent middle cerebral artery occlusion (MCAO). TNF_α mRNA (Northern blot) analysis revealed enhanced TNF_α mRNA expression as early as 1 hr post MCAO, peak (104±8, vs control, 2±1, n=4, $p<0.01$) at 12 hr followed by partial decline over a 5 d period. Immunocytochemical (hamster anti-murine TNF_α Mab) analysis using double fluorescence technique revealed TNF_α positive immunofluorescence coinciding with neurofilament (neuronal) but not GFAP (astrocytes) or vimentin (microglia) immunofluorescence. Neuronal TNF_α originated in the ischemic zone at 12 hr, but at 24 hr was primarily localized around the core zone. These data indicate that very early on in focal cerebral ischemia, TNF_α transcripts and peptides are produced. Since TNF_α possesses pro-inflammatory and pro-thrombotic actions on endothelium and direct cytotoxic effects, our data suggest a potential role of TNF_α in the evolution of brain damage following stroke.

678.7

IN SITU NICK TRANSLATION: A NOVEL APPROACH TO IDENTIFY NEURONAL INJURY AFTER ISCHEMIC LESIONS. L. MacKenzie, F.G. Szele, and M-F Chesselet* Dept of Pharmacology, U. of Pennsylvania, Philadelphia, PA 19104

The identification of neuronal injury after ischemic insults has been hindered by the lack of reliable markers at the single-cell level. We have used in situ nick translation to identify increases in DNA strand breaks in individual neurons after ischemic lesions of the cerebral cortex induced by thermocoagulation of pial blood vessels. This procedure induces a progressive loss of cortical tissue, with a marked decrease in neuronal density already visible 6 hrs after surgery, and complete cortical atrophy by 7 days post-surgery (Szele and Chesselet, submitted). Rats were perfused with paraformaldehyde under anesthesia 6 hrs after surgery. Tissue sections (10 µm) were cut on a cryostat and incubated with ^{35}S-dTTP in the absence or in the presence of DNA polymerase for 120 min. at 16° C. Dense clusters of silver grains were observed at the periphery of the lesion in sections incubated in the presence of DNA polymerase I. In addition, numerous neurons within the ischemic area showed moderate labelling. These neurons did not show marked alterations in morphology, suggesting that increased DNA strand breaks is an early event in post-ischemic neuronal death. No specific labelling was observed in the striatum or in cortical regions distal from the ischemic lesion, which did not show neuronal loss at later time points. No labelling was observed in sections from rats witout lesions, or in sections incubated without DNA polymerase. Supp. by NS-29230 and the Hereditary Disease Foundation.

678.8

ISCHEMIC BUT NOT EXCITOTOXIC CELL DEATH IS ASSOCIATED WITH INTERNUCLEOSOMAL DNA CLEAVAGE. C. Charriaut-Marlangue, F. Dessi, H. Pollard, A. Heron, J. Moreau, M. Schumacher* and Y. Ben-Ari. INSERM U29, 123 Bld de Port-Royal, 75014 Paris, France.

Transient forebrain ischemia induces in several species a delayed cell death of pyramidal neurons in the CA1 region of the hippocampus. Previous investigations, using *in vitro* systems, have demonstrated that anoxia induces an early increase of protein synthesis (C. Charriaut-Marlangue et al., Eur. J. N., 1992, 4, 766) and that protein synthesis inhibitors *in vivo* and *in vitro* reduces the CA1 neuronal death (Goto et al., Brain Res., 1990, 534, 299; Papas et al., Eur. J. N., 1992, 4, 758). In contrast, in cerebellar culture transcriptional and translational inhibitors do not prevent glutamate-induced neuronal death (Dessi et al., J. Neurochem, 1993, 60, in press). Physiological cell death or apoptosis which requires RNA and protein synthesis is characterized by the internucleosomal cleavage of DNA by a Ca^{2+}, Mg^{2+}-dependent endonuclease. Our purpose was to analyze the integrity of genomic DNA, by agarose gel electrophoresis, in a transient global ischemia model or after excitotoxic cell death in "*in vitro*" models. Electrophoresis of DNA extracted from the Ammon's horn and the striatum from ischemic rats showed a "ladder" pattern whereas no DNA degradation was observed in dentate gyrus and in cerebellum, which are less vulnerable. No DNA degradation was observed after NMDA (100 µM)-induced neuronal death in hippocampal slices or glutamate treatment (100 µM) in cerebellar culture. Our results were confirmed by DNA nick ends of histological sections from post-ischemic brains which were also labelled in CA1, cortical and striatum neurons. These results suggestthat activation of an endonuclease is involved in ischemic- but not in excitotoxic-induced neuronal death.

678.9

IN VITRO NEUROPROTECTIVE ACTIVITY OF INHIBITORS OF POLY-ADP-RIBOSE POLYMERASE. M.S. Miller*, C. Zobre, and M. Lewis. Pharmaceuticals Research Division, Sterling Winthrop Inc., Collegeville, PA 19426.

Poly-ADP-ribose polymerase (PARP) is a 116 kD nuclear metalloenzyme which is activated by DNA strand breaks and utilizes intracellular NAD^+ to ribosylate numerous proteins. Excitotoxicity-associated increases in intracellular Ca^{++} may activate Ca^{++}-dependent nucleases to produce DNA fragmentation. Protein ribosylation and/or reduced NAD^+ by PARP may contribute to excitotoxic neuronal injury. To investigate this potential mechanism, a small series of substituted benzamides with varying potencies for inhibiting PARP were evaluated for the ability to attenuate NMDA-induced neurotoxicity (LDH release) in primary cultures of mouse fetal cortex. Potency (IC50) for attenuating NMDA neurotoxicity varied within the series from 70-400 uM. A correlation (r=0.904) was observed between reported potency to inhibit PARP and potency for neuroprotection. Efficacy was greatest when PARP inhibitors were coadministered with NMDA. Data suggest that selective inhibition of PARP may represent a useful neuroprotective mechanism.

678.10

EVIDENCE OF APOPTOTIC LADDERED DNA FRAGMENTS IN BRAIN FOLLOWING ISCHEMIA. J.P. MacManus[1], J. Rasquinha[1], E. Preston[1], H. Li[2], D. Xue[2], Z.G. Huang[2] and A. Buchan[2]*. [1]Institutes for Biological Sciences and Biodiagnostics, National Research Council Canada, Ottawa, Ontario, Canada K1A 0R6 and [2]Ottawa Civic Hospital, Ottawa, Ontario, Canada K1Y 4E9, from the *Canada/Fisons Fight Stroke* program.

Biochemical evidence was sought for apoptosis in rat brain *in vivo* in three separate models of ischemia using a sensitive radioactive end-labelling technique to quantitate DNA breaks. Neocortex, hippocampus and striatum were sampled 24 and 48 hr following global ischemia induced by occlusion of two vessels (both common carotid arteries and hypotension to 50 mmHg) (2VO) or four vessels (common carotid and vertebral arteries) (4VO) for 5 to 30 min. Gel autoradiography showed greater laddered DNA fragmentation in the CA1 layer of the hippocampus than in the CA3. There was extensive DNA damage in the striatum after both 2 or 4VO. Little DNA fragmentation was seen in the neocortex. The extent of DNA damage in both hippocampus and striatum was dependent upon the duration of the ischemic episode in both 2 and 4VO models. Laddered DNA fragmentation was also shown 24 hr after temporary focal ischemia produced by occlusion of the middle cerebral artery (MCAO). The amount of DNA damage was greater in the core of the neocortical infarct than in the penumbra. Again, the extent of damage was proportional to the duration of MCAO (0.5 to 3 hr). Thus, laddered DNA fragmentation correlated with the known regional and temporal patterns of neuronal death in three established models of ischemic brain damage in the rat.

It is concluded that there is an apoptotic component to the cell death following ischemia.

678.11

HYPOXIA INDUCED PROGRAMMED CELL DEATH IN CULTURED NEURONS. D. M. Rosenbaum*, J. Kalberg, D. K. Batter and J. A. Kessler. Depts. of Neurology and Neuroscience, Albert Einstein College of Medicine, Bronx, NY 10461.

Apoptosis, a form of cell death ("programmed" cell death) in which the nucleus and cytoplasm shrink and often fragment, serves to eliminate excessive or unwanted cells during remodeling of embryonic tissues, during organ involution, and in tumor regression. In acute pathologic states, such as ischemia, the cells tend to swell and lyse - a process called necrosis. We hypothesize that the delayed neural death associated clinically with hypoxia may, in part, represent apoptosis. A tissue culture model of hypoxia was employed using sympathetic neurons. Pretreatment with an endonuclease inhibitor (Aurintricarboxylic), a protein synthesis inhibitor (ActinomycinD), depolarizing conditions (55 mM KCl) as well as antisense c-myc were tried in order to ameliorate cell death (all have been shown to prevent apoptosis). In addition, cell nuclei were stained with propidium iodide (a DNA marker) to demonstrate nuclear chromatin condensation. After 24 hrs of hypoxia Aurintricarboxylic acid decreased cell death by 39%, depolarizing conditions decreased cell death by 33%, and Actinomycin by 26%. Antisense c-myc had no effect. Fluorescent staining demonstrated chromatin condensation. These data suggest that apoptosis may play a role in hypoxic cell death and that in this paradigm expression of c-myc is unnecessary. This would suggest a new approach to our understanding of hypoxia and open new strategies to lessen neuronal damage secondary to this process. We are currently studying this hypothesis utilizing an *in vivo* model.

678.12

GLUTAMATE NMDA RECEPTOR mRNA EXPRESSION AFTER FOCAL ISCHEMIA IN THE RAT. C.Gonzales*, R.Franco, T.L.Sailer and J.A.Moyer. CNS Pharmacology Division, Wyeth-Ayerst Research, Princeton, NJ 08543-8000.

Neuronal cell death that occurs after focal ischemia in the rat is widely believed to involve overactivation of the NMDA subtype of the glutamate receptor. Recently, cloning of subunits of the NMDA receptor has been described. The NMDAR1 receptor subunit is expressed in most neuronal cells and possesses electrophysiological properties associated with the NMDA receptor. The NMDAR2A-D subunits, which are differentially expressed in brain regions, do not exhibit these properties, but when co-expressed with the NMDAR1 subunit, they potentiate NMDA receptor activity (Nakanishi, Science 258:597-603,1992). The molecular cloning of the rat NMDA receptor subunits makes it possible to design specific oligonucleotide probes to study the role of NMDA receptor subunit expression in ischemia induced cell death. We have used *in situ* hybridization histochemistry to look at the expression of three subunits of NMDA receptor mRNAs that are expressed in regions susceptible to ischemic damage in our model of rat focal ischemia. Sham operated rats or rats subjected to tandem occlusion of the right common carotid and right middle cerebral arteries were sacrificed 1, 3, or 24 hrs after surgery. Sections were processed for *in situ* hybridization for the NMDAR1, 2A, and 2B subunits and synapsin (control). Sections were apposed to film and optical densities between sham and lesioned cortices were compared. Our results show that after 24 hrs, mean NMDAR1, 2A and 2B expression in the lesioned hemisphere was reduced 37% from sham operated animals, 30% after 4 hrs., and there was no significant decrease 1 hr after ischemia. Decreases in NMDA mRNA followed the pattern of cell death seen with adjacent Nissl-stained sections and prior to cell death, no changes in mRNA were observed for either NMDA or synapsin. These results are consistant with decreasing mRNA levels being directly related to cell death.

678.13

RESPONSE OF IONOTROPIC GLUTAMATE RECEPTOR (GluR) SUBUNITS DURING EARLY STAGES OF REGIONAL BRAIN ISCHEMIA. C.A. Pardo, L.J. Martin, L.H. Monsein, C.D. Blackstone and R.N.Bryan*. The Johns Hopkins Univ. Sch. of Med., Balto., MD 21205

The response of non-NMDA ionotropic AMPA-GluR subunits in early stages of regional ischemia (3-18 hours) were studied in a baboon model. Ischemic injury by occlusion of the middle cerebral artery, using interventional neuroradiological techniques, was used to produce either complete ischemia (CI; n=5) or incomplete ischemia with reperfusion (IIR; n=4). Antibodies that detect GluR1, GluR2/3, and GluR4 subunits [Martin et al., *J. Neurosci.* **13**:782, 1993] of AMPA receptors were used to study the cellular expression of GluR in striatum and cortex. Changes in AMPA receptor immunoreactivity were correlated with neuronal-dendrite markers (e.g., microtubule-associated protein-2 [MAP-2]) that defined necrotic-perinecrotic areas. In the dorsolateral and posterior caudate/putamen, perinecrotic regions in the CI model showed decreased GluR1 and GluR2/3 and increased neuronal expression of GluR4. Different subtypes of striatal neurons appeared to be affected equally, and the GluR1 response was similar temporarily in striosomes and matrix in the CI model. However, in the IIR model, matrical neurons showed decreased GluR1 before striosomal neurons. In cortical perinecrotic areas, responses of GluR1, GluR2/3, and GluR4 were similar to striatal changes. In cortical penumbra areas, superficial layers showed reduced expression of GluR2/3 before deeper layers. In both striatum and cortex, early neuronal injury was correlated with dendritic collapse (reduced MAP-2 expression) and loss of specific AMPA receptor subunits. These studies show that AMPA receptor subunits are altered differentially during ischemic injury, perhaps as a result of cytoarchitectonic changes.

ISCHEMIA: NEONATAL

679.1

THE NEUROPROTECTIVE EFFECTS OF MK-801 AND GM1 GANGLIOSIDE ON INJURY TO THE NEWBORN RAT CORTEX. G.D. Rosen*, E.A. Sigel, G.F. Sherman, and A.M. Galaburda. Beth Israel Hospital and Harvard Medical School, Boston, MA, 02215

Placement of a freezing probe on the skull of newborn rats results in the formation of a neuropathologic malformation resembling focal microgyria (Humphreys et al., *J Neuropath Exp Neurol* **50**:145, 1991), a minor malformation seen in the brains of some dyslexics (Galaburda et al., *Ann Neurol* **18**:222, 1985). The freezing injury likely acts as an ischemic/hypoxic event that leads to neocortical reorganization resulting in and the formation of the microgyria (Rosen et al., *J Neuropath Exp Neurol* **51**:601, 1992). We hypothesized that neuroprotective agents, which ameliorate the effects of hypoxic/ischemic insults, might also reduce the effects of a freezing lesion and prevent or modify the reorganization of the developing cortex.

Newborn rats were given injections of GM1 ganglioside (30 mg/Kg b.w.) as either a pretreatment (6 h before freezing injury), posttreatment (2, 16, 40, and 64 h after freezing injury), or a combination of both. A second group of rats received treatment with MK-801 (1 or 2 mg/Kg b.w.) 0.5 h before the freezing injury and 6 and 14 hours afterward. Controls were given either vehicle (saline) injections along with freezing lesions or injections of neuroprotective agents only.

GM-1 ganglioside and MK-801 had significant ameliorating effects on the formation of microgyria which is most apparent in those animals receiving pre- and post-treatments.

This work was supported, in part, by NIH grant HD20806.

679.2

PHENYTOIN AND CI 953 ATTENUATE HYPOXIC-ISCHEMIC BRAIN DAMAGE IN NEONATAL RATS M.G. Vartanian* , J.J. Cordon, N.C. Kupina, J. Schielke, and C.P. Taylor. Dept. of Neuroscience Pharmacology, Parke-Davis Research Division, Warner-Lambert Co., Ann Arbor, MI. 48105.

Phenytoin and CI 953 (Urea N-(2-chloro-6-methylphenyl)-N'-4-pyridinyl-are anticonvulsant compounds that modulate voltage-dependent sodium channels (Rock et al., *Epilepsy Res.* 8:197-203,1991). Both compounds are neuroprotective in vitro against hypoxic damage in hippocampal slices (Taylor & Weber, Soc. Neurosci. Abstr., 18:1135, 1992, and unpublished data).

We tested phenytoin and CI 953 using an in vivo model of hypoxic-ischemic brain injury in neonatal rats. To induce brain injury, seven day old Sprague-Dawley rats were anesthetized with ether and the left carotid was ligated. Pups were allowed recovery for 2 hours before to hypoxia for 3 hours (humidified 8% oxygen, balance nitrogen) in a sealed chamber. Temperature was accurately controled before, and during hypoxia. Immediately before hypoxia, 60 pups recevied i.p., either phenytoin (30 mg/kg), CI 953 (30 mg/kg; 3 times, separated by 1.5 hours), or vehicle. Animals were sacrificed two weeks after hypoxia, and right and left hemispere weight disparities were compared as percent damage ([(L - R)/R] x 100). Phenytoin was most effective in reducing neuronal damage (vehicle treated, 38.06 ± 4.74% vs. 6.30 ± 3.22%; $p < 0.001$). Multiple dosing of CI 953 resulted in a significant, (vehicle treated, 35.79 ± 2.28% vs. 20.36 ± 6.50%; $p < 0.04$) but less effective inhibition of cerebral damage when compared to phenytoin. These data suggest that compounds that modulate voltage-dependent sodium channels may reduce injury from hypoxic-ischemic brain insult.

679.3

THE LAZAROID U-74389G AMELIORATES ISCHEMIC NEURONAL INJURY IN INFANT PIGS. J. Deshpande*, W.O. Whetsell, Jr., R. England, J. Stewart and C. Elkins, Depts. of Pediatrics, Anesthesiology, Neuropathology and Cardiac Surgery, Vanderbilt University Medical Center, Nashville, TN 37232.

U-74389G is a 21-amino-steroid antioxidant that inhibits lipid peroxidation. Related lazaroids have shown some efficacy in reducing ischemic CNS damage. This study was designed to investigate the ameliorative effects of U-74389G upon ischemia in hippocampal pyramidal cells in the infant mammalian brain. Three-week-old pigs, fasted overnight, were anesthetized with pentobarbital, intubated and mechanically ventilated. Anesthesia as maintained with isoflurane in N_2O/O_2. MABP and blood gases were monitored via femoral artery and venous catheters. Bitemporal EEG activity (Neurotrack) was monitored during all experiments. Ischemia was induced in one group of animals (n=6) by occlusion of the innominate trunk bilaterally for 18 minutes. In a second group (n=8), ischemia was induced in the same manner and U-74389G (3 mg/kg) was administered intravenously immediately and at two hours post-ischemia. All animals were extubated when they could sustain adequate spontaneous ventilation. Neurological examinations were performed daily. All animals were maintained for 7 days then sacrificed and brains were harvested and studied histologically. In both groups, neurologic function showed greatest impairment at 24 hours post-ischemia and improved over the remaining study period. There was no difference between groups. Histologic examination at 7 days showed significantly greater loss of hippocampal pyramidal cells ($P < 0.05$, Wilcoxin's RS Test) in the untreated animals. Results indicate that U-74389G may be effective in reducing ischemic CNS damage. [U-74389G was kindly supplied by Upjohn, Inc.]

679.4

HYPOGLYCEMIA ABOLISHES HYPOXIC DILATION OF PIAL ARTERIOLES IN NEWBORN PIG T.S. Park, E.R. Gonzales, A.R. Shah, R.G. Dacey*, and J.M. Gidday. Department of Neurosurgery, St. Louis Children's Hospital and Washington University School of Medicine, St. Louis, MO 63110.

Compensatory increases in cerebral blood flow occur in hypoglycemic adult animals in response to cerebral hypoxia, but it is not known if such a preservation in cerebrovascular reactivity to hypoxia occurs in hypoglycemic neonates. Therefore, we investigated the effects of systemic hypoglycemia (blood glucose < 25 mg/dl; non-isoelectric EEG) on the ability of pial arterioles of newborn pigs to dilate in response to systemic hypoxia. Arterioles 25-50 μm in diameter under baseline conditions were studied by videomicroscopy in isoflurane-anesthetized piglets equipped with closed cranial windows over the parietal cortex. Systemic hypoxia (PaO_2=26±1 mmHg) increased mean arterial blood pressure (MABP) 31±6% (p=0.002) and dilated pial arterioles 57±9% (p=0.0007; n=8) in normoglycemic animals. Induction of hypoglycemia did not change MABP, but dilated pial arterioles 31±5% (p=0.002; n=7), significantly less (p=0.025) than the vasodilation resulting from hypoxia. When hypoxia (PaO_2=28±1 mmHg) was subsequently induced in these animals after 40 min of sustained hypoglycemia, MABP increased 22±11% (p=0.095), but pial arterioles did not dilate further (35±5% relative to baseline; p=0.339 vs. hypoglycemia alone). In summary, the arteriolar dilation in response to hypoxia in hypoglycemic animals was significantly less (p=0.045) than that occurring in normoglycemic animals. Thus, these results indicate that hypoglycemia impairs the ability of the pial microcirculation to dilate to hypoxia, suggesting that dysfunctions in cerebrovascular reactivity can result from hypoglycemia in the neonate.

679.5

POTENTIATION OF ENDOGENOUS ADENOSINE PROTECTS THE NEWBORN BRAIN FROM HYPOXIC-ISCHEMIC INJURY. J.M. Gidday*, J.C. Fitzgibbons, A.R. Shah, and T.S. Park. Departments of Neurosurgery, St. Louis Children's Hospital and Washington University School of Medicine, St. Louis, MO 63110.

While the ischemic cerebroprotective actions of adenosine have been investigated in adult animal models, comparable studies in neonates are lacking. We utilized the well-established 7-day old rat hypoxia-ischemia model (unilateral carotid ligation and 3h hypoxia) to separately test the neuroprotective potential of propentofylline (HWA 285), an adenosine transport inhibitor, and deoxycoformycin (DCF), an adenosine deaminase inhibitor, which were administered immediately after hypoxia. Injury analyses were undertaken one week later. Animals treated with HWA (10 mg/kg ip; n=14) showed a 31% decrease ($p<0.03$) in hemispheric necrosis ipsilateral to the carotid ligation, compared to saline-treated controls (n=15). Regression analysis showed a significant correlation ($r>0.9$) between these data and morphological determinations of cross-sectional area in coronal sections, which yielded improvements of 32% and 29% at the level of the striatum and dorsal hippocampus, respectively. Histological analyses of Richardson-stained coronal sections revealed a significant reduction ($p<0.03$ Mann-Whitney) in striatal neuronal injury in animals treated with HWA. DCF (2.5 mg/kg ip; n=12) yielded a 29% improvement ($p<0.02$) in necrosis relative to controls (n=13), a 21% and 22% increase in striatal and hippocampal cross-sectional areas ($p<0.03$), respectively, and a marked reduction in cellular injury in striatum and hippocampal CA4 region ($p<0.03$). These findings indicate that the potentiation of extracellular adenosine levels by HWA and DCF affords significant protection from hypoxic-ischemic injury in the brain of the newborn.

679.6

CHRONIC HYPOXIA CAUSES DOWN-REGULATION OF GLUCOSE TRANSPORTER I mRNA IN ADULT BUT NOT IN FETAL RAT BRAIN. Y. Xia*, J. B. Warshaw and G.G. Haddad. Section of Respiratory Medicine, Department of Pediatrics, Yale University School of Medicine, New Haven, CT 06510

Recent work from our laboratory and others has shown that chronic hypoxia can up- or down-regulate membrane proteins such as sulfonylurea receptors and sodium channels. Since there are virtually no glucose stores in the brain, glucose transport becomes crucial for cell survival under stress and could therefore be regulated. As a first step, we asked whether glucose transporter isoform I (GlutI) mRNA was changed during chronic hypoxia in the brain. Since metabolism in fetal brain depends proportionally less on glucose as a substrate than the adult brain, we studied brain GlutI mRNA at birth and in the adult after exposure of pregnant rats (from E10 to E22) or adult rats (from P90 to P120) to hypoxia (8-9%, FiO_2) using Northern blot analysis with total RNA isolated from whole brain.

Our results show that i) GlutI mRNA was less than about 35% of adult level at birth and increased with age; by 30 days, GlutI mRNA was about 80% of adult level; ii) chronic hypoxia caused a decrease of 70% in GlutI mRNA in the adult brains and iii) no decrease of GlutI mRNA was seen in exposed fetal brains (100% vs 115%).

Our data suggest that 1) the increase in GlutI mRNA with age is a reflection of the increase in O_2 consumption in brain tissue and 2) although both the fetus and the adult may down-regulate brain metabolism during O_2 deprivation, glucose transporters in the adult seem to be more sensitive to hypoxic stimuli than in the fetus.

679.7

PERINATAL HYPOXIA CAUSES REDUCED ACCUMBENS DOPAMINE AND ALTERED RECEPTOR DENSITIES IN THE ADULT RAT STRIATUM. S.B. Schwarzkopf*, L. McCoy, E.K. Richfield, and M. Hadjiconstantinou. Depts. of Psychiatry & Neurology, University of Rochester, Rochester, NY 14627; Depts. of Psychiatry & Pharmacology, Ohio State University, Columbus OH, 43210.

Perinatal hypoxia causes immediate and long term alterations in behavior and neurotransmitters in cortical and subcortical brain regions. Excitatory amino acid neurotoxicity mediated through NMDA receptors has been implicated in these effects. Previous findings indicated cortical changes in cholinergic, serotonergic, and dopaminergic indices 3 weeks following exposure to perinatal hypoxia. In this study we examined levels of striatal dopamine, its metabolites, and dopamine receptor and reuptake complex densities in adult rats exposed to perinatal hypoxia. Animals were male Sprague-Dawley rats exposed for 2 hours at age 7 days to 8% oxygen (HYPOX, N=26) and a control group (CTL, N=24). At 120 days of age animals were sacrificed and brains rapidly dissected and frozen. High performance liquid chromatography with electrochemical detection was used to measure dopame (DA), 3,4-dihydroxyphenylactic acid (DOPAC) and homovanillic acid (HVA) levels in the dorsal striatum (DSTR) and nucleus accumbens (NAC). DA autoradiography was used to assess striatal D1 and D2 receptors and DA reuptake complex (DAUC) densities. HYPOX animals exhibited decreased DA and DOPAC in the NAC but not in the DSTR. HYPOX animals also showed significantly increased DAUC and D1 densities compared to CTLs, with a highly significant reduction of D2 receptor binding. These results show that a relatively brief exposure to hypoxia during the perinatal period leads to marked alterations in striatal DA and DA receptor characteristics in adulthood. Findings suggest a compensatory increase in DA terminals (or diminished "pruning") in response to perinatal neurotoxicity. Diminished D2 density might indicate greater vulnerability to hypoxic injury of GABAergic neurons belonging to the "indirect" striatal efferent pathway. *(Supported by MH00859 and MH40381)*

679.8

THE AGE-RELATED EFFECTS OF TEMPERATURE RESPONSES AND BRAIN DAMAGE FOLLOWING HYPOXIA-ISCHEMIA(HI) TO ANESTHETIZED RATS. J.A. Thornhill, E. West, M.C. Chubb*, A. Shuaib and J. Yager Saskatchewan Stroke Research Center, Saskatoon, Canada S7N 4J9.

Younger animals are believed to be more resistant to H-I than their adult counterparts. To study this hypothesis, male, Wistar rats (10 days, 3, 6 and 9 weeks), were anesthetized with halothane and rectal temperatures kept at 37°C via a heating blanket. Ipsilateral and contralateral temporalis muscles were measured using thermistor probes to indicate changes in brain temperatures. Hypoxia-ischemia was induced by ligation of the right common carotid artery followed by exposure to hypoxia (12% O_2) for 35 min. (modified Levine model). Animals were sacrificed 7 days later, brains perfusion-fixed and neuropathological damage assessed histologically. Ipsilateral and contralateral temporalis temperatures fell spontaneously with hypoxia in all groups except the 10-day old group, with greater decreases in the ipsilateral side. Brain damage was most severe and consistent in the 9 & 3 week old animals compared to the 10 day and 6 week old animals ($p > 0.05$). Thus, a direct correlation between age and brain damage following hypoxia-ischemia is not apparent.

Supported by the Sask. Health Research Board and the Sask. Heart and Stroke Foundation.

679.9

AGE-DEPENDENT NEUROTOXIC RESPONSE TO L-CYSTEINE.
J.G. Csernansky, M.E. Bardgett, J. Labruyere, J.J. Jackson, J.W. Olney. Psychiatry Dept., Wash. Univ. Sch. of Med., St. Louis, MO 63110 & Psychology Dept., Univ. of MO-St. Louis, St. Louis, MO 63121.*

The common amino acid, L-cysteine, is neurotoxic to the developing rodent brain when administered orally or subcutaneously to a pregnant dam or her newborn offspring. The pattern of brain damage (hippocampus, neocortex, caudate, thalamus) and the mechanism (pathological stimulation of *N*-methyl-D-aspartate (NMDA)-type glutamate receptors) of L-cysteine neurotoxicity is similar to the pattern and mechanism of perinatal hypoxic/ischemic brain damage. Given its similarity to hypoxia/ischemia, L-cysteine neurotoxicity offers a tool to study developmental excitotoxic insults which may ultimately result in psychopathology. Since the neurotoxic response to NMDA injection or hypoxia/ischemia in the rat brain peaks at one week of age (Ikonomidou et al., J. Neurosci., 9:2809, 1989), we have examined initially whether the neurotoxic response to L-cysteine demonstrates a similar age-dependent profile. Sprague-Dawley pups were injected subcutaneously with 0.9 mg/kg of L-cysteine on either postnatal day 1, 4, 7, or 10, and killed 6 hrs later. Relative to the younger pups, pups at postnatal day 7 were rated as exhibiting greater damage (edematous degeneration, pyknotic cells, and/or overt cell loss) in the retrosplenial granular cortex, the cingulate cortex, the deep layers of piriform cortex, and mediodorsal and ventrolateral thalamic nuclei. All of the postnatal day 10 pups died 2-3 hrs after injection. These preliminary results indicate that the neuropathological sequelae of L-cysteine neurotoxicity differs as a function of age of exposure. We are currently examining the neurotoxic response to L-cysteine at other ages and after different doses. Supported by a grant from the Theodore and Vada Stanley Foundation to J.G.C. and M.E.B. M.E.B. was supported by a N.I.M.H. Training Grant (MH14677).

679.10

NEONATAL ANOXIA REDUCES *C-FOS* EXPRESSION IN THE RAT HIPPOCAMPUS. M.E.Dell'Anna*[1,2], M.C.Geloso[1], M.Magarelli[1]. Institutes of Neurology[1] and Human Physiology[2], Catholic University, Rome (Italy).

The early expression of the proto-oncogene *c-fos* has been proposed as a marker of neuronal anoxic damage. Hippocampal sectors show different sensitivity to neonatal anoxia and CA1 neurons are the most damaged while CA3 cells appear less affected. In the present study we analyzed the expression of *c-fos* in hippocampal neurons at different time-points following neonatal anoxia (N_2 100% for 25 minutes at 30 hours after birth - P2). Sagittal 40 µm sections, obtained from animals sacrified at 1, 2, 4 and 6 hours and at 7 and 21 postnatal days after anoxia, were processed with ABC immunocytochemistry for *c-fos* (policlonal rabbit antibody, kind gift of Dr. Jadarola and Draisci). At all stages of life studied, *c-fos* immunoreactivity (IR) was expressed in many neurons of CA1, CA2 and CA3 hippocampal sectors in both control and anoxic animals. In gyrus dentatus, only few neurons expressing *c-fos* were present at P2 and P7 while they were numerous at P21 in both groups. One hour after N_2 exposure, the number of *c-fos* IR neurons was significantly reduced in CA1 and CA2 sectors of anoxic rats in comparison with the controls, while it clearly rised in CA3. In the following hours, the number of neurons expressing *c-fos* decreased significantly in all CA regions. At P7, the number of *c-fos* IR neurons was still reduced in all hippocampal sectors of anoxic rats, while no clear changes were present at P21. In gyrus dentatus no effects of neonatal anoxia were seen at the different time-points.

The present data, in contrast with several observations in adult anoxic conditions, are suggestive for a prolonged inhibition of neuronal expression of *c-fos* after neonatal anoxia. Furthermore, the results obtained in this study indicate that the time-course of changes in *c-fos* IR in the CA1, CA2 and CA3 sectors correlates with the specific sensitivity of the different hippocampal regions to neonatal anoxia and suggest the reduction of *c-fos* expression, in early stages after neonatal anoxia, as a possible predicitive index of the neuronal damage.

679.11

SELECTIVE LOSS OF PURKINJE CELLS FOLLOWING HYPOTHERMIC CARDIAC BYPASS AND ARREST IN THE NEONATAL PIG J. Brasko,* H. Kasowski, and D.T. Ross Head Injury Center, Division of Neurosurgery, University of Pennsylvania, Philadelphia, PA 19104

Cerebellar Purkinje cells are selectively vulnerable to ischemic degeneration following cardiac arrest or global ischemia in man and animals. We examined whether hypothermia during cardiac arrest/bypass was sufficient to prevent Purkinje cell loss.

Deeply anesthetized neonatal (21 day) pigs were placed on cardiopulmonary bypass, cooled to body temperatures of 15-27°C, and the bypass pump turned off. The pump was restarted 45 minutes to an hour later, the animal taken off bypass, and recovered. Animals survived for 7 days prior to sacrifice and transcardial perfusion. Four parallel sets of 40µm serial sections were cut; one was stained with cresyl violet, and the others were processed for immunohistochemistry using antisera to the calcium binding protein parvalbumin, heavily phosphorylated epitopes common to the heavy and medium neurofilament proteins (SMI-31).

In the cerebella from the animals arrested at 24-27°C the folia throughout the vermis and hemispheres were characterized by extensive selective loss of Purkinje cells particularly from the depths and sides of the folia. The density of granule cells in the affected foliar regions appeared normal and there was no evidence of focal infarction in the molecular layer. Spared Purkinje cells at foliar crests were frequently abnormal in appearance, with a marked decrease in cytoplasmic basophilia, a ballooned appearance with perikaryal and nuclear swelling and nucleolar eccentricity, and perikaryal neurofilament phosphorylation. In cerebella from animals arrested at colder temperatures Purkinje cell loss was present but less extensive. These results suggest that hypothermic neuroprotection alone may not be sufficient to prevent the loss of Purkinje cells following hypothermic cardiac arrest open heart surgery procedures. Augmentation with the prophylactic administration of pharmacological agents may be necessary for insuring complete protection. (Supported by The University of Pennsylvania Research Foundation, and NS-28852).

679.12

IN UTERO HYPOXIA AND CHOLINERGIC SIGNAL TRANSDUCTION IN THE DEVELOPING BRAIN. K. Hersey, Z.Y. Hu, P.K. Rudeen* and G.Y. Sun. Depts Child Health and Biochem., Univ. Missouri, Columbia, MO 65212.

Hypoxic-ischemic insult to neonates at birth is associated with learning deficits and other functional abnormalities during brain development. In this study, a rat model was used to examine the effects of this type of insult on the cholinergic signal transduction activity of the developing brain. Pregnant rats at term were anesthetized with diethyl ether and the gravid uterus surgically exposed. Hypoxic treatment was carried out by clamping the uterine blood vessels to the individual fetuses for 5, 10 and 15 min. The fetuses were delivered and resuscitated, and were fostered until two-weeks of age. Prior to the brain slice experiment, each pup was injected intracerebrally with [^{3}H]inositol (10 µCi in 10 µL) and the label was equilibrated in brain for 16 hr. Carbachol stimulation of poly-PI signaling activity was assessed by incubating the labeled brain slices in the presence of LiCl (10 mM) and measuring the levels of labeled inositol monophosphates (IP). Results indicated no obvious changes in labeled IP in pups that experienced a 5 min hypoxic treatment but an obvious decrease (20-30%) in this label could be found in pups that experienced 10 or 15 min hypoxic treatment at birth. This study clearly shows that hypoxic-ischemic insult at birth can lead to an alteration of the cholinergic signaling activity during brain development.

679.13

THE EFFECT OF PRENATAL HYPOXIC INSULT ON REGIONAL BRAIN WEIGHT AND BEHAVIOR IN RATS.
Z. Binienda*, S. A. Ferguson, F.D. Racey, N.R. Taylor, W. Slikker, Jr. and R.R. Holson. NCTR/FDA, Jefferson, AR 72079.

The effects of prenatal hypoxia on early behaviors, cognitive functions and regional brain weight were studied in rats. Hypoxic conditions were produced at gestational day 21 by submerging one isolated uterine horn in warm saline for 15 min. Fetuses were then delivered and cross-fostered as hypoxic pups. Fetuses of the adjacent horn (controls) were delivered by cesarean section and cross-fostered to the same surrogate mothers as the hypoxic pups. On postnatal day (PND) 30, regional brain weights were obtained. Negative geotaxis, olfactory discrimination, open field activity and complex maze performance were measured in hypoxic and control rats. Caudate nucleus and brain stem weights were significantly reduced in PND 30 hypoxic rats, to 95% of control values. Hypoxia was associated with lower activity on day 1 of open field testing (PND 18); other behavioral measures indicated control levels of performance in the hypoxic rats. These data, while preliminary, would suggest that a 15 min hypoxic insult has few dramatic effects on behavior.

679.14

BRACHIOCEPHALIC ARTERY OCCLUSION IN SHEEP: A MODEL FOR PERINATAL HYPOXIC-ISCHEMIC BRAIN DAMAGE. K.J. Anderson*[1] and D.J. Burchfield[2]. Depts. of Physiological Sciences[1], Neuroscience[1] and Pediatrics[2], University of Florida, Gainesville, FL 32610.

To better delineate the roles of ischemia and hypoxia on cerebral injury in the fetal and newborn brain, we developed an animal model for cerebral ischemia using lambs. The major advantages of using sheep as a model are two-fold; first, a single brachiocephalic artery arises from the arch of the aorta and its occlusion provides ischemia to the head, while leaving other vital organs in a state of complete perfusion. Second, the fetal lamb is remarkably resistant to *in utero* surgical manipulations and will not be aborted by the ewe. This is important because a large portion of cerebral palsy is of prenatal origin and use of fetal sheep will allow us to study the neurotoxicity of excitatory amino acids during this critical time period. After performing a left thoracotomy in newborns, we placed a pneumatic cuff occluder around the base of the brachiocephalic artery, a pre-calibrated ultrasound flow probe around a carotid artery in the neck and electrodes on the dura for the measurement of ECoG. The wires, cable and tubing were tunneled subcutaneously and exteriorized, the wounds closed, and the animal allowed to awaken. Two days later, the pneumatic cuff occluder was inflated so that the carotid flow fell to between 10-20% of baseline values and the animal was made to breathe an hypoxic gas mixture of 10-12% oxygen. After several minutes, the ECoG went flat and the animal became apneic. This was maintained for 1-2 min while providing artificial ventilation to the animal, then the lambs were resuscitated with release of the occlusion and administration of 100% oxygen. A similar procedure was used to provide hypoxia-ischemia to fetal lambs. In both cases, the lambs were allowed to recover for 4 days, euthanized and their brains were examined for evidence of injury. These lambs showed clinical evidence of neurological deficit for 12-36 hours after the insult. and histologic examination of their brains showed clear evidence of neuronal damage in several brain regions including parasaggital regions of frontal and parietal cortex and area CA1 of the dorsal hippocampal formation. This pattern of injury is very similar to that seen in newborns having succumbed to asphyxia. This model therefore has the potential to help researchers understand the pathophysiology of perinatal hypoxic-ischemic encephalopathy.

680.1

EFFECT OF CHRONIC NITRIC OXIDE SYNTHASE INHIBITION ON HYPOTHALAMIC BLOOD FLOW IN RAT Z. Benyó*, M. H. Velkei and P. Sándor Experimental Research Department - 2nd Institute of Physiology, Semmelweis University of Medicine, H-1446 Budapest, POB 448, Hungary

Tonic basal release of endothelium derived nitric oxide (NO) contributes to the maintenance of normal cerebral blood flow. In a previous study the NO-synthase (NOS) inhibitor N^G-nitro-L-arginine (30mg/kg bolus iv. followed by 1 mg/kg/min infusion for 15 min) increased mean arterial blood pressure (MAP) by 43 mmHg and reduced hypothalamic blood flow (HBF) by 53% in cats (Kovách et al.; J.Physiol.(Lond.)1992;449:183-196). In the present study the effect of chronic oral administration of the NOS inhibitor N^G-nitro-L-arginine-methyl-ester (L-NAME) was investigated on the HBF in rats.

The experiments were carried out in 2 groups of male Sprague-Dawley rats: the first received tap water, while the second received 0.1 mg/ml L-NAME solution to drink. After 1 week the animals were anesthetized (1.3g/kg Urethan, ip.), and the HBF (ml/g/min) was determined by the H_2-clearance method. The MAP (mmHg), arterial CO_2 and O_2 tensions (mmHg) and pH were also determined during the HBF measurement. The hypothalamic vascular resistance (HVR) was calculated as MAP/HBF.

The daily fluid intake did not differ in the 2 groups of animals, the L-NAME intake was 8.5±0.4 mg/kg/day in the treated rats. The other measured parameters were as follows: *=p<.02; **=p<.001

Treatment	MAP	HBF	HVR	apCO2	apO2	apH
Control (n=9)	94±12	.977±.056	98±7	40.2±1.5	84.8±2.5	7.39±.01
L-NAME (n=7)	129±6**	.903±.078	155±22*	41.0±1.2	85.0±3.4	7.38±.01

The results indicate a significant increase of HVR following chronic NO blockade. A significant fall of HBF, however, was counteracted by the simultaneous substantial increase of the MAP.

(SUPPORTED BY OTKA 1/3-1371, ETT 4-280 AND NIH NS 10939 GRANTS)

680.2

INHIBITION OF NITRIC OXIDE (NO) SYNTHESIS ATTENUATES THE RISE OF CYTOSOLIC CALCIUM ($[Ca^{2+}]_i$) EXHIBITED BY CEREBELLAR GRANULE CELLS UNDER HYPOXIC CONDITIONS. J.M.Mei*, W.M.Chi#, M.W.Smith#, C.U.Eccles, and B.F.Trump#. Dept. of Pharmacol. and Toxicol. and Dept. of Pathol.#, Univ. of Maryland, Baltimore, MD 21201.

NO has been proposed as a neuronal messenger molecule in hypoxic/ischemic cell injury. We conducted preliminary studies in a model of O_2 deprivation (simple hypoxia) using cultured rat cerebellar granule cells. Experiments were designed to test the hypothesis that sustained elevations of $[Ca^{2+}]_i$ and NO generation act in concert to trigger neuronal injury after anoxic insult. A hypoxic state was achieved by perfusing the cells with medium pre-equilibrated with argon gas. $[Ca^{2+}]_i$ was monitored using digital-imaging fluorescence microscopy in cells loaded with fura-2 AM. Under short-term hypoxic conditions, cells displayed a gradual and sustained increase of $[Ca^{2+}]_i$ which returned to near basal levels upon restoration of O_2-containing medium. Prolonged hypoxic conditions (>60 min) often caused irreversible elevation of $[Ca^{2+}]_i$ followed by disruption of cell membrane integrity as indicated by severe swelling and leakage of dye. Pretreatment with N^G-nitro-L-arginine (N-arg, 100 uM), a specific NO synthase inhibitor, markedly delayed the onset and intensity of the rise of $[Ca^{2+}]_i$. The hypoxia-induced elevation of $[Ca^{2+}]_i$ was also greatly attenuated if N-arg (100 uM) was added to the argon-perfused medium before the cells demonstrated signs of irreversible injury. Prolonged or repeated hypoxic conditions, however, caused a rapid and intense increase of $[Ca^{2+}]_i$ which could not be blocked by the inhibition of NO synthesis.

680.3

REACTIVE ASTROCYTES EXPRESS INDUCIBLE NITRIC OXIDE SYNTHASE AFTER ISCHEMIA. M. Endoh*, K. Maiese, and J. A. Wagner. Neurol./Neurosci.; Cornell Univ. Med. College, 1300 York Ave., NY, NY 10021

We have recently demonstrated that astrocytes in the hippocampus CA1 region are NADPH diaphorase positive after transient forebrain ischemia (Endoh, et al, Neurosci. Lett.), and that nitric oxide is involved in the mechanism of cultured hippocampal neurons death after ischemia (Maiese, et al, J. Neurosci.). To determine whether reactive astrocytes express NOS, we studied pathological change of rat hippocampus by immunohistochemistry after ten minutes of transient forebrain ischemia which results in the selective delayed death of CA1 pyramidal cells and marked gliosis in the CA1 subfield. In the normal hippocampus, astrocytes do not show NADPH diaphorase activity nor express inducible NOS. After ischemia a population of reactive astrocytes (GFAP+ cells) are observed to express NADPH diaphorase activity by double staining. Moreover, the similar population of reactive astrocytes are stained with both anti-mouse macrophage NOS antibody (Drs. C. Nathan, J. Weinder, R. Mumford) and anti-GFAP antibody by double immunofluorescent histochemistry. This change is observed one day after ischemia and increases in prominence from one week to one month. Thus, we conclude that inducible NOS is present in reactive astrocytes after transient forebrain ischemia. This increase in NOS expression and, presumably, NO production by reactive astrocytes may contribute neuronal death after ischemia.

680.4

SELECTIVE LOSS OF NITRIC OXIDE SYNTHASE-CONTAINING NEURONS IN THE DENTATE GYRUS AFTER TRANSIENT FOREBRAIN ISCHEMIA IN GERBILS. G. Lanzino*, S.C. Hong, J. Collins, N.F. Kassell, K.S. Lee. Dept. of Neurosurgery, University of Virginia, Charlottesville, VA 22908

Nitric oxide derived from nitric oxide synthase-containing (NOS+) neurons is suggested to participate in pathological damage to CNS neurons. Consistent with this hypothesis are observations that NOS+ neurons are selectively resistant to excitotoxic and ischemic damage. The present study further investigated this issue by examining the response of NOS+ neurons in the gerbil dentate gyrus to temporary ischemia. Transient forebrain ischemia was induced by reversibly occluding the carotid arteries for 10 minutes (n=9). Sham-operated animals (n=7) served as a control group. Seven days following surgery, NOS+ neurons were identified in the infragranular zone of the dentate gyrus using NADPH-diaphorase histochemistry. The number of NOS+ neurons in the infragranular zone of the dentate gyrus was reduced by approximately 50% following ischemia ($p<0.001$, Student t-test). Sham operated animals exhibited 6.47±0.62 cells/mm while the post-ischemic animals showed only 3.22±0.29 cells/mm. NOS-negative granule cells located nearby were resistant to this level of ischemia.

These observations demonstrate that NOS-containing neurons in the dentate gyrus are selectively vulnerable to brief ischemia. These findings stand in contrast to previous observations in which NOS+ neurons in the striatum were shown to be selectively resistant to ischemic damage. The hypothesis that NOS+ neurons may serve as 'killer' cells in CNS pathology does not appear to be applicable to ischemic death in the dentate gyrus.

680.5

ACCUMULATION OF FREE FATTY ACIDS AND EDEMA FORMATION IN FOCAL ISCHEMIA. M. R. Prasad*, C. Ramiah, D. Donaldson, Y. Hu and R. J. Dempsey. Department of Surgery, University of Kentucky Chandler Medical Center, Lexington, KY 40536

The increase of free fatty acids in the brain has been well demonstrated in several models of ischemia in rodent species. Free fatty acids and arachidonate metabolites have been implicated as some of the factors that contribute to edema formation in brain ischemia. We examined the levels of free fatty acids in the cortices of cats after 8 hours of middle cerebral artery occlusion, and we correlated these levels with the water content of the cortex. Fourteen adult cats were subjected to 8 hours of right middle cerebral artery occlusion. Seven cats were administered difluoromethyl ornithine, an inhibitor of ornithine decarboxylase and polyamine synthesis (250 mg iv at 30 minutes before occlusion and 4 hours after occlusion). Eight hours after occlusion, a 300-mg section of cortex was quickly removed from both the right parietal (ischemic) and the left parietal (nonischemic) regions of each cat. These sections were frozen in liquid nitrogen and analyzed for free fatty acids. A square millimeter of cortex was also removed from these brain regions to measure specific gravity. The levels of palmitic, stearic, oleic, and docosahexenoic acids were two to three times higher ($p<0.05$) in the ischemic regions than in the nonischemic regions. The water content of the ischemic regions was 80.6±0.5% whereas that of the nonischemic regions was 77.48±0.3% ($p<0.05$). Administration of difluoromethyl ornithine did not significantly reduce either the free fatty acid level or the water content in the ischemic region of the cortex. These results suggest that free fatty acids may be a factor in edema formation of focal cerebral ischemia in nonrodents also. (Supported by funds from NIH NS 31816 and NS 2800.)

680.6

DIFFERENTIATION OF INTRINSIC AND SYSTEMIC DIFFUSION PROPERTIES OF RAT NEOCORTEX FOLLOWING *IN VIVO* AND *IN VITRO* ISCHEMIA E. Syková, M. Pérez-Pinzón*, N. Zoremba, A. Lehmenkühler, O. Gil, L. Tao and C. Nicholson. Acad. Sci. Czech Rep, 180 85 Prague 8, Czech Republic; University. of Münster, 48149 Münster, Germany; NYU Medical Center, New York, NY 10016.

Intrinsic and systemic properties of the brain extracellular micro-environment during and after ischemia were compared. We analyzed extracellular volume fraction (α) and tortuosity (λ) using tetra-methylammonium (TMA^+) in the somatosensory neocortex of the adult rat *in vivo* and *in vitro*. Concentration-time profiles of TMA^+ applied by iontophoresis were quantitatively analyzed (Nicholson and Phillips, J. Physiol. 321:225, 1981). Transient ischemia *in vivo* was induced by clamping the carotid arteries for 10 min. and by concomitant reduction of inspiratory oxygen content to 6 % in nitrogen. After releasing the clamps the animals were ventilated with pure oxygen again. *In vivo* ischemia was induced in submerged slices (400 µm thick), by replacing the oxygenated 10 mM glucose medium with aglycemic and anoxic medium for about 10 min. Normal values of α and λ averaged 0.20 and 1.60, respectively in both preparations. During re-oxygenation λ recovered from an immediate post-ischemic value of 1.8 to normal in 15 min. in both preparations. Differences between *in vitro* and *in vivo* preparations occurred in the behavior of α. *In vivo*, α increased in all layers up to a maximum of about 0.28 at 10 min. after ischemia then recovered during the next hour. *In vitro*, α simply recovered from a reduced value during ischemia to a normal value in about 30 min. The *in vitro* and *in vivo* differences during re-oxygenation point toward systemic mechanisms of injury such as edema in this phase. Partly funded by NIH grant NS 28642.

680.7

SOURCE OF EXTRACELLULAR ADENOSINE DURING OXYGEN/GLUCOSE DEPRIVATION IN CULTURED CORTICAL NEURONS. D. Lobner* and D.W. Choi. Dept. of Neurology and Center for the Study of Nervous System Injury, Washington Univ. School of Medicine, St. Louis, MO 63110.

Extracellular adenosine accumulates during reduced energy conditions in the brain, but the source of this adenosine is uncertain. Murine cortical cell cultures exposed to oxygen-glucose deprivation for 45-60 min, showed an increase in extracellular glutamate (1-3 μM) and adenosine (0.5-2 μM), and submaximal neuronal death by the next day. 10 μM dipyridamole, an adenosine transport inhibitor, decreased this adenosine accumulation by about 25% while increasing glutamate accumulation and neuronal death. In contrast, dipyridamole increased the comparable increases in extracellular adenosine induced by 45 min exposure to 10 μM NMDA, or 300 μM kainate plus 10 μM MK-801, without affecting glutamate accumulation or neuronal death.

These results support the idea that the extracellular adenosine accumulation induced by oxygen-glucose deprivation may be mediated, at least in part, by adenosine transporters. Inhibition of these transporters by drugs like dipyrimadole may worsen neuronal injury by reducing extracellular adenosine accumulation, and thereby permitting greater release of synaptic glutamate.

Supported by NIH training grant NS 07205 to DL, and grants NS 30337 and NS 26907 to DWC.

680.8

EFFECT OF HYPOGLYCEMIA ON LOCAL CORTICAL BLOOD FLOW AND INTERSTITIAL ADENOSINE CONCENTRATION DURING POST-ISCHEMIC REPERFUSION Y.B. Kim**, T.S. Park,* A.R. Shah, E.R. Gonzales, and J.M. Gidday. Departments of Neurosurgery, St. Louis Children's Hospital and Washington University School of Medicine, St. Louis, MO 63110, and **Chung-Ang University, Seoul, Korea.

Cerebral ischemia often occurs in hypoglycemic (HG) neonates. The effect of HG on changes in neonatal cerebral blood flow (CBF) induced by ischemia-reperfusion are not known. We induced 10 min global cerebral ischemia in 32 isoflurane-anesthetized newborn pigs by occlusion of subclavian and brachiocephalic arteries; cortical CBF and interstitial adenosine concentration ([ADO]) were evaluated using hydrogen clearance and microdialysis. HG (blood glucose <25 mg/dl; induced by regular insulin; n=16) increased pre-ischemic CBF and [ADO] relative to normoglycemic (NG) animals (n=16). At 60 min of post-ischemic reperfusion, a significant hypoperfusion (42 % reduction in CBF) associated with a significant 43 % reduction in [ADO] was observed in the NG group. These post-ischemic changes in CBF and [ADO] were significantly exacerbated by HG.

	CBF (ml/min/100g)		ADENOSINE (nM)	
	NG	HG	NG	HG
Pre-Ischemia	38±2	51±4#	142±13	203±50#
Reperfusion	22±2**	17±1#**	80±7**	50±10#*

(#p<0.05 vs NG; *p<0.05, **p<0.001 vs pre-ischemia). Thus, HG increases the magnitude of post-ischemic hypoperfusion, which may potentiate reperfusion injury in HG neonates. The concomitantly low [ADO] suggests that pharmacologic augmentation of [ADO] may improve post-ischemic CBF.

680.9

Ca^{2+} or NMDA ANTAGONISM DOES NOT ALTER THE EFFLUX OF ADENOSINE FROM RAT HIPPOCAMPAL SLICES DURING *IN VITRO* ISCHEMIA. J.C. Fowler*. Department of Physiology, Texas Tech University Health Sciences Center, Lubbock, TX 79430.

Submerged hippocampal slices release large amounts of adenosine into the medium within minutes of exposure to in vitro ischemia (ie. glucose-free medium equilibrated with 95% N_2 / 5% CO_2). Exposure to in vitro ischemia is also associated with NMDA receptor activation and enhanced Ca^{2+} influx, two conditions which elevate adenosine efflux under normoglycemic, normoxic conditions. Therefore, the effect of Ca^{2+} and NMDA antagonism on ischemic adenosine efflux was examined.

Hippocampal slices were initially superfused at 2 ml/min with physiological medium maintained at 33 - 34°C and equilibrated with 95% O_2/ 5% CO_2. Extracellularly recorded DC and evoked synaptic potentials were recorded. Slices were pre-exposed to medium that contained Co^{2+} (500 µM), the NMDA antagonist MK-801 (10 µM) or was Ca^{2+}-free. Slices were then exposed to in vitro ischemic conditions until one minute after an anoxic depolarization occurred. One ml aliquots of superfusate were collected at 2 min intervals and adenosine was measured using absorbance HPLC.

While recovery of synaptic transmission was not apparent during reoxygenation of slices exposed to normocalcemic, drug-free in vitro ischemia, exposure to Co^{2+}, MK-801 or Ca^{2+}-free medium during ischemic-like conditions did result in significant recovery upon reoxygenation. Ca^{2+}-free medium also significantly shortened the time to anoxic depolarization observed during in vitro ischemia. In spite of these differences in electrophysiology and functional recovery, levels of ischemic adenosine efflux were not different between treatments.

680.10

CYANIDE MEDIATED ADENOSINE RELEASE FROM RAT HIPPOCAMPAL NEURONS

J.M. Kurbat, R.J. Buchanan, S.C. Wulff, and K.-W. Yoon*. Division of Neurosurgery and Surgical Research Institute, Saint Louis University, St. Louis, MO 63110-0250.

Ischemic neuronal cell damage is mediated in part by glutamate. Glutamate is released by ischemic brain parenchyma by a synaptic mechanism and activates glutamate receptors which mediate neuronal cell death. Specific glutamate receptor antagonists (e.g. MK801) attenuate neuronal injury due to ischemia in several experimental models. Ischemic brain, in addition to glutamate, release adenosine. By activating the presynaptic adenosine (A1) receptors, adenosine may modulate the amount of glutamate released in the extracellular space. However, the origin of adenosine released in ischemia has not been well established. Rat glial cell cultures with or without dissociated hippocampal neurons were perfused continuously with artificial CSF and exposed to an ischemic condition by addition of potassium cyanide (KCN, 1mM, 1hr) (Sturm, et al., J of Neurosurg., in press). The perfusate was analyzed every 15 minutes with HPLC for adenosine. In four out of four glial cell cultures without neurons, the baseline adenosine level did not change with KCN. However, in cultures with glia and neurons there was a dramatic but reversible increase in baseline adenosine concentration (269% of control, n=6, p<0.005) This observation suggests that adenosine is released by ischemia and the origin of this adenosine is neuronal.

680.11

CHANGES IN DIALYSATE AMINO ACIDS AND PURINES DURING CEREBRAL ISCHEMIA: CHRONICALLY VERSUS ACUTELY IMPLANTED MICRODIALYSIS PROBES. M.C. Grabb*, V.M. Sciotti and D.G.L. Van Wylen. Departments of Physiology and Pathology, SUNY-Buffalo School of Medicine and Biomedical Sciences, Buffalo, NY 14215.

Although recent studies suggest that microdialysis probes should be implanted for 24 hours before the initiation of protocols, the responsiveness of the tissue surrounding chronically implanted probes is debatable. The purpose of this study was to compare the ischemia-induced changes in interstitial fluid purines and amino acids as determined by either acutely or chronically implanted microdialysis probes. Changes in dialysate levels of adenosine (ADO), inosine (INO), glutamate (GLU), serine (SER), and glycine (GLY) were determined during 90 min of cerebral ischemia (4-vessel occlusion). Probes were implanted bilaterally in the caudate nuclei of rats either 24 hours prior (CHRONIC, n=7) or 2.5 hours prior (ACUTE, n=7) to ischemia in rats whose vertebral arteries were previously cauterized. Baseline dialysate levels of all amino acids were higher in CHRONIC than in ACUTE, while both ADO and INO were 10x lower in CHRONIC. In ACUTE, ADO, INO and GLU increased within the first 20 min of ischemia (26x, 11x, 17x respectively) and remained elevated throughout ischemia. In CHRONIC, ADO and INO increased within the first 20 min of ischemia (22x, 13x respectively); however, GLU did not increase until after 60 min of ischemia (5x). SER and GLY levels tended to remain stable during ischemia in both ACUTE and CHRONIC. These data demonstrate that the responsiveness of tissue surrounding the probe varies with the duration of probe implantation, and suggest that dialysate profiles obtained with acute probe implantation best reflect ischemia-induced changes. Supported by NIH HL-40878.

680.12

EFFECT OF ISCHAEMIA AND REPERFUSION ON THE EXTRA- AND INTRACELLULAR DISTRIBUTION OF AMINO ACIDS IN THE RAT HIPPOCAMPUS. R. Torp, B.S. Meldrum,† J. Storm-Mathisen*, O.P. Ottersen Dep. of Anatomy, University of Oslo, P.O. Box 1105 Blindern N-0317 Oslo, Norway.†Dep. of Neurology, Inst. of Psychiatry, London SE5 8AF, U.K.

The redistribution of neurotransmitter amino acids resulting from 20 min of forebrain ischaemia was studied in the rat hippocampus by quantitative, EM immunocytochemistry and in vivo microdialysis in the same animals. Changes in the distribution of glu, gln, asp and GABA in various cell compartments of CA1 were analyzed immediately after ischaemia or after 60 min of reperfusion, by incubating ultrathin sections with antisera raised against the respective amino acids and subsequently with a secondary antibody coupled to colloidal gold particles. The extracellular concentrations of glu, asp and GABA showed the expected increase during ischaemia and returned rapidly to normal during reperfusion. Glu-like immunoreactivity was reduced in pyramidal cell somata both immediately after ischaemia and after 60 min of reperfusion. Ischaemia caused no consistent changes in terminals. The ratio between the intracellular levels of glu and gln was assessed by double labelling immunocytochemistry, using two different gold particle sizes. The glu/gln ratio in glial cells was several fold increased after ischaemia (glu up, gln down), but recovered to normal level within 1h of reperfusion. The present results suggest that postsynaptic neuronal elements as well as glial cells contribute to the extracellular overflow of excitatory amino acids during an ischaemic event: postsynaptic elements by leaking or releasing glu and asp, and glial cells by losing their ability to effectively convert glu to gln. The temporal association between the changes in the glial contents of glu and gln, and the extracellular amino acid fluctuations recorded by microdialysis in the same animals, underlines the strategic role of glia in regulating the extracellular level of glu.

680.13

WHITE MATTER GABA-B RECEPTOR MEDIATED AUTOPROTECTION INVOLVES PROTEIN KINASE C ACTIVATION. R. FERN* [1,2] S. G. WAXMAN[1,2] AND B. R. RANSOM[1] Department of Neurology[1], Yale University School of Medicine, New Haven, CT, and Neuroscience Research Center[2], VA Medical Center, West Haven. CT.

We have recently found that GABA, the major inhibitory neurotransmitter of the brain, acts to protects CNS white matter from the effects of anoxia. Thus, 1 μM GABA increases recovery in the rat optic nerve following a 60 min period of anoxia from a control of 36% (+/-2.6%) to 55% (+/-3.1%), as determined by recording the evoked compound action potential. The effect of GABA was bicuculline insensitive, was not mimicked by selective GABA-A agonists but was mimicked by the selective GABA-B agonist baclofen (recovery=55% +/-5%). Thus GABA is acting via GABA-B receptors. Block of GABA uptake with nipecotic acid also protected against anoxia, while block of GABA-B receptors with phaclofen significantly reduced post-anoxic recovery. We have suggested a model where release of endogenous GABA during anoxia activates GABA-B receptors to limit subsequent white matter dysfunction via an unknown mechanism.

We now report that protein kinase C activation has a similar protective effect to that of GABA, increasing post-anoxic recovery to 57% (+/-7%). Furthermore, inhibition of protein kinase C by H-7 completely blocked GABA-mediated protection. It appears, therefore, that protein phosphorylation by protein kinase C is an essential step in the white matter GABA-B receptor mediated autoprotective reflex. Protein phosphorylation of a number of possible sites could act to reduce white matter anoxic injury, including axolemma ion channels and ion exchangers. We are currently investigating which of these sites is important in this novel system. Supported by NIH (B.R.R) and VA Med. Res. Serv. (S.G.W).

680.14

HIPPOCAMPAL NEURON LOSS AND PEPTIDE CHANGES FOLLOWING GLOBAL ISCHEMIA IN THE RAT. T.M. Wengenack*[1], R. Slemmon[2,1], W.P. Dunlap[3] and P.D. Coleman[1]. Depts. of [1]Neurobiol. & Anat. and [2]Biochem., Univ. of Roch., Rochester, NY 14642 and [3]Tulane Univ., New Orleans, LA 70118.

Global cerebral ischemia causes selective neurodegeneration and gliosis in the hippocampus. This has become of intense interest as a model for identification of molecular events of neurodegeneration and the plasticity of surviving neurons in response to injury. The specific aim of this study was to compare the postischemic progression of hippocampal neuron loss and molecular events which may play a critical role in neuronal vulnerability and plasticity in response to injury.

Rats were sacrificed 1, 3, or 7 days after 30 min of 4-VO global ischemia or sham surgery. Crude peptide fractions were isolated from supernatants of denatured hippocampal homogenates using gel and ion exchange chromatography and then separated by RP-HPLC. Peaks that exhibited a 25% or greater change after 4-VO were sequenced and identified. In two rats from each group, CA1 neurons were counted in three regions of CA1, in two sections of the dorsal hippocampus.

The number of CA1 pyramidal neurons was not decreased significantly on postischemic day 1, but was on days 3 and 7. Comparisons of postischemic differences in hippocampal peptides indicated significant changes in peptides of cellular and vascular origin on postischemic day 1, before neuron loss was histologically apparent, as well as on days 3 and 7, demonstrating a temporal dissociation between CA1 neuron loss and early peptide changes. For example, decreases were observed for the calcium-binding protein calmodulin. This may have reflected down-regulation in response to postischemic calcium influx and accumulation, which is believed to be a major cause of ischemic neuron death. Increases in peptides of vascular origin suggested increases in blood-brain barrier permeability that may also contribute to neurodegeneration.

Supported by NIH AG 00107 and LEAD Award AG 09016 to P.D.C.

680.15

MODIFICATIONS OF OXIDATIVE ENERGY METABOLISM INDUCED BY A UBIQUINONE-LIKE MOLECULE IN RAT CEREBRAL CORTEX SYNAPTOSOMES. D. Curti* and E. Izzo. Institute of Pharmacology, University of Pavia, 27100 Pavia, Italy.

Oxidative energy metabolism was evaluated by measuring: 1) the oxygen consumption rate (QO_2), a measure of the rate of ATP synthesis by oxidative phosphorylation in basal conditions and of the potential activity of the respiratory chain to cope with increased energy demands in stimulated states; 2) the activity of pyruvate dehydrogenase (PDHc), a mitochondrial multienzyme complex with critical functions in providing acetyl groups for energy metabolism and acetylcholine synthesis. PDHc is present in an active, dephosphorylated form (PDHa) and in an inactive, phosphorylated form. The fraction of active form over the total gives a measure of the activation state of mitochondrial energy metabolism. Synaptosomes of rat cerebral cortex have a QO_2 of 3.5 nmol/min/mg of protein and less than 60% of the PDHc in the active form. Persistent stimulation of energy consumption, induced by veratridine, increases both the QO_2 and the conversion of PDHc into the PDHa form. A structural analog of ubiquinone, idebenone (1 μM), does not affect the QO_2 in basal conditions but counteracts the veratridine-stimulated increase of QO_2. The drug does not affect the increase of QO_2 induced by an uncoupler of oxidative phosphorylation. The effect of idebenone on veratridine-stimulated QO_2 is slightly enhanced by a preactivation of the Na^+ channels. Idebenone (1-10 μM) is also able to totally counteract veratridine-induced increase of PDHa. The observed effects of idebenone could result in some protection of the cerebral tissue against ischemic/hypoxic insults, in fact a slowing down of energy utilization might delay the depletion of energy stores and thus protect the cerebral tissue against the development of irreversible damage.

680.16

INFLUENCES OF AGE ON MAINTENANCE AND RECOVERY OF SYNAPTIC TRANSMISSION AND ION HOMEOSTASIS IN HIPPOCAMPAL SLICES DURING AND AFTER ANOXIA. E.L. Roberts, Jr.[1]* and Z.-C. Feng. Department of Neurology, University of Miami School of Medicine, and [1]Geriatric Research, Education, and Clinical Center, Miami VA Medical Center, Miami, FL 33136

Aging diminishes the capacity of brain tissue to maintain ion homeostasis during anoxia, and to recover ion homeostasis and synaptic transmission after anoxia (E.L. Roberts, Jr. *et al.*, *Brain Res. 514* (1990) 111-118). The goals of this study were to determine (1) whether this capacity is constantly diminished with age and (2) whether age-related declines in glycolytic or oxidative metabolism underlie these changes. Experiments were carried out in hippocampal slices from young adult (6-9 month old), adult (16-19 mon.) and aged (26-29 mon.) Fischer-344 rats. Slices were exposed to physiological solutions containing 2.5-20 mM glucose or 5-40 mM sodium lactate. Lactate supports oxidative phosphorylation only in brain slices. Slices were exposed to anoxia (95% N_2, 5% CO_2), and returned to normoxia (95% O_2, 5% CO_2) one minute after catastrophic loss of ion homeostasis (anoxic depolarization). Extracellular K^+ concentrations (K^+_o) and magnitudes of orthodromically-evoked pyramidal cell population spikes were assessed in hippocampal subfield CA1 before, during, and up to one hour after anoxia. Maintenance or recovery of K^+_o homeostasis during and after anoxia in slices from adult rats was similar to that in slices from aged, but not young adult, rats. Age-related alterations in oxidative phosphorylation were not involved in these findings because recovery of K^+_o homeostasis and synaptic transmission following anoxia showed no differences between age groups and resembled that in 2.5 mM glucose. (Supported by research grants from NIA (AG08710) and the American Federation for Aging Research).

680.17

DIFFERENTIAL EFFECTS OF GLOBAL CEREBRAL ISCHEMIA/ REPERFUSION ON HIPPOCAMPAL [^{3}H]NOREPINEPHRINE (NE) AND STRIATAL [^{3}H]DOPAMINE (DA) RELEASE IN CANINE BRAIN SLICES. L.L. Werling*, K.J. Hurt, R.E. Rosenthal and G. Fiskum. Depts. of Pharmacology, Biochemistry and Molecular Biology, and Emergency Medicine, The George Washington Univ. Med. Ctr., Washington, DC 20037.

Striatum and hippocampus are selectively vulnerable to ischemic damage. While DA is thought to contribute to neuronal damage, NE is thought possibly to play a neuroprotective role in ischemia. We sought to compare changes in NE release from hippocampus in the same model used for the DA studies.

Canines were divided into one of five experimental groups: non-arrested controls (NA); global cerebral ischemia induced by 10 min cardiac arrest (CA); CA followed by 30min, 2hr, or 24hr restoration of spontaneous circulation (ROSC). Slices of brain were prepared, washed, and incubated with [^{3}H]catecholamine. Striatal slices prepared from all groups accumulated about the same amount of [^{3}H]DA. Hippocampal slices from CA accumulated less [^{3}H]NE than did those from NA. Slices were washed and loaded into chambers of a superfusion apparatus. Release of DA or NE was stimulated by a 2 min exposure to 20 mM K^+ or 50μM NMDA ± 100 μM GLY. Release of [^{3}H]DA from striatum was more sensitive to K^+-stimulation immediately following CA, and to NMDA or NMDA/GLY at all times after CA, but sensitivity of [^{3}H]NE release from hippocampus was unchanged until 24hr ROSC, at which time it was greatly increased to K^+-stimulation. These data suggest that changes in NE systems exhibit a different time course than changes in DA systems. Supported by Sigma Tau, S.p.A. and Souers Stroke Fund.

680.18

CHANGES IN BRAIN LIPID METABOLISM INDUCED BY ISCHEMIA-REPERFUSION. J-P. Zhang, P.M. Wixom* and G.Y. Sun Biochem. Dept., Univ. Missouri, Columbia, MO 65212.

Cerebral ischemic insult in brain is associated with the release of free fatty acids (FFA) although the exact source for the increase is not well understood. Using a rat model in which focal cerebral ischemia was induced by ligation of the middle cerebral artery (MCA), we observed previously an increase in FFA in the MCA cortex with time during ischemia and at 16 - 24 hr after a 60 min insult. The increase in FFA at 16 hr after reperfusion was correlated to a rise in levels of diacylglycerols (DG) and triacylglycerols (TG). In this study, [^{14}C]arachidonic acid (AA) was used to trace the metabolism of glycerolipids in brain at different times after a 60 min ischemic insult. Labeled AA was incorporated into phospholipids as well as the neutral glycerides and labeled TG constituted about 20% of the total glycerolipids. At 8, 16 and 24 hr after a 60 min ischemic insult, incorporation of labeled AA into DG and TG in the ischemic right MCA cortex was higher than that in the left MCA cortex. The increase in labeling of TG could be further correlated to an increase in labeled phosphatidic acid, the precursor for glycerolipid biosynthesis. These results suggest an increase in DG and TG biosynthesis due to a disturbance in the de novo lipid synthesis. The sustained increase in DG may have an implication on protein kinase C activity, which in turn, may play an important role in the mechanism of delayed neuronal cell death.

680.19

MODIFICATION OF HYPOXIC INJURY TO CEREBRAL ENDOTHELIAL CELLS BY FRUCTOSE-1,6-BISHOSPHATE BUT NOT GLUTAMATE RECEPTOR ANTAGONISTS. G.T. Gobbel, T.Y-Y. Chan, G.A. Gregory, P.H. Chan*. CNS Injury & Brain Edema Res. Center, Dept. Neurol., & Brain Tumor Res. Center, Dept. Neurosurg., Univ. of Calif., S.F., CA 94143.

Fructose-1,6-bisphosphate (FBP) has been shown to reduce the damage to astrocytes induced by hypoxia; similarly, antagonists of some of the glutamate receptors have been shown to ameliorate hypoxic injury to neurons. To determine whether FBP or glutamate receptor antagonists might also protect cerebral endothelial cells from hypoxic injury, cerebral endothelial cells were isolated from 2 week old Sprague-Dawley rats. At 5-7 days after plating, cells were treated with 48 hours of complete hypoxia ± FBP (3.5 mM), MK-801 (50 μM), Kynurenic Acid (KA; 0.5 mM), or 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo (F) quinoxaline (NBQX; 1 or 10 μM). Hypoxia increased the release of lactate dehydrogenase (LDH) into the media relative to total LDH (released and intracellular) from 0.28±0.14 (mean±SD) to 0.93±0.02. The ratio of dead to total cells (ethidium bromide and fluorescein diacetate staining) also increased from 0.03 to 0.34. None of the glutamate receptor antagonists, MK-801, KA, or NBQX, altered the amount of damage measured by either LDH release or viability staining. In contrast, LDH release in FBP treated cells was significantly reduced by 72% from 18.7±2.8 U/mg protein in untreated hypoxic controls to 5.2±1.1. FBP treatment significantly increased the level of ATP under hypoxic conditions by 48% (6.4±1.5 vs. 4.4±0.8 μmole/mg protein) but did not alter hypoxia-induced GSH depletion. These results suggest that glutamate receptor antagonists to the NMDA (MK-801 and KA), AMPA (NBQX), and kainate subtypes (NBQX) are not effective in ameliorating hypoxic injury to cerebral endothelial cells. However, FBP is effective, and this effect may be related to the increase energy charge induced by FBP, an effect similar to that seen in astrocytes. Supported by NIH Grants NS 14543, AG 08938, NS 25732, and the American Brain Tumor Association.

680.20

(3H)-D-ASPARTATE EFFLUX FROM RAT HIPPOCAMPAL SLICES DURING IN VITRO ISCHEMIA IS MIMICKED BY XANTHINE/XANTHINE OXIDASE EXPOSURE AND IS NOT BLOCKED BY DIHYDROKAINATE. V.Roettger* and P.Lipton. Dept. of Physiol., Univ. of Wisconsin, Madison, WI 53706

Excess glutamate release most likely contributes to ischemic brain damage. The release mechanism is unknown although reversal of the Na-dependent glutamate transporter has been suggested. Free radical formation has also been implicated in ischemic-induced release.

At 37°C, slices loaded with (3H)-D-Aspartate (ASP, transporter substrate) were exposed to a combination of in vitro ischemia (IVI) and veratridine (VER, 50uM). By 10 min, ASP efflux increased x6 over basal level; IVI or VER alone increased ASP efflux x3 or x4, respectively. Dihydrokainate (DHK, transporter inhibitor, 0.5mM) blocked VER-induced ASP efflux but did not block efflux induced by IVI alone or by the combination of IVI and VER.

The free radical generating system of xanthine (X, 0.5mM) and xanthine oxidase (XOD, 20mU/ml) increased ASP efflux x3 by 10 min; XOD alone produced the same increase in efflux. This suggests that endogenous X may be adequate to induce the observed effect. VER combined with either X/XOD or XOD alone increased ASP efflux x7 by 10 min. DHK did not block ASP efflux when XOD was present.

Therefore, X/XOD mimicked IVI in the following ways: (1) both conditions alone increased ASP efflux, (2) IVI or X/XOD-induced efflux was insensitive to DHK, (3) IVI or X/XOD-induced efflux was additive with VER-induced efflux and (4) IVI or X/XOD prevented DHK from blocking the VER-induced increase.

Increased ASP efflux may be due to IVI or X/XOD-induced Na influx. The loss of DHK sensitivity of VER-induced efflux may be due to effects of the conditions on the transporter itself or on DHK. An alteration in the transporter itself may enhance ASP efflux during IVI or X/XOD. These possibilities are being tested.

ISCHEMIA: NEUROCHEMISTRY II

681.1

TYROSINE PHOSPHORYLATION AND MAP KINASE ACTIVITY IS ENHANCED IN RESISTANT AREAS OF THE BRAIN FOLLOWING TRANSIENT CEREBRAL ISCHEMIA. Bing Ren Hu and Tadeusz Wieloch* Laboratory for Experimental Brain Research, Lund Hospital, Lund University, S-221 85 Lund.

Activation of trophic factor receptors stimulate tyrosine phosphorylation on proteins and support neuronal survival. We report, that in the recovery phase following reversible cerebral ischemia, tyrosine phosphorylation increases in the membrane fraction of the resistant hippocampal CA3/dentate gyrus (DG) region, while in the sensitive CA1 region or striatum, tyrosine phosphorylation is less marked or decreases. In the cytosolic fractions, a 42 kD protein, identified as the mitogen-activated protein (MAP) kinase, is markedly phosphorylated and activated immediately following ischemia, in particular in CA3/DG, but not in striatum. In the CA1 region, phosphorylation of MAP kinase is less intense and decreases later during reperfusion, which could explain the delay of neuronal degeneration in this structure. The data suggest that in resistant neurons, the growth factor receptor signalling cascade is stimulated, may support neuronal survival following ischemia. In vulnerable cells the cascade is depressed.

Supported by the Swedish Medical Research Council.

681.2

CHANGES IN EXTRACELLULAR STRIATAL DOPAMINE(DA) IN THE RAT DURING GRADED CEREBRAL ISCHEMIA: RESPONSE TO POTASSIUM STIMULATION AND DA REUPTAKE BLOCKADE. S.H. Lee*, T.Kondoh, R.C. Heros, W.C. Low. Depts. of Neurosurgery and Physiology, Uiv. of Minnesota Medical School, Mpls., MN 55455.

The excitotoxic release of DA in the rat striatum is closely related to the severity of ischemia. Cerebral ischemia was induced by bilateral occlusion of the common carotid arteries with hemorrhagic hypotension. To study the kinetics of DA metabolism, potassium(100mM) and nomifensin, a DA reuptake blocker, (1mM) were infused through a microdialysis probe for 10 min. and DA changes were measured during graded ischemia. In control animals, high K^+ resulted in a 20.4 fold increase in DA efflux. This returned to basal levels after one hour. During three hours of moderate ischemia, where CBF was maintained at 20-50% of basal levels, DA efflux showed a 28.6 fold increase. This returned to basal level after three hours. At this time, high K^+ induced a two fold increase; this persisted for two hours. In the mild ischemia group, DA increased insignificantly after induction of ischemia but increased 72.6 fold after K^+ injection at three hours. Severe ischemia, which showed 146.2 fold increase of DA efflux, revealed no additional increase after K infusion. In the same setting, response to nomifensin in control conditions showed a 14.9 fold increase in DA release. In comparison, the response during moderate ischemia increased 1.4 fold while in mild ischemia it increased 20 fold. In severe ischemia, no response occurred. In conclusion, in moderate ischemia, DA levels may return to normal after some time but the response to drugs affecting DA metabolism differs from control, suggesting underlying pathophysiological changes. (This work supported in part by grants from the American Heart Association and PHS grants NS-24464 and NS-23682).

681.3

INVOLVEMENT OF NMDA RECEPTORS IN THE REBOUND RELEASE OF 5-HT AFTER HYPOXIA FROM RAT HIPPOCAMPAL SLICES. K. E. Lee, D. W. Kang, D. G. Kim* and Y. S. Ahn. Dept. of Pharmacology, Yonsei Univ. College of Medicine, Seoul 120-752, Korea.

We have previously demonstrated that ischemia, induced by partial ligation of both carotid arteries, increased the concentration of 5-HT in rat hippocampus after 24 hours. Therefore, in the present study, we aimed to investigate whether hypoxia and/or NMDA was able to stimulate, directly, release of 5-HT from hippocampal slices. After 30 min's of preincubation, the slices were incubated for 20 min in a buffer containing [^{3}H]-5-HT (0.1 mM) for uptake, and washed for 10 min. To measure the release of [^{3}H]-5-HT from the buffer, the incubation medium was drained off and refilled every 10 min through a sequence of 13 tubes. When 1 mM NMDA was added at 5th tube, [^{3}H]-5-HT release was increased, and NMDA-induced [^{3}H]-5-HT release was prevented by adding AP5 (30 mM) 10 min before NMDA administration(4th tube). When slices were exposed to hypoxia, induced by gassing 95% N_2/5% CO_2 for 20 min, [^{3}H]-5-HT release was markedly decreased (about 47 %) during hypoxic period. Curiously, a rebound release of [^{3}H]-5-HT was observed 30 min after hypoxic period. The hypoxia-induced decrease in [^{3}H]-5-HT release was not affected by treatment with AP5 while the rebound release was blocked by treatment with AP5. These results suggest that the rebound release of [^{3}H]-5-HT during post-hypoxic period was mediated, at least in part, by the NMDA receptor.

681.4

ELEVATION OF N-ACETYL-ASPARTATE-GLUTAMATE (NAAG) IN ACUTE BRAIN ISCHEMIA: ANOTHER SCAVENGER METABOLIC PRODUCT OF GLUTAMATE? H. Igarashi, I. L. Kwee* and T. Nakada. Neurochem. Res. Lab., VANCSC, Martinez, CA 94553 and Dept. of Neurology, Univ. of Calif., Davis, CA 95616.

Excitatory amino acid accumulation, especially that of glutamate, is believed to play a significant role in the generation of ischemia induced brain damage, especially delayed neuronal death. Nevertheless, to date, little is known regarding the metabolic fate of glutamate accumulated *extracellularly*. The two principal anaerobic enzymatic activities which consume glutamate during ischemia are alanine transaminase and glutamic acid decarboxylase forming alanine and γ-amino-butyric acid (GABA), respectively. Both, however are *intracellular* reactions, indicating that they play a minor role, if any, in the fate of *extracellular* glutamate. In this study, we demonstrated that N-acetyl-aspartate-glutamate (NAAG) was significantly elevated in acute brain ischemia (maximum ca. 4 hours post ischemia and slowly declining thereafter) in rat, utilizing proton nuclear magnetic resonance (NMR) spectroscopy. The finding strongly indicates that the N-acetyl-aspartate-linked acid dipeptidase (NAALAD) reaction is likely to be an important *scavenger* metabolic pathway for *extracellular* glutamate during brain ischemia.

681.5

THE ROLE OF cGMP ON THE NEURONAL INJURY INDUCED BY HYPOXIA-HYPOGLYCEMIA IN THE ORGANOTYPIC CULTURE OF RAT HIPPOCAMPUS. X. Sun*, M. Colberg and C. Shin. Departments of Pharmacology and Neurology, Mayo Clinic/Mayo Foundation, Rochester, MN 55905

Excitatory amino acid (EAA), glutamate, is thought to play a key role in the development of neuronal injury through excitotoxic mechanism. Glutamate and the EAA analogues also induce elevation of cGMP in neurons. Since the cyclic neucleotides are known to modulate many cellular signalling pathways, one could hypothesize that rise in intracellular cGMP could be a part of the excitotoxic process underlying ischemia induced neuronal injury. We tested this hypothesis by using methylene blue, which has been shown to prevent the activation of guanylate cyclase.

The organotypic cultures were made from 6 day old rat pup hippocampus. At 10 DIV, they were subjected to ischemic condition by exposure to 2-deoxyglucose substituted HBSS equilibrated with 95% N_2 plus 5% CO_2 atmosphere. Methylene blue was apllied for 30 min before, during and for 24 hours after ischemia. At 24 hours, neuronal damage was assessed by propidium iodide fluorescence and quantitated by assigning a severity index (0=none, up to 4=severe) to the intensity of fluorescence.

With 40 min exposure to ischemia, neuronal injury was induced in the pyramidal layers of hippocampus. The treatment with methylene blue (100 μM) blocked the ischemia induced neuronal injury as judged by propidium iodide fluorescence. Methylene blue (100 μM) by itself without ischemic treatment did not alter the histologic appearance of the cultures.

The results of the present investigation suggest that cGMP may play a significant role in the development of neuronal injury in brain produced by ischemic insults.

681.6

TIME-COURSE OF NEUROCHEMICAL CHANGES IN INSULAR CORTEX AND AMYGDALA FOLLOWING FOCAL ISCHEMIA. R.T.F. Cheung, T. Diab and D.F. Cechetto*. Robarts Research Institute, University of Western Ontario, London, Ontario, Canada, N6A 5K8.

Occlusion of the middle cerebral artery (MCAO) using bipolar coagulation at two points above and below the rhinal fissure in anesthetized male Wistar rats reproduces stroke-associated autonomic changes that are observed clinically. There is evidence to suggest that these are due to involvement of insular cortex (IC). The amygdala (AMG) which receives afferents from the IC and projects to brain stem autonomic centers, may also be involved. We previously observed a striking increase in the immunoreactivity (IR) of neuropeptide-Y (NPY), leu-enkephalin (leu-ENK) and neurotensin (NT) over ipsilateral IC and ipsilateral basolateral AMG or central nucleus of AMG at 5 days after MCAO. In this investigation we studied the time-course of these changes. At 6 hours after MCAO, there was an increase in the density of NPY labelled fibers and terminals in IC. The change, however, was not quantifiable by a computerized-microscopic imaging system. Data from the imaging system showed that the changes in IR of NPY, leu-ENK and NT became just detectable at 1 day, peaked around 3-5 days and returned toward baseline at 10 days following MCAO.

Our results provide evidence for the time-course in neurochemical changes in IC and AMG following MCAO. The finding of an increase in NPY-IR in IC at 6 hours suggests a mechanism by which early autonomic changes can occur following MCAO in rats and in patients clinically, while the delayed changes in the AMG may have implications for longer term changes in autonomic variables. (Supported by Heart & Stroke Foundation of Ontario)

681.7

TYROSINE PHOSPHORYLATION OF GLYCOPROTEINS DURING ISCHEMIA IN RAT HIPPOCAMPUS. K. Au, J. Soulliere and J.W. Gurd*. Scarborough Campus, University of Toronto, West Hill, Ont, M1C 1A4.

The effects of post-decapitative ischemia and in vitro ischemia on the tyrosine phosphorylation of hippocampal glycoproteins (gp) were investigated. Rats were sacrificed by decapitation, the heads immediately frozen in liquid N_2 and the hippocampus analyzed for the presence of tyr(P)-proteins by immunoblotting with α-tyr(P) antibodies. Under these conditions 10 tyr(P)-proteins and 2 major tyr(P)-Con A^+ gp of app. Mr 110K (GP110) and 180K (PSD-GP180) were detected. Subcellular fractionation studies showed that GP110 was enriched in synaptic membranes. Maintaining brains at room temperature prior to freezing resulted in the rapid loss of tyr(P) from GP110 ($t_{1/2}$ < 30 sec.) and the slower dephosphorylation of PSD-GP180 ($t_{1/2}$ = 5-10 min.) and proteins of Mr 60K and 39K. The tyr(P) of other proteins remained unchanged. The effect of ischemia on tyr(P) in hippocampal slices was determined by preincubating slices for 60 min. prior to transfer to O_2 and glucose-free media. Fresh slices contained tyr(P)-PSD-GP180 but low levels of tyr(P)-GP110. Incubation in oxygenated media resulted in a rapid increase in the tyr-phosphorylation of GP110 ($t_{1/2}$ < 1 minutes) as well as of p60, p39 and an additional protein, p116. Ischemia led to the loss of tyr(P) from GP110, PSD-GP180, p116, p60 and p39. Reperfusion with O_2 and glucose increased the tyr(P) of GP110 (t1/2 < 1 min.), but not of PSD-GP180 and resulted in a transient increase in tyr(P)-p39. The results demonstrate ischemia induced changes in the tyr(P) of several hippocampal proteins and identify GP110 as a synaptic gp capable of rapid changes in tyr-phosphorylation. Supported by NSERC.

681.8

NEUROCHEMICAL ALTERATIONS IN HIPPOCAMPUS CA1 PYRAMIDAL CELLS AND INTERNEURONS AFTER SPONTANEOUS STROKE IN SHR-STROKE PRONE RATS. G.I. De Jong, E.A. Van der Zee, B. Bohus and P.G.M. Luiten*. Dept. of Animal Physiology, University of Groningen, P.O.Box 14, 9750 AA Haren, The Netherlands.

GABA-mediated inhibition plays an important role in regulating the activity of pyramidal cells in the hippocampus CA1, which are the major output cells of the hippocampus. Previously, we demonstrated that activated CA1 pyramidal neurons express high levels of PKCγ-immunoreactivity (Van der Zee et al.; J. Neurosci. 12:4808, 1992). CA1 pyramidal cells are selectively vulnerable to abnormal brain perfusion (like ischemia), which was frequently attributed to an overexcitation due to an increased glutamatergic input. In the present study we examined the relationship between pyramidal cells and their inhibitory input after decreased brain perfusion by the spontaneous development of strokes in SHR-SP rats. Immunoreactivity for the γ-isoform of protein kinase C (in pyramidal cells) and parvalbumin and GAD (in interneurons) was determined in the dorsal hippocampus CA1 by way of immunocytochemistry. Because chronic treatment with the calcium antagonist nimodipine prevents the development of strokes in SHR-SP rats (Luiten et al.; Drugs in Development, in press), we compared SHR-SP animals (stroke) with age-matched nimodipine treated rats (non-stroke). After stroke in control animals, we observed a strikingly enhanced protein kinase Cγ-immunoreactivity in CA1 pyramidal cells, when compared to nimodipine treated rats, which can be interpreted as the result of an increased activation of these cells. This pathological increase of PKCγ-immunoreactivity in symptomatic SHR-SP rats was accompanied by a reduced level of parvalbumin- and GAD-immunoreactivity in interneurons innervating these pyramidal cells. Since parvalbumin and GAD are present in a subset of GABAergic inhibitory interneurons, these data suggest that increased activity of CA1 pyramidal cells after spontaneous stroke may partially be related to a decreased inhibitory input upon these cells.

681.9

ICAM-1 AND IL-6 CHANGES IN THE HIPPOCAMPUS FOLLOWING TRANSIENT FOREBRAIN ISCHEMIA IN THE RAT. W.M. Clark, J.D. Lauten, B.M. Coull, N.J. Cherry, W.R. Woodward*. Depts. of Neurology and Biochemistry, Oregon Health Sciences Univ., Portland, OR 97201.

Cytokine production and adhesion molecule expression appear to play important roles in the pathogenesis of CNS reperfusion injury. The rat two vessel occlusion model was used to investigate the time course of changes for IL-6 and ICAM-1 immuno-staining in hippocampus following ischemic injury. Using a polyclonal antibody against IL-6, low levels of immunoreactivity were seen in sham surgery controls in the dentate granule and CA1 pyramidal cell layers. Staining increased in these areas at 24 hours post ischemia, but the CA1 staining had disappeared by four days. This pattern and time course was similar to that of Nissel stained sections with greater than 90% of CA1 neurons lost between days one and four. For ICAM-1, no immunoreactivity was seen with a monoclonal anti-ICAM-1 in sham controls. At 24 hours post ischemia a small increase in staining in the CA1 pyramidal layer was observed; however by four days there was a marked increase in immunoreactivity throughout CA1 and the dentate hilus. This time course and pattern was similar to that observed in sections stained with a specific microglial antibody (OX42). These results suggest that following CNS ischemia, increased IL-6 production occurs predominately in neurons, whereas ICAM-1 expression is increased in the microglial cell population.

Supported in part by a National Stroke Association/ Allied Signal Inc. Award, a NIH:CIDA award; and NIH NS17493.

681.10

IMMUNOCYTOCHEMICAL LOCALIZATION OF THE NEURON-SPECIFIC GLUCOSE TRANSPORTER (GLUT3) IN RAT BRAIN. M.Moholt-Siebert, A.van Bueren, N.J.Cherry, W.R.Woodward, F.P.Eckenstein* and A.McCall. Depts. of Cell Biol. & Anat. and Neurol., Oregon Health Sci. Univ. & VA Medical Ctr., Portland, OR 97201

Although GLUT3 has been shown to be neuron-specific in the CNS, immunolocalization of this glucose transporter has not been possible because the available antipeptide antisera have been of low affinity. To circumvent this problem and to ascertain the CNS localization of GLUT3, we produced a polyclonal antipeptide antiserum (ALM3-C) by a special immunization procedure with a GLUT3 C-terminal peptide. Based on detection of 25 ng of this peptide on a slot blot, the antibody titer was 1:8000. By Western blot analysis of rat brain membranes, ALM3-C identifies a 44 kD protein band, identical to that detected by two other GLUT3 antisera, but does not detect proteins in muscle or red blood cells, tissues devoid of GLUT3. Staining is completely blocked in Western blots and immunocytochemistry by pre-incubation of ALM3-C with 10 μg/ml of GLUT3 peptide but is unaffected by pre-incubation with an unrelated, GLUT1 peptide. This high affinity antiserum was used for immunocytochemical localization of GLUT3 in frozen coronal sections of rat brain. GLUT3 staining is seen in cerebellar molecular and granular layers but not in the Purkinje cell layer, and in hippocampal molecular layers but not in the pyramidal cell or dentate granule cell layers. In cortex more intense staining is observed over layers 1, 4 and 6 than in intermediate layers. The pattern of neuropil localization and paucity of perikaryal staining suggests that GLUT3 may provide the energy needed locally for synaptic transmission. Support: NIH Grants NS22213 and NS17493.

681.11

PROGRESSIVE HIPPOCAMPAL LOSS OF IMMUNOREACTIVE GLUT3 AFTER GLOBAL FOREBRAIN ISCHEMIA. A.McCall*, M. Moholt-Siebert, A. van Bueren, N.J. Cherry, N. Lessov, and W.R. Woodward. Depts. of Neurol., Pharmacol., and Cell Biol. & Anat., Oregon Health Sci. Univ. and VA Medical Ctr., Portland, OR 97201.

Brain damage from global forebrain ischemia is modified by glycemia; furthermore, ischemia/hypoxia itself may modify glucose transport. To assess whether GLUT3, the neuron specific glucose transporter, is affected by cerebral ischemia, we investigated the time course and pattern of GLUT3 immunoreactivity in the rat 2-vessel occlusion model of global forebrain ischemia. We used a newly generated, specific, C-terminally directed polyclonal antiserum against GLUT3 to stain coronal frozen sections. Preincubation of the antiserum with the C-terminal peptide used to generate the antiserum (10 μg/ml) abolished all specific staining. The microglial marker, OX42, indicated the extent of ischemic damage in hippocampus and correlated with GLUT3 loss. One day after ischemia, a small decrease in hippocampal immunoreactivity was observed; at 4 days the loss was more pronounced; and at 7 days loss of GLUT3 staining was maximal. The greatest loss of GLUT3 staining was in stratum oriens and stratum radiatum of Ammon's horn, with sparing in other hippocampal neuropil and in dense fibers in the lateral aspect of CA3. By contrast, GLUT3 staining was undiminished in the stratum lacunum moleculare and in all but the inner-most portion of the molecular layer of dentate gyrus, immediately adjacent to the granule cells. These data are consistent with the pattern of neuronal loss and microglial activation in hippocampus. Loss of GLUT3 may affect the availability of glucose to and possibly the viability of ischemically damaged neurons. Supported by NIH Grants NS22213 and NS17493.

681.12

MECHANISM OF THE POST-ISCHEMIC RISE IN HIPPOCAMPAL SLICE CYCLIC GMP. C.M. Jenkins, D.H. Pennington, T.L. Hoffman, W.D. Lust* and T.S. Whittingham. Department of Neurosurgery, Case Western Reserve University, Cleveland, OH 44106.

Both cyclic GMP (cGMP) and cyclic AMP levels increase during recirculation following ischemia in vivo. These increases are thought to be involved in mediating the recovery of the tissue, with the rise in cGMP potentially producing reactive hyperemia. We initially found that 80% of the rise in hippocampal cGMP following carotid artery occlusion was blocked by antagonists of nitric oxide synthase (NOS). We have subsequently investigated what may trigger the activation of NOS using the hippocampal slice model of in vitro ischemia.

Transverse hippocampal slices were prepared from male mongolian gerbils and allowed to recover in control artificial cerebrospinal fluid (ACSF) for 60 minutes. Slices were transferred to ACSF devoid of oxygen and glucose (In vitro ischemia) for 10 minutes, and then returned to control ACSF for recovery. Cyclic GMP levels were measured by radioimmunoassay and are expressed as femptomoles per mg protein.

Cyclic GMP levels peaked within 6 minutes of recovery (rising from 0.5 to 9.5 fmol/mg protein) and began to fall at 14 minutes. Inclusion of 100 μM N-nitro-L-arginie (NNLA) to inhibit NOS before, during and after the ischemic insult reduced the cGMP response to 2.1 fmol/mg protein ($p < 0.05$). A similar inhibition of the cGMP response was obtained if MK-801 was used instead of NNLA, or if the ischemic insult and recovery period were performed in the absence of extracellular calcium. Taken together, these results suggest that glutamate release during ischemia results in NMDA receptor stimulation, accumulation of intracellular calcium, and resulting stimulation of NOS. However, the inclusion of 1 mM glutamate or 100 μM NMDA in the ACSF with no ischemic insult failed to trigger an increase in tissue cGMP levels in hippocampal slices, indicating the possibility that there is an additional permissive effect produced by ischemia which allows the post-ischemic triggering of cGMP via glutamate NMDA receptor stimulation.

681.13

FREE RADICAL MEDIATED INHIBITION OF CANINE CORTEX PYRUVATE DEHYDROGENASE (PDH) FOLLOWING ISCHEMIA/REPERFUSION: EFFECTS OF Ca^{2+} AND Mg^{2+}. Y.E. Bogaert, R.E. Rosenthal, and G. Fiskum*. Depts. of Biochemistry and Molecular Biology and Emergency Medicine The George Washington University Medical Center, Washington, DC USA 20037

This study tested the hypothesis that failure of post-ischemic brain tissue to establish normal energy metabolism may be due to inhibition of key regulatory enzymes, e.g. PDH, by free radical-mediated mechanisms. Anesthetized beagles were subjected to either; sham surgery; 10 min of cardiac arrest or; 10 min arrest followed by resuscitation and 0.5, 2, 4 or 24 hr of reperfusion. At the end of the experiment a sample of frontal cortex was freeze-clamped. Following tissue homogenization, PDH activity was measured isotopically and lactate dehydrogenase (LDH) was measured spectrophotometrically. PDH activity was unchanged following ischemia but was decreased by 30-70% following 0.5, 2, 4 or 24 hr of reperfusion. LDH activity remained unchanged throughout. Experiments were also performed to determine if PDH activity could be inhibited by an in vitro free radical generating system. The presence of a Fenton reagent (0.25 mM $FeSO_4$ + 0.5 mM H_2O_2 for 10 min, 37°C) resulted in a 45% inhibition of purified PDH activity but had no effect on the activity of purified LDH. The omission of 2 mM Mg^{2+} or the addition of 0.1 mM Ca^{2+} increased the extent of PDH inhibition observed with the Fenton reagent by 31.9% and 20.4%. The results of these experiments indicate that the post-ischemic inhibition of PDH may be due to its relative sensitivity to site-specific protein oxidation. Supported by Sigma Tau,S.p.A.

681.14

CEREBRAL EXTRACTION OF OXYGEN (CEO) AND ARTERIOJUGULAR DIFFERENCES IN CARBON DIOXIDE IN PATIENTS WITH SUBARACHNOID HEMORRHAGE. J.B. Bederson, R. Horowitz, J. Oropello and D. Weisz*. Department of Neurosurgery, Mount Sinai Medical School, New York, New York 10029.

Delayed ischemia after subarachnoid hemorrhage can be difficult to diagnose in patients who are already stuporous from their bleed. In this study, indwelling 4 - French catheters were placed in the right jugular bulb to measure venous pH, oxygen content, CO_2 and lactate. Results were compared with radial artery samples and were correlated with neurological grade and angiography.

Seven patients (age 59 ± 1.2y) were monitored for 5 - 9 days with 188 simultaneous arteriovenous comparisons. Average initial CEO, defined as the arteriojugular difference, was 5.5 ± 0.1 ml/dl. Two patients developed delayed ischemia and their CEO rose significantly to 8.5 ± 0.3 ml/dl ($p<.05$, ANOVA). CEO was inversely correlated with arterial pCO_2 in the patients with vasospasm ($p<.001$), indicating increased oxygen extraction during hyperventilation. The arteriojugular difference in CO_2 (A-JdCO_2), a measure of ischemia, was positively correlated with CEO in all patients ($p<.001$). A-JdCO_2 was 9.2 ± 0.6 mm Hg on admission and rose to 12.2 ± 0.8 in the patients with vasospasm ($p<.001$, ANOVA). Both CEO and A-Jd CO_2 were inversely correlated with neurological grade $p<.0001$, Kruskal-Wallis test).

Jugular bulb monitoring can be a useful bedside method of detecting the onset of cerebral ischemia in stuporous patients with subarachnoid hemorrhage.

681.15

WITHDRAWN

681.16

HIBERNATION INDUCED CHANGES IN BRAIN AND PLASMA PROTEINS. J.E.Joy*[1], K.U.Frerichs[2], G.J.Creed[1], C.R.Merril[1], J.M.Hallenbeck[2]. [1]Laboratory of Biochemical Genetics, NIMH, St.Elizabeths Hospital, Washington D.C. 20032 and [2]Stroke Branch, NINDS, NIH, Bethesda, MD 20892.

Mammalian hibernation is characterized by a metabolic suppression so profound as to be lethal in non-hibernating species. Cerebral blood flow is reduced to ischemic levels during entrance into hibernation and yet hibernating animals show no neuropathological changes. As part of a larger study investigating factors that enable hibernating animals to tolerate ischemia, we analyzed brain and plasma protein changes in thirteen-lined ground squirrels during hibernation. Plasma samples were serially collected through an indwelling jugular catheter from animals before and during hibernation bouts. Brains were collected from active and hibernating animals. The total protein concentration of plasma was not significantly different between samples collected before or during hibernation. We found several several plasma proteins that were altered during hibernation. One of these showed an increase during hibernation and two (one of which appears to be *B*-haptoglobin) showed decreases. In the brain samples, two proteins were twofold more abundant in the brains from hibernating animals and another protein was twofold less abundant.

681.17

SPECIES-DEPENDENCE OF ASCORBATE AND GLUTATHIONE LEVELS IN BRAIN: ASCORBATE IS HIGH IN TURTLE; GSH IS HIGH IN GUINEA PIG. M. E. Rice* & Y. Choy, Depts. Neurosurgery and Physiology and Biophysics, NYU Medical Center, New York, NY 10016.

Ascorbate (Asc) and glutathione (GSH) are water soluble antioxidants and free-radical scavengers. In rat brain, both are about 2 mM. We have reported (Pérez-Pinzón et al. *Neurosci. Abstr.* 18: 154) that the Asc content of turtle brain is much higher, reaching 5-6 mM in cerebral cortex. The present studies addressed two follow-up questions: 1) are high levels of Asc in anoxia-resistant turtle unique? and 2) is turtle GSH also high? We determined CNS Asc and GSH levels for several species, using HPLC with electrochemical detection. In all species, Asc varied regionally, with highest levels in cortex or hippocampus. GSH distribution was similar, but with much less variation. Data for cortex (μmoles/g tissue wet wt) are summarized below (mean ± S.E.M.):

SPECIES NAME	ASCORBATE	GSH	(N)
Painted turtle (*Pseudemys scripta*)	5.59 ± 0.35	2.35 ± 0.14	(12)
Box turtle (*T. carolina triunguis*)	5.00 ± 0.29	1.98 ± 0.13	(10)
Garter snake (*Thamnophis sirtalis*)	3.72 ± 0.20	1.83 ± 0.04	(8)
African clawed toad (*Xenopus laevis*)	2.37 ± 0.16	2.53 ± 0.10	(12)
Rat (Sprague-Dawley)	2.46 ± 0.05	1.91 ± 0.06	(34)
Guinea pig	1.71 ± 0.03	2.66 ± 0.06	(37)

Asc content varied among species (2-3-fold, depending on region), while GSH was again more nearly constant. Relatively high GSH accompanied the exceptionally low Asc of guinea pig brain. These data suggest that the brain of air breathing vertebrates requires an intracellular antioxidant concentration of about 5 mM and that complementary regulation of Asc and GSH provides this.

This study was supported by NINDS Grant NS-28480.

ISCHEMIA: NEUROPHYSIOLOGY

682.1

DIFFERENT RESPONSES OF BRAINSTEM AND CORTICAL NEURONS TO GRADED HYPOXIA. J.P. O'Reilly*, C. Jiang and G.G. Haddad. Depts. of Biology and Pediatrics, Yale Univ. and Yale Sch. of Med., New Haven, CT 06510

It is known that different areas of the central nervous system exhibit different sensitivities to O_2 deprivation. However, the responses in the initial period of hypoxia are not well documented when neurons from different areas of the brain are compared. In order to better define these responses, we studied hypoglossal (XII) and neocortical (layer 2/3) neurons in the rat (21-35 days) during hypoxia ($20\%O_2/75\%N_2/5\%CO_2$) and anoxia ($95\%N_2/5\%CO_2$) using the *in vitro* slice preparation. Although all neurons studied showed a membrane depolarization (ΔVm) and a decrease in input resistance (Rn) during both hypoxia and anoxia, major differences in the responses were evident. After three minutes in hypoxia, ΔVm in cortical neurons was 4.0±3.2 mV, whereas XII neurons showed a ΔVm 3x greater (12.2±10.7 mV). Similarly, the reduction in Rn (% change from control) for the XII neurons was 3x greater than in the cortex (XII=31.4±23.6%; cortex=9.3±6.1%). After two minutes in anoxia, XII neurons depolarized 29.2±20.2 mV compared to only 7.2±4.0 mV for the cortex.The change in Rn was again greatest (4x) in the XII (81.1±4.2%) when compared with the cortex (17.9±14.9%).The pattern of ΔVm with graded hypoxia was characterized as a biphasic depolarization. An initial slow phase (1-5 mV/min) was followed by a rapid, major ΔVm (>15 mV/min). The slow phase in the cortex lasted 2x-6x times longer (4-18 mins) when compared with the XII (<3 mins). Hypoxia elicited the fast ΔVm (2-5 mins) in 50% of the XII cells studied, while all of the cortical cells studied failed to show the fast ΔVm during hypoxia (10-20 minutes). We conclude that cortical neurons are more tolerant than brainstem neurons to reduced O_2 availability. The mechanism for this increased tolerance may involve intrinsic membrane properties, better maintenance of energy stores, and/or extrinsic influences such as synaptic input and extracellular milieu.

682.2

EFFECTS OF K_{ATP}-CHANNEL AGONIST AND ANTAGONISTS ON ANOXIC OUTWARD CURRENT OF CA1 HIPPOCAMPAL NEURONS J.M. Godfraind, G. Erdemli, A.T. Tan* and K. Krnjević, Anaesthesia Research Dept., McGill University, Montréal, Québec H3G 1Y6 Canada.

If the outward current (I_{OUT}) induced by anoxia is mainly an ATP-sensitive K current, this I_{OUT} and the associated increase in input conductance (G_N) should be occluded by a K_{ATP}-channel **opener**, such as cromakalim (CROM), and suppressed by sulphonylurea **blockers**. Indeed, bath applications of CROM (50-300μM) reduced anoxic I_{OUT} - both at constant V (in 8/10 cells) and when evoked by depolarizing ramps (in 5/5 cells) - as well as anoxic G_N increases (in 8/10 cells). However, in the same cells, CROM produced no consistent changes in holding current (-31 ± 72pA) or G_N (2.8 ± 12.1%, n=11), and there was no correlation between CROM-induced changes in holding current and anoxic currents (r=-0.089) or the corresponding changes in G_N (r=-0.060). Of the sulphonylureas blockers of K_{ATP} channels, glyburide (GLYB 10-30 μM) had little effect on anoxic I_{OUT} (in 5/5 cells); but tolbutamide (TOLB 0.5-2mM) caused a major reduction of both I_{OUT} and G_N increase in all 10 cells tested with anoxia. In most cells, both GLYB and especially TOLB potentiated the suppression of steady anoxic I_{OUT} (and G rise) - but they tended to restore large I_{OUT}`s evoked by depolarizing ramps.

Judging by the inconstant effects of CROM and GLYB, "classical" K_{ATP} channels are not prominent in CA1 cells, nor do they contribute greatly to the anoxic I_{OUT}. On the other hand, the marked suppression of I_{OUT} by TOLB may indicate the presence of K_{ATP} channels of the type observed by Ashford et al. 1990 (Br. J. Pharm. 101: 531) in some hypothalamic and neocortical neurons.

Supported by MRC of Canada, FRSQ (J.M.G.) and a Nato Fellowship(G.E).

682.3

INFLUENCES OF TISSUE OXYGENATION ON RECOVERY OF EVOKED POTENTIALS AFTER GLOBAL ISCHEMIA IN RAT CEREBRAL CORTEX. Y.S. Zagvazdin, Z.C. Feng, T.J. Sick* and M. Rosenthal, Dept Neurology, Univ of Miami Sch. of Med., Miami, FL 33101

Prior studies suggested that increasing the fraction of inspired oxygen (FiO_2) may impair acute recovery of evoked potentials after global cerebral ischemia; while transiently decreasing FiO_2 may enhance EP recovery. In those studies, EPs were elicited by simultation of the facial nerve. Present studies were conducted in rats anesthetized with pentobarbital during (and for 3 hrs after) 20 min cerebral ischemia (4-vessel occlusion). Goals were to determine whether early EP recovery was indicative of such recovery when the animals were reanesthetized 3 days later. Stimulating electrodes were implanted in the VPL nucleus of the thalamus and EPs were recorded in a somatosensory projection area. In contrast to recovery of evoked potentials provoked by stimulation of the facial nerve, we found that increasing FiO_2 from 0.3 to 1.0 during the first 30 minutes of reperfusion did not influence recovery of EPs provoked by stimulation of the VPL nucleus at 3 hrs or 3 days after ischemia. Differences between data derived from stimulation of the facial nerve or VPL nucleus may be due to the differences in EP pathways or to the intensity of ischemia (the effects of changes in FiO_2 on facial nerve EPs were greater after 30 than 20 min ischemia). Present data suggest that acute hyperoxygenation may not impair the recovery of at least one type of evoked potential, at least after short (20 min) ischemia.

682.4

PROTECTIVE EFFECT OF SPREADING DEPRESSION(SD) IN CARDIAC ARREST CEREBRAL ISCHEMIA(CACI). N. Kawahara, L. Penix, C.A.Ruetzler, H.G.Wagner, I. Klatzo*, Stroke Branch, NINDS, NIH, Bethesda, MD 20892

A deleterious effect of spreading depression has been recently demonstrated. However, our recent studies on cardiac arrest cerebral ischemia revealed an unexpected protective effect of SD when it was applied several days preceding ischemic insult. The present study represents an effort to elucidate the nature of this phenomenon. Cortical SD was elicited in male Sprague-Dawley rats on the left side 3 days prior to CACI(10 min.) by applying 5M KCl over the intact dura for 1 hr. This method induced more than 10 DC deflections which were confined to the ipsilateral cortex. The rats were sacrificed 14 days after the ischemia, sections stained with cresyl violet, and surviving CA1 pyramidal neurons were counted in the SD and sham groups. In the second experiment, SD was elicited in the left hippocampus by microdialysis with 100 mM K+ perfusion for 1 hr., which was associated with elevation of ECF glutamate. The rats were processed the same way as in the cortical SD group. Our study indicated that induction of cortical SD protected CA1 neurons bilaterally by 20 %, and decreased the incidence of audiogenic seizure(33% vs.100%). Induction of hippocampal SD protected CA1 neurons by more than 40 % on the ipsilateral side when compared to the contralateral side and sham group. Our results demonstrated a neuroprotective effect of SD when it was induced 3 days prior to ischemia.

682.5

GABA-INTERNEURONS ARE FUNCTIONALLY DISCONNECTED BY ANOXIA FROM EXCITATORY INPUTS IN RAT HIPPOCAMPUS. R.N.Khazipov, P.Bregestovski, G. Barbin*, Y. Ben-Ari. INSERM Unite 29, Paris, France 75014.

The effects of brief anoxic episodes on the inhibitory and excitatory postsynaptic currents (EPSC and IPSC) were studied in adult rat hippocampal CA1 neurons of adult rats using whole-cell patch-clamp technique. In keeping with earlier studies with conventional 'distant' stimulation of Schaffer collaterals, anoxia preferentially inhibited GABA-mediated polysynaptic IPSC prior to the glutamatergic EPSC. The former recovered also slower than the latter after reoxygenation. We then examined the effects of anoxia on the $GABA_A$ and $GABA_B$ monosynaptic response evoked by 'close' (<0.5mm) stimulation in the stratum radiatum in presence of CNQX (20μM), APV (50μM) to block glutamate receptors and CGP35348 (100μM) or bicuculline (15μM) to record pure $GABA_A$ or $GABA_B$ receptors mediated components. Monosynaptic $GABA_A$-mediated component and responses to pressure application of isoguvacine ($GABA_A$-agonist) were not changed significantly during and after anoxia. Monosynaptic $GABA_B$ receptors mediated component and responses to baclofen ($GABA_B$-agonist) were strongly but reversibly suppressed by anoxia. We conclude that $GABA_A$ receptors mediated component of monosynaptic IPSCs is in fact highly resistant to anoxia. Thus, the block by anoxia of the polysynaptic IPSCs evoked by 'distant' stimulation is due to an impairment of the excitatory input to GABA interneurons. Furthermore, anoxia preferentially impairs by a postsynaptic mechanism $GABA_B$ receptors mediated currents.

682.6

FUNCTIONAL MODIFICATIONS IN MOUSE NEOCORTEX FOLLOWING PERMANENT OCCLUSION OF THE MIDDLE CEREBRAL ARTERY.

T. Mittmann and H.J. Luhmann*. Inst. of Neurophysiol., D-5000 Cologne 41, FRG.

The middle cerebral artery (MCA) of tribromethanol-anaesthetized adult male mice (>8 weeks) was unilaterally permanently occluded to induce a focal cerebral infarct. After survival times of 1-3 (n=7) and 28 (n=5) days, neocortical slices of 400 µm thickness were analyzed with conventional electrophysiological *in vitro* techniques. Untreated age-matched mice served as controls. Nissl staining revealed massive necrosis in the parietal and partly in the frontal neocortex. At lateral distances between 1.5 and 2 mm from the lesion center neuronal activity was suppressed. With increasing distances the orthodromically evoked field potential (FP) responses rose to 1.6 ± 0.5 mV (mean ± STD, n=34). After 28 days survival time, the FP duration was significantly ($p<0.001$, t-test) larger in ischemic animals (25.4 ± 12.3 ms, n=34) as compared to controls (17.9 ± 1.1 ms, n=24). Intracellular recordings revealed no significant difference in the resting membrane potential (V_m) and input resistance (R_N) between ischemics (V_m=-79.6 ± 10.5 mV, R_N=45.5 ± 18.1 MΩ; n=18) and controls (V_m=-81.8 ± 11.21 mV, R_N=43.4 ± 18.1 MΩ; n=29). Biphasic IPSPs could be observed in every cell of both experimental groups to suprathreshold synaptic stimulation of the afferent pathway. After 28 days survival time the peak conductance of the $GABA_B$-mediated l-IPSP was significantly ($p<0.05$) increased in cells of ischemic animals (23.9 ± 5.2 nS, n=14) as compared to control neurons (12.2 ± 2.1 nS, n=19). Furthermore, the reversal potential of the l-IPSP and the $GABA_A$-mediated f-IPSP was significantly ($p<0.01$ and $p<0.001$, respectively) different in cells recorded in ischemic (f-IPSP: -65 ± 1.9 mV, n=15; l-IPSP: -81.3 ± 2.1 mV, n=14) and control cortex (f-IPSP: -77.1 ± 1.7 mV; l-IPSP: -94.5 ± 3.2 mV, n=19). These data indicate functional alterations in the intracortical GABAergic system. However, in contrast to other models of cerebral ischemia, the efficacy of IPSPs is enhanced and hyperexcitability is only weakly expressed in this stroke model.

682.7

TRANSHEMISPHERIC ELECTROPHYSIOLOGIC DIASCHISIS DURING THE HYPERACUTE PERIOD OF FOCAL CEREBRAL ISCHEMIA. **N Panahian, GK Steinberg*, Department of Neurosurgery, Stanford University School of Medicine, Stanford, CA 94305**

Although transhemispheric diaschisis has been well described days to months following focal cerebral ischemia, few studies have examined the contralateral electrophysiological functions within the first few hours after ischemia. We studied 20 rabbits under halothane anesthesia, following 2-hour occlusion of the internal carotid, middle cerebral and anterior cerebral arteries followed by four hours of reperfusion. Bilateral somatosensory evoked potentials (SEPs) were recorded using median nerve stimulation. The ischemic hemisphere (ipsilateral) SEPs in 10 rabbits were completely abolished within five minutes of arterial occlusion ("severely ischemic group"). In another 10 rabbits the ipsilateral SEPs did not decrease below 35% of their pre-ischemic values ("mildly ischemic group"). Within five minutes of arterial occlusion the contralateral SEPs in the severely ischemic group increased to 163±40% of preischemic values, while the control group showed no change (p=.012). This elevation in contralateral SEPs persisted for 90 minutes following induction of ischemia and subsequently returned to preischemic values (p=0.028). This study suggests that disinhibition or facilitation of contralateral electrophysiologic activity during the first 90 minutes of focal, cerebral ischemia is a manifestation of transcallosal diaschisis that is closely linked to the degree of cortical ischemia.

682.8

REDUCTION OF CORTICAL INHIBITION IN THE BRAIN TISSUE SURROUNDING PHOTOCHEMICALLY INDUCED CORTICAL INFARCTS IN THE RAT

O.W. Witte, R. Domann, M. Kraemer, G. Hagemann, H.-J. Freund (Spon.: European Brain and Behaviour Society). Neurologische Klinik, Heinrich Heine Universität, 40001 Düsseldorf, Germany

The correlation between the size of ischemic lesions and the ensuing functional deficits is often poor. Such observations indicate that the functional deficits are not solely caused by the damaged area, but also by functional alterations of neurons in the vicinity of such lesions. An example for such alterations is the occurrence of focal epileptic seizures in 5 to 15 % of cerebral lesions due to trauma or ischemia.

The aim of the present experiments was to investigate functional changes in brain areas surrounding focal cerebral infarctions. Small infarctions in the parietal cortex of Wistar rats were produced photochemically using the Rose Bengal technique. The infarctions evoked reproducible cortical lesions of about 2 mm diameter. In the surrounding brain tissue changes in electrophysiological responses occurred. Whereas in control animals without infarction a paired-pulse inhibition could be evoked all over the neocortex, in infarcted animals the paired-pulse inhibition was significantly reduced or even absent within an area extending up to 5 mm lateral from the lesion center. The changes in paired-pulse inhibition were already present on the first day and persisted at least up to 60 days after infarction. Such functional changes may contribute to neurological deficits occurring after cerebral infarcts.

Supported by DFG Wi 840/4-2

682.9

CHANGES IN MEMBRANE CURRENT FLUCTUATIONS OF HIPPOCAMPAL NEURONS INDUCED BY ANOXIA. P. Miu*, G. Erdemli, M. Glavinovic, and K. Krnjevic. Anaesthesia Research and Physiology Depts., McGill Univ., Montreal, Que. H3G 1Y6 Canada.

Short intervals of anoxia are associated with hyperpolarization of pyramidal cells. The underlying membrane changes can be complex and may be caused by opening or closing of various ion channels, but also by activation of ion pumps - the transport systems that extrude or accumulate ions against an electrochemical gradient. To obtain more information on the nature of membrane changes associated with brief anoxia (2-3 min of 95% N_2 + CO_2), CA1 neurons were voltage-clamped (with single, 3 M KCl electrodes) in the presence of TTX, and membrane currents and current fluctuations were recorded and analyzed. The experiments were done at a 33ºC, in slices from Sprague-Dawley rats. The anoxic outward currents evoked at holding potentials of -70 to -30 mV were associated with a change in variance (δ^2) of the current fluctuations. Power spectra analysis of current fluctuations revealed that, as reported previously, anoxia elicits opening and closing of certain ion channels. A non-Lorentzian component of power spectra (with a minor contribution at low, but a major one at high frequencies) can also be observed, suggesting the activation of a transport mechanism or a pump (Läuger 1984 *Eur. Biophys. J.* 11:117; Glavinovic & Trifaro 1991 *Soc. Neurosci. Abstr.* 7:779).

Supported by M.R.C. of Canada and a NATO Fellowship to G.E.

682.10

ENHANCED UTILIZATION OF NMDA RECEPTOR IN AFTERDISCHARGES OF CA1 PYRAMIDAL CELLS FOLLOWING TRANSIENT CEREBRAL ISCHEMIA IN THE RAT HIPPOCAMPUS IN VIVO. M. Furuichi, T. Kano, A. Yoshino, T. Kawamata*, S. Miyazaki, Y. Katayama, T. Tsubokawa. Department of Neurological Surgery, Nihon University School of Medicine, Tokyo, Japan 173.

We previously demonstrated post-ischemic potentiation (PIP) of synaptic efficacy in hippocampal Schaffer collateral/CA1 responses of the rat beginning at 6-8 hours following 10-15 min transient cerebral ischemia in vivo. The present study demonstrated that repetitive stimulation, which produces a short-lasting non-NMDA receptor-mediated afterdischarges (ADs) in sham-controls, results in a prolonged ADs at the same period after ischemia. Pre-treatment with systemic administration of MK-801 prevented the development of the prolonged ADs as well as PIP, indicating that NMDA activation is required for initiation of these phenomena. The PIP was not affected, however, by AP5 administered via microdialysis at 6 hours post-ischemia. The same procedure blocked the prolonged ADs after ischemia. These observations indicate that, during reperfusion period, while there is no NMDA receptor-mediated component in the increased synaptic efficacy, NMDA receptor can be activated more easily than in normal condition by appropriately timed multiple synaptic inputs. If the increased synaptic efficacy underlies ischemia-induced hippocampal damage, it appears that delayed treatment with NMDA antagonists may not be as potent as other drugs which blocks more upstream synaptic events in protecting CA1 pyramidal cells.

682.11

MEMBRANE CURRENTS AND CALCIUM TRANSIENTS INDUCED BY HYPOXIA-AGLYCEMIA IN RAT HIPPOCAMPAL NEURONS AND GLIAL CELLS Y. Fujito*, A. Mizuguchi, H. Shimizu, Y. Nakazono and M. Aoki. Dept. Physiology, Sapporo Medical University, School of Medicine, Sapporo 060, Japan.

We examined the effects of hypoxia-aglycemia (HA) on membrane currents (Ims), membrane potentials and $[Ca^{2+}]i$ of neurons and glial cells in the CA1 of rat hippocampal slices at 34 ± 0.5° C. Ims were measured by single-electrode voltage clamp (SEVC) with KCl, K acetate and Cs acetate electrodes. Fifteen min of HA elicited an initial slow hyperpolalization and a succeeding depolarization which caused an irreversible depolarization and disappearance of action potentials in CA1 pyramidal neurons. In SEVC recording with K electrodes at a holding potential adjusted to each resting potential from -60 to -70 mV, 6-8 min HA elicited a slow outward current with an increase in membrane conductance and a succeeding inward current in CA1 pyramidal neurons. The outward current in CA1 pyramidal neurons induced by HA was almost disappeared with Cs electrodes, indicating that the outward current is, at least in part, K current. By contrast, in glial cells, SEVC recording showed a slow inward current without initial outward currents during HA at a holding potential adjusted to each resting membrane potential from -75 to -85 mV. When exposed to 8-15 min HA, $[Ca^{2+}]i$ increased significantly and returned to the prehypoxic levels after reoxygenation both in neurons and glial cells. However, in some glial cells, $[Ca^{2+}]i$ began to decrease in 10-13 min of HA. These results indicate the differential responses to HA in membrane currents and $[Ca^{2+}]i$ between neurons and glial cells.

682.12

LOSS AND RECOVERY OF SYNAPTIC RESPONSE IN HIPPOCAMPAL AREA CA1 PARTIALLY PROTECTED FROM ISCHEMIC DAMAGE. M.M. Okazaki*, K.H. Neill, B.J. Crain and J.V. Nadler. Depts. Pharmacology, Pathology and Neurobiology, Duke Univ. Med. Ctr., Durham, NC 27710.

Area CA1b of the gerbil hippocampus can be partially protected from ischemic damage by interruption of glutamate pathways, glutamate receptor antagonists and cerebral hypothermia. These protected CA1b pyramidal cells can remain intact for at least 4 weeks after ischemia, but they appear histologically abnormal. We now report that abnormal histologic appearance predicts a loss of response to excitatory synaptic input and that both morphologic and physiologic defects appear to be reversible. Gerbils were subjected to a 5-min carotid occlusion under halothane anesthesia with maintenance of body temperature. Responses to stimulation of the commissural input to area CA1b were recorded simultaneously in stratum radiatum and the pyramidal cell body layer.

In sham-operated gerbils, commissural input-output curves remained essentially unchanged for 3 days after surgery. In gerbils subjected to ischemia without neuroprotective intervention, synaptic responses never recovered to baseline values and could hardly be evoked 3 days after ischemia. In animals partially protected from ischemic damage, the remaining CA1b pyramidal cells were histologically abnormal and the loss of synaptic response markedly exceeded the loss of cells. When cases of partial protection were studied 4 months after ischemia, however, the great majority of CA1b pyramidal cells appeared histologically normal and the loss of synaptic response was proportional to cell loss. These results suggest that neuroprotective interventions arrest the process of ischemic degeneration at a stage of damage from which the cell eventually recovers. (Supported by NIH grant NS 06233.)

682.13

CHANGES OF NEURONAL ACTIVITY AND IMMUNOHISTOCHEMICAL REACTIONS ASSOCIATED WITH PHOTOCHEMICALLY-INDUCED THROMBOSIS IN CAT VISUAL CORTEX. U.T. Eysel*, U. Kretschmann & R. Schmidt-Kastner. Department of Neurophysiology, Faculty of Medicine, Ruhr-Universität Bochum, D-44780 Bochum, F.R. Germany.

Changes of neuronal activity occur in the surrounding of cortical lesions and the resulting dysfunction can spread far beyond the region of actual cell death. In the present study we have used photochemically-induced thrombosis as a model of an ischemic infarction in the visual cortex of adult cats (n=3). Local illumination (2 mm diameter, 570 nm, 5 minutes) of the visual cortex 3 minutes after systemic administration of Rose Bengal (10 mg/kg) led to circumscribed ischemic lesions sparing the lower cortical layers. Single cell recordings were performed with glass micropipettes in the surrounding and below the lesions between 18 and 24 hours following thrombosis. The recording sessions were immediately followed by perfusion fixation with 4% paraformaldehyde solution. Histology and immunohistochemistry revealed thrombosis of small vessels and infarction in the core region. A narrow borderzone showed distorted neurons and abnormal processes of parvalbumin-positive interneurons, damaged astrocytes (GFAP-stain), and vasogenic edema (serum-proteins). Single cell recordings (n=77) showed increased maintained activity often associated with epileptiform high-frequency burst discharges (N = 29, 38%). Stimulus-evoked responses to moving light-bars were significantly increased ($p<0.05$) and orientation and direction specificity reduced ($p<0.05$). Increased glutamate release and reduced inhibition in the surrounding of infarction may contribute to the effects. (Supported by DFG Ey 8/13-2).

682.14

SQUARE WAVES SIGNAL IMPEDANCE METHOD FOR TISSUE WATER CONTENT MEASUREMENT.
M., Itkis, Ph.D., * L, Roberts, M.S., R.,Hariri, M.D.,Ph.D., L, Ghajar M.D., Ph.D. Dept. of Surgery, Aitken Neurosurgery Lab. Cornell University Medical College, New York, NY 10021

It was shown that low frequency electrical signals propagate only through extracellular space; while high frequency signals can propagate through the cells membrane as well, due to the membranes high capacitance. Presently, using a sine wave signal with changing frequency, one could obtain information of both extra- and intracellular resistance. We found that using the square wave signal in lieu of the sine, will help avoid the need for expensive frequency control and amplitude-phase measuring devices, and to ease on-line data processing.

The isolating-calibration device developed for the present studies was used to condition and stabilize current through the brain tissue. This device was also used for impedance calibration before and/or after experiments. For generation, amplification and recording signals a Macintosh based data acquisition system was used.

Preliminary experiments were carried out with a 0.49 mm stainless-steel electrode tetrapolar system on the rat brain. This study showed good linearity between stimulating current I = 5 - 25 microA and drop of voltage, which was measured by an inner pair of electrodes. Results of the device and program accuracy test, shown in the paper, allow us to propose a method for use in the animal research, for analyses of intra - and extracellular water content.

682.15

HYPOXIC DEPOLARIZATION AND CORRELATED HISTOLOGICAL INJURY IN HIPPOCAMPAL SLICES IN VITRO: NEUROPROTECTION WITH LIDOCAINE. M.L. Weber* And C.P. Taylor. Parke-Davis Pharmaceutical Research, Div. of Warner-Lambert Co., Ann Arbor, MI 48105

Hypoxic depolarization (HD) has been associated with irreversible neuronal injury in CNS tissue. We have previously shown that EPSPs are irreversibly lost in hippocampal slices in vitro within ~2 min of HD from oxygen/glucose deprivation. Here we demonstrate a correlation between loss of EPSPs and morphological damage in hippocampal slices using histology as an endpoint. Rat hippocampal slices were maintained in a Haas-type superfusion chamber at 36°C with oxygenated artificial CSF (95% 0_2, 5% CO_2, 10 mM glucose). EPSPs were evoked each minute in CA1 stratum radiatum and extracellular DC potential was recorded to show HD. Slices were subjected to 12 min of oxygen-glucose deprivation (95% N_2, 5% CO_2, 0 mM glucose) with or without 200 μM lidocaine and then returned to oxygenated media with glucose for 3.5 hr before fixation, sectioning and staining. All untreated hypoxic slices had HD with irreversible loss of EPSPs and had grossly swollen or lysed perikarya with pyknotic nuclei in CA1. Treatment with lidocaine (200 μM) did not markedly reduce EPSPs but prevented HD and irreversible loss of EPSPs from hypoxia; cell bodies were also well-preserved (6 of 14 slices). In cases with lidocaine treatment that had HD and EPSPs that did not recover (6 of 14 slices), CA1 perikarya were grossly damaged. Treated slices with HD near the end of the hypoxic period with EPSPs that recovered only slightly (2 of 14 slices) had extensive damage to some but not all CA1 cell bodies. Continuously oxygenated slices did not have HD or histological damage. Our experiments demonstrate that HD with permanent loss of evoked EPSPs in hippocampal slices correlates closely with severe histopathological damage to CA1 pyramidal cells.

682.16

IONIC MECHANISMS UNDERLYING NEOCORTICAL ANOXIC DEPOLARIZATION. A. S. Rosen* and M. E. Morris. Dept. of Pharmacology, Univ. of Ottawa, Ottawa, Canada K1H 8M5.

In submerged rat neocortical slices, brief anoxia (4-6 min) induces a moderate membrane depolarization (2-5 mV) of pyramidal neurons (*Rosen & Morris 1991, Neurosci. Lett., 124:169-173*). This anoxic depolarization (AD) is recorded at resting potentials between -55 and -100 mV and is reversible upon reoxygenation. In slices superfused with 1 μM tetrodotoxin, AD amplitude was reduced to almost 50%. A 68% reduction of AD amplitude was obtained when $[Na^+]_o$ was decreased to 28% of control, by substituting NaCl in the superfusate with an equimolar concentration of choline chloride. These results, together with the sensitivity of AD to the non-specific excitatory amino acid (EAA) antagonist, kynurenic acid (1 mM), suggest that Na^+ influx -- possibly through voltage-dependent channels, but mainly through EAA-activated synaptic channels -- is strongly implicated in the production of AD. The finding that the NMDA antagonist, DL-aminophosphonovaleric acid (DL-APV) (100-250 μM) had no effect on AD amplitude, strengthens the evidence for a Na^+ influx through non-NMDA channels, (rather than one of Ca^{2+} through NMDA channels), as being the major cation influx during brief anoxia in neocortical pyramidal neurons.

(Supported by the Medical Research Council of Canada)

682.17

MECHANISM OF ANOXIA-INDUCED SUPPRESSION OF INHIBITORY CURRENTS IN THE HIPPOCAMPUS. A.N. Katchman S. Vicini and N. Hershkowitz*, Georgetown University Medical Center, Dept. Neurol. and FGIN, Washington DC 20007.

While the mechanism of anoxia-induced depression of excitatory postsynaptic currents (EPSCs) results from a presynaptic reduction in transmitter release, the mechanism of depression of the inhibitory postsynaptic currents (IPSCs) is less clear. Whole-cell patch clamp recordings were obtained from CA1 neurons of hippocampal slices in an interface chamber. Anoxia was induced by switching perfusion and ambient gas from a 95%O_2/5%CO_2 to a 95%N_2/5%CO_2 mixture. Using a K gluconate micropipette, containing QX-314 (1mM), a $GABA_A$ IPSC was recorded following stimulation to interneurons in the stratum radiatum in the presence of the glutamate blockers, CNQX (50 uM) and CPP (20 uM). I/V curves were constructed during control and at various time periods following the onset of anoxia in 10 cells. These plots indicated that while conductance was not significantly altered, the IPSC reversal potential exhibited a significant positive shift (from -74 mV to -47 mV) following 5 min of anoxia. Miniature IPSCs (mIPSCs) were also examined as spontaneous inward currents, using a KCl filled micropipette containing QX-314 (V_H=-60 mV), from slices preincubated in CNQX (50 uM), CPP (20 uM) and tetrodotoxin (1uM). 2 min following the onset of hypoxia a moderate increase in mean peak amplitude (from 21.7 pA to 26.3 pA) and a small decrease in half decay time (from 11.0 mS to 9.2 mS) of the mEPSCs were observed in 11 cells. These data suggest that the apparent early anoxia-induced suppression of the IPSC is a result of a shifting of the reversal potential for $GABA_A$ current. This observation is consistent with those made by other investigators of an anoxia-induced decrease in extracellular chloride concentration. (Supported by NINDS grant NS 14600-02).

682.18

RESPONSES OF RAT NEOSTRIATAL AND HIPPOCAMPAL NEURONS TO TRANSIENT GLOBAL ISCHEMIA: AN *IN VIVO* INTRACELLULAR RECORDING STUDY. Z. C. Xu* and W. A. Pulsinelli Dept. of Neurology, University of Tennessee Memphis. Memphis, TN 38163.

Specific populations of CNS neurons, such as spiny neurons in the neostriatum and CA1 pyramidal neurons in the hippocampus, are highly vulnerable to transient ischemia. The pathogenesis of such selective injury after ischemia may involve alternations of these neurons' electrophysiological properties. We examined, *in vivo*, the changes of neuronal and synaptic activities in rat neostriatal and hippocampal neurons after transient global ischemia with intracellular recording techniques.

We evoked the synaptic potential of neostriatal or hippocampal neurons by stimulation of frontal cortex or commisural and perforant path fibers respectively in the anesthetized Wistar rat. Severe forebrain ischemia was induced by 4-vessel occlusion in normothermic animals for 5 minutes. The recorded neurons were identified by intracellular iontophoresis of neurobiotin.

Approximately 3 minutes after the onset of forebrain ischemia, the baseline membrane potential depolarized from ~ -70 mV to ~ -20 mV and remained at that level throughout the ischemia. One to two minutes after reperfusion, the membrane potential quickly returned to the control level. Spontaneous synaptic activities were reduced immediately after occlusion and were abolished shortly before anoxic depolarization. Although both inhibitory (IPSP) and excitatory (EPSP) postsynaptic potentials were suppressed by ischemia, the IPSPs disappeared earlier and recovered later than the EPSPs. The membrane input resistance increased and the time constant decreased after reperfusion. These results begin to characterize the effects of *in vivo* ischemia on electrophysiology of vulnerable neurons.

682.19

ACUTE ELECTROPHYSIOLOGICAL CONSEQUENCES OF BCO IN THE GERBIL: A CORRELATION WITH DELAYED BEHAVIORAL AND ANATOMICAL CHANGES. James D. Stittsworth, Jr.*, E.J. Drower and T.H. Lanthorn Neurological Diseases Research, Searle, Skokie, IL 60077.

Few studies have investigated the electrophysiological consequences of BCO in the gerbil. Of these studies the primary focus has been in the cortex. However, the most susceptible structure to 5 minute BCO is the hippocampus. This study investigated the electrophysiological consequences of 1 min and 5 min BCO on responses recorded from the CA1 region of the gerbil hippocampus in vivo under two different anesthetic conditions and two different temperature conditions. In animals anesthetized with urethane, loss of the hippocampal population spike was observed within one min of BCO. An ischemic depolarization was observed in the D.C. trace at 4.63 ± 1.1 min following the loss of the population spike. The mean amplitude of the depolarization was 23.1 ± 1.4 mV (n = 9). Animals anesthetized with 0.5% halothane/60% nitrous oxide also lost synaptic function within one min of the onset of BCO. However, an ischemic depolarization was observed at 0.30 ± 0.76 min following loss of the population spike with a mean amplitude of 24.4 ± 2.4 mV (n = 10). Hyperlocomotion was observed in all BCO animals, however only in those animals whose temperature was maintained at 37°C was a significant difference observed. This temperature effect was also important for neuronal damage.

ISCHEMIA: TEMPERATURE

683.1

EFFECT OF INTRAISCHEMIC HYPOTHERMIA ON ISCHEMIA-INDUCED CHANGES IN REGIONAL LEVELS OF INOSITOL 1,4,5-TRISPHOSPHATE (IP_3). R. Busto*, M.Y.-T. Globus, E. Martinez, I. Valdés, and M.D. Ginsberg. CVD Research Center, Univ. of Miami, Sch. of Med., Miami, FL, 33101.

Extracellular glutamate release and histopathological outcome following ischemia are sensitive to intraischemic brain temperature. Since 1,4,5-trisphosphate (IP_3) is implicated in glutamate receptor-mediated events and intracelluar calcium homeostasis, the relationship between intraischemic brain temperature and regional IP_3 levels following ischemia was evaluated. Twenty min of 2-vessel occlusion plus hypotension was induced in rats whose intraischemic brain temperature was maintained at 30°C or 37°C. Using a D-myo-IP_3[^{3}H] assay system, IP_3 was determined in hippocampal, striatal, cortical, and thalamic homogenates at the end of ischemia or following 1h of recirculation. Significant ($p<0.05$) decreases of IP_3 were observed during normothermic ischemia in hippocampus (by 74%), striatum (81%) and cortex (66%), whereas thalamic levels were unchanged. During recirculation, hippocampus, striatum and cortex showed increases over ischemic levels though not fully to control. Thalamic levels remained unaltered. Following hypothermic ischemia, only the striatum showed a significant ($p = 0.02$) decrease in IP_3 levels. Our results demonstrate that following ischemia and recirculation, there is a decrease in IP_3 levels in vulnerable brain regions, a process which is partially sensitive to intraischemic hypothermia. These results do not confirm that breakdown of phosphatidylinositol and the generation of IP_3 occur at the end of ischemia or early recirculation (1h).

683.2

EFFECT OF MILD HYPERTHERMIA AND ANOXIA ON PHYSIOLOGICAL RECOVERY, SODIUM, POTASSIUM, CALCIUM AND ATP IN THE CA1 REGION OF THE RAT HIPPOCAMPAL SLICE. P. Amorim, I.S. Kass*, J.E. Cottrell and G. Chambers. Anesthesia Dept., SUNY Health Science Center, Brooklyn, NY 11203.

It is well known that large changes in temperature affect neuronal damage. Recently small changes in temperature have also been examined. Mild hypothermia improves neurological recovery from anoxia/ischemia; mild hyperthermia has the opposite effect. We decided to examine the effect of a small increase in temperature on anoxic damage.

Adult rat hippocampal slices were incubated in artificial CSF, aerated with 95%O_2+5%CO_2 (normoxia) and subjected to anoxia (95%N_2+5%CO_2) either at 37 or 39°C. Recovery of the CA1 population spike (PS) after anoxia was used as an indicator for anoxic damage. After 5 min of anoxia at 37°C the PS recovered to 56±13% of its pre-anoxic amplitude; when the temperature was increased to 39°C the PS recovered to only 13±5% ($p<.05$). ATP measured in the CA1 region (luciferin-luciferase assay) after 5 min of anoxia at 37°C was 2.8±.2 nM/mg; when the temperature was increased to 39°C, ATP fell to 0.9±.1 ($p<.05$). Intracellular Na and K were measured in the CA1 region by flame-photometry after 5 min of anoxia. Na increased from 171±16 uM/g at 37°C to 253±24 at 39°C ($p<.05$) and K fell from 130±10 uM/g at 37°C to 97±12 at 39°C ($p<.05$). Ca^{45} influx, measured in the CA1 region after 10 min of anoxia (the method is less sensitive to shorter periods of anoxia), increased from 9.15±.32 nM/mg at 37°C to 9.95±.29 at 39°C ($p<.1$).

Our results demonstrate that a mild increase in temperature significantly worsens anoxic damage in the in-vitro hippocampal slice. The marked depletion in ATP levels and the significant increase in Na and decrease in K intracellular levels observed with hyperthermia are likely explanations for its harmful effect.

683.3

DOES HYPOTHERMIA CONTRIBUTE TO THE NEUROPROTECTIVE ACTION OF NBQX? S.M. Nurse* and D. Corbett. Div of Basic Medical Sciences, Fac of Med, Memorial University of Newfoundland, St. John's, NF, Canada, A1B 3V6.

Hypothermia during or within the first 30 min of an ischemic event is neuroprotective. Studies using putative anti-ischemic agents have been confounded by hypothermia during this period. However, little attention has been paid to potential confounding effects of hypothermia when drug administration is delayed.

We found that NBQX (30mg/kgx3) reduces brain temperature ~3°C for several hours, in normal rats and gerbils. We tested whether these temperature alterations contributed to its protective action against ischemic insults of 3 or 5 min in a gerbil model of global ischemia. NBQX (30mg/kgx3) was administered 1 or 6 hr after carotid artery occlusion. Brain temperature was either uncontrolled, or regulated to simulate the pattern of ischemic controls for 24 hr post-occlusion. NBQX provided greater protection of CA1 neurons against a moderate insult (3 min occln) versus a severe insult (5 min occln). NBQX at 1hr post-occlusion was more beneficial than delaying treatment for 6 hr. However, regulating brain temperature decreased the protection afforded by NBQX. Thus, the potential confound of hypothermia should be ruled out even when drug administration is delayed several hours after the ischemic event.

Supported by the Medical Research Council of Canada.

683.4

DELAYED AND PROLONGED POSTISCHEMIC BRAIN HYPOTHERMIA IS NEUROPROTECTIVE IN GERBILS. F. Colbourne* and D. Corbett. Memorial University of Newfoundland, St. John's, NF, CANADA A1B 3V6

A brief period of global ischemia in the gerbil produces extensive delayed neuronal death in the hippocampal CA1 sector with a concomitant spatial mapping impairment. Intra- or immediate postischemic hypothermia (PH) lessens this injury. We assessed whether PH (brain temp. = 32°C) initiated one hour after occlusion and maintained for 12 hr was neuroprotective in gerbils exposed to either 3 or 5 min of normothermic ischemia. CA1 integrity was assessed at either 10 or 30 days after ischemia and spatial mapping ability was determined by an open field test given 3, 7 and 10 days after ischemia. A 5 min occlusion in control animals (no hypothermia) produced complete CA1 cell loss at days 10 and 30. PH slowed and attenuated this death, such that there was about 50% savings at day 10, but < 20% with 30 day survival. Three min of ischemia also produced severe CA1 loss (~70%) in controls, which PH greatly attenuated at both days 10 and 30. Finally, PH reduced associated spatial mapping deficits caused by both 3 and 5 min ischemic episodes.

Research supported by the MRC of CANADA.

683.5

THE EFFECT OF PREISCHEMIC SPINAL CORD HYPOTHERMIA ON AMINO ACID RELEASE AND NEUROLOGICAL RECOVERY INDUCED BY REVERSIBLE AORTIC OCCLUSION IN RAT. M.Marsala*, L.S.Sorkin, T.L.Yaksh, Dept. of Anesthesiology, University of California, La Jolla, San Diego, CA 92093

Preischemic brain cooling has been shown to suppress glutamate release during global cerebral ischemia and to provide neurological protection for extended periods of reflow. In the present work, we characterized the neurological changes and amino acid release that occur in spinal cord after 20 min of transient normothermic or hypothermic ischemia in rat. Extracellular changes in spinal cord amino acid concentration were measured using microdialysis in conjunction with HPLC and UV detection. In the hypothermic group (n=12) temperature was lowered using an underbody cooling system to 34.2± 0.7°C 2 min before spinal cord ischemia was induced by the occlusion of the descending thoracic aorta. After ischemia, the animals survived 2 hr (microdialysis study) or 8 hr (behavioral study) of normothermic reperfusion. In normothermic animals, glutamate concentration was significantly elevated during the ischemic and immediate reperfusion periods. Taurine increased significantly 0.5 hr postocclusion and remained elevated for the full 2 hr. No consistent changes in aspartate or asparagine concentration were detected. In the hypothermic group, no significant changes in glutamate concentration were detected during the ischemic or reperfusion periods. Taurine was significantly elevated 30 min after reperfusion and returned close to baseline after 60 min of reperfusion. In contrast to the normothermic group in which majority of animals displayed spastic paraplegia and extensive hypersensitivity, hypothermic animals showed no deficit of motor or sensory function at any time of reperfusion. The data from the present study showed that mild spinal cord hypothermia can significantly suppress glutamate release and prevent neurological deficits otherwise induced by normothermic spinal cord ischemia of the same duration. Supported by NS 16541 (TLY), Fogarty International Center (MS; Fellowship No.1 F05 TWO 464901 NSS), California Paralysis Project (MM, LS).

683.6

HYPO- AND HYPERTHERMIA AFFECT THE NEURONAL SURVIVAL SEVERAL HOURS AFTER BRAIN ISCHEMIA IN RATS Cicero Coimbra, Fredrik Boris-Möller*, Mikael Drake and Tadeusz Wieloch. Laboratory for Experimental Brain Research, Lund University, Lund Hospital, S-221 85 Lund.

Hypothermia (HT) is recognized as a powerful protectant when induced intraischemically, but is generally considered not to improve neuronal survival if initiated as late as 30min into recirculation (R) phase. We report that HT (33.0°C) with a minimal duration of 5 hrs reduced the neuronal damage in the lateral hippocampal CA1 region by approximately 50%, when initiated up to 12hr-R from 10min of 2VO + hypotension in rats. When initated at 2hr-R, 5h-HT also provided protection to regions with fast maturation of damage, such as the striatum. Shorter periods provided minor or no protection and, when the cooling was delayed for 24hrs, 5hr-HT did not protect the brain. The effect on CA1 neurons was reduced by prolonging HT for 10hrs from 2hr-R. On the other hand, as opposed to the 24hr-cycle observed in normal rats, the mean core temperature (CT) was spontaneously held above 38.5°C from 21 to 63hr-R (39.2°C peak at 33hrs). When administered during this period, the antipyrogen dipyrone depressed CT to normothermic values and diminished the neuronal damage. The results point to an optimal duration of moderate HT induced within 2-12hrs after transient ischemia to achieve neuronal protection, and indicate that hyperthermia occurring quite late after recirculation may be detrimental to neuronal survival. Prolonged CT control seems to be required for proper evaluation of drugs in ischemia models. Supported by the Swedish MRC and Brazilian CNPq.

683.7

TEMPERATURE MODULATION OF NMDA-INDUCED HISTOPATHOLOGICAL DAMAGE IN THE ADULT RAT BRAIN. M.Y.-T. Globus*, L. ElDeiry, W.D. Dietrich, I. Valdés, and R. Busto, CVD Research Center, Univ. of Miami, Sch. of Med., Miami, FL, 33101.

Extracellular glutamate release and histopathological outcome following ischemia are sensitive to intraischemic brain temperature. However, whether postsynaptic NMDA mediated events are sensitive to mild temperature alterations is unknown. We evaluated the relationship between brain temperature and the magnitude on NMDA-induced histopathological damage in the adult rat's brain. Excitotoxic lesions were performed by unilateral striatal injections of NMDA (50 or 150 nmols). Animals were divided into three groups in which brain temperature was maintained at 30°C (n = 7), 37°C (n = 8), or 39°C (n = 6) five min prior to and for a period of three hours following injection of NMDA. The extent of hemispheric lesion was quantitated 3 days following injections by morphometric analysis of lesion area. No association was observed between brain temperature and the extent of the lesion induced by 150nmols of NMDA (Integrated lesion volumes were: mean±SD, mm^3; 30°C = 57.7±18.5; 37°C = 44.4±13; 39°C = 61.57±20). Hypothermia significantly *increased* lesion volume induced by 50nmols of NMDA (30°C = 33.08±12.8; 37°C = 19.92±5.1; 39°C = 32.5±18.6). Results indicate that postsynaptic effects of NMDA and the subsequent neuronal damage are not attenuated by mild hypothermia. Therfore, the modulatory effects of hypothermia on the excitotoxic process during ischemia involves mainly presynaptic mechanisms.

683.8

INTRAISCHEMIC HYPOTHERMIA REDUCES TISSUE INJURY BY REDUCING OXIDATIVE STRESS AFTER TEMPORARY FOCAL ISCHEMIA IN RATS. H. Karibe, S. F. Chen, G. J. Zarow, J. Gafni, S. H. Graham, P. H. Chan and P. R. Weinstein*. Neurosurgery and Neurology, Univ. of California San Francisco and V.A.M.C., San Francisco, CA 94121

To investigate the influences of intraischemic hypothermia on oxidative injury after prolonged focal ischemia followed by reperfusion, 32 Sprague-Dawley rats underwent 3 hours middle cerebral artery occlusion followed by 3 hours reperfusion, with hypothermia (brain temperature = 33°C) or normothermia (37°C) maintained throughout ischemia. Normothermia was maintained throughout reperfusion for all animals.

In the first study (n=8/group), intraischemic hypothermia suppressed the reduction of tissue concentrations of endogenous antioxidants, ascorbic acid ($p \leq 0.05$) and glutathione ($p \leq 0.05$), in ischemic cortex. In a parallel second study (n=8/group), laser-Doppler estimates of cortical blood flow revealed that intraischemic hypothermia significantly attenuated early post-ischemic hyperperfusion ($p \leq 0.01$) and delayed post-ischemic hypoperfusion ($p \leq 0.01$). Hypothermia also reduced infarct volume as estimated from semi-serial Nissl stained slides ($p \leq 0.01$), blood-brain barrier disruption as estimated from the extravasation of Evans blue dye ($p \leq 0.01$), and brain edema as calculated by subtracting the volume of the non-ischemic hemisphere from that of the ischemic hemisphere ($p \leq 0.01$).

These results demonstrate that intraischemic hypothermia reduces oxidative stress as well as tissue injury following prolonged temporary ischemia. Reduction of oxidative stress may protect tissue through the attenuation of both postischemic hypoperfusion and vasogenic edema.

683.9

HYPOTHERMIA DECREASES ASTROGLIAL INJURY AND ARACHIDONATE RELEASE DURING COMBINED GLUCOSE-OXYGEN DEPRIVATION. SE Haun*, VL Trapp, and LA Horrocks. Depts. of Pediatrics and Medical Biochemistry, The Ohio State University, Columbus, OH 43210.

Hypothermia provides significant protection when intiated before or after cerebral ischemia *in vivo*. However, the mechanisms producing this protective effect are not known. We studied the effect of hypothermia on lactate dehydrogenase (LDH) efflux and [^{3}H]arachidonic acid metabolite ([^{3}H]AAM) release from prelabeled cultures of astroglia during combined glucose-oxygen deprivation (CGOD). Four groups (n=12, each group) were studied: control and CGOD at 37°, 34°, and 31°C. Hypothermia reduced cellular injury, i.e. LDH efflux (expressed as a percent of total LDH) after 12 h of CGOD was 0.8 ± 0.4, 84.2 ± 6.2, 64.5 ± 24.3, and 31.4 ± 15.8 (mean ± SD) in the control, CGOD 37, CGOD 34, and CGOD 31 groups, respectively. [^{3}H]AAM release was also reduced by hypothermia. [^{3}H]AAM release (expressed as dpm/culture x 10^{-4}) after 6 h of CGOD was 7.4 ± 0.6, 13.1 ± 0.5, 9.6 ± 0.8, and 6.9 ± 0.4 (mean ± SD) in the control, CGOD 37, CGOD 34, and CGOD 31 groups, respectively. We speculate that inhibition of phospholipid degradation may be one mechanism, but not the only mechanism that contributes to the neuroprotective effect of hypothermia. Supported by NIH K08 NS 01523 and CHRF 020-886.

INFECTIOUS DISEASES

684.1

NEUROTROPIC CAPABILITY IN JC VIRUS COINCIDES WITH VIRAL REGULATORY REGION REARRANGEMENT IN THE HOST. G.S. Ault and G.L. Stoner*. Lab. of Exp. Neuropathology, NIH, Bethesda, MD 20892

The ubiquitous polyomavirus JCV is the agent of PML, a rare demyelinating disease in the immunocompromised host. Comparison of the promoter/enhancer structure of isolates from 11 PML brains revealed that sites of strand breakage in the promoter were not random; four or five preferred sites or areas exist. Alignment of the unduplicated archetypal promoter, isolated from kidneys of these patients, with the prototype strain defines six blocks of sequence, A through F, which are delineated by the preferred sites of strand breaks. Region A, containing the TATA box, the first half of region C, and region E, were consistently retained. Region B, the 23-bp "insertion" in archetype relative to the prototype, was also retained in all 11 isolates. Region D, the 66-bp "insertion", was retained in 3 isolates. Regions A and D were never duplicated, whereas regions C and E usually were duplicated or triplicated. All regions contain transcriptional promoter or enhancer elements, indicating that the virus can operate with various combinations of elements, certain ones of which are required while others are expendable or interchangeable. Variation in the exact breakage point within the preferred sites, alternate use of the sites in different isolates, and occasional short deletions at other sites result in sequences which are unique in each case. At the same time, the limited choice of break sites and the characteristic fates of the regions themselves result in three broad patterns of duplication and deletion. The patterns do not correspond to our previously described viral genotypes 1 and 2 defined by coding region base changes, and do not appear to be a stable feature of the virus. Rather, neurotropic genomes appear to arise infrequently within an individual host as unique rearrangements of a basic archetypal sequence.

684.2

PSEUDORABIES VIRUS INFECTS AND REPLICATES IN CELLS OF NEURONAL ORIGIN . R.J. Wyborski‡*, G. Subramanian#, & A.O. Fuller#,‡Biotechnology Department, Parke-Davis Pharmaceuticals & #Department of Microbiology, University of Michigan Medical School , Ann Arbor, MI 48105.

Pseudorabies virus (PRV) naturally infects swine and cattle and is closely related to herpes simplex virus (HSV). In studies to understand the mechanism of entry and determinants of tropism for these herpesviruses, we have determined that PRV replicates poorly in several common cultured human cell-lines that efficiently replicate HSV (Subramanian & Fuller, submitted). We were interested in determining if human neuronal cells are also capable of being infected with PRV, but are not competent for viral replication. As one model cell for this study we utilized the human neuroblastoma cell line SY5Y. When cells were infected with PRV at low multiplicity, harvested and titered after 48 hrs, titers from SY5Y cells were comparable to the high titers normally seen from Vero cells and all porcine cells tested. Of other human cell lines tested, HEL, HEp-2, and 549 produced very poor virus yields while E1A transformed human 293 cells produced yields comparable to Vero, porcine, and SY5Y cells. These results suggest that PRV is capable of infecting a wide variety of human cells *in vitro* but is not capable of productive infections in all of these cells. Further examination will focus on the susceptibility of neuronal cells, both primary and transformed, for productive PRV infection and if PRV can be developed as a possible vector for gene delivery to neurons.

684.3

IMMUNE-MEDIATED MECHANISMS OF RESISTANCE TO DEMYELINATING DISEASE IN BALB/c SUBSTRAINS. S.M.Nicholson‡,J.L.Hoeft,C.E. Elliott,W.J.Karpus,S.D.Miller,M.C. Dal Canto, and R.W. Melvold. Department of Microbiology-Immunology, Northwestern University Medical School,Chicago,IL,60611.

Theiler's Murine Encephalomyelitis Virus (TMEV), upon access to the central nervous system (CNS), induces a demyelinating disease, similar clinically and histopathologically to multiple sclerosis, in susceptible strains of mice. TMEV-induced demyelinating disease (TMEV-IDD) is believed to result from bystander damage mediated by TMEV-specific CD4+ T lymphocytes homing to the CNS. These T cells recruit activated macrophages to the CNS which, while attempting to clear the virus, damage myelin. We have previously reported that BALB/c substrains are differentially susceptible to TMEV-IDD. We have further shown that an active mechanism may mediate resistance to TMEV-IDD in resistant BALB/cByJ, as low dose γ irradiation administered 2 days prior to intracerebral inoculation of virus converts this substrain to 90-100% susceptible. Resistance can be restored by the transfer of spleen cells from unirradiated, TMEV-infected BALB/cByJ. The same population of cells also protects susceptible BALB/cAnNCr from TMEV-IDD.

We report here that protection to TMEV-IDD appears to be mediated by T cells from unirradiated, TMEV-infected BALB/cByJ. Experiments are underway to determine the precise T cell subset responsible.

684.4

CYTOKINE RECEPTOR GENE EXPRESSION IN LYMPHOCYTIC CHORIOMENINGITIS (LCM) A. Stalder and I. L. Campbell*, Department of Neuropharmacology, The Scripps Research Institute, La Jolla, CA 92037

Intracerebral (ic) inoculation of immunocompetent adult mice with Lymphocytic Choriomeningitis virus (LCMV) results in a vigorous immune response in the brain culminating in death of the host around day 6 - LCM represents a prototypic model for viral meningitis. In recent studies we have shown that LCM is accompanied by the coordinate expression of multiple cytokine genes. To further define the role of cytokines in the molecular pathology of LCM, in the present study we analyzed cytokine receptor gene expression in mice following ic infection with LCMV.

To analyze cytokine receptor gene expression a RNAse protection assay (RPA) was developed which permits the simultaneous detection of IL-1 p60 and p80, TNF p60 and p80, IL-6 and IFNγ receptor mRNAs. PolyA$^+$RNA was isolated from brain and kidney at day 3 and day 6 post infection and analyzed in the RPA. In non-infected mice levels of TNF p60, TNF p80, and IFNγ receptor mRNA were higher in kidney than in brain. IL-1 p60 and IL-6 receptor transcripts were expressed at comparable levels. The levels of IL-1 Rp60, TNF Rp80 mRNA were upregulated both in kidney and brain by day 6 of infection. IL-1 p80 receptor mRNA was only detectable at day 6 of infection in brain and kidney. Parallel studies in nude mice, which do not develop LCM revealed a generally lower expression for all the cytokine receptor mRNAs, and no regulation was observed following LCMV infection. Particularly the IL-1 p80 receptor transcript was not detectable at any time pre or post infection.

These findings indicate significant upregulation in the expression of specific cytokine receptor genes in the development of LCM. The absence of such a response in nude mice suggests the upregulation seen in LCM is linked to the immuno- inflammatory response. The anatomic distribution of cytokine receptor mRNA expression in LCM is not defined and is the subject of ongoing studies.

684.5

PRODUCTION OF GRANULOCYTE-MACROPHAGE COLONY STIMULATING FACTOR BY HUMAN T-LYMPHOTROPIC VIRUS TYPE I-INFECTED HUMAN GLIOMA CELLS. Y. Nishiura, T. Nakamura, S. Shirabe*, K. Ichinose, M. Tsujihata and S. Nagataki
First Department of Internal Medicine, Nagasaki University School of Medicine, Nagasaki, Japan 852.

We investigated the production of granulocyte-macrophage colony stimulating factor (GM-CSF) by human T-lymphotropic virus type-I (HTLV-I)-infected human glioma cells (KG-1-C and T98G). When glioma cells were co-cultured with HTLV-I producing T-cell lines (HCT-1 and MT-2) after which treated with mitomycin C, GM-CSF were detected in the culture supernatant by ELISA. In co-cultures using Millicell (Millipore Products Div.) and in the presence of anti-HTLV-I antibody, there was suppression of GM-CSF production. Double-label immunostaining showed that about 10% of the glioma cells co-cultured with HTLV-I producing T-cells were stained by monoclonal antibody to HTLV-I gag protein (GIN 14) and the cells stained by antibody to GM-CSF always were stained by GIN-14, indicating that HTLV-I infection of glioma cells caused GM-CSF production. These data showed that human glial cells infected with HTLV-I gain the ability to produce cytokines, suggesting infection of HTLV-I to astrocytes in vivo may play a part of role in pathogenesis of HTLV-I associated myelopathy.

684.6

NEUROBIOLOGY OF VACCINE-INDUCED PROTECTION FROM HERPES SIMPLEX VIRUS TYPE 1 (HSV-1)IN MICE. JR Martin*[1], WJ Mitchell[1], DB Henken[1], P Gressens[1], E Kern[2], [1]Lab Exptl Neuropath, NIH, Bethesda MD; [2]U Alabama, Birmingham AL.

Neurotropic HSV-1, which spreads cell-to-cell to and within the nervous system, is a target of vaccine development. As a measure of vaccine efficacy, prevention of virus spread was examined in tissue sections. Mice were immunized with vaccinia virus recombinants expressing HSV-1 glycoprotein D (gD) or control β-galactosidase genes (Science 228:737), and 1 month later were intranasally challenged with a lethal HSV-1 dose. HSV-1gD immune but not control mice survived, had high HSV antibody titers, and much reduced virus titers in olfactory bulb, cerebrum, brainstem, cord, and trigeminal and superior cervical ganglia. HSV antigen tests on decalcified tissue sections indicated 4 neural pathways for virus spread from the nasal sinus:(i) olfactory: ethmoid epithelium to olfactory bulb to limbic structures (ii) sensory: trigeminal ganglion to brainstem (iii) sympathetic: superior cervical ganglion to thoracic cord (iv) parasympathetic: to pterygopalatine ganglion. In controls, peripheral and neural tissues were extensively infected but in HSV immunes, infection was minimal and neural spread appeared restricted to trigeminal ganglia and brainstem. By this approach, HSV spread in otherwise relatively inaccessible tissues and vaccine efficacy could be evaluated.

684.7

ELECTROPHYSIOLOGICAL STUDIES OF FELINE IMMUNODEFICIENCY VIRUS (FIV)-INFECTED CATS. O. Prospéro-García*, N. Herold, T. Phillips, J. Elder and S. J. Henriksen. Dept. Neuropharmacology, The Scripps Research Inst. La Jolla, CA 92037.

We have evaluated visual and brainstem auditory evoked potentials (VEP and BSAEP) and sleep recordings of FIV-infected cats to characterize comparative neurological deficits observed in HIV-1-infected humans. Two groups of specific pathogen free cats were systemically infected with 1000 $TCID_{50}$ units of the Mount Airy strain. The first group (2 controls and 4 infected cats) was studied 13 months post-inoculation of the virus whereas the second group (4 controls and 9 infected cats) was evaluated six months post-inoculation. All infected cats were virus isolation positive by eight weeks post-infection. FIV cats developed a persistent reduction in CD4/CD8 antigen ratio, lymphadenopathy, anisocoria and abnormalities in pupilary reflexes. In the FIV group, BSAEP revealed a delay in the latency of the waves P1, P2 and P3. Likewise VEP's exhibited prolonged latencies, and a reduction in the amplitude. The sleep-wake cycle as assesed by 3 consecutive 8 hour recordings also exhibited deficits, showing a prolonged latency to sleep onset and a significant reduction in REM sleep in the FIV group. In addition, preliminary data has shown that a surface fragment of the FIV envelope protein, selectively reduces REM sleep in rats when injected intraventricularly. As these electrophysiological findings resemble those observed in HIV-1 infected patients, the FIV model shows potential as a small animal model of lentivirus effects on CNS.

Supported by MH47680 granted to SJH

684.8

REACTIVE ASTROCYTES IN CONTIGUITY WITH HIV INFECTED HUMAN LYMPHOCYTES IN THE RECONSTITUTED SCID MOUSE. F. J. Denaro.* Dept. of Neurology, Texas Tech University Health Sciences Center, Lubbock, TX 79430.

Careful histochemical studies in humans have demonstrated that the HIV infected cell types in the brain are restricted to monocytes, macrophages, microglia and CD4+ cells (T cells). Neuronal infection with HIV and its effect have not been identified. Consequently, alternate hypotheses of HIV induced pathology have had to be proposed. One hypothesis is that the infected lymphocytes release toxic substances: cytokines, viral proteins or as yet unidentified agents. It is these hypothesized substances that induce the pathology which is now attributed to HIV infection. In the present study, HIV infected human CD4+ cells or monocytes/macrophages have been used to reconstitute the SCID mouse. At varying time points post-reconstitution, the brains were examined for evidence of neuropathology. Double label studies have demonstrated HIV infected monocyte/macrophages to be in contiguity with reactive astrocytes. Uninfected cells in control animals have not been found to induce the responses. Further studies will map the distribution patterns of HIV infected human cells and the topography of the reactive gliosis. Such a correlational study will reveal the magnitude of the observed trend and thus provide evidence of HIV induced pathology in a model system. Examination for other neuropathologic changes is underway.

684.9

NO EFFECT OF HIV gp120 ON CHOLINERGIC CURRENTS IN CULTURED HUMAN NEURAL CELLS. Edward. K. Stauffer* and Richard. J. Ziegler. Depts. of Med. & Molec. Physiol. and Med. Microbiol. & Immunol., Univ. Minn.-Duluth, Duluth, MN 55812

HIV infection often produces neurological disorders which occur despite the lack of viral presence in neurons. This situation has lead to the hypothesis that viral products from other infected cells might be disrupting neural function. Indeed, a recent report (*FEBS Lett.* **311**:115) shows that gp120 can block the binding of α-bungarotoxin (Bgt) in TE671 cells indicating that gp120 might be attaching to the acetylcholine receptor (AChR). We have investigated whether gp120 affects ionic currents associated with activation of AChRs. Whole-cell patch clamp experiments were performed on human SH-SY5Y (neuronal AChRs) and TE671 (muscle AChRs) cells, both of which produce pronounced inward currents when nicotine (100 μM) is superfused over the cells with micropressure ejection. No membrane currents were observed when gp120 (0.1 nM) was applied to the surface of either cell line. When gp120 (0.1 nM) was present in the bath, there was no inhibition of nicotine-evoked inward currents (NEICs) in either cell line. In the presence or absence of gp120, Bgt completely abolished all NEICs. If gp120 is, in fact, attaching to the Bgt-binding site of the AChR, our results indicate that it has no effect on the ionic current commonly associated with these ligand-gated channels. (Supported by NIH NS29320, Minn. Med. Found. and Duluth Clinic)

684.10

HIV gp120 EFFECTS ON TYROSINE PROTEIN PHOSPHORYLATION AND $[Ca^{+2}]_{int}$ IN HUMAN NEURAL CELLS. R.J. Ziegler*, J. DiSalvo, G. Trachte, L. Semenchuk, and M. Sneve. Depts. of Medical Microbiology & Immunology and Medical and Molecular Physiology, Univ. of Minnesota Sch. of Med., Duluth, MN 55812

AIDS dementia complex, a clinical syndrome consisting of specific neurological signs and symptoms, is the result of human immunodeficiency virus (HIV) invasion of the central nervous system (CNS). The causative mechanisms underlying the dementia remain unknown. Virological and pathological data, however, suggest that the observed neurological impairment is not the result of HIV infection of the functional elements of the CNS: neurons, oligodendrocytes and astrocytes. Several mechanisms have been proposed. They include possible toxic, inhibitory, or stimulatory effects of HIV gene products, or activated immune cell or HIV-infected microglial cell products on these functional cells. We have studied possible effects of recombinant strain IIIB and SF-2 gp120 on several processes including electrogenic mechanisms and second messenger systems in human neuroblastoma (SK-N-MC and SK-N-SY5Y) and astrocytoma (U-373 MG) cells which are known to bind this HIV envelope glycoprotein. We have observed no gp120-induced effect on electrogenic mechanisms in the SK-N-MC and SY-5Y cells and no induction of the second messenger cAMP, IP_3 and NO in any cells. However, 10^{-8} M SF-2 gp120 (Chiron Corp.) induced a ~7-fold increase in $[Ca^{+2}]_{int}$ in the U-373 MG cells, but had no effect on the neuroblastoma cell lines. IIIB gp120 (AGMED, Inc.) had no effect. 1.0 μg/ml of IIIB gp120 did induce a significant increase in tyrosine protein phosphorylation in several SK-N-MC cellular substrates. This increase was maximum one minute following gp120 exposure. Possible similar increases in U-373 MG cells have not been studied yet. These two gp120-induced effects document two mechanisms by which gp120 may cause functional impairment of human neural cells. (Supported by NIH NS29320.)

684.11

GP-120 ADMINISTERED INTRATHECALLY INDUCES MONOPARESIS IN RATS. J.L. Steinman*, B.R. Komisaruk, L. Gomez, R. Cueva-Rolon, C. Pert and M. Ruff. Rutgers-The State Univ. of New Jersey, Newark, NJ 07102, USA.

Neurological deficits have been observed in neonatal rats following systemic administration of gp-120 (Hill et al, 1992, *Brain Res.*), an envelope protein of HIV. Furthermore, direct injection of gp-120 to the cerebral ventricles results in memory impairments in adult rats (Glowa et al, 1992, *Brain Res.*). The present study found that direct administration of gp-120 to the spinal cord via intrathecal (IT) catheters induced forelimb monoparesis in adult female rats.

IT catheters (PE-10 tubing, 7 cm length) were implanted under anesthesia 7-10 days prior to testing. Motor performance (hindlimb and forelimb use, walking patterns) was videotaped before and up to 12 hrs after rats received either gp-120 (1 ng/5·µl saline) or vehicle alone. The experimenter was blind to treatment.

Seven of 10 rats receiving IT gp-120 showed monoparesis of either the left or right forelimb; none of the saline control rats exhibited paresis ($Chi^2 = 6.39$, df=1, $p < 0.02$ comparing the two treatment groups). The onset of monoparesis was variable (mean ± sem= 22.2 ± 7.4 min), appearing as early as 4 min post IT injection. The mean recovery time was 6 hrs. In 3 of the 7 rats, the monoparesis appeared intermittently during the first two hours, then persisted for the next 3-4 hrs; in the other 4 rats, monoparesis was continuously present from its onset. In 5 of the 7 rats, forelimb monoparesis was accompanied by hindlimb rigidity during walking or when pressure was placed manually against the footpad.

At autopsy, it was found that the monoparesis was ipsilateral to the side of the spinal cord where the tip of the IT catheter was positioned. This points to the specificity of the effect of gp-120 in acting locally, at or near the level of the motoneurons, to produce forelimb paresis. These findings demonstrate that an envelope protein of HIV induces discrete motor deficits when administered directly to the spinal cord, providing a unique model for studying gp120-induced neuronal deficits. Findings using rotorod performance to assess motor deficits will be presented.

684.12

CIRCULATING gp120-INDUCED NEUROTOXICITY. W. Carragher III,M. Ruff, Candace Pert* and Ian Creese. Center for Molecular and Behavioral Neuroscience, Rutgers-Newark, NJ 07102.

The paradox of neuroAIDS is that frank neuronal damage occurs in patients who show neurocognitive and motor symptoms although neurons themselves do not appear to be directly infected with HIV. A current hypothesis is that infected CNS macrophages and microglia are responsible for the concurrent neurotoxicity. One potential mechanism is that free viral envelope protein, gp120, is a neurotoxic agent. Indeed, neuronal culture systems have demonstrated that addition of gp120 can lead to neuronal death, if glial cells are present, suggesting an indirect glial-mediated neurotoxicity. However, cell culture tells us what could happen *in vivo* - but does not tell what does happen. We have found that 1) in the neonatal rat, systemic administration of exogenous gp120 (5ng/day for 1 week) is neurotoxic, 2) that its neurotoxicity can be reduced by co-administration of the novel drug Peptide T, and 3) that administration of serum (equivalent dose of 1-10µl/day for 1 week) from HIV+ve patients with neurocognitive deficits shows a similar Peptide T-reversible neurotoxicity. Neurotoxicity was studied with a silver stain, modified from Gallyas, which detects the acute morphological consequences of neuronal damage over a window of about 5 days. Most "dark" neurons were concentrated in cortical areas, especially entorhinal and perirhinal, layer II and the hippocampus, areas previously reported to be rich in CD_4 receptors as visualized with monoclonal antibodies (Pert, C.B. et al, *P.N.A.S. USA* 83:9254-58, 1986). Only scattered "dark" neurons were found in subcortical areas. This indicates that frank neuronal killing occurs in the neonatal animal and not simply reduced dendritic arborization as has been reported previously. These studies suggest a potential critical role for a maternal circulating neurotoxic agent (gp120) that can cause neonatal CNS damage, with or without frank HIV infection of the infant, and that Peptide T may be a beneficial treatment.

684.13

EFFECTS OF CYTOKINES ON *TOXOPLASMA GONDII* MULTIPLICATION IN HUMAN MICROGLIA. S. Hu*, G. Gekker, P. K. Peterson and C. C. Chao. Minneapolis Medical Research Foundation and the University of Minnesota, Minneapolis, MN 55404

Microglia may play an important role in host defense of the central nervous system against *Toxoplasma gondii*. Cytokines released from these glial cells may participate in their host defense activity against *T. gondii*. In the present study, the anti-toxoplasma activity of human fetal microglia was investigated. The RH strain of *T. gondii* multiplied readily after 20 h incubation in these cells (155±6 tachyzoites/100 cells). Activation of microglia with interferon (IFN)-γ plus lipopolysaccharide suppressed ($P<0.01$) *T. gondii* growth (68±7 tachyzoites/100 cells). Greater than 90% of the anti-toxoplasma defense of activated microglia was blocked ($P<0.01$) by neutralizing antibodies to tumor necrosis factor (TNF)-α and interleukin (IL)-6, but not to IL-1, suggesting that these cytokines play a role in the inhibitory process. Consistent with this hypothesis, treatment of microglia with TNF-α or IL-6 (in the presence or absence of IFN-γ) inhibited ($P<0.01$), in a dose dependent manner, *T. gondii* multiplication. Since N^G-monomethyl-L-arginine (500 µM) did not affect cytokine-mediated anti-toxoplasma activity of microglia, the finding suggests that nitric oxide is not involved in this inhibitory process. The present study suggests that the host defense function of human microglia against *T. gondii* is dependent primarily upon the activating properties of IFN-γ, TNF-α, and IL-6.

684.14

AN INTRAOCULAR MODEL MODEL OF HUMAN CYTOMEGALOVIRUS (CMV) RETINITIS. D.A. DiLoreto, Jr.*, L.G. Epstein, E. Lazar, W. Britt and M. del Cerro. University of Rochester School of Medicine, Rochester, New York, 14642.

CMV infection of the human retina is a devastating complication in immunocompromised patients, especially those with HIV-1 infection. AIDS related CMV retinitis leads to blindness in 20 to 25% of these patients prior to death. *In vitro* viability of CMV is largely limited to human fibroblast cultures. There is an acute need to develop a model basesd on the infection of retinal cells in order to better understand and treat the disease. Here we report on a model based on grafting human fetal retina into the anterior chamber of immunedeficient mice.

We transplanted 2nd trimester human fetal retina into the anterior chambers (A.C.) of 15 SCID mice. One week later 15 of 30 grafts were infected in the A.C. with CMV strain AD169. Infected and non-infected specimens were compared for histopathological changes using H&E staining. Polyclonal antibodies to GFAP and PGP 9.5 were used to characterize the effects of virus on glial and nervous elements of the retina. Virus was characterized using monoclonal antibodies to various replicative phases of CMV.

At 30 days, healthy control grafts were seen within the A.C. consisting mostly of photoreceptor cells in the characteristic rosette pattern that is seen in A.C. retinal transplants. Infected grafts also showed a rosette pattern, but of noted difference was the fact that they also included larger cells, both giant and multinucleated, that had intranuclear and intracytoplasmic inclusions pathognomonic of CMV infection.

We have now achieved a model of CMV infectivity of human retinal tissue in a small animal model. This will allow the study of the replicative properties of CMV and the specific effects of CMV on the human retina. In addition to this, we can now assay multiple types of drug therapy to combat CMV infection of the human retina. This *in vivo* model will allow a greater understanding CMV retinitis and permit the study of various anti-CMV agents. Supported by NIH 05262, The Markey Foundation, Strong Children's Research Center, and private gifts.

684.15

PURIFICATION AND BIOLOGICAL ACTIVITIES OF AN INTERFERON-γ IMMUNOREACTIVE MOLECULE IN SENSORY GANGLIA. T. Olsson, M. Bakhiet, C. Edlund, B. Höjeberg, S. Kelic and K. Kristensson*. Department of Neurology, Huddinge Hospital and Department of Neuroscience, Karolinska Institutet, Stockholm, Sweden.

Two monoclonal antibodies (mabs DB-1 and DB-16) directed against non-competetive epitopes of rat lymphocyte derived interferon-γ (IFN-γ) cross-react immunohistochemically with an antigen in small neurons in trigeminal and dorsal root ganglia of the rat. A homogenate was prepared from rat trigeminal ganglia and centrifuged. The supernatant was applied to two consecutive affinity columns, one immobilized with mab DB-1, the other with mab DB-16. The eluate was concentrated and then diluted in phosphate buffered saline, pH 7.2.

This doubly affinity purified preparation shared biological activities with lymphocyte derived IFN-γ: a/ it induced major histocompatibility complex antigens class I and II in macrophage cultures, b/ it stimulated the proliferation of Trypanosoma brucei brucei in vitro, and c/ it enhanced the number of myotubes in cultures of rat skeletal muscle. In the latter cultures, both lymphocyte derived IFN-γ and the purified molecule caused an increased myoblast proliferation and a decrease in number of dorsal AChR aggregates in myotubes. SDS-electrophoresis of the affinity purifed preparation displayed three bands after silverstaining between 54 and 62 kD in size. No bands corresponding to the size of IFN-γ molecules, 14-25 kD, could be detected.

684.16

QUANTITATIVE ANALYSIS OF HERPES SIMPLEX VIRUS (HSV) PERSISTENCE AND RNA EXPRESSION IN LATENTLY INFECTED CELLS OF RAT BRAIN. D.J. Fink*, G. Jiang, M. Levine, and R. Ramakrishnan. Dept. of Neurology, GRECC VAMC, and Dept. of Human Genetics, University of Michigan, Ann Arbor, MI 48104.

Previous studies have demonstrated that when attenuated herpes simplex viruses are injected into focal brain regions, viral DNA persists for at least 1 year (Fink *et al.*, 1992). In order to study this phenomenon quantitatively, we have determined the amount of viral DNA, represented by the gB gene, and latency associated transcript (LAT) RNA by competitive quantitative PCR and rt-PCR respectively, at 2d, 7d, and 8wk after injecting 5 µl containing 1 x 10^6 pfu of a ribonucleotide reductase deletion mutant HSV into rat hippocampus.

DNA and RNA were extracted from tissue scraped from the hippocampus of 10 µm frozen sections of brain, and serial dilutions of known amounts of a standard template, identical in sequence to the target except for an altered internal restriction site, were co-amplified with the extract, using the same set of primers.

The number of viral genomes in the hippocampus decreased approximately 4-fold from 2d to 7d post-injection, but then remained constant out to 8wk. No evidence for viral replication was seen. The amount of LAT RNA in the same region remained constant from 7d to 8wk post-injection, and represents approximately 16 LAT RNA molecules/HSV-1 genome equivalent.

We conclude that latency in the brain has many similarities to that in peripheral ganglia, and that once established, the amounts of viral DNA and RNA remain constant in the brain over at least several months.

684.17

UNALTERED PROTEIN PATTERNS OF CULTURED POSTMITOTIC SYMPATHETIC NEURONS AFTER INFECTION WITH WILD-TYPE HSV-1: FURTHER EVIDENCE THAT NEURONS DO NOT UNDERGO VIRION-INDUCED HOST SHUTOFF. P.F. Nichol*, P.D. Olivo, and E.M. Johnson, Jr. Washington University School of Medicine, St. Louis, MO 63110.

Previously, we reported that the rate of methionine incorporation does not decrease in sympathetic neurons after infection with HSV-1. This suggests that neurons are resistant to the HSV phenomenon of virion-induced host shutoff or VHS. The VHS phenomenon, as mediated by the viral gene UL 41, occurs in a wide variety of dividing cells after infection with HSV-1. Genetic studies indicate that the UL 41 gene product induces the disaggregation of polyribosomes and promotes degradation of transcripts. These effects result in a decrease in the rate of protein synthesis and methionine incorporation of the infected cell. We have observed that no such decrease is detectable in neurons in the first 30 hours after infection with wt HSV-1. The effects of the UL 41 gene product is also manifested by a change in the pattern of ^{35}S-methionine-labeled protein seen on denaturing gels. This pattern is characterized by the disappearance of cellular proteins and the appearance of viral proteins. We report here that such an alteration did not occur in the protein pattern of ^{35}S-methionine-labeled sympathetic neurons after infection. Viral proteins were detectable by 36 hours after infection on SDS/acrylamide gels, but there was no alteration in the pattern of cellular proteins. We are presently in the process of confirming viral infection of these cells by immunoprecipitation. We take this as further evidence that peripheral neurons are resistant to the VHS phenomenon as mediated by the UL 41 gene product and that the time course of expression of viral proteins is much slower in peripheral neurons than in mitotic cells. These two phenomena may be important in the establishment of viral latency in these neurons. (Supported by the American Paralysis Foundation and the Monsanto Corporation.)

684.18

MECHANISM OF IMMUNOSUPPRESSION IN THE BRAIN BY A LETHAL STRAIN OF HSV-1. G. Lewandowski*, K.Kogel and F.E. Bloom, Dept. of Neuropharmacology, The Scripps Research Institute, La Jolla, CA 92037.

Recently we reported on a lethal strain of Herpes simplex virus type-1 (HSV-1) that produces a widespread infection in the CNS of mice following intraocular inoculation. The immune response to HSV-1 infection in the brain is characterized by recruitment of CD4+ and CD8+ cells to the viral infection centers, and an unusual *intracellular* expression of MHC class II antigens (*Lewandowski, et al PNAS 90:2005 1993*). With two color fluorescence confocal microscopy we determined that MHC class II antigens were expressed primarily in the nucleus in cells that were also positive for HSV-1 antigens and at the cell surface of cells that were negative for viral antigens. The nuclear localization of MHC class II antigen expression was confirmed by co-localization with the nuclear stain, DAPI. Furthermore, the induction of synthesis and nuclear sequestering of MHC class II antigens was reproduced *in vitro*, following the infection of trigeminal nerve explants with the HSV-1 mutants. We postulate that HSV-1 is causing suppression of the normal immune response in the brain by inhibiting viral antigen presentation to the recruited CD4+ cells. We are currently investigating the pattern of cytokine induction in the CNS following HSV-1 infection in order to determine whether recruited CD4+ cells become activated.

Immunoblot analysis of extracts from trigeminal ganglion nerves and the superior colliculus of mice infected with either the non-lethal control strain of HSV-1 or the lethal HSV-1 strain indicated that the HSV-1 mutant does not interfere with the induction of synthesis or post-translational processing of the MHC class II protein. Further investigation of the nature of the HSV-1-MHC class II complex is underway.

(Supported by NIH training grant T32MH19185-02)

684.19

SUBCELLULAR COMPARTMENTALIZATION OF MHC CLASS II AND SIALOADHESIN PROTEINS IN BRAINS INFECTED WITH HSV-1. M. Morales*, G. A. Lewandowski and F. E. Bloom. The Scripps Research Institute, Neuropharmacology. 10666 N. Torrey Pines Rd., La Jolla CA 92037.

The immune response to herpes simplex virus type 1 (HSV-1) infection in the brain has been studied in detail: intraocular inoculation of mice with wild type HSV-1 resulted in a latent infection with cell surface expression of MHC class II proteins. In contrast, inoculation with a mutant strain resulted in a lethal infection and intracellular accumulation of MHC class II proteins (Lewandowski et al., PNAS '93). We have now extended our observations on this model to the ultrastructural level. Six days after viral inoculation, mouse brains were fixed by perfusion and sections of the superior colliculus (SC) were incubated with polyclonal antibodies against viral antigens and examined by fluorescence microscopy. Sections containing viral antigens were processed for the ultrastructural immunodetection of MHC class II and sialoadhesin proteins. Viral particles are abundant in SC cells of brains infected with the mutant strain but are absent in brains infected with the wild type. The SC of infected material (both groups) contained microglia and macrophages with sialoadhesin protein in their plasma-membrane (PM). In wild type infected animals, MHC class II was located on the PM of macrophages and microglia. In contrast, the microglia of animals infected with the mutant strain contain both virus and MHC class II-immunoreactivity in their nuclei, macrophages here lack virus in their nuclei but contain MHC class II on their PM. Through serial sections, we observed an apparent association between the virus and the MHC class II marker. The expression of sialoadhesin protein on the PM of infected microglia indicates that MHC class II in the nuclei is not due to non-specific accumulation of proteins in the nuclei of moribund cells but rather represents a selective transport of MHC class II to the nuclei. Our results support the hypothesis that evasion of immune response by viral interference with antigen presentation results in lethal infection. (Antibodies against sialoadhesin were provided by Dr. S. Gordon, University of Oxford, Supported by AIDS center MH 47680 and AA 06420).

684.20

ALTERED VIRAL mRNA EDITING IN MEASLES VIRUS INFECTIONS OF THE CENTRAL NERVOUS SYSTEM. J.A. Vanchiere*, J.G. Wood, W.J. Bellini. Respiratory and Enterovirus Branch, Centers for Disease Control and Neuroscience Program, Emory University, Atlanta, GA 30333.

As a model for the study of measles virus infection of the nervous system, the Hamster Neurotropic Strain (HNT-1) of measles virus was derived by serial passage of the Philadelphia 26 (Ph26) wild type isolate in suckling hamster brain and then passaged in Vero cells to produce a persistent infection (HNT-2). In order to assess the distribution of phosphoprotein (P) mRNA editing in these viruses, a fragment of PCR-amplified DNA reverse-transcribed from poly-A(+) RNA was cloned and sequenced for 208, 121, and 116 clones of Ph26, HNT-1, and HNT-2, respectively. As these clones were derived from poly-A(+) RNA, the statistics of editing *in vivo* are reflected in the distribution of edited clones. The distribution is nearly identical for HNT-1 and HNT-2 ($p=0.63$), and both are different from that of Ph26 ($p<0.001$). Compared to Ph26, HNT-1 and HNT-2 editing is shifted toward a higher proportion of unedited transcripts (66.9% and 72.4% vs. 61.5%). Insertions of more than one G are 2.4% of the clones from Ph26 and represent 11.6% and 10.3% of the clones from HNT-1 and HNT-2. These editing distributions are unlike those previously reported for measles virus derived by other methods. The shift in editing may play a role in persistence, as decreased expression of the V and W proteins may favor transcription over replication.

684.21

DANTROLENE PREVENTS MUMPS VIRUS-INDUCED NEURODEGENERATION *IN VITRO*.
T. Andersson* and K. Kristensson. Department of Neuroscience, Karolinska Institutet, S-104 01 Stockholm, Sweden.

Persistent mumps virus infection of dorsal root ganglia neurons cause a transient, early reduction in calcium influx during the action potential. The virus-induced neurodegeneration in these cells was markedly enhanced by increasing the calcium concentration of the medium and could be almost totally inhibited by the dihydropyridine calcium channel antagonist Nifedipine. Dispersed primary cultures of embryonic rat hippocampal neurons, prepared as described by Rothman (J. Neuroscience, 4, 1884-91, 1984), were grown in Petri dishes and infected with the hamster adapted RW-strain of mumps virus. The virus replication was restricted with expression of nucleocapsid, but not envelope proteins as determined by immunohistochemistry. The neurons in well defined areas of the dishes were counted daily in a phase-contrast microscope. During the first 4 days of infection, 30-40% of the neurons died, but later there was no further cell loss. The effects on the degeneration of antagonists of voltage-dependent and receptor-operated calcium channels were studied, as was the effect of Dantrolene, which inhibits mobilization of intracellular calcium stores. It was found that 30 µM Dantrolene in the medium significantly reduced the neurodegeneration caused by the virus. Our observation indicates that a virus infection of neurons may lead to a disturbed calcium homeostasis.

685.1

HEAT-SHOCK PROTEINS AND NEUROTROPHINS IN THE HIPPOCAMPAL FORMATION FOLLOWING METHYLMERCURY, AS REVEALED BY IN SITU HYBRIDIZATION. H. Lindström, E. Lindqvist, J. Luthman* and L. Olson. Dept. of Histology & Neurobiology, Karolinska Institute, Stockholm, Sweden.

Methylmercury (8 mg/kg i.p.) was injected in adult Sprague-Dawley male rats. After 4 h animals were decapitated, and brains were dissected out and frozen on dry ice. Coronal sections at the dorsal hippocampal level were hybridized in situ with oligonucleotide probes for heat-shock protein 70 (HSP70), heat-shock cognate protein (HSC70) or nerve growth factor (NGF) family members. Heat-shock proteins may function to protect cells from the adverse consequences of heat and other stresses. HSC70 mRNA was observed in most nerve cell populations of control brains. In contrast, only little HSP70 mRNA was found in control hippocampus. No significant effect on the induction of HSP70 mRNA was seen after the methylmercury administration, although a tendency to reduced HSC70 mRNA was observed in the dentate gyrus (DG). Growth factors are known to exert striking effects on neuronal survival and neurite outgrowth. Specific sets of neurons in the brain have been demonstrated to synthesize proteins belonging to the NGF family, namely NGF, BDNF (brain-derived neurotrophic factor) and NT-3 (neurotrophin 3). BDNF mRNA was highly expressed in pyramidal neurons in hippocampus as well as in neurons of the DG. NGF mRNA was primarily expressed in neurons scattered throughout the pyramidal cell layer and stratum oriens as well as in the hilar region of the DG. NT-3 mRNA was abundantly expressed in the DG and the CA2 pyramidal cell area. No significant alterations in NGF and NT-3 mRNA hybridization patterns were observed, although a decrease in BDNF mRNA was seen in the DG. The local reduction in BDNF mRNA 4 h after methylmercury administration indicates that the DG may be more vulnerable than other hippocampal regions to an acute injection of methylmercury. Cerebellum is particularly vulnerable to exposure to methylmercury. Further investigations are under way to determine the effects of methylmercury in cerebellum at 1, 4 and 24 hours after different dosages.

685.2

VOLTAGE GATED CALCIUM CHANNEL CURRENTS IN RAT DRG AND *APLYSIA* NEURONS ARE REDUCED BY MERCURY (Hg^{2+}); D. Büsselberg*, Pekel, M., and B. Platt; Universität Düsseldorf, Physiology II, Moorenstraße 5, 4000 Düsseldorf, FRG.

The effects of inorganic mercury (Hg^{2+}) on voltage-gated calcium channel currents were investigated in two different preparations. We used the whole-cell patch-clamp technique with cultured rat dorsal-root-ganglion (DRG) neurons and the two-electrode voltage-clamp method for measuring calcium channel currents of abdominal ganglion neurons of *Aplysia california*. All voltage activated calcium channel currents were irreversibly reduced by Hg^{2+} in both preparations but with different time courses.

In rat neurons there was a rapid and concentration-dependent decrease of the L/N-type current to a steady state with an IC_{50} of 1.1 µM and a Hill coefficient of 1.3. T-type currents were blocked by Hg^{2+} in the same concentration range (0.5-2 µM). Effects with effective concentrations this low of Hg^{2+} on voltage activated calcium channels have not been reported before. The IV relation of the calcium currents shifted to more positive values, suggesting a binding of Hg^{2+} to the channel protein and/or modifycation of gating properties. With increasing Hg^{2+} concentrations a slow membrane current was additionally activated which was most obvious at concentrations over 2 µM Hg^{2+}. This current was not reversible and might be due to the opening of other (unspecific) ion channels by Hg^{2+}.

In *Aplysia* neurons a continuos decrease of the calcium channel currents was observed during application even with low concentrations of Hg^{2+} (5 µM). A steady state was never reached. There was no obvious influence on resting membrane currents even with high concentrations (up to 50 µM) and no shift of the current-voltage (IV) relation.

We conclude that neurotoxic effects of inorganic mercury could be partially due to a reduction of voltage-activated calcium currents.

685.3

EFFECTS OF DEVELOPMENTAL AND CONTINUOUS EXPOSURE TO EXCESS DIETARY ALUMINUM ON THE AUDITORY STARTLE RESPONSE OF MICE. M.S. Golub*, B. Han, C.L. Keen, and M.E. Gershwin. Dept. Internal Medicine, Univ. California Davis Sch. of Med., Davis, CA 95616.

Swiss Webster mice were exposed to a high aluminum diet (1000 µg Al/g, Al as Al lactate) from conception to weaning (20 days of age) or from conception through adulthood. Controls were fed 7 µg Al/g diet. Amplitude and habituation of an auditory startle response were assessed in separate groups of mice (6-7 males and 6-7 females per group) at 22 and 52 days of age using an automated apparatus (San Diego Instruments; 50 msec, 120 db noise; 8 sec inter-trial interval; 50 trials). Body weight was not influenced by diet. At 22 days of age, startle response amplitude was lower in Al-exposed mice than controls ($p<.02$); effects on habituation rate could not be assessed because the response had habituated by the second trial in all groups. At 52 days, response amplitude was not significantly lower in Al-exposed groups but initial habituation was more rapid than in controls. Response amplitude decreased between the first and second 5-trial block in the continuously exposed group ($p<.005$) but not in controls ($p>.10$). The developmentally exposed group had an intermediate decrease ($p<.05$). This finding supports previous reports of reduced activity and responsiveness in mice fed excess dietary aluminum. Supported by ES04190.

685.4

ALUMINUM-BLOCKADE OF VOLTAGE-ACTIVATED CALCIUM CHANNELS: MODULATION BY EXTRACELLULAR PH B.Platt, M.Thewissen*, H.L. Haas and D. Büsselberg Univ. Düsseldorf, Physiology II, Moorenstr. 5, 4000 Düsseldorf, FRG.

Mammlian voltage-activated calcium channels are blocked by Aluminum (Al) in the micromolar range. The mechanism of this blockade is not yet entirely clear. A dependency of Al-toxicity on its chemical properties is considered. Therefore we examined the influence of different pH-values (pH 6.7, 7.3 and 7.7) of the extracellular solution on this effect. Significant changes of extracellular H^+-concentration may occur during neuronal activity and under a variety of pathological conditions. Voltage-activated calcium channels were studied in rat dorsal-root ganglion (DRG) cells using the whole-cell patch clamp technique.

For pH 7.3 threshold concentration for reduction of the peak calcium channel was about 20 µM Al, with concentrations above 200 µM a blockade over 80 % was obtained. The IC_{50} for blockade of calcium channel currents was 83 µM Al and the Hill slope was calculated to be 2.2. For pH 7.7 a decrease of blockade was found with an IC_{50} of 115 µM and an Hill slope of 2.8. Increasing the H^+-concentration to pH 6.7 induced an dramatic shift of the concentration-response relation to lower concentrations. The IC_{50} was calculated to be 21µM and the Hill slope to be 1.5. Steady state of the reduced calcium channel currents was achieved within 1 to 5 min. In general, a faster stabilization was found for increasing Al-concentrations as well as for increasing H^+-concentrations. Washout of the Al-containing solution lead to a recovery between 15 to 30%.

Modulation of the Al-effect on voltage activated calcium channels by H^+ might be related to its neurotoxicity. Beside the findings of the specificity of Al for voltage activated calcium channels, its use-dependence and the partial irreversibility of the block, the pH-modulation is another hint to the complex and wide-ranging action of Aluminum in the nervous system.

685.5

ALUMINUM TOXICITY IN DISSOCIATED RAT HIPPOCAMPAL NEURONS

S.R. Brenner* and K.-W. Yoon, Department of Neurology and Division of Neurosurgery, St. Louis University School of Medicine, St. Louis, MO 63110–0250

Aluminum toxicity was investigated in rat hippocampal cells upon exposure to concentrations from 10^{-3} to 10^{-12}M of $AlCl_3$. The toxicity was assessed 24 hours after a one hour exposure to the artificial CSF solution containing $AlCl_3$ by counting the cells that were stained by Trypan Blue. At 1mM, there was only minimal toxicity. However, the neuronal toxicity increased as the concentration of aluminum was decreased to 10^{-4} to 10^{-9}M, and gradually decreased though persisting even at 10^{-12}M. The neuronal toxicity to potassium cyanide which is blocked by MK801, an NMDA receptor antagonist (Sturm, et al., J. of Neurosurg., in press) was also blocked by the high concentration (10^{-3}M) of $AlCl_3$. At 10^{-12}M $AlCl_3$, MK801 (10µM) blocked the toxicity. It appears that aluminum is toxic to the rat hippocampal neurons. However at the high concentration of 10^{-3}M it exerts a protective effect against cyanide and itself. Since MK801 reduces cell death, NMDA receptor may be involved in aluminum toxicity at lower concentrations while at high concentrations aluminum may protect the cells from cyanide and its own toxicity by attenuating NMDA mediated cell injury.

685.6

ORAL ALUMINUM ADMINISTERED DURING PREGNANCY AND LACTATION PRODUCES GASTRIC AND RENAL LESIONS IN RAT MOTHERS AND DELAY IN CNS DEVELOPMENT OF THEIR PUPS. B.K.Poulos,[a,b]M. Perazzolo,[b]V.M.-Y Lee,[c]H.M.Wisniewski[a,b] and D. Soifer[a,b]*, [a]CSI/IBR Ctr. for Dev. Neurosci.,[b]Ctr. for Trace Metal Studies and Environmental Neurotoxicol.,Staten Island,NY 10314,[c]Univ. Penna., Phila.,PA 19104.

The heavy neurofilament protein [NF-H] is developmentally regulated. For example, a monoclonal antibody (RMO 24.9) directed against a phosphorylated epitope in the tail domain of NF-H, binds to specific tracts within the rat brain stem on postnatal day one, but does not bind to tracts within the diencephalon until postnatal day 12 [P12]. A diet providing 300mg/kg/d Al (as aluminum lactate) to rat dams throughout gestation causes behavioral deficits in their offspring (Bernuzzi *et al.* '89). We repeated this regimen by substituting 120 mM aluminum lactate (pH 6.5) for drinking water during gestation and lactation and looked at the distribution of binding of RMO 24.9 by the developing pup CNS. Tracts within the diencephalon that normally bind RMO 24.9 on P12 did not bind the monoclonal antibody until P15. Aluminum treatment of the mothers seemed to cause a developmental delay in the expression of phosphorylated NF-H in the pups. However, the neuropathological deficits we have found - and those reported by other investigators using similar aluminum levels - may not be due to the direct effects of aluminum on the pup but rather secondary to its effects on the dam. Throughout lactation, treated dams appeared progressively more cachexic. Unlike the viscera of pair-watered controls, the stomachs of treated dams were ulcerated and their kidneys contained stones. These lesions suggest that the ability of the treated dams to absorb nutrients and to excrete toxins and regulate water and electrolytes were compromised and, by extension, that their ability to provide their pups with an adequate diet might also be compromised.

685.7

DIFFERENTIAL EFFECTS OF LOW-LEVEL LEAD ON SUSTAINED AND TRANSIENT RETINAL GANGLION CELLS IN DEVELOPING RATS. D.Y. Ruan*, L.X. Tang and C. Zhao, Dept. of Biology, Univ. Sci. Tech. China, Hefei, Anhui 230026 P.R. China

Neonatal rats were exposed to Lead from parturition to weaning via the milk of dams drinking 0.2% Lead acetate solution.The alterations in excitability and temporal response properties of retinal ganglion cells from optic tract in adult rats following developmental lead exposure were studied. 45 sustained and 24 transient cells in 11 control rats and 45 sustained and 32 transient cells in 10 lead-exposed rats were recorded. Compared with controls, electrophysiological alterations of retinal ganglion cells in lead- exposed rats showed that the mean peak response increased 83% for sustained cells and 215% for transient cells, mean optimal temporal frequency decreased 27.5% for sustained cells and 38.7% for transient cells, mean bandwidth at half amplitude decreased 29.1% for sustained cells and 40.8% for transient cells, mean temporal resolution decreased 27.5% for sustained cells and 38.7% for transient cells. No significant difference in response phase was observed between control- and lead- sustained cells. However, 0.1 cycle response phase of 37.5% transient cells in lead-exposed rats was advanced. These results indicate that lead-induced alterations in excitability and temporal response properties of transient cells were greater than that of sustained cells in retinal ganglion cells. Supported by NSFC grant 39070287 (DYR).

685.8

SELECTIVE TOXICITY TO CENTRAL SEROTONERGIC NERVOUS SYSTEM IN PRENATALLY AND POSTNATALLY LEAD-EXPOSED RATS. D.O. Seo, E.Y. Jung, J.H. Cheong, C.Y. Shin, U.T. Oh and K.H. Ko.*
Department of Pharmacology, College of Pharmacy, Seoul National University, Seoul 151, Korea.

Possibility whether lead ingestion can cause selective toxicity to central serotonergic nervous system in rats was tested. Three groups of wistar rats; 1) Control, 2) Low dose and 3) High dose groups, were prepared. In prenatally lead-exposed rats, until parturition from dams, rat pups were intoxicated via placenta of mother rats having received drinking water containing either 0% (control), 0.05% (low dose) or 0.2% (high dose) of lead acetate respectively. In postnatally lead-exposed rats, right after parturition from dams rat pups received drinking water containing either 0% (control), 0.05% (low dose) or 0.2% (high dose) of lead acetate. At 2, 4, 6 and 8 weeks of age, tryptophan hydroxylase (TPH) activity and Na^+/K^+-ATPase activity were measured in 4 areas of rat brain; Telencephalon, Diencephalon, Midbrain and Pons/Medulla. TPH activities were assayed by modified method of Beevers *et al.* (1983) using L-[5-^{3}H]-tryptophan as substrate. TPH activity was determined as a criterion of lead poisoning to central serotonergic nervous system and Na^+/K^+-ATPase activity as a criterion of nonspecific lead poisoning to any kinds of tissues. Selective toxicity of lead poisoning to central serotonergic nervous system was evaluated by the changes of TPH activities without concomitant changes of Na^+/K^+-ATPase activities. In prenatally lead-exposed rats, this selectivity was found in Telencephalon (2 weeks of age), Diencephalon/Midbrain (2 weeks of age), Midbrain (4 and 6 weeks of age), Pons/Medulla (2, 4 and 6 weeks of age) in rats exposed to low dose of lead and Pons/Medulla (2 weeks of age) to high dose of lead. In postnatally lesd-exposed rats, this selectivity was found in Telencephalon (8 weeks of age), Diencephalon (8 weeks of age), Pons/Medulla (6 and 8 weeks of age) in rats exposed to low dose of lead and Pons/Medulla (8 weeks of age) to high dose of lead. These results suggest that lead poisoning may exhibit selective toxicity to central serotonergic nervous system.

685.9

INTERACTIONS OF GLUTATHIONE AND LEAD (PB) IN CULTURED ASTROGLIA. L. A. Schneider, M. E. Legare, R. Barhoumi, R. C. Burghardt, and E. Tiffany-Castiglioni*. Dept. Vet. Anatomy and Public Health. College Station, TX 77840.

We have previously shown that astroglia in culture take up and store Pb intracellularly without significant cytotoxicity, suggesting that astroglia adapt to the presence of Pb. One possible mechanism of adaptation is an upregulation in the synthesis of glutathione (GSH), a tripeptide abundant in astroglia. We have recently shown by interactive laser cytometry that the cytosolic GSH content of cultured astroglia is initially depleted after brief exposure to 0.1 or 1.0 μM Pb, after which it becomes elevated above normal levels. We also noted a decrease in mitochondrial membrane potential (MMP), which may be causally related to the transient GSH depletion. We tested the hypothesis that the a cell permeant GSH monoester would elevate intracellular GSH content as a first step in protecting astroglia from the Pb-induced decrease in MMP. The monoester failed to elevate GSH, suggesting a non-causal relationship to altered MMP.

685.10

ATTENTIONAL AND MNEMONIC EFFECTS OF LOW-LEVEL LEAD EXPOSURE. B.J. Strupp*, B. Saelens, T. Meyer, D. Levitsky, S. Alber and J. Barnabe. Dept. of Psychology and Div. of Nutritional Sciences, Cornell Univ., Ithaca, NY 14853.

Despite the increasing evidence that low-level lead (Pb) exposure is associated with small, but significant, IQ deficits in humans, relatively little is known about the specific cognitive processes that are affected. The animal studies that have identified specific areas of cognitive dysfunction have generally examined blood Pb (BPb) levels much higher than those commonly seen in humans. The present study was designed to study the effects of mild-moderate elevations in BPb level on several specific functions in the rat: sustained attention, distractibility, impulsivity, short-term memory, and long-term memory. Preliminary analyses revealed robust increases in impulsivity and distractibility in the lead-exposed animals, as well as a transient deficit in a delayed spatial alternation (DSA) task when delays were imposed. The attentional deficits were seen in rats with chronic BPb levels averaging as low as 19μg/dL, while the DSA impairment was only seen in the higher exposure group averaging 39μg/dL. This study substantiates anecdotal evidence that children with even modest elevations in BPb level often display symptoms characteristic of Attention Deficit Hyperactivity Disorder.

Supported by grants from NIEHS (T2ES05950A) and the March of Dimes Birth Defects Foundation (#12-0515).

685.11

A CHRONIC INCREASE IN CALCIUM BUT NOT SODIUM INTAKE IS OBSERVED AFTER A SINGLE SUBCUTANEOUS INJECTION OF LEAD CHLORIDE, BUT NOT BY MERCURY CHLORIDE, IN FEMALE RATS. G.F. Alheid[1], C.A. Beltramino[2], J. Savory[3]. [1]Psychiatric Medicine, [2]Otolaryngology, and [3]Pathology, Univ. of Virginia, Health Sciences Center, Charlottesville, Va. 22908

Heavy metals differ in crossing the blood-brain barrier depending upon their chemical species, but most metals do accumulate in circumventricular organs of the brain. Since these areas are known to be important in a variety of regulatory and reproductive functions, we are examining the impact of metal loading on a subset of these behaviors. Sodium appetite is known to be under the control of forebrain mechanisms proximal to circumventricular areas, while the chemical similarity of lead and calcium suggested the inclusion of the test for calcium ingestion. Injections of inorganic lead (25-200 mg/kg) or mercury (0.5-2.5mg/kg) were made in adult female rats. Intake of water, concentrated sodium or calcium solutions was measured during in the post-injection period. Sodium intake was unchanged from baseline, while a delayed dose-related increase in calcium ingestion occurred following only the lead injections, and which persisted for the remainder of the experiment (approximately 3 months). Since it has been reported that calcium deprived animals increase their intake of lead solutions, the present result suggests that lead intoxication could lead to a destructive positive feedback loop. Supported by USPHS NS17743.

685.12

GLUCOCORTICOID TREATMENT MODIFIES THE ASTROCYTES OF CHRONICALLY LEAD-EXPOSED RATS. A. Selvín-Testa, F.C. Loidl, E.M. López, J. Pecci Saavedra * Instituto de Biología Celular, Facultad de Medicina, U.B.A., Paraguay 2155, (1121) Buenos Aires, República Argentina.

Chronic lead exposure (1g% lead acetate solution-started when rat pups were 7 days old) increased the concentration of glial fibrillary acidic protein (GFAP) in astrocytes of the hippocampus. Glucocorticoids regulate the expression of the GFAP in physiological conditions, but it is not clear how the excessive exposure to hydrocortisone (HC) affects hypertrophic astrocytes in the hippocampus. 3-month lead-intoxicated male rats were injected daily with HC (5 mg/-100g s.c.) or its vehicle for 15 days. Rats were sacrificed at 1, 7, 14, 21, and 30 days after the last dose of HC. GFAP immunohistochemistry and computerized image analysis were used to study astroglial morphology. After 1, 7 and 14 days of HC administration, the positive-GFAP cells showed a shape and distribution similar to that of hypertrophic astrocytes (mean area: 191.78±5.01μm^2). The astroglial area decreased after HC administration to 150.61±2.50μm^2. The decrement of GFAP was similar to that seen in the normal HC treated astrocytes: 80.67±4.54μm^2 versus 131.74±2.50μm^2 in control rats. Such decrease of GFAP in the hypertrophic astrocytes after HC treatment showed values close to those seen in normal ones. The observed HC effects probably improved specific glial functions.
(Supported by CONICET and U.B.A., Argentina)

685.13

MANGANESE TOXICITY: EXCITATORY AMINO ACIDS RECEPTORS IN MOUSE BRAIN. G. Cano and E. Bonilla*. Fundacite-Zulia and Inst. de Inves. Clínicas, Univ. del Zulia, Maracaibo, Venezuela.

Chronic manganese poisoning is characterized by neuronal destruction of the striatum, as well as degeneration of substantia nigra and cortex. Since it is known that primary corticostriatal neurotransmitters are excitatory amino acids (EAA), we analyzed the (^{3}H)-Glutamate (Glu) binding to the NMDA, Quisqualate (Quis) and Kainate (Kai) receptor subtypes and also the total Glu receptors on mouse brain, using "in vitro" quantitative autoradiography.

Male albino mice were injected i.p. with manganese chloride (5mg Mn/Kg/day) five days per week, during eight weeks. Control animals were treated with saline. Binding procedures were carried out using (^{3}H)-Glu 20 nM as a ligand, in the presence or absence of NMDA (100 uM), Quis (2.5 uM) and Kai (10 uM). Non specific binding was determined using 1mM Glu. Among all the regions studied, Glu receptors were significantly decreased in cortex, olfactory nucleus (ON), lateral septum (LS), striatum, hippocampus (Hp) and cerebellum of intoxicated animals. NMDA receptors were reduced in olfactory bulb, ON, cingulate cortex, LS and Hp. Quis receptors were decreased in orbital and frontal cortex, globus pallidum, Hp and cerebellum. It was difficult to quantify Kai receptors due to the weak signal obtained.

The decrease in EAA binding could be caused by neuronal destruction, a decreased receptor synthesis in these areas or by down-regulation of receptor binding sites due to increased glutamatergic activity. Given the potential neurotoxicity of Glu, the changes in the EAA receptor densities found in different regions may help to explain the damage in the brain of manganese treated animals.

685.14

SELECTIVE DEGENERATION OF CA1 PYRAMIDAL CELLS BY CHRONIC APPLICATION OF BISMUTH. Michael Müller*, Lotty Rietschin, Franziska Grogg, Peter Streit and Beat H. Gähwiler. *Brain Research Institute, University of Zurich, Switzerland.*

Many neurodegenerative disorders in the brain are characterized by the loss of particular populations of neurons in specific brain regions. We report a new type of selective neuronal degeneration which is induced by a heavy metal and which shares some common aspects with those seen following hypoxia and ischemia. Continuous application of 3 µM bismuth tartrate to organotypic slice cultures of rat hippocampus resulted after 2 - 3 weeks in a selective degeneration of CA1 pyramidal cells, while only few CA3 pyramidal and subicular cells were affected. At 10 µM bismuth, the cytoarchitecture of the culture was completely destroyed. Incubation with comparable concentrations of sodium tartrate did not induce degeneration. Furthermore, acute application of 100 µM bismuth did not change the electrophysiological properties of CA1 pyramidal cells. Interestingly, an important subclass of hippocampal interneuron, the GABAergic cells, were slightly less sensitive to bismuth than neighbouring pyramidal cells as assessed by post-embedding immunocytochemistry on thin sections.

685.15

EFFECTS OF NMDA ANTAGONIST AND NON-NMDA ANTAGONIST ON TRIMETHYLTIN INDUCED BRAIN DAMAGE.
N.Ishida, H.Kanai, N.Kato*, A.Masui, M.Akaike and S.Tsutsumi. Dept. of Psychiatry, Shiga Univ. of Med. Science, Otsu 520-21 and Pharma Res Labs., Hoechst Japan Ltd., Kawagoe 350, Japan.

Male rats were treated with trimethyltin hydroxide (TMT, 9mg/kg, p.o.) and then MK-801 as a NMDA antagonist (0.5 or 1.0 mg/kg, i.p.) or an analogue of Joro spider toxin as a putative non-NMDA antagonist (1-naphthyl acetyl spermine; 1-NA-Spm, 20ug, icv) was injected. Depth EEG electrode was implanted into the right hippocampus and the seizure susceptibility was tested by subthreshold dose of pentylenetetrazol (PTZ, 30mg/kg, i.p.). The susceptibility for PTZ- induced seizure elevated after TMT administration with the maximal effect at 4 post-TMT days. Histological examinations revealed marked loss of hippocampal CA 3/4 pyramidal cells after TMT. While MK-801 showed no antagonizing effect on PTZ-seizure, 1-NA-Spm exacerbated the seizure susceptibility to PTZ exposure. The loss of pyramidal cells was less marked in 1-NA-Spm injected side than in the contralateral side. It is suggested that the hippocampal lesion induced by TMT is mediated by non-NMDA receptors.

685.16

Effects of neonatal exposure to trimethyltin on auditory and associative processes in the rat: Evidence from reflex modification procedures. Ellen S. Goldey and Kevin M. Crofton*. Neurotoxicology Div., MD-74B, U.S. EPA, RTP, NC, 27711.

Trimethyltin (TMT) is an organotin compound which is neurotoxic to adult and developing rats. The present study assessed the effects of neonatal TMT exposure on auditory thresholds, auditory temporal acuity and associative conditioning. Long-Evans male and female rat pups were acutely exposed to 0 (vehicle control) or 6 mg/kg TMT on postnatal day (PND) 5 and were tested as young adults (PND 60 - 70). Auditory thresholds for 1, 5 and 40 kHz tones were determined using reflex modification audiometry. In a separate group of animals, auditory temporal acuity was assessed using an associative conditioning paradigm. Animals were tested over 7 consecutive days by pairing an eliciting stimulus (120 dB, 13 kHz) with an antecedent gap in background noise (80 dB, white noise) of variable duration (0, 2, 4, 6, 8, 10 and 20 msec; 190 msec inter-stimulus interval).

Whereas rats given TMT as adults showed only high-frequency hearing loss (Crofton et al., 1990), early postnatal TMT exposure resulted in a 20-30 dB increase in auditory thresholds at all frequencies tested. This finding suggests that the developing auditory system may be more sensitive to TMT ototoxicity than the mature system. By the seventh day of gap testing, control and TMT animals showed very similar response inhibition across all gap durations, indicating that TMT did not affect auditory temporal acuity. However, whereas control animals demonstrated significant gap inhibition by day two, and maximal inhibition by day 4, TMT-exposed rats showed significantly less inhibition than controls until day 7. These findings support earlier work suggesting that a learning component is involved in gap perception (Crofton et al., 1990), and indicate that relative to controls, TMT-exposed animals required more training before achieving maximal performance in this task. Whether impaired performance in the associative conditioning procedure is causally related to the auditory deficits remains to be determined.

685.17

DISTRIBUTION OF GLIAL FIBRILLARY ACIDIC PROTEIN (GFAP) IMMUNOREACTIVE ASTROCYTES IN RATS EXPOSED TO TRIMETHYLTIN (TMT) AT SEVERAL POST-EXPOSURE TIME INTERVALS. R. L. Qualls, R. H. Baisden and M. L. Woodruff*. Department of Anatomy, J. H. Quillen College of Medicine, East Tennessee State University, Johnson City, TN 37614.

Based largely on the effects of TMT in rodents expression of GFAP by astrocytes has been proposed as a sensitive indicator of exposure to neurotoxins. But a detailed description of TMT-induced GFAP within specific neocortical areas of the rat brain has not been reported. The areal and laminar distribution of GFAP-immunoreactive astrocytes within the cortex of rats (5 per time period and 5 controls) 3,5,10,15,20, and 30 days after exposure to 6 mg/kg TMT (p.o.) was studied. The ABC immunocytochemical procedure was used to visualize GFAP. The number of GFAP-immunoreactive astrocytes increased from day 3 through day 15, and decreased by day 30. The orbital frontal, pyriform and entorhinal cortices showed more GFAP-positive astrocytes than other cortical areas and superifical layers of cortex had more GFAP than deeper layers at early time points. These results confirm that GFAP is a good marker for dynamic changes occurring in cortex after TMT. (Supported by PHS Grant ES 04070-07 to MLW)

685.18

THE EFFECT OF LEAD ON CAMK OF RAT HIPPOCAMPAL SLICES. Y.Z. XU*, N.LIU, Y.B.JIANG, Y.WU, D.Y.RUAN DEPT. OF BIOLOGY, UNIV. OF SCIE- & TECH- OF CHINA, HEFEI, ANHUI, P.R.CHINA.

Pregnant rats were treaded with lead (0.2% lead acetate) daily. The newborns received lead through milk from 1 day until 28 days of age. Compared with latent period of the slices of the control and the poisoning rats. The control rats was 2.33 ± 0.85 ms(n=6), but the poisoning was 3.82 ± 0.85 ms (n=9). The excitatility of the lead poisoning rats was more than that of the control rats. Before tetanic stimulation, the PS amplitude of the control was 0.98 ± 0.33 mv (n=4), but that of the poisoning was 1.42 ± 0.23 mv (n= 4) . After tetanic stimulation 20 minutes, the amplitude of PS of the control rats increased by 3.03 times, but the lead poisoning slices increased by 1.87 times. These results suggested that the amplitude of LTP of the lead poisoning rats was less than that of the control rats. For the lead poisoning rats, after LTP maintaining 20 minutes the amplitude of PS decreased. According to the results, there are effects of lead on the acceptor of the postsynapse membrane, and therefore on the depolarization of it, that resulted in the delayed latent period. According to the mitochondrion hypothesis, there are effects of lead on CAMK and therefore the PS amplitude is reduced during LTP.

686.1

REPEATED EXPOSURE TO CYANIDE ATTENUATES PENTYLENTETRAZOLE (PTZ) SEIZURES AND FOS INCREASE IN RATS. G. Pavlakovic*, A. Kanthasamy and G. E. Isom, Department of Pharmacology and Toxicology, Purdue University, West Lafayette, IN 47907.

Prolonged exposure to cyanide leads to pathohistological changes in select brain areas. These changes may alter CNS response to physiological and pharmacological stimuli. In order to test this hypothesis, the ability of rats treated for 8 days with KCN (5 mg/kg, ip.) or saline to respond to a convulsant challenge (PTZ) was assessed. The ED_{50} for PTZ-induced clonic seizures was almost doubled in the cyanide pretreated animals, while ED_{50} for clonic-tonic seizure induction was increased by 50%. In control, but not cyanide-treated animals, PTZ (60 mg/kg, ip.) produced an increased expression of Fos in pyriform and cingulate cortex and dentate gyrus of hippocampus. These data show that multiple dosing of cyanide increases the threshold for PTZ-induced seizures. Further, the ability of cells in select brain areas to increase their Fos levels is reduced in cyanide-treated animals. (Supported by NIH Grant ES04140)

686.2

CYANIDE-INDUCED DOPAMINERIGIC TOXICITY IN MICE A.G. Kanthasamy*, J.L. Borowitz, J.J Turek[1], G. Pavlakovic and G.E. Isom, Dept of Pharmacol & Toxicol., [1]Dept of Vet. Anatomy, Purdue Univ., W. Lafayette, IN 47907.

Our previous studies demonstrate that cyanide produces a dose-dependent release and depletion of dopamine (DA) in PC12 cells. In humans, cyanide can produce a delayed and progressive Parkinsonism following acute intoxication. These observations suggest that cyanide interacts with the central dopaminergic system. Preliminary studies indicated that subchronic cyanide treatment produces dopamine depletion in striatum and induces locomotor dysfunction in mice. To study whether cyanide produces neuronal damage in basal ganglia, mice were treated with cyanide (KCN, 6 mg/kg, sc) twice a day for 7 days and 16 hrs after the last dose histological observations were made in striatum and substantia nigra. Brain sections were made at 10 and 50 micron thickness and processed for thionin nissl staining and immunohistochemical staining using tyrosine hydroxylase (TH) antibody. Thionin staining in striatum showed a bilateral vacuolation indicating neuronal damage. TH immunohistochemical examination of brains from cyanide treated mice had a reduced number of TH-positive cells in the substania nigra, indicating loss of dopaminergic cell bodies. These changes were noted in 30-35% of cyanide treated animals, suggesting a sub population of animals are susceptible to neurotoxic lesions. These findings indicate that the loss of dopaminergic neurons are associated with DA depletion and behavioral changes following subchronic cyanide exposure. (supported by NIH grant ES04140).

686.3

A NEUROTOXIC REGIMEN OF METHAMPHETAMINE DAMAGES STRIATAL DOPAMINE TERMINALS AND DECREASES RADIOLIGAND BINDING TO STRIATAL GLUTAMATE RECEPTORS. S.J. O'Dell*, F.B. Weihmuller, A.J. Eisch, J. Ulas, C.W. Cotman and J.F. Marshall. Dept. of Psychobiology, University of California, Irvine, CA 92717.

Multiple injections of methamphetamine (m-AMPH) [4 mg m-AMPH/kg (sc) at 2 hr intervals for a total of 4 injections] produces large increases in striatal dopamine (DA) overflow and subsequent damage of striatal DA terminals. The involvement of striatal glutamate synapses in this process is suggested by: 1) neurotoxic schedules of m-AMPH produce a delayed but prolonged increase in striatal glutamate overflow and 2) NMDA receptor antagonists can protect against m-AMPH-induced striatal DA terminal damage. We used quantitative receptor autoradiography to determine the relationship between m-AMPH-induced damage to striatal DA terminals and alterations in radioligand binding to striatal glutamate receptors (NMDA and kainate subtypes). Rats given a neurotoxic regimen of m-AMPH showed marked reductions in [3H]mazindol-labeling of DA terminals in the ventral (-35%) and lateral (-43%) regions of the striatum with sparing of nucleus accumbens DA terminals. Adjacent striatal sections from these animals showed decreases in radioligand binding to NMDA and kainate receptors, with the greatest reductions found in the dorsal (-27%) and medial (-25%) striatum. Regional mismatches between losses of [3H]mazindol binding and decreases in striatal glutamate receptors suggest that the changes seen in glutamate receptors are not a direct consequence of DA terminal injury.

686.4

ALTERATIONS IN STRIATAL GLUTAMATE RECEPTORS FOLLOWING 6-OHDA-INDUCED DAMAGE TO NIGROSTRIATAL DOPAMINE NEURONS. F.B. Weihmuller*, S.J. O'Dell, and J.F. Marshall. Dept. of Psychobiology, Univ. of California, Irvine, CA 92717 USA

Recent evidence suggests that an important modulatory relationship exists between the nigrostriatal dopamine (DA) and corticostriatal glutamate (GLU) systems. This relationship has recently been highlighted by the finding that L-[3H]glutamate binding to the NMDA receptor subtype is elevated in Parkinsonian striatum. The present study used the 6-OHDA-treated rat to determine whether loss of nigrostriatal DA neurons induces GLU receptor changes in the striatum. Rats were given unilateral intracerebral injections of 6-OHDA, sacrificed 1 week or 1 month later and their brains processed for quantitative receptor autoradiography using [3H]mazindol (15 nM) to label striatal DA terminals, L-[3H]glutamate (200 nM) to label NMDA receptors and [3H]kainate (50 nM) to label kainate receptors. In the 6-OHDA-injected hemisphere, rats had 95% or greater loss of [3H]mazindol binding to striatal DA terminals accompanied by a small (5-10%) but statistically reliable ($p < .01$) reduction of glutamate binding to NMDA receptors as compared to the intact hemisphere. Moreover, these animals had a small but statistically significant increase (5%; $p < .01$) in striatal [3H]kainate binding in the 6-OHDA-injected as compared to the intact hemisphere. These results suggest either that a small population of NMDA receptors (5%) exist on striatal DA terminals or that the DA terminals help maintain postsynaptic NMDA receptor expression. The bases for differences between 6-OHDA-treated rats and Parkinsonian tissue will be discussed.

686.5

THE ONTOGENY OF 3,4-METHYLENEDIOXYMETHAMPHETAMINE (MDMA)-INDUCED NEUROTOXIC SENSITIVITY IN THE RAT. H.W. Broening*, L. Bacon, W. Slikker, Jr. Div. of Toxicology, Univ. of Arkansas Medical Science, Little Rock, AR 72205 and Div. of Neurotoxicology, Nat. Center Toxicological Res., Jefferson, AR 72079.

The ontogeny of neurotoxic sensitivity to MDMA was studied in the rat to determine if the development of such sensitivity coincided with the development of the serotonergic neurotransmitter system. Sprague Dawley rats were dosed orally with 40 mg/kg MDMA at various stages of development and adulthood. 5-HT and 5-HIAA levels and [^{3}H]paroxetine (PRX) receptor binding (5-HT reuptake carrier) were evaluated at several time points after treatment. MDMA administered at postnatal day (PND) 10 acutely reduced 5-HT and 5-HIAA levels by 78% and 62%, respectively, in frontal cortex (FC), which recovered to control levels within 72 hours. No changes in PRX binding were observed. MDMA administered at PND 40 acutely reduced 5-HT and 5-HIAA levels by 89% and 76%, respectively, in FC. At one week, 5-HT and 5-HIAA levels were still reduced by 40% and 32%, and PRX binding was decreased by 21%. MDMA administered at PND 70 acutely reduced 5-HT and 5-HIAA levels by 87% and 76%, respectively, in FC. At one week, 5-HT and 5-HIAA levels were still depleted by 59% and 51%, and PRX binding was decreased by 40%. The development of the serotonergic neurotransmitter system (characterized by the ontogeny of 5-HT levels, 5-HT receptor populations, tryptophan hydroxylase activity, 5-HT reuptake sites, etc.) is approximately 70%-80% complete by PND 21 in the rat. These results demonstrate that MDMA can acutely deplete 5-HT and 5-HIAA at an early age in the rat, but that neurotoxic sensitivity to MDMA develops only after the majority of serotonergic development has occurred.

686.6

NEUROTOXICITY PROFILES OF SUBSTITUTED AMPHETAMINES IN THE C57/B16/J MOUSE. J. P. O'Callaghan* and D.B. Miller. U.S. Environmental Protection Agency, Research Triangle Park, NC 27711.

Dopaminergic (DA) and serotonergic (5-HT) neurons have been implicated as the primary targets of amphetamine-induced neurotoxicity, however, unequivocal evidence of damage to these systems has been difficult to obtain. Here, we evaluated the potential neurotoxic effects of methamphetamine (D-METH), methylenedioxyamphetamine (D-MDA), methylenedioxymethamphetamine (D-MDMA) and fenfluramine (D-FEN) in the C57/B16/J mouse. Astrogliosis and argyrophila were taken as indices of amphetamine-induced neural damage. Astrogliosis was quantified by assaying glial fibrillary acidic protein (GFAP); the silver degeneration stain of de Olmos was used to detect argyrophilic neurons. Assays of tyrosine hydroxylase (TH), DA and 5-HT were used to assess effects on DA and 5-HT systems. Mice received either D-METH (10 mg/kg), D-MDA (20 mg/kg), D-MDMA (20 mg/kg) or D-FEN (25 mg/kg) every 2 hours for a total of 4 s.c. inj. D-METH, D-MDA and D-MDMA caused a large (300%) increase in striatal GFAP that resolved by 3 weeks and a 75% decrease in TH and DA that did not resolve. D-METH, D-MDA and D-MDMA also caused terminal degeneration in striatum as revealed by silver staining. D-FEN did not affect any parameters in striatum. D-METH, D-MDA and D-MDMA also increased GFAP in cortex, effects which were associated with small (10-25%) and transient decrements in cortical 5-HT. D-FEN caused prolonged (weeks) decrements (20%) in cortical 5-HT but did not affect any other measures. The effects of D-METH, D-MDA and D-MDMA were blocked by pretreatment with MK-801. The L-forms of all compounds did not affect any measure. D-METH, D-MDA and D-MDMA, but not D-FEN, appear to be toxic to neural elements of mouse striatum and cortex.

686.7

ENVIRONMENT-, STRESS- AND DRUG-INDUCED ALTERATIONS IN BODY TEMPERATURE AFFECT THE NEUROTOXICITY OF SUBSTITUTED AMPHETAMINES IN THE C57/Bl6/J MOUSE. D. B. Miller and J. P. O'Callaghan*, U.S. Environmental Protection Agency, Research Triangle Park, NC 27711

The putative toxic effects of substituted amphetamines (AMPs) in the rat have been linked to an elevation of core temperature (CT). Here, we examined the influence of CT changes on the potential neurotoxic effects of methamphetamine (D-METH), methylenedioxymethamphetamine (D-MDMA) and fenfluramine (D-FEN) in the C57Bl6/J mouse. Astrogliosis, quantified by immunoassay of glial fibrillary acidic protein (GFAP), was taken as an index of AMP-induced neural damage. Assay of dopamine (DA) was used to assess effects of AMPs on the striatal DA system. Female mice were given D-METH, D-MDMA or D-FEN at a dosage (s.c., as the base) of 20.0, 10.0, or 25.0 mg/kg, respectively. An inj of AMPS or saline vehicle was given every 2 hrs beginning at 11:30 AM for a total of 4 inj. Mice were restrained in plastic 50 ml centrifuge tubes at 22°C or single-housed with no bedding at 15 or 22 °C. In mice housed at 22°C, D-METH and D-MDMA elevated CT 1.0 °C and D-FEN and restraint lowered CT by as much as 3°C. At this housing temp (22°C), D-METH and D-MDMA caused a large (300%) increase in striatal GFAP and a large (75%) decrease in striatal DA at 72 hrs after the last inj.; D-FEN and restraint did not alter striatal GFAP or DA. In mice housed at 15°C, D-METH and D-MDMA did not elevate CT, increase GFAP or decrease DA. Restraint or administration of D-FEN prior to each inj of D-MDMA blocked (restraint) or attenuated (D-FEN) the D-MDMA-induced increase in GFAP and the decrease in DA in mice housed at 22°C. The glutamate antagonist, MK-801 (1.0 mg/kg x 2 inj.), which blocked the D-METH and D-MDMA increase in GFAP and decrease in DA at 22°C, did not alter CT when given alone. When MK801 was given in combination with D-METH and D-MDMA, however, CT fell by as much as 3°C. The data suggest that an elevation in CT is an important determinant of the dopaminergic neurotoxicity of D-METH and D-MDMA in the mouse.

686.8

6-HYDROXYDOPAMINE (6-OHDA or 6-HDA) NEUROTOXICITY: INVESTIGATIONS INTO THE MODE OF ACTION. RUSSELL J. LEWIS, SU MA, DONG-DONG ZHANG, R. RHAGAVAN, ROLAND E. LEHR, and C. LeROY BLANK*, Department of Chemistry & Biochemistry, University of Oklahoma, Norman, OK, USA, 73019.

Following a previous report on the peripheral neurotoxic effects of various 6-HDA analogs (J.-P. Tranzer & H. Thoenen, *Experientia*, (1973) **29**, 314-5), we have synthesized and tested a series of 6-HDA related species to, hopefully, establish relationships between the observed destruction and the chemical and/or physical nature of the toxins. The extent of destruction afforded by a toxin was measured as both the ability to create long-term depletion of the endogenous transmitter substance (NE, DA, and 5-HT) and the ability to create long-term disruption in the transport of the endogenous transmitter. The chemical properties examined to establish quantitative relationships to these long-term effects included (1) the ability to effect in vitro blockade of the transporter for the transmitter, (2) the ease of oxidation of the toxin, (3) the reactivity of the oxidized form of the neurotoxin with respect to attack by nucleophiles, (4) the autoxidation of the neurotoxin and concomitant production of malevolent oxygen species, and (5) similar effects for oxidation products of the parent toxin. Initial results indicate surprisingly poor correlations between the extent of destruction and either the ability of the neurotoxin to block transport or the ease of oxidation of the neurotoxin. Uptake blockade is, thus, a necessary but not sufficient condition for the neurotoxic properties of these compounds. Quantitative predictions of the degree of neuronal loss afforded by such neurotoxins may require a more complex combination of a number of the chemical properties possessed by such species.

686.9

THE ROLE OF BODY TEMPERATURE IN METHAMPHETAMINE (METH) LETHALITY AND NEUROTOXICITY, AND IN THE EFFECTS OF COMPOUNDS WHICH PROTECT AGAINST SUCH NEUROTOXICITY. R.R. Holson*, B. Gough, G. Newport, W. Slikker Jr. and J.F. Bowyer. Div. of Develop. Toxicol. and Neurotoxicol., NCTR/FDA, Jefferson, AR 72079-9502.

Repeated exposure to METH (5mg/kg, 4 times at 2 hr intervals) increases body temperature, sometimes producing extreme hyperthermia (41°F and above) and lethality, and depletes striatal dopamine (DA). The magnitude of striatal DA depletions correlates ($r = -0.58$) well with the peak body temperatures attained during METH administration. This correlation may be causal, since increased room temperature (27°C) substantially increases METH-induced body temperature and DA depletion. Further, several agents, including haloperidol and diazepam, diminish the METH-induced hyperthermia, DA depletions and lethality. The correlation between maximum body temperature and DA depletion remains high even with these protective agents present, and the DA depletion for a given maximum temperature is almost that of METH alone. Therefore, protection from METH-induced DA depletion by these compounds may be solely through reducing METH-induced hyperthermia. At 50 mg/kg the interleukin-1 receptor antagonist (IL-1ra, from Synergen Inc.), decreased the incidence of lethal hyperthermia produced by METH without substantially reducing striatal DA depletion, or the correlation between body temperature and DA depletion. Therefore, the thermogenic effects of METH may play a role in the lethality and striatal DA depletions produced by METH, and pharmacological protection against these effects often involves a reduction in the hyperthermia produced by METH.

686.10

EFFECTS OF NITRIC OXIDE SYNTHETASE (NOS) INHIBITION AND ADENOSINE (AD) RECEPTOR ANTAGONISM ON EXTRACELLULAR DOPAMINE (DA) LEVELS DURING METHAMPHETAMINE (METH) EXPOSURE. [1]B. Gough*, [1]R.R. Holson, [2]W. Slikker Jr. and [2]J.F. Bowyer. [1]Div. of Repro. & Develop. Toxicol. and [2]Div. of Neurotoxicol., NCTR/FDA, Jefferson, AR 72079-9502.

When 100 μM nitro-arginine (N-ARG, a NOS inhibitor) was present in the microdialysis buffer for 2 hrs no effects on the extracellular levels of DA, DOPAC or HVA in the striatum were observed. However, the subsequent increase in extracellular DA produced by 4 sequential doses of METH (5 mg/kg i.p./2 hrs) was reduced by approximately 50%. The inclusion of 100 μM l-ARG in the microdialysis buffer did not markedly enhance DA levels in the absence or presence of METH. In contrast, neither N-ARG or l-ARG (1 to 100μM) affected either 5 or 50 μM METH-evoked release of [^{3}H]DA from striatal slices. Therefore, *in vivo* the N-ARG inhibition of METH-evoked DA release, possibly due to NOS inhibition, may be mediated by either multi-neuronal activity or changes in blood and CSF circulation. The effects of the AD receptor antagonist 8(p-sulfophenyl)theophylline (at 2 to 200 μM) were similar to that of N-ARG in that *in vivo* it had no effect on extracellular DA release in the absence of METH but decreased extracellular DA levels during METH exposure. Other investigators have observed that adenosine inhibits DA release via presynaptic mechanisms *in vitro*, therefore, the effects of the AD antagonist *in vivo* appear to again be mediated by either multi-neuronal activity or changes in blood and CSF circulation. The results of these studies indicate that multi-neuronal pathways may play a role in the *in vivo* release of DA evoked by METH.

686.11

Differential effects of amfonelic acid on dopaminergic nerve terminal degeneration and reactive astrogliosis induced by methamphetamine in rat striatum C. Pu, J. E. Fisher, C. V. Vorhees*, Children's Hosp. Res. Found. and Neurosci. Prog, Univ. Cincinnati, Cin., OH 45229.

Administration of methamphetamine (MA) induces degeneration of dopaminergic terminals and astroglosis, such as hypertrophy and increase in apparent number, in the neostriatum. In this experiment adult rats were treated with MA (10 mg/kg, i.p.) 4 times in one day at 2 hour intervals. Amfonelic acid, a dopamine reuptake blocker, was administered (20 mg/kg, i.p.) at the same time the last MA dose was given. Three days later, dopaminergic terminals and astrocytes were examined immunohistochemically. The contents of striatal dopamine and its metabolites were analyzed by HPLC 7 days after treatment. The results showed that MA alone induced the typical depletion of dopaminergic terminals, reduction of dopamine content and astrogliosis in the neostriatum. Amfonelic acid treatment completely prevented the effects of MA on the dopaminergic system, both morphologically and biolochemically. However, the reaction of astrocytes remained in the region where the most severe depletion of dopaminergic terminals was seen in MA treated animals (ventral lateral portion of neostriatum). The results support the concept that dopamine reuptake is involved in the MA-induced dopaminergic nerve terminal degeneration. The results also indicate that blocking DA reuptake cannot prevent the reaction of astrocytes in the neostriatum, which indicates that the astrocytic reaction can be induced by factors other than degeneration of dopaminergic terminals in this region.

686.12

THE PRETREATMENT OF RATS WITH ACETONE POTENTIATES THE SEROTONIN DEPLETING EFFECT OF 3,4-METHYLENE-DIOXYAMPHETAMINE (MDA). R. E. Michel* and W. J. George, Department of Pharmacology, Tulane University School of Medicine, New Orleans, Louisiana, 70112.

The neurotoxicity associated with the administration of 3,4-methylenedioxyamphetamine (MDA) is thought to be produced by a metabolite of the parent compound and not by MDA directly. The objective of this study was to investigate the role of cytochrome P450 activity in the mediation of the neurotoxicity induced by MDA. Cytochrome P450 activity was induced by pretreating animals with 1% acetone that was added to their drinking water. Acetone induces several P450 isozymes including P4501A2, P4502B1/2, and P4502E1. Male Sprague Dawley rats were exposed to acetone through their drinking water for seven days. Exposed animals were compared to a control group that received tap water without acetone. Following the seven day pretreatment period, animals in both groups received 20 mg/kg MDA, subcutaneously. The animals were sacrificed 7 days post drug administration (day 14) and selected brain regions were analyzed for serotonin and 5-hydroxyindole acetic acid (5-HIAA) content. Animals that were pretreated with 1% acetone had significantly lower serotonin and 5-HIAA levels than control animals in all brain regions assayed (5-40% of control values). The present findings indicate that P4501A2, P4502B1/2 or P4502E1 may be involved in the formation of a neurotoxin responsible for the serotonergic toxicity associated with the use of MDA.

686.13

PREFERENTIAL INJURY TO TYROSINE HYDROXYLASE IMMUNOREACTIVE FIBERS OF THE VENTRAL CAUDATE-PUTAMEN [CPu] AFTER A NEUROTOXIC METHAMPHETAMINE REGIMEN. A.J. Eisch*, F.B. Weihmuller, J.F. Marshall. Dept. of Psychobiology, University of California, Irvine, CA 92717.

Repeated administration of moderate doses of methamphetamine [m-AMPH] injures dopaminergic afferents to the striatum, as indicated by changes in striatal dopamine [DA] content, DA metabolites, DA uptake sites, tyrosine hydroxylase [TH] activity and TH-immunoreactivity. Of particular interest to us is the fact that striatal subregions are differentially vulnerable to the neurotoxic effects of m-AMPH (Eisch et al., Br. Res. 598: 321-6, 1992). The ventral CPu, but not the nucleus accumbens or the dorsal CPu, shows a significant loss of DA content and DA uptake sites one week after m-AMPH administration. Here, we extend the characterization of m-AMPH-induced neurotoxicity by examining TH-immunoreactivity and DA content separately in the two hemispheres of rats given m-AMPH (4 s.c. injections of 4 mg/kg, 2 hrs apart). A regionally-selective decrease in striatal DA content was accompanied by a similar pattern of TH-immunoreactivity one week after the m-AMPH regimen. Specifically, both measures were decreased in the ventral CPu, while the nucleus accumbens and dorsal CPu remained relatively intact. We believe the striking susceptibility of the ventral CPu will be important in understanding the processes underlying m-AMPH neurotoxicity. Several hypotheses concerning the preferential vulnerability of this region will be discussed.

686.14

IN VIVO TRANSLOCATION/ACTIVATION OF PROTEIN KINASE C (PKC) BY MDMA: A POSSIBLE LINK TO NEUROTOXICITY. H.K. Kramer, J.C. Poblete and E.C. Azmitia. Dept. of Biology, New York University, New York, NY 10003.

3,4-methylenedioxymethamphetamine (MDMA) is a substituted amphetamine found to elicit its psychotropic and neurotoxic effects through its ability to release serotonin (5-HT) from central neurons. Interestingly, MDMA also increases the uptake of Ca^{2+} into rat brain synaptosomes, an effect independent of those which stimulate 5-HT release. Elevated levels of intracellular calcium, possibly leading to the destabilization of $[Ca^{2+}]_i$ homeostasis, has been observed in several models of delayed neuronal death. The disruption of normal $[Ca^{2+}]_i$ levels may begin a cascade of metabolic events that eventually contribute to neuronal degeneration. One of these events is the translocation/activation of the phospholipid and Ca^{2+}-dependent protein kinase C (PKC). It was our desire to examine if acute MDMA administration had an effect on the translocation/activation of PKC *in vivo*, in order to elucidate the precise metabotropic events that underlie MDMA-induced neuropathology.

Male Sprague-Dawley rats were injected twice (10 hrs. apart) with either saline or MDMA (25 mg/kg, s.c.). 24 and 48 hours after the last injection the animals were sacrificed, their brains removed, and subsequently assayed for PKC translocation by high-affinity [^{3}H]phorbol-12,13-dibutyrate (^{3}H-PDBu; 20.0 Ci/mmol) binding to cortical membrane fractions.

At 24 hrs., ^{3}H-PDBu binding demonstrated a K_d of 11.44 nM and a B_{max} of 7.58 pmol/mg prot. (saline treated) and 18.94 nM and 13.81 pmol/mg prot. (MDMA treated). MDMA treatment produced an 82.2% increase in the amount of membrane-bound ^{3}H-PDBu binding sites. By 48 hrs., no significant difference was observed between the two treatment groups.

Our results indicate that acute, *in vivo* MDMA exposure produces a transient increase in the translocation of PKC from the cytosol to the plasma membrane (believed to be an indicator of PKC activation). Pathological PKC translocation/activation has been hypothesized to contribute to glutamate excitotoxicity in cerebellar neurons by allowing the enzyme prolonged proximity to many of its Ca^{2+}-dependent substrate proteins (Favaron et al., 1990). These preliminary results provide further information on the metabolic effects of MDMA. (NIDA contract # 271-90-7403)

686.15

ATTENUATION OF 1-METHYL-4-PHENYLPYRIDINIUM (MPP+) NEUROTOXICITY BY DEPRENYL (DP) IN ORGANOTYPIC CULTURES OF CANINE SUBSTANTIA NIGRA. **D.E. Schmidt*, J.C. Lynn, M.H. Ebert and W.O. Whetsell, Jr., Depts. of Pathology and Psychiatry, Vanderbilt University School of Medicine, Nashville, TN 37232.**

The mechanisms responsible for the neuroprotective effects of DP in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyrindine (MPTP) models of Parkinsonism are not clear. To determine the role of MAO-B mediated conversion of MPTP to MPP+, mature (43 day) organotypic cultures of canine substantia nigra were directly exposed to 100 nM MPP+ for 3 days in the presence or absence of 10 μM DP. Additional cultures were incubated with 10 μM DP for 4 days prior to similar MPP+ exposure. Dopamine metabolism was measured by determining HVA release (fmoles/culture/day). Morphological alterations were assessed by light microscopy. MPP+ exposure alone reduced HVA release by 90% as compared to pretreatment release. In contrast, HVA release in cultures previously incubated with DP, then exposed to MPP+, was reduced by 35%; HVA release from cultures simultaneously exposed to MPP+ and DP was reduced by 50%. Microscopic examination demonstrated that MPP+ exposure alone produced widespread neuronal damage. Such damage was reduced by simultaneous presence of DP in the culture media and this effect was greater when DP treatment preceded MPP+ exposure. These in vitro data indicate that, like previous reports of the effect of DP in vivo (J Neurosci Res 30:666, 1991), dopaminergic neuroprotection by DP in the MPTP model does not depend on blockade of conversion of MPTP to MPP+.

686.16

PHARMACOKINETICS AND NEUROTOXICITY OF MPTP IN THE BRAIN OF CHICK EMBRYO. A. B. Naini, H. Jiang, L.J. Côté* and S. Przedborski. Dept. of Neurology, College of P & S of Columbia Univ., N.Y., N.Y. 10032.

The administration of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) to mammals produces a profound disruption in the nigrostriatal dopaminergic pathway, which is similar to that observed in Parkinson's disease. We have investigated the pharmacokinetics of MPTP in the brain of the chick embryo. MPTP (100 μg) dissolved in saline, was injected into the fertilized egg at 14 days of gestation and the level of MPP+ formed was measured at 7 different time points within 48 hr post-injection. The concentration of MPP^+ in the brain increased rapidly, reached its maximum level at about 8 hr post-injection and was still detectable after 48 hr post injection. This is in contrast to the observation in mice, in that MPP^+ is not detectable after 12 hr post i.p. injection. The brain 5-HT and DA levels measured at 5 days post MPTP injection were decreased by 86% and 92%, respectively. Pre-treatment of the fertilized egg with Selegiline (L-deprenyl), a specific MAO-B inhibitor, profoundly decreased the rate of MPP^+ formation and its effects on the levels of 5-HT and DA in the chick brain. Herein, we report the pharmacokinetics of MPTP in the brain of the chick embryo and its profound effects on the biogenic. The chick embryo is a promising model for studying the effects of MPTP on the developing brain.

686.17

MPP^+-LIKE NEUROTOXICITY OF A PYRIDINIUM METABOLITE OF HALOPERIDOL. J. Bloomquist*[1], E. King[2], A. Wright[1], C. Mytilineou[3], and N. Castagnoli, Jr.[2] Departments of Entomology[1] and Chemistry[2], Virginia Polytechnic Institute and State University, Blacksburg, VA 24061 and Department of Neurology[3], Mount Sinai Medical Center, New York, NY 10029.

The neurotoxic action of HPP^+, a pyridinium metabolite of haloperidol, was compared to that of MPP^+ using cell culture and synaptosomal techniques. In 2 hr exposures of rat mesencephalic cultures to 25 μM HPP^+, dopamine uptake was inhibited approximately 30% and serotonin uptake about 75%. In 4 hr incubations at 50 μM and 1 hr incubations at 100 μM, both toxins inhibited dopamine uptake at least 80%, and this effect was accompanied by a generalized cell loss with HPP^+, but not with MPP^+. These data suggest that HPP^+ may be a less selective neurotoxin at high concentrations. The interaction of MPP^+ and HPP^+ with aminergic transporters was studied in more detail in mouse brain synaptosome preparations. The IC_{50} values for inhibiting dopamine uptake by MPP^+ and HPP^+ were 4 and 25 μM, respectively, and for inhibiting serotonin uptake were 3 and 4 μM, respectively. The parent compound haloperidol was much less effective, showing <20% inhibition of dopamine uptake at 300 μM and an IC_{50} for inhibiting serotonin uptake of 200 μM. These results demonstrate that HPP^+ posseses *in vitro* neurotoxic properties similar to MPP^+, and these actions may contribute to the development of tardive diskinesia observed in some patients given haloperidol.

686.18

EFFECTS OF A SALEN-MANGANESE COMPLEX, A SOD-MIMIC, IN VARIOUS MODELS OF NEURONAL PATHOLOGY. **A. Bruce*, W. Musleh, B. Malfroy† and M. Baudry. Neuroscience Program, USC, Los Angeles, CA 90089-2520, and † Eukarion Inc., Arlington, MA 02174.**

Oxygen free radicals (OFRs) have long been proposed to play an important role in most forms of neuronal injury. To test this hypothesis, we evaluated the effects of EUK-8, a salen-manganese complex with Superoxide Dismutase (SOD) activity, on various markers of neuropathology in different models of neuronal damage. Adult rat hippocampal slices were subjected to a brief period of anoxia which resulted in an irreversible alteration of synaptic responses in field CA1. Incubation of slices in the presence of 50 μM EUK-8 provided a significant degree of protection against anoxia-induced reduction of evoked synaptic responses. The dopaminergic neurotoxins, MPTP and 6-OHDA were administered to mice (i.p and i.c.v., respectively) to produce a selective degenerescence of dopaminergic neurons, as indicated by the decreased binding of ^{3}H-mazindol, a ligand for the dopamine transporter in membranes or tissue sections. Treatment of mice with EUK-8 (i.p., 66 mg/kg, for 3 days in the MPTP model; or 1.8 μg, i.c.v. in the 6-OHDA model) resulted in a significant reduction in the decreased binding of ^{3}H-mazindol elicited by the toxins, one week after toxin administration. The results indicate that OFRs participate in both acute and long-term consequences of neuronal insults and suggest potential benefits for the use of low molecular weight SOD-mimics after brain injury. (Supported by a grant from Eukarion, Inc.)

687

SYMPOSIUM. VIEW OF A NEURAL SYSTEM IN THE BLINK OF AN EYE. THE EYEBLINK REFLEX: CONTROL, LEARNING, AND CELLULAR MECHANISMS. J. R. Bloedel (Chairperson); N. H. Donegan, Yale Univ.; R. F. Thompson, Univ. Southern Cal.; V. Bracha, Barrow Neurol. Inst.; J. F. Disterhoft, Northwestern Univ.; C. Evinger, SUNY-Stony Brook.

This Symposium will present an overview of the eyeblink reflex emphasizing its modification during adaptation and associative conditioning and the role of specific central systems in mediating these phenomena. Dr. Donegan will characterize the properties of the unconditioned reflex system as well as changes in stimulus processing resulting from the pairing of the conditioned and unconditioned stimuli. Next, Dr. Thompson will present evidence supporting the hypothesis that the cerebellum and its associated circuitry constitute the essential circuit for classical delay conditioning and that the "memory trace" for this conditioning is formed and stored in a localized region of the cerebellum. Based on the effects of modifying interactions in cerebellar and brainstem nuclei and the responses of simultaneously recorded brainstem neurons obtained during conditioning, Dr. Bracha will address the role of the cerebellum and associated brainstem structures in the "on-line" control of both the conditioned and unconditioned eyeblink reflexes. Dr. Disterhoft will discuss the modifications in the membrane and receptor properties of hippocampal neurons produced during trace conditioning and new experiments in humans showing PET images of specific central structures during delay conditioning. In the final lecture Dr. Evinger will focus on the mechanisms and brainstem sites which mediate eyeblink reflex adaptation induced by altered properties of the motor units responsible for eyelid closure and the relation of these observations to pathological conditions observed in human patients.

688

SYMPOSIUM. THE CONTRIBUTION OF IDENTIFIED NEURONS TO NEUROSCIENCE: A TWENTY-FIVE YEAR RETROSPECTIVE. J.L. Leonard, Oregon State Univ. (Chairperson); R.R. Hoy, Cornell Univ.; R.C. Eaton, Univ. of Colorado; S.S. Kater, Colorado State Univ.; A.I. Selverston, Univ. of Calif., San Diego.

It is now roughly 25 years since uniquely identifiable neurons, neurons that can be identified from one individual and/or species to another, became an established tool in neuroscience research and we are now at a point where the information from these studies is causing reappraisal of our ideas about the organization of neural circuits. Studies of identified neurons, *in vivo*, *in vitro*, and in cell culture, have produced a wealth of information on regeneration, synapse formation and function, neuromodulation, multifunctionality of neurons, and the dynamic rewiring of circuits as a normal part of behavioral organization that have lead to a reevaluation and redefinition of fundamental concepts of neuroscience. This symposium will focus on two subthemes: 1) The organization of systems of neurons for behavior; 2) Dynamic and plastic properties of nervous systems. Four speakers will describe the progress made in each of four popular and powerful model systems. *Dr. Hoy* will discuss what identified neurons have taught us about the organization and control of behavior using a neuroethological perspective and focussing on insects. *Dr. Eaton* will describe how studies of the Mauthner cell and its role in the brainstem escape network of lower vertebrates have lead to changes in the command cell concept. *Dr. Kater* will focus on the use of gastropod identified neurons in cell culture in understanding the mechanisms of establishment and modulation of neural circuits. *Dr. Selverston* will address the issue of how the study of small systems of identified neurons, particularly the crustacean stomatogastric ganglion, has changed our ideas of neural "wiring diagrams" and the function of neural circuits.

NEUROTROPHIC FACTORS: BIOLOGICAL EFFECTS IX

690.1

NGF Reduces Striatal Excitotoxic Neuronal Loss Without Affecting Concurrent Neuronal Stress or NMDA Receptors. O. Isacson*, D. M. Frim, U. Wullner, W. M. Yee. Neuroregeneration Laboratory, McLean Hospital, Belmont, MA, 02178, and Program in Neuroscience, Harvard Medical School, Neurology and Neurosurgery Services, Massachusetts General Hospital, Boston, MA, 02114.

Nerve Growth Factor (NGF) has protective effects against striatal excitotoxic injury in the adult brain. To help define the mechanism of NGF-mediated sparing, we determined the effects of biologically delivered NGF on the degree of neuronal stress, NMDA receptor binding and the development of excitotoxic lesions in the rat striatum. Immortalized fibroblasts genetically altered to secrete NGF (NGF[+]), or control fibroblasts (NGF[-]), were stereotactically implanted near the striatum 7 d before striatal infusion of an NMDA-receptor agonist. Two days after excitotoxin infusion, the volume of neuronal loss was reduced by 34% ($P<0.01$) in the NGF[+] group when compared to the NGF[-] group; however, there was no difference in the volume of 72 kD heat shock protein (HSP72) immunoreactivity expressed in the two groups after 2 d. The final volumes of neuronal loss at 10 d were significantly greater than seen at 2 d, with the volume of neuronal loss in the NGF[+] group reduced by 20-40% ($p<0.01$) when compared to the NGF[-] group. Interestingly, the volume of neuronal loss at 10 d in the NGF[-] group, but not the NGF[+] group, closely approximated the HSP72 immunoreactive volumes seen at 2 d. These results suggest that the cell stress marker, HSP72, is predictive of neuronal loss after striatal excitotoxic insult and while NGF treatment does not alter the overall HSP72 response, it significantly reduces subsequent neuronal loss. NGF[+] grafts also did not affect striatal [^{3}H]glutamate binding or NMDA receptor mRNA levels as measured by *in situ* histohybridization. We conclude that NGF-mediated neuroprotective mechanisms alter neuronal response to injury without affecting the primary cell stress response to NMDA-receptor activation.

690.2

Effects of biologically delivered NGF, BDNF, and bFGF on striatal degeneration caused by 3-nitropropionic acid induced mitochondrial blockade. D.M. Frim*, T.A. Uhler, M.P. Short, X.O. Breakefield, and O. Isacson. Neuroregeneration Laboratory, McLean Hospital, Belmont, MA, 02178; Molecular Neurogenetics Unit, Neurosurgery and Neurology Services, Massachusetts General Hospital and Program in Neuroscience, Harvard Medical School, Boston, MA

Consistent with the notion that a defect in cellular energy metabolism can be a cause of human neurodegenerative disease, systemic treatment with the mitochondrial complex II inhibitor, 3-nitropropionic acid (3-NPA) closely models the striatal neurodegeneration seen in Huntington's disease. Previously, we have found that Nerve Growth Factor (NGF) and Basic Fibroblast Growth Factor (bFGF), but not Brain-Derived Neurotrophic Factor (BDNF), can protect *in vivo* against striatal degeneration induced by infusions of high doses of glutamate receptor agonists. We now report that implantation of NGF-secreting fibroblasts 7 days before systemic 3-NPA treatment reduces the size of adjacent striatal 3-NPA lesions by an average of 64% ($p<0.001$). bFGF causes a similar decrease in striatal lesion size. BDNF, however, did not affect striatal 3-NPA-mediated degeneration. We conclude that biologically delivered NGF and bFGF protect striatal neurons against excitotoxicity and mitochondrial blockade--both energy depleting processes. This observation implies that appropriate neurotrophic support in the adult brain could protect against striatal neurodegeneration caused in part by energy depletion.

690.3

BDNF AMELIORATES AMPHETAMINE-INDUCED ROTATIONAL ASYMMETRY IN HEMI-PARKINSONIAN RATS. S.J. Wiegand,* C. Alexander, C. Jackson, K.D. Anderson, R.M. Lindsay, C.A. Altar and C. Hyman. Regeneron Pharmaceuticals, Inc., Tarrytown, NY 10591.

The ascending nigrostriatal dopamine (DA) system was lesioned by injecting 6-OHDA*HBr (8 µg/2 µl) into the right medial forebrain bundle of adult rats. Rotational bias in response to injections of apomorphine (APO, 1 mg/kg) and amphetamine sulfate (AMPH, 4.5 mg/kg) was assessed one and two weeks following 6-OHDA administration. Animals were assigned in a counterbalanced manner to experimental or control groups, and BDNF (12 µg/day) or normal saline vehicle (VEH) were infused via osmotic minipumps into the region of the substantia nigra on the side of the lesion. APO- and AMPH-induced rotations were again assessed one and two weeks following the initiation of BDNF or VEH treatment. Animals were then sacrificed and DA and DA metabolite levels determined by HPLC. In animals exhibiting the greatest loss of striatal DA (99 ± 0.2 %), administration of BDNF but not VEH significantly attenuated AMPH-induced rotations towards the side of the lesion (317 ± 40 vs. 153 ± 69 net ipsiversive rotations/hr, pre- vs. post-BDNF). Animals with moderate DA depletions (88 ± 2 %) exhibited a reversal in the direction of AMPH-induced rotational bias, from ipsiversive (113 ± 45) to contraversive (-40 ± 50) following BDNF treatment. AMPH did not induce asymmetric rotation in animals with the smallest depletions of striatal DA (36 ± 3%) either before or after BDNF or VEH treatment. Rotational behavior exhibited in response to APO was not significantly altered by BDNF treatment in any group. These findings suggest that in hemi-Parkinsonian rats BDNF infusion into the vicinity of the substantia nigra modulates the effect of amphetamine on rotational behavior through non-dopaminergic mechanisms.

690.4

PLATELET-DERIVED GROWTH FACTOR (PDGF): EXPRESSION AROUND INTRACEREBRAL LESIONS AND GRAFTS AND EFFECTS ON DOPAMINE NEURONS IN VITRO. P. Odin[1]*, A. Othberg[1], M. Grabowski[1], A. Smits[2], W.-M. Duan[1], P. Brundin[1], H. Widner[1], C.-H. Heldin[2], B. Johansson[1], O. Lindvall[1] and K. Funa[2]. [1]Restorative Neurology Unit, University Hospital, S-221 85 Lund, Sweden. [2]Ludwig Institute for Cancer Research, Biomedical Centre, University of Uppsala, S-751 24 Uppsala, Sweden.

The level of platelet-derived growth factor (PDGF) shows dynamic changes in and around intrastriatal grafts of fetal dopamine (DA)-rich mesencephalic tissue in a rat model of Parkinson's disease. Furthermore, PDGF-BB but not PDGF-AA increases the survival of rat and human DA neurons and the neurite outgrowth from human DA cells *in vitro*. The objective of the present study was to characterize in more detail the changes of PDGF expression following insults to the brain and after intracerebral neural grafting and to further analyze the mechanism of action of PDGF-BB on DA neurons. Immunohistochemical, *in situ* hybridization and primary cell culture techniques were used. The PDGF beta-receptor was found to be expressed on neurons in cultures of fetal mesencephalic cells including DA neurons. This indicates that the effect of PDGF-BB on DA neurons could be direct and not mediated via other cell types. Using different concentrations of PDGF-BB, maximal effect on DA cell survival was found at 30 ng/ml of PDGF-BB. We have also studied the effects of PDGF-BB and brain-derived neurotrophic factor (BDNF), separately and in combination, on DA neurons in culture. Survival of DA neurons was comparable after addition of each of the factors but no additive effects were seen when combining them. Following a focal ischemic infarction, increased expression of PDGF was observed in the tissue surrounding the lesion. Taken together with the role of PDGF in other systems, these findings indicate that PDGF could be important both as a survival factor and in wound healing processes in the central nervous system.

690.5

HYPOTHALAMIC ASTROCYTES RESPOND TO TRANSFORMING GROWTH FACTOR ALPHA (TGFα) WITH PROSTAGLANDIN E_2 (PGE_2) RELEASE.
K. Berg-von der Emde, Y.J. Ma, M.E. Costa and S.R. Ojeda*. Div. Neurosci., Oregon Regional Primate Research Center, Beaverton, OR 97006.

Previous studies have suggested that TGFα stimulates the release of luteinizing hormone-releasing hormone (LHRH), the neurohormone controlling sexual development, via an indirect mechanism that involves an action of *TGFα* on glial cells, followed by the release from these cells of neuroactive substance(s) that act on LHRH neurons to stimulate their secretory activity. The ability of *TGFα* to stimulate PGE_2 release from the median eminence and the inhibition of *TGFα*-induced LHRH release by blockade of cyclooxygenase activity suggested that PGE_2 may be one of the neuroactive substances secreted by astroglial cells in response to *TGFα*. To examine this hypothesis, hypothalamic (H) astrocytes cultured in defined medium were exposed to either *TGFα* (50 ng/ml) or a phorbol ester (TPA, 10 ng/ml) for 4,8,16 and 24h and the effect of these agents on PGE_2 release was assessed by RIA. Astrocyte cultures from the cerebellum (Cb), a brain region not involved in neuroendocrine regulation, were used as controls. While H astrocytes responded to *TGFα* with a 2-3-fold increase in PGE_2 release after 8-16h of exposure, Cb astrocytes failed to respond. Activation of protein kinase C, an intracellular mediator of *TGFα* biological effects, increased PGE_2 release 10-fold from H astrocytes, but only 1.5-fold from Cb astrocytes. These results and the previous finding that *TGFα* increases its own gene expression in H but not in Cb astrocytes support the notion that brain astrocytes are regionally specialized and functionally diverse. They also indicate that PGE_2 may be one of the glial signals involved in mediating the stimulatory effect of *TGFα* on LHRH release. (NIH Grants HD-24870, RR-00163, HD-18185).

690.6

IMPLANTATION OF GENETICALLY-MODIFIED FIBROBLASTS PRODUCING NEUROTROPHIN-3 INTO THE DAMAGED BRAIN.
Ernest Arenas* and Håkan Persson. Lab. of Molecular Neurobiology, Karolinska Institute, Box 60400, S-10401 Stockholm, Sweden.

Neurotrophin-3 (NT3) is a member of the neurotrophin family of trophic factors that support the survival of vertebrate neurons. Little is known about their function in the central nervous system *in vivo*. NGF prevents the degeneration of lesioned septal cholinergic neurons in the brain. BDNF supports the survival of developing spinal cord motoneurons and prevents the death of axotomized motoneurons and of a partial population of septal cholinergic neurons. No function *in vivo* has been described for NT3 or for the recently isolated, NT4/5.

In order to investigate the role of NT3 *in vivo*, we established a stable cell line producing recombinant NT3. Using the calcium phosphate transfection technique, Fisher rat 3T3 fibroblasts were cotransfected with the neomycin-resistance gene and a rat NT3 cDNA insert in the OVEC-expression plasmid. Transfected cells resistant to the neomycin analogue G-418 were analyzed by Southern blots, Northern blots and bioassays. The cell line designated F3A-NT3 contained more than one hundred copies of the NT3 gene and expressed high levels of NT3 mRNA. Conditioned media from the F3A-NT3 cell line promoted survival of dissociated E9 chick sensory neurons in a manner indistinguishable from purified recombinant NT3 protein. Dose response curves in comparison to pure NT3 protein revealed that the F3A-NT3 cells produced 150-300 ng of NT3 protein/10^6 cells/day. We are currently implanting this cell line after brain insults, at different developmental stages, in order to determine whether recombinant NT3 prevents neuronal death *in vivo*.

690.7

DIFFERENTIAL REGULATION OF AXONAL BRANCHING OF DENTATE GYRUS NEURONS BY NGF, BDNF AND BASIC FGF.
M. N. Patel* and J. O. McNamara. Duke University and VA Medical Centers, Durham, N C 27710.

The axons of dentate granule cells (mossy fibers) undergo morphological rearrangement (sprouting) following experimental seizures and in human epilepsy. The molecular mechanisms underlying mossy fiber sprouting are unknown. Recent evidence indicates that the growth factors NGF, BDNF and bFGF are differentially expressed by seizure activity *in vivo* and promote neurite-outgrowth *in vitro*, suggesting that these might be involved in the molecular events of mossy fiber sprouting. To test this idea we developed an *in vitro* model in which dentate gyrus neurons were maintained in low density culture in defined media (Brewer and Cotman; Brain Res. 494:65-74, 1989). MAP2 and GAP43 immunocytochemistry was used to distinguish axons from dendrites. Axonal branching, an early step in the sprouting response, was measured using a digital image analyzer. Phase-contrast images of axons and dendrites extended from neurons grown in the presence of varying concentrations of NGF, BDNF and bFGF were quantitated. We find that BDNF and bFGF produce selective increases in axonal but not dendritic branches. In contrast, NGF has no effect on either axonal or dendritic branching of dentate gyrus neurons. The selectivity and specificity of growth factor action may provide clues to the molecular basis of mossy fiber sprouting following seizures *in vivo*.

690.8

NERVE GROWTH FACTOR CAUSES ACTIVITY-DEPENDENT SYNAPTIC MODIFICATION IN ADULT CAT VISUAL CORTEX.
Q. Gu*, Y.L. Liu and M.S. Cynader. Department of Ophthalmology, University of British Columbia, Vancouver, British Columbia, Canada.

We have been testing the hypothesis that NGF, in addition to its known functions such as for neuronal survival, growth and differentiation, also plays a role in synaptic modification in the CNS. Our previous studies indicate that NGF-treatment could increase ocular dominance plasticity in adult cat visual cortex (Gu *et al.* 1992), suggesting that NGF is involved in activity-dependent synaptic modification. Intracortical NGF-infusion into adult cat visual cortex coincident with monocular deprivation could induce an ocular dominance shift toward the deprived eye, implying that under NGF-treatment, synaptic modification favours the less-active input. To investigate further the mechanism of NGF-effects, single unit recordings were performed during the NGF-infusion period, since it has been shown that complete blockade of postsynaptic activity in kitten visual cortex by muscimol-infusion could cause an ocular dominance shift toward the deprived eye (Reiter and Stryker, 1988, PNAS 85:3623). We found that cortical neurons did not show abnormal responsiveness to visual stimulation while NGF was infused, and that the postsynaptic activity was not blocked by NGF-infusion. Thus, the possibility of an acute pharmacological effect of NGF similar to that of muscimol may be excluded. These results, together with anatomical evidence (Liu *et al.* Soc. Neurosci. Abstr. 1993) support the idea that NGF plays a trophic role in activity-dependent synaptic modification.

690.9

NGF AND BDNF MODULATE EXCITATORY SYNAPSES IN RAT VISUAL CORTICAL NEURONS.
G. Carmignoto*, A. Negro, and S. Vicini. FGIN, Georgetown Univ., Washington DC.

Whole-cell patch-clamp recordings from layer IV visual cortical neurons in coronal slices from 13-18 days old rat brain were used to analyze the effect of Nerve Growth Factor (NGF) and Brain Derived Neurotrophic Factor (BDNF) on spontaneous as well as evoked excitatory post-synaptic currents (EPSCs). Whole-cell recordings of synaptic currents were performed with patch-pipette filled with a Cs MethylSulfonate solution, in the presence of picrotoxin (50 μM) and glycine (10 μM). NMDA and AMPA receptor-mediated EPSCs were recorded in response to focal stimuli applied through a bipolar tungsten electrode in appropriate experimental conditions such as various holding voltages, the presence and the absence of Mg^{2+} and selective receptor antagonists. Spontaneous and evoked EPSC's amplitude and kinetics were studied before and after perfusion for 15 minutes with either NGF (100 ng/ml) and BDNF (10-50 ng/ml). Both neurotrophins consistently increased the amplitude of spontaneous EPSCs recorded in the presence of tetrodotoxin (TTX, 1 μM). Probably due to the large heterogeneity of neuronal types and to the uneven distribution of neurotrophin receptors in visual cortex, in some neurons we failed to observe any effects of NGF and BDNF. In other neurons, instead, the increase of spontaneous EPSC amplitude was accompanied by an increase of evoked EPCS's amplitude. The AMPA and NMDA-mediated component contributing to the peak amplitude of the average of at least 20 evoked EPSCs were differentially affected in distinct neurons. Our results suggest a functional role of NGF and BDNF in modulating excitatory synaptic transmission in visual cortical neurons. These results may represent the molecular mechanism of the reported effects of NGF in the modulation of visual cortical plasticity observed in developing rat (Domenici et al. '91, PNAS 88;8811) and kitten visual cortex (Carmignoto et al.' 93; J.Physiol. 464:343). Supported by NIH grant PO1 NS28130-04.

690.10

EFFECTS OF NGF AND BDNF ON VOLTAGE-GATED CALCIUM CHANNELS IN EMBRYONIC BASAL FOREBRAIN NEURONS.
E. S. Levine*, M. B. Plummer, C. F. Dreyfus and I. B. Black. Dept. Neurosci. & Cell Biol., UMDNJ/Robert Wood Johnson Med. Sch. and Dept. Biol., Rutgers Univ., Piscataway, NJ 08854.

Nerve growth factor (NGF) and other members of the neurotrophin family have well characterized effects on the survival and differentiation of basal forebrain neurons. Recent studies have suggested that some trophic molecules may also modulate the excitability of neuronal membranes. The present studies therefore investigated the effects of both NGF and brain-derived neurotrophic factor (BDNF) on voltage-gated calcium channels of neurons in the basal forebrain.

Whole-cell patch clamp recordings of calcium currents were obtained from embryonic day 17 basal forebrain neurons grown in culture for 4 - 6 days. Neurons were grown in serum-free medium (SFM) with either NGF, BDNF, or a vehicle control added at the time of plating. Peak calcium currents were obtained using a -90 mV holding potential and a +10 mV test potential. In neurons grown in SFM alone, the mean calcium current density was 46.5 ± 5.62 pA/pF. Addition of NGF elicited a 40% increase in the mean calcium current density (mean = 62.9 ± 7.92 pA/pF; $p < 0.01$). This increase appeared to reflect a change in both the sustained and inactivating components of the recorded current. In contrast, the current density of neurons grown in the presence of BDNF was not significantly different from control. These studies indicate that NGF modulates the voltage-gated calcium conductance in basal forebrain neurons, most probably by increasing channel number. Consequently, calcium entry may mediate some of the important neurotrophic effects of NGF.

(Supported by NIH grants HD 23315 and NS 09137)

690.11

DIFFERENT TREATMENT EFFECTS WITH NERVE GROWTH FACTOR IN NBM-LESIONED RATS USING IBOTENIC ACID, QUISQUALIC ACID, AND AMPA. J. Winkler, and L.J. Thal*. Dept. of Neuroscience & Neurology, UCSD & VAMC, San Diego, CA 92161.

Rats received bilateral lesions of the nucleus basalis magnocellularis by infusion of ibotenic (Ibo, n=20), quisqualic (Quis, n=17) and AMPA (n=20). Two weeks after the lesion, osmotic minipumps were implanted that released 5 µg human recombinant nerve growth factor (NGF) or 0.3 µg cytochrome c per day through intraventricular cannulas. Starting one month after the lesion, spatial learning of the animals was tested using the Morris water maze. Lesions with all 3 toxins impaired acquisition of the task, but NGF reduced the average latency to find the platform significantly for the Ibo-lesioned animals only. The 3 toxins reduced Chat activity in frontal/parietal cortex: Ibo 31%/18%, Quis 38%/32%, and AMPA 52%/49%. Six weeks of NGF treatment increased Chat activity for all 3 toxin-treated groups in the hippocampus by about 30%. A significant increase in Chat activity in frontal/parietal cortex was seen in Ibo- (25%/52%) and Quis- (62%/40%) lesioned rats and in frontal cortex (17%) only in AMPA-lesioned rats. These data suggest that treatment with NGF results in an improvement of spatial learning for Ibo-lesioned animals. In addition, Chat activity can be restored for Ibo as well as Quis-lesioned animals possibly because these toxins did not lower Chat activity to the same extent as AMPA did.

690.12

ASSESSMENT OF LEARNING AND MEMORY PERFORMANCE IN MICE HETEROZYGOUS FOR NERVE GROWTH FACTOR DELETION. K. S. Chen *, M. C. Nishimura, S. Broz, C. Crowley, and H. S. Phillips. Dept. of Neurosciences and Dept. of Cell Genetics, Genentech Inc., 460 Point San Bruno Blvd., South San Francisco, CA 94080.

The cholinergic system has been shown to be important in learning and memory function. Lesions of basal forebrain cholinergic neurons or their afferent pathways produce severe memory deficits. These cholinergic neurons have been also shown to respond to nerve growth factor (NGF) and to contain receptors for NGF. Intraventricular infusions of NGF can rescue lesioned cholinergic cells and reverse the observed memory deficits. Additionally, NGF infusions can ameliorate age-related memory deficits as well as reverse the degeneration of basal forebrain cholinergic cells associated with the memory deficits. In the present study heterozygous mutant mice were generated that contained only one normal copy of the NGF gene. These mice did not exhibit any gross morphological or behavioral abnormalities. At 5 months of age the heterozygous mice and normal littermates were tested on the water maze task, a test of spatial learning and memory. The heterozygous mice were significantly impaired in the acquisition of the location of a hidden platform compared to their normal littermates, although the heterozygotes were able to learn the platform location during the two week acquisition period. The deficit exhibited by the heterozygotes in retention of the hidden platform location following a two week retention interval was not as great as the aquisition deficit. Quantitative morphometric analysis of ChAT-positive cells in the basal forebrain will be presented for these animals.

690.13

NGF GENE DELETION PRODUCES SEVERE DEFECTS IN SENSORY NEURONS. H. S. Phillips*, M. C. Nishimura, M. P. Armanini, L. H. Ling, S. B. McMahon, S. Pitts-Meek, A. Levinson, S. Broz, and C. Crowley, Depts of Neuroscience and Cell Genetics, Genentech, Inc. S.S.F., CA 94080 and Dept. Physiology, UMDS, London SE1 7EH.

Homologous recombination was employed to generate mice with a deletion in the coding sequence of the nerve growth factor (NGF) gene. Animals heterozygous for the NGF gene deletion were indistinguishable from normal littermates by visual inspection, but displayed a slight prolongation of tail flick latency. At birth, animals homozygous for the deletion displayed no gross morphological abnormalities and displayed normal spontaneous behavioral patterns. However, homozygotes could be readily distinguished by their failure to respond to noxious mechanical stimulation (tail pinch) and by their reduced size. As homozygous animals exhibited reduced viability, examination of the sensory nervous system was confined to the first few days of postnatal life. Animals homozygous for NGF gene deletion demonstrated an absence of small cells within dorsal root ganglia (DRG), with an approximate cell loss of 70% for the 4th and 5th lumbar ganglia. DRG neurons remaining in these animals expressed trkB and trkC, but not trkA. Homozygotes displayed a complete loss of CGRP-immunoreactive afferents to both the dorsal horn of spinal cord and the epidermis of hairy and glabrous skin. These results confirm earlier antibody-deprivation studies by demonstrating that selective deprivation of NGF by gene deletion has marked selective effects on development of primary sensory neurons.

NEURAL-IMMUNE INTERACTIONS: NEUROENDOCRINE CONTROL OF IMMUNE RESPONSE

691.1

DECREASED TYPE II GLUCOCORTICOID RECEPTOR GENE EXPRESSION IN TRANSGENIC ANIMALS ALTERS THE DEVELOPMENT OF NEUROIMMUNE CONNECTIONS. B. Marchetti*, M.C. Morale, N. Batticane, A. Peiffer and N. Barden. Dept. of Pharmacology, University of Catania Medical School, 95125 Catania Italy, and Molecular Psychogenetics, CHUL, Québec, G1V4G2, Canada.

In the immune system, glucocorticoids inhibit the cascade of immune and inflammatory responses at multiple levels, both directly via action on immunocompetent cells, and indirectly via effects on leukocyte migration and release of inflammatory mediators. To study the glucocorticoid feedback effect on the hypothalamic-adrenal-immune axis, we have used a transgenic mouse with impaired type II glucocorticoid receptor (GR) function caused by GR antisense RNA expression (Pepin et al. 1992, Nature 355:725). Northern blot analysis of endogenous type II GR mRNA indicates a maximal 50-70% decrease in the hippocampus and a 40-60% decrease in the thymus in animals bearing the antisense RNA transgene. In intact mice, age-and sex-dependent changes of type II GR mRNA levels within the thymus accompanied the development of cell-mediated immune response, while transgenic mice showed a significant shift in blastogenic potential, with an almost 3-fold increase in maximal proliferative capacity, and a higher degree of proliferative activity after sexual maturation. While normal adult mice displayed a time-dependent down-regulation of thymic GR mRNA following an antigenic stimulus, in transgenic mice basal immune reactivity was at high levels, and a shift in the time-course of the immune response followed immunogenic challenge, suggesting that type II GR is a critical component of the immune system-hypothalamic-pituitary-adrenal axis loop.

691.2

CHANGES IN LYMPHOCYTE SUBPOPULATIONS IN RESPONSE TO CRH IN NORMAL SUBJECTS. C.M. Bamberger[1], A.M. Bamberger[1], H. Sheng*[2], J. Barth[1], H. Elsen-Ziem[1], H.M. Schulte[1]. [1]I. Medizinische Klinik, University of Kiel, Germany, [2]LDN, NICHD, NIH, Bethesda, Maryland 20892

Acute stress results in activation of the hypothalamic-pituitary-adrenal (HPA) system. It is also known that stress induced activation of the HPA axis is accompanied by changes in the immune response. We previously demonstrated activation of the HPA axis and elevation of serum interleukin-1 alpha (IL-1) and interleukin-2 (IL-2) levels in patients with coronary heart disease undergoing angioplasty as well as in normal subjects receiving CRH by intravenous injection. The purpose of this study was to investigate the influence of CRH on lymphocyte subpopulations in normal subjects. Furthermore, IL-1 levels were measured in CRH-incubated blood monocyte cultures in order to determine the origin of IL-1 demonstrated in our in vivo studies. Ten normal volunteers (20-28 years) received 100 µg human CRH intravenously at 6:00 p.m. Blood was drawn at -15, 0, and 15 min. intervals. Lymphocyte subpopulations (IL-2 receptor positive cells, CD-3, CD-4, CD-8, natural killer (NK), and B cells) were distinguished by immune peroxydase reactions. For the in vitro studies CRH (1 nmol/l) was added to cultured monocytes isolated from venous blood of normal volunteers. IL-1 concentrations were measured in the culture medium in 2 h intervals up to 24 h. In response to intravenously administered CRH, the relative number of IL-2 receptor positive lymphocytes rose from 3.9±1.2 to 6.2±1.6% (n=10, mean±SEM, $p<0.05$). CD-3 cells increased from 74.5±1.6 to 78.3±2.0% ($p<0.05$). No significant change was observed in the other lymphocyte subsets. In vitro, no significant difference in IL-1 concentrations was measurable between CRH-incubated and control cultures. We conclude that CRH administration in normal subjects results in significant changes in the immune response. Our in vitro data suggest that the origin of increased IL-1 levels in vivo may be different to blood monocyte sources. In summary, our studies demonstrate a major role of CRH in neuroendocrine-immune interactions during stress.

691.3

CORTISOL INFLUENCES IN A DIFFERENT WAY LYMPHOCYTE SUBSETS DISTRIBUTION IN ANXIETY AND AFFECTIVE DISORDERS. GI Perini *, Preti A, Zara M, Carraro C, Gava F, Tosin C. Department of Psychiatry, Padua Univ. Sch. of Med., Italy, 35128 PD

The aim of our study was to determine if the distribution of subsets of the major lymphocytes population shows a specific pattern in distinct mental illness. We studied 15 patients with Major Depression, 7 with Panic Disorder, 6 with Generalyzed Anxiety Disorder that were drug free and without concomitant medical diseases. MD patients showed significantly higher T helper % (46 ± 6) compared to PD (38 ± 5) and GAD (34 ± 4) patients (ANOVA: F = 9.18, p = 0.001). T suppressor % was higher in PD patients (31 ± 9) than in MD (24 ± 6) (ANOVA: Fisher post hoc = 6.54, p = 0.05). There was a trend toward higher Urinary Free Cortisol levels in MD (467 ± 348 nmol/24 hrs) compared to PD (217 ± 119 nmol/24 hrs) and GAD (198 ± 128 nmol/24 hrs) patients. In MD patients UFC levels correlate negatively with percentage of T helper cells (Spearman rank test: r= -.73, p = 0.03), whereas in PD patients they correlate positively with T helper % (Spearman rank test: r = .88, p = 0.04). Activation of HPA axis has been described as a characteristic marker in MD. We speculated that the distribution of T cell subsets in MD and in PD patients is influenced in an opposite way by different levels of cortisol.

691.4

TWENTY-FOUR-HOUR CONCENTRATIONS OF INTERLEUKIN-2 IN HEALTHY WOMEN EXHIBIT EPISODIC FLUCTUATIONS: ANALYSIS OF INTEGRATED BASAL LEVELS AND DISCRETE PULSE PROPERTIES. J. Licinio*, M.-L. Wong, M. Altemus, P. B. Bongiorno, A. Bernat, L. Tamarkin, P. W. Gold. Department of Psychiatry, Yale University School of Medicine, West Haven V.A.M.C./116A, West Haven, Connecticut 06516.

Circulating interleukin-2 (IL-2) levels have been measured at one time point in healthy subjects and in patients with a wide variety of inflammatory disorders. It has not been determined if IL-2 is present in the circulation throughout the 24-h period, and whether IL-2 levels are constant or show episodic fluctuation (pulsatility). We examined these points by measuring 24-h levels of IL-2 in six healthy females. Blood samples were collected every 15 min for 24 h, starting at 0800. Cluster analysis was used to identify pulses in the IL-2 time; immunoreactive IL-2 was detectable in all 582 time points, IL-2 concentrations exhibiting pulsatility throughout the 24-h period. The total integrated IL-2 concentration (IIL2C) was 6788±3142 min.μg/L (mean±SD); the integrated pulse IL-2 concentration was 93.1±28.9 min.μg/L; the overall pulsatile IIL2C was 1205±319 min.μg/L, or 20±7% of the total IIL2C. The mean IL-2 concentration was 4.71±2.18 μg/L; mean pulse frequency was 13.3±2.3; mean pulse height was 6.16±2.44 μg/L, and mean pulse width was 75.7±13.7 min. Pulsatility of circulating IL-2 throughout the 24-h period suggests that IL-2 may function as a classical hormone, being secreted in the blood stream and acting at distant sites. Future studies of IL-2 blood levels should consider these findings that circulating IL-2 levels are pulsatile.

691.5

ASTROGLIAL CELLS IN PRIMARY CULTURE RELEASE FACTORS THAT PROMOTE THE GROWTH OF GT1-1 LHRH NEURONAL CELLS AND STIMULATE LEUKOCYTE PROLIFERATION. F. Gallo*, R. Avola, A. Costa & B. Marchetti. Depts of Pharmacology and Biochemistry, University of Catania Medical School, 95125 Catania, Italy.

Glial mechanisms are hypothesized to be involved in the marked changes in synaptic and interneuronal organization that occur under various physiological conditions. Recent evidences indicate that astrocytes are immunologically competent cells that share many important functional characteristics with macrophages, including the ability to both produce and respond to a broad spectrum of soluble immune mediators. The hormonal sensitivity of the glia and its involvement in estrous cycle-related changes in synaptic circuitry prompted us to study the trophic interactions between astroglial cells and the luteinizing hormone-releasing hormone (LHRH) neuron. For this aim, an immortalized hypothalamic LHRH neuronal cell line (Mellon et al. 1990, Neuron 5: 1) was used. In this experimental design LHRH neuronal cells (GT1-1 subclone) were cultured alone, in the presence of astroglial cells, or in medium conditioned (CM) from developing astroglial cell cultures. Media from cultured LHRH neurons were collected 2, 4, 6 and 8 days following coculture with glial cells obtained from cerebral hemispheres of newborn rats, or after addition of astroglial cell CM. A significant stimulation of GT1-1 neuronal cell proliferation and increased LHRH secretion followed coculture with astrocytes at late stages of differentiation. Moreover, a marked stimulation of cell proliferation followed CM addition to leukocyte cultures, indicating that the astroglial cells produce trophic factors that participate in CNS intercellular communications and in the interactions between the nervous and immune systems.

691.6

PAVLOVIAN CONDITIONING OF MORPHINE-INDUCED IMMUNE ALTERATIONS: EVIDENCE FOR INVOLVEMENT OF THE ß-ADRENERGIC SYSTEM Mary E. Coussons*, Linda A. Dykstra, and Donald T. Lysle. Departments of Psychology and Pharmacology, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599-3270

Previous work in our laboratory has shown that reexposure to an appetitive stimulus developed through pairings with morphine can elicit profound conditioned changes in immune status. The observed pattern of conditioned immunomodulation is very similar to that of a conditioned aversive stimulus previously paired with electric shock. Work in our laboratory has clearly demonstrated that the ß-adrenergic system is involved the immunomodulatory effects elicited by an aversive conditioned stimulus. The present studies were aimed at determining the involvement of this system in the establishment and expression of morphine-induced conditioned immune alterations. During the training phase, rats received conditioning sessions during which injections of morphine were paired with exposure to a distinctive environment. On the test day, animals were reexposed to the distinctive environment or remained in their home cages prior to sacrifice. Saline or nadolol (0.002, 0.02, 0.2, or 2.0 mg/kg) was administered either prior to training or to test. Administration of nadolol prior to training did not diminish the conditioned changes in immune status; nadolol administration prior to testing antagonized a subset of the conditioned immune alterations elicited by exposure to the conditioned appetitive stimulus. These findings suggest that the ß-adrenergic system is involved in the expression, but not the establishment, of conditioned morphine-induced immune alterations. (Supported by PHS grants DA02749, DA07481, DA05522 and MH46284.)

691.7

Cocaine and Cocaine/ß-Endorphin Mixtures: Effects on Transmembrane Signaling in Normal Human T Cells. F. Chiappelli* P. Frost & C. Bender. Anatomy & Cell Biology, UCLA-1763, Los Angeles, CA 90024; Human Immunol. Psycho-neuroimmunol., Brentwood VAMC (151).

Cocaine abuse is associated with a significant modulation of cellular immune responses. We have shown that cocaine alters the expression of several membrane markers of T cell activation in vitro (CD25, CD71 and HLA-Dr). Because cocaine administration leads to activation of the pituitary-adrenal axis in vivo in both experimental animals and in control human subjects, and because we previously showed that ß-endorphin modulates membrane signaling events during human T cell activation, we have characterized the effects of cocaine and cocaine/ß-endorphin mixtures on certain transmembrane signaling outcomes in human T cells during activation with T cell mitogens, or via the CD3/TcR pathway either in the absence or in the presence of CD28 co-stimulus. Our experiments include testing the small GTP-carrying protein, $p21^{ras}$, because of its important role during T cell activation. (NIDA 07683).

691.8

NE TURNOVER IN SPLEENS FROM YOUNG AND OLD F344 RATS. D.L. Bellinger*, N. Costello, S.Y. Felten, & D.L. Felten. Dept. Neurobiol. & Anat., Univ. Rochester Sch. Med., Rochester, NY 14642.

With normal aging, the frequency of viral infections, cancer, and autoimmune diseases increases, cell-mediated immune responses decline, degenerative brain changes occur, and psychosocial factors have a greater impact on health. Studies from our laboratory, as well as others, have revealed a role for noradrenergic (NA) sympathetic innervation of primary and secondary lymphoid organs in modulation of immune function. Further, we have demonstrated a striking decline in the presence of NA nerves and norpinephrine (NE) content in spleens from aged F344 rats that parallels the time course of age-associated decline in cell-mediated immunity in these rodents. The present study examines NE turnover in spleens from 3- and 21-mo-old rats by measuring the rate of splenic NE degradation following α-methyl-p-tyrosine inhibition of tyrosine hydroxylase, the rate-limiting enzyme in the synthesis of NE. Mean NE turnover rate was elevated in aged rats compared with their young adult counterparts. This finding suggests an increase in the activity of NA nerves in spleens from aged F344 rats. Increased NE metabolism probably occurs as a compensatory mechanism resulting from the loss of NA nerve terminals that occurs with aging. Enhanced NA nerve activity with age may be contributory to further decline in NA innervation of spleen through elevated oxidative metabolites, like 6-hydroxydopamine, that are neurotoxic to NA nerves. Supported by R29 MH47783-02, and Sandoz Foundation for Gerontological Research.

691.9

LOCALIZATION AND CELLULAR DISTRIBUTION OF INTERLEUKIN-1 RECEPTOR ANTAGONIST mRNA IN RAT BRAIN. M.-L. Wong*, J. Licinio. Department of Psychiatry, Yale University School of Medicine, West Haven V.A.M.C./116A, West Haven, Connecticut 06516.

Interleukin-1 (IL-1) is a potent inflammatory cytokine with a vast range of biological effects. There are three forms of IL-1, namely IL-1α and IL-1ß, which occupy IL-1 receptors and elicit biological responses, and IL-1 receptor antagonist (IL-1ra), which occupies IL-1 receptors with the same affinity as IL-1α and IL-1ß, but elicts no biological responses, being a pure endogenous IL-1 antagonist. IL-1 antagonism by means of exogenous IL-1ra administration has been shown to protect neurons from ischemia. We therefore hypothesized that IL-1ra might be present in brain, possibly as an endogenous neuroprotective agent. The recent cloning of rat IL-1ra mRNA has permitted us to conduct *in situ* hybridization histochemistry (ISHH) studies using a rat riboprobe to localize IL-1ra mRNA in rat brain. We performed ISHH with a ^{35}S-labelled antisense riboprobe for rat IL-1ra mRNA and localized IL-1ra mRNA in hippocampus, hypothalamus, choroid plexus, and cerebellum. Control hybridizations revealed no specific binding to those or other regions. Anatomical distribution of the probe at the cellular level was performed by dipping the slides in photographic emulsion, exposing for 45 days, developing, and counterstaining. IL-1ra mRNA was localized in neurons in glia, in the same regions where IL-1ß mRNA has been described. We conclude that endogenous IL-1ra exists in brain, where it might function as an endogenous neuroprotective agent.

691.10

ELECTRICAL STIMULATION OF THE RAT MIDBRAIN PERIAQUEDUCTAL GRAY SUPPRESSES PERIPHERAL BLOOD NATURAL KILLER CELL ACTIVITY. **M.K. Demetrikopoulos*, S.E. Keller, S.J. Schleifer, and A. Siegel Dept. of Neurosciences, Grad. School of Biomedical Science, and Dept. of Psychiatry, N.J. Med. School, UMDNJ, Newark, NJ 07103**

It has been previously reported that electrical stimulation of the periaquaductal gray (PAG) leads to suppression of splenic natural killer cell (NK) activity (Weber & Pert, Soc. for Neuroscience Ab., 1990). The present study examined the effect of PAG stimulation on both splenic and peripheral blood NK function as well as the blastogenic response of lymphocytes to phytohemagglutinin (PHA) mitogen. Male Sprague-Dawley rats were implanted with bipolar electrodes in rostral aspects of the dorsal PAG. Two weeks following recovery, bipolar electrical stimulation was delivered once per min. for 30 min. to the freely moving rats [biphasic, rectangular electrical pulses (0.4 mA, 62.5 Hz, 1ms half cycle duration) from a Grass S-88 stimulator]. Peripheral blood NK activity was determined by use of a modified 4 hr chromium release 'whole blood' micro-assay, and splenic NK activity was determined by a standard 4 hr chromium release assay. There was no effect of PAG stimulation on blastogenic response to mitogen. While PAG stimulation did not suppress splenic NK activity, PAG stimulation produced a marked decrease in peripheral blood NK response ($F = 5.49$, $p < .03$). Differences from previous reports may relate to stimulation localization, sampling time, and cell purification. These results suggest that the central mechanisms involved in the modification of NK function may differ for peripheral and splenic subsets.

CONTROL OF POSTURE AND MOVEMENT XI

692.1

ENTRAINMENT OF RAPID WRIST MOVEMENT BY ESSENTIAL TREMOR. R. J. Elble*, C. Higgins and L. Hughes. Dept. of Neurology, Southern Illinois Univ. Sch. of Med., Springfield, IL 62794-9230.

The effect of 4- to 7-Hz essential tremor on the timing of rapid wrist flexion was examined in ten patients with moderate to severe disability, using a computer-controlled torque motor and manipulandum. The mean reaction time and motor time of the patients did not differ from the mean values of 10 healthy age- and sex-matched controls. The latencies of the triphasic agonist and antagonist EMG bursts did not differ between patients and controls. The initiation of movement was time-locked to the tremor cycle in all patients. The activation of the agonist muscle occurred in phase with the rhythmic bursts of EMG, but the onset of rapid wrist flexion occurred during the wrist extension phase of the tremor cycle, when the momentum of essential tremor opposed the volitional movement. Similar phase relationships occur with Parkinson tremor (Staude et al., Z. EEG-EMG 1992;23:108-113), but normal people extend their index finger with the momentum of the 8- to 12-Hz physiologic tremor, as though physiologic tremor served to facilitate movement (Goodman and Kelso, Exp. Brain Res. 1983; Exp. Brain Res. 49:419-431). The rhythmic entrainment of motor units dictates the timing of volitional muscle activation in all three forms of tremor. The timing of movement with respect to the joint oscillation will be determined by the motor time, the tremor period (frequency), and the phase between EMG and joint tremor. The seemingly beneficial phase relationship between volitional movement and physiologic tremor is possibly a fortuitous result of the latter two factors. (Supported by NINDS NS20973).

692.2

PROGRESSIVE SPECIFICATION OF RESPONSE AMPLITUDES IN A POINTING TASK. W. A. Hening*[1,2], M. Rolleri[1], and J. Gordon[3]. Neurology Depts, VAMC[1], Lyons, NJ & UMDNJ-RWJohnson Med Sch[2], New Brunswick, NJ; and Ctr for Neurobiology & Behavior[3], Columbia Univ, NY, NY

In a reaction time (RT) task, RTs were modulated by instructions about how soon subjects should initiate their responses (Favilla et al, Neurosci Abstr 13:13). Isometric jerks aimed at predictable targets (Simple Condition, SIM) were accurate at all RTs. However, responses to unpredictable targets (Choice condition, CHO) made at short RTs were deficiently specified and their amplitudes clustered near the center of the target range while, with increasing RT, amplitudes were progressively more accurately specified. We have now examined how subjects specify the accuracy of a pointing movement. With arms hidden, 3 normal adult subjects moved a cursor over a digitizing tablet to match 4 visual targets requiring 2.4 to 24 cm movements. Their SIM responses were accurate at all RTs, but they gradually improved specification of CHO responses with longer RTs. 2 subjects achieved this by increased preprogramming of responses, evidenced by peak accelerations highly correlated with target amplitude (R^2>75%) at RTs greater than 300 ms. One subject used a strategy of adaptive modification of late trajectories to achieve accuracy. Subjects almost invariably overshot the small target at RTs <200 ms, but could extend movements to match larger targets. In conclusion, the subjects showed a progressive specification of CHO responses, but they used a somewhat different means for achieving accuracy than observed with isometric jerks. Early matching of large targets was facilitated by the longer duration of these arm movements.
This research supported by the Dept of Veterans Affairs Medical Research Council.

692.3

THE CENTRAL COMMAND FOR VOLUNTARY MOVEMENT: DOES IT EXPRESS A NOMINAL FORCE OR A VIRTUAL TRAJECTORY? G. L. Gottlieb*. Rush Medical Center, Chicago, IL, 60612.

The voluntary movement of a single joint or a single limb can be regarded as a realization of some command by the central nervous system, expressed at the segmental motoneuron pools. This command is carried out by the contraction of the activated muscles, interacting with the physical load. One school of thought has regarded the command as intrinsically spatial in nature, specifying the kinematic trajectory of a moving equilibrium point, towards which the limb is attracted. An alternative to this is to consider the plan as a nominal force trajectory, interacting compliantly with the load. In either case, movements of a limb are always sufficiently slow that reflexes of many sorts may potentially play a role in the activation of the muscles but the degree and timing of this role have long been a source of dispute.

We will present data on both single and multi joint arm movements, performed under different speed, load and distance conditions. These movements can be interpreted as emerging from central force plans that rely little on concurrent reflex action. This perspective provides a more comprehensive description of and better insight into the mechanisms of voluntary motor control than any current version of the equilibrium point hypotheses.

This work was supported by NIH grants AR 33189, NS 28176 and NS 28127.

692.4

MOVEMENT PERFORMANCE IN A DOUBLE-STEP POINTING PARADIGM. O.Bock*. Human Perf.Lab, Inst.f.Space&Terrestr.Sci., York Univ., Toronto, Ont. M3J 1P3, CANADA.

The present study investigates how double-step pointing responses in different orientations with respect to Earth's field of gravity.

Human subjects pointed, without seeing their arm, at mirror-viewed visual targets presented in a frontal plane at eye level 40 cm ahead. Targets could be displaced after 25-100 ms, requiring a modification of movement direction in the frontal plane. Index fingertip position was measured contact-free by the WATSMART® system.

If the time between target displacement and movement onset (RT2) was brief, initial movement direction (ID) was towards the original target; with longer RT2, ID gradually changed towards the displaced target. This confirms earlier studies that internal target representation changes gradually. The slope of ID versus RT2 was 270 deg/s if ID changed from horizontal to oblique, and 420 deg/s if ID changed from vertical to oblique, whether the changes were assisted or opposed by gravity. Direction early *after* movement onset didn't remain constant but rather changed at a rate of 580 deg/s (hor. to oblique) and 360 deg/s (vert. to oblique); values were smaller when the changes were resisted, and larger when they were assisted by gravity.

We conclude that the gravity vector provides a spatial reference for movement preparation, that initial differences between hor. and vert. directions are adjusted early in the movement, and that gravitational force is not fully compensated when movement direction is changing.

Supported by the Can.Space Agency, MITT Ontario, and NSERC.

692.5

NORMAL DEVELOPMENT OF HUMAN LIMB CONTROL REQUIRES FOVEAL INPUTS FROM AREA V_1. M. Rizzo*, W. Darling. Department of Neurology, University of Iowa, Iowa City, IA 52242.

We studied the reaching movements in a 21 year old man with severe bilateral occipital lobe lesions acquired at birth. MR imaging showed that the right occipital lobe was nearly absent; the lesion on the left fully damaged the foveal representation. Our aim in the current study was to evaluate how the loss of a foveal representation affected limb control during reaching tasks. Visual acuity was 3/500 and the patient could navigate without bumping into objects. This relied on extrafoveal fixation which was mediated by a region of intact left superior visual cortex. Hand movements were recorded using an optoelectronic technique. The patient was asked to reach for different targets under a variety of conditions. Self-directed movements were normal with both hands. Right hand movements to suprathreshold visual targets showed near normal velocity and accuracy. However, there were curved handpaths and high endpoint variability with the left hand. Moreover, in comparison to normal control subjects, the patient made hand movements to remembered visual targets that showed large overshoot errors for both far and near targets. Azimuth and elevation errors were also noted. The pattern of findings suggests that the neural system that transforms visual representation of target locations into a kinesthetic coordinate system to program reaching movements requires the influence of foveal cortex for normal development.

692.6

VISION OF THE LIMB ALLOWS DEAFFERENTED PATIENTS TO CONTROL MULTIJOINT DYNAMICS R.L. Sainburg, M.F. Ghilardi, H. Poizner, and C. Ghez* Columbia Univ., Ctr. for Neurobio. & Behav., NY.10032; CMBN, Rutgers U., Newark, NJ 07120.

We have recently shown that proprioceptive deafferentation produces severe deficits in interjoint coordination due to failure to control interaction torques that develop across joints (Neurosci. Abs. 18:647). We now ask whether these deficits are reduced when patients view their limb. We analyzed muscle activation patterns and joint torques at the elbow during the performance of two-joint, horizontal arm movements in two patients with large-fiber sensory neuropathy and 5 control subjects. Subjects were to trace a series of straight paths in different directions from a central starting position and to reverse direction without stopping. Target paths were chosen to keep elbow joint excursion constant while varying shoulder excursion. Whether or not they viewed their limb, control subjects made straight, accurate movements that reversed direction sharply. They synchronized shoulder and elbow joint movements by varying the time and magnitude of muscle activation, utilizing or dampening the effects of interaction torques to regulate elbow kinematics. Without vision of their limbs, the patients were unable to temporally coordinate the movements of their elbow and shoulder joints at movement reversals. This resulted from failure to control large interaction torques at the elbow due mainly to shoulder deceleration. Muscle patterns were not adapted to direction-dependent variations in interaction torques and showed excessive co-contraction. When patients viewed their limbs during movement, reversal errors and interjoint coordination deficits were dramatically reduced. The timing of muscle activities were better adapted to direction-dependent variations in interaction torque. We conclude that visual information about limb movement can be used to partially control limb multijoint dynamics, but proprioceptive information is critical for optimal control. Supported by Grants NS 227715, JSMF 91-45, McKnight Fnd.

692.7

DIRECTIONAL BIASES IN TARGETED ARM MOVEMENTS RESULT FROM DISTORTIONS IN THE REPRESENTATION OF THE WORKSPACE. M.F. Ghilardi, J. Gordon*, C. Ghez. Ctr. for Neurobiol. & Behav., Prog. in Phys. Ther., Columbia Univ. and NYS Psych. Inst., New York, NY 10032.

We have previously shown that, when subjects use a computer screen to aim hand movements on a horizontal digitizing tablet, movements show directional biases that vary with the initial position of the hand. The nature of these biases suggested that in planning movement direction the nervous system underestimates the distance of the hand from the midline. In the present report, we further characterized these errors.

The directional biases are independent of the location of the computer screen; they vary systematically with the mediolateral distance of the initial position of the hand from the midline but are independent of its anteroposterior distance from the body. They disappear when targets are presented directly within the workspace. Therefore, these biases initially appeared to represent errors in transforming the direction of the required movement from screen to hand coordinates. However, the same errors occurred when targets were not presented on the screen and subjects were verbally instructed to perform straight movements in one of the four cardinal directions without viewing their limb. Thus, the error results from the transformation of a distorted representation of space into actual workspace coordinates. Similar biases were present in visual discrimination and proprioceptive matching tasks performed in the horizontal plane.

We conclude that subjects' representation of peripersonal space is distorted and proprioception does not provide adequate information to accurately plan movement direction. (NS 22715)

692.8

MINIMUM-JERK PRINCIPLE, ISOCHRONY, AND THE TWO-THIRDS POWER LAW: CONVERGING APPROACHES TO MOTOR PLANNING. P. Viviani* and T. Flash. Dept. of Psychobiology, FAPSE, University of Geneva, Switzerland; INB-CNR, Milan, Italy[¶] and The Weizman Institute, Rehovot, Israel[§]

One approach to the logic of motor planning is based on the assumption that voluntary movements comply with an optimality criterion concerning their smoothness. It was shown (Hogan, 1984; Flash & Hogan, 1985) that a minimum-jerk constraint, associated with appropriate boundary conditions, predicts both trajectory and kinematics of point-to-point movements. An independent line of research (Viviani & Schneider, 1991) has demonstrated that most end-point movements are constrained by two empirical covariations between kinematic and geometrical parameters; the *Isochrony Principle* states that average velocity is an increasing (power) function of the linear extent of the trajectory, while the *Two-thirds Power Law* relates the instantaneous values of velocity and curvature. A body of experimental data on drawing movements was used to test whether the minimum-jerk logic predicts these empirical covariations. With the help of a digitizing table (sampling: 100 Hz.), we recorded the motor performances of adult subjects who traced continuously three complex, closed trajectories (cloverleaf, asymmetric lemniscate and oblate limaçon), at three imposed rhythms. We found that the convergence of the two approaches is satisfactory insofar as the relation between instantaneous velocity and curvature is concerned. Moreover, the durations of the movement sub-units - which, according to the isochrony principle, scale with the length of the corresponding segments of trajectory - is also accurately predicted by the minimum-jerk model. By contrast, the global scaling of average velocity as a function of total trajectory length cannot be derived from the optimality hypothesis. The implications of the this pattern of convergence vis à vis the general issue of movement planning are discussed with an emphasis on the format in which the motor control system represents the intended trajectories.

692.9

GEOMETRIC STRUCTURE OF THE ADAPTIVE CONTROLLER FOR ARM MOVEMENTS. R Shadmehr*, and FA Mussa-Ivaldi. Dept. of Brain and Cognitive Sciences, MIT, Cambridge, MA 02139.

In order to investigate how the CNS adapts a motor behavior, we considered a task which required subjects to interact with a virtual mechanical environment. The environment was a force field produced by a robot manipulandum. The subjects made reaching movements while holding the end-effector of this manipulandum. Initial movements in the field were grossly distorted compared to movements in free space, but practice led to convergence of the hand trajectories to a path very similar to that observed in free space. This indicated that for reaching movements, there was a kinematic plan independent of task dynamics. In order to investigate how subjects adapted to the force field, for some movements during the practice trials the field was temporarily removed (unbeknown to the subject). The resulting trajectories, named *after-effects*, were approximately mirror images of those observed in the field, suggesting that the motor controller was gradually composing an *internal model* of the virtual environment. In order to explore the structure of this internal model, we asked whether adaptation to a force field, as presented in a small region, led to after-effects in other regions of the workspace. We found that there were after-effects in workspace regions where no exposure to the field had taken place, i.e., there was transfer. From the coordinate system in which this transfer took place we inferred the geometric structure of the internal model. The transfer was consistent with an internal model which represented the field in an intrinsic, e.g., joint centered, coordinate system. Our observations are in line with a theoretical framework where the process of adaptive control is viewed as that of composition of an internal model through force field approximation. This approximation may be accomplished by the CNS through co-activation of a small set of pattern generators, each producing a time varying force field. The result is a highly structured adaptive controller which learns interaction with a novel environment through co-activation of a set of elementary behaviors. Supported by NIH: NS09343 & AR26710, ONR: N00014/K/0372, and a McDonnell-Pew fellowship.

692.10

THE GRASPING HAND: AN OPPOSITION SPACE MODEL OF PREHENSION. T. Iberall* and C. L. MacKenzie. Computer Science, U. of Southern Calif., L.A., CA 90089 Kinesiology, Simon Fraser U., Burnaby, B.C., Canada V5A 1S6

Using a triangle strategy to integrate conceptual, computational, and experimental models, we study the nature of prehension. Prehension is the application of functionally effective forces to an object for a task, given numerous constraints. These constraints include: task requirements, object properties, environmental characteristics, and the performer's biological structure and past experience. Reaching and grasping are controlled by parallel distributed processing in the CNS within a multidimensional constraint space, conceptualized by Arbib's coordinated control program (1981) and expanded in MacKenzie and Iberall (in press). An opposition space, as a collection of virtual fingers, defines the hand's functional capabilities for executing stable grasp and object manipulation. This abstraction focuses on key dimensions of task requirements (force, movement, sensation). Real fingers group together into virtual fingers to apply functionally effective force using pad, palm and/or side opposition. See MacKenzie and Iberall.

Supported by NSF#BCS-9009934 and NSERC.

692.11

AN OPPOSITION SPACE MODEL OF PREHENSION: THE GRASPING HAND. C. L. MacKenzie* and T. Iberall, Kinesiology, Simon Fraser U., Burnaby, B.C., Canada V5A 1S6 Computer Science. U. Southern Calif., Los Angeles, CA 90089

MacKenzie and Iberall (in press) identify unique phases of prehension: planning, setting up, using, and releasing an opposition space by the grasping hand. For each phase, we present experimental evidence and computational models of the role of information from various sensory modalities, levels of motor commands, triggering events, and coordinate frames of reference. For example, in using an opposition space, active touch information (dominant over vision) combines with mechanical characteristics of skin (adhesion and friction, e.g., by papillary ridges and eccrine sweat glands) to enable the neuromuscular system to apply appropriate forces for grasping and manipulative stability, demonstrating anticipation of task mechanics. Integrating conceptual, computational, and experimental models of prehension, we identify areas for further experimentation and modelling. See Iberall and MacKenzie.

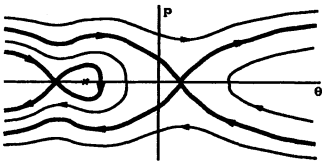
Supported by NSERC and NSF#BCS-9009934.

692.12

THE DYNAMICS OF RISING FROM A SITTING POSITION. P.Roberts and G.McCollum*, R.S.Dow Neurological Sciences Institute, Good Samaritan Hospital & Medical Center, Portland, OR 97209.

Rising from a sitting position is an important task for stroke and trauma patients to master during rehabilitation. Although the movement involved can be functionally described as moving from one position of stability to another, the physical details of the movement may vary broadly. For instance, physical therapists teach patients to lean far forward in the chair, *nose over toes*, so that the center of mass is brought over the ankles and stable stance results from extending the knees. In contrast, healthy people often utilize the *momentum method* in which they flex the abdominal muscles to give momentum to the upper trunk before extending the knees and thereby "hop" from stable sitting to standing.

In this study we take advantage of global analysis used in the modern approach to non-linear dynamics, and apply these techniques to the problem of body movement. After writing a set of coupled first order differential equations describing the possible movements, we are able to take relevant slices of the resultant phase space diagrams to elucidate the different strategies available to rise from sitting. The objective is to unite continuous physical properties of a multijointed system with discrete functional properties found in goal directed behavior.



Nose over toes (angular momentum *vs.* ankle angle)

AUDITORY SYSTEM

693.1

RESPONSES OF SACCULAR NERVE FIBERS AND TORUS SEMICIRCULARIS UNITS OF THE GOLDFISH TO TWO-TONE STIMULI. Z. Lu* and R. R. Fay. Parmly Hear. Ins. and Dept. Psychol., Loyola Univ. Chicago, 6525 N. Sheridan Rd., Chicago, IL 60626.

Responses of single units were recorded from saccular nerve (SN) and torus semicircularis (TS) of the goldfish. Frequency-response functions (FRF), PSTHs, and rate-level functions were obtained in response to single- and two-tone stimuli. For all units, a single-tone FRF was determined as an iso-level, spike-rate function of tone frequency (75-1250 Hz). Rate level functions were determined at characteristic frequency (CF). In two-tone experiments, FRFs at three levels above CF threshold were determined in the presence of a simultaneous probe tone at CF. Two-tone rate-level functions were determined for the CF tone in the presence of "suppressor" tones fixed at various frequencies and levels. The magnitude of two-tone suppression or inhibition was defined as the horizontal distance (in dB) between the single-tone and two-tone rate-level functions. For some torus units, FRFs for both single- and two-tone stimuli resemble those of saccular nerve fibers, and exhibit features due to single-tone suppression, two-tone suppression, and response entrainment to the stimulus frequency. However, qualitative and quantitative differences were observed in FRFs, PSTHs and rate-level functions between SN and the TS units. Some TS units exhibit complex inhibitory areas above and below CF that are not observed in SN units. For suppressor tones above CF, the magnitude of suppression in torus units is generally greater than SN units, suggesting that the FRFs of some torus units are shaped by inhibition in the auditory CNS. [Work supported by a Center Grant to Parmly from NIDCD.]

693.2

COCHLEAR NUCLEUS ENHANCEMENT OF AMPLITUDE MODULATION ENCODING IS PRESERVED IN BACKGROUND NOISE. R.D. Frisina*, K.J. Karcich, and J.P. Walton. Otolaryngology Div., Physiology Dept., U. Rochester Sch. of Medicine & Dentistry, Rochester, NY 14642.

Sound envelope temporal fluctuations are important for effective processing of biologically-relevant acoustic information including speech, animal vocalizations, sound-source location and pitch. Amplitude modulation (AM) of sound envelopes can be encoded in quiet with high fidelity by many auditory neurons including those of the auditory nerve (AN) and cochlear nucleus (CN). From both neurophysiological and clinical perspectives, it is critical to understand the effects of background masking noise on the processing of AM. To further this goal, single-unit recordings were made from 106 CN units and 36 AN fibers in 18 anesthetized chinchillas. Units were classified and located in the CN according to PSTH response patterns, 1st spike latencies, shape of best-frequency (BF) rate-intensity functions, regularity analyses and mapping with extracellular HRP injections. BF pure-tone bursts, wideband noise bursts, and AM (10 to 500 Hz) BF tone bursts were employed as stimuli at several sound levels, both in quiet and in the presence of a continuous wideband noise. Data analyses indicate that 1) CN units can enhance their AM coding relative to the AN even in the presence of loud (0 or 6 dB S/N) background noise and at high sound levels (up to 75 dB SPL); 2) In the dorsal CN this enhancement is achieved by lowering the average firing rate and increasing the synchronous (fundamental frequency) response; 3) For AN fibers and in some dorsal CN units, the AM coding stays the same or declines in the presence of the background noise. In these cases, the synchronous and average responses both decline. These findings suggest that part of a CN unit's abilities to preserve or enhance AM coding in the presence of a continuous masking noise results from shifts in the operating ranges of AN fibers whereas part comes from intrinsic neural circuitry or cellular mechanisms within the CN.

[Supported by NIH-NIDCD Grant #: R29 DC00408 and the International Center for Hearing & Speech Research - RICHS, Rochester NY]

693.3

CNS REPRESENTATION OF ACOUSTIC CLICK TRAINS IN A SONIC FISH. J.D. Crawford.*Psychology Dept., U. of Pennsylvania, Philadelphia, PA 19104.

In the African mormyrid fish Pollimyrus isidori, acoustic signals are important in communication. These animals produce 5 different low frequency (< 1 KHz), pulsatile, sounds. Neurophysiology shows that these fish are sensitive to sound, and specialization of the inner ear seems to allow sound pressure detection: the sacculi (otolithic organs) are each attached to one of a bilateral pair of gas bladders in the head. Since the temporal spacing of clicks, comprising fish sounds, appears to carry important communication information, I have conducted experiments to examine responses of neurons in relation to inter-click-interval (ICI) and level.

Extra-cellular, recordings were made in the auditory mesencephalon (nucleus MD) while fish were 30 mm sub-surface in a water-filled, calibrated, tank. Each neuron was initially stimulated with tones to estimate its best excitatory frequency (BEF), best excitatory sensitivity (BES), temporal response characteristics at BEF, and BEF rate-level-function. Subsequently, 400 ms click-trains were presented, with 8 ICIs ranging from 6 to 80 ms. Within an experiment, level and click waveform were constant, and ICI sequence was randomly determined. By the end of 256 stimulus presentations, each ICI was used 32 times. For neurons that appeared to have a preferred ICI, additional data were collected with different stimulus levels.

Over 40 neurons have been examined, about a third showed clear ICI-dependent responses. Several distinct response types have been found, including highly click-locked responses, non-locked responses with a dominant inter-spike-interval (ISI, ISI ≠ ICI), and non-locked responses without a dominant ISI. Among these, ICI-tuned responses, ICI long-pass, and ICI short-pass profiles have been observed. ICI-tuned neurons most often respond best in the 20-40 ms range, but several best ICIs have been in the 7-10 ms range. ICI-selective neurons are probably important in processing sounds that these animals produce (R01DC1252-01A1 & DC00293-06).

693.4

ABSTRACTION OF THE MISSING FUNDAMENTAL FOLLOWING BILATERAL LESIONS OF AUDITORY CORTEX **J.I. Bharucha, M.J. Tramo* & R.L. Zatorre Dept of Psychology, Dartmouth Coll, Hanover, NH; Dept of Neurobiology, Harvard Med Sch, Boston, MA; Dept of Neuropsychology, Montreal Neurological Inst**

The pitch of a harmonic complex tone corresponds to the pitch of its fundamental frequency (F0), even when energy at F0 is missing. While information about this "virtual pitch" is present in the temporal discharge pattern of auditory nerve fibers (Delgutte & Cariani 1992), dichotic studies indicate that the abstraction process is central (Houtsma & Goldstein 1972). One study of anterior temporal lobectomy patients has demonstrated that, in most cases, right-sided excisions extending posteriorly into Heschl's gyrus impair the abstraction of virtual pitch (Zatorre 1988). Employing a similar experimental design, we examined a 34 year old stroke patient with bilateral lesions of Heschl's gyri and portions of the superior temporal gyri (Tramo et al. 1990). Judgments of the relative pitch of two harmonic complex tones presented at 75 dB SPL were significantly less accurate when energy at F0 was missing (65%) than when energy at F0 was present (92%). Although the patient's performance was significantly above chance for stimuli missing F0, it was significantly worse than that of right temporal lobectomy patients with Heschl's gyrus excisions. These results suggest that: 1) normal abstraction of virtual pitch relies on the integrity of primary auditory cortex; 2) the magnitude of the deficit may depend on the amount of the damage to primary and non-primary auditory cortical areas; and 3) in the chronic stage following ischemic stroke, the contributions of non-primary cortical areas and/or subcortical structures are sufficient to sustain above-chance performance. Supported by NINDS NS17778, NSF DBS-9222358, NIDCD DC00071, MRC MT11541

693.5

PATTERNS OF RESPONSE PLASTICITY IN THE RECEPTIVE FIELD OF THE RAT AUDITORY CORTEX AFTER CONDITIONING. M.E.Taylor and H.K. Rucker. Meharry Medical College, Nashville, Tn. 37208.

The specific aim of these studies was to characterize the patterns of response plasticity in receptive fields of the auditory cortex of rat after conditioning. During conditioning, acoustic stimulation was paired with rewarding medial forebrain bundle (MFB) stimulation. Twenty-one neuronal multiunit clusters isolated in the non-primary auditory cortex of urethane anesthetized were characterized with regard to their frequency receptive fields. Neuronal activity was recorded after tone presentations from 4 kHz to 28 kHz (increments of 4) at 80 dB to establish a baseline. After establishing the baseline, neuronal activity was recorded and characterized after sensitization and after conditioning. Prior to sensitization training, neurons exhibited increased firing at best frequency (BF) and decreased firing at other frequencies. After sensitization, there were either a generalized increase in neuronal firing throughout the frequency receptive field or no significant changes in firing from baseline. After the conditioning training, the receptive fields were analyzed for changes in neuronal activity at BF and at the conditioned stimulus (CS) frequency. After conditioning, approximately 48 % (8/18) neurons exhibited a decrease in neuronal firing at BF with an increase in firing at CS frequency. There were differences in the incidence of increase neuronal firing at CS frequency with decreased BF if the CS frequency came before rather than after the BF. Approximately, 37% (3/8) demonstrated an increased CS frequency firing with decreased BF firing when the CS frequency was before the BF; while, 63% (5/8) exhibited the same response patterns when the CS frequency was after the BF. When the BF and the CS frequency were the same (3/3), there were no significant changes in the receptive field profile; increased firing remained at best frequency. Overall, the CS exerted significant effects on neuronal firing at that frequency. 67% (12/18) of neurons exhibited an increase in neuronal firing at CS; while, 11% exhibited a decrease in firing. However, the CS caused no significant changes in 22% (4/18) of neuronal firing at that frequency. These results are interpreted to indicate that plasticity of information processing, herein represented as frequency receptive field alterations, can occur in a context-specific manner in the rat auditory cortex. (Supported by NSF-RCE RII871-4805, NIH S06-GM08037, and ONR N00014-92-J-1372)

693.6

SERIAL AND PARALLEL PROCESSING IN MACAQUE AUDITORY CORTEX. J.P. Rauschecker*, B. Tian, T. Pons and M. Mishkin. Lab. of Neurophysiology, NIMH, Poolesville, MD 20837 and Lab. of Neuropsychology, NIMH, Bethesda, MD 20892.

Electrophysiological recording techniques were used to map the exposed supratemporal plane in six anesthetized rhesus monkeys with both pure-tone and digitized broad-band complex stimuli. We confirmed the existence of at least three tonotopic areas (AI, RL, and CM; Merzenich and Brugge, 1973), plus several surrounding areas where neurons responded best to broad-band complex sounds and a tonotopic organization was not obvious. Neurons in the three tonotopic areas also responded to some of the complex sounds, most frequently in CM and least in AI. After mapping, area AI was aspirated in four of these monkeys and the remainder of the cortex on the supratemporal plane was remapped. Pure-tone responses in area CM were abolished in all animals but one, in which tonal responses were restricted to the high-frequency range. Some CM sites were still responsive to complex stimuli in all four monkeys. In contrast to the effects on CM, no significant changes were detectable in RL after the AI ablation, with most RL neurons maintaining brisk pure-tone responses.

After mapping the exposed supratemporal plane with pure tones in four additional anesthetized monkeys, injections (0.3-1.0 µl) were made with different tracers (fast blue, diamidino yellow, and fluoro ruby) into matched best-frequency regions of AI, RL, and CM in each monkey. Injections in AI and RL led to dense retrograde labeling of neurons in all three major subdivisions of the medial geniculate nucleus (MGv, MGd, and MGm), whereas CM injections led to only sparse labeling of neurons in a restricted zone of the lateral portion of MGd. In one case with an injection of DY into the border zone between AI and CM, cells were also labeled in MGv.

The combined results suggest that the main nucleus of the medial geniculate body (MGv) sends a direct projection in parallel to areas AI and RL, through which neurons in both areas are driven by tonal stimuli. Pure-tone responses in area CM, on the other hand, seem to be driven by serially relayed input from AI. The direct input to CM from MGd may only be capable of mediating responses to broad-band complex sounds.

693.7

SOUND LOCATION REPRESENTED IN THE FIRING PATTERNS OF AUDITORY CORTICAL NEURONS. J. C. Middlebrooks*, A. E. Clock, and D. M. Green. Depts. of Neuroscience, Otolaryngology, and Psychology, Box 100244, University of Florida, Gainesville, FL 32610-0244.

We trained an artificial neural network to classify the firing patterns of neurons according to the location of a sound source. We recorded from single units in auditory field AES in chloralose-anesthetized cats. Noise bursts were presented from the horizontal plane, ranging through 360° of azimuth in 20° steps. Units typically responded with a 10-to-30-msec burst that varied in spike number and temporal pattern according to the sound location. The network took spike trains as input and produced locations in degrees azimuth as output. For each unit, we trained a network with spike trains from half of the trials, then used the network to classify the other half of the trials. In the first 39 units studied, the network correctly identified the stimulus location in as many as 23% of trials (average 11.1% across 39 units; chance performance would be 5.6%), and median values of the magnitude of errors were as small as 25° (average 54.5°; chance=90°). Performance showed no systematic relationship to conventional measures of sharpness of spatial tuning. When stimulus sound pressure roved among levels 10, 25, and 40 dB above threshold, classification of stimulus locations was comparable to that under fixed-level conditions. Classification perform-ance was roughly constant across locations. That is, the spike train from a single unit could "point to" a target about equally well throughout 360° of azimuth. These results offer a new way to think about spatial representation in the auditory cortex, in which a stimulus location is coded, not by the location of activity within a map of sharply tuned neurons, but by the firing patterns of individual neurons. (supported by NIH grant DC000420)

693.8

EFFECT OF INTERAURAL TIME DIFFERENCE ON THE MIDDLE LATENCY AND LATE AUDITORY EVOKED MAGNETIC FIELDS. L.K. McEvoy, J.P. Mäkelä*, M. Hämäläinen. Low Temp. Lab., Helsinki Univ. of Techn., 02150 Espoo, Finland

To determine if middle latency and late auditory evoked fields differ in their sensitivity to perceived sound location, we recorded the neuromagnetic responses from 5 subjects using a 122-channel whole-head magnetometer. Binaural clicks were delivered with interaural time differences (ITDs) of 0.8 ms favoring the left or right ear, or with no ITD. Current dipole models were used in data interpretation. The N100m was largest to contralaterally-perceived clicks. The N100m latency was not affected by ITD. The 35-ms middle latency deflection was 3-5 ms later to stimuli perceived at the left than to stimuli perceived at the right or midline. The amplitude of this response was slightly but not significantly larger to contralaterally- than to medially- or ipsilaterally- perceived clicks. The 60-ms deflection was not affected by ITD. The generators of the N100m response are sensitive to ITD and may play a role in sound localization. The middle latency responses do not demonstrate a clear ITD specificity, and may be generated by neurons in auditory cortex which are not involved with sound localization.

693.9

NEURONAL RESPONSES TO VARIATIONS IN BINAURAL CROSS-CORRELATION IN BARN OWLS. Yehuda Albeck and Masakazu Konishi* Division of Biology 216-76, California Institute of Technology, Pasadena, CA 91125

Barn owls localize phantom sound sources in response to dichotically presented broad-band binaural signals containing interaural time and intensity differences. Similarly, the space-specific neurons in the external nucleus of the owl's inferior colliculus translate combinations of interaural time and intensity differences into two dimensional spatial locations or receptive fields. Just as owls fail to respond to uncorrelated signals delivered to the two ears, so do the space-specific neurons. We synthesized broad-band dichotic signals in which the coefficient of binaural cross-correlation could be systematically varied. Using these signals, we recorded the responses of space-specific neurons and their precursors to interaural time differences. When the signals contained neurons' preferred combination of interaural time and intensity differences, the rate of firing increased monotonically with the coefficient of binaural cross-correlation. The rate augmented non-linearly, but it did not indicate any abrupt changes that could be regarded as threshold of detection. This non-linear response function resembles the cross-correlogram of half-wave rectified versions of the stimulus aveforms, but exceptions to this pattern were also found.

693.10

SPACE-SPECIFIC NEURONS IN THE BASAL GANGLIA OF THE BARN OWL: BINAURAL CHARACTERISTICS. Y.E. Cohen* and E.I. Knudsen. Dept. Neurobiology, Stanford University, Stanford, CA 94305

Recent behavioral studies in our laboratory have demonstrated that the forebrain contributes to sound localization in the barn owl. In order to determine the forebrain pathway responsible for sound localization, we surveyed a number of auditory fields for evidence of neural tuning to binaural localizing cues: interaural timing differences (ITD) and interaural level differences (ILD). We found a region in the basal ganglia, paleostriatum augmentum (PA), that almost exclusively contains units sensitive to both ILD and ITD.

We recorded extracellular activity of PA neurons during dichotic stimulation with digitally synthesized noise or tone bursts. Almost all PA cells were broadly tuned for frequency and were sensitive to ITD and ILD. The majority (125/155) of PA units were sharply tuned for both ITD and ILD. Overall sound level had little effect on either ITD or ILD tuning. When measured at 20 dB above threshold, average ITD tuning width, at 50% of maximum, was 69 µsec (range 13 to 199 µsec) and average ILD tuning width was 22 dB with a range from 5 to 53 dB. Although, best ITDs and ILDs ranged from ipsi 72 µsec to contra 128 µsec and from ipsi 12 dB to contra 19 dB, most PA units were tuned to ITD and ILD values near zero corresponding to the frontal region of space.

The large proportion of binaurally-tuned cells in PA suggests that this forebrain region plays an important role in sound localization.

This work was supported by grants from the NIH (NS09278 and R01 DC00155).

693.11

TINNITUS-RELATED ABNORMALITIES OF THE SPONTANEOUS ACTIVITY IN THE INFERIOR COLLICULUS IN RATS INDUCED BY SALICYLATE. P.J. Jastreboff*, G-D. Chen. Dept. of Surgery, University of Maryland School of Medicine, Baltimore, MD 21201.

The neuronal activity that yields the perception of tinnitus remains elusive. In search for a neuronal basis of tinnitus, the spontaneous activity of single units from the inferior colliculus (IC) was examined in 11 anesthetized pigmented rats (Nembutal 50 mg/kg) before and after injection of sodium salicylate, in a dose (233 mg/kg) shown, by our behavioral paradigm, to induce tinnitus.

358 units were recorded from the IC with 131 units recorded before, and 137 after salicylate; 46 before and 44 after saline. The characteristic frequency (CF) of each unit was determined and 5 minutes of spontaneous activity collected for off-line analysis. Cells with bimodal interval histograms with a Short Interval (SI) mode were marked.

The injection of salicylate resulted in an increased proportion of SI units from 12 to 56% for cells with CF between 8 and 12 kHz, whereas this ratio decreased from 18 to 7%, and from 38% to 19% for units with CF of 4 to 8 kHz, and above 12 kHz, respectively. Importantly, tinnitus pitch evaluated by behavioral paradigm was 10 kHz, which corresponded to CF range where a high increase in proportion of abnormal activity is observed. Consistent with these findings temporal analysis revealed that SI units exhibited high frequency, epileptic-like bursting discharges, with the intervals between spikes down to around 1.5 ms. These data show the presence of salicylate-induced abnormal bursting activity which may be related to tinnitus.

Supported by NICDC/NIH grant DC00299

GENETIC MODELS OF NERVOUS SYSTEM DISORDERS III

694.1

CATECHOLAMINERGIC CELL ATROPHY IN A TRANSGENIC MOUSE OVEREXPRESSING MAO-B IN NEURONS. J.K. Andersen*, D.M. Frim, O. Isacson, and X.O.Breakefield. Neurosci. Ctr., Mass. Gen. Hosp., Charlestown, MA 02129, Harvard Med. Sch., Boston, MA 02115, and Neuroregen. Lab., McLean Hosp., Belmont, MA 02178.

Transgenic mice were created to neuronally overexpress the mitochondrial enzyme monoamine oxidase B (MAO-B). MAO-B is normally expressed in the brain predominately in astrocytes and serotoninergic neurons. MAO has been hypothesized to play a role in dopaminergic cell death both during normal aging and in neurodegenerative disease: 1) MAO levels in the brain increase with age, 2) the oxidant species hydrogen peroxide is produced during the enzymatic reaction, 3) MAO-B can convert substances like MPTP to neurotoxins resulting in a Parkinsonian-like syndrome in mammals, and 4) deprenyl, an MAO-B specific inhibitor, appears to slow the course of Parkinson's disease.

A human MAO-B cDNA placed under the control of the neuron-specific enolase (NSE) promoter was microinjected into early mouse embryos by standard transgenic techniques. Resulting offspring were evaluated for genomic insertion of the transgene by PCR amplification of tail DNA and positives were confirmed by Southern blot analysis. Transgenic mice were bred to create new lines, and expression of MAO-B in these lines was analyzed using a RT-PCR based method. Transgene-specific transcripts were found to be brain-specific and MAO-B protein expression by immunocytochemistry mimicked that found for endogenous NSE. One line, MAOB20, was found to have a 3-4 fold increase in brain MAO-B activity compared to normal littermates. Numbers and sizes of tyrosine hydroxylase immunopositive (TH+) cells in the substantia nigra and locus coeruleus from MAO-B transgenic animals were compared with controls at 4 months of age. TH+ cell number was not decreased in the MAO-B transgenics, however, TH+ perikaryon size was decreased by 20% ($p<0.001$). In contrast, there was no change in cell size of non-TH+ neurons. These results suggest that the overexpression of MAO-B in neurons results in atrophy of cells containing enzyme substrate due to an increased production of hydrogen peroxide. These mice are currently being analyzed for their susceptibility to the neurotoxin MPTP.

694.2

ABNORMAL ACCUMULATION OF THE SUBUNIT *c* OF MITOCHONDRIAL ATP SYNTHASE IN THE MOTOR NEURON DEGENERATION (*Mnd*) MUTANT MOUSE. B.A. Rabin[1], C.A. Pardo[1], D.N. Palmer[2], J.D Glass[1]* and D.L. Price[1]. [1]The Johns Hopkins Univ. Sch. of Med., Balto, MD 21205 and [2]Massey Univ., New Zealand.

The *Mnd* mutant mouse is a mutant with an autosomal dominant degenerative disease characterized by progressive loss of motor activities and accumulation of lipofuscin-like material in neurons and nonneural cells. Ultrastructurally, the storage material is composed of multilamellar, fingerprint, and granular profiles surrounded by limiting membrane and associated with degenerating mitochondria. Immunocytochemical, Western blot, and ultrastructural studies demonstrate that this storage material is associated with the lysosomal accumulation of subunit *c* of mitochondrial ATP synthase in an age-dependent pattern. The accumulation of subunit *c* first appears in neurons of the thalamus, hippocampus, and cortex and eventually involves virtually all nerve cells, including the retina and enteric nervous system. Subunit *c* also accumulates in extraocular muscle fibers, myocardium, pancreas, kidney, and other nonneural cells. Heterozygous *Mnd/Ola* and *Mnd*/wild-strain mice show the accumulation of subunit *c*, but the course of disease is slower than *Mnd/Mnd* mice. The morphological and biochemical findings in *Mnd/Mnd* are similar to those described in some human forms of neuronal ceroid lipofuscinosis (NCL) or Batten's disease and neurodegenerative disorders in children and young adults. These findings suggest that an abnormal assembly or degradation of the ATP synthase complex is responsible for the accumulation of subunit *c* in *Mnd* mutant mouse and, rather than a model for amyotrophic lateral sclerosis, *Mnd* is an excellent animal model to study the neurodegenerative and pathogenic processes that occur in NCL.

694.3

CELL-TYPE SPECIFIC NEUROFILAMENTOUS ACCUMULATIONS IN TRANSGENIC MICE EXPRESSING THE HUMAN MID-SIZED NEUROFILAMENT SUBUNIT. J.C. Vickers*1, R.A. Lazzarini2, V.L. Friedrich Jr1,2, W.G. Janssen1, G.A. Elder2 and J.H. Morrison1 1 Fishberg Research Center for Neurobiology, 2 Brookdale Research Center for Molecular Biology, Mount Sinai School of Medicine, New York, NY 10029.

The pathological processes underlying many human neurodegenerative diseases involve an alteration in neurofilament (NF) triplet proteins and subsequent formation of hallmark filamentous inclusion bodies. Age-associated accumulations of NFs also occur in the central nervous system of the transgenic mouse line NF(M)27, which contains an 8.5 kb DNA fragment of the human mid-sized NF subunit gene. Furthermore, only specific neuronal subpopulations develop these accumulations and the intracellular localization, morphological features and immunoreactivity for specific NF epitopes of these pathologic structures varied in a cell-type specific manner. For example, NF accumulations resembling neurofibrillary tangles were localized to the subpopulation of cortical pyramidal cells that normally contain abundant NF triplet proteins. In contrast, many of the cortical neurons that normally lack NF triplet proteins developed spherical NF accumulations in their cell bodies that could be further distinguished from the tangle-like pathologic structures by their immunoreactivity for phosphorylation-dependent NF epitopes normally present in axons. Ultrastructural studies demonstrated that the spherical NF accumulations consisted of intertwining bundles of intermediate filaments. Other examples of pathologic heterogeneity include the presence of proximal axonal swellings in cerebellar Purkinje cell axons and tangle-like neurofilamentous accumulations in the perikarya of cranial motor neurons as well as the alpha ganglion cells of the retina. These studies indicate that there are widespread and yet cell-type specific alterations in the cytoskeleton of neurons in this transgenic mouse line, which partially resemble homologous perturbations of NFs that occur in several human neurodegenerative diseases. Supported by the N.I.H. and the American Health Assistance Foundation. J.C.V. is an Australian N.H.&M.R.C. C.J.Martin Research Fellow.

694.4

OVEREXPRESSION OF THE HUMAN DOPAMINE D3 RECEPTOR IN THE BRAIN OF TRANSGENIC MICE. B.G. Sahagan*, R.I. Focht, D.S. Conklin, K. Sperle, B. Levant, M.J. Turner, R.S. Senick, S. Ebner, D.E. Grigoriadis, and G.J. Ionak. CNS Diseases Research and Biotechnology, The Du Pont Merck Pharmaceutical Co., Wilmington, DE. 19880-0400.

Using an FVB/N strain of mice, transgenic mice were constructed with the human dopamine D3 receptor cDNA regulated by the murine *thy*-1 promoter. By Northern blot analysis, D3 receptor mRNA was detected in the brains of transgenic mice from five independent lines. Only a very low level of expression of the transgene was detected in the spleen and none in the liver or the kidney. By receptor autoradiography with the D3 selective ligand [^{3}H]7OH-DPAT, dense binding was found throughout the brains of transgenic animals. Control animals exhibit the expected pattern of binding in olfactory tubercles, nucleus accumbens and Islands of Calleja. In contrast to control animals, mice from several independent transgenic lines manifest elevated aggressive and/or circling behaviors. The penetrance of these phenotypes varies among the transgenic lines, and between females and males. In females, up to 40% are aggressive and 25 % circle. In males, up to 70% are aggressive and 10% circle. The behaviors were temporarily suppressed upon administration of the dopamine antagonist eticlopride. The age of onset of phenotypes also varies. For example, male transgenics show aggressivity towards their cagemates as early as 3-6 weeks after birth. The onset of the circling phenotype is at the age of 1-2 months. The aggressive and circling phenotypes severely hinder mating performance of both sexes. These transgenic mice are a valuable model in further studying the human dopamine D3 receptor and the *in vivo* binding and functional properties of subtype specific dopaminergic ligands.

694.5

HISTOLOGICAL ANALYSIS OF THE MALE-STERILE PHENOTYPE SEEN IN THE NEUROLOGICAL MOUSE MUTANT *WEAVER*. S.M.W. Harrison* and S. Roffler-Tarlov. Program in Neurosciences & Dept. of Anatomy and Cell Biology, Tufts Univ. Sch. of Med., Boston, MA 02111.

The *weaver* mutation affects the terminal differentiation of several distinct cell populations in the brain. Granule cells in the cerebellum fail to differentiate or migrate properly and die during early postnatal development. Purkinje cells in the cerebellum have an abnormal morphology and some of these cells also die. Dopamine-containing cells of the substantia nigra, pars compacta and the retrorubral nucleus die, also during postnatal development, resulting in the absence of projections to the caudoputamen. These neurons are the mouse homologues of the dopamine-containing neurons lost in patients with Parkinson's disease.

Sterility in homozygous mutant males is also associated with the *weaver* mutation. We have begun a detailed histological analysis of the weaver's testis in an effort to describe the basis for this sterility and to gain clues about the molecular defect underlying all of the phenotypes associated with this mutation. Testicular and early germ cell development in the weaver male are indistinguishable from the wild-type mouse. However, as the first wave of spermatids progress through spermiogenesis, the spermatogenic process in weaver males is slowed with respect to normal animals. The onset of delay occurs between 21 and 28 days after birth. Although sperm in the weaver develop to the late spermatid stage, mature sperm were never seen to be released from the testes at any age in the weaver. In addition, gradual degeneration of germ cells occurs culminating in the virtual absence of cells within more than half of adult weaver's seminiferous tubules. There is no detectable spermatogenic defect in the heterozygous mutant male. The lack of mature sperm produced by this mutant explains the infertility of homozygous *weaver* males and suggests that *weaver* may be a gene shared by testes and brain. NIH NS 20181.

694.6

A RAT MUTANT WITH BRAIN AMYLOID DEPOSITS, LOW NMDA RECEPTOR BINDING AND BEHAVIORAL DEFICITS: A PRELIMINARY REPORT. P.Romanelli, L.DiMatteo, M.P.Pellicano and A.G.Sadile*, Dipt. Fisiologia Umana "F. Bottazzi", 2nd Univ. of Naples (SUN), Naples, Italy.

A new rat mutant has been obtained by genetic techniques, the NLE/AD, that features at 12 months of age some histochemical, neurochemical and behavioral aspects of Alzheimer's disease (AD). Histochemistry: Congo red staining on paraffin embedded brain sections revealed deposits of amyloid substance varying in size in the hippocampus mainly in CA1, amygdala and somatosensory cortex. They are birefringent at polarized microscope and immunoreactive for a β-APP polyclonal antibody. NMDA binding: twenty μm thick unfixed cryostat sections were incubated with 150nM ^{3}H-L-glutamate (56 Ci/mmol) at 4 °C alone or in presence of 100μM N-methyl-D-aspartate (NMDA) or 2.5μM quisqualate (QA). Non specific binding was determined in presence of 1mM unlabelled glutamate. The sections were exposed to tritium-sensitive films for 3 weeks at 4 °C. Quantitative analysis revealed (i) lower levels of ^{3}H-glutamate binding in NLE/AD in the somatosensory cortex, hippocampal CA1, dentate gyrus and amygdala; (ii) in the hippocampus, the displacement by NMDA was lower in NLE/AD. Behavior: rats were tested in a Làt-maze, in a six-arm tunnel maze, and in a shuttle box. NLE/AD rats showed (i) lower activity in the Làt-maze with impaired short-term and long-term habituation; (ii) poor working and reference memory in the tunnel maze; (iii) no retention of two-way avoidance. In conclusion, the NLE/AD rats with amyloid deposits, lower NMDA receptor binding and a distorted behavioral profile, feature some of the aspects of AD and may help understanding the relationships between amyloid deposits and dementia.

Supported by a CNR grant on Biotechnology.

694.7

GENOTYPIC DIFFERENCES IN THE EFFECT OF CHRONIC COCAINE ADMINISTRATION ON GABAERGIC FUNCTION. R. Marley & K. Shimosato*, Genetics Sec., Mol. Neurobio. Branch, NIDA-Addict. Res. Ctr., Box 5180, Baltimore, MD 21224.

Repeated injections of subconvulsant doses of cocaine result in changes in the susceptibility of rodents to cocaine's convulsant properties. The nature of these changes in susceptibility to cocaine seizures is under genetic control. We have identified 3 strains of mice that differ qualitatively in their response to repeated cocaine administration. Five daily injections of 50 mg/kg of cocaine result in the development of sensitization and tolerance to cocaine-induced seizures in SJL/J and C57BL/6J mice, respectively. This same treatment regimen has no effect on the susceptibility of BALB/cByJ mice to cocaine seizures. We have been using these animal models to examine possible biochemical mechanisms underlying these changes in seizure susceptibility. In the present study, we examined the effect of this treatment regimen on GABA-stimulated $^{36}Cl^-$ uptake into cortical and cerebellar membrane preparations from SJL, C57 and BALB mice. Five daily injections of 50 mg/kg cocaine resulted in an increase in GABA-stimulated $^{36}Cl^-$ uptake into cortical tissue from the SJL mice and into cerebellar tissue from the C57 mice. No changes in $^{36}Cl^-$ uptake were observed in either region in tissue preparations from BALB mice. These results suggest an association between the development of sensitization and/or tolerance to cocaine seizures and changes in GABAergic function.

694.8

STRUCTURE/FUNCTION ANALYSIS OF MEC-4: A PROTEIN INVOLVED IN MECHANOSENSORY NEURON FUNCTION AND NEURODEGENERATION. M. Driscoll* and K. Hong, Dept. of Molecular Biology and Biochemistry, Rutgers University, New Brunswick, NJ 08855

mec-4 is a member of a family of genes, termed degenerins, that can mutate to cause swelling and degeneration of specific groups of neurons in *C. elegans*. Because death-inducing *mec-4(d)* alleles appear to alter osmotic balance, we have suggested that MEC-4 may normally function to regulate membrane permeability. The deduced primary structure of MEC-4, which includes two hydrophobic domains (MSDI and MSDII), is consistent with the idea that MEC-4, expressed in and required for the function of mechanosensory neurons, might be a subunit of a mechanosensory ion channel. The cloning of a member of the degenerin gene family from rat (*Nature* 361:467) by virtue of its ability to induce an amiloride-sensitive Na^+ current in frog oocytes supports the hypothesis that MEC-4 is a channel component.

We have conducted a structure/function analysis of MEC-4 to gain insight into normal MEC-4 function and into how this protein can be altered to induce inappropriate death. Our analysis has highlighted the functional importance of charged and polar amino acid residues that align on one face of the predicted α helix that spans MSD2. We have introduced various point mutations in *mec-4(+)* or *mec-4(d)* that alter amino acids in MSD2 to test for disruption of normal function or degeneration, respectively.

We have identified two additional regions where amino acid substitutions disrupt MEC-4 function. One frequently altered region is within a Cys-rich domain and the other affects a putative intracellular region that may be phosphorylated by cAMP kinase.

694.9

A HERPES SIMPLEX VIRUS FOR GENE TRANSFER INTO NEURONS IN ORGANOTYPIC HIPPOCAMPAL SLICE CULTURES. B.A. Bahr*[1], J. Sharp[1], R.L. Neve[2], A.I. Geller[3] & G. Lynch[1], [1]Center for Neurobiology of Learning and Memory, Univ. of California, Irvine, CA 92717, [2]McLean Hospital, Harvard Medical School, Belmont, MA 02178, [3]Children's Hospital, Harvard Medical School, Boston, MA 02115

Enhanced-expression and antisense-intervention are both good strategies to target specific gene products in order to elucidate mechanisms of neurophysiology. Viral vectors have proved useful in this respect to deliver sense or anti-sense mRNAs to brain neurons. In this study, a defective herpes simplex viral vector (pHSVlac) containing a gene (*lacZ*) encoding β-galactosidase (β-gal) was used to infect organotypic hippocampal slice cultures. Cultured slices were utilized since they retain many *in vivo* features and are comparable to intact hippocampus with regard to anatomy, circuitry, electrophysiology, synaptic plasticity, pathology, and synaptic composition. Slices infected with pHSVlac (5,000-150,000 infectious particles) expressed the β-gal enzyme in a dose-dependent manner as measured on immunoblots; no β-gal was detected in slices infected with control virus (pHSVsyn) lacking the *lacZ* gene. β-Gal was evident as early as 2 h post-infection with pHSVlac and was maximal after ~35 h. Expression of the *lacZ* gene (mRNA and protein) persisted for at least 12 days as measured by immunoblots and by reverse transcription-PCR using primers specific for the recombinant *lacZ* RNA. The level of the intrinsic synaptic marker synaptophysin was not affected by the virus. Staining the infected slices with the β-gal substrate X-gal revealed that the density of β-gal-positive blue cells is highest in CA3 and dentate gyrus and lowest in CA1. X-Gal also stained somata and dendrites of infected interneurons. Double labeling with anti-MAP2 confirmed that most of the infected cells were neurons. In conclusion, HSV allows transfer of foreign genes with no evident cytopathology (NIA AG00538 supported).

694.10

TRANSGENIC MICE WITH ABNORMAL ACETYLCHOLINE RECEPTOR (ACHR) CHANNELS: THE SLOW CHANNEL PHENOTYPE. CM Gomez*, B Bhattacharyya, EH Lambert Dept. of Neurology, Univ. of MN, Minneapolis, MN 55455.

The slow channel syndrome (SCS) is an autosomal dominant progressive myopathy in which abnormal neuromuscular transmission (NT) and degenerative changes in the postsynaptic membrane are postulated to arise from an inherited abnormality of AChR causing delayed closure of AChR ion channels of the neuromuscular junction. To test this hypothesis we generated transgenic mice using a DNA construct that expresses an AChR δ subunit with a mutation (δS6T) that prolongs mean single channel open time 3-fold. Three founder lines have several-fold higher levels of muscle mRNA for δS6T than for wild type subunits. We studied miniature endplate currents (mepc) and endplate currents (epc) in diaphragm *in vitro* using 2 electrode voltage clamp. Both mepc and epc of δS6T transgenic mice have significantly increased decay time constants (τ) (Control τ_{mepc}: 1.21±0.05ms vs transgenic τ_{mepc}: 2.7±0.17ms, $p<0.01$; Control τ_{epc}:1.94±0.23 vs transgenic τ_{epc}: 5.6±0.84, $p<0.01$). Mepc *amplitudes* of transgenics are also significantly reduced (Control: 2.57±0.1nA vs transgenic: 1.76±0.1 nA, $p<0.01$). To test whether increased τ_{epc} affects NT in transgenic mice, we recorded compound muscle action potentials (CMAP) evoked by direct stimulation of sciatic nerve *in vivo*. All transgenic mice tested (N=18) had *repetitive* CMAP after a single nerve stimulus, while no control mice (N=9) had repetitive CMAP. Thus, expression in muscle of a mouse AChR δ subunit with a single amino acid substitution causes altered synaptic currents, reduced mepc amplitudes, and repetitive CMAP following single nerve stimuli, as are seen in the SCS. Supported by NIH, MDA.

695.1

DRD4 DOPAMINE RECEPTOR ALLELE FREQUENCIES IN ALCOHOLICS AND CONTROLS FROM VARIOUS POPULATIONS.

M. Adamson, J. Kennedy, A. Petronis, M. Dean, M. Virkkunen, M. Linnoila, D. Goldman*, Lab. of Neurogenetics, NIAAA, Bethesda, MD 20892

The DRD4 gene contains a 48 bp repeated segment in Exon III of the coding region (*Nature* 358, pp 149-152; 1992). Alleles coding for 2 to 8, and 10 repeats have been found. Varying the numbers of repeated segments changes the length, structure and function of the receptor, which makes this gene an intriguing candidate as a genetic determinant of dopamine-related behaviors.

Using a PCR method developed by Kennedy et al (in press), the frequency of DRD4 repeat alleles was determined in a population of Finnish alcoholics and controls, and in four other populations (Blacks, Pima Indians, Cheyenne Indians, and Jemez-Pueblo Indians). Finnish alcoholics were of the early onset, impulsive type. The homogeneity of this population and of their alcoholic phenotype would allow us to detect an association to alcoholism. However, these 98 Finnish alcoholics were not significantly different from 96 ethnically matched controls. The distribution of repeat alleles (2-8) in this more isolated Finnish population was similar to that seen in Caucasians (Kennedy et al). Caucasians, Finns, and Jemez-Pueblo Indians were multiallelic but Pima Indians, Cheyenne Indians, and Blacks showed less variability and lacked the shorter repeat alleles. Selection or random genetic factors, including founder effects and drift, may have determined these differences in allele frequency. Further investigation of the DRD4 variants may elucidate whether these population differences are behaviorally significant.

695.2

PREPUBERTAL CASTRATION AUGMENTS AMPHETAMINE INDUCED DOPAMINE INCREASE IN ADULT MALE RATS.

E. Murzi*, L. E. González, X. Páez, T. Baptista and L. Hernández. Laboratory of Behavioral Physiology, Medical School, Los Andes University, Mérida 5101, Venezuela.

The impact of puberty on the mesolimbic dopamine system was assessed in male rats castrated before puberty. The dopamine function was evaluated by a pharmacological challenge with amphetamine. Eight male rats, three weeks old were castrated by escrotal incision. Seven controls only had the surgical wound (sham group). After two months guide shafts aimed to the nucleus accumbens were permanently implanted in each rat. Seven days after surgery, microdialysis probes were inserted in the accumbens with the technique described elsewhere[1]. Dopamine, DOPAC and Homovanillic Acid were measured in the dialysates by high pressure liquid chromatography and electrochemical detection. After obtaining a stable base line, amphetamine sulfate (1 mg/kg) was injected i.p. Dopamine increased three folds more in castrated than in normal rats. This difference was statistically significant. Dopac and HVA decreased more in the castrated rats than in the normal rats. These results indicate that androgens might increase dopamine receptor sensitivity lowering the response to amphetamine in normal rats. If this is correct, these results might provide an explanation for the onset of schizophrenia symptoms after puberty and the greater severity of those symptoms in males than in females[2].

1) Hernández, L. et al. *Life Sci.* 1986
2) Castle and Murray. *Psychol. Medicine*, 1991.

695.3

THREE-METHOXYTYRAMINE (3MT) IS THE MAJOR METABOLITE OF RELEASED DOPAMINE (DA) IN THE RAT FRONTAL CORTEX (FC), NUCLEUS ACCUMBENS (NAC) AND STRIATUM (ST): REASSESSMENT OF THE EFFECTS OF NEUROLEPTICS ON DOPAMINE RELEASE AND METABOLISM. F. Karoum*, S.J. Chrapusta and R.J. Wyatt. Neuropsychiatry Branch, NIMH Neuroscience Center at St. Elizabeths, Washington, D.C 20032

3MT and DOPAC rates of formation were estimated in the FC, NAC and ST of rats 60 min. after the administration of 0.4 mg/kg haloperidol (HAL), and 10 mg/kg clozapine (CLZ). The rates of formation were calculated from the respective increase and decrease in 3MT and DOPAC concentrations following 75 mg^{-1}, I.P. pargyline. 3MT rate of formation (53 $\pm$ 2 fmol/mg protein/min.) was found to be comparable to that of DOPAC (39 $\pm$ 4 fmol/mg protein/min) in the FC. In contrast, 3MT rates of formation in the NAC and ST were about 10% of the rates of formation of DOPAC. Both HAL and CLZ significantly increased 3MT and DOPAC production in the FC, indicating that DA release and metabolism were stimulated. While 3MT and DOPAC rates of formation were significantly increased by HAL in the ST and NAC, only DOPAC production was elevated by CLZ. It is concluded that: 1) O-Methylation is a prominent step in the catabolism of DA in the FC under both physiological conditions and following acute treatment with antipsychotics; 2) While stimulation of DA release in the FC by neuroleptics may be involved in the antipsychotic properties of these drugs, HAL stimulation of DA release in the ST and perhaps in the NAC may underlie its extrapyramidal effects; 3) most DOPAC in the NAC and ST apparently originates from intraneuronal deamination of unreleased DA.

695.4

DO VTA DOPAMINE NEURONS RELEASE A GLUTAMATE-LIKE COTRANSMITTER? D. Sulzer* and S. Rayport Dept Psychiatry, Ctr Neurobiology & Behavior, Dept Anatomy & Cell Biology, Columbia Univ; Dept Neuropathology, NYS Psychiatric Inst, NY 10032.

Immunocytochemical studies suggest that ventral tegmental area (VTA) neurons are mostly inhibitory, releasing either dopamine or GABA. However, in VTA cultures derived from postnatal rats, neurons recorded in whole cell patch clamp mode commonly receive excitatory input that can be blocked by glutamate receptor antagonists, including the AMPA antagonist CNQX (10 μM), the NMDA antagonist APV (30 μM), or the broad-spectrum glutamate antagonist kynurenate (500 μM). The EPSPs appear to have both AMPA and NMDA components.

To determine whether dopamine neurons might be the source of the excitation via the release a glutamate-like cotransmitter, we cultured single VTA neurons on 100 μm diameter glial islands surrounded by an agar substrate inhospitable to neurite outgrowth (Segal & Furshpan, J Neurophysiol 64:1390, 1990). In these single neuron cultures, where all synaptic input must be autaptic, neurons frequently produced stimulation-dependent EPSPs that could be abolished by glutamate receptor antagonists. A subset of these neurons were dopaminergic by tyrosine hydroxylase immunocytochemistry. Therefore, some dopamine neurons both release and respond to a glutamate-like transmitter.

These results are consistent with immunolabeling studies in the intact brain that suggest that a classical neurotransmitter other than DA may be released from midbrain dopamine neurons (Hattori et al, J Comp Neurol 309:391, 1991; Kaneko et al, Brain Res 507:151, 1990). Constrained by the expression of autoreceptors, the single neuron microculture technique provides a novel method to identify the transmitters released by identified mammalian neurons.

695.5

Fe^{2+} CATALYZES THE OXIDATION OF 3,4-DIHYDROXYPHENYLALANINE (DOPA) TO AN EXCITOTOXIN T.A. Newcomer* & E. Aizenman, Dept. of Neurobiology, Univ. of Pittsburgh School of Medicine, Pittsburgh, PA 15261.

2,4,5-Trihydroxyphenylalanine (TOPA)-containing solutions oxidize to form a quinone derivative that is a non-NMDA agonist and excitotoxin (Rosenberg et al., *P.N.A.S.* 88: 4865;1991). We have recently reported that TOPA quinone exists as the dominant species in TOPA-containing physiological salt solutions. In addition, we observed that DOPA partially autoxidizes at neutral pH to form TOPA compounds (Newcomer et al., *J. Neurochem.* in press). In this study, we have examined the influence of Fe^{+2} and Fe^{+3} on the rate and amount of this conversion. In Hanks' balanced salt solution neither 1 mM $FeNO_3$ (Fe^{+3}) nor 100 μM ascorbate alone had any effect over baseline conversion rates (0.2% at 3 hours). However, in the presence of 100 μM ASC, 1 mM Fe^{+3} increased the amount of conversion to 1.3% at 3 hours. In contrast, the effects of 1 mM $FeCl_2$ (Fe^{+2}) were independent of ascorbate and significantly faster (1.7% conversion at 24 min). These data suggest that Fe^{+2} can catalyze the conversion of DOPA to TOPA, and that Fe^{+3} must be reduced to Fe^{+2} by ascorbate to produce this oxidation. These results may have implications for disorders involving high levels of transition metals near catecholaminergic neurons such as in Parkinson's disease.

695.6

IDAZOXAN-SPECIFIC ANTISERUM RECOGNIZES AN ENDOGENOUS CLONIDINE-DISPLACING SUBSTANCE (CDS) IN HUMAN SERUM AND CSF. H. Wang*, D. McGowan, S. Regunathan, S. Bramwell, G. Li and D.J. Reis. Div. Neurobiol., Dept. Neurol. & Neurosci., Cornell Univ. Med. Coll., New York, NY 10021.

Clonidine-displacing substance (CDS) is an endogenous, non-peptidergic, low molecular weight (<500 Da) material present in brain, other tissues and serum which, like clonidine, binds to both α_2-adrenergic and imidazoline receptors (IRs). CDS may be an endogenous ligand for IRs. It also cross-reacts with antibodies to PAC (although with low sensitivity and some non-selectivity) (Meeley et al., *Hypertension*, 13:341, 1989). Therefore, we sought to develop a more selective, sensitive and clinically applicable radioimmunoassay (RIA) for CDS based on its ability to cross react with idazoxan. Antibodies were produced in rabbits against an idazoxan-hemocyanin conjugate. The anti-idazoxan antiserum had high affinity for unconjugated ^{3}H-idazoxan (Kd=26nM) in RIA. In competition experiments of various drugs and relevant endogenous molecules, only idazoxan potently (IC_{50}<100nM) inhibited ^{3}H-idazoxan binding. A few drugs weakly (IC_{50}>1mM) inhibited binding (cirazoline>UK 14304>guanabenz>amiloride). Other relevant agents including clonidine, naphazoline, moxonidine, rauwolscine, histamine and catecholamines did not inhibit binding even at 1mM. CDS highly purified from bovine brain dose-dependently and totally inhibited specific ^{3}H-idazoxan binding. The antiserum was more sensitive than the anti-PAC antiserum (4 idazoxan RIA Unit=1 PAC RIA Unit) and more selective. CDS activity was also detected by the antibody in human (0.9-4.1U/ml) and rat sera (1-2U/ml). Human CSF samples tested contained CDS activity ranging from undetectable to 1.3U/ml. We conclude that a RIA using an anti-idazoxan antibody can detect CDS activity in human serum and CSF.

695.7

EXPRESSION OF IMIDAZOLINE RECEPTOR PROTEIN IN HUMAN BRAIN AND LIVER. P.V. Escriba, M. Sastre, H. Wang, S. Regunathan, D.J. Reis* and J.A. Garcia-Sevilla. Lab of Neuropharmacol., Univ. of Balearic Islands, SPAIN and Div. of Neurobiol., Cornell Univ. Med. Coll., New York, NY 10021.

Clonidine, idazoxan and associated ligands bind to both α_2-adrenergic receptors and a nonadrenergic binding site, the imidazoline receptor (IR). We have purified, by idazoxan-affinity chromatography, an imidazoline receptor binding protein (IRBP) from bovine adrenal chromaffin cells (Wang et al., *Mol. Pharmacol.* 42:792, 1992) and generated antibodies (Ab) thereto (Wang et al., *Mol. Pharmacol.* 43:509, 1993). Ab specificity was established by immuno-(Western) blotting, inhibition of ligand binding, immunoprecipitation and immunocytochemistry. We examined whether the IRBP is expressed in human and rat cerebral cortex, caudate nucleus, hypothalamus, medulla oblongata, cerebellum, and liver and also rat adrenal medulla, tissues expressing IRs by ligand binding. Tissues were processed for Western blotting using HRP-linked donkey anti-rabbit IgG detected by enhanced chemiluminescence. All expressed a double band of 29-30kDa, and a lesser band of 47kDa (with the exception of cerebellum, liver, and adrenal). A less intense band of 66kDa was seen in most rat tissues (except cerebellum and liver) but only in human liver. Human medulla oblongata had bands of 44 and 50kDa and rat adrenal bands of 58 and 86kDa. We conclude that (a) human brain and liver express an IRBP which is immunochemically similar to that of rat; (b) the IRBP exists in several molecular forms representing either receptor isoforms or degradation products; (c) the IRBP of brain and liver share comparable elements; (d) the IRBP may be common to the two major subclasses of IRs, the I-1 and I-2, since these are differentially concentrated in medulla oblongata and adrenal medulla/liver. Since adrenal medulla expresses only IRs, the results further indicate that the IR is molecularly distinct from α_2-adrenergic receptors.

695.8

L-DOPA FORMATION AND PHOSPHORYLATION OF TYROSINE HYDROXYLASE (TH) IN AtT-20 CELLS EXPRESSING WILD TYPE TH OR A SERINE 40 MUTANT OF TH. M. Goldstein,* J. Wu, D. Tang and J. Haycock. Neurochem. Res. Labs., N.Y.U. Med. Ctr., N.Y., N.Y.10016 and Dept. Biochem. Mol. Biol., LSU Med. Ctr., New Orleans, LA 70119

Substitution of Leu for Ser-40 in tyrosine hydroxylase (TH-S40L) produces an enzyme with *in vitro* properties similar to wild type TH (TH-WT) that has been phosphorylated by protein kinase A (PKA) (Wu, J. et al. J. Biol. Chem. 267:7373, 1992). In the present study we have determined the *in situ* activities of TH-WT and TH-S40L in stably transfected AtT-20 cells by measuring DOPA accumulation in the presence of 50 μM NSD 1015 (30 min, 37°C) to inhibit DOPA catabolism. The majority of DOPA was present in the incubation medium. In separate experiments, site-specific phosphorylation of TH was determined after preincubation with ^{32}P.

DOPA formation in AtT-20 cells expressing TH-S40L was 6-8 fold higher than that in the cells expressing TH-WT. Addition of 10 μM forskolin, an activator of adenylyl cyclase, increased DOPA synthesis in the cells expressing TH-WT, but slightly decreased DOPA formation by the cells expressing TH-S40L. TH-WT was phosphorylated at Ser-8, -19, -31, and -40, whereas ^{32}P incorporation into TH-S40L was restricted to Ser-8, -19 and -31. Basal phosphorylation of TH-S40L was approx. 2-fold higher than that of TH-WT, due primarily to increased S-31 phosphorylation. Whereas forskolin increased TH-WT phosphorylation (primarily at S-40), TH-S40L phosphorylation was decreased by forskolin. While the data suggest that S-40 mediates PKA-dependent activation of TH *in situ*, they also suggest that phosphorylation of the other sites is important in TH regulation. Supported by NIMH Grants 02717 and 43230 and NS 25134.

695.9

NEUROPEPTIDE-PROCESSING ENZYME PEPTIDYLGLYCINE ALPHA-AMIDATING MONOOXYGENASE (PAM) AND CHOLECYSTOKININ (CCK) EXPRESSION IN THE CATECHOLAMINERGIC SYSTEM FOLLOWING AXOTOMY. T.C. Wessel*, T.A. Houpt, H. Baker, and T.H. Joh. Laboratory of Molecular Neurobiology, Burke Medical Research Institute, and E.W. Bourne Behavioral Research Laboratory, Cornell Univ. Medical College, White Plains, NY 10605.

Medial forebrain bundle (MFB) transection is a model system for degenerative brain disorders such as Parkinson's disease, which are characterized by a decline in neurotransmitter-synthesizing enzymes and ultimately cell death of specific neuronal populations. We have previously shown a close correlation between resumption of tyrosine hydroxylase (TH) gene expression after MFB transection and long-term cell survival: the majority of substantia nigra compacta (SNc) neurons lose TH gene expression and gradually degenerate, while locus ceruleus (LC) neurons regain basal TH expression between 2 and 4 weeks after axotomy.

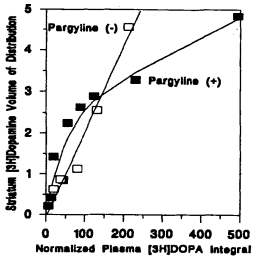
These experiments determined whether the neuropeptide CCK, the neuropeptide-processing enzyme PAM and the catecholamine-synthesizing enzyme TH exhibit differential spatial and temporal regulation following MFB transection. The production of bioactive CCK and galanin in the SNc and LC, respectively, is dependent on PAM which is co-expressed with TH in these neurons. Rats were examined with *in situ* hybridization and immunohistochemistry at various time points from 4 days to 6 weeks after unilateral MFB transection: in the SNc, TH and CCK mRNA and protein levels as well as PAM mRNA levels decreased irreversibly over 6 weeks, although a small number of neurons were resistent to the effects of unilateral axotomy. In contrast, PAM mRNA levels in the LC followed a similar time course to that previously described for TH mRNA and protein levels. These results suggest that TH and PAM gene expression may be co-regulated in degenerating and regenerating axotomized catecholaminergic neurons. Supported by MH44043.

695.10

DETERMINATION OF IN VIVO DOPA DECARBOXYLASE ACTIVITY IN RAT STRIATUM: THE EFFECT OF PARGYLINE. P. Cumming*, H. Kuwabara, and A. Gjedde. Montreal Neurological Institute, McGill University, Montreal, Canada H3A 2B4.

Experiments were conducted to test for regulation of DOPA decarboxylase in striatum of Wistar rats. Conscious rats were treated with saline or pargyline (50 mg/kg, i.p.) 30 minutes prior to [^{3}H]DOPA injection (200 μCi, i.v.) which circulated for 3-240 minutes. Radioactivities in arterial plasma and striatum were fractionated by HPLC. The distribution volume for [^{3}H]dopamine in striatum was plotted as a function of the normalized plasma [^{3}H]DOPA input. Results indicate that DOPA decarboxylase activity declines after prolonged blockade of [^{3}H]dopamine metabolism with pargyline. The finding also suggests that pargyline initially prevents efflux of dopamine metabolites.

Experimental Condition	DOPA Decarboxylase Activity [ml hg^{-1} min^{-1}]
pargyline (−)	
initial rate	2.1±0.2
final rate	2.1±0.2
pargyline (+)	
initial rate	5.4±1.7
final rate	0.5±0.2



Supported by MRC SP-30 and the Quebec Heart and Stroke Foundation.

695.11

EGR1 ACTIVATES EXPRESSION OF THE RAT PNMT GENE S.N. Ebert*, S.L. Balt and D.L.Wong, Department of Psychiatry and Behavioral Sciences, Stanford University School of Medicine, Stanford, CA 94305

Phenylethanolamine N-methyltransferase (PNMT) catalyzes the conversion of norepinephrine to epinephrine and is subject to neural and hormonal controls. However, the intracellular factors which mediate the effects of these stimuli on PNMT gene expression remain undefined. Examination of rat PNMT genomic sequences revealed consensus DNA-binding sites for Egr1, an immediate-early transcription factor which is known to be rapidly and transiently induced by a variety of neural stimuli. In order to evaluate the potential Egr1 interactions with the PNMT gene, the rat PNMT promoter/regulatory DNA sequence was fused to the firefly luciferase reporter gene and co-transfected into transformed PC12 (RS1) cells with a vector which provides strong constitutive expression of Egr1. The presence of Egr1 caused a marked stimulation (300-400%) in PNMT promoter-dependent luciferase activity. RNase protection assays show that the endogenous PNMT gene was also activated by Egr1. The consensus DNA-binding sites for Egr1 are located approximately 45 and 165 bp 5' of the PNMT transcriptional initiation site. Deletion and site-directed mutagenesis of the PNMT promoter/regulatory region in combination with transient co-transfection assays suggest that maximal Egr1 stimulation of the PNMT promoter is dependent on the integrity of both of the putative Egr1 binding sites, although either site alone appears capable of conferring partial Egr1 responsiveness. Gel mobility-shift assays confirm that Egr1 binds to both sites. These results demonstrate that Egr1 can activate PNMT gene expression and that this activation is likely to occur through direct binding of Egr1 to its target DNA sequences within the promoter/regulatory region of the PNMT gene. The fact that Egr1 is itself induced by neural stimulation suggests that it may participate in neural activation of PNMT gene expression.

695.12

PHENYLETHANOLAMINE N-METHYLTRANSFERASE GENE ACTIVATION: EGR-1 AND GLUCOCORTICOID RECEPTOR SYNERGY. D. L. Wong,*S. N. Ebert, S. L. Balt and J. P. B. Hunter. Department of Psychiatry and Behavioral Sciences, Stanford University School of Medicine, Stanford, CA 94305.

Rat adrenal phenylethanolamine N-methyltransferase (PNMT) is neurally controlled through its synthesis while glucocorticoids (GC) control its degradation. GCs and the GC receptor (GR) also may be important in PNMT transcription, consistent with a GRE in the PNMT gene. However, GCs will not induce premature PNMT expression developmentally, and only weakly stimulate its expression in vitro in cells with the potential for PNMT expression. Recently, we have demonstrated that the transcriptional factor Egr-1 activates reporter gene and endogenous PNMT from the PNMT promoter using a PC12 derivative cell line (RS1). The transcriptionally activated cells show high nuclear expression of Egr-1. Similarly, in vivo, Egr-1 is markedly elevated in adrenal medullary cell nuclei in rats administered reserpine (10 mg/kg i.p.), a drug elevating PNMT mRNA 8-fold through neural mechanisms. Reserpine elevates adrenal corticosterone 11-fold coincident with transcriptional activation, suggesting interaction between neural and hormonal controls. Transient co-transfections assays have been used to further investigate this interaction. In the presence of the functional Egr-1 binding sites (-45 and -165 bp) and GRE (-513 bp) in the PNMT promoter/regulatory sequences, dexamethasone (1 μM) enhances reporter gene activity from PNMT-luciferase constructs 2-fold above induction seen with Egr-1 alone. When the GRE or GRE and distal Egr-1 binding site are eliminated dexamethasone will not similarly activate luciferase although Egr-1 activation of luciferase still occurs. Thus, Egr-1 appears to be an important activator of PNMT gene transcription, with the GR playing a modulatory role in controlling this catecholamine enzyme.

696.1

EVIDENCE FOR A NEUROPEPTIDE Y → GALANIN →β-ENDORPHIN PATHWAY IN THE RAT HYPOTHALAMUS. Horvath, T.L.,[1] S.P. Kalra,[3] F. Naftolin*[1] and C. Leranth[1,2]. [1]Dept. of Ob/Gyn, and [2]Section of Neurobiology, Yale University, Sch. of Med., New Haven CT. 06510 and [3]Dep. of Ob/Gyn, University of Florida, Gainesville, FL 32610.

We provided morphological evidence of an NPY→β-endorphin connection (Horvath et al. Endocrinology 1992), suggesting an indirect effect of NPY on gonadotropin secretion. This hypothesis was confirmed by a pharmacological experiment, which revealed that naloxone blocks the inhibition of gonadotropin release induced by NPY. On the other hand, communication between the hypothalamic NPY and galanin systems has been postulated to play a role in the active feeding cycle. Furthermore, galanin was demonstrated to induce prolactin release, which involves opioidergic mechanisms. This study was designed to discern the morphological interconnections between NPY and galanin, and galanin and β-endorphin neurons. Correlated light and electron microscopic double-immunostaining analysis demonstrated synaptic contacts between NPY boutons and galanin cells in the arcuate-, paraventricular-, and supraoptic nuclei. The density of these synaptic contacts was highest in the paraventricular nucleus and lowest in the supraoptic nucleus. Furthermore, galanin-immunoreactive fibers were found in synaptic connection with a population of mediobasal hypothalamic β-endorphin neurons. These results indicate that: 1) in the paraventricular nucleus NPY probably influences galanin-producing neurons, shifting the animal's preference for macronutrients; 2) galanin, at least in part, exerts its effect on prolactin secretion via β-endorphin cells of the arcuate nucleus; and 3) that in the rat hypothalamus, there is an NPY→galanin→opiate pathway, which is involved in both reproduction and ingestive behavior in a site specific manner. Supported by NIH grants HD-23830 (to C.L.), HD-13587 (to F.N.), and HD-08634 (to S.P.K.).

696.2

BOTH HIGH AND LOW AMBIENT TEMPERATURES ELEVATE HYPOTHALAMIC CYCLO(HIS-PRO) (CHP) CONTENT IN *MASTOMYS NATALENSIS*. R. Shukla[1], H. Mizuma[2], C.W. Hilton[2], R.C. Srimal[1] and C. Prasad*[2]. Div. Pharmacology[1], CDRI, Lucknow, and Dept. Medicine[2], LSUMC, New Orleans, LA.

Many neuropeptides, including CHP, have been suggested to regulate body temperature through undefined mechanism(s) that may involve the central thermostat, the set-point mechanism or the heat production or loss pathways. To understand the role of CHP in thermoregulation, we have examined regional changes in brain CHP in *M. natalensis*, a rodent capable of maintaining normothermia during extreme changes in environmental temperatures. *M. natalensis* were maintained at 24°, 40° and 10° C for four hours at a relative humidity of 50%. After treatment, CHP levels in 7 brain regions were measured. At 24°C, CHP was unevenly distributed throughout the brain with striatal level being over 8 times higher than that of mid-brain. Both at 40°C and 10°C, the total brain CHP content decreased significantly ($p<0.01$). What is more interesting, however, is that at both temperatures, there was a rearrangement in regional distribution with hypothalamus (Ht) becoming the region with the highest CHP level. It is tempting to speculate that the rise in Ht CHP may attenuate the sensitivity of the trigger responsible for setting off the changes in the heat balance.

696.3

INHIBITORY PARASYMPATHETIC NERVES BECOME EXCITATORY FOLLOWING ORBITAL SYMPATHETIC DENERVATION P. G. Smith* and C. L. Beauregard. Department of Physiology, University of Kansas Medical Center, Kansas City, KS 66160

Autonomic nerves can regulate target activity directly through effects on postjunctional cells and indirectly through actions on other nerves. Parasympathetic innervation normally mediates its actions on orbital smooth muscle solely indirectly by prejunctionally inhibiting adjacent sympathetic nerves. The present study investigated alterations in orbital parasympathetic function following removal of sympathetic nerves.

Adult Sprague-Dawley rats were sympathectomized by unilateral superior cervical ganglion excision. Five weeks later, parasympathetic innervation to the intact or sympathectomized orbit was stimulated by an electrode placed stereotaxically in the superior salivatory nucleus and changes in superior tarsal smooth muscle tone were measured.

Stimulation of parasympathetic innervation to the intact muscle caused a small decrease in resting tension (-106±8 mg). In contrast, parasympathetic stimulation of the sympathectomized side caused a large contraction (619±76 mg), which was abolished by atropine. Excitation after sympathectomy is not attributable to changes in smooth muscle muscarinic receptor sensitivity, as responses to the muscarinic agonist bethanechol were not altered significantly.

We conclude that following sympathetic denervation, changes occur in cholinergic neurotransmission that result in the functional conversion of orbital smooth muscle parasympathetic nerves from prejunctional inhibition to postjunctional excitation. Supported by NS23502.

696.4

GLP-1(7-36)AMIDE BINDING SITES IN RAT BRAIN - PUTATIVE ROLE FOR GLP-1 IN THE BRAIN-GUT AXIS.

R. Göke, P.J. Larsen, J.d. Mikkelsen, M. Treiman* and S.P. Sheikh
Depts. of Clinical Biochemistry, Medical Anatomy & Physiology, University of Copenhagen, 2200 Copenhagen, Denmark

The preproglucagon-derived gut hormone gucagon-like peptide 1(7-36) amide (GLP-1) is present in the A-cells of the pancreas and the L-cells of the gut. GLP-1 has also been found in discrete regions in the brain. However, little is known about brain GLP-1 receptors.

Using autoradiography binding sites for [^{125}I]-GLP-1 were identified on frozen sections of rat brain. Highest concentration of GLP-1 binding sites (10-18 pmol/mg tissue) were fund in the following regions: In the hypothalamus and the arcuate nucleus and the median eminence; in the thalamus, especially in the laterodorsal nucleus and the posterior nuclear group; in the lateral part of the interpenduncular nucleus; in the posterodorsal tegmental nucleus; in the subfornical organ; in the brain stem, especially in the area postrema, the inferior olive and the nucleus of the solitary tract; in the neuropituitary. Binding of [^{125}I]-GLP-1 was inhibited by unlabeled GLP-1 and the structurally related exendin-4 but not by glucagon or vasoactive intestinal peptide (VIP).

Cross-linking studies with hypothalamic membranes revealed a molecular mass of the GLP-1 receptor of 66,000 dalton. These results indicate that GLP-1 may have a role as neurotransmitter in the brain. Furthermore, the presence of binding sites for GLP-1 in regions which are out of the blood-brain barrier (subfornical organ, median eminence, area postrema, neuropituitary) suggests that GLP-1 may also play a role in the brain-gut axis.

696.5

PERIPHERAL GASTRIC EFFECTS OF CCK-8 ON BRAINSTEM UNITARY RESPONSES IN THE *IN VITRO* NEONATAL RAT. C.S. Yuan and W.D. Barber*. Dept. of Anatomy, College of Medicine, University of Arizona, Tucson, AZ. 85724.

Subdiaphragmatic vagally-evoked unitary responses were recorded extracellularly in the nucleus tractus solitarius (NTS) in an *in vitro* neonatal rat brainstem-gastric preparation. The preparation was maintained in a chamber containing a superfused Kreb's solution. A partition divided the chamber into a gastric and a brainstem compartment. A suction stimulating electrode was placed on the vagi at the gastroesophageal junction. The peripheral gastric effects of sulfated CCK-8 were evaluated on 93 evoked NTS responses in 0-6 days old rats. Peripheral gastric application of CCK-8 (300 nM) increased the excitability (283 + 9.8%, mean ± S.E.) in a majority of NTS neurons tested. Surgical removal of the distal stomach containing the pylorus significantly decreased the effect of the peptide on the NTS neurons. The peripheral gastric effects of CCK-8 on the subdiaphragmatic vagal evoked responses in NTS were also evaluated in three age groups between 0 and 6 days of age. The gastric effects of CCK-8 produced a 30% greater increase in the NTS response in the 5-6 day old age group than in the < 1 or 2-3 day old age groups. These results suggest that gastric receptors, served by vagal afferents, responded to CCK-8 resulting in an increase in NTS neuronal activity. The peripheral gastric effects of the peptide on NTS activity were more pronounced in 5-6 day old rats. (Supported by USPHS Grant DK 36289).

696.6

INNERVATION OF THE RAT PYLORIC SPHINCTER BY VAGAL AFFERENTS AND EFFERENTS, AND BY VIP, CGRP, GAL AND NADPH-DIAPHORASE-POSITIVE FIBERS. H.-R. Berthoud*, Q. Lin, M. Kressel and W.L. Neuhuber, Pennington Biomedical Research Center, LSU, 6400 Perkins Rd., Baton Rouge, Louisiana, 70808 USA, Anatomy Institute, Univ. of Zürich, Switzerland, and Anatomy Institute, Univ. of Erlangen, Germany.

The pyloric sphincter with its ability to control gastric emptying plays important roles in the rate of nutrient absorption, the regulation of food intake, and pathological states such as duodenal ulceration and obesity. Because of this strategic role, we used specific vagal labeling, and immunohistochemical techniques to better characterize its afferent and efferent innervation.

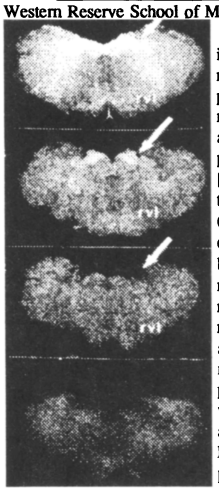
Vagal efferent terminal-like structures were present in small ganglia within or just outside the circular sphincter muscle, in myenteric ganglia of the distal antrum and proximal duodenum, and in a few submucosal ganglia. The sphincter muscle contained very dense networks of varicose GAL, VIP and NADPH-diaphorase positive fibers with GAL and NADPH-diaphorase positive cell bodies in myenteric and small intramuscular and VIP positive cell bodies mainly in submucosal ganglia, delineating the potential vagal postganglionic innervation. Vagal afferents formed many trellis-like structures with numerous varicose endings running parallel to the circular muscle bundles, and were often in close anatomical contact with fibrocyte-like cells. Afferent endings were also present in the large myenteric ganglia of distal antrum and proximal duodenum, in the modified small intramuscular ganglia, in submucosal ganglia, and throughout the mucosal lining. The branching patterns of some vagal afferents suggested that individual axons produced multiple collaterals in different compartments, serving local reflex action. The large majority of CGRP-positive fibers in the sphincter muscle, submucosa and mucosa was not of vagal origin, but most likely represented the dorsal root afferent innervation.

Roche Foundation and Hartmann-Müller Stiftung No. 439.

696.7

AUTORADIOGRAPHY OF I_1-IMIDAZOLINE AND α_2-ADRENERGIC SITES IN RAT BRAINSTEM: RETICULAR LOCALIZATION OF I_1-SITES

P. Ernsberger* & L.A. Collins Depts of Medicine & Neuroscience, Case Western Reserve School of Medicine, Cleveland OH 44106



The cardiorespiratory actions of imidazolines in the rostral ventrolateral medulla (rvl) may be mediated not only by α_2-adrenergic but also by putative I_1-imidazoline receptors. In order to map the distribution of I_1 relative to α_2 sites, adjacent 15 µm sections of rat brainstem were preincubated 1h, then incubated 1h with 0.5nM [^{125}I]*p*-iodoclonidine and either vehicle (top, total binding), 0.1mM cimetidine to mask I_1 (second), 10µM epinephrine to mask α_2 (third), or 0.1mM BDF-6143 to define nonspecific binding (bottom). Labeling in NTS (arrows mark solitary tract) and other dorsal nuclei is mainly α_2, since it is blocked by epinephrine but not cimetidine. I_1-Imidazoline sites (third) are absent in NTS but are widely distributed across reticular areas, including rvl. Similarly, in the pons the locus coeruleus expresses mainly α_2 while I_1 are present in A5 and ventral tegmental areas. I_1-Imidazoline sites are putative receptors located in the reticular formation which may participate in autonomic control.

696.8

ACTIVATION OF NORADRENERGIC NEURONS IN THE LOCUS COERULEUS BY CORTICOTROPIN-RELEASING HORMONE.

C. Schulz, M. Kretz, A. Bock, J. Beyer, H. Lehnert* Dept. of Endocrinology, University Hospital of Mainz, FRG

The locus coeruleus (LC) is involved in the behavioral activating and anxiogenic effects of corticotropin-releasing hormone (CRH). Stress increases CRH in the LC and CRH dose-dependently increases the firing rate of noradrenergic neurons in the LC.

The aim of our present study was to examine the activating effects of different doses of CRH on the noradrenergic neurons of the LC. A microdialysis probe was implanted in one projection area of these neurons, the parietal cortex. To rule out the influence of locomotor activity, the experiments were performed in urethane anesthetized male Wistar rats. The probe was perfused with artificial CSF, samples were collected in 20 min intervals. Substances (0.17, 0.51 nmol CRH, saline) were applied via a stainless steel canula (250 µm od) into the LC. The samples were analysed by HPLC-ECD for noradrenalin (NA) and it's metabolite MHPG.

While after administration of saline the level of NA in the parietal cortex remained stable at the baseline level (0.0279 pmol/sample), both doses of CRH increased the firing rate of noradrenergic neurons, as documented by a marked augmentation of NA release (0.51 nmol CRH: from 0.0200 to 0.0274 pmol/sample). The amount of MHPG released did not change after application of either saline or 0.17 nmol CRH. Only following 0.51 nmol CRH a slight increase of MHPG could be observed.

The results of our experiments clearly demonstrate an activation of noradrenergic LC-neurons by CRH.

696.9

TIME-DEPENDENT INHIBITION OF SOMATIC AFFERENT-EVOKED ACTIVITY IN NUCLEUS TRACTUS SOLITARIUS (NTS) NEURONS INVOLVES REDUCED EXCITATORY POST SYNAPTIC POTENTIAL (EPSP) MAGNITUDE

G.M. Toney* and S. W. Mifflin Dept. of Pharmacol. University of Texas Health Science Center, San Antonio, TX 78284.

In previous studies, extracellular recording techniques were used to demonstrate time-dependent inhibition of NTS cell activity following repetitive stimulation of visceral and somatic afferent inputs. In this study, experiments were performed in anesthetized, paralyzed, rats using intracellular recording techniques to examine possible synaptic mechanisms underlying this inhibition. NTS cell activity was evoked by electrically stimulating the contralateral tibial nerve (skeletal muscle afferents) with 500-600 µA conditioning stimuli followed 50 msec later by identical test stimuli. Among NTS cells responding to tibial nerve stimulation (n=20), the resting membrane potential (RMP) was 52.7±1.8 mV. Membrane potential responses to conditioning stimuli were characterized by discrete EPSPs. The magnitude (area) and dV/dt of test EPSPs were significantly ($p<0.001$) reduced (50.7±14.2 % and 65.5±15.5 % respectively) compared to responses following initial conditioning stimuli. In 18 cells, the reduction in EPSP magnitude occurred with no change in RMP. In 2 NTS cells, EPSPs were followed by inhibitory postsynaptic potentials (IPSPs) of 5.25±0.75 mV. The lack of IPSPs in the majority of NTS neurons tested indicates that the synaptic mechanisms responsible for time-dependent inhibitory interactions between somatic afferent inputs to NTS involve disfacilitation of excitatory inputs and is consistent with results obtained for visceral afferent inputs to NTS in cats. Taken together, this indicates that in both species similar synaptic mechanisms mediate time-dependent inhibitory interactions and that similar synaptic mechanisms appear to underlie the integration of multiple types of sensory inputs within NTS. (Supported by HL 36080).

INGESTIVE BEHAVIORS VI

697.1

BUTORPHANOL DECREASES RATS' LATENCY TO BEGIN LEVER PRESSING AND INCREASES AMOUNT OF FOOD CONSUMED IN OPERANT CHAMBERS.

J.M. Rudski*, C.J. Billington, J.P Cleary, & A.S. Levine, VAMC, Minneapolis, MN 55417.

Opiate administration typically reduces food-reinforced operant responding, yet increases food intake under conditions of free-access. Butorphanol, a mixed opioid agonist-antagonist, produces robust feeding in free access experiments, but its characteristic effects on responding in operant chambers have not been well documented. In Experiment 1, 8.0 mg/kg butorphanol administered for 4 consecutive days 1 hour before sessions significantly increased pellets (45 mg) eaten under an FR 10 reinforcement schedule over 60 minutes (vehicle= 74.7±8.1, butorphanol= 148.6±13.5; $F_{(1,1)}$= 17.42, $p<0.002$). Experiment 2 examined whether butorphanol affected initiation or maintenance of food intake. Butorphanol's effects (0, 0.3, 1.0, 3.0, and 10.0 mg/kg) on responding under an FR 80 (1st pellet) FR 3 (all subsequent pellets) reinforcement schedule were examined. Each rat received each dose for 4 consecutive days. Food intake above baseline was increased (range = -0.38 pellets for vehicle to +48.0 pellets for 10.0 mg/kg; $F_{(4,368)}$= 11.4, $p<0.0001$)) and latency of the first response was decreased (range = +112.8 sec for vehicle to -363.1 sec for 10.0 mg/kg; $F=3.15$, $p< 0.02$) by butorphanol, but amount of time to complete the FR 80 following commencement of responding was not. Repeated drug administration increased intake ($F_{(3,368)}$= 4.98, $p<0.001$). In sum, butorphanol, in contrast to other opiates, increases lever pressing contingent food intake in addition to increasing free feeding.

697.2

NALOXONE'S ANORECTIC EFFECT IS DEPENDENT UPON LENGTH OF DEPRIVATION AND PALATABILITY OF FOOD.

A. S. Levine*, D.T. Weldon, M. Grace, J.P. Cleary and C.J. Billington. VA Medical Center and University of MN., Minneapolis, MN 55417.

Blockade of opioid receptors results in decreased food intake. In the current study we evaluated the effect of peripherally administered naloxone on intake induced by chronic deprivation (80% of body weight), 24 and 48 hour food deprivation; and by offering rats a palatable food (laboratory chow containing sucrose). To control for schedule-induced feeding effects of the chronic deprivation, a group of rats were given food ad libitum for 2 hours per day. Naloxone was injected subcutaneously at doses ranging from 0.001 to 10 mg/kg and ED_{50}'s were calculated from log/linear plots (table):

	Hour 0-0.5	Hour 0-1	Hour 0-2
24 hr. deprive	5.7 mg/kg	5.6 mg/kg	6.7 mg/kg
48 hr. deprive	5.8 mg/kg	7.0 mg/kg	>10 mg/kg
Chronic deprive	>10 mg/kg	>10 mg/kg	>10 mg/kg
Schedule fed	>10 mg/kg	>10 mg/kg	>10 mg/kg
Sweet/satiated	0.03 mg/kg	0.03 mg/kg	0.05 mg/kg
Sweet/deprive	0.8 mg/kg	1.3 mg/kg	>10 mg/kg

Naloxone was most effective in decreasing food intake induced by palatability (sweetened chow) and least effective when rats were extremely "hungry". Although not deprived, the schedule fed rats ate a large amount of food in a short time and thus simulated the consummatory behavior of a chronically deprived rat. These data suggest that opioids are involved in taste perception and palatability (reward) rather than nutrient seeking behavior.

697.3

2-MERCAPTOACETATE (MA) AND 2-DEOXY-D-GLUCOSE (2DG) INDUCE QUALITATIVELY DIFFERENT MACRONUTRIENT APPETITES. L. Cromer, F.H. Koegler and S. Ritter*, Dept of VCAPP, Washington State University, Pullman, WA 99164.

2DG and MA block glucose utilization and fatty acid oxidation, respectively, and stimulate feeding by these distinct metabolic actions in brain and periphery. In this study, we examined the effects of 2DG and MA on macronutrient selection. Rats were maintained until intakes had stabilized (~3 wks) on a diet consisting of 3 separate macronutrient sources: carbohydrate (corn starch), protein (casein) and fat (corn oil), supplemented with vitamins, minerals and fiber. Intake of these diets in response to MA (400 and 600 umol/kg, ip), 2DG (100 and 200 mg/kg, sc) and saline was then measured in 4-hr tests with all 3 macronutrients present. MA produced a highly selective increase in protein intake, while 2DG increased intake of all 3 macronutrients. In vagotomized rats, however, 2DG increased carbohydrate intake selectively, as did injection of the glucoprivic agent into the 4th ventricle. Vagotomized rats did not eat in response to MA. Finally, in collaboration with M. Friedman we found that 2,5-anhydro-D-mannitol, which impairs carbohydrate metabolism peripherally but does not enter the brain, selectively increased protein intake. Results suggest that glucoprivation elicits a carbohydrate appetite that is centrally-mediated. MA-induced protein intake and intake of protein and fat after systemic 2DG are vagally-mediated.

697.4

COMPARISON OF THE EFFECTS OF ADRENALECTOMY AND RU486 ON FOOD INTAKE AND WEIGHT GAIN OF DIET INDUCED OBESE SPRAGUE-DAWLEY RATS. Q.Trocki,D.J.Baer and T.W.Castonguay*, Dept of Human Nutrition and Food Systems, Univ. of Maryland, College Park, MD 20742 and USDA Beltsville, MD 20705

The effects of adrenalectomy (ADX) or RU486 on food intake and body weight were investigated in Sprague-Dawley rats made obese by giving them free access to Crisco in addition to AIN-76 diet. Control groups were given free access to AIN-76 diet only. Animals were given their respective diets for 4 wk before they were randomly assigned to one of 7 treatments. Rats in the dietary control groups adrenalectomized (C-ADX) or given sham operations (C-SHAM). Rats assigned to the dietary obese groups received ADX (O-ADX), sham operation (O-SHAM), ADX with corticosterone replacement (O-CORT, 2mg/d s.c.), RU486 (O-RU, 20mg/kg/d s.c.) or vehicle of RU486 (O-VEH). Daily food intake and body weight were monitored for additional 3 wk. ADX significantly decreased weight gain ($p<0.01$) but the effect was greater in animals that had the fat option (O-ADX vs C-ADX, $p<0.05$). CORT replacement significantly increased weight gain of ADX animals ($p<0.01$). RU486 significantly decreased weight gain ($p<0.01$) but not as much as ADX. Body composition analysis showed that ADX, not RU486, decreased carcass fat content ($p<0.01$). Both ADX and RU486 decreased daily caloric intake, and food intake ($p<0.01$). RU486 also decreased fat intake ($p<0.01$). These results indicate that the effect of ADX on weight gain is greater than RU486. (Supported in part by NIH grant DK44486).

697.5

DIETARY INDUCED OBESITY IN AGING FEMALE C57BL/6J MICE: SENSORY AND HYPOTHALAMIC NORADRENERGIC RESPONSES. JA Grinker*, R Lipman and M. Jhanwar-Uniyal. Sch Pub Health, Univ. MI, Ann Arbor MI 48109; HNRC USDA Tufts Univ. Med. Cent. Boston, MA 02111 and Rockefeller Univ., NY, NY 100021.

Variability and stability in weight/fatness to palatible diets were examined in C57BL/6J female mice (retired breeders). Mice were 10 months (27 g) at the start of dietary manipulations [(S) high CHO: lab chow +30% Borden's sweetened condensed milk; 3.26 kcal/g)]; [(O) high fat: lab chow +15% wt/wt corn oil: 5.01 kcal/g)]; lab chow: (3.44 kcal/g). Mice became obese in 4-6 wks with wts of S mice plateauing by 10 wks (peak wt.=36 g). O mice continued to gain wt. (46.3g at end). At sacrifice brains were removed for determination of medial hypothalamic α_2-noradrenergic receptor binding to [3H] p-amino- clonidine. Results show that sensory responsiveness on chow (Grinker & Block, 1992) was associated with increased wt/fatness on O but unrelated to gain on S diet. Mice on both palatabile diets consumed similar calories (S: 20.5 kcal/day; O: 20.6 kcal/day). No decrements in body wt/fatness occurred with return to chow after 16 wks (confirmed by tissue and carcass analysis). The enhanced adiposity and heightened variability in wt gain were greatest in O fed mice compared with S fed mice. Higher hypothalamic α_2-noradrenergic receptor binding occurred in S ($S=231.95 \pm 38.6$ fmoles/mg protein; +205%; $P<0.05$) and O (284 ± 23.4 fmoles/mg protein; +251%; $P<0.01$) compared to chow (113 ± 9.1 fmoles/mg protein). Ongoing research clarifies whether higher noradrenergic receptors are related to diet, degree of wt gain or sensory predisposition. Current results demonstrate that hypothalamic receptors are involved in the hyperphagia and enhanced wt gain in dietary induced obesity.

697.6

ALPHA$_2$ NORADRENERGIC RECEPTORS IN THE PREPIRIFORM CORTEX DECLINE WITH AMINO ACID IMBALANCE. D.W.Gietzen* and M.Jhanwar-Uniyal. Dept. VM:Physio.Sci. and Food Intake Lab., Univ. Calif. Davis, CA 95616 and The Rockefeller Univ. N.Y., N.Y., 10021.

The anorectic responses to imbalanced amino acid diets (IMB) have been associated with decreased norepinephrine (NE) and cAMP in the prepiriform cortex (PPC), an area essential for the initial feeding responses to IMB. Also, the α_2 agonist, clonidine, injected into the PPC, increased intake of IMB. Therefore, we measured α_2-noradrenergic receptor binding in the PPC of rats fed threonine IMB or a control diet, either low-protein (BAS) or corrected (COR). After prefeeding BAS for 10 days, rats were given IMB, BAS or COR for 2.5 hours. The PPC (N=6), anterior cingulate cortex (AC, N=7), ventromedial hypothalamus (VMH, N=3) and lateral hypothalamus (LH, N=3) were assayed. Binding of [^{3}H]*p*-aminoclonidine to α_2 receptors was determined using radioligand binding techniques. Non-specific binding was determined in the presence of 10 μM phentolamine. Data were calculated as fmol/mg protein. In the PPC, α_2 binding expressed as a % of AC (a control brain area that does not show NE changes with IMB) was decreased over 6 fold in the IMB group compared with the BAS group, with COR intermediate. Values were BAS: 94.7 ± 25.6 (%AC, X $\pm$ SE), IMB: 15.6 ± 4.9, COR: 50.2 ± 8.5, $p = 0.005$. α_2 binding in the PPC, but not AC, was correlated with food intake, PPC: $r^2 = 0.61$, $p < 0.001$, AC: $r^2 = 0.31$, NS. There were no significant changes in LH or VMH, but binding in VMH tended to decrease with IMB. These data support our suggestion that NE acts at the α_2 receptor in the PPC in mediating the anorectic responses to IMB diets.

Supported by USDA CSRS 90-37200-5440, NIH DK35747 & DK42274.

697.7

ACCUMBENS D_1 AND D_2 RECEPTORS SUPPORT SHAM-FED SWEET REWARD. **L.H. Schneider*, C.A. Sikorsky, E.A. Rauhofer and G.P. Smith Department of Psychiatry, N.Y. Hospital-Cornell Med. Center, Bourne Lab, White Plains, NY 10605, and Dept. of Chemistry, New York University, NY NY 10003**

We have previously reported that dopaminergic (DA) mechanisms are necessary for the positive reinforcing effect of sucrose to sustain sham feeding in the rat. In these studies, selective D_2 antagonists were administered intraperitoneally or infused into the lateral ventricle. In the present study, we attempted to identify a central DA site at which the blockade of D_2 receptors is critical for the positive reinforcing effect of sucrose to maintain sham feeding. Rats (n=9) were chronically implanted with a gastric cannula for sham feeding and bilateral brain cannulas aimed stereotaxically at the nucleus accumbens (NACC). Rats were adapted to 30-min tests sham feeding a 40% sucrose solution following an 18h food deprivation and bilateral infusions of 0.5ul/side of artificial csf, raclopride, or SCH 23390. Raclopride at 2.5, 10 and 40 ug/rat produced a significant dose-related inhibition in the animals tested [$F=6.9(3,9)$; $p<.01$)] from saline baseline [45.9 ± 2.8 ml/30 min) of 35%, 41% and 55%, respectively. The inactive (+)-enantiomer or raclopride was without effect at the highest dose (40 ug/rat) tested. The selective D_1 antagonist SCH 23390 produces similar effects in a dose-related manner, indicating that either D_1 or D_2 mechanisms are required in the NACC. Analyses of the microstructure of licking in the sucrose sham feeding rat as well as behavioral time-sampling support the conclusion that blockade of NACC DA receptors reduces the reinforcing potency of the sucrose solution without interference with rats' ability to lick and swallow the sweet solution.**[Supported by (LHS) NIH R29 NS232781 and (GPS) NIMH MH15445,MH00149.]**

697.8

COMPARATIVE ANORECTIC EFFICACY OF SEROTONERGIC ANTIDEPRESSANT AGENTS ASSESSED IN SUCROSE SHAM-FEEDING RATS. R.B. Murphy*, L. Bajdeki, S.M. Feldman, and L.H. Schneider, Dept. of Chemistry and Center for Neural Sciences, New York University, NY NY 10003

We have employed the model of the sucrose sham-feeding rat to examine the anorectic actions of various serotonergic (5-HT) reuptake blockers and amphetamines. These included (+)-fluoxetine, sertraline, d-fenfluramine, and d-amphetamine. In this preparation, all of these agents produced anorexic actions in a highly significant dose-dependent manner; the ED_{50} doses were 9 mg/kg, 6.7 mg/kg , 2 mg/kg, and 1.0 mg/kg, respectively. Microstructural analysis of each agent indicated characteristic differences in rates and patterns of licking. For example, the size of bursts of licks was significantly reduced only under sertraline, whereas the number of intercluster intervals was significantly reduced only under d-fenfluramine. The distribution of interlick intervals was not significantly shifted at the doses of these agents which were used. These results indicate that the sucrose sham-feeding rat may be a useful system in which to explore activity of novel anorexic agents [Supported by INNOVA BIOMED INC].

697.9

SUCROSE AND SACCHARIN DRINKING BOUT ANALYSIS: EFFECTS OF D-FENFLURAMINE. S.J. Cooper* and D.A. Morris. School of Psychology, Univ. Birmingham, Edgbaston, Birmingham B15 2TT, U.K.

Studies have shown that a primary effect of d-fenfluramine (d-fen) in suppressing sucrose solution consumption is to reduce the duration of drinking bouts. In the present work, analysis of bout duration (and rates of licking) was conducted for both saccharin and sucrose drinking to investigate the effects of i) manipulating solution concentrations, and ii) 1.8 mg/kg, i.p. d-fen on drinking bout durations. D-Fen reduced the consumption of both types of solution, and the overall mean bout duration appeared to be more sensitive to d-fen's effects in rats consuming sucrose solutions. There was a strong relationship between the duration of early bouts of drinking sucrose and the concentration of the solutions. These long bout durations rapidly diminished as the test proceeded. Bout duration, in the case of sucrose drinking, appears to be sensitive to both palatability and satiety factors. Since d-fen did not affect the duration of the initial drinking bout for either solution, but did reduce the average bout duration of the first 5 min of the test, we summise that d-fen did not reduce solution palatability directly, but may have affected it secondarily.

697.10

BEHAVIORAL ANALYSIS OF A KETANSERIN-SENSITIVE ANORECTIC ACTION OF LYSERGIC ACID DIETHYLAMIDE (LSD) IN RATS. **K. J. Simansky*, J. F. Kampherstein and W. Kachelries. Dept. Pharmacology, Medical College of Pennsylvania, Philadelphia, PA 19129.**

LSD and other hallucinogens disrupt operant responding maintained by appetitive reinforcement. The present study: 1) assessed the potency of LSD to reduce food intake in male rats consuming sweetened milk diet after 18 h food deprivation; 2) analyzed the effects of LSD on the gross pattern of prandial behaviors; 3) determined the ability of ketanserin to block LSD-induced anorexia; and 4) characterized the action of LSD on the distribution of licking during gastric sham-feeding. Tests were conducted for 30-min with behaviors rated by a time-sampling observational method and licking detected using on-line computer acquisition. LSD (0, 0.1, 0.33 and 1.0 μmol/kg, i.p., 6 min before access to milk, n=6-7/grp) reduced intake (control = 34.0 ± 2.4 ml) in a dose-related manner ($p<0.01$) with an approx. ED_{50}≈0.33 μmol/kg. Rats began feeding normally after all treatments but the two highest doses of LSD decreased the incidence of resting normally observed in satiety and produced an abnormal prone posture. Ketanserin (5.0 μmol/kg) blocked completely the anorectic effect, the prone posture and the inhibition of resting. LSD also reduced milk intake when high rates of consumption were sustained during sham-feeding without satiation (control = 50.1 ± 3.3 ml). During sham-feeding, 0.33 μmol/kg LSD did not significantly disrupt feeding behavior but did reduce intake by 40%. Rats given this dose displayed smaller bursts of licking and decreased efficiency of licking. This pattern is also observed after fenfluramine and fluoxetine and may reveal an action to reduce the palatability of the tastant (Asin et al., 1993). Thus, the data suggest that, besides disrupting appetitive patterns sustained during behavior, LSD may probe physiologically important processes involved in feeding. Further studies will better define the role of 5-HT_2 receptors in these processes. Supported by NIMH 41987

697.11

AGE DEPENDENT EFFECTS OF CCK AND DEVAZEPIDE ON FOOD INTAKE IN BOTH MALE AND FEMALE RATS. C.F. Salorio, P.B. Hammond, G.J. Schwartz, P.R. McHugh* and T.H. Moran. Dept. of Psychiatry, Johns Hopkins Univ. School of Medicine, Baltimore, MD. 21205, Dept. of Environmental Health, Univ. of Cincinnati, Cincinnati, OH 45267.

Peripheral administration of the brain gut peptide CCK has been demonstrated to inhibit food intake in a variety of species and recent data have demonstrated that administration of a specific type A CCK receptor antagonist increases food intake in a variety of experimental paradigms. The potency of CCK to inhibit food intake varies across deprivation schedules and can be shown to be significantly lessened under conditions of elevated food intake (i.e. genetic obesity, lactation and during the long photoperiod in Siberian Hamsters). To examine the potential for differences in the relative role of CCK in feeding control at different ages, we examined the effects of administration of CCK (1-16 μg/kg) and the CCK antagonist devazepide (32-320 μg/kg) on 1 hr glucose consumption in male and female rats at 45-70 and 110-130 days of age. CCK was more potent in older rats as measured by the absolute magnitudes of suppression at any dose of CCK and the lowest dose at which significant suppressions were obtained. Administration of the CCK A antagonist devazepide had no effect on intake in the younger rats but significantly increased intake in both male and female older rats in a dose dependent fashion. These data demonstrate that during a period of rapid growth and high levels of food intake relative to body weight, adolescent rats are relatively insensitive to exogenous CCK and endogenous CCK does not appear to play a significant role in controlling their intake. (DK19302)

697.12

EFFECTS OF PREWEANLING LOSORTAN TREATMENT ON HUMORAL SYSTEMS REGULATING BODY FLUID BALANCE. **R. Kirby*, A. Alt, C. Novak, L. Flanagan, M. Henry & A. K. Johnson. Dept. of Psychology Univ. Iowa, Iowa City, IA. 52242.**

We have found that blockade of the renin angiotensin system (RAS) during preweanling development produces long-term increases in water intake and urine output. The present study examined the humoral mechanisms responsible for body fluid regulation following preweanling RAS blockade. Male WKY pups were administered daily the angiotensin receptor antagonist Losortan potassium (10 mg/kg) or vehicle subcutaneously on postnatal days 11-20. On day 22 animals were weaned to metabolism cages with water and chow available. Fluid intake and urine output were measured on a weekly basis. Blood was sampled for hormone measurement from day 22 to adulthood. Preweanling Losortan treatment led to a 600% increase in plasma renin activity on day 22. However, levels were comparable between Losortan and vehicle treated animals from day 29 to adulhood. Losortan did not alter vasopressin or aldosterone levels at any age examined. These results suggest that increased renin release may be involved initially in the increased water intake and urine output following early RAS blockade. However, other mechanisms must be involved as the increases in plasma renin activity are present only briefly following blockade while increased intake and output persist into adulthood.

EXCITATORY AMINO ACIDS: EXCITOTOXICITY VI

698.1

TRANSNEURAL PROPAGATION OF EXCITOTOXICITY BY L-CCG-IV FOR BILATERAL DEATH OF CA1 NEURONS. T. Shigeno*[1], M. Sasaki[2], G. Kato[3], Y. Yamasaki[4], M. Miyamoto[5], M. Ishida[5], and H. Shinozaki[5]: [1]Neurosurgery, Saitama Medical Center, [2]Fuchu Metropolitan Hospital, [3]Chiba College of Medical Science, [4]Taiho Pharmaceuticals, [5]The Tokyo Metropolitan Institute of Medical Science, Bunkyo, Tokyo 113, JAPAN

We have previously reported that intracerebral injection with a potent NMDA receptor agonist, the (2S,3R,4S) isomer of α-(carboxycyclopropyl)glycine (L-CCG-IV) induced selective death of the CA1 neurons in the rat hippocampus. Although the injection site was unilateral CA1, neuronal death frequently occurred in the bilateral CA1 sectors. However, when combined with severance of dorsal hippocampal commissure together with corpus callosum, there was no neuronal death in the bilateral CA1 sectors. This indicates a presence of transneural mechanism for selective death of CA1 neurons by glutamate excitotoxicity. Therefore, we investigated EEG changes recorded from bilateral CA1 sectors before and after injection of L-CCG-IV (50 nmole in 1μl vehicle) into the unilateral CA1. We observed immediate spike discharges in the ipsilateral CA1 that propagated to the contralateral CA1 in a few minutes. The rats showed intermittent seizure and wet-dog shaking. By contrast with severance of dorsal hippocampal commissure and corpus callosum, no spike discharges were seen in both CA1 sectors. We further investigated whether or not NMDA itself could cause the same neuropathology as observed by L-CCG-IV. NMDA was injected from 10 to 100 nmole in 1μl vehicle in a separate series of rats. Beyond the dose of 30 nmole, most of rats died after severe seizure. However, death of CA1 neurons was only observed in the ipsilateral side to injection. Severance of dorsal hippocampal commissure and corpus callosum did not ameliorate the death of ipsilateral CA1 neurons. These findings indicate that (1) receptor for L-CCG-IV distributes differently from NMDA and (2) neural connection governed by L-CCG-IV is different from that of NNDA. The intrinsic neuronal circuitry in and around the hippocampus would underlie this phenomenon.

698.2

STUDY OF LATHYRISM:SOME NEW FINDINGS. A.Haque* F.Lambein,M.Hossain,J.DeReuck, J.K.Khan,Y.H.Kuo. Institute of Postgraduate Medicine & Research, Dhaka 1000 and State University of Gent.B-9000.

Lathyrism is a type of human spastic paraparesis caused by an excitatory aminoacid ODAP present in Lathyrus sativus L.cultivated and consumed in the tropics. 882 lathyrism patients were studied between 1991-92 in Bangladesh to assess epidemiological,clinical and some biochemical aspects of lathyrism. 80% of the patients were below 30 yrs during onset. The disease is prevalent in male(87%). The disease is static in 73% for 19±4 yrs and 27% started deterioration after a static state for 14±2 yrs showing features of Amyotrophic lateral sclerosis. The deterioration does not correlate with habit of lathyrus consumption($p. <.001$). Radiological investigation revealed features of osteolathyrism in 2 of 60 patients x-rayed. Study for HTLV-1 association showed 4% positive results. Serum Zn level was .78±.24mg/L compared to .92±.24mg/L in control raised suspicion of low Zn level and affinity to ODAP.Tolperisone Hcl gave symptomatic improvement in 60% ($p <.01$). Analysis of CSF aminoacid revealed raised glycine and glutamate level.

698.3

LITHIUM-PILOCARPINE-INDUCED STATUS EPILEPTICUS PRODUCES CHANGES IN EXTRACELLULAR AMINO ACID CONCENTRATIONS IN THE RAT AMYGDALA. D.G. Fujikawa*, J.S. Kim, A.H. Daniels and A.F. Alcaraz. Exp. Neurol. Lab., VA Med. Ctr., Sepulveda, CA 91343 and Dept. of Neurology and Brain Res. Inst., UCLA Sch. of Med., Los Angeles, CA 90024.

It has been hypothesized that excessive release of glutamate (GLU) *in vivo* produces seizure-induced neuronal damage. The amygdala is one of many limbic structures which are damaged by lithium-pilocarpine-induced status epilepticus (SE). We measured extracellular amino acid concentrations in this structure during SE. Six Wistar rats underwent stereotaxic placement of microdialysis probes into basolateral amygdaloid nuclei. Probes were perfused with Krebs-Ringer-bicarbonate solution, and dialysate samples were obtained during a 2-h baseline period, 3-h SE and a 2-h recovery period. Diazepam and phenobarbital were given 3 h after SE onset to stop the seizures. Extracellular GLU (GLU_e) decreased to 64-70% of baseline levels during the first 60 min of SE and at 150 min of SE. Extracellular glutamine (GLN_e) decreased to 55-71% of baseline during the first 150 min of SE. Extracellular taurine (TAU_e) increased to 166-194% of baseline at 60 min and 120-180 min of SE and remained elevated at 209% of baseline 2 h later. Aspartate, glycine and serine were unchanged. Increased TAU_e occurs when extracellular osmolarity increases, as a result of cell swelling and a decrease in the size of the extracellular space (ECS). GLN_e probably falls because of increased presynaptic uptake of this GLU precursor. Decreased GLU_e in the face of a smaller ECS suggests that the rate of GLU uptake may exceed its rate of release during SE. GLU_e measured by MD probes do not necessarily reflect synaptic concentrations, and GLU_e alone is not adequate for assessing *in vivo* activation of postsynaptic GLU receptors. Other strategies are necessary for *in vivo* confirmation of the excitotoxic hypothesis for SE.

698.4

POSTNATAL DEVELOPMENT OF NEURONAL VULNERABILITY TO INDIRECT AND DIRECT EXCITOTOXINS: A COMPARATIVE STUDY OF γ-ACETYLENIC GABA AND QUINOLINIC ACID IN THE RAT HIPPOCAMPUS. O.G.McMaster*, F. Du and R. Schwarcz. Maryland Psychiatric Research Center, Baltimore, MD 21228.

Indirect and direct excitotoxins can produce axon-sparing brain lesions via excitatory amino acid-mediated mechanisms (Ann. Neurol. 31: 119, 1992). We have now studied, in a comparative fashion, the development of vulnerability of rat hippocampal neurons to focal injections of γ-acetylenic GABA (GAG) and quinolinic acid (QUIN). At 10, 14, 18, 22 and 28 days of age, rats were injected intrahippocampally with 30 nmol QUIN or 240 nmol GAG - doses which produce virtually identical patterns of neurodegeneration in hippocampal CA1 and CA3 fields in the adult rat. Three days later, the animals were perfused, and their hippocampi were examined by light microscopy with Nissl staining. GAG did not produce cell death until postnatal day 22, when relatively mild neuronal damage was observed in CA1 and CA3. Notably, the 18 day-old rat was even resistant to 360 nmol GAG. By day 28, GAG produced an adult-like hippocampal lesion. QUIN did not cause neuronal damage until day 14 when cell loss was observed in CA1. By day 18, QUIN caused neuronal loss in both CA1 and CA3. These data confirm that the mechanism of GAG toxicity is distinct from classical ("direct") excitotoxicity and may depend on processes which have not developed in the hippocampus of 18 day-old rats.

Supported by USPHS grants NS 16102 and NS 28236.

698.5

PHENCYLIDINE INDUCES THE HSP70 HEAT SHOCK GENE IN INJURED CORTICAL NEURONS VIA MULTIPLE RECEPTORS: DISINHIBITION AS A MECHANISM OF INJURY. F.R. Sharp*, M. Butman, K. Aardalen, R. Nakki, J. Koistinaho, S. Massa, S.M. Sagar. Dept. Neurology, Univ. of California and SFVAMC, San Francisco, CA 94121.

Phencyclidine and other NMDA receptor antagonists induce the HSP70 heat shock gene in injured, vacuolated rat cingulate cortical neurons. PCP (50mg/kg) induces hsp70 mRNA and HSP70 protein in neurons in cingulate cortex, neocortex, insular cortex, piriform cortex, amygdala and hippocampus. Induction of HSP70 by PCP could be prevented by haloperidol (D2), clozapine (D4), valium (GABAa), SCH 23390 (D1), muscimol (GABAa), scopolamine (M1), and rimcazole (sigma). Baclofen had no effect. Nifedipine blocked HSP70 induction in some regions (cingulate and neocortex) and not others.

These results are interpreted to mean that PCP injures cortical pyramidal neurons by disinhibiting them because PCP blocks NMDA receptors on GABAergic neurons in cortex that would normally inhibit the pyramidal neurons. This would make cortical pyramidal neurons sensitive to injury produced by excitatory inputs from dopamine acting on D1, D2, and D4 receptors, ACh acting on M1 receptors, actions at sigma receptors, and by actions of other transmitters at other excitatory receptors. Injury is mediated by calcium entry through L-type voltage gated calcium channels in some brain regions. In addition, GABAa but not GABAb agonists decrease the injury produced by PCP, with GABA actions at the benzodiazepine site appearing to be more effective than at the GABA (muscimol) site itself.

698.6

CEREBELLAR TOXICITY OF PHENCYCLIDINE. R. Näkki, L.J. Noble, J. Koistinaho, F.R. Sharp, S.M. Sagar*, Depts. of Neurology and Neurosurgery, VA Medical Center and University of California, San Francisco, CA 94121.

Phencyclidine (PCP), as well as other NMDA antagonists, are toxic to cortical neurons, especially in the cingulate and retrosplenial cortex. To determine if other neurons are damaged, the distribution of microglial activation following the administration of PCP, 50 mg/kg i.p., was studied immunohistochemically using the monoclonal antibody OX-42. In addition to microglial activation in cingulate and retrosplenial cortex, coronal sections of cerebellar vermis of PCP-treated rats contained vertical stripes of activated microglia in the molecular layer. In sagittal sections, the microglial activation occurred in irregularly shaped patches, consistent with damage to Purkinje cells. Only a small minority of Purkinje cells were associated with activated microglia. In contrast to the cingulate cortex, where PCP induces the synthesis of the HSP70 heat shock protein, no HSP70 immunoreactivity was detected in the cerebellum after PCP administration. However, *in situ* hybridization histochemistry demonstrated the expression of hsp70 mRNA widely in the Purkinje cell layer 6 hr after PCP, 50 mg/kg i.p.

These observations indicate that PCP is toxic to cerebellar Purkinje cells and support the usefulness of microglial activation and heat shock protein induction as markers of neurotoxicity. The findings raise the clinically important question of whether NMDA antagonists other than PCP are also toxic to the cerebellum.

698.7

ANTI-PSYCHOTIC AGENTS PREVENT NMDA ANTAGONIST NEUROTOXICITY JW Olney*, NB Farber, MT Price, J Labruyere, J Nemnich, H St. Peter. Washington Univ., St. Louis MO 63110.

PCP (phencyclidine), MK-801 and ketamine -- all PCP receptor ligands (PRL) and antagonists of NMDA glutamate receptors -- acutely injure cerebrocortical neurons when administered subcutaneously to adult rats. Normal humans anesthetized with PCP or ketamine experience acute psychotic episodes sometimes termed an "emergence reaction," and patients with quiescent schizophrenia respond to PCP by relapsing into a protracted acute psychotic state. We have proposed that the neurotoxic and psychotic effects of PRL may represent morphological and psychological manifestations of the same toxic process, since both of these effects show similar patterns of age dependency (onset of susceptibility in early adulthood) and can be prevented by the same drugs (benzodiazepines and barbituates). A possible link to schizophrenia might also be proposed in view of the early adult onset of schizophrenia. Consistent with this proposal, we now report that the neurotoxic effects of PRL in the adult rat can be prevented by pretreatment with various drugs that have either putative or proven anti-psychotic activity (e.g., haloperidol, thioridazine, DTG, rimcazole and clozapine). A sigma receptor may be involved in the neural circuitry mediating PRL neurotoxicity since all of the above agents, with the exception of clozapine, are sigma receptor ligands. Because clozapine is not a sigma receptor ligand, it is difficult to identify a single characteristic that all of the effective agents have in common other than anti-psychotic potential. In summary, PRL cause psychotic symptoms in humans and cerebrocortical neuronal injury in rats, and we now find that various agents with proven or potential antipsychotic activity in schizophrenia prevent PRL from injuring neurons in rat brain. We propose that PRL neurotoxicity is a promising animal model for studying mechanisms underlying human psychotic processes. Supported by DA 05072, DA 06454, AG 05681 and RSA MH 38894 (JWO).

698.8

ELECTROENCEPHALOGRAPHIC SEIZURE ACTIVITY PRODUCED BY MK-801 BUT NOT BY THE GLY-SITE NMDA ANTAGONIST L-687,414. F.C. Tortella* and +R.G. Hill. Walter Reed Army Inst. Res., Washington, DC 20307 and +MSDRL Neurosci. Res. Ctr., Harlow, UK, CM20 2QR.

L-687,414 is a novel partial antagonist for the glycine site of the NMDA receptor that displays anticonvulsant and neuroprotective properties *in vivo* (Foster et al., Mol. Neuropharm. 2: 97, 1992). In a differential study of EEG and behavior in adult rats L-687,414 (5-100 mg/kg as salt, i.v.) was compared to MK-801 (0.1-0.5 mg/kg, i.v.) and a weaker glycine site partial agonist, (+)-HA966 (12.5-100 mg/kg as salt, i.v.). MK-801 produced typical head-weaving and locomotor ataxia along with intermittent EEG spike complexes and associated myoclonic responses even at the lowest doses tested. In contrast, high doses of (+)-HA966 caused sedation accompanied by disruption in normal EEG patterns seen as high-amplitude, slow-wave synchronizations centered on the 4-5 Hz band. L-687,414 caused mild locomotor ataxia at the highest doses tested, but otherwise behavior and EEG patterns were indistinguishable from controls. These results suggest that L-687,414 is likely to be free of some of the undesirable properties of MK801. In addition, there appear to be differences between the actions of (+)-HA966 and L-687,414 on the EEG of the conscious rat.

698.9

GLUCOCORTICOIDS AND STRESS EXACERBATE KAINATE-INDUCED ANTIGENIC ALTERATIONS IN TAU AND NEURON LOSS IN RAT HIPPOCAMPAL NEURONS. B.A. Stein-Behrens*[1], I. Chang[1], M.P. Mattson[2], R.M. Sapolsky[1]. [1] Dept. Biological Sci., Stanford Univ., Stanford CA, 94305. [2] Sanders-Brown Center on Aging, U. Kentucky, Lexington KY 40536.

Glucocorticoids (GCs), the adrenal steroids released during stress, can endanger hippocampal neurons such that an insult (seizure, hypoxia/ischemia, hypoglycemia) results in more neuron damage in the presence of GCs. These steroids have been shown to exert their compromising effects by increasing levels of extracellular glutamate and free cytosolic calcium concentrations in the post-synaptic neuron. If GCs enhance calcium mobilization induced by neurological insults to the hippocampus, the hormones should enhance Ca^{++}-dependent degenerative events as well. In support of this, we have previously observed that GC concentrations at the peak physiologic range, as compared to very low GC controls, exacerbate kainate-induced degenerative events such as antigenic changes in tau and spectrin proteolysis. In the present study, we found that this GC exacerbation occurs under more subtle physiological conditions. We found that increasing GC concentrations from low- to high-basal and low- to high-stress values as generated by exogenous GC pellet implantation caused incremental increases in neuronal immunoreactivity with tau antibodies 5E2 and ALZ-50 and neuron loss after kainate infusion to the hippocampus. We then found that stress makes kainate-induced tau alterations and neuron loss worse. Interestingly, we have also found that stress alone, in the absence of kainate excitation, results in some tau accumulation. This is the first evidence that stress itself, rather than exogenous GCs, can exacerbate neurodegenerative events in the hippocampus.

698.10

PREGNENOLONE SULFATE ATTENUATES EXCITOTOXIC NEURONAL INJURY IN MURINE CORTICAL CELL CULTURES. M.B.Wie*, C. Tian and D.W.Choi. Dept. of Neurology, and Center for the Study of Nervous System Injury, Washington Univ. School of Medicine, St. Louis, MO 63110.

The ability of the endogenous neurosteroid pregnenolone sulfate (PS) to modulate CNS glutamate receptor function, NMDA receptor-gated currents while reducing AMPA and kainate receptor-gated currents has been recently reported (Wu et al., Mol. Pharmacol. 40:333, 1991). We therefore examined the ability of PS to modify excitotoxicity in murine cortical cell cultures.

Cultured cortical cells exposed to 100 μM NMDA for 5 min or 10 μM AMPA for 24 hr developed widespread submaximal neuronal death by the next day. Addition of 1 - 100 μM PS to the exposure solution produced a modest, concentration-dependent attenuation of NMDA-induced neuronal death, with 30-50% reduction at 10 μM PS. Somewhat weaker attenuation was seen of AMPA-induced neuronal death. These results support the possibility that endogenous PS may modify excitotoxic neuronal death - at least that mediated by NMDA receptors - under certain pathological conditions.

Supported by NIH grant NS 30337.

GENE STRUCTURE AND FUNCTION VI

699.1

IDENTIFICATION OF NOVEL cDNA CLONES CONTAINING CAG OR CCG TRINUCLEOTIDE REPEATS FROM HUMAN BRAIN. S-H. Li, R.L. Margolis, M.G. McInnis, C.A. Ross*. Laboratory of Molecular Neurobiology, Departments of Psychiatry and Neuroscience, Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205

A new form of human mutation, expansion of trinucleotide repeats, has recently been shown to cause Huntington's disease, myotonic dystrophy, Kennedy's disease, and fragile X syndrome, diseases with neuropsychiatric features and a pattern of inheritance known as anticipation. Identification of additional genes containing trinucleotide repeats may provide clues to other diseases exhibiting anticipation, including bipolar affective disorder and possibly schizophrenia and autism. We have previously identified eight novel clones containing CAG or CCG repeats (Li et al, *Genomics*, in press); we now report the isolation of additional novel cDNA clones containing long CAG and CCG repeats. A human cerebral cortex cDNA library was screened at high stringency using CAG_{60} or CCG_{60} oligonucleotides as probes. Clones representing 28S ribosomal RNA were excluded with a pooled 28S rRNA oligo probe. Clones likely to have brain selective expression were identified by excluding clones reacting strongly with a probe made from human liver poly A^+ RNA. So far, we have identified seven CAG- and eleven CCG-containing novel clones. The nature and length of the repeat within each novel clone was determined by sequencing through the repeats. All seven novel CAG-containing clones have eight or more consecutive repeats and all 3 of CCG-containing clones which have been sequenced through the repeats have more than nine consecutive repeats. Work is in progress to further characterize these clones. The novel sequences that we have identified should provide useful linkage markers for the human genome project, and are candidate genes for bipolar affective disorder and other neuropsychiatric conditions.

699.2

IDENTIFICATION OF NOVEL cDNA CLONES CONTAINING AAT OR CCA TRINUCLEOTIDE REPEATS. R.L. Margolis*, S-H. Li, Melvin G. McInnis, C.A. Ross. Departments of Psychiatry and Neuroscience, Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205

A new form of human mutation, expansion of trinucleotide repeats, has recently been shown to cause Huntington's disease, myotonic dystrophy, Kennedy's disease, and fragile X syndrome, diseases with neuropsychiatric features and a pattern of inheritance known as anticipation. Identification of additional genes containing trinucleotide repeats may provide clues to other diseases exhibiting anticipation, including bipolar affective disorder and possibly schizophrenia and autism. We have previously identified eight novel clones containing CGG or CAG repeats (Li et al, *Genomics*, in press); we now report the isoloation of novel cDNA clones containing AAT and CCA repeats. A human cerebral cortex cDNA library was screened at high stringency using CCA_{30} or AAT_{60} oligonucleotides as probes. After plaque purification, plasmid isolation, and partial sequencing, the nature and length of the repeat within each novel clone was determined. Preliminary data indicate that at least two of eleven novel clones with eight or more consecutive repeats are polymorphic, containing at least four different alleles. Work is in progress to further characterize these clones. The novel sequences that we have identified should provide useful linkage markers for the human genome project, and are candidate genes for bipolar affective disorder and other neuropsychiatric conditions.

699.3

HZF-3, A NOVEL NUCLEAR HORMONE RECEPTOR RELATED TO NGFI-B S. Peña de Ortiz, R. Pun* and G.A. Jamieson Jr. Toxicology Program, Department of Environmental Health, University of Cincinnati College of Medicine, Cincinnati Ohio 45267-0056.

We hypothesize novel members of the nuclear hormone receptor superfamily play important roles in signal-transcription coupling in the hippocampus. To isolate rat hippocampal cDNAs encoding novel nuclear hormone receptors, a systematic search for members of this family expressed in the hippocampus was initiated. We report here the isolation of HZF-3, a novel member of the superfamily related to the inducible orphan nuclear receptor NGFI-B. The sequence of HZF-3 predicts a protein 69Kd in size which overall is 58% homologous to NGFI-B. Highest homology between HZF-3 and NGFI-B is observed in their DNA and ligand binding domains, 92% and 72% respectively, suggesting they may interact at a functional level. Reverse transcription coupled to PCR analysis determined HZF-3 mRNA within the brain to be preferentially localized to the hippocampus (hippocampus >>> cortex > cerebellum). *In situ* hybridization experiments will define the precise neuroanatomical localization of HZF-3. Studies aimed at identifying stimuli which regulate HZF-3 expression in PC12 cells have determined that unlike NGFI-B, the expression of HZF-3 is not induced by NGF, TPA, or calcium ionophore treatments. Additional experiments seek to determine whether HZF-3 expression is induced in the rat brain subsequent to seizures and whether HZF-3 interacts with NBRE, the NGFI-B DNA target site. If so this would suggest a complex set of interactions between HZF-3 and NGFI-B serve to regulate transcriptional responses in the hippocampus.

Supported by ONR N00014-90-J-1898, NIMH Minority Fellowship, Ford Foundation Minority Dissertation Fellowship.

699.4

CLONING AND REGIONAL DISTRIBUTION OF THE RAT MAP2d, A NOVEL MAP2c SPLICE VARIANT ENCODING FOUR MICROTUBULE BINDING DOMAINS, WHOSE EXPRESSION IS REGULATED BY bFGF. M. Khrestchatisky*, L. Ferhat, H. Pollard, A. Bernard, G. Charton, J. Moreau, G. Barbin and Y. Ben-Ari. INSERM U-29, 123 Bd de Port Royal, 75014 Paris, France.

High and low molecular weight forms of MAP2 proteins, MAP2a-MAP2b and MAP2c respectively coexist with different ratios in the embryonic and postnatal rat brain. MAP2c is predominantly expressed in the immature brain. MAP2 variants are encoded by distinct mRNAs of 9 and 6 kb, both transcribed from a single gene. Using reverse transcription-coupled PCR (RT-PCR) and various primer pairs, we have characterized MAP2d, a novel MAP2c transcript comprising a 93 bp insertion encoding 31 additional amino acids in the carboxy terminal region. This insertion, absent from the MAP2b mRNA is highly homologous to the three microtubule binding domains found in MAP2 proteins and to the fourth microtubule binding domain encoded by the alternatively spliced exon 10 of the Tau gene. Splicing of the additional exon is developmentally regulated, the MAP2d being predominantly expressed over the MAP2c in the young and adult rat brain. A study of the regional distribution of both isoforms, using RT-PCR and In Situ Hybridization reveals that MAP2c/MAP2d ratios differ in various structures of the CNS. Finally, we show that in primary cultures of hippocampal neurons, bFGF exposure promotes the MAP2c-MAP2d switch. The MAP2c/MAP2d ratios found at the mRNA level suggests assuming ratios are conserved at the protein level- that in the young adult brain, a major proportion of low-molecular weight MAP2 comprises 4 rather than 3 microtubule binding domains.

699.5

THE SAXITOXIN-BINDING PROTEIN, SAXIPHILIN, IS A NEW MEMBER OF THE TRANSFERRIN FAMILY. M.A. Morabito* and E.G. Moczydlowski. Departments of Pharmacology and Cellular and Molecular Physiology, Yale University School of Medicine, New Haven, CT 06518.

Saxitoxin (STX) is a potent neurotoxin that inhibits electrical excitability by blocking voltage-dependent Na-channels. Saxiphilin is a ~91 kd soluble protein that binds STX with high affinity ($K_D \sim 0.2$ nM) and has been previously purified from bullfrog (*Rana catesbeiana*) plasma (Li and Moczydlowski, 1991. J. Biol. Chem. 266: 15481-7). We cloned saxiphilin cDNA from bullfrog liver by combining PCR amplification with screening of a cDNA library. We isolated a cDNA clone with an open reading frame of 845 amino acids. Identity of the clone with saxiphilin is verified by the presence in the cDNA of the amino terminal sequence and the sequence of five tryptic fragments of native saxiphilin. Primary sequence analysis of saxiphilin reveals significant homology to the transferrin family of proteins with amino acid identity as high as 51% for transferrin from *Xenopus laevis* and 44% for human transferrin. A 19-residue signal secretion sequence, internal duplication characteristic of transferrin and 14 disulfide bonds common to the transferrin family are conserved in saxiphilin. In contrast to transferrins, saxiphilin does not bind Fe^{3+} which is explained by mutation in saxiphilin of nine out of ten highly conserved residues in the two homologous Fe^{3+}-binding sites of transferrin. In addition, saxiphilin contains a unique insertion of 144 residues, a region of which is highly homologous (61%) to a 46-residue domain of nidogen, an ubiquitous cell matrix protein. Tissue distribution analysis of saxiphilin mRNA in the bullfrog shows the highest level of expression in liver, followed by lung, pancreas and brain. The unique features of saxiphilin and strong homology to transferrin identify saxiphilin as a new member of the transferrin family that does not function in iron transport but may bind a different ligand, such as saxitoxin, that interacts with the nervous system. [Supported by NIH and USAMRDC.]

699.6

IS THE NEURON-SPECIFIC SPLICING MECHANISM OF CLATHRIN LIGHT CHAIN B EXON EN USED BY OTHER NEURON-SPECIFIC EXONS? S. Stamm[1], D. Casper[2]* and D. M. Helfman[1] [1]Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724, [2]Fishberg Research Center for Neurobiology, Mount Sinai School of Medicine, One Gustave L. Levy Place, New York, NY 10029

Clathrin light chains are components of clathrin coated vesicles which are involved in endocytosis and membrane recycling. The light chain B (LCB) gene encodes two isoforms, termed LCB2 and LCB3, via an alternative RNA splicing mechanism. The neuron-specific LCB2 isoform is generated by the inclusion of a single exon, termed exon EN. We analyzed *cis* elements involved in splice site selection by transfecting minigene constructs into primary neuronal and HeLa cell cultures. Both the 5' and 3' splice sites of exon EN deviate from the vertebrate consensus sequences. Changing either the 3' or 5' splice site of exon EN into the consensus sequence leads to the use of exon EN in HeLa cells. If both splice sites are converted into the consensus sequence, exon EN is constitutively used in HeLa cells. We noticed sequence identity between exon EN and other neuron-specific exons. Changing this shared sequence eliminates the use of EN in neurons. UV cross linking experiments detect specific RNA-binding proteins in brain nuclear extract that bind to EN but not to EN probes lacking the sequence motif shared by several neuron-specific exons. We demonstrated that one of the brain-specific exons sharing sequence identity (exon 9c of the α tropomyosin gene) is also neuron-specific and is first expressed at the same time (E16) as EN during rat brain development. We propose that the use of exon EN is regulated as follows: Exon EN is not spliced in HeLa cells because its 3' and 5' splice sites differ from the consensus. In neurons, a protein factor binds to EN and promotes its use. We postulate that a developmentally regulated neuron-specific exon binding factor is partially responsible for the regulation of a variety of neuron-specific exons, such as LCB exon EN and α tropomyosin exon 9c.

699.7

REGULATION OF POMC TRANSLATION BY POMC STEM-LOOP RNA-BINDING PROTEINS IN AtT-20 CELLS. C.M. Spencer* & J.H. Eberwine. Dept. of Pharmacology, University of Pennsylvania School of Medicine, Philadelphia, PA 19104

Regulation of proopiomelanocortin (POMC) gene expression in the anterior pituitary is an important component of the stress response. There is evidence to suggest that translational control is involved in the acute response. POMC mRNA contains a stable (-45 kcal) stem-loop structure that may play an important role in its translational control. We have previously reported that cytoplasmic extracts of mouse corticotroph AtT-20 cells interact specifically with a 60-nt riboprobe corresponding to this stem-loop structure. We are presently investigating the role of these RNA-protein interactions in the translational control of POMC gene expression. In this study we examined the effects of POMC stem-loop RNA and various cytoplasmic extracts on the translatability of full-length POMC RNA in a rabbit reticulocyte lysate *in vitro* translation system. Increasing amounts of AtT-20 cytoplasmic extract reduce the amount of POMC protein synthesized *in vitro*, but do not affect the translation of BMV control RNA. Cytoplasmic extracts of rat pituitary also inhibit POMC translation; however, COS cytoplasmic extracts have no effect on the amount of POMC protein synthesized. These data suggest that cytoplasmic factor(s) present in rat pituitaries and AtT-20 cells, but absent in green monkey kidney COS cells, are involved in the inhibition of POMC translation. Furthermore, this inhibition can be attenuated in a dose-dependent manner by the addition of the 60-nt POMC stem-loop RNA, suggesting that POMC stem-loop RNA-binding proteins are able to modulate the translation of POMC.

699.8

HUD BINDS TO THE STABILITY ELEMENT OF FOS MRNA. J. Liu, E. Wong and H. M. Furneaux*. Lab. of Mol. Neuro-Oncology, Sloan Kettering Institute for Cancer Research, N.Y., N.Y. 10021.

Hud is a human neuronal-specific RNA binding protein which is the target antigen in autoimmune encephalitis sensory neuronopathy. By virtue of its high homology to the Drosophila protein, Elav, it is thought that Hud regulates neuronal development and maintenance. Many intermediate early gene products (such as Fos Jun Myc) which are involved in neuronal signal transduction pathways are regulated at the post transcriptional level by an element in their 3' untranslated region which controls message stability. Using North Western Blot and gel retardation assay we have shown that Hud selectively binds to the stability element of fos mRNA. No complex was detected with a deleted fos mRNA which lacked the stability element. Deletions of Hud have shown that the third RNA binding domain is essential for complex formation. These results suggest that Hud may control neuronal development and maintenance, at least in part, by regulation of cfos expression.

699.9

IDENTIFICATION OF CHROMOSOME 16 cDNA CLONES FROM A MOUSE NEWBORN *wv*/+ LIBRARY. M.E. Hodes, L. Sangameswaran, Y. Wang, S. Dlouhy and B. Ghetti*. Indiana University School of Medicine, Indianapolis, IN 46202.

The weaver mutation was mapped previously to mouse chromosome 16. Our approach to weaver gene identification is by mapping and characterizing cerebellar cDNAs that have been obtained by different screening methods. In previous studies, we described a number of novel cDNAs that were obtained by immunoscreening an expression library and we are in the process of genetically mapping them. In addition, we have now screened a newborn *wv*/+ cerebellar cDNA library with a probe prepared by subtracting cDNA from a *wv*/+ neonatal library with cRNA from a P31 *wv*/*wv* library. This was done because the expression of the weaver gene might be transient, or, in adult cerebellum, might be limited primarily to granule cells. In the first of a series of experiments, we screened 200,000 phage and pulled 27 positive plugs. PCR analysis indicated that the plugs consist of pools of phages that contain different inserts. These amplified inserts were evaluated by Southern blot analysis with a hamster x mouse cell line that contains mouse chromosome 16. Four of the 27 pools contain an insert(s) that appears to derive from mouse chromosome 16. These clones are being characterized further. We conclude that this approach will lead to cDNAs derived from chromosome 16. (Supported by grant PHS P01-NS27613)

699.10

QUANTITATIVE MEASUREMENT OF CALRETININ AND ß-ACTIN mRNA FROM RAT BRAIN MICROPUNCHES WITHOUT PRIOR ISOLATION OF RNA. K.I. STRAUSS* & D.M. JACOBOWITZ, Lab of Clinical Science, NIMH, Bethesda, MD 20892.

A microdissection technique for quantitation of neurochemicals in discrete brain nuclei has been coupled with a quantitative lysate ribonuclease protection assay (RPA). Discrete microdissected nuclei and other brain regions (10-500 μg protein) are solubilized in 6 M guanidine thiocyanate solution and directly hybridized with riboprobes. Concurrent detection of mRNA(s) of interest with the ß-actin "housekeeping" gene and total sample protein permits normalization and quantitation in terms of these internal controls. This method eliminates the necessity for isolation of RNA from solid tissue. Assumptions regarding RNA recovery are not necessary since solid tissue specimens are solubilized, hybridized and treated with RNase in a single tube. We have determined the mRNA levels of calretinin, a predominantly neuron-specific calcium binding protein in discrete rat brain nuclei and developing embryos. Calretinin mRNA ranged from 281 ± 35 fg/μg protein in the thalamic paraventricular nucleus to 2.3 ± 0.5 fg/μg protein in the cerebral cortex. Calretinin:ß-actin ratios for the same specimens ranged from 79.9 ± 9.3% to 1.3 ± 0.1%, respectively. The "micropunch RPA" bypasses the uncertainties associated with RNA isolation from solid tissue; is quantitative for detection of high and low abundance mRNAs from microdissected tissue specimens; and enables the analysis of large numbers of samples in 2-3 days.

699.11

TRANSIENT EXPRESSION OF GLIAL FIBRILLARY ACIDIC PROTEIN (GFAP) mRNA IN RETINAL MULLER CELLS.
V. Sarthy*, H. Egal and L. Verderber, Department of Ophthalmology, Northwestern University, Chicago, IL 60611.

The Glial Fibrillary Acidic Protein (GFAP) gene is not normally expressed in Müller (glial) cells in the mouse retina. The GFAP gene is, however, activated in response to retinal injury, degeneration and detachment. Recently, we examined the time course of GFAP mRNA and GFAP formation in mice with light-induced retinal degeneration. BALB/c mice were maintained under constant light source for one to six weeks to induce photoreceptor degeneration. GFAP accumulation was examined by immuno-cytochemistry while GFAP mRNA formation was determined by *in situ* hybridization and Northern blotting. GFAP immunostaining in Müller cells was first observed on day 4 and reached maximal intensity after two weeks of light exposure. GFAP was present in Müller cells even after two months. GFAP transcripts were first noted in Müller cells around day 4 and GFAP mRNA levels were increased after two weeks. Surprisingly, no GFAP transcripts were detected in Müller cells after four weeks of light exposure. The retinal astrocytes expressed high levels of GFAP mRNA in all cases examined. In RNA blotting studies, a small amount of GFAP mRNA was present initially in the mouse retina. Following two weeks of light damage, GFAP mRNA levels were elevated about 5-fold. After six weeks in constant light, little GFAP mRNA was found in the retina. Our results show that GFAP is found in Müller cells long after initial synthesis probably as a consequence of its low turnover in the Müller cell cytoskeleton while GFAP mRNA levels rise and decline rapidly due to transient induction of the GFAP gene.

CIRCUITRY AND PATTERN GENERATION V

700.1

THE ROLE OF GLUTAMATE IN THE SWIM NEURAL CIRCUIT OF *TRITONIA*. G.D. Brown* and A.O.D. Willows. Friday Harbor Laboratories, Friday Harbor, WA 98250

Predatory starfish elicit an escape swimming response from the sea slug *Tritonia diomedea*. The neural circuit which underlies this rhythmic behavior is comparatively well understood (Getting, 1983). We are currently trying to identify the neurotransmitters used in this circuit and to determine their role in pattern generation. We report here that glutamate may have a role in activating the escape swim in *Tritonia*.

Tritonia brains were isolated and perfused with filtered sea water. The swim neural program (SNP) can be activated in this preparation by brief electrical stimulation of a nerve root. The SNP was monitored with intracellular electrodes in identified swim circuit neurons. We found that puffing 100 µl of 10 mM glutamate onto the cerebral-pleural ganglion reliably activated the SNP. Lower concentrations were sometimes effective as well. We also monitored cells in the swim neural circuit during addition of several common glutamate agonists. NMDA most closely mimicked the SNP response to glutamate. Quisqualate also activated the SNP and produced tonic activity in some of the cells. Kainate given at a much lower dose (25 µl of 1 mM) produced tonic activity in many, if not most cells in the brain. Glutamate and each of these agonists inhibited SNP activation by nerve root stimulation when applied in sufficient amounts (see also McClellan et al, 1993).

The glutamate receptor antagonist CNQX applied at concentrations of 200-500 µM blocked activation of the SNP by nerve root stimulation. Our observations indicate that this block occurs prior to the central pattern generator which produces the rhythmicity in this system, but CNQX may have effects further downstream as well.

700.2

SEROTONIN FROM DSI NEURONS ACTS AS BOTH A NEUROTRANSMITTER AND AN INTRINSIC NEUROMODULATOR IN THE *TRITONIA* ESCAPE SWIM CPG CIRCUIT. P.S. Katz* and W.N. Frost, Dept. of Neurobiol. & Anat., Univ. of Texas Health Science Center, Houston, TX 77030.

The dorsal swim interneurons (DSI) are integral components of the swim pattern generating circuit in the mollusc, *Tritonia diomedia*. In addition to its conventional synaptic actions, DSI also evokes neuromodulatory effects, enhancing the strength of connections made by the C2 interneuron onto other CPG neurons and motor neurons (Frost & Getting, Soc.Neurosci.Abstr. 1989, Brown & Willows, Soc.Neurosci.Abstr. 1991). We are interested in the role of this "intrinsic neuromodulation" in the functioning of the network. As a first step, we have investigated the role of serotonin (5-HT), since the DSIs had been shown to be immunoreactive to 5-HT (Getting et al, Soc. Neurosci.Abstr. 1985). In the present study, several lines of evidence indicate that 5-HT may be responsible for both the synaptic and modulatory actions of DSI. First, we found that 5-HT applications mimicked and occluded both the synaptic and modulatory actions of DSI. Second, the 5-HT antagonist, gramine (0.1mM), blocked the rapid synaptic potential evoked by DSI onto a motor neuron as well as the depolarization caused by pressure-applied 5-HT; we have not yet tested gramine's effects on the modulation. Third, within a few hours of a brief exposure of the ganglion to 5-HT or its precursor, 5-hydroxy-L-tryptophan, both the synaptic and modulatory effects of DSI were greatly enhanced, consistent with the possibility that these treatments increased the quantity of 5-HT released by DSI. These results suggest that DSI utilizes 5-HT both to produce conventional synaptic effects and to simultaneously modulate network properties.

Supported by: NIMH R01 MH49563 to PSK.

700.3

A NETWORK OF CELLS INITIATING PATTERNED ACTIVITY IN THE BUCCAL GANGLIA OF *APLYSIA*. I. Hurwitz, R.S. Goldstein, & A.J. Susswein*. Dept Life Sci, Bar Ilan Univ, Ramat Gan, Israel 52 900.

The B31/B32 cells in the buccal ganglia of *Aplysia* initiate a patterned burst of activity in many cells. The pattern resembles that caused by CBI-2, a feeding command neuron. Brief depolarization of B31/B32 initiates a sustained, regenerative depolarization, followed by a hyperpolarization that is correlated with excitation of cell B4. We have now identified a number of cells that are activated by B31/B32, and that in turn feed back onto it. The input from these cells onto B31/B32 can account for much of the activity pattern seen in B31/B32 during bursts of buccal ganglia activity.

B34 is probably involved in coordination between the 2 buccal hemi-ganglia. It is depolarized along with B31/B32, and monosynaptically excites contralateral B31/B32 cells with facilitating EPSPs. Firing B34 initiates a burst in the buccal ganglia.

B63 is part of a positive feedback loop with B31/B32. The cell is excited by B31/B32 activity, and also monosynaptically and polysynaptically excites B31/B32. Monosynaptic and polysynaptic EPSPs show facilitation. B63 is active during the sustained depolarization of B31/B32, and firing it initiates a burst in the buccal ganglia.

B64 causes the large hyperpolarization at the end of a B31/B32 burst. It is active during the hyperpolarization. Firing it produces IPSPs in B31/B32 and in B34, and EPSPs in B4 resembling those seen during feeding bursts. Firing B64 can prevent B31/B32 from initiating a burst. B64 is apparently unable to fire single action potentials: depolarization sufficient to cause firing always elicits a train of spikes. Depolarization of B64 does *not* initiate a feeding burst in the buccal ganglia. An intracellular fill of B64 revealed a ~20 mm bipolar cell, with one process entering the buccal commissure. The morphology and position of B64 suggest that it may be a previously-identified dopaminergic neuron.

Future studies will be required to determine whether these connections can account for the entire activity pattern of B31/B32 during feeding bursts.

700.4

DIFFERENT FORMS OF GATING OF A PEPTIDERGIC MECHANOAFFERENT NEURON BY CENTRAL PATTERN GENERATOR NEURONS IN THE FEEDING SYSTEM OF *APLYSIA*. **S. C. Rosen*, M. W. Miller, K. R. Weiss, and I. Kupfermann, Cntr. Neurobiol. & Behav., NYS Psychiat. Inst., New York, NY 10032; Inst. of Neurobiol., Univ. of Puerto Rico, San Juan, PR 00901; Dept. Physiol. & Biophys., Mount Sinai Sch. of Med., New York, NY 10027.**

B21 is a low threshold radula mechanoafferent neuron containing the neuropeptides SCPa and SCPb. It makes different kinds of plastic chemical synaptic connections and electrical synaptic connections to motor neurons that control radula closure (e.g., B8a, B8b, B15, B16). B21 also makes a nonrectifying electrical synapse with CPG interneuron B19. B19 is a powerful regulator of the biting command-like interneuron CBI-2. B21 activity can potentiate the biting motor program via the B21, B19, CBI-2 loop. To determine whether signals across the B21-B19 electrical synapse might be transmitted in the other direction so that phase information generated by the CPG might gate B21's synaptic output, we manipulated B19's membrane potential while recording synaptic potentials evoked by the sensory cell in its motor neuron followers B8a and B15. Depolarization of B19 dramatically enhanced the chemical synaptic output of B21 to its follower cells, whereas hyperpolarization had the opposite effects. Moreover, we found that multiaction, CPG neuron B4 produced monosynaptic IPSPs in B19 and B21, as well as in motor neurons B8a and B15. When B4 was fired in bursts it also gated the sensory input from B21 to the motor neurons. The data indicate that the output of a single sensory neuron can be gated by multiple CPG elements utilizing different mechanisms and that electrical synapses might produce subtle forms of bidirectional gating between sensory cells and CPG neurons.

700.5

MULTIPLE INTRACELLULAR RECORDINGS FROM IDENTIFIED NEURONS IN *APLYSIA* DURING EVOKED FEEDING-LIKE ACTIVITY

P. J. Church* and P. E. Lloyd. Committee on Neurobiology, The University of Chicago, Chicago, Il 60637.

Consummatory feeding behavior in Aplysia consists of biting, swallowing, and rejection. It has been postulated that the neuronal control of these behaviors is hierarchically organized with the stimulation of a few command-like neurons necessary and sufficient to elicit buccal motor programs (BMPs). Previous studies suggest that neurons located in the cerebral ganglia may be responsible for the initiation and modulation of these BMPs and that motor neurons in the buccal ganglia generate the cyclic motor output underlying ingestion and egestion. We have identified a large number (>25) of these buccal motor neurons and have characterized the conventional transmitters and peptide cotransmitters which they express. In several cases, the stimulation paradigms necessary for release of those transmitters has been determined. In order to determine the behavioral firing patterns of these neurons we have recorded intracellularly from identified buccal neurons during evoked feeding-like BMPs. By recording simultaneously from up to seven neurons we were able to determine the relative firing rates and phase relationships of many identified neurons. In addition this activity can be correlated to specific aspects of feeding behavior. We have found that during evoked BMPs these neurons fire in paradigms capable of releasing peptide cotransmitters. Supported by NRSA 1-F31-MH10240 to PJC.

700.6

A POPULATION OF CEREBRAL INTERNEURONS IN APLYSIA MEDIATES THE COORDINATION OF VASCULAR RESPONSES WITH "SKELETOMOTOR" RESPONSES INVOLVING HEAD MOVEMENTS.

Y. Xin, K.R. Weiss, and I Kupfermann*. Center for Neurobiology and Behavior, Columbia University, 722 W. 168 St. New York, NY 10032 and Fishberg Center, Mt. Sinai School of Medicine, New York, NY 10029.

As an approach toward locating cerebral interneurons that might be involved in head turning responses we backfilled the cerebral pedal and pleural connectives. A population of neurons in the C cluster were identified which produce synaptic effects on a wide variety of pedal and pleural-ganglion neurons. CC5 receives unilateral input from the lips and produces unilateral synaptic effects to presumptive motor neurons of the body wall, and most notably a monosynaptic input to Pas, a motor neuron previously identified by Skelton and Koester, which produces shortening of the ipsilateral pedal artery. Tactile stimuli applied to the tentacle evokes spikes in the ipsilateral CC5, together with a contraction of the ipsilateral pedal artery. Hyperpolarization of CC5 blocks the contraction, and firing of CC5 produces a similar contraction, suggesting that under some circumstances CC5 may be acting as a true command neuron for reflex contraction of the pedal artery. CC5 and the Pas motor neuron also appear to be activated during locomotion, at the phase in which head shortening occurs, during feeding when the buccal mass moves backward, and perhaps during an ipsilateral turn of the head during various behaviors. Other C cluster neurons similarly produce responses appropriate for coordinating movements of the buccal mass, body wall and foot with responses of the circulatory system.

700.7

FUNCTIONAL MODIFICATION OF RHYTHMIC MOTOR ACTIVITY BY A MODULATORY PROJECTION NEURON.

M.J. Coleman*, B.J. Norris and M.P. Nusbaum, Neurobiology Research Center and Dept. of Physiology & Biophysics; Univ. of Alabama at Birmingham; Birmingham, AL 35294.

In the stomatogastric ganglion (STG) of the crab, *Cancer borealis*, the pyloric and gastric mill motor patterns are produced by distinct but overlapping subsets of STG neurons. The Inferior Cardiac (IC) neuron participates in both patterns, so that when these two rhythms occur concurrently, the impulse bursts in this neuron are time-locked to both rhythms. However, its synaptic effects on the other STG network neurons tend to be relatively weak. We have recently identified Modulatory Commissural Neuron 7 (MCN7) and have found that it selectively enhances IC neuron activity. MCN7 projects an axon from its soma in the commissural ganglion to the STG, via the superior oesophageal and stomatogastric nerves.

MCN7 enhances the membrane potential oscillations in the IC neuron, causing it to fire more intense impulse bursts. During these times, IC has stronger synaptic effects on all other gastric mill and pyloric neurons. Moderate activation of MCN7 enhances the pyloric-timed impulse bursts in IC. Stronger activation of MCN7 causes IC to become the pacemaker for a distinct gastric mill motor pattern in which there is a co-activation of two gastric mill neurons that otherwise exhibit an alternating activity pattern. This enhanced IC neuron activity also rhythmically suppresses the pyloric motor pattern by inhibiting the pyloric pacemaker neuron ensemble. This MCN7-evoked rhythm resembles the IC-driven gastric mill rhythm resulting from bath-application of the peptide proctolin (Heinzel et al., J. Neurosci. 13:1793-1803), suggesting that MCN7 is proctolinergic. We are examining whether MCN7 does indeed contain proctolin and, if so, why its effects differ from those of the other two proctolin neurons that influence the STG networks. Supported by NS29436 & HFSP (MPN).

700.8

MODIFICATION OF RHYTHMIC MOTOR ACTIVITY BY RECRUITMENT OF A NEWLY IDENTIFIED PROJECTION NEURON.

B.J. Norris*, M.J. Coleman and M.P. Nusbaum. Neurobiology Research Center & Dept. of Physiology & Biophysics; Univ. of Alabama at Birmingham; Birmingham, AL 35294.

Modulatory transmitters enable single neural networks to produce many neural activity patterns by altering neuronal membrane properties and/or synaptic efficacies. We have been studying how modulatory substances influence the gastric mill network, in the stomatogastric ganglion (STG) of the crab, *Cancer borealis*. The STG is one of four ganglia of the stomatogastric nervous system (STNS). Bath-application of the muscarinic agonist oxotremorine (OXO: 10^5 M) to the isolated STNS evokes either of two distinct gastric mill rhythms. The rhythm that is elicited is determined by whether an additional neuron, located in the commissural ganglion (CoG), is sufficiently excited to enable it to participate in the gastric mill rhythm. This newly identified neuron, called Modulatory Commissural Neuron 2 (MCN2), projects to the STG via the superior oesophageal and stomatogastric nerves.

The gastric mill rhythm that includes MCN2 activity is characterized by MCN2 firing together with the LG and GM neurons, and in alternation with the DG and IC neurons. When MCN2 does not participate in the gastric mill rhythm, IC fires along with LG and GM. MCN2 is instrumental in producing this motor pattern by way of its excitatory synaptic effects on LG and GM and its inhibitory synaptic effects on DG and IC. Interestingly, the rhythmic activity in MCN2 results partly from effects of OXO on MCN2 membrane properties and partly from synaptic input that MCN2 receives from one of the gastric mill neurons. Additionally, in the presence of OXO, an MCN2-type gastric mill rhythm can be elicited by rhythmic (but not tonic) stimulation of MCN2. Thus, modulatory transmitters can elicit a novel motor pattern by recruiting activity in an additional neuron. Supported by NS29436 and HFSP (MPN).

700.9

REAL TIME IMAGING OF ANTIDROMIC, SYNAPTIC AND RHYTHMIC ACTIVITY OF MOTONEURONS IN THE ISOLATED SPINAL CORD OF THE NEONATAL RAT.

A. Lev -Tov* and M. O'Donovan[1], Dept. of Anatomy, Hebrew University Medical School, Jerusalem Israel and [1]The .Lab. of Neural Control, NIH, NINDS, Bethesda, MD, 20892.

L3-L6 motoneurons in the lumbar cord of the neonatal rat (P1-P4) were retrogradely filled with the fluorescent calcium indicator calcium green (25% w/v) coupled to dextran (O'Donovan et. al., J.Neurosci. Meth., 91-106, 1993). Fluorescent responses were monitored from the cut face of the spinal cord using real-time intensified video microscopy during antidromic stimulation of ventral roots, orthodromic stimulation of dorsal roots or pharmacologically-induced rhythmic motor activity. Backlabelled motoneurons were restricted to lamina IX and often formed 2-3 discrete clusters of cells. Fluorescent changes could be detected in motoneurons following a single antidromic stimulus and progressively increased in amplitude during stimulus trains (1-5 sec) at frequencies from 5-50Hz. Synaptic responses were detected in motoneurons following stimulation of either ipsi- or contralteral dorsal roots. Rhythmic motor activity was induced by acetylcholine (Ach, 50-100μM) and edrophonium (100-500μM), strychnine(10μM), bicuculline (10-20μM) or 4-Aminopyridine (4-AP; 10μM-2.5mM). Rhythmic oscillations of free intracellular calcium were detected during rhythmic activity induced when Ach, Edrophonium and one of the inhibitory antagonists was applied or when 4-AP was applied alone. Our results indicate that elevations of free intracellular calcium accompany several forms of motoneuronal activity in the neonatal spinal cord of the rat and suggest that this technique may be useful for optical studies of single cell and network function in this preparation.

700.10

MODIFICATION OF THE LEECH SWIM OSCILLATOR PERIOD.

C.G. Hocker and W.O. Friesen*. Center for Biological Timing and Department of Biology, University of Virginia, Charlottesville, VA 22901

Swimming in the leech, *Hirudo medicinalis*, is an example of a fixed complex behavioral act that is created within the CNS. The leech propels itself through water by undulating its body wall. The movement is generated by a traveling wave of motor neuron activity, which sweeps from the anterior to the posterior end of the nerve cord. The pattern repeats with a period of 0.4 to 2 s. To examine the nature of the dynamic control of the CNS network underlying the coordination of the swimming behavior, several methods for comparing time series (graphic, spectral, and statistical) were applied to the fictive swims of the isolated leech CNS (20 midbody ganglia (M2-M21) plus the tail ganglion). Swimming activity occurred spontaneously or by shocking the dorsal posterior nerve of M16, M17 or M18. The time series data are composed of bursts of action potentials recorded extracellularly (spike events) from the dorsal excitor motor neuron (cell 3) of four midbody ganglia. Careful examination of the structure of the swim period variation was done with actograms created by plotting the time of spike events along a vertically stacked set of horizontal lines with a fixed time width. Upon converting the spike events to instantaneous frequency vs. time, the approximate entropy statistic of the continuous time series was found to be useful for comparing the regularity of ten second intervals of data.

For both spontaneous and induced fictive swims, the two most reproducible features, which could be an expression of a dynamic control within the CNS, are the abrupt shifts in the mean period and the frequency modulation (FM) with a period of about 10 swim oscillations. The FM of the posterior ganglia tends to precede ganglia more anterior. Unambiguous identification of the FM peak in frequency spectrum estimates was achieved.

700.11

EFFECTS OF LOCAL OSCILLATOR FREQUENCIES ON PHASE LAG IN SIMULATED CHAINS OF COUPLED OSCILLATORS. T.L.Williams* and K.A.Sigvardt. Physiology Dept, SGHMS, Univ London, SW17 0RE, UK, and Neurology, Univ California-Davis, VAMC, Martinez CA 94553.

The lamprey spinal CPG for locomotion can be considered as a chain of coupled oscillators, in which each oscillator has an intrinsic frequency that can be modified by coupling from its neighbors. Theoretical work indicates that chains with uniform intrinsic frequencies have uniform phase lags along the chain if coupling is asymmetric (Kopell and Ermentrout, SIAM J Appl Math 50:1014, 1990). Changing the intrinsic frequency in the rostral or caudal half of a chain changes the phase lags in one half but not the other. The half in which the phase lag changes depends not on which half has the higher intrinsic frequency but on whether ascending or descending coupling is dominant. We will compare the behavior of simulated chains of oscillators with the theory and with experimental data on the lamprey spinal cord. Our results indicate that ascending intersegmental coupling is dominant for setting intersegmental phase lags along the cord.

700.12

COMPUTER SIMULATION OF MOTOR PATTERN GENERATION FOR VARIABILITY IN SNAPPING IN ANURANS. J.S. Liaw, A. Weerasuriya and M.A. Arbib*. Center for Neural Engineering, University of Southern California, Los Angeles, CA 90089 and School of Medicine, Mercer University, Macon, GA 31207.

Anuran prey capture, released by specific stimuli, consists of a sequence of motor synergies. Snapping the consummatory event of this sequence has been thought to be a ballistic motor pattern. We have developed a model to address the variability recently observed in our laboratory of this rapidly executed behavior (<200 msec). Current evidence suggest that the motor pattern generator (MPG) for snapping is located bilaterally in the rostral portion of the medial reticular formation of the medulla oblongata. A biologically constrained neural network model for such an MPG is proposed. This model incorporates separate but interacting modules for the control of the depressor mandibuli (jaw opener), genioglossus (tongue protractor), and hyoglossus (tongue retractor) muscles. Activation of this MPG produces spatio-temporal patterns of activity in the above three motoneuron pools that are strikingly similar to EMG patterns recorded from these muscles during prey capture. A push-pull mechanism built into each module facilitates the coordination of the motoneurons and also provides a flexibility to motoneuron activation. Two schemes, mediation and modulation, for achieving this flexibility are explored to simulate variable tongue extension by having a 'closeness' signal to control the synchronization of tongue protractor and retractor. In the mediation scheme, the signal is used to control the onset time of the retractor, whereas in the modulation scheme, it changes the time-constant of the retractor thus leading to a variation in its rate of peaking.

BIOLOGICAL RHYTHMS AND SLEEP VI

701.1

QUANTITATIVE ANALYSIS OF Per PROTEIN CYCLING IN NUCLEI AND CYTOPLASM OF *Drosophila* PHOTORECEPTORS. M.G. Folwell, W. Bug*, and K.K. Siwicki. Biology Department, Swarthmore College, Swarthmore, PA 19081

A circadian rhythm in the expression of the *Drosophila period* gene apears to underly the effects of *per* mutants on behavioral rhythms. Evidence for rhythms in *per* mRNA and Per protein, which are opposite in phase, suggests a feedback loop in which *per* transcription is inhibited when nuclear Per levels are high. We have developed a photometric method using indirect immunofluorescence to characterize the kinetics of Per cycling in specific cell types. We use a cooled CCD camera (Photometrics, Inc.) to acquire two digital images of each eye section: nuclear staining (DAPI) and Per immunofluorescence. Using IPLab software (Signal Analytics) on a Macintosh Quadra 800, the edges of the nuclei are defined with the DAPI image, then superimposed on the Cy3 image to obtain the average value of anti-Per fluorescence within the nuclei. When applied to photoreceptors of per^+ flies, this analysis revealed robust daily and circadian rhythms in nuclear Per, confirming previous results. Levels of nuclear Per were lowest near dusk, and increased 4-fold to peak near dawn. The combination of sensitivity and spatial resolution of this method have revealed, for the first time, significant levels of cytoplasmic Per. In photoreceptors of flies from 12:12 LD, cytoplasmic Per levels were lowest near dawn, and highest near dusk (i.e., opposite in phase to the nuclear Per rhythm). Thus, cytoplasmic Per increased several hours earlier than nuclear Per, suggesting that nuclear translocation may be regulated. Experiments are in progress to examine the effects of entrainment cues and *per* mutations on the relative levels of cytoplasmic vs. nuclear Per.

701.2

GENETIC ANALYSIS OF A CIRCADIAN PACEMAKER OUTPUT PATHWAY. F.R. Jackson* and L.M. Newby. Worcester Foundation For Experimental Biology, Shrewsbury, MA 01545.

All circadian-period mutations of *Drosophila* affect the temporal patterning of locomotor activity and pupal eclosion, consistent with the idea that a single neural pacemaker governs the properties of both endogenous rhythms. Evidence suggests, however, that genetically separable neuroendocrine output pathways couple the pacemaker to eclosion and activity. To understand the physiology of a specific pacemaker output pathway, we are studying several mutations which alter the daily timing of eclosion but do not perturb the expression of the activity rhythm. One mutant named *gate* has completely arrhythmic patterns of eclosion in constant darkness but normal activity rhythms, indicative of a lesion in the output pathway controlling pupal eclosion. Studies are underway to cytogenetically map the *gate* mutation in order to facilitate molecular analyses of the relevant gene. A different mutant named *lark* has been identified, for which daily peaks of eclosion occur abnormally early in either light/dark or temperature cycles. The temporal distribution of locomotor activity, however, is normal in *lark* mutants, as is the free-running period of the circadian pacemaker. Interestingly, the *lark* mutation has a dominant effect on the timing of eclosion, but causes a recessive embryonic lethal phenotype. Both the behavioral and essential functions of the *lark* gene are encoded by a single transcription unit which expresses mRNAs throughout the embryonic central nervous system. Studies are in progress to document the expression pattern in pupae to understand how LARK protein contributes to the timing of eclosion. Based primarily on the observed mutant phenotypes, we propose that the LARK product might mediate the response of neural tissues to an endocrine signal.

701.3

INTRACELLULAR CALCIUM RESPONSES OF CIRCADIAN PACEMAKER NEURONS MEASURED WITH FURA-2. M. Geusz*, S. Michel, and G. Block. NSF Center for Biological Timing, Department of Biology, Gilmer Hall, University of Virginia, Charlottesville, VA 22903.

A neural pacemaker in the eye of the mollusk *Bulla gouldiana* generates a circadian rhythm in optic nerve activity. The basal retinal neurons (BRNs) of the eye contain the pacemaker and retain circadian rhythmicity while in cell culture. Light and other depolarizing stimuli shift the phase of the pacemaker in the eye through a process that is blocked by $[Ni^{2+}]_o$ or low $[Ca^{2+}]_o$. To test if an influx of Ca^{2+} is present throughout depolarizing treatments that produce phase shifts, dissociated BRNs in cell culture were loaded with the calcium-sensitive dye Fura-2 AM, and then depolarized with elevated $[K^+]_o$.

Calcium levels in the BRNs remained elevated during treatments with 50 mM $[K^+]_o$ lasting at least 1 h, a stimulus capable of phase shifting the pacemaker. $[Ca^{2+}]_i$ reached a peak near 1 μM within minutes of beginning the treatment, and then persisted at a lower plateau level until the stimulus ended (n=23 cells). Elevated $[K^+]_o$ produced significantly larger Ca^{2+} responses (p=0.03) when given during the subjective night (n=9) versus the subjective day (n=14). EGTA treatments administered during depolarization blocked the sustained rise in $[Ca^{2+}]_i$, verifying that the elevation requires a Ca^{2+} influx. The $[Ca^{2+}]_i$ increase during depolarization was also prevented with 50 mM Ni^{2+}, which blocks phase shifts to depolarization, but not with 5 mM Ni^{2+}, which does not block phase shifts. The Ca^{2+} changes occurred throughout the cell body, suggesting that a Ca^{2+} signal due to depolarization spreads into the nucleus. The results show that a phase-shifting stimulus generates a prolonged Ca^{2+} signal, and that BRNs in cell culture retain a critical part of the entrainment mechanism. *Supported by NS15264, NS08806 and the NSF Center for Biological Timing.*

701.4

TWO CHANNEL CANDIDATES UNDERLYING CIRCADIAN RHYTHMOGENESIS IN ISOLATED PACEMAKER NEURONS OF *APLYSIA* RETINA. J.W. Jacklet*[1] and S. Barnes[2], Department of Biological Sciences, SUNY Albany, NY 12222[1] and Neuroscience Research Group, University of Calgary, Alberta, Canada, T2N 4N1[2].

Several cell types (including pigmentless monopolar and bipolar neurons, pigmented support cells and pigmented large and small photoreceptors) are obtained from enzymatic dispersion of the *Aplysia* retina. Whole-cell tight-seal recordings in current-and voltage-clamp were made with 2-5 Mohm KCl, CsCl or K gluconate-filled electrodes from small (15-20 μm dia.) monopolar neurons, the putative circadian pacemaker neurons. They had resting potentials near -40 mV and, if neurites had grown out, produced spikes (60 mV) spontaneously at under 1 Hz. Depolarizing current injections increased the rate of firing. Hyperpolarizing current injections revealed inward rectification with slowly decaying (1-3s) inhibition following the current step. Under voltage-clamp, five ionic currents were apparent including a rapidly-inactivating Na current, a Ca current too small to be seen except during dialysis with CsCl electrode solution, and a delayed rectifier-type K current that activates positive to -10 mV. Two other currents have kinetic properties that suggest a possible role in modulating the resting potential and spontaneous spike generation. The A-current, although transient (inactivation time constant of 250 ms at -30 mV), has overlapping activation and steady-state inactivation curves, giving rise to window current in the voltage range between -90 and -50 mV. Dialysis of the cells with low free calcium solution also induces within minutes the appearance of inward rectifier Cl current, activating negative to -30 mV. Due to the low capacitance (9.5 $\pm$ 3.3 pF, n=16, SD) and high input resistance of the cells in this voltage range (6.5 $\pm$ 6.6 Gohm), even small membrane currents exert strong influence on excitability. We thank N. Syed, K. Lukowiak and A. Bulloch for help culturing cells. Supported by AHFMR, MRC and NSF.

701.5

PUTATIVE CIRCADIAN PACEMAKER NEURONS IN *APLYSIA* EYE ARE IMMUNOREACTIVE TO *APLYSIA* NEUROPEPTIDE Y.

S.M. Rajpara, D.F. Owens, V. L. Begnoche and E. Mayeri*. Dept. of Physiology, University of California, San Francisco, CA 94143-0444.

We recently identified a neuropeptide Y homolog in *Aplysia* (apNPY) that produces excitatory and prolonged inhibitory responses in neurons in the abdominal ganglion. In the present study we used an affinity purified antibody specific to the amino terminus of apNPY to investigate immunoreactivity (IR) in the *Aplysia* eye. Neurons and fibers in the outer layer of the retina were intensely IR for apNPY. The labelled neurons were secondary neurons, which correspond to the putative circadian pacemaker neurons. ApNPY IR was also present in the pacemaker neuropil, located near the initial portion of the optic nerve, and in a large bundle of fibers in the optic nerve. Staining was eliminated by antibody preadsorption with apNPY, but not by preadsorption with FMRFamide. Distribution of IR-apNPY in the eye appears to correlate well with IR previously described by Siwicki et al., 1989, for the *period* gene product. The *period* gene product has been implicated in the function of the circadian clock of *Drosophila*. Since circadian pacemaker neurons project to the central nervous system, the results raise the possibility that apNPY released from these cells may have a role in imposing a daily cycle of activity on central neurons and the animal's behavior.

701.6

CIRCULATING CORTICOSTERONE (CORT) AND HYPOTHALAMIC NEUROPEPTIDE Y (NPY): ANALYSES OF GENE EXPRESSION, PEPTIDE LEVELS AND RECEPTOR BINDING SITES.

S. F. Leibowitz*[1], A. Akabayashi[1], N. Levin[2], J. Roberts[2], Y. Watanabe[1] and X. Paez[1]. The Rockefeller Univ., N.Y., N.Y. 10021[1] and Mt. Sinai School of Medicine, N.Y., N.Y. 10029[2].

The present experiments support the existence of a close, positive relationship between circulating CORT, NPY gene expression in the arcuate nucleus (ARC), and NPY projections to the parvocellular paraventricular nucleus (pPVN), which is functionally expressed at the onset of the natural feeding cycle when CORT peaks to activate type II receptors. Results in intact rats demonstrate a clear light/dark rhythm of NPY gene expression (via solution hybridization/nuclease protection assay) and NPY levels (via RIA) in relation to circulating CORT, characterized by: 1) a rise in CORT, from <1.0 μg% to 5.0 μg%, starting at 6 hrs before dark onset; 2) an increase 2 hrs later in NPY mRNA in the mediobasal hypothalamus (MBH, containing the ARC), which returns to basal levels within 4 hrs; 3) a rise in NPY levels in the mediodorsal hypothalamus (MDH, containing the PVN) at dark onset, associated with a marked decline in NPY levels within the MBH; and 4) peak levels of CORT at dark onset, which are positively correlated with NPY mRNA or peptide levels ($r=+0.90$, $p<0.05$) in the MBH. Results in ADX rats suggest that CORT, at levels which activate type II glucocorticoid receptors, may directly contribute to this natural rise in NPY gene expression and peptide transport to the pPVN. While basal NPY gene expression in the ARC (via in situ hybridization) and peptide levels (via RIA) in the cell bodies and most terminal sites are unaffected by the absence of CORT, NPY levels in the pPVN, dorsomedial nucleus and preoptic area, which have high concentrations of type II receptors, are reduced by ADX and restored by CORT (>5 μg%). In ADX rats, CORT also potentiates NPY mRNA in the ARC, as well as the locus coeruleus, and tends to raise the Bmax for ^{125}I-PYY receptor binding sites in whole hypothalamus, suggesting its positive action at the NPY receptor site as well.

701.7

EVIDENCE FOR THE DIRECT INVOLVEMENT OF TRANSCRIPTION IN THE TIMING MECHANISM OF A CIRCADIAN PACEMAKER.

S.B.S. Khalsa*, D. Whitmore, B. Bogart and G.D. Block.
NSF Center for Biological Timing, Department of Biology, Gilmer Hall, University of Virginia, Charlottesville, VA 22903.

Using long pulses of translation and transcription inhibitors the *Bulla* ocular circadian pacemaker has been shown to exhibit a phase-dependent requirement for translation (PNAS, 89:10862, 1992) and transcription (Soc. Neurosci. Abstr. 16:640). Pacemaker motion is stopped with inhibitor at the sensitive phase in the late subjective night, and resumes only when the inhibitor is withdrawn.

With additional inhibitor pulses applied over a range of durations and at different phases we have determined that the transcription-sensitive phase (late subjective night / entire subjective day) is longer than the translation-sensitive phase (late subjective night / early subjective day), i.e. for the region of the circadian cycle in the mid to late subjective day the pacemaker is transcription-sensitive but not translation-sensitive. The transcription inhibitor 5,6-dichlorobenzimidazole riboside (DRB; 100 μM) applied at ZT 6-12 yields a 7 hr phase delay, whereas 10 mM cycloheximide, which is sufficient to stop the pacemaker at the translation-sensitive phase, is ineffective at this phase.

To assess the timecourse of the DRB-induced delay, light pulses were applied at either ZT 13-16, 20-23, or 4-7 following a DRB pulse at ZT 6-12. Comparison of the resultant shifts with those to light pulses alone (no DRB pre-pulse) suggests that the delay to DRB had occurred during or immediately following the DRB treatment, i.e. the light-induced phase shifts following DRB were consistent with a pacemaker phase which was delayed by 7 hr. The observation of a transcription-sensitive phase which is translation-insensitive, together with the observation that DRB appears to stop the pacemaker at this phase, suggests that transcription is an integral component of the circadian timing loop, as opposed to simply a phase-dependent requirement. *Supported by NS15264*

701.8

USE OF PHOSPHOPEPTIDE-SPECIFIC ANTIBODIES AGAINST CREB AND SRF IN THE SUPRACHIASMATIC NUCLEUS.

J. M. Kornhauser[1]*, D. D. Ginty[2], K. E. Mayo[1], M. E. Greenberg[2] and J. S. Takahashi[1]. [1]NSF Center for Biological Timing, Northwestern Univ., Evanston, IL 60208; [2]Dept. of Microbiology and Molecular Genetics, Harvard Univ., Boston, MA 02115.

The principal mammalian circadian pacemaker is located in the hypothalamic suprachiasmatic nucleus (SCN). Light stimuli which phase shift circadian rhythms also induce the expression of a set of genes, including c-*fos*, in the SCN, suggesting a role for their gene products in photic resetting of the circadian clock. Several key molecules in signal transduction pathways regulating c-*fos* transcription undergo specific phosphorylation events concomitant with their functional activation. We are investigating the intracellular signaling pathways by which light regulates gene expression in the SCN, using antibodies specific for the phosphorylated forms of regulatory proteins. Regulation of c-*fos* transcription by both calcium and cAMP is mediated by the cAMP response element-binding protein (CREB). We have demonstrated, using immunocytochemistry with an antibody specific for the phosphorylated/activated form of CREB, that light during subjective night, but not subjective day, rapidly induces CREB phosphorylation in the hamster SCN. Another signaling pathway regulating c-*fos* utilizes the serum response element (SRE). A complex of factors binds to the SRE, including the serum response factor (SRF), which becomes newly phosphorylated on serine^{103} following extracellular stimulation. We used an antibody which recognizes ser^{103}-phosphorylated SRF (P-SRF) to ask if light activates SRF in the SCN. P-SRF immunoreactivity is constitutively high in the SCN, and is not significantly increased by light. This may be surprising, since NMDA receptors, which are important for photic signaling to the SCN, act through the SRE to activate c-*fos* transcription in neurons *in vitro*. Thus, light may instead regulate a different protein in the SRF complex or may not signal through the c-*fos* SRE. Antibodies specific for the phosphorylated forms of transcription factors and kinases should help to elucidate further neuronal signal transduction pathways in the SCN and throughout the brain.

701.9

FOS EXPRESSION IN THE RAT DIENCEPHALON DURING SLEEP (S) AND WAKEFULNESS (W) IN BASAL CONDITIONS AND EXPERIMENTAL TRYPANOSOMIASIS.

M. Bentivoglio[1]*, G. Grassi-Zucconi[2], Z.-C. Peng[1], G. Bertini[1], J. Harris[1], A. Bassetti[2] and K. Kristensson[3]. *Institutes of [1]Anatomy, University of Verona, [2]Cell Biology, University of Perugia, Italy, and [3]Clinical Research Center, Karolinska Institutes, Huddinge Hospital, Sweden.*

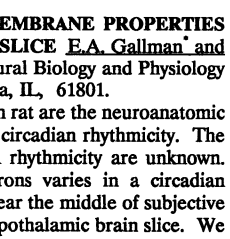
We recently reported that expression of the Fos protein, encoded by the immediate early gene *c-fos*, occurs in different brain areas during spontaneous S and W documented by EEG monitoring and behavioral control (12h-12h light/dark cycle). The present study focuses on the diencephalon, where Fos expression occurs - consistently but not exclusively - in the paraventricular nucleus (Pv) of the thalamic midline and suprachiasmatic nucleus of the hypothalamus (SCN). Fos-immunoreactive (ir) neurons are present in Pv during both S and W, being more numerous in the W cases. On the other hand, Fos-ir neurons are mainly found in the SCN in the S animals. Given the projections of SCN to Pv, these data indicate that the link between the circadian pacemaker and the thalamic midline could play a substantial role in the sequence of gene expression underlying the S and W sequence. Pv efferents reach different limbic targets (such as the amygdala and nucleus accumbens), and we are at present verifying whether Fos expression during S and W occurs selectively in one of these cell populations. Rats infected with *Trypanosoma brucei brucei*, that produces in humans the African sleeping sickness, were matched with non-infected controls. A considerable fragmentation of the sleep pattern was observed in the infected animals. No significant differences were detected in Pv, whereas the pattern of spontaneous Fos induction in the SCN was mostly reversed in the infected animals with respect to controls: Fos-ir neurons were detected in the SCN in many of the infected W cases, but in only a few of the S infected animals. These changes in gene expression suggest that Trypanosomiasis could be underlied by selective diencephalic dysfunctions.

701.10

CIRCADIAN MODULATION OF MEMBRANE PROPERTIES OF SCN NEURONS IN RAT BRAIN SLICE

E.A. Gallman* and M.U. Gillette. Depts of Cell & Structural Biology and Physiology & Biophysics, Univ. of Illinois, Urbana, IL, 61801.

The suprachiasmatic nuclei (SCN) in rat are the neuroanatomic substrate for a pacemaker underlying circadian rhythmicity. The mechanisms responsible for circadian rhythmicity are unknown. Mean firing frequency of SCN neurons varies in a circadian pattern, with peak activity occurring near the middle of subjective day, both *in vivo* and in the *in vitro* hypothalamic brain slice. We employed whole cell recording in the rat brain slice to examine possible mechanisms for modulation of firing rate, as such mechanisms may reflect underlying organization of the circadian clock. We found statistically significant changes in membrane potential and specific membrane conductance which paralleled the changes in firing rate. Membrane potential was more negative at CT 14-18 than at CT 6-10. Specific membrane conductance was low at CT 6-10 and high at CT 14-18 (Figure). We are investigating the ionic conductances underlying these observations. AFOSR-90-0205 & PHS NS 22155.



701.11

Morphology and distribution of retinal ganglion cells (RGC) projecting to the suprachiasmatic nucleus in the sheep.

H.M. Cooper*, A.Tessonneaud, A. Caldani, A. Locatelli, S. Richard and M.C. Viguier-Martinez. *INSERM U-371, Cerveau et Vision, 69500 Bron; Lab. Neuroendocrinologie, Univ. de Tours, Fac. Sciences, 37200 Tours; INRA Lab. Neuroendocrinologie Sexuelle, 37380 Nouzilly; FRANCE.*

Several lines of evidence suggest that the retinohypothalamic pathway arises from a particular class of RGC's. In the sheep, the large size of the SCN, combined with a trans-sinusal surgical approach allowing direct visual access to this structure, allowed us to accurately place single or multiple injections of retrograde fluorescent tracers (FR, FB, DY). Single injections in the SCN labels a sparse population of RGC's (< 1000), homogeneously distributed in homologous regions of each retina, above the horizontal streak. The projection of these regions into visual space covers a wide area including the entire ventral part of the visual field. Multiple, bilateral injections further show a lack of precise topographic organization. RGC's from a given sector of the retina project to all parts of the SCN, and conversely, each part of the SCN receives a projection from all regions of both retinas. RGC's were of two morphological types. One unusual class of cell had a small to medium sized elongated soma (10-15 μm diam), and an asymmetrical organization with two or three long slender dendrites (>250 μm). The second class, resembling W-type cells, had a small soma (<10 μm diam) and more restricted but highly branched dendritic arborization. These results show that the spatial distribution of retinohypothalamic GC's forms a mosaic independent of thalamic and tectal projecting ganglion cells. The sparse number and widespread naso-temporal distribution of RGC's simultaneously reduces spatial resolution and increases the available sampling area. The lack of retinotopic connections (high degree of divergence and convergence) further blurs the retinal image by potentially annulling spatial contrasts, enabling whole field integration of light. Modeling this organization illustrates how retinal and optical constraints combine to increase the efficiency of this system for the detection of diffuse, ambient light levels.

SOMATOSENSORY CORTEX AND THALAMOCORTICAL RELATIONSHIPS V

702.1

Clonazepam suppresses $GABA_B$ inhibition in relay cells through actions in the reticular nucleus J.R. Huguenard* and D.A. Prince. Dept. of Neurology & Neurological Sciences, Stanford Univ. Med. Ctr., Stanford, CA 94305.

Recurrent feedback within the intrathalamic circuit is thought to underlie various thalamocortical oscillations including those involved in absence epilepsy. Thalamocortical relay cells (TCs) excite GABAergic neurons of nucleus reticularis thalami (nRt) leading to recurrent IPSPs onto relay neurons. These hyperpolarizing IPSPs can trigger rebound low-threshold spikes (LTSs) in TCs which, in turn, lead to reexcitation of nRt and a maintained thalamic oscillation. Thus, drugs which directly (succinimides) or indirectly ($GABA_B$ antagonists) decrease LTS generation can eliminate the pathological oscillations and absence seizures, while barbiturates, which enhance $GABA_A$ inhibition, may exacerbate them. In this context, the beneficial actions of the $GABA_A$ agonist, clonazepam, are difficult to explain, and were the focus of these experiments. Stimulation of nRt evoked robust biphasic ($GABA_A$-$GABA_B$) IPSPs in rat somatosensory TCs recorded in thalamic slices with patch-clamp techniques. Bath application of clonazepam (300 nM) reduced the amplitude of the $GABA_B$ component with only slight effect on the $GABA_A$ component. In contrast, the $GABA_A$ antagonist bicuculline methiodide (BMI, 2-10 μM), blocked the $GABA_A$ component but enhanced the $GABA_B$ component, even when excitatory amino acid receptors were blocked. Possible sites for these $GABA_A$ modulatory actions were 1) intra-nRt inhibitory circuits or 2) presynaptic inhibition of nRt terminals impinging onto TCs. Voltage-clamp recordings coupled with local perfusion revealed that $GABA_B$ responses were enhanced only with BMI application to nRt, thus ruling out the latter possibility. These data are consistent the hypothesis that nRt cells are mutually inhibitory during synchronous stimuli such as those which occur with extracellular stimulation and during absence seizures. This points to a promising therapeutic target for anti-absence drugs. Supported by NIH grants NS06477 and NS12151.

702.2

SPATIAL ORGANIZATION OF INHIBITORY SYNAPTIC RESPONSES ONTO PYRAMIDAL NEURONS OF RAT NEOCORTEX A. Nicoll*, H.G. Kim & B.W. Connors. Dept. Neuroscience, Brown University, Providence, R.I. 02912.

Neocortical pyramidal cells receive GABA-mediated synaptic inputs from local inhibitory neurons, but the pattern of their postsynaptic GABA responses and the spatial extent of local inhibitory interactions is unclear. These issues were investigated with whole-cell recordings from pyramidal cells in coronal slices of rat somatosensory cortex, in the presence of bath-applied glutamate antagonists APV and DNQX. Spontaneous, picrotoxin-sensitive IPSPs were examined in 8, CsCl-loaded layer 5 pyramidal cells. They displayed a wide range of 10-90% rise times (1-34 ms), not correlated with amplitude (0.2-18 mV), suggesting that they arose over a range of dendritic locations. 'Proximal' and 'distal' IPSPs (~1 mV) were evoked in 10 CsCl-loaded layer 5 pyramidal cells using extracellular electrodes placed simultaneously in layers 5/6 and 2/3, separated by a horizontal cut made in layer 4. Evoked IPSPs were also blocked by picrotoxin. In 7 out of 10 of the averaged IPSP pairs, the distal waveform had a longer 10-90% rise time than the proximal one. The proximal IPSP could be reversed by membrane depolarization when the distal one could not (n=4/4). These results suggest that GABA-A responses can be evoked in the apical dendrites of pyramidal cells. The spatial organization of inhibitory inputs onto pyramidal neurons was mapped in layers 2/3 and 5. Cells were recorded with K^+-filled pipettes in the presence of glutamate antagonists, and monosynaptic IPSPs were evoked by focal pressure ejections of 1-10 mM acetylcholine. Trials were made sequentially at 100 μm intervals over horizontal distances of 100-1000 μm from the postsynaptic cell, which was held at c. -60 mV by current injection. The frequency of IPSP occurrence declined with distance in both layers. IPSPs could be evoked from a horizontal distance of 500 μm for 26 layer 2/3 cells, but up to 800 μm for 30 layer 5 cells. Furthermore, within layer 5, the probability of observing IPSPs in intrinsically-bursting cells was significantly lower than in the regular-spiking cells. These results suggest that the spatial organization of inhibition varies across different layers and cell types.

Supported by grants from NIH and ONR to B.W.C.

702.3

CORTICAL PROJECTIONS OF THE VENTRAL PERIPHERY OF THE CAT'S THALAMIC VENTRAL POSTEROMEDIAL NUCLEUS (VPM_{vp}). C. Vahle-Hinz* and E. Oertle. Physiologisches Institut, Univ.-Krankenhaus Eppendorf, D-20246 Hamburg and Physiologisches Institut, Univ. Würzburg, D-97070 Würzburg, Germany.

Neurons of the VPM_{vp} in the cat are involved in processing of information about noxious events affecting the skin and the viscera. The response properties of VPM_{vp} neurons and their location in the lateral thalamus indicate that they may subserve the sensory-discriminative aspect of nociception and pain. In order to reveal the cortical areas of projection of VPM_{vp} neurons, the anterogradely transported neuronal tracer biotin-dextran was injected into this region and visualized by the avidin-biotin complex method using diaminobenzidine with nickel enhancement as chromogen.

The injection sites were small (100-300 μm in diameter) lying well within the borders of VPM_{vp}. Labeled fibers traveled rostrally in the internal capsule towards their cortical fields of termination. Terminal arbors with bouton-like endings were found in areae 1, 2, 3a, 3b, 4, 4b, 5, 6, 14 and 43. The terminals were located in layers III and IV as well as layer I. Apart from the anterograde label, a few retrogradely labeled somata of neurons in layer VI were present in the same cortical areae.

The results indicate that information about noxious stimuli, which is relayed in the thalamic VPM_{vp}, is represented in the primary and secondary somatosensory cortices as well as in parts of the motor, the insular and orbitofrontal cortices.

Supported by the Deutsche Forschungsgemeinschaft.

702.4

THE SEQUENCE OF ACTIVATION OF VB THALAMIC AFFERENTS, CORTICAL EFFERENT NEURONS AND PUTATIVE INTERNEURONS IN RABBIT SOMATOSENSORY CORTEX H. A. Swadlow*, Dept. of Psychology, University of Connecticut, Storrs, CT 06269

Previous studies of rabbit S-1 (Swadlow, H. A., J. Neurophysiol., 62:, 288-308, 1989; 63:, 1477-1498, 1990) examined the properties of several classes of efferent neurons and a class of suspected interneurons (SINs) which responded to VB thalamic stimulation with a high frequency (> 600 hz) burst of very short-duration spikes. Because SINs were the first cells in S-1 to respond synaptically to thalamic stimulation, it was suggested that they were involved in feed-forward inhibition of efferent neurons. However, the above studies could not eliminate the possible synaptic activation of the SINs via the recurrent collaterals of descending corticofugal axons. The present work examines this question in the cutaneous facial representation of VB thalamus and S-1 of awake rabbits by monitoring the sequence of activation in identified elements following activation of the receptive field center with a brief (< 0.6 ms rise time) air-puff.

VB projection neurons (antidromically identified, N=27) responded at a median latency of 5.1 ms (range = 4.2 - 5.8 ms) and had a median antidromic latency of 0.94 ms (range = 0.74 - 1.3 ms) to S-1 stimulation. The estimated arrival time of air-puff generated impulses at thalamo-cortical terminals (the latency of the VB neuron to the air-puff plus the cortico-thalamic antidromic latency) was 5.98 ms (median value, range = 5.18 - 6.84). SINs were the first cells in S-1 to respond synaptically to both the thalamic stimulus (median latency = 1.45 ms, range = 1.25 - 3.1 ms) and the air-puff stimulus (median latency = 6.7 ms (range = 6.05 - 13.0, N=56). Most SINs responded to the air-puff with several spikes. These data suggest a powerful monosynaptic activation of most SINs by VB thalamic afferents. In contrast, most cortico-cortical and descending corticofugal efferent neurons responded at considerably longer latencies to both the thalamic (median = 3.3 ms, range = 2.25 - 4.9) and air-puff (median = 10.65 ms, range = 7.55 - 22.9 ms) stimulation. (Supported by NSF grant IBN-9213451)

702.5

THE DEVELOPMENT OF THALAMOCORTICAL AND INTRACORTICAL CONNECTIVITY IN RAT SOMATOSENSORY "BARREL" CORTEX IMAGED WITH OPTICAL RECORDING. M.C. Crair*, Z. Molnar**, S. Higashi, T. Kurotani and K. Toyama, Dept. of Physiol., Kyoto Pref. Univ. of Med., Kyoto 602 Japan and ** Univ. Lab. of Physiol., Oxford, OX1 3PT, UK.

The somatosensory (SI) cortex of rodents contains an easily identifiable functional and cytoarchitectural somatotopic map of the sensory periphery. In particular, the so called "barrels" are a classic example of a functional cortical column. This makes SI an ideal area for studying the development of laminar specific (radial) and area specific (tangential) functional connectivity patterns. We have used a new high spatial (128 X 128 pixels) and temporal (0.6 msec) resolution optical recording apparatus in conjunction with conventional electrophysiological techniques on an in-vitro thalamo-cortical slice preparation (Agmon and Connors, 1991) to study the development of barrel columns. This new device, used with a voltage sensitive dye (RH482), allows us to image the spatio-temporal pattern of signal propagation from the thalamus to SI and within SI in response to stimulation of the thalamus (VB). In newborn rats (P0-P2) the response of SI, as reflected in the optical signal and current source density analysis, has a long initial latency, slow time course, and is radially and tangentially broad. In slightly older animals (P3-P5), as layer IV neurons differentiate from the cortical plate and cytoarchitectural barrels are formed, the response is quicker and grows more patchy, presumably representing excitation of individual barrels. This barrel related response becomes more well defined as the supragranular layer matures (P6-P9), and the initial thalamocortical response in layer IV barrel hollows propagates to layer II/III and to inter-barrel regions in a column specific pattern. By the end of the second postnatal week (P10-P14), a relatively mature excitation-propagation pattern exists. In summary, we have been able to image the response of discrete barrel columns in rat SI cortex to stimulation of the VB, and study the temporal and spatial development of thalamocortical and intracortical connectivity in an in-vitro slice preparation.

702.6

STRUCTURE-FUNCTION RELATIONSHIPS EXAMINED IN RAT BARREL CORTEX USING INTRINSIC SIGNAL OPTICAL IMAGING THROUGH THE SKULL. S. A. Masino* M. C. Kwon, Y. Dory, R. D. Frostig, Department of Psychobiology, U.C. Irvine, Irvine, CA 92717.

We used intrinsic signal optical imaging through a thinned skull to study the functional representations of the mystacial vibrissae (whiskers) in rat barrel cortex. Each whisker has a cytoarchitectonically distinct representation in cortical layer IV (Woolsey, T. A., and Van der Loos, H. 1970. *Br. Res.*, 17: 205-242), as well as a functional column spanning from the pial surface to the white matter (Welker, C. 1976. *J. Comp. Neur.*, 166: 173-190). This columnar arrangement combined with the unique cytoarchitecture provides an opportunity to investigate the relationship between structure and function within a discrete cortical representation.

We stimulated individual whiskers continuously for two seconds at theta rhythm (5Hz). In response to the stimulation of any whisker we obtained a discrete area of activity in the cortex. We verified that this activated area determined by optical imaging corresponds precisely with the response area of single units in the cortex to that whisker. Lesions placed in the center of the optical activity within cortical layer IV always corresponded with the center of the anatomical representation of that whisker within layer IV as determined by cytochrome oxidase staining.

In addition, we stimulated a 3 x 3 matrix of nine neighboring whiskers to obtain a map of their functional representations in barrel cortex. We compared this optically-determined functional map with the anatomical map of cytochrome oxidase staining of the same nine whiskers and found that the distances between the centers of the whiskers on the optical map versus the anatomical map were highly correlated (r=.99). The striking correspondence between imaging, physiology, and anatomy found in this study makes this system ideal to investigate the ongoing dynamic relationship between structure and function in the neocortex.

Supported by the Beckman Foundation and NIMH grant 5 T32 MH14599-17

702.7

PARALLEL PROCESSING OF TACTUAL INFORMATION IN THE POSTCENTRAL GYRUS OF RHESUS MONKEYS. I. Danielsson*, J. Hahm and T.P. Pons, Lab Neuropsychology, NIMH, Bethesda, MD 20892.

In Old World monkeys, including macaques, primary somatosensory cortex is comprised of four cytoarchitectonically distinct areas, 3a, 3b, 1 and 2. Each of these areas is known to receive direct inputs from the thalamic ventroposterior nuclear complex. A recent study in Marmosets (a New World monkey), however, demonstrated that cutaneous activation of area 1 was dependent upon inputs from areas 3a and 3b, rather than on a direct projection from the thalamus (Garraghty et al. 91, JCN). In the present experiment we determined whether direct inputs from the thalamus were sufficient to drive cutaneous responses in areas 1 and 2 of macaques in the absence of areas 3a and 3b.

In 4 monkeys ablations of the area 3a and 3b hand representations were made immediately prior to electrophysiological mapping of the spared hand representations in areas 1 and 2. In each case it was possible to record neuronal responses to cutaneous stimulation at numerous sites across areas 1 and 2, though a slight reduction in cutaneous inputs was noted. In an additional case we removed the hand representation in areas 3a, 3b and 1 and mapped in area 2. Again we found cutaneous activation of sites across the entire area 2 hand representation with no increase in the ratio of "deep" to cutaneous recording sites. We conclude that direct thalamic projections to areas 1 and 2 are sufficient to provide cutaneous input to these regions in macaques and that there may be fundamental differences in cortical processing of tactual information in New and Old world monkeys.

702.8

THE ORGANIZATION OF LATERAL SOMATOSENSORY CORTEX IN THE MACAQUE MONKEY: WHERE IS SII? L. Krubitzer*, I. Clarey, R. Tweedale, G. Elston, and M. Calford. Vision, Touch and Hearing Research Centre, University of Queensland, Australia, 4072

The organization of somatosensory cortex lateral to SI was investigated in three macaque monkeys (*Macaca fascicularis*) using multiunit mapping procedures. Over 2,300 recording sites were obtained, and reconstruction of receptive fields, neural response properties, and stimulus preference of neurons allowed us to reconstruct comprehensive maps of this region of cortex. Cortex was flattened, cut parallel to the cortical surface and stained for myelin, and myeloarchitecture was related to electrophysiological mapping results. We found clear evidence for at least two complete repre-sentations of the body surface in the region of cortex traditionally desig-nated as SII. We termed these fields SII and the parietal ventral area, PV, because their overall organization, relative position, and architectonic appearance were similar to SII and PV as described in other mammals. Evidence for two additional fields was also obtained. One field caudal to SII we termed 7b, because its location and architectonic appearance were similar to 7b described previously in macaque monkeys. The other field was ventral to SII and PV, and we termed this the ventral somatosensory area, VS, because the topography and relative position were similar to VS described in owl monkeys and flying foxes. Since a number of fields were demonstrated in the region of cortex traditionally defined as SII, it is logical to ask which field we should term SII. By comparing our results in the macaque monkey with those in other mammals, a clearer picture of which field is "SII" has begun to emerge. This question has important implications for the interpretation of reorganization plasticity reported in "SII" following cortical or peripheral lesions.

702.9

POLYSENSORY EVOKED POTENTIALS IN RAT PARIETO-TEMPORAL CORTEX: COMBINED AUDITORY AND SOMATOSENSORY RESPONSES D. S. Barth*, B. Brett and S. Di, Department of Psychology , University of Colorado, Boulder, CO 80309-0345 (U.S.A).

In a previous report from our laboratory, separate vibrissal and auditory click stimulation produced epicortical potentials centered on the granular areas of primary auditory (AI) and somatosensory (SI) cortex in the rat. However, it was noted that the auditory (AEP) and somatosensory (SEP) evoked potentials also overlapped in a region of dysgranular cortex separating these areas, suggesting a polysensory cortical site.

The present experiment was concerned with mapping epicortical potentials in this presumed polysensory cortex, evoked by combined somatosensory and auditory stimulation (ASEP). While recording from an 8x8 microelectrode array covering a 3.5 x 3.5 mm^2 area, the AEP, SEP and ASEP were mapped, and their areal patterns compared to cytochrome oxidase stained tangential sections of layer IV. Horse radish peroxidase (HRP) was then injected into SI, AI, or the polysensory region to trace thalamocortical projections.

Our results demonstrate a consistent response in dysgranular cortex separating AI and SI during the ASEP, that is not modeled by a simple sum of the AEP and SEP waveforms. While HRP injections into AI and SI stain the lateral divisions of the medial geniculate and ventrobasal thalamic nuclei, injections into the polysensory zone stain a more medial region at the juncture between the posterior nuclei and the medial division of the medial geniculate, suggesting that polysensory integration may occur at both the cortical and subcortical level.

702.10

COMPARISON OF NEURONAL ACTIVITY IN THE PRIMARY SOMATOSENSORY CORTEX (SI) DURING ACTIVE AND PASSIVE TACTILE TEXTURE DISCRIMINATION. F. Tremblay* and C.E. Chapman, CRSN, Univ. Montréal, Canada, H3C 3J7.

While there is ample evidence from psychophysical studies that the ability to discriminate suprathreshold tactile stimuli is not affected by movement, electrophysiological experiments have clearly established that the transmission of cutaneous inputs to the SI cortex is decreased during movement, including exploratory movements (Chapman and Ageranioti, 1991). In the present study, we addressed this issue by looking at the influence of movement on 1) the ability of SI neurones to encode graded changes in surface texture and 2) the ability of monkeys to discriminate such changes in texture. Neuronal activity and performance measured during active scanning of textured surfaces (monkey scans surface with its digit tips) were compared with similar measures made during passive exploration of the same surfaces (surface displaced under digit tips). In both modes of touch, the monkey had been trained to discriminate, after a single scan, a standard surface (raised dots, 2 mm spatial period (SP) over the entire length) from 3 other surfaces in which, over the second half, SP was proportionally increased (3, 4 or 5 mm SP). In each task, the monkey indicated the presence or absence of a change in surface texure by pushing (no difference) or pulling (difference) a lever with the opposite hand. A preliminary analysis performed on a sample of 70 units with cutaneous fields on the digit tips contacting the surface, indicated that 23% of cells were texture-related (mean firing rate related with SP). Of these, the majority signalled the differences in texture during passive touch (9/16) but not during active touch. Only a few cells were texture-related in both tasks (n=4). In parallel with the unit data, analysis of the monkey's task performance indicated that it was actually better during passive, than during active, touch. The monkey made significantly fewer errors ($p< 0.05$) in discriminating the surface with the smallest increase in SP (3mm) during passive, as compared to active, touch (respectively, 5 and 23 % error rate). Thus, these preliminary results suggest that the gating influences associated with movement may interfere with the ability of SI neurones to signal differences in texture as well as with the ability to discriminate small differences in surface texture.(Supported by MRC and FRSQ).

702.11

DISCRIMINATION OF THE DIRECTION OF MOTION ON THE HUMAN HAND. *E.P. Gardner* and B.F. Sklar* Dept. of Physiology and Biophysics, NYU Medical Center, New York, NY 10016

We evoke sensations of apparent motion across the skin by pulsing sequential rows on the OPTACON with independent control of spacing and timing. We previously showed that the ability of humans to discriminate direction of motion depends mainly on spatial properties. A 3-factor repeated measures ANOVA confirms that distance and spacing significantly affect discriminability d' (P=0.0001), yielding an increase of 0.2 d' /mm path length and a drop of 1.04 d' /row spacing. Pulse rate has a much weaker effect (P=0.011) characterized by higher d' at 50 Hz than at 25 or 100 Hz. The effect of spacing is not linked to stimulus proximity on the skin, but to the *total number* of pulses in a sweep. Greater accuracy related to path length results from increased information transmitted centrally, rather than the wider separation between start and stop points. Randomly varying the total pulses presented and their spacing confirms a linear relation between number of pulses and d' with slope=0.84 (P=0.0001). Total pulses account for 89% of stimulus-linked variance, while pulse spacing contributes only 5.6% of variance (P=0.012), and is optimal at 2.4 mm. Stimuli to two points are insufficient for accurate discrimination regardless of whether they activate overlapping or distinct populations of cutaneous afferents. Threshold discrimination occurs with three pulses; d' is only slightly lower when the stimulated fields overlap than when they are distinct. Accuracy greater than 90% requires at least 5 pulses, which shift the activated population beyond its original boundaries to a completely new group of afferents. *(Supported by NIH: NS11862).*

702.12

REORGANIZATION OF SOMATOSENSORY CORTEX, AND CHANGES IN THE PERIPHERAL NERVE INNERVATION PATTERNS IN SPINAL CORD AND BRAIN STEM AFTER AMPUTATION OF THE HAND IN MONKEYS. S.L. Florence*, N.Jain, P.D. Beck, J.H. Kaas, Dept. of Psychology, Vanderbilt Univ., Nashville, TN.

To investigate plasticity mechanisms underlying cortical changes produced by long-term sensory deprivation, we mapped primary somatosensory cortex and labeled peripheral nerve inputs to the spinal cord and brain stem of 2 owl monkeys (Aotus trivirgatus) and 1 macaque monkey (Macaca mulatta) with long-standing amputations. Amputation of the hand (macaque and 1 owl monkey) or forearm (1 owl monkey) all resulted from medical treatment of injuries rather than experimental protocol. Microelectrode mapping of area 3b in the owl monkeys revealed that most of the presumptive hand representation was activated by cutaneous stimulation of the arm, forearm, and face. In the macaque, cortex normally activated by the distal digits was largely silent. However much of the region where the palm is typically represented (along the 3b/1 border) contained a new, precise representation of the forearm. Additionally, the representation of the face appeared to be somewhat enlarged. Multiple injections of HRP conjugated cholera toxin in the skin of the forearm (macaque) or arm (1 owl monkey) proximal to the amputation resulted in much more extensive labeling in both the dorsal horn of the spinal cord and the cuneate nucleus of the brain stem than matched injections in the intact arms. Based on the normal topography of inputs to these structures, the changes in the labeled peripheral nerve input patterns was coincident with the somatotopic reorganizations of area 3b. For example, in the macaque, label from the forearm which is normally lateral-most in the dorsal horn and dorsal-most in the cuneate extended medially in the spinal cord and ventrally in the cuneate nucleus where inputs from the palm normally are situated. The label did not encroach upon the regions where the distal digits are represented. Thus, somatotopic changes seen in area 3b may reflect sprouting or an increased density of previously sparse peripheral nerve inputs. Supported by NS16446 & NICHDHD15052

702.13

PHANTOM BREAST AS PERCEPTUAL CORRELATE OF NEUROPLASTICITY IN ADULT HUMAN BRAIN. **S. Aglioti , F. Cortese , C. Franchini, S. Zamboni. Inst. of Hum. Physiology and SRRF , I-37134, Verona (SPON : European Neuroscience Association).**

Upper limb amputees report sensations in the phantom limb upon tactile stimulation of other cutaneous regions (Ramachandran et al. , NeuroReport, 3, 583-6, 1992). These regions (called 'reference fields') were mainly clustered on the lower face, ipsilateral to the amputation. Since hand and face are represented on contiguous zones in the somatosensory cortex, reference fields could indicate that deprived cortical and/or subcortical zones were invaded by contiguous cerebral zones. According to this interpretation, reference fields would suggest neural rearrangements on an amazing large scale in the adult human brain. Data have now been obtained from breast amputees supporting and extending this 'heretic' hypothesis. We provided evidence for such reference fields in 32.5 % of an unselected population of 40 females who had undergone breast amputation for cancer treatment. Far from being randomly distributed, reference fields were located on cutaneous regions which were represented on zones of the somatosensory cortex contiguous to the zone where the amputated breast is presumably mapped. The present study reports perceptual indices of reorganisation in the somatosensory system, even after breast amputation that causes only limited areas of deprivation, in comparison to limb amputation. Therefore, our data suggest that neuroplasticity in the adult human brain is potentially much higher than previously suspected, thus offering new opportunities in the field of rehabilitation.

LONG-TERM POTENTIATION VIII

703.1

POSTSYNAPTIC EXPRESSION OF CONSTITUTIVELY ACTIVE CaMKII OCCLUDES LTP IN THE HIPPOCAMPAL SLICE. D.L. Pettit*, S. Perlman & R. Malinow. Neuroscience Program, and Pediatrics Department and Physiology & Biophysics Department, University of Iowa.

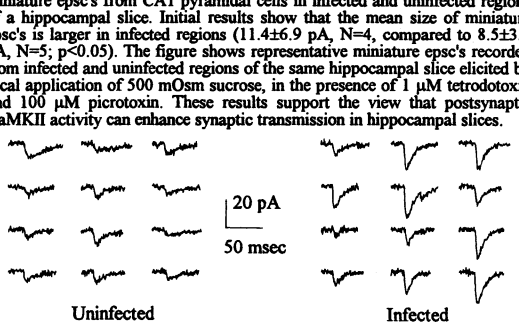
Long-term potentiation (LTP) can be prevented by postsynaptic block of calcium/calmodulin-dependent protein kinase II (CaMKII). Does increasing CaMKII activity mimic and thereby occlude LTP? To test this we have generated a recombinant vaccinia virus that produces the constitutively active CaMKII(1-290) driven by a strong early/late vaccinia promoter. A luciferase expression assay in cultured cells confirms expression of a functionally active CaMKII enzyme.

To determine the effect of increased postsynaptic CaMKII activity on LTP, we have infected CA1 pyramidal neurons in hippocampal slices. Synaptic transmission onto CA1 neurons in infected regions was elicited by stimulating electrodes placed in the stratum radiatum and recorded with whole-cell voltage clamp. After monitoring baseline transmission, LTP was attempted by pairing stimulation with postsynaptic depolarization. At depolarized potentials slow synaptic currents, indicating NMDA-receptor mediated transmission, were present in all cells. Following this pairing protocol, transmission was 101% of baseline at 5 min. (n=10) and 99% of baseline at 30 min. (n=6). LTP was observed in only 1 of 10 cells. Parallel experiments with a virus that expresses β-galactosidase showed levels of potentiation comparable to uninfected slices. These results, in conjuction with the following abstract (Shirke, et al), support the view that increased postsynaptic CaMKII activity is sufficient to mimic and occlude LTP.

703.2

MINIATURE EPSC SIZE INCREASES WITH POSTSYNAPTIC EXPRESSION OF CONSTITUTIVELY ACTIVE CaMKII. A.M. Shirke*, D.L. Pettit, S. Perlman & R. Malinow. Dept. of Physiology & Biophysics, Neuroscience Pgm., & Dept. of Pediatrics, U. of Iowa, Iowa City.

To study the effect of postsynaptic CaMKII activity on synaptic transmission, we have generated a recombinant vaccinia virus that produces constitutively active CaMKII (1-290). If CaMKII activation is sufficient to increase postsynaptic responsiveness, as suggested by recent experiments showing CaMKII-mediated regulation of kainate-elicited ion currents (Nature, 362: 640-642), then the size of miniature epsc's should be greater in cells expressing constitutive CaMKII than in uninfected cells. We measured miniature epsc's from CA1 pyramidal cells in infected and uninfected regions of a hippocampal slice. Initial results show that the mean size of miniature epsc's is larger in infected regions (11.4±6.9 pA, N=4, compared to 8.5±3.8 pA, N=5; $p<0.05$). The figure shows representative miniature epsc's recorded from infected and uninfected regions of the same hippocampal slice elicited by focal application of 500 mOsm sucrose, in the presence of 1 μM tetrodotoxin and 100 μM picrotoxin. These results support the view that postsynaptic CaMKII activity can enhance synaptic transmission in hippocampal slices.



703.3

MECHANISM OF PROTEIN KINASE C ACTIVATION DURING INDUCTION AND MAINTENANCE OF LTP. E. Klann*, S.-J. Chen and J.D. Sweatt, Division of Neuroscience, Baylor College of Medicine, Houston, Texas, 77030.

We have previously described a persistent increase in basal (Ca^{2+} and cofactor-independent) PKC activity in the maintenance phase of LTP. Additionally, we have observed an increase in both basal and total (Ca^{2+} and cofactor-dependent) PKC activity in the induction phase of LTP. These data suggested the possibility of different mechanisms of PKC activation associated with LTP. To investigate the biochemical mechanisms underlying these phenomena, LTP of the Schaffer collateral input into area CA1 of rat hippocampal slices was studied. Slices were frozen, dissected, and homogenized either 2 min (induction) or 45 min (maintenance) after the final tetanic stimulation. Western blots for PKC with control and LTP homogenates at the 45 min time point indicated an LTP-associated decrease in PKC immunoreactivity (69±5% of control, n=21). Bath-applied AP5 (50 μM) inhibited the decrease in PKC immunoreactivity (92±8% of control, n=4). These results suggest that the maintenance of LTP is associated with a persistent modification of PKC and that NMDA receptor activation is necessary for this modification. Incubation of control and LTP homogenates with phosphatase could reverse the LTP-associated decrease in PKC immunoreactivity. A 45-min incubation resulted in a 33% reversal (minus phosphatase, 52±6% of control; plus phosphatase, 68±4% of control , n=5) and a 90-min incubation resulted in a 64% reversal (minus phosphatase, 56±5% of control; plus phosphatase, 84±5% of control , n=7). These data are consistent with a persistent increase in PKC phosphorylation and suggest this as a mechanism for the persitent PKC activation observed in the maintenance of LTP. Additional experiments indicated that the induction phase of LTP was associated with an increase in both basal and total PKC activity. This is consistent with previous reports indicating the necessity of PKC activity during LTP induction and suggest that the mechanism of PKC activation in LTP induction is distinct from that in LTP maintenance. Supported by an NIH NRSA (E.K.) and the McKnight and Klingenstein Foundations and NIH MH 48186(J.D.S.).

703.4

ACTIVATION OF PROTEIN KINASE C IN NMDA RECEPTOR-INDEPENDENT LTP: TIME COURSE AND MECHANISM *C. M. Powell*, D. Johnston, & J. D. Sweatt*, Division of Neuroscience, Baylor College of Medicine, Houston, TX 77030

We reported previously that a persistent increase in basal protein kinase C (PKC) activity is associated with the maintenance phase of NMDA-receptor-independent LTP (NMDA-R-*indep* LTP) induced by application of tetraethylammonium (TEA; LTP_K). This increase in PKC activity was measured 45 min after TEA washout in area CA1 of hippocampal slices. To test whether this increase in PKC activity is specific to potentiated slices, we blocked LTP_K with the Ca^{2+} channel antagonist nifedipine. Blocking LTP_K with nifedipine prevented the increase in PKC activity after TEA washout. Low frequency stimulation alone did not alter PKC activity. If this increased PKC activity is involved in LTP maintenance, then the increase in PKC activity should persist as long as LTP is maintained. To test this we induced LTP_K and monitored potentiation for 3 hours after TEA washout. Three hours into the maintenance phase of NMDA-R-*indep* LTP, PKC activity was increased in potentiated slices. Thus the time course of the increase in basal PKC activity is consistent with the hypothesis that this increase is involved in LTP_K maintenance.

Preliminary western blot analysis suggests that proteolytic activation contributes to the persistent activation of PKC in NMDA-R-*indep* LTP. Persistent activation of PKC has been reported for NMDA-R-*dep* LTP in area CA1 of the hippocampus (Klann et al, JBC 266; 24253-6) and this increase is thought to be mediated by phosphorylation of PKC (Klann et al, PNAS, submitted). When compared to these studies of NMDA-R-*dep* LTP, the present studies suggest that these two forms of LTP achieve a common biochemical end via two distinct pathways. (supported by MH10338-02, MH44754, McKnight and Klingenstein Foundations).

703.5

MAINTENANCE OF LTP IS ASSOCIATED WITH AN INCREASE IN THE PHOSPHORYLATION OF RC3/NEUROGRANIN PROTEIN. S.-J. Chen*, E. Klann, and J.D. Sweatt. Division of Neuroscience, Baylor College of Medicine, Houston, TX 77030.

We have previously observed that NMDA-receptor dependent long-term potentiation (LTP) in hippocampal area CA1 is associated with an increase in the phosphorylation of an endogenous protein, P17, using a post-hoc phosphorylation assay. The phosphorylation of P17 in vitro was greatly enhanced by the addition of calcium and lipid cofactors and was sensitive to inhibition by $PKC_{(19-36)}$, indicating that P17 is an endogenous PKC substrate protein. Two-dimensional gel analysis revealed a pI of ~5.5 for P17, similar to that of postsynaptic calmodulin-binding protein, RC3/neurogranin. In this abstract, we present direct evidence that P17 is identical to RC3. First, phosphorylation of P17 is attenuated by the addition of calmodulin. Second, phosphorylated P17 remained soluble in 2.5% perchloric acid. Third, an anti-RC3 antibody recognized a 17 kDa protein in hippocampal homogenates which comigrated with phosphorylated P17 and phosphorylated P17 can be immunoprecipitated with an anti-RC3 antibody. In addition, back-phosphorylation experiments were carried out to determine the *in situ* phosphorylation state of RC3 protein in the maintenance of LTP. Hippocampal homogenates were prepared from control and LTP slices 45-60 minutes after the high frequency stimulation. Samples were heat-inactivated and used as substrates for purified PKC. We observed a decrease in the back-phosphorylation of RC3 protein in homogenates from LTP samples (n=8). The immunoreactivity with anti-RC3 antibody showed no difference in the amount of RC3 protein between control and LTP samples. These results indicate that the maintenance phase of LTP is associated with an increase in the phosphorylation of RC3 protein in situ. As RC3 protein has been snown to be localized postsynaptically, our results are biochemical evidence for postsynaptic modifications in LTP. (Supported by McKnight and Klingenstein Foundations)

703.6

PKMζ, A CONSTITUTIVELY ACTIVE KINASE INCREASING IN THE MAINTENANCE OF CA1-LTP, IS SPECIFIC TO BRAIN. P. Osten*, H. Valsamis, E. Sublette, T.C. Sacktor Dept. of Pharmacology and Neurology, SUNY-Health Sci. Ctr. Brooklyn, NY 11203.

We have previously demonstrated that PKMζ, the free catalytic domain of PKCζ, increases in the maintenance phase of LTP in CA1. We now find that the increase in PKMζ requires NMDA-receptor activation. The increase in PKMζ can be demonstrated in rat hippocampal slices homogenized and fractionated into cytosol and pellet or in slices immediately boiled in SDS, indicating that *in vitro* proteolysis is unlikely to be generating the PKM form. Immunoprecipitation demonstrates that PKMζ is the major *in vitro* autophosphorylating protein in homogenates of hippocampus. Analysis of the tissue distribution shows that while PKCζ is ubiquitous (see M. Naik *et al.*, these abstracts), the PKM form is found in all regions of the brain tested, but not in non-neuronal tissues. These results suggest that PKMζ may participate in long-term modifications of neuronal activity, both in hippocampus and in other neural structures.

703.7

A NOVEL, HIGH-MOLECULAR WEIGHT PROTEIN RELATED TO PKCζ INCREASES DURING MAINTENANCE OF CA1-LTP. E. Sublette*, P. Osten, H. Valsamis, T.C. Sacktor Dept. of Pharmacology and Neurology, SUNY-Health Sci. Ctr. Brooklyn, NY 11203.

We report the detection of higher molecular weight proteins related to the PKCζ isoform, in rat hippocampus. On immunoblot, our antiserum against the carboxy-terminal region of PKCζ recognizes the native isozyme (70 kD), PKMζ (51 kD) and several higher molecular weight bands, all of which are blocked by pre-incubation of the antiserum with immunizing peptide. The most prominent of these proteins, 160 kD, is found under a variety of homogenization conditions, and by peptide mapping appears closely related structurally to PKC and PKMζ. In CA1 of rat hippocampal slices, the 160 kD protein increases 30 min after tetanization (27.4% ± 9.5, SEM, $p<0.05$). Like PKMζ, which increases during LTP maintenance (see P. Osten *et al.*, these abstracts), this high-molecular weight form of ζ may also be involved in regulation of constitutive phosphotransferase activity in neurons, following synaptic activation.

703.8

DEFICIENCY OF HIPPOCAMPAL LONG-TERM POTENTIATION IN PKC-γ MUTANT MICE IS COMPARABLE TO THE EFFECT OF NMDA RECEPTOR ANTAGONIST. C.Chen*, A.Abeliovich, Y.Goda, C.F.Stevens and S.Tonegawa, Howard Hughes Med. Inst.,MIT, Cambridge, MA 02139 and Salk Institute, La Jolla, CA.

Activation of PKC is necessary for induction of hippocampal LTP (Malenka et al., 1986; Malinow et al., 1989). In mutant mice which lack the γ isoform of PKC, LTP in CA_1 synapses is found deficient, while excitatory synaptic transmission seems to be normal (Abeliovich et al., this volume). We tested with field potential and whole cell patch recording techniques whether this deficiency in mutant mice is similar to the effect of NMDA receptor antagonists. We found that the accumulative probability of long-term synaptic changes after the tetanus in the mutant mice (normal saline) is not significantly different from wild-type mice in the presence of APV, a specific NMDA receptor antagonist. In addtion, approximately 10-20% of mutant slices did exhibit LTP after the tetanus, and this residual LTP could not be prevented by APV. Therefore, the deficiency of LTP in the mutant mice is comparable to the effect of NMDA receptor blockage.

We also found that these mutant mice have deficiencies in such cerebellum-dependent motor behaviors as climbing a inclined plank and hind leg reflex. We will examine synaptic transmission and plasticity in the cerebellum.

703.9

CYCLIC-AMP-MEDIATED ENHANCEMENT AND LTP AT MOSSY FIBER SYNAPSES IN THE HIPPOCAMPUS.

M. G. Weisskopf*, R. A. Zalutsky and R. A. Nicoll Depts. Pharmacol. and Physiol., UCSF, San Francisco, CA 94143-0450.

In the hippocampal slice preparation it has been shown that forskolin, an activator of adenylyl cyclase, causes an enhancement of evoked responses at the mossy fiber-CA3 synapse (Hopkins and Johnston, J. Neurophysiol., 59:667, 1988). We have used field potential recordings from guinea pig hippocampal slices to investigate further this action. This enhancement, which averages 4-fold, is specific for mossy fiber-CA3 synapses as it is not seen in responses evoked by associational/commissural afferents in the same experiment. The effect is not mimicked by the inactive isomer 1,9-dideoxyforskolin, while Sp-cAMPS, a membrane permeable activator of cAMP dependent protein kinase, does produce enhancement. Both of these observations support the role of cAMP in the enhancement. This effect is likely to result, at least in part, from a presynaptic action , since it is associated with a decrease in paired-pulse facilitation and responses to iontophoretically applied glutamate are unaltered during the enhancement of the evoked mossy fiber response.

The similarity between the effects of forskolin and previously reported aspects of long-term potentiation (LTP) at mossy fiber-CA3 synapses suggested that the two mechanisms might share a common step. Therefore, the effect of forskolin was examined both before and after an LTP-inducing tetanus, and vice-versa. The forskolin-induced enhancement was reduced at synapses that had previously undergone LTP, and LTP was reduced at synapses exposed to forskolin.

Our data suggests that cAMP-mediated enhancement and LTP at mossy fiber synapses share a common step.

703.10

CYCLIC AMP-DEPENDENT PROTEIN KINASE IS ACTIVATED DURING THE INDUCTION OF LTP.

Erik D. Roberson* and J. David Sweatt. Division of Neuroscience, Baylor College of Medicine, Houston, TX 77030.

Although the activity of protein kinases is essential for the induction of LTP, the identity of the specific enzymes involved remains unclear. We have examined the possibility of a role for the cyclic AMP-dependent protein kinase (PKA) in long-term potentiation (LTP) in area CA1 of the hippocampus. Following the induction of LTP by 100 Hz stimulation, slices were frozen and the CA1 region was dissected and homogenized. PKA activity was determined *in vitro* using the synthetic PKA substrate Kemptide. All phosphorylation was blocked by a selective inhibitor of PKA (PKI_{1-20}, 10 μM). Basal PKA activity (determined in the absence of added cyclic AMP) was increased 20% above control 2 min after LTP-inducing high-frequency stimulation ($p<0.005$). No change in total PKA activity (determined with 10 μM exogenous cyclic AMP) was detected, consistent with activation of PKA by dissociation of the catalytic subunit. The LTP-associated increase in PKA activity was completely blocked by the *N*-methyl-D-aspartate (NMDA) receptor antagonist D,L-2-amino-5-phosphonovaleric acid at concentrations which blocked the induction of LTP (50 μM). Activation of NMDA receptors also induced activation of PKA; NMDA led to a dose-dependent increase in PKA activity with an EC_{50} of approximately 50 μM. These results demonstrate that activation of PKA occurs during the induction of LTP, and that stimulation of NMDA receptors is both necessary and sufficient to produce the effect. In light of the fact that PKA can enhance current flux through kainate receptors, activation of PKA may lead to potentiation of synaptic efficacy during the early stages of LTP. (Supported by the Life and Health Insurance Medical Research Fund and the McKnight and Klingenstein Foundations.)

703.11

NITRIC OXIDE SYNTHASE INDEPENDENT LTP IN AREA CA1 OF HIPPOCAMPUS.

J. David Sweatt*, Dane Chetkovich, and Eric Klann. Division of Neuroscience, Baylor College of Medicine, Houston, TX 77030.

While the induction of LTP is dependent upon biochemical events in the postsynaptic neuron, recent evidence implicates the involvement of the presynaptic neuron in the maintenance of LTP. This implies the necessity of a retrograde messenger to carry a signal from the postsynaptic to the presynaptic neuron. The labile, membrane-permeant compound nitric oxide (NO) has been proposed to perform such a retrograde messenger function. We have previously observed that LTP-inducing stimuli elicit an increase in the activity of guanylyl cyclase, an effect blocked by inhibitors of NO synthase. We have chosen to capitalize on these data, which document the effectiveness of NO synthase inhibitors in this system, to determine if the activity of NO synthase is necessary for LTP induction under our conditions. When Schaffer collateral inputs into area CA1 were tetanized at a stimulus intensity that gave about one-half the maximal postsynaptic response (measured using extracellular field recordings, bath temp. 32 deg C), LTP was produced in 12/13 slices. Addition of the NO synthase inhibitor nitro-arginine (NO-Arg, 50 µM) decreased the probability of LTP induction (7/13 slices) and decreased the magnitude of LTP (148±8% LTP in control slices, 114±4% LTP in NO-Arg treated slices). When tetanic stimuli were delivered under conditions eliciting the maximal EPSP, more robust LTP was observed (177±8% LTP, n=6) and no significant effect of NO-Arg was observed on the magnitude or probability of induction of LTP. These data suggest a modulatory role for NO in LTP induced using low-intensity tetanic stimulation, but demonstrate that the activity of NO synthase is not necessary for LTP induction under all conditions. (Supported by the McKnight and Klingenstein Foundations.)

703.12

PHOSPHATASE INHIBITORS BLOCK THE DEPRESSION OF POTENTIATED SYNAPSES BY THETA-FREQUENCY STIMULATION.

T.J. O'Dell* and E.R. Kandel, HHMI, Center for Neurobiology & Behavior, Columbia University College of Physicians & Surgeons, New York, NY 10032.

We investigated the modulation of excitatory synaptic transmission in the CA1 region of hippocampal slices by theta-frequency stimulation. Three minutes of 5 Hz stimulation produced a small (13.6 ± 2.4% above control, $\bar{x}$ ± SEM, n = 6), persistent (> 30 minutes) potentiation of synaptic transmission. However, 15 minutes after the induction of long-term potentiation (LTP), 3 minutes of 5 Hz stimulation depressed synaptic transmission (responses were 41.7 ± 1.4% above control (n = 7) 1 hour after inducing LTP vs. 95.5 ± 1.3% of control (n = 7) in control experiments. 5 Hz stimulation did not inhibit potentiated synaptic transmission if delivered in the presence of 50 µM APV (N = 9) or if delivered more than 50 minutes after inducing LTP (n = 7). This suggests that theta-frequency stimulation acts via an NMDA receptor-dependent mechanism and interferes with early processes required for the induction and stabilization of LTP.

To determine whether protein phosphatases mediate the depression of potentiated synapses, we examined the effects of the phosphatase inhibitor okadaic acid (OA). 1.0 µM (n = 8) but not 0.5 µM OA (n = 4) completely prevented the inhibition of LTP by 5 Hz stimulation delivered 15 minutes post-tetanus, suggesting that NMDA receptor activation during 5 Hz stimulation can activate protein phosphatases which can interfere with protein kinase activity required for normal LTP.

GENESIS OF NEURONS AND GLIA IV

704.1

A NOVEL MECHANISM FOR NEUROGENESIS IN THE ADULT BRAIN.

M.-F. Cheng* and M. Zuo. Institute of Animal Behavior, Rutgers University, Newark, NJ 07102

In a study designed to examine the kinetics of glial responses to thalamic lesion, Altman (1962) observed that intracranial injection of ^{3}[H]thymidine not only labeled glial cells but also labeled some neurons in the adult rat. We have reexamined this long forgotten observation, using adult ring dove, a species in which we have previously shown to exhibit low level neurogenesis dispersed within the telencephalon. Two experiments were conducted. The first experiment was designed to determine the rate and distribution of cell proliferation in the ventricular zone. One year old adult ring doves were treated with a single injection of ^{3}[H]thymidine 24 hr after the unilateral or sham lesions in the avian visual thalamus (n. Rt.). The 2nd experiment was designed to determine lesion-induced neurogenesis. The doves received the same treatment as in the 1st experiment except that they received 7 consecutive injections of ^{3}[H]thymidine 24 hr after unilateral electrolytic or sham lesions. Lesions promoted not only cell proliferation in the normally active ventricular zone but also induced proliferation activity in the 3rd ventricle that is normally quiescent in the intact birds. Evidence of neurogenesis is subjected to a series of critical tests for verification. Supported by Hoechst-Celanese Award.

704.2

GENERATION OF NEW CELLS IN THE VENTRICULAR ZONE DECLINES DURING DEVELOPMENT IN ZEBRA FINCHES.

S.D. Brown*, S.W. Bottjer. Biology Dept., Univ. of Southern California, Los Angeles CA 90089-2520.

Neurogenesis continues in the songbird telencephalon into adulthood. During song learning in juvenile zebra finches, larger numbers of newly-generated neurons are incorporated into brain regions controlling song than in adulthood, when song-learning is complete (Nordeen & Nordeen, 1988). We report that the proliferative activity of stem cells located in the ventricular zone of the lateral ventricles, the source of new neurons in the telencephalon, is extremely high at 20 days of age (the beginning of song learning) but declines dramatically by adulthood when song learning is complete. Male and female zebra finches aged 20 days to adult (> 100 days) received a single injection of [^{3}H]-thymidine and were perfused 24 hours later. We chose a short survival time in order to measure proliferation rates per se before processes such as cell death or migration could intervene. The brains were embedded in paraffin or glycol methacrylate and processed as autoradiograms. The amount of labeling found over cells within the ventricular zone, an index of the total mitotic activity of cells within this region, was compared for each age group. The amount of thymidine labeling found within the ventricular zone of the youngest birds (20 days) far exceeded that of adults. The dramatic addition of new neurons to song-control regions during song learning may be due at least in part to an overall higher level of neurogenesis at young ages. This finding makes possible the investigation of naturally occurring down-regulation of cellular proliferation in a post-hatch, adult-sized animal model.

704.3

IN VITRO NEURONAL AND GLIAL PRODUCTION AND DIFFERENTIATION BY PRECURSOR CELLS DERIVED FROM THE ADULT HUMAN BRAIN B. Kirschenbaum, M. Nedergaard, A. Preuss, K. Bahramian, S. A. Goldman,* Dept. of Neurology & Neuroscience, Cornell Univ. Medical College, NY, 10021.

A variety of subprimate vertebrates have been found to harbor neural precursor cells in adulthood, which retain the potential for neuronal production *in vitro*. We sought to determine whether the human brain might retain such precursors, by culturing adult temporal lobe under conditions permissive for neuronal differentiation. Fresh surgical samples of human temporal cortex, subcortical white matter, and periventricular zone (VZ) were either explanted or dissociated into cell suspensions, to which ^{3}H-thymidine was added. The cultures were incubated for 7-28 days, fixed, immunostained for one of several neuronal or glial antigens, and autoradiographed. Neuron-like cells were found in both explant cultures and single cell dissociates of the VZ; these cells expressed neuronal antigens including MAP-2, MAP-5, NF and N-CAM, and were GFA$^-$. Such cells were only rarely found in dissociates of subcortex or cortex, and were not observed in explant cultures lacking VZ. Cultures from each region were also loaded with the calcium-sensitive dye Fluo-3, and exposed to 60 mM K$^+$ during confocal microscopy, so as to detect depolarization-induced calcium increments. A subpopulation of neuron-like cells responded to K$^+$ depolarization with rapid and reversible increases in cytosolic calcium, which were substantially greater than those observed in co-cultured astrocytes. Among MAP-2 or MAP-5 VZ-derived neurons, a small number incorporated ^{3}H-thymidine, suggesting their origin from precursor cell mitosis *in vitro*. Most were unlabeled by ^{3}H-thymidine, and may have arisen from precursors induced to differentiate by the culture conditions. Thus, the adult human forebrain may harbor precursor cells which retain the potential for neuronal and glial production, once removed from their local environment. (Supported by NIH NS01316, R29NS29813, the Mathers and Lookout Foundations, the American Paralysis Association, and the Hirschl Trust).

704.4

POSTNATAL DEVELOPMENT OF THE MAGNOCELLULAR HYPOTHALAMIC NEURONS IN THE BRAZILIAN OPOSSUM. J. Iqbal, J. K. Elmquist, L. R. Ross, M. R. Ackermann[1] and C. D. Jacobson*. Dept. of Veterinary Anatomy and Neuroscience Program, Iowa State University, Ames, IA 50011 and [1]National Animal Disease Center, Ames, IA 50010.

Our laboratory has been studying the ontogeny of neuropeptide systems in the Brazilian opossum, *Monodelphis domestica*. Monodelphis is a small, pouchless marsupial whose young are born in an extremely immature state with a protracted postnatal period of nervous system differentiation. Presently, we are studying the development of the hypothalamic paraventricular (PVN) and supraoptic (SON) nuclei in the developing opossum. We have utilized tritiated thymidine (*thy) autoradiography and 5-bromo-2-deoxyuridine(BrDU) immunohistochemistry to study hypothalamic neurogenesis. To this end, postnatal pups were given a subcutaneous injection on one of the days between postnatal (PN) days 1 & 13 and killed on PN 60. We have found that neurogenesis of these nuclei continues to take place postnatally. Analysis of the slides revealed a high proportion of BrDU or *thy labeled neurons in the PVN area following injection on days 6 or 7 PN. Whereas only one third of the neurons were labeled in the SON area following injection on 4 or 5 PN. The number of labeled neurons appeared to decrease with increasing age of exposure. Injection on day 10 or 11 PN resulted in no labeling in the PVN and SON areas. We have also found that arginine vasopressin-like (AVP) immunoreactivity was present as early as 1 PN in the developing hypothalamic magnocellular neurons, whereas oxytocin-like (OT) immunoreactivity first appeared at 5 PN. The early appearance of AVP and OT immunoreactivity indicate that these peptides may be playing a role in the ongoing development of the brain. Since neurogenesis and peptide expression of the magnocellular neurons occurs during the postnatal period, we believe the opossum is an excellent model for developmental and physiological studies of the magnocellular system of the hypothalamus.

704.5

ALZ-50-IMMUNOREACTIVE CELLS IN HUMAN FETAL CNS. L.A. Mattiace*, K.M. Weidenheim, W.D. Lyman, W.K. Rashbaum, P. Davies, Depts. of Pathology and Ob-Gyn, Albert Einstein College of Medicine, Bronx, NY 10461

Alz-50 immunolabeled cells have been reported in the subplate region of the human fetal cortex by 36 wks of gestation. The present study was designed to determine whether Alz-50-immunoreactive cells are present in normal human fetal CNS prior to 36 wks. Serial hemisections from 16 wk to 24 wk abortuses of HIV-seronegative females were examined immunocytochemically with antibodies developed to cytoskeletal proteins including Alz-50, AP14 (MAP-2) and NP-18 (neurofilament). Cresyl violet staining on separate sections was used for anatomical localization.

In preliminary data, clusters of intensely stained Alz-50-immunoreactive cells were found in a number of subcortical cell groups at 23 wks. These areas included the corpus striatum, hypothalamus, thalamus and amygdala. Morphologically, many of these cells were bipolar and/or multipolar, depending upon the subcortical area. Scattered Alz-50-immunoreactive cells were also found in the subplate region throughout the longitudinal extent of the cortex, but in smaller numbers than previously reported in the 36 wk fetus. Scattered and sparse, but often intensely stained, Alz-50-immunoreactive beaded processes were also seen throughout the cortical layers including the marginal zone.

Because Alz-50 has been suggested to be a marker for programmed cell death, the presence of Alz-50-immunoreactive cells suggests that certain subcortical cell groups are undergoing programmed cell death in the latter part of the second trimester in the human fetus. Given that programmed cell death occurs during neuronal differentiation and maturation, this may be additional evidence that certain subcortical cell groups undergo developmental changes prior to those seen in cortical regions.

704.6

PROLIFERATIVE BEHAVIOR OF PROGENITORS IN THE GANGLIONIC EMINENCE OF MICE. P. G. Bhide* and T. Takahashi, Department of Neurology, Massachusetts General Hospital, Boston, MA 02129.

Ganglionic eminence is the embryonic source of cells destined for a variety of telencephalic structures including the neostriatum. The proliferative behavior of cells in this region was studied in mice during the interval embryonic day 11 (E11; first 24 h after conception = E0) to E16 using bromodeoxyuridine as the S-phase marker. Two proliferative populations, the pseudostratified ventricular epithelium (PVE, corresponding to the ventricular zone) and the secondary proliferative population (SPP, corresponding to the subventricular zone), were distinguished based upon the spatial distribution of cells in S-phase. PVE borders the ventricular cavity and SPP is located lateral to the PVE, away from the ventricular border. Nuclei of proliferating cells in the PVE display an oscillatory movement such that nuclei in M-phase are located along the ventricular border and those in S-phase are located away from the ventricular border in the "S-phase zone". Nuclei of proliferating cells in the SPP do not display such an oscillatory movement. PVE dominates the proliferative activity during the early part of cytogenesis - until E12-13. After that time, cell proliferation in PVE begins to decrease whereas that in SPP begins to increase. By E16, PVE is virtually completely replaced by SPP. Postmitotic cells - apparently of PVE origin - accumulate lateral to the PVE as early as on E11. Those cells may be among the earliest generated cells of the telencephalon and/or the precursors of SPP which are temporarily in a resting (Go) state. The PVE and SPP of the ganglionic eminence share several characteristics with their neocortical counterparts. A marked spatio-temporal gradient of cell proliferation was apparent when the overall duration of the cell cycle and the duration of the individual phases of the cell cycle were estimated separately for rostral, middle and caudal ganglionic eminence for each of E11 to E16.

704.7

PROLIFERATION AND SURVIVAL OF RAT SENSORY NEURON PRECURSORS IN VITRO. Stacey J. Piszczkiewicz* and Alison K. Hall, Dept. Neurosciences, Case Western Reserve University School of Medicine, Cleveland, OH 44106.

The processes that regulate the development of neurons from their precursors are essentially unknown. We have developed a tissue culture system in which precursors to E12 lumbar rat dorsal root ganglion (DRG) neurons divide and subsequently differentiate. Cells were initially undifferentiated and polygonal, and after two days, numerous bipolar neurons with long processes were detected. This time period corresponds well to sensory neuron differentiation in vivo. The effects of specific additives on neuron precursor division and neuronal survival were assayed. Dissociated E12 DRG were labeled with the thymidine analog, BrdU, and subsequently analyzed for the presence of neuron-specific tubulin and BrdU. NGF enhanced proliferation compared with control conditions containing rat serum. BDNF potentiated the effect of NGF compared with control. LIF alone enhanced proliferation and in combination with BDNF and NGF had an additive effect compared with control. Thus, specific factors enhanced division of sensory neuron precursors. The percentage of neurons that survived 48 hr was enhanced by BDNF, NGF and LIF relative to control values. However, the addition of all three factors did not increase neuronal survival compared with BDNF alone. The lineage relationships between the neurons and glial cells that mature in these cultures is under investigation.(BDNF supplied by Regeneron; Supported by Ohio Board of Regents and Basil O' Connor Starter Scholar Research Award 5-FY92-1178)

704.8

NESTIN EXPRESSION IN HUMAN FETAL BRAIN IN VIVO AND IN VITRO. P.T. Cheung†, C. Spenger*, L. Hotayt, L. Studer, N. Herschkowitz† and R.W. Seiler Dept. of Neurosurgery and Dept. of Pediatrics†, Inselspital, CH 3010 Bern, Switzerland.

Human neural cell cultures are potential sources of neural cells for transplantation. Nestin, an intermediate neurofilament expressed by neuroepithelial stem cells, has been used as a marker to identify cells capable of proliferating and differentiating into both neurons and glial cells in embryonic and adult mouse brain cell cultures (Reynolds et al., 1992). Nestin is also expressed in human fetal neural tissues.

To define specific human fetal brain region(s) suitable for providing neuroepithelial stem cells, the expression of nestin mRNA in various brain regions was examined by Northern hybridisation using a rat nestin cDNA probe generated by RT-PCR. Nestin mRNA was detected in rhombencephalons at all ages examined [5weeks+6days (w+d) post conception (p.c.) to 9w+6d p.c.; n=9] with a decline of signal intensity after 7-8 w. p.c. It was also found in cortices [7w+4d to 9w+6d p.c.; n=4] and in cerebellum [9w+6d p.c.; n=1]. Signals in spinal cords were lower than those of rhomencephalon and cortex from the corresponding embryos [7w+4d and 8w+6d; n=2].

Rhombencephalon was then chosen to be evaluated as an in vitro source of human neuroepithelial stem cells using a free floating micro culture system. In these cultures cell proliferation was demonstrated by incorporation of bromodeoxyuridine and tritiated thymidine, and mitotic figures seen in histologic sections for up to 3 weeks in culture. In addition, the continual presence of neuroepithelial stem cells was suggested by the detection of nestin mRNA by Northern hybridisation. Therefore, further investigations to determine whether progenitor cells actively proliferate in these cultures are warranted. Supported by SNF-Grants No 31-36243.92 and No 3-30909.91

704.9

SERUM-FREE DERIVED MOUSE EMBRYO CELLS EXPRESS NESTIN AND SELECT NEURONAL MARKERS: REGULATION BY SERUM. D.T. Loo*, M.C. Althoen and C.W. Cotman. Irvine Research Unit in Brain Aging and Dept. of Psychobiology, *University of California, Irvine, CA 92717.*

We previously reported the derivation of a serum-free mouse embryo (SFME) cell line from E17 embryos using a basal medium supplemented with insulin, transferrin, epidermal growth factor, high density lipoprotein and fibronectin (D. Loo et al., Science 236, 200 [1987]). SFME cells have been cultured for more than 200 generations without undergoing growth crisis, have retained a diploid karyotype and are nontumorigenic *in vivo*. SFME cells are dependent on epidermal growth factor for survival and are reversibly growth inhibited by serum. Additionally, cells with properties similar to SFME cells have been isolated from adult mouse brain. Initial experiments aimed at defining the cell type represented by SFME cells showed that treatment of SFME cells with serum or transforming growth factor beta induced the expression of glial fibrillary acidic protein, suggesting that SFME cells represent an astrocyte precursor cell.

To further define the phenotypic character and explore the differentiation potential of SFME cells we are using genetic and immunocytochemical approaches to assess the expression of neuronal- and glial-specific cell markers. We found that SFME cells express the gene encoding nestin, a neuroepithelial stem cell marker. Expression of nestin is down-regulated by serum. SFME cells also express the gene encoding neuron-specific microtubule associated protein 2 (MAP2), and are immunoreactive for MAP2, MAP5 and neurofilaments. This work suggests that SFME cells represent a neural precursor cell type which retains the capacity to express markers of both neuronal and glial cells.

704.10

CONNEXIN EXPRESSION IN EMBRYONAL CARCINOMA CELLS. D.J. Belliveau* and C.C.G. Naus. Department of Anatomy, The University of Western Ontario, London, Ontario, Canada, N6A 5C1.

The P19 embryonal carcinoma cell line represents an ideal model of early neural differentiation events. We have examined the expression of connexin genes in the neural cell derivatives of differentiated P19 cells in an attempt to better understand the role gap junctions play during CNS development. P19 cells were treated for 96 hr with retinoic acid (RA; 0.3 μM) causing them to differentiate into numerous cell types including neurons and astrocytes. Untreated P19 cells expressed abundant connexin43 (cx43) and to a lesser degree connexin26 (cx26) immunoreactivity for up to 12 days in culture. Results from polymerase chain reaction experiments on reverse transcribed mRNA revealed the presence of cx26, 32, 37, and 43 gene products, although in Northern blot analysis only cx26 and cx43 mRNA were detected. In RA treated cells, neurons were the first neural cell type to develop, noticeable within 24 hours, and were maintained for as long as 8 days in culture. In 5 day cultures, cx26 immunoreactivity could be seen on processes and cell bodies in some populations of neuron-like cells. Astrocytes (positive for glial fibrillary acidic protein [GFAP]) were first detected in small numbers after 7 days in culture and by 10 days, cx43 immunoreactivity was found to be associated with the astrocytes. Not all cx43 immunoreactivity was correlated with GFAP positive cells, much of it was found in other, yet unidentified, cells in the culture. These results show that the P19 embryonal carcinoma cells express multiple connexin genes and, therefore, will prove to be a valuable tool in examining the function of specific gap junction genes in differentiation of neural cells. Supported by the Medical Research Council of Canada and the Vice President (Research) Funds from The University of Western Ontario.

704.11

NEURON-SPECIFIC CLASS III BETA-TUBULIN IS EXPRESSED BY DIVIDING NEURON PRECURSORS. Alison K. Hall*, Anthony Frankfurter# and Stacey J. Piszczkiewicz. Dept. Neuroscience, Case Western Reserve Univ. School of Medicine, Cleveland, OH 44106 and #Depts. Biology and Neuroscience, University of Virginia, Charlottesville, VA, 22908.

The differentiation of precursor cells into neurons is likely to involve specific molecular and morphological changes. The expression of class III neuron-specific beta tubulin, as detected by the monoclonal antibody, TuJ1, is initiated very early in neuronal maturation (Moody et al., 1989, J. Comp. Neurol. 279: 567-580; Easter et al., 1993, J. Neurosci. 13: 285-299). We have found that the E12 rat ventral lumbar spinal cord possessed TuJ1 immunoreactive cells that, in some cases, spanned the neuroepithelium. To investigate the expression of TuJ1 in neuron precursors, embyronic rat cultures were pulsed with the thymidine analog BrdU, and assayed by double label immunocytochemistry for their expression of BrdU and TuJ1. In cultures of E14 sympathetic ganglia and E12 lumbar dorsal root ganglia or spinal cord, numerous neurons bearing long neurites and TuJ1 expression incorporated BrdU during a 24 hr pulse. These data are consistent with a precursor incorporating BrdU during division, and the postmitotic neuron subsequently elaborating TuJ1+ processes. Intense TuJ1 expression was also detected in the spindle of mitotic cells, and in pairs of cells joined at cytokinesis, indicating that TuJ1 expression is initiated before the final mitosis of a neuronal progenitor. Therefore, class III tubulin expression may represent the earliest detectable molecular event in neuronal commitment exhibited by both CNS and PNS precursors. (Supported by Basil O'Connor Starter Scholar Research Award 5-FY92-1178 to AKH).

704.12

PROMOTER ACTIVITY OF GLIAL FIBRILLARY ACIDIC PROTEIN GENE IN EMBRYONIC BRAIN CELLS. K. Nakahira, K. Nakajima, N. Morita, K. Mikoshiba and K. Ikenaka*. Natl. Inst. Physiol. Sci., Myodaiji, Okazaki, 444 Japan.

We developed an assay system using retroviral vector for measuring promoter activity of neural tissue-specific gene in embryonic cells. The vector, pIP200, has a cloning site followed by bacterial beta-galactosidase gene (lacZ) as a reporter. Promoter region of glial fibrillary acidic protein (GFA) gene (2.5kbp; it is thought to be sufficient to confer astrocyte-specific expression) was inserted in this vector, and its activity was examined in primary cultured cells from embryonic day 13 (E13) murine brain hemispheres. Infected cells were sequentially fixed and stained with X-gal and anti-GFA antibody to identify cell type. Although there were few GFA immunoreactive cells in the culture of this stage, some X-gal positive cells were observed one day after infection. After 3 - 5 days *in vitro*, increasing number of GFA immunoreactive cells appeared, however, many of X-gal positive cells were GFA-negative. After 10 days *in vitro*, all of X-gal positive cells became GFA-positive. This result suggests that GFA promoter is activated several days before GFA becomes detectable immunohistochemically, and activation of the promoter is a potent early marker for investigating astrocyte differentiation.

704.13

GENE TRANSFER IN A HIGH DENSITY NEURON-ENRICHED CULTURE FROM HUMAN FETAL BRAIN. F.-C. Chiu, C. Bassallo, W. Lyman, H.J. Federoff* Albert Einstein Coll. Med., Bronx, NY 10461.

To investigate the feasibility of gene transfer into human fetal neurons that might be suitable for transplantation, we have prepared high density cultures that are enriched in neurons and transduced them with defective herpes simplex viral (HSV) vector. Fetal brains at gestation week 20-24 were dissociated within 1-2 hrs of elective termination, resuspended in a growth medium containing 10% fetal calf serum and rapidly seeded at 0.5 x10^6/cm^2 on culture plates and slides previously coated with laminin and poly-ornithine. Over 60% of plated neural cells survived. At 2 days *in vitro* (DIV), cytosine arabinoside (6 μM) was added. Within 3 DIV, the culture became confluent and consisted of small cells with phase-dark cell bodies that accounted for >85% of the total population and a dense network of fine processes. These small cells and fine processes were immuno-positive for the 66 kD neurofilament (NF-66) protein that is abundant in the human fetal brain but were immuno-negative for the astrocyte-specific glial fibrillary acidic protein (GFAP). Larger cells, representing <15% of the total population, had thick processes and were GFAP+. At 7-10 DIV, cultures were transduced with HSVlac, a vector containing the E. Coli β-galactosidase (Lac Z) gene under the control of the HSV IE 4/5 promoter. Robust expression of Lac Z as measured by X-gal histochemistry was found in both NF-66+ and GFAP+ cells. Our data demonstrate the feasibility of gene transduction into a neuron-enriched population of cells from human fetal brain and suggest the possibility of genetically modifying these cells prior to transplantation. Supported by PHS Grants NS23840, NS23705 (FCC), MH47667 (WL), HD27226 (HJF).

705.1

IDENTIFIED NEURONS FROM EMBRYONIC LEECHES CULTURED WITH AND WITHOUT POTENTIAL TARGET TISSUES. C. Pfahlert, W.B.Kristan, Jr., and K.A. French*. Dept. of Biology, UCSD, La Jolla, CA 92093-0322.

Retzius (Rz) neurons in *Hirudo medicinalis* acquire certain identifying characteristics - such as the production of the neurotransmitter serotonin - as a result of early events in their development. Other characteristics are acquired relatively late in development, after their processes have grown into the periphery. These properties depend upon whether or not embryonic reproductive ducts (located in mid-body segments 5 and 6 - MS 5 and MS6) are present in the segment; the properties include certain identified synaptic inputs and how the cell responds to acetylcholine (ACh) ejected onto the soma. Experimental manipulations in embryos have shown that the final phenotype of Rz neurons depends upon association between Rz processes and reproductive ducts and that Rz neurons may become committed during a relatively brief period of time very soon after Rz processes first contact the duct mesenchyme.

To study directly this interaction between embryonic Rz neurons and the reproductive ducts, we have begun to remove individual Rz neurons from *Hirudo* embryos and to culture them in the presence or absence of potential target tissues - reproductive ducts or muscles from the body wall of hatchling leeches. These embryonic neurons survive at least 3 days in culture, which allows us to compare them with adult Rz neurons cultured under the same conditions, as well as with Rz neurons *in vivo*. We should now be able to determine whether interactions between processes of embryonic Rz neurons and reproductive duct muscle *in vitro* can modify Rz neuron development as they do *in vivo*. This research was supported by funding to CP from the Education Abroad Program and NIH research grant NS25916 to WBK.

705.2

EXPRESSION OF THE CHOLECYSTOKININ GENE IN SENSORY CORTEX SLICE CULTURE OF RAT. D.K. Meyer, C. Olenik and B. Heimrich*#. Dept. of Pharmacology and #Institute of Anatomy, Freiburg University, D-7800 Freiburg, FRG.

The preprocholecystokinin gene is expressed in a subpopulation of GABAergic interneurons in rat cortex. In vivo experiments indicate that the expression is regulated by neuronal afferents in a complex manner. Thus, we looked for an in vitro model which is organotypic and allows the analysis of neuronal interactions. Slices of rat somatosensory cortex were explanted at postnatal day 1 and kept in static cultures for 12 ± 2 days (Stoppini et al., J. Neurosci. Meth. 37: 173 (1991)). In these slice cultures, two layers of neurons containing cholecystokinin-mRNA were found with in situ hybridization in an arrangement which paralleled the distribution pattern in situ. Immunocytochemistry was performed with an antiserum against cholecystokinin8-sulfate. Examination with light- and electron-microscopy showed immunopositive cells with small round somata and a dense network of immunoreactive fibers. The immunoreactive terminals formed only symmetric synapses. All these morphological features are typical for cholecystokinin neurons in situ. In a first series of experiments, the GABAA receptor antagonist bicuculline (70 µM) was applied for 90 min. Twelfe hours later, the levels of cholecystokinin-mRNA were unchanged. The finding indicates the absence of GABAergic inhibition via GABAA receptors.

Taken together, cholecystokinin neurons in slices of rat somatosensory cortex retain several biochemical and morphological properties, when cultivated in vitro. Organotypical slice cultures provide a model to analyze the effects of neurotransmitters on the regulation of the cholecystokinin gene in interneurons.

Supported by DFG grant SFB 325/A3 and B10.

705.3

RELATIONSHIPS BETWEEN NEUROMORPHOGENESIS & CYTOCHROME OXIDASE (CO) ACTIVITY IN RAT AUDITORY AND VISUAL CORTICES, HIPPOCAMPUS & CEREBELLUM AS DEMONSTRATED WITH METAL-INTENSIFIED CO HISTOCHEMISTRY. G.H. Kageyama* and R.T. Robertson. Department of Anatomy and Neurobiology, Univ. of California, Irvine, CA 92717.

The original CO histochemical method described by Wong-Riley ('79) utilizes the catalytic oxidation of DAB as the chromagen in the presence of cytochrome c. At the light microscopic level, the reaction product is typically orange to orange-brown in color. Lighter orange stained sections often require the use of a dark blue (Wratten 47B) filter for optimal B/W photography. We have used two modifications: (1) 0.05 M Tris buffer, pH 7.6 and (2) the addition of nickel ammonium sulfate, to improve the contrast and shorten the time of the reaction without loss of specificity as determined by the EM localization of reaction product within the intracristate space and outer mitochondrial compartment. In auditory and visual cortices, elevated CO was localized in the apical dendrites of cortical plate (CP) neurons and in interneurons and subplate (SP) neurons of neonatal animals. Immature neurons migrating to the CP contained few mitochondria and exhibited very little CO activity, indicating that CO becomes elevated in neurons after they reach the CP and extend apical dendrites into the marginal zone where they receive their first synaptic inputs. The dendrites of SP neurons were particularly well stained, indicating their high metabolic activity, probably resulting from convergent excitatory synaptic input from thalamocortical and other axons. Dentate granule cells and hippocampal pyramidal cells exhibited elevated CO activity in their distal dendrites where they receive their most intense excititory synaptic input from perforant path axons (Kageyama & Wong-Riley,'82). The dendrites and somata of various interneurons were also well-stained. In the cerebellum, Purkinje cell dendritic arbors and somata were clearly delineated. This method clearly demonstrated that most of CO staining throughout the CNS of postnatal rats was localized predominantly in dendrites and somata of specific classes of neurons rather than in axons or terminals. Oligodendrocytes were also quite reactive compared with astrocytes. Support: NIH grants DC 00450 and NS 30109.

705.4

LIMITED MORPHOLOGICAL DEVELOPMENT OF AVIAN PURKINJE CELLS IN AN IN VITRO CO-CULTURE SYSTEM P.L. Jeffrey*, J. Meaney, O. Tolhurst and R.P. Weinberger, Children's Medical Research Institute, Wentworthville, N.S.W. Australia 2145.

As an approach to investigating the expression of developmentally regulated neuronal antigens and the development of neuronal polarity this laboratory has developed a unique co-culture system for the survival and differentiation of avian Purkinje cells (PC). Dissociated PC from ED chick cerebellum were plated on laminin and poly-L-lysine coated coverslips. Astrocytes from various ages were grown in Millicells and placed above the PC coated coverslips in culture dishes containing neuronal medium. Purkinje cells of purity greater than 90% were identified by calbindin-D, cyclic GMP protein kinase and NF200 immunohistochemistry. The Purkinje cells also stained positive for NSE, A2B5 and parvalbumin. After one day in culture most PC exhibit a "simple fusiform" morphology (Type I, Armengol, J.A. & Sotelo, C. Dev. Brain Res 64 (1991) 95). A generalised MAP-2 and Thy-1 staining is seen throughout processes. Following 21 days in culture the majority of the PC are arrested at the Type II complex fusiform morphology. Purkinje cells with Type III (regressive atrophic dendrites) morphology are rarely seen up to 21 days in culture. No cerebellar granule cells are present in this culture system which are necessary for the development of dendritic processes (Baptista, D.H. et al. Soc Neurosc.: 17,1991,38). A population of cells with a phenotype representing early stages of PC development has been defined which is suitable for studies of cellular interactions and effect of extracellular components on PC survival and differentiation.

705.5

AXONAL AND DENDRITIC OUTGROWTH OF INFRAPYRAMIDAL GRANULE CELLS IN THE DEVELOPING DENTATE GYRUS. P. Alba, O. Rahimi, P. A. Brewer*, and B. J. Claiborne. Division of Life Sciences, University of Texas at San Antonio, San Antonio, TX 78249.

Previous results indicate that dendritic trees of granule neurons in the suprapyramidal blade of the developing dentate gyrus mature after the axons reach their target area in the CA3 region of the hippocampus (Claiborne et al., Neuro. Abstract, 17:35; personal communication). Because granule neurons in the infrapyramidal blade develop later than those in the suprapyramidal blade, we have investigated the relationship between axonal and dendritic outgrowth of infrapyramidal granule cells. DiI crystals were placed on the CA3 region in fixed 400 um thick slices of hippocampi dissected from rats ranging in age from 2 to 9 days, thereby retrogradely labeling granule neurons whose axons have reached their target area. Using a low-light camera attached to a light microscope, labeled dendritic trees were videotaped. Confocal microscopy was used to obtain three-dimensional images of representative neurons.

Results showed that granule neurons in 2 to 4 day-old rat pups displayed immature characteristics: a long primary apical dendrite, large varicosities, basal dendrites, and filamentous projections. Neurons from 5 to 6 day-old animals had a reduced number of these immature characteristics but had developed spines. In most cells from 7 to 9 day-old rats, spines became more prevalent and the number and frequency of immature characteristics decreased. These results show that the dendritic trees of infrapyramidal granule neurons mature after their axons reach area CA3, as was found previously for suprapyramidal granule neurons. (Supported by NIH/NIGMS GM01894 and the Texas Higher Education Coordinating Board)

705.6

EFFECTS OF ANTINEOPLASTICS ON THE DEVELOPMENT OF HIPPOCAMPAL NEURONS IN CULTURE: A MORPHOLOGICAL STUDY. J-S. Cloix, R.L. Kenigsberg, R. Collu* and Y. Théorêt. Centre de Recherche, Hôpital Ste-Justine, Montréal, Québec, Canada, H3T 1C5.

Although remarkable advances over the last twenty years in cancer therapy have been met with higher survival rates for children, this success unfortunately has been accompanied by a high incidence of neurological disorders. It is likely that chemotherapy-induced neuronal effects are the results of alterations at the cellular level. However, ethical and methodological considerations limit severely the direct study of these structural alterations in human subjects following chemotherapeutic intervention. Consequently, the proposed study aims to determine the effects of therapeutically relevant levels of selected antineoplastics on the morphological characteristics of dendrites and axons, as well as on the time course of neurite growth. Hippocampal neurons were isolated from rat embryos (E17-18), plated at low density (1,000 to 10,000 cells/cm²) in modified multiwell dishes and co-cultured with astrocytes. These cultures were maintained in chemically defined serum-free media. At different times after plating, cells were treated with the following agents: 6-mercaptopurine (0.05-5µM), methotrexate (0.1-10mM), ara-C (1-100µM) or 4-OOH-ifosfamid (0.02-20µM). Using an inverted microscope (Carl Zeiss) and SigmaScan (Jandel Scientific), the following parameters were measured: 1) axon length, 2) total axonal and dendritic length, 3) number of axonal and dendritic branches/neuron, 4) number of dendritic intersections, and 5) degree of branching. Our initial results indicate that the kinetics of neuronal development can be altered by antineoplastic agents. This supports the hypothesis that morphological alterations may underly some of the neuronal deficits observed in children treated for cancer. (Supported by the National Cancer Institute of Canada and Telethon of Stars).

705.7

THE INFLUENCE OF THE NOTOCHORD ON A SPECIFIC POPULATION OF SPINAL CORD CELLS. A. Chen* and R.D. Heathcote, Department of Biological Sciences, University of Wisconsin, Box 413, Milwaukee, WI, 53201.

Little is known about how spinal cord neurons become organized during development. In the frog *Xenopus laevis*, two longitudinal columns of catecholaminergic interneurons gradually form a nonrandom pattern within the floor plate region of the spinal cord. The notochord is involved in several aspects of spinal cord development. It induces the neural plate, the ventral floor plate and the longitudinal columns of motor neurons. Therefore, the role of the notochord in establishing the catecholaminergic cell pattern was examined. Ultraviolet (UV) irradiation of fertilized eggs produced a range of phenotypic abnormalities including reduction or loss of the notochord. Animals without organized notochord, had no tyrosine hydroxylase (TH) immunoreactive interneurons. Thus notochord appeared to be necessary for induction of the catecholaminergic population of cells. In other experiments, an additional notochord was transplanted next to the developing neural tube before the initial appearance of TH cells. These experiments resulted in an increased density of catecholaminergic cells whose columnar pattern was disrupted. When the size of the neural plate was reduced by inserting a cellulose filter between the neural folds, the spinal cord formed two branches. One branch was notochordless and showed signs of atrophy. The other branch, which had a greater ratio of notochord to neural plate than normal, exhibited many of the same properties as notochord transplants. Thus, manipulation of the notochord affected the density of catecholaminergic cells as well as their longitudinal columns. These experiments provide the opportunity to further test the role of the notochord in regulating the formation and organization of a specific population of spinal cord cells.

705.8

SPINAL CORD MORPHOGENESIS IN A SIMPLE VERTEBRATE. J. Frey, A. Chen and R.D. Heathcote*, Department of Biological Sciences, University of Wisconsin, Box 413, Milwaukee, WI, 53201.

All cells of the spinal cord originate from the germinal or ependymal layer that surrounds the central canal. In the frog *Xenopus laevis* this ependymal layer contains differentiated neurons including a cerebrospinal-fluid (CSF) contacting population that is immunoreactive for tyrosine hydroxylase. During development, the catecholaminergic population gradually formed two longitudinal columns that subsequently "converged" at the ventral midline. During this period cell bodies decreased in size and underwent changes in shape, number and position. In order to understand the mechanisms involved in these changes we began a study of the spinal cord focusing on the ependymal layer. Ultrastructural evidence of ependymal cell differentiation including cytoplasmic projections into the central canal, non-motile neuronal cilia (those lacking the two central singlet microtubules), and motile cilia were present within a few hours of neural tube closure. Thus ependymal cells began differentiating at the same time as the earliest neurons in the spinal cord. Quantitative analysis of developing spinal cords at the level of the seventh somite showed extensive remodeling early in development. Starting at neural tube closure the number of cells increased while their size decreased. Although the central canal initially increased dramatically in size, it remained roughly constant until hatching (st 35/36). The tapered spinal cord resulted from reduced spinal cord size and cell numbers in regions one millimeter caudal to the seventh somite. Unlike the more rostral region, there were no major changes in the size and number of cells throughout the developmental stages studied. Thus, cellular remodeling occurs differentially along the length of the spinal cord. In rostral regions changes in cell size, shape, number and position accompany the morphogenesis of catecholaminergic interneurons.

705.9

DIFFERENTIAL EXPRESSION OF β-NADPH DIAPHORASE IN SOMATIC AND AUTONOMIC MOTOR NEURONS OF DEVELOPING RAT SPINAL CORD. R. Wetts* and J. E. Vaughn, Div. of Neurosci, Beckman Res. Inst. of City of Hope, Duarte, CA, 91010.

Although somatic and autonomic motor neurons (SMNs & AMNs) share certain characteristics, they differ in others. For example, the β-NADPH diaphorase histochemical reaction, which correlates with nitric oxide synthase expression, is present in AMNs, but not SMNs. Determining when this difference arises during development may provide insights into the mechanisms that control the separate fates of AMNs and SMNs.

At upper thoracic levels, AMNs stained for diaphorase activity as early as E14. These cells remained positive for the rest of development and into adulthood, whereas SMNs never stained at this spinal level. In contrast, some developing cervical SMNs were diaphorase-positive, exhibiting intense staining from E15 until E19. Subsequently, this staining gradually decreased and disappeared by birth. Thus, in one type of motor neuron (AMNs), diaphorase staining (and hence nitric oxide synthase) is continuously expressed into adulthood, whereas in a subset of another type (cervical SMNs), the staining is transient. The differential and transient nature of diaphorase expression implies that nitric oxide may have developmental roles that differ from its function(s) in the adult. *Supported by NINDS grants NS18858 and NS25784.*

705.10

INFLUENCE OF NOTOCHORD AND SURROUNDING TISSUE ON OLIGODENDROCYTE DIFFERENTIATION IN XENOPUS SPINAL CORD. C.E.Maier* and R.H.Miller. Dept. of Neurosciences, Case Western Reserve Univ., Cleveland OH 44106.

During the development of Xenopus spinal cord oligodendrocyte differentiation occurs in a ventral-dorsal sequence. Differentiated oligodendrocytes first appear in the ventral spinal cord at stage 44/45. By stage 54/55 differentiated oligodendrocytes are found in the lateral region of the spinal cord, while at stage 60/61 differentiated oligodendrocytes can be found in the dorsal spinal cord.

To determine if this distinct pattern of oligodendrocyte development in the spinal cord is influenced by extrinsic factors, the notochord was ablated and the surrounding somites and skin disrupted over a 3-5 somite distance caudal to the ear placode in embryos at stage 26/28 or stage 34/35. Operated animals were allowed to develop until stage 49. The differentiation of oligodendrocytes along the rostral-caudal axis of the CNS was then determined using Olig, a monoclonal antibody specific for myelinating frog oligodendrocytes. In control animals differentiated oligodendrocytes were found only in ventral regions of brain and spinal cord at stage 49. After ablation at stage 26/28, differentiated oligodendrocytes were absent from the area associated with notochord ablation but present in the ventral spinal cord rostral and caudal to that area. Similarly, after ablation at stage 34/35, differentiated oligodendrocytes were seen in lateral and dorsal spinal cord in the area associated with the ablation but only in ventral spinal cord rostral and caudal to the ablation site. These results imply that the notochord or surrounding tissue may play an important role in the regulating the timing and initial location of oligodendrocyte differentiation and myelination.

PROCESS OUTGROWTH, GROWTH CONES, AND SPROUTING IX

706.1

IMMEDIATE EARLY GENE EXPRESSION IN DRG NEURONS UNDERGOING STIMULATION-ENHANCED COLLATERAL SPROUTING K.M. Mearow*. Dept of Biomedical Sciences, McMaster University Medical Centre, Hamilton, Ont. L8N 3Z5

Intact cutaneous sensory neurons will undergo collateral sprouting to reinnervate adjacent areas of denervated skin. Sprouting is NGF-dependent and electrical stimulation (in vivo) of the DRG neurons results in an acceleration of the growth response, advancing it by up to one week. In an effort to discern neuronal events which may be involved in the stimulation-induced acceleration of the sprouting response, I have examined expression of several immediate early genes, including the c-jun family, c-fos and NGFIA (KROX 24)

The following experimental paradigms were set up: 1. Normal sprouting -cutaneous nerve fields were "isolated" (by surrounding denervation); 2. Accelerated sprouting - at the time of isolation the intact nerves were electrically stimulated; 3. Stimulation only -nerves were stimulated, but not isolated. Subsequently (6 hrs, 1, 2, 4, 6, 14, and 21 days) animals were anaesthetized and the appropriate DRGs were removed and prepared for cryosectioning. Immunocytochemistry (ICC) was carried out using polyclonal antibodies against cJUN, JUNB, JUND, FOS, KROX24 (Santa Cruz Biotech), while in situ hybrid-ization was performed using ^{35}S-labeled oligonucleotides complementary to the IEGs.

The results indicate that c-jun expression is increased in the sprouting neurons by 2d postop, peaking by 1 week and subsequently declining to control levels. While stimulation alone does not appear to influence c-jun expression, the combination of stimulation plus isolation results in increased expression apparent by 6 hrs and peaking 1-2 days earlier than in the absence of stimulation. Preliminary results suggest that neither Jun D nor NGFIA expression is significantly altered under any of these experimental conditions. Supported by NSERC.

706.2

NGF RECEPTOR mRNA EXPRESSION IN COLLATERALLY SPROUTING SENSORY NEURONS - THE INFLUENCE OF STIMULATION. Y. Kril*, J. Diamond and K. Mearow. Dept. of Biomedical Sciences, McMaster University, Hamilton, Ont. L8N 3Z5.

The collateral sprouting of intact cutaneous neurons has been shown to be NGF-dependent. Furthermore, electrical stimulation of these neurons advances the sprouting by almost one week. We have previously shown that NGF receptor (both p75 and trkA) and GAP43 mRNA expression is upregulated in the sprouting neurons, and have now extended this work to examine the influence of electrical stimulation on gene expression in sprouting neurons.

In the sprouting paradigm, alternate pairs of dorsal cutaneous nerves (DCNs) were cut in adult rats; the remaining intact DCNs then undergo collateral sprouting into the denervated areas of skin. Two further groups of animals were examined - one in which the surviving nerves were electrically stimulated, and another in which the nerves were stimulated, but denervations were not done. Subsequently (6hrs, 1, 2, 4, 6, 14, 21 days) the appropriate DRGs were removed and frozen. Cryosection of these DRGs were taken and used for in situ hybridization (ISH) with ^{35}S-labeled oligonucleotides complementary to p75, trkA and GAP43.

The results show that, as expected, the expression of NGFR (p75 and trkA) and GAP43 mRNA increases in the normal sprouting neurons, with the increases apparent by 2-4 days postop. In the stimulated sprouting neurons, however, the increase in trkA is apparent by 6 hrs, in p75 and GAP43 by 24 hrs. Stimulation in the absence of denervation resulted in an increase in only trkA expression, which was significantly less then in the combined stimulation and sprouting mode. Supported by the NCE on Neural Regeneration (JD, KM) and NSERC (KM).

706.3

PRENATAL DENDRITIC DEVELOPMENT OF RAT SYMPATHETIC PREGANGLIONIC NEURONS. K.G. Ruit* and Y.F. McKee. Dept. of Anatomy & Cell Biology, Univ. of North Dakota School of Medicine, Grand Forks, ND 58202.

We have utilized the neuroanatomical tracer DiI in fixed tissues to describe the embryonic development of the dendritic arborizations of sympathetic preganglionic neurons (SPNs) in the rat spinal cord. SPNs were labelled by placing small crystals of DiI within upper thoracic sympathetic chain ganglia or within upper thoracic ventral roots beginning in embryonic day 13 animals (E13) and continuing through the first postnatal week. SPNs were visualized by fluorescence microscopy in transverse, horizontal or sagittal sections, photoconverted using diaminobenzidine, and drawn by camera lucida.

Between E13 and the day of birth, SPNs change from a bipolar to a multipolar shape and their dendritic arborizations become mediolaterally-oriented, extending laterally to the pial surface of the cord and medially across the midline; by the end of the first postnatal week, contralaterally-projecting dendritic bundles reach the opposite intermediolateral cell column. Rostrocaudally-oriented dendritic bundles develop during the first postnatal week. The development of the dendritic arborizations of SPNs may be related to the development of innervating afferent systems and/or support by centrally or peripherally-derived trophic molecules.

706.4

CALLOSAL AXONS SHOW AN EXTENDED PERIOD OF MORPHOGENESIS IN HAMSTERS. C.Hedin-Pereira* & S.Jhaveri. Dept.Brain and Cognitive Sciences, M.I.T., Cambridge, MA 02139

We have studied the developmental changes in the morphology of callosal axons originating in the parietal cortex. Biocytin and PHA-L were used as anterograde tracers in P6,P9,P12, P20, P24 and adult hamsters. A standard immunohistochemical procedure was used to visualize labeled axons. Axons were serially reconstructed using a computerized 3D reconstruction system (Neurotrace). At P6, most fibers with homotopically projecting arbors arise from trunk axons that continue on laterally, sometimes as far as the rhinal fissure. Typically, axons have two or three sprouts along their trajectory, including in the perirhinal region. Some axons extend a collateral into the homotopic cortex and also into the striatum, a non-cortical target. Homotopic arbors have few ramifications within the gray matter. From P7 to P17, there is a dramatic increase in the number of arbor branches; these are mostly focused in the supra and infragranular layers but also send collaterals to other laminae. A heterotopic projection to the perirhinal region with arbors exhibiting a complexity similar to homotopic arbors, is consistently seen and persists to adulthood. By P25, arbors acquire two types of distribution: sharply bilaminar (layers II/III and V) or only supragranular. The lateral continuation of the trunk axon disappears. These observations document that the sculpting of callosal axons in the tangential as well as radial dimension continues at least into the fourth week of postnatal life.

Support : NIH grant EY 05504; CNPq, Brazil.

706.5

GROWTH CONE-TARGET CELL INTERACTIONS IN LIVE HIPPOCAMPAL SLICES: FLUORESCENCE LABELING AND TIME-LAPSE CONFOCAL IMAGING M.E. Dailey* & S.J Smith, *Dept. of Molecular & Cellular Physiology, Stanford University Medical School, Stanford, CA 94305.*

We are exploring the cellular mechanisms of target cell selection and synapse formation in tissue slices of developing mammalian brain. Specifically, we are studying the formation of giant *en passant* synapses between mossy fibers (MFs) and pyramidal cell dendrites within the rat hippocampus. The ability of MFs to form new giant synapses within isolated tissue slices was previously established by a series of experiments involving Synapsin-I immunostaining, electron microscopy and intracellular recordings. In an effort to characterize the cellular dynamics related to synapse formation, the MFs and target pyramidal cells in living brain slices were stained with a fluorescent membrane dye, DiI or DiO, and imaged over time. Imaging was performed with a custom-built scanning laser confocal microscope that afforded collection of Z-axis stacks of confocal images at time intervals of a few minutes. Active filopodial protrusions were observed not only at the leading growth cone of extending MFs but also at small varicosities along the axonal shaft. Moreover, some preliminary observations suggest that target cell dendrites may likewise exhibit a dynamic morphology: filopodialike structures were occasionally seen to emerge from dendritic shafts of some putative target cells. These studies suggest a possible dynamic interaction of axonal and dendritic processes during targeting and synapse formation.

Supported by NIH grants NS09027 & NS28587.

706.6

ONTOGENY OF MOSSY FIBERS IN THE RAT HIPPOCAMPUS: A TIMM'S HISTOCHEMISTRY STUDY. J.P. Leite, T.L. Babb*, J.K. Pretorius and G.W. Mathern. Brain Research Institute, UCLA School of Medicine, Los Angeles, CA 90024.

The organization of the mossy fiber (MF) system which projects from granule cells of fascia dentata to the hilus, CA4, and CA3 cells was studied at postnatal (PN) days 2, 7, 14, 16, 18, 20, 22, 24, 26, 28, 30 and 42 in normal Sprague-Dawley rats. Mossy fibers and terminals (MFTs) were stained with neo-Timm histochemistry and counterstained for neurons with cresyl violet. Coronal sections at 16 "equidistant" levels covering the entire septo-temporal length of the hippocampus (HC) were examined.

At each PN age there were greater differences in the MF distribution that depended on the septal/temporal axis than the age difference within a given axis. At the septal level, MFs mostly projected to the suprapyramidal CA3 but also penetrated the neuron layer of the stratum pyramidale ("intrapyramidal") as early as PN 7. At later ages, these intrapyramidal MFs appear to extend and form MFTs on the basilar dendrites of CA3. These infrapyramidal (stratum oriens) MFTs in CA3 were also more prevalent at the septal one-third of the HC by PN 14. In the temporal sections, intrapyramidal and infrapyramidal MFTs were sparse or absent. At PN 16, MFTs appear more highly concentrated in the suprapyramidal zone (stratum lucidum) at all septal-temporal sections, and by PN 20-22 the entire hilus, CA4 and CA3 MF distributions are virtually identical to the adult HC; although Timm staining is less intense and MFTs appear smaller. This study demonstrates that during development, MFs project diversely to several strata (PN 7-14) and later (PN 20) develop specialized MFTs on the dendritic zones, stratum lucidum (septal and temporal) and oriens (septal only). Supported by NIH Grant NS02808 and a CNPq Fellowship (Brazil) to JPL.

706.7

SYNAPSIN I GENE EXPRESSION DURING DEVELOPMENT AND IN RESPONSE TO SELECTIVE LESIONS OF THE RAT CNS. R.H. Melloni, Jr.*, P.J. Apostolides, J.E. Hamos, and L.J. DeGennaro, University of Massachusetts Medical Center, Worcester, MA 01655.

Synapsin I is the best characterized member of a family of nerve terminal-specific proteins implicated in the regulation of synaptic function. To better understand the regulation of synapsin I gene expression during synapse development, we first examined the expression of the synapsin I gene in the granule cell neurons of the dentate gyrus. The results indicate a significant difference between the temporal expression of high levels of synapsin I mRNA in dentate granule somata and the appearence of protein in their mossy fiber terminals during the postnatal development of these neurons. Next, we examined the expression of synapsin I mRNA and protein following lesions of hippocampal circuitry. These studies show marked changes in the pattern and intensity of synapsin I protein staining in the dendritic field of dentate granule cell neurons following perforant path transection. In contrast, changes in synapsin I mRNA expression in target neurons, and in those neurons responsible for the reinnervation of the hippocampus, were not found to accompany new synapse formation.

On a molecular level, developmental and lesion data suggest that expression of the synapsin I gene is tightly regulated in the CNS, and that considerable changes in synapsin I protein may occur in neurons without concommitant changes in the levels of its mRNA. Finally, our results suggest that the appearance of synapsin I protein in developing and sprouting synapses coincides with the acquisition of function by those central synapses. Supported by NIH NS25050 and NS27833 to L.J.D.

706.8

INTRAHIPPOCAMPAL COLCHICINE ADMINISTRATION INDUCES STATHMIN mRNA IN THE DENTATE GYRUS. N. Mori*, T. Himi, M. M. Dugich-Djordjevic, M. Cao, and F. Hefti Division of Neurogerontology, Andrus Gerontology Center, University of Southern California, Los Angeles, CA 90089.

Microinjection of colchicine into the hippocampal formation selectively destroys granule cells of the dentate gyrus and their axons over several days, while sparing pyramidal cell populations. Granule cell degeneration is triggered primarily by microtubule depolymerization, however little is known about the precise mechanisms for selective granule cell death that occurs following colchicine administration. To approach this, we examined the expression of mRNAs encoding neural specific or enriched phosphoproteins, i.e. stathmin, SCG10, and synapsin I. Expression of these molecules are associated with neuronal differentiation, regeneration, and synaptic formation. Colchicine or control saline was injected unilaterally into the dentate gyrus of male Sprague-Dawley rats (2 months old), and the animals were sacrificed at 1, 3, and 7 days following injection. Quantitative *in situ* hybridization histochemistry demonstrated that SCG10 and synapsin I mRNAs expression was no longer evident in the ipsilateral dentate gyrus 3 days after the colchicine injection, suggesting that the dentate granule cells degenerate by this time. In CA3 pyramidal neurons, SCG10 and synapsin I mRNA expression decrined by day 3, but recovered to control levels by day 7. In contrast, stathmin mRNA was upregulated (+35%) 1 day following colchicine administration in the ipsilateral dentate gyrus, but not in the contralateral side. By day 3, stathmin mRNA was also induced in the contralateral dentate gyrus (+20%). Stathmin mRNA was increased (+25%) in the ipsilateral CA1 and CA3 pyramidal cells, but was decreased in the contralateral side (-50%). Stathmin mRNA expression was not altered at any time in saline injected animals. Our results demonstrate that stathmin, a putative signal transduction molecule, is regulated by events associated with neurodegeneration and/or reactive synaptogenesis following the selective destruction of dentate gyrus granule cells in the adult hippocampus. It is important to determine mechanisms of the induction of p19/stathmin gene for the further understanding of synaptic reorganization process within the hippocampus.

706.9

3D RECONSTRUCTION OF INTRACELLULARLY-STAINED CA1 HIPPOCAMPAL NEURONS FROM NORMAL AND KAINIC-ACID LESIONED RATS. G.K. Pyapali*, J. Bradley and D.A. Turner. Neurosurgery and Neurobiology, Duke Univ. and Durham VAMC, Durham, NC, 27710.

Unilateral kainic acid (KA) lesions of the CA3 region of the hippocampus lead to denervation of ipsilateral CA1 neurons, particularly in the stratum radiatum. To assess these changes quantitatively, CA1 neurons were recorded *in vitro*, stained with neurobiotin and then reconstructed using a computerized 3D system (Neurolucida).

The CA1 neurons from the lesioned animals showed an elevated input resistance but otherwise there was no difference in cell health (3 - 42 days post-lesion). Total dendritic length was slightly longer in the denervated CA1 cells: normal = 8.84 ± 0.82 mm (n = 6) versus KA = 11.0 ± 4.85 mm (n = 6), comparable to previous reconstruction estimates of 8.38 ± 2.27 mm (n = 6). However, the dendrites in the mid-stratum radiatum appeared to be increased in number in the KA-lesioned cells as compared to control neurons. Total apical dendritic length revealed that control = 5.93 ± 1.47 mm versus KA = 7.92 ± 3.38 mm (NS). Sholl analysis for density of terminals and branches in 75 μm lamina was performed at various distances from the pyramidal cell layer in the apical dendrite: at 75-150 μm control value = 36.3 ± 9.7 while the KA value = 38.8 ± 15.3 (NS). However, at 225-300 μm normal = 14.3 ± 9.7 and the KA (all) = 29.8 ± 11.1 ($P < 0.05$ different). The KA lesions at 5 days and later showed even more significant changes in this denervated zone in the apical dendrites (34.0 ± 10.7, $P < 0.01$). However, at 375-450 μm control = 10.7 ± 5.4 while the KA cells showed 10.5 ± 9.2 (NS). Likewise, the basal dendrites showed control = 9.0 ± 6.5 and KA-lesioned cells 12.7 ± 8.0 (NS).

These findings indicate that there is a selective and specific increase in the number of terminals and dendritic branches in the denervated zone of the apical dendrite, suggestive of dendritic sprouting in the stratum radiatum following the lesion. **Supported by NINDS #29482 (DAT) and VA Merit Review Award (DAT).**

706.10

EMBRYONIC N-CAM IS RAPIDLY EXPRESSED IN DENERVATED DENTATE GYRUS: ULTRASTRUCTURAL ANALYSIS

P.D. Miller*[1,2], S.D. Styren[3], C.F. Lagenaur[1], S.T. DeKosky[1,3,4], Depts. Neurobiology[1], Neurological Surgery[2], Psychiatry[3], and Neurology[4], University of Pittsburgh School of Medicine and Western Psychiatric Institute and Clinic, Pittsburgh, PA 15213

Entorhinal cortex (ERC) lesions denervate the outer two thirds of the hippocampal dentate gyrus molecular layer (ML). The denervation depletes 85% of the synapses in the outer ML, which is followed by a brisk lesion-induced synaptogenesis. The embryonic form of neural cell adhesion molecule (eN-CAM) is reexpressed in the outer ML following ERC lesion; the eN-CAM is localized on cell membranes of granule cell dendrites and occasionally axons within the denervated zone. Because eN-CAM is expressed within 2 days after ERC lesion, we were interested in the temporal sequence of expression. Denervated hippocampi (12, 18, 24, and 48 hours post ERC lesion) were stained with anti-eN-CAM (epitope: polysialic acid) prior to processing for electron microscopy. By 18 hours post lesion, cell membranes of both dendrites and axons throughout the inner and outer ML exhibited moderate eN-CAM staining; by 24 hours post lesion, staining intensity had increased significantly and labeled more profiles. eN-CAM staining disappeared from the non-denervated inner ML by 48 hours post ERC lesion, but remained intense and widely distributed in the outer ML. In the absence of cytoplasmic or Golgi staining with e-N-CAM antibody in the dendrites following ERC lesion, we speculate that the rapid appearance of eN-CAM after lesion may be from modification of adult N-CAM, perhaps via membrane-associated sialyltransferases. These enzymes may glycosylate newly arriving or endogenous membrane N-CAM, aiding in afferent segregation during reinnervation.

706.11

TRANSIENT INCREASED EXPRESSION OF CYTOSKELETON PROTEINS PRECEDES SPROUTING AND SYNAPTOGENESIS IN EPILEPTIC RATS. Represa A.*, Pollard H., Moreau J., Ghilini G., Khrestchatisky M., Jorquera I., Diabira D. and Ben-Ari Y., INSERM U29 123 Bd. Port Royal 75014 Paris (France).

One of the most striking changes seen in epileptic brains is the sprouting and synaptogenesis of the hippocampal mossy fibers (mf, the axons of dentate granule cells), which make ectopic synaptic contacts with hippocampal granule cells dendrites. We examined here the effects of kainate-induced seizures, upon the hippocampal expression of tubulins (α and ß) and microtubule associated proteins (MAP2 and Tau). mRNAs encoding for these proteins increase in the dentate granule cell bodies 3 days after KA (MAP2 mRNA also increased in the dendrites). mRNA levels peaked on day 12. These changes were correlated with an enhanced tubulin (α and ß) immunoreactivity (IR), in both granule cells dendrites and mf, already detectable 4 days after KA. 6-12 days afterKA, MAP2 IR was also selectively increased in the dendrites of granule cells, whereas Tau IR was enhanced in mf and granule cell bodies. The levels of mRNA and protein IR returned to control values by 20-30 days after KA, respectively. These changes developed before and during axonal mf sprouting which starts around 12 days after KA. We suggest that the production and stabilization of tubulin polymers with Tau contribute to the axonal growing and side-branching of mf. The dendritic increase of tubulin and MAP2, which also develop during the period of synapse formation may be related to a local synthesis involved in the formation of postsynaptic specializations.

706.12

EFFECTS OF CASTRATION AND ESTRADIOL ON GRANULE CELL DENDRITE SPINE LOSS AFTER PERFORANT PATH TRANSECTION. J.R. Day*, P.E. Schauwecker, N.J. Laping, C.E. Finch and T.H. McNeill. Andrus Gerontology Center, USC and Biology Department/Gerontology Center, Penn State University, University Park, PA 16802-5301.

Unilateral perforant path transection removes a large percentage of presynaptic elements in the outer 2/3's of the ipsilateral dentate molecular layer. Gonadal and adrenal steroids have been shown to alter granule cell morphology in rats. The purpose of this study was to assess the effects of castration on granule cell dendritic spine density during synaptic reorganization after perforant path transection. Male Fisher 334 rats were castrated and implanted with estradiol-filled or empty silicone elastomer implants (5mm X 2mm) for 17 days prior to unilateral perforant path transection. Four days after lesion (21 days after castration) whole brains were collected for Cox-Golgi processing. The spines were counted on the distal 80-100 μ of a granule cell dendritic branch in the dorsal blade of the rostral hippocampus. In this preliminary study, 10 to 20 cells per side were counted from 2-3 rats per group. Ipsilateral dendrites of a separate group of uncastrated, lesioned males exhibited a 50% decrease in spines compared to the contralateral side (8.5±3.6 vs 16±1 spines/20μ, n=3). Castrated males showed no difference in spine density between ipsilateral and contralateral granule cells 4 days after lesion (16.6±1.3 vs 16.8±1.3, n=2). Perforant path lesion of E2-implanted castrates resulted in a 15% decrease in spine density in the ipsilateral hippocampus (14.7+0.1 vs 17.2+0.6, n=2). The results show that the initial phases of lesion-induced synaptic reorganization in the male rat hippocampus are effected by removal of the testes, and that E2 can partially reverse this effect. The absence of a change in spine density after lesion in castrates could represent a delay in the sequence of reactive synaptogenesis and/or be evidence of plasticity in response to castration-induced atrophy. (Supported by: JD & CT MacArthur Program in Successful Aging and NIA 07909 to CEF; NS 30426 to THM)

706.13

AXONAL AND GLIAL RESPONSES TO CORTICAL DEAFFERETATION OF THE STRIATUM. F.G. Szele* and M.-F. Chesselet, Dept. of Pharmacology, U. of Pennsylvania, Philadelphia., PA 19104.

Whereas denervation-induced sprouting has been demonstrated in the hipocampus, less is known about this phenomenon in the striatum. Thermocoagulatory lesions of pial vessels overlying the cerebral cortex were performed in adult rats to induce a gradual loss of the massive cortical input to the striatum. Rats were sacrificed six hours to six weeks after surgery and brains processed for immunohistochemistry. Both GAP-43 and synaptophysin (indices of axonal sprouting) were transiently (1 week) increased under the corpus callosum but not in the rest of the striatum. Because astrocytes have been shown to produce molecules which can modulate sprouting, the expression of glial fibrillary acidic protein (GFAP), basic fibroblast growth factor (bFGF), tenascin, and chondroitin sulfate proteoglycan (CSPG) was examined after denervation. Despite a rapid and prolonged increase in GFAP, immunoreactivity to bFGF was not increased in the striatum, in contrast to both ispsi and contralateral cortices. Tenascin expression was increased bilaterally in the corpus callosum six hours to three weeks and in the dorsolateral striatum five to seven days after surgery. CSPG was increased to a lesser extent in the corpus callosum. Taken together these results suggest a lack of major sprouting in the striatum which may be related to the absence of bFGF induction by striatal astrocytes and the increase in adhesion molecules serving as barriers. Supp. by PHS grant NS 29230.

706.14

EFFECT OF FUNCTIONAL AXON SPROUTING ON OXIDATIVE METABOLISM AND SIZE OF MOTONEURON SOMA. R.N. Michel*†, N. Neil, and G. Parker. Depts. of Human Movement† and Biology, Laurentian Univ., Sudbury, Ontario.

When a skeletal muscle is partially denervated, motoneurons surviving the injury respond by extending supernumerary extensions to reinnervate vacated post-synaptic sites. To accommodate this growth, the motoneuron soma must rapidly increase its proteo-synthetic drive and regulate its metabolism to meet the increased demand for transport of membrane, cytoplasmic and synaptic constituents to the new axon terminals. We measured the size and oxidative capacity of identified motoneurons at two stages of this response. Plantaris muscle fibers in one hindlimb of 10 rats were denervated by cutting radicular nerve L4. The extent of functional sprouting was assessed 10 or 28 days later by the ratio of force responses evoked via stimulation of the sprouting L5 nerve to that produced by its counterpart in the contralateral limb. L5 motoneurons on both sides were labeled with 5% Fast Blue, injected (10 μL) into the plantaris 7 days previous to the contractile measurements. Quantitative histochemistry for succinate dehydrogenase (SDH) activity in fresh-frozen sections was used as an indicator of oxidative metabolic capacity. The progression of the SDH reaction in labelled somas from both limbs was recorded using microphotometric imaging. Motor units increased their functional size 2 to 13-fold in response to partial denervation. Sprouted motoneuron soma sizes were not different from non-sprouted counterparts (1711 vs 1658 μm^2; total n = 173). Similarly, SDH activity in 10 and 28 day sprouted motoneurons was not different from control (12.5 vs 12.1 O.D./min x 10^{-3}). Interestingly, total SDH content correlated with soma size (r from 0.542 to 0.619; $P<0.01$) in all but the 28 day sprouted motoneurons. Our results suggest that the increased motoneuronal work associated with rapid terminal field expansion does not require significant modulation (i.e. up-regulation) of cell body oxidative metabolism. Indeed, as in cerebral cortex tissue, motoneurons may rely on alternate metabolic pathways (i.e to lactate) to meet the new energy requirements associated with this growth.

Supported by NSERC Canada (RNM).

706.15

VOMERONASAL ORGAN TRANSPLANTATION INTO THE BRAIN CORTEX. P.P.C. Graziadei* and E.E. Morrison. Dpt. Biological Science, Florida State University, Tallahassee, FL 32306 and Dpt. Anatomy & Embryology, Auburn University, Auburn, AL 36489

Vomeronasal organs of rat pups (P1-P10) were transplanted into the parietal cortex of same age littermates. The organs developed as a series of tubules and vesicles not always interconnected. The vesicles were lined by sensory and non sensory epithelia. From the latter bundles of axons originated and penetrated the surrounding brain parenchima. The course of the bundles appeared to be random. At variable distance from the originating sensory epithelia (from 50 microns to 1 mm) a complex plexus of sensory fibres developed. Among the bundles of the plexus we consistently observed discrete glomeruli-like structures (from 50 to 150 microns in diameter). We observed glial nuclei in the plexiform structure, however, around the glomeruli we did not observe periglomerular cells nor a special glial envelope. The occurrence of glomerular structures was also observed when the plexiform mass freely protruded into the lateral ventricle. Our study was based on histological preparations.

It is concluded that the vomeronasal organ of postnatal rats can survive and develop when transplanted into the brain parenchima and that the axons generated by the organ are able to form a plexus and glomeruli-like structures in the absence of obvious postsynaptic neurons.

Supported by NIH grant NS20699 to PPCG).

AXON GUIDANCE MECHANISMS AND PATHWAYS VIII

707.1

TOPOGRAPHIC SIMILARITIES BETWEEN SYMPATHETIC FIBERS AND NGF IMMUNOSTAINING IN THE HIPPOCAMPUS FOLLOWING SEPTAL LESIONS. C. Yu and K. A. Crutcher.* Department of Neurosurgery, University of Cincinnati School of Medicine, Cincinnati OH 45267.

The ingrowth of sympathetic fibers into the hippocampus following cholinergic denervation may involve NGF-like activity (Crutcher, 1987). The suggestion that an NGF-like signal arises from dentate granule cells and their axons (mossy fibers) was originally based on the similarity in distribution between sympathetic axons, visualized with catecholamine histofluorescence, and mossy fibers, visualized with the Timm stain (Crutcher and Davis, 1982). NGF-like staining was recently reported to be localized to the dentate hilus and stratum lucidum of CA_3 (Conner et al., 1992), areas normally occupied by mossy fibers. In the present study, comparisons were made between the distribution of NGF-like staining (polyclonal antibody kindly provided by J. M. Conner and S. Varon) and the topography of sympathetic fibers [monoclonal antibody against the low-affinity NGF receptor (NGFR), generously provided by E.M. Johnson, Jr] in the hippocampus following cholinergic deafferentation.

Mature female Sprague-Dawley rats received medial septal lesions (5 mA for 30 sec) or served as sham controls. They were perfused with 0.2% parabenzoquinone and 2% paraformaldehyde 4 weeks after surgery. Visualization of NGF-like immunoreactivity and NGFR-positive fibers was carried out on adjacent sections to permit topographic comparisons. As described by Conner et al. (1992), NGF-like staining was present in the dentate hilus and stratum lucidum of CA_2 and CA_3, in both normal and lesioned animals. On adjacent sections, thick, NGFR-positive fibers, arising from penetrating blood vessels, were distributed within the same areas only in animals with septal lesions. These findings support the hypothesis that the topography of sympathetic ingrowth is regulated by the distribution of an NGF-like signal associated with granule cell axons.

707.2

REGENERATING GOLDFISH CNS NEURONS CHOOSE INAPPROPRIATE PATHWAY INTO THE PNS. S.J. Zottoli* and A.P. Bentley. Dept. of Biology, Williams College, Williamstown, MA 01267.

Damaged neurons within the central nervous system of the adult goldfish are thought to regrow to appropriate target areas with resultant recovery of swimming behavior (Bernstein, Exp. Neurol., 9:161). However, after a whole spinal cord crush at the spino-medullary level (SML crush), approximately 50% of surviving goldfish do not recover all behavior. Brain neurons regenerating past a SML crush have a choice between the spinal cord and the first ventral root. To determine whether novel pathway choices were made by these regenerating CNS neurons, the normal pool of motoneurons that enters the first ventral root was defined by application of HRP to the root and compared to the pool that enters this root 70-210d after a SML crush. The experimental pool differed from the normal pool, which was exclusively ipsilateral, in three fundamental ways; 1) cell bodies were labelled on the contralateral side, 2) the rostro-caudal extent of the ipsilateral regenerated pool was greater , and 3) new classes of CNS neurons, including vestibulospinal and reticulospinal cells, were labelled on both the ipsilateral and contralateral sides. Anterograde filling of regenerated CNS neurons by application of HRP rostral to the normal pool showed that at least 60% chose the pathway into the first ventral root. We conclude that some regenerating neurons do not make the same pathway choice as they made during development, and that shunting of these fibers away from their normal target areas may limit behavioral recovery in adult goldfish. These results indicate that the goldfish PNS may present a more permissive environment to regenerating fibers than the CNS, as is the case in mammals. Therefore, we suggest that the goldfish is a better model for mammalian regeneration than previously thought. Supported by NSF grant BMS8809445.

707.3

DOPAMINERGIC DENERVATION OF THE STRIATUM BLOCKS SCG-10 mRNA RESPONSE TO CORTICOSTRIATE LESION J.P. Cogen*, H.W. Cheng, P.E. Schauwecker, and T.H. McNeill. Andrus Gerontology Center and Department of Biological Sciences, Univ. of Southern California, Los Angeles, CA 90089.

Previous studies have shown that the growth-associated neuronal protein SCG-10 is abundant during developmental synaptogenesis, and its mRNA is upregulated during synaptic remodeling in the adult rodent after unilateral aspiration of the sensory motor cortex. On northern blot analysis, SCG-10 mRNA was increased 8-fold in contralateral cortex 3 days after the lesion and declined to near-control levels by 27 days postlesion, a time course corresponding to rapid and robust praterminal sprouting of homologous fibers from the intact contralateral side. In contrast, there was no change in mRNA for GAP-43, a protein associated with neurite outgrowth. In addition, unilateral 6-OHDA lesions produced no change in SCG-10 or GAP-43 mRNA in the ipsilateral cortex at 3, 10, and 27 days postlesion when assessed by northern blot analysis.

In order to investigate the role of the nigrostriatal synapse in the synaptic remodeling capabilities of the striatum, 6-OHDA injections were made in the substantia nigra before or at the same time as cortical ablation. There were no changes in SCG-10 or GAP-43 mRNA in contralateral cortex after 3, 10, and 27 days on northern blot analysis. These results suggest that the maintenance of dopaminergic synapses on medium spiny striatal neurons may be critical in preserving corticostriatal synaptic plasticity. Supported by AG 09794, NS 30426 and the National Parkinson Foundation.

707.4

EFFECT OF AGE ON THE EXPRESSION OF SCG-10 AND P-19 mRNA DURING REACTIVE SYNAPTOGENESIS H. W. Cheng*, T. Jiang, N. Mori and T.H. McNeill. University of Southern California, Andrus Gerontology Center, Los Angeles, CA 90089

Previous studies suggest that aging is associated with a delay in the response of surviving afferent axons to reinnervate deafferented target neurons during reactive synaptogenesis. However, whether this age-related delay is generalized to all regions of the brain or is more characteristic of specific brain areas such as the hippocampus is unclear. To address this issue we used an experimental model of striatal (ST) deafferentation and northern blot and in situ hybridization analysis to assess changes in mRNA prevalence for two proteins thought to be involved in neurite outgrowth (SCG-10, P-19) in young adult (3-4mos.) and aged (24 mos.) rat brain.

We found that there is no difference in the basal expression of both SCG-10 and P-19 mRNA in the cortex and ST of young adult and aged rats and that the time course of the SCG-10 and P-19 mRNA response to the cortical lesion is the same in both age groups. However, while the time course of the response is the same, the magnitude of the response is less in aged rats. To what degree this response affects neurite outgrowth and new synapse formation in the aged ST is yet to be determined. However, these data suggest that in the ST the initiation and time course of the mRNA response to a cortical lesion is similar in the young adult and aged rats and that the age-related delay characteristic of reactive synaptogenesis in the hippocampus may not be a generalized phenomenon in the aged brain. Supported by AG 09794, NS 30426 and the National Parkinson Foundation.

707.5

MORPHOMETRIC ANALYSIS OF IPSILATERAL AND CONTRALATERAL STRIATAL NEURONS FOLLOWING A UNILATERAL CORTICAL LESION S.A. Brown, H.-W. Cheng and T.H. McNeill* University of Southern California, Andrus Gerontology Center, Los Angeles, CA 90089

While numerous studies have examined the response of ipsilateral target neurons to a unilateral deafferentation lesion, few have addressed changes that occur in the morphology and time course of the response of target neurons ipsilateral vs contralateral to the lesion. To address this issue we used Golgi impregnation and computer assisted morphometry to investigate the bilateral effect of a unilateral cortical lesion on medium spiny I (MSI) striatal neurons in young adult Fischer 344 rats. We found that there was a significant (40%) reduction in both total dendritic length (TDL) and spine density in the ipsilateral striatum (ST) at 3 days postlesion, which reached a maximum (50%) at 10 days postlesion. In contrast, spine loss was not observed in the contralateral ST until 10 days postlesion (18%), with no change in TDL. Partial recovery of both spine density and TDL was found in the ipsilateral ST at 20 days postlesion, and spine density reached 80% and TDL 100% of intact values at 27 days postlesion. In contrast, spine loss in the contralateral ST remained decreased (20%) compared to intact values at 27 days postlesion but was equivalent to the "recovered" spine density found in the ipsilateral ST. These data suggest that the plastic response of MSI neurons to a unilateral cortical lesion differs between the ipsilateral and contralateral ST and that one possible role for a differential response is to maintain symmetry in the number of target neuron synapses following a unilateral lesion. Supported by AG 09794, NS 30426 and the National Parkinson Foundation.

707.6

ENHANCED SPROUTING OF AFFERENT AXONS AND IMPAIRED RECOVERY OF DENTATE GRANULE CELL SPINE DENSITY FOLLOWING A COMBINED ENTORHINAL CORTEX/FIMBRIA-FORNIX (EC/FF) LESION. P. Elyse Schauwecker* and T.H. McNeill. Andrus Gerontology Center and Department of Biological Sciences, University of Southern California, Los Angeles, CA 90089

This study examined the degree to which the loss of multiple synaptic input to the dentate gyrus affects the sprouting of surviving afferent axons and the recovery of dendritic spine density on dentate granule neurons. Specifically, we used a comparison of entorhinal cortex (EC), fimbria fornix (FF) and combined EC/FF lesion models to assess axonal sprouting in the commissural/associational (C/A) fiber plexus and dendritic spine density of dentate granule neurons. Based on data obtained using the Holmes fiber stain we found that similar to previous studies, there was no change in the width of the C/A fiber plexus following a FF lesion and only a transient 25-30% increase after an EC lesion. In contrast, following the EC/FF lesion there was an enhanced (40-45%) but delayed (30 days postlesion) increase in the width of the fiber plexus which remained elevated at 60 days postlesion. By comparison, data from our Golgi analysis suggests that the enhanced sprouting of C/A axons coincides with the impaired recovery of spine density on dentate granule neurons. Specifically we found that spine loss (45%) occurred as early as 4 days postlesion, was maximum at 10 days postlesion and reached 80% of intact values by 30 days post-lesion. However, in strong contrast to the EC lesion the recovery of spine density was transient and was again significantly decreased (50%) at both 45 and 60 days postlesion. Supported by AG 09794 and NS 30426

707.7

REGENERATION OF CEREBROSPINAL FLUID CONTACTING NEURONS (CSFCN) IN THE REGENERATED TAIL SPINAL CORD OF THE LIZARD *ANOLIS CAROLINENSIS*. M.T. Duffy, A. Hawrych, D.R. Liebich and S.B. Simpson, Jr.* Dept. Biological Sciences, University of Illinois at Chicago, IL 60680.

Recent work in our lab has focused on the role of CSFCNs in the regenerated tail spinal cord of *Anolis* lizards. We now report results of retrograde labeling of neurons by spinal transections at either midthoracic, low lumbar or two segments rostral to regenerated spinal cord. HRP placed at thoracic levels (n=3) and lumbar levels (n=3) revealed no labeled cells in the regenerated tail spinal cord while HRP (n=2) and lucifer yellow (n=1) placed rostral to regenerates revealed an average of 16 ± 4 retrogradely labeled CSFCNs in the regenerated spinal cord. In all three positive cases, the labeled cells were clustered in the distal regenerate. However, examination of serial longitudinal sections of regenerated tail spinal cord revealed the presence of CSFCNs throughout the regenerate.

These results indicate that regeneration of ascending CNS axons is limited to a distal subset of regenerated CSFCNs. Furthermore, these ascending axons do not appear to regenerate further than the last few segments rostral to the regenerate. We have previously shown that this area of spinal cord rostral to the regenerate is the origin of the vast majority of regenerated and sprouted descending axons (Duffy et al., 1990 & 1992). While the function of CSFCNs in general remains unknown, it is possible that a subset of CSFCNs exhibiting ascending axonal regeneration might play a key role in CNS regeneration in the lizard. This might occur by extension of pioneer-type axons, secretion of neurotrophic substances or some other unstated mechanism.

NEUROTRANSMITTERS AND CHANNELS: CHANNELS

708.1

L-TYPE Ca^{2+} CHANNEL IS INVOLVED IN THE REGULATION OF SPONTANEOUS TRANSMITTER RELEASE AT DEVELOPING NEUROMUSCULAR SYNAPSES. W.M. Fu* and F.L. Huang. Department of Pharmacology, College of Medicine, National Taiwan University, Taipei, Taiwan.

Involvement of L-type Ca^{2+} channel in the regulation of spontaneous transmitter release was studied in Xenopus nerve-muscle cultures. Spontaneous synaptic currents (SSCs) are detectable from the innervated muscle cell with the whole-cell voltage-clamp recordings. The frequency of SSCs, which reflect impulse- independent acetylcholine (ACh) release from the nerve terminals, showed marked increase in high-K^+ medium or after treatment with a phorbol ester, TPA, a drug that activates protein kinase C and depolarizes the presynaptic neuron. The potentiation effect of high K^+ and TPA requires Ca^{2+} influx through L-type Ca^{2+} channel in the plasma membrane, since it was significantly reduced by the presence of nifedipine, verapamil or diltiazem and enhanced by Bay K 8644, a L-type Ca^{2+} channel agonist. It was shown recently that adenosine 5'-triphosphate (ATP) markedly potentiates the spontaneous ACh release at these synapses through the binding of P_2-purinoceptors and the activation of protein kinase C. We found that potentiation effect of ATP is inhibited by L-type Ca^{2+} channel blockers, suggesting that L-type Ca^{2+} channel is responsible for the positive regulation of spontaneous ACh secretion by ATP at the developing neuromuscular synapses.

708.2

TARGET TISSUES REGULATE THE EXPRESSION OF K+-CURRENTS IN CHICK CILIARY GANGLION NEURONS DEVELOPING *IN SITU*. M. M. Dourado[1*], C. Brumwell[2], M. E. Wisgirda[1], M. H. Jacob[2] and S. E. Dryer[1], [1]Department of Biological Science, Florida State University, Tallahassee, FL 32306. [2]Worcester Foundation for Experimental Biology, Shrewsbury, MA 01545.

Neurons of the chick ciliary ganglion are known to express a voltage-activated, transient A-type K^+ current (I_A) and Ca^{2+}-activated K^+ currents ($I_{K[Ca]}$). These currents first appear, and undergo a large increase in amplitude and density, during the developmental stages at which ciliary ganglion neurons form synapses with their target tissues in the eye. The expression of I_A and $I_{K[Ca]}$ was examined in acutely-isolated E13 chick ciliary ganglion neurons that developed *in ovo* following surgical removal of the optic vesicle at E2, prior to the formation of the ciliary ganglion. I_A was expressed at equal amplitude by neurons developing *in situ* in the absence (OV-) or presence (OV+) of target tissues. However OV- neurons expressed an I_A with activation and inactivation kinetics that were 2-4 times faster than those expressed by OV+ neurons. There were no differences in the voltage-dependence of activation or steady-state inactivation of I_A in OV+ and OV- neurons. The amplitude of macroscopic $I_{K[Ca]}$ was reduced by 75% in OV- neurons compared to OV+ controls. These results indicate that target tissues play a role in regulating the properties of voltage- and Ca^{2+}-activated ionic currents in developing vertebrate neurons.

Supported by NIH Grants NS-27013, NS-21725, and a grant from the Pfeiffer Foundation.

708.3

TRANSCRIPTIONAL REGULATION OF K^+ ION CHANNEL EXPRESSION IN DEVELOPING CEREBELLAR PURKINJE NEURONS. Y. Li Muller and Andrea J. Yool*. Dept. Physiology, AHSC, Univ. of Ariz., Tucson, AZ 85724.

During the postnatal development of the cerebellum of rat, Purkinje neurons in culture grow from immature cells (day 1-6) to large mature neurons with dendritic outgrowth (day14-21) *(1)*. This morphological change is associated with the activation of K^+ ion channels that contribute to the increased complexity of the firing pattern of neurons *(2)*. We hypothesize that the transcriptional regulation of the various channel conductances (K, Ca and Na) influences the differentiation of Purkinje cells. This hypothesis was tested using Xenopus oocytes to express polyA-enriched RNA isolated from postnatal rats at different ages (day 1, day 8 and 6 months) using single channel and voltage-clamp analyses. The regulation of the expression of ion channels at the transcriptional level also was analyzed by Northern blot using low stringency hybridization with randomly-primed cDNA from other cloned channels. Based on previous work *(2)*, we predict that the delayed rectifier K^+ channel (27pS) is expressed during the immature stage (day 1-6); this channel is not sensitive to 0.5 mM TEA. However, the developmentally delayed appearance of Ca^{2+}-dependent K^+ channel (K_{Ca}, 100pS) during differentiation (day 7-9) is hypothesized to show a different pattern of transcriptional regulation. As shown previously *(2, 3)*, K_{Ca} currents were half-blocked at 0.5 mM TEA and sensitive to the intracellular Ca^{2+} concentration. Ongoing experiments will test the hypothesis that the developmental expression of the K_{Ca} conductance plays an essential role in the maturation of Purkinje neurons.

References: *(1)* Yool, AJ, and DL Gruol. 1987. *Brain Res.* 420: 205-219. *(2)* Yool, AJ, VE Dionne and DL Gruol. 1988. *J. Neurosci.*, 8: 1971-1980. *(3)* Gruol, DL, T Jacquin and AJ Yool. 1991. *J. Neurosci.*, 11: 1002-1015.

708.4

ANALYSIS OF THE ELECTROPHYSIOLOGICAL CONSEQUENCES OF AXOTOMY OF BULLFROG SYMPATHETIC B-NEURONS. B.S. Jassar*, P.S. Pennefather & P.A. Smith. Dept. Pharmacol., Univ. Alberta, Edmonton, T6G 2H7 and Fac. Pharmacy, Univ. Toronto, Ontario, M5S 2S2, CANADA.

Axotomy of B-neurones in adult bullfrog sympathetic ganglia promotes an increase in spike height and width as well as an attenuation of the amplitude and duration of the afterhyperpolarization which follows the action potential (a.p; Gordon *et al., J. Physiol.*, **392**, 213, 1987). The mechanism of this effect was was analyzed using whole-cell recording from dissociated control neurones and neurones dissociated from ganglia 11-13d after cutting the postganglionic nerve. After axotomy, I_{Ba} was reduced by 50%. Most of this effect was due to enhanced inactivation of N-type Ca^{2+} channels. L-type channels, which are responsible for 10-20% of the total I_{Ba} were unaffected. The kinetics of activation/deactivation of I_{Ba} and I_{Na} were little changed yet I_{Na} amplitude increased to about 170% of control. Changes in Ca^{2+}-dependent K^+ currents, I_C and I_{AHP} were the consequence of the reduction in I_{Ca}. Axotomy increased M-conductance to about 135% of control and slowed its deactivation kinetics whereas the delayed rectifier K^+ current was reduced to about 62% of control amplitude with little change in kinetics. These data account for the axotomy-induced changes in a.p. configuration and show that *i)* different ion channels are affected in different ways following disruption of neuron-target contact and *ii)* the compliment of ion channels expressed after axotomy is different from what might be expected in an immature neurone. Titmus & Faber (*Prog. Neurobiol.* **35**, 1, 1990) have documented the variability of the effect of axotomy on a.p. configuration in various neuronal types. The present results will allow the formulation of a general hypothesis for the effects of axotomy on neuronal electrophysiological properties and provide a basis for investigation of the molecular mechanisms involved. Supported by MRC of Canada

708.5

EFFECTS OF TETRAETHYLAMMONIUM ON ONE PULSE - INDUCED RELEASE OF NOREPINEPHRINE AND $[Ca^{2+}]_i$ IN SYMPATHETIC NEURONS ALONE AND CO-CULTURED WITH TARGET CELLS V. Mashalkar, T. D. Wakade, S.V. Bhave, D.A. Przywara , A. R. Wakade & P. E. Knapp* Dept. of Pharmacol., Wayne State Univ.Sch.of Med., 540 E. Canfield, Detroit, MI 48201.

Application of 1 pulse (1 msec, 80 mA) evoked release of ^{3}H-norepinephrine (^{3}H-NE) in chick sympathetic neurons (SN) which lasted up to 60 sec & increased $[Ca^{2+}]_i$ in neuritis from about 100 to 300 nM. $T_{1/2}$ for decay of $[Ca^{2+}]_i$ was about 2 sec.Tetraethylammonium (TEA,10 mM) prolonged duration of action potential by about 2 fold but did not significantly modify 1 pulse -induced changes in ^{3}H-NE release & $[Ca^{2+}]_i$. After co-culturing SN with cardiac cells or hepatocytes but not with skeletal muscle cells or sensory neurons 1 pulse did not increase either $[Ca^{2+}]_i$ or ^{3}H-NE release. However, in presence of TEA, 1 pulse facilitated ^{3}H-NE release & $[Ca^{2+}]_i$ over 20 fold in co-cultured SN. $T_{1/2}$ for $[Ca^{2+}]_i$ decay in TEA treated co-cultured SN was about 10 sec.TEA prolonged action potential duration of co-cultured SN by 8 fold. We draw 2 conclusions. Target cells are essential for full expression of TEA effects on $[Ca^{2+}]$ mobilization & transmitter release in SN. TEA interferes with extrusion of $[Ca^{2+}]_i$, in addition to blockade of K^+ currents, to account for enhanced & prolonged $[Ca^{2+}]_i$ & ^{3}H-NE responses.

708.6

TROPHIC EFFECTS OF CARDIAC CELLS ON K^+ CURRENT, $[Ca^{2+}]_i$ AND TRANSMITTER RELEASE IN CULTURED SYMPATHETIC NEURONS.

S.V. Bhave*, D.A. Przywara, T.D. Wakade & A.R. Wakade. Dept. of Pharmacology, Wayne State University, Detroit, MI 48201.

The pharmacologic behavior of sympathetic neurons (SN) in culture is different from their counterparts growing in the body. The K^+-channel antagonist tetraethylammonium (TEA) has little effect on evoked release of [^{3}H]norepinephrine ([^{3}H]NE) from cultured SN, but acts in the expected manner seen in mature neuroeffector organs when SN are co-cultured with cardiac cells (Wakade & Wakade, Neuroscience 27:1007, 1988). We examined this phenomenon in SN cultured from embryonic chick. TEA (10 mM) caused less than a 2 fold increase in [^{3}H]NE release evoked by 1 Hz stimulation in SN, but caused a 6 fold increase when SN were co-cultured with cardiac cells. Stimulation-induced increase in $[Ca^{2+}]_i$ in SN was only little affected by TEA, but was facilitated more than 5 fold in SN co-cultured with cardiac cells. Action potentials (AP) of SN and co-cultured SN had similar shape and duration (3.7 ± 1 and 3.0 ± 0.4 ms at 50% repolarization). TEA caused an approximate 2 fold increase in AP duration in SN grown alone. In co-cultured SN, TEA caused up to 10 fold increase in AP duration. When $[Ca^{2+}]_i$ in SN was set to $\leq$ 100nM using Ca^{2+}-EGTA in the patch pipette, TEA increased AP duration as in co-cultured SN. Chick SN provide a unique model to study trophism between cardiac cells and SN. The results indicate that target cells regulate transmitter release and AP repolarization via modulation of $[Ca^{2+}]_i$.

708.7

MECHANISMS UNDERLYING THE DEVELOPMENT OF AFTERDISCHARGE IN THE BAG CELL NEURONS OF *APLYSIA*. T.A. Nick*, L.K. Kaczmarek, and T.J. Carew. Interdept. Neurosc. Prog., Depts. of Pharm. and Psychol., Yale Univ., New Haven CT 06520.

The bag cell neurons (BCNs) of adult *Aplysia* generate a long-lasting afterdischarge which triggers egg laying. We have examined the developmental onset of the afterdischarge in the intact CNS and found that the percentage of preparations exhibiting an afterdischarge (to stimulation of the pleuro-abdominal connectives or TEA; Conn and Kaczmarek, 1988) increases systematically as a function of the weight of juvenile animals: 2-11g=2.6% (N=39); 11.1-17g=40% (N=15); 17.1-50g=80% (N=5); >50g=100% (N=11). Consistent with Leibowitz and Castellucci (1983), BCNs of preparations that failed to show an afterdischarge were normally silent but, in response to connective stimulation, exhibited single spikes of low amplitude (<40 mV) and long duration (100 msec). In juvenile BCNs that did not afterdischarge, TEA in concentrations (25-100 mM) that would stimulate a discharge in adults produced recurrent depolarizations, each ~30 mV in amplitude and 3-500 sec in duration. The onset of each depolarization was marked by a single 50-70 mV spike (0.5-1 sec duration). In low calcium (2 mM), the duration of TEA-induced recurrent depolarizations significantly increased (from $\bar{x}$=7.9 sec to $\bar{x}$=49.6 sec; p<.01; N=6) suggesting that the depolarizations may normally be terminated by a calcium-activated outward current or calcium-inactivated inward current.

We are currently investigating the development of afterdischarge capacity in cultured juvenile BCNs by measuring ionic currents with whole-cell patch clamp. Although the magnitude of net outward currents across a wide activation range is less than in adults, positive voltage steps from different holding potentials reveal at least 3 outward currents which resemble I_A, $I_{K(Ca)}$, and I_{KV} described in adults. It will be of considerable interest to further characterize these and other ionic currents and determine their contribution to the development of the afterdischarge in the BCNs.

708.8

N-TYPE CALCIUM CHANNELS IN NEURODEVELOPMENT - AN AUTORADIOGRAPHIC STUDY. J. M. McIntosh*, A. Schapper, S. R. Naisbitt, B. M. Olivera and F. Filloux. Depts. of Biology, Psychiatry, Pediatrics and Neurology, University of Utah, Salt Lake City, Utah, 84112.

Intracellular calcium ion concentration plays a critical role in nervous system function in general, and has more recently been implicated in neurodevelopmental processes. N-type calcium channels in particular appear to play a role in synaptogenesis and neuronal migration. We have used [^{125}I]ω-conotoxin GVIA to autoradiographically study the neurodevelopmental expression of N-type calcium channels in rat brain. In striking contrast to L-type calcium channels which are reported to gradually increase to adult levels, N-type calcium channels appear and disappear in complex temporal and spatial patterns. Developmental patterns include: gradual increase to adult levels (cortex); transient expression (pons); substructure differentiation (cerebellum); and selective depletion (medulla). These results suggest that N-type calcium channels may be important for specific neurodevelopmental sequences. In addition, ω-conotoxins may serve as useful tools for studying disorders with possible neurodevelopmental pathophysiologies such as schizophrenia and Sudden Infant Death Syndrome.

708.9

SYNAPTOSOMAL $^{45}Ca^{++}$ UPTAKE PARALLELS SEIZURE SUSCEPTIBILITY IN EPILEPTIC MICE. A.F. Burroughs, J.R. Abbott, M.S. Esplin, and M.J. Litzinger* Department of Pediatrics, University of Utah, Salt Lake City, UT 84132

The N-type voltage sensitive calcium channels (VSCC) on the presynaptic neuronal membrane are involved in neurotransmitter release and action potential propagation. Evidence from ω-conotoxin binding data suggests a difference in VSCC development between genetically related audiogenic seizure mice (DBA/2J) and their non-epileptic counterpart (C57/Bl). Our goal was to determine if presynaptic Ca^{++} uptake differed as did VSCC development. $^{45}Ca^{++}$ uptake into synaptosomes was monitored from postnatal days (PND) 3-60 using fresh DBA and C57 whole brains. Synaptosomes were depolarized with a 50 nM potassium solution to induce Ca^{++} flux across the membrane. The C57 mice showed an increase in Ca^{++} uptake from PND 9 through PND 17 where it peaked. The amount of Ca^{++} uptake plateaued between PND 25-60. The DBA mice also showed a Ca^{++} uptake increase beginning at PND 9 which peaked at PND 25. Preliminary data shows Ca^{++} uptake for the DBA mice between PND 17-60 to be >50% in comparison with those values obtained for the C57 Ca^{++} uptake at that age. We found that the pattern of increase in $^{45}Ca^{++}$ uptake from synaptosomes through development followed the profile for the juvenile onset of audiogenic seizure susceptibility in DBA mice. This increase in susceptibility to depolarization and potential loss of Ca^{++} homeostasis may play a critical role in the audiogenic seizure onset in DBA mice.

708.10

ALTERATION OF PEPTIDERGIC AND MUSCARINIC CALCIUM SIGNALING IN TRANSFECTED N1E-115 CELLS. Jay S. Coggan*, Ildikó Kovács and Stuart H. Thompson. Hopkins Marine Station, Department of Biological Sciences, Stanford University, Pacific Grove, CA 93950.

In murine N1E-115 neuroblastoma cells muscarinic and bradykinin receptors are linked to calcium (Ca^{++}) release from internal stores and Ca^{++} influx. Responses to agonists were measured with fura-2/AM fluorescence video microscopy. Undifferentiated cells respond to bradykinin but not carbachol, whereas 2% DMSO differentiated cells respond to both. N1E-115 cells were stably transfected with an antisense sequence to the binding region of the type 1 IP_3 receptor. The aminoglycoside G418 was used to select for transfected clones. After selection, if G418 was present during DMSO differentiation, greater than 90% of normal sensitivity to carbachol, but not bradykinin, was lost. Those differentiated without G418 retained normal muscarinic calcium release. Acute incubation with G418 had no effect on the muscarinic responses. In one of eight undifferentiated, antisense-transfected clones, responses to bradykinin were 99% attenuated by removing extracellular calcium. This result indicates that Ca^{++} release in this clone is significantly reduced, leaving only the Ca^{++} influx component. In differentiated clones responses to bradykinin were as in normal cells. Supported by NIH NS14519.

708.11

QUANTIFICATION AND QUALITATIVE ASSESSMENT OF DEVELOPMENTAL BRAIN Na^+CHANNEL mRNA EXPRESSION BY RNASE PROTECTION ASSAY COMBINING A COMMON PROBE WITH SPECIFIC 3' END PROBES. J.L. Walewski, D.F. Catanzaro, J.R.Sparrow*, and E. Recio-Pinto, Cornell University Medical College, Depts. of Anesthesiology, Physiology and Ophthalmology, New York, NY 10021.

To quantify various brain mRNAs encoding voltage-dependent Na^+ channels we generated a 161b cRNA probe which recognizes a segment of high homology between the three known genes. Therefore in RNAse protection assays this probe gives rise to three protected fragments, corresponding to mRNAs encoding either type I (107b); type II (71b) or type III (161b) Na^+ channels. Quantification with this common probe, when compared to the specific 3' probes for mRNA encoding Na^+ channel types I, II or III, allowed us to investigate whether there were additional Na^+ channel mRNAs. Additional mRNAs could be grouped based on their resemblance to one of the known Na^+ channel types. The combination of differential recognition of various mRNAs by using a single common cRNA probe, together with the use of specific 3' cRNA probes provides a strong tool for quantifying mRNAs encoding known Na^+ channels as well as detecting and quantifying other putative Na^+ channel mRNAs. We have begun using this approach to provide new information regarding changes in brain Na^+ channel mRNAs under different developmental conditions. JLW is an Aaron Diamond Foundation Fellow and this work was supported in part by a grant from The Aaron Diamond Foundation. Additional support was provided by the Depts. of Anesthesiology and Surgery, Cornell University Medical College.

708.12

EXPRESSION OF CALCIUM CHANNEL TRANSCRIPTS IN THE EMBRYONIC AMPHIBIAN NERVOUS SYSTEM. M. A. Cecchini* and A. B. Ribera, Neuroscience Program and Department of Physiology C-240, University of Colorado Health Sciences Center, Denver, CO 80262.

Amphibian spinal neurons initially fire long duration calcium-dependent action potentials. Immature neurons are spontaneously active, generating transient intracellular calcium elevations which regulate aspects of neural differentiation. Impulse duration subsequently shortens due to increased voltage-dependent potassium conductance. The molecular basis for this program of electrical excitability is being analyzed using probes for voltage-dependent ion channel genes. Given the developmental requirement for calcium influx, our efforts focus on calcium channels.

A partial clone encoding a *Xenopus* calcium channel gene has been isolated by homology screening with rat neural-specific calcium channel sequences (kindly provided by Dr. T. Snutch). The 6 kb clone contains 3 kb of coding region spanning part of domain III and all of domain IV plus ~3 kb of 3' untranslated sequence. It is most related to the mammalian rbA calcium channel sequences and thus called XCalA. *In-situ* hybridization assays indicate that XCalA transcripts are detectable throughout the central nervous system. In addition, the mRNA is detected in a region within the middle of each myotome. However, one day later this signal is reduced in intensity.

The temporal pattern of transcription of XCalA is consistent with a putative role in regulating excitability of developing neurons. Furthermore, these results indicate a transient pattern of expression for XCalA mRNA in peripheral structures.

708.13

EXPRESSION OF *SHAB* TRANSCRIPTS IN THE EMBRYONIC *XENOPUS LAEVIS* NERVOUS SYSTEM. C. Burger* and A.B. Ribera. Programs in Cellular & Developmental Biology and Neuroscience, University of Colorado Health Sciences Center, Denver, CO .

Voltage-activated potassium channels belong to a multigene family that includes the *Shaker, Shal, Shaw and Shab* gene subfamilies. Delayed rectifier potassium currents regulate the differentiation of electrical excitability in amphibian spinal neurons. To understand the molecular basis of potassium channel gene expression in these neurons, we are identifying relevant members of the potassium channel gene family. We describe here the characterization of *Shab* genes expressed in the embryonic *Xenopus* nervous system.

Degenerate oligonucleotide primers and PCR generated *Xenopus Shab* products that were used as probes for the screening of a *Xenopus* tadpole brain cDNA library. We isolated three different *Xenopus Shab* cDNAs: XShab 6, 9 and 12. Partial sequence analysis of XShab 6, 9 and 12 confirms their identity as *Shab* homologues. Over the region spanned by the S2 and S5 transmembrane domains, XShab 6, 9 and 12 are 88%, 77%,and 95% identical, respectively, at the amino acid level to mammalian *Shab* genes. Additional sequence information indicates that although XShab 6 has its 5' end within the coding region, both the 5' and 3' ends of XShab 9 and 12 appear to contain untranslated sequences. Thus XShab 9 and 12 are likely to contain the entire coding region. Functional expression in oocytes will extend the interpretation of sequence data.

In situ hybridization studies indicate that XShab is widely expressed throughout the amphibian spinal cord during the period when the endogenous delayed rectifier current has been functionally characterized. Supported by NIH NS25217 and NS01531.

708.14

EXPRESSION OF Kv3.1 mRNA ENCODING A VOLTAGE-GATED K^+ CHANNEL DURING RAT CEREBELLAR DEVELOPMENT. S. Verma* and R.H. Joho. Department of Cell Biology and Neuroscience, The University of Texas Southwestern Medical Center, Dallas, TX 75235-9111.

Kv3.1(Kv4/NGK2) is a voltage-gated K^+ channel found in different brain areas but it is particularly highly expressed in the granule and Purkinje cell layer in the cerebellum. Northern blot analyses with mRNA from postnatal days P1, P3, P7, P11, P15, and P20, and from adult rat brain showed that Kv3.1 expression started around P11 and increased steadily up to and past P20 to reach adult levels. We used *in situ* hybridization on sections of the developing cerebellum (P7, P11, P15, and P20) to study mRNA expression in the external germinal layer (EGL), the molecular layer (ML), the Purkinje cell layer, and in the granule cell layer (GCL) during postnatal cerebellar development. In particular, we addressed the question whether Kv3.1 mRNA expression can be detected in migrating granule cells, or whether its expression is confined to postmigratory cells located in the granule cell layer.

Expression levels (numbers of silver grains/area) were similar in EGL, ML, and GCL on P7. An increase of Kv3.1 mRNA expression in granule cells in GCL was first observed around P11. At P20 there was at least a five-fold increase in grains in GCL over EGL. Even during the peak of granule cell migration, the number of grains/area in EGL and ML never exceeded the number found at P7. From visual inpection, it appears that the premigratory cells in EGL and the migrating granule cells in ML do not express Kv3.1. (Supported by NS28407 to R.H.J.)

708.15

DEVELOPMENT OF Ca^{2+}_i HOMEOSTASIS MECHANISMS IN EMBRYONIC RAT CEREBRAL CORTICAL CELLS USING FLUO-3 AND FLOW CYTOMETRY. I. Maric*, D. Maric, S.V. Smith and J.L. Barker. Laboratory of Neurophysiology, NINDS, NIH, Bethesda, MD 20892

We have previously utilized flow cytometry and a voltage-sensitive fluorescence indicator dye in order to describe the development of resting and excitable plasma membrane properties of rat cerebral cortical cells during embryonic (E) histogenesis. Here we report on the development of Ca^{2+} homeostasis of the same cell preparations isolated over E11-22 using a Ca^{2+}-sensitive fluorescence indicator dye, Fluo-3. Acutely dissociated single cell suspensions were loaded with 1μM Fluo-3 and their resting and stimulated cytosolic calcium (Ca^{2+}_c) levels were recorded at the rate of ~1000 cells/sec using a flow cytometer. Ca^{2+}_c levels were estimated with ionomycin, a Ca^{2+} ionophore, and $MnCl_2$. The results reveal a characteristic cellular distribution of resting Ca^{2+}_c, which we have classified into three primary subpopulations: (1) relatively low Ca^{2+}_c (80-150nM), (2) moderate Ca^{2+}_c (150nM-600nM) and (3) relatively high Ca^{2+}_c (>600nM). The percentage of cells with each of these Ca^{2+}_c levels was age-dependent with many cells exhibiting higher Ca^{2+}_c levels (>150nM) over E11-14 and >90% of cells having relatively low Ca^{2+}_c during E15-22. Ca^{2+} homeostasis in these subpopulations depended on the presence of Na^+_c-Ca^{2+}_c exchange mechanisms, pools of sequestered Ca^{2+}_i and their voltage-sensitivity, and voltage-dependent and/or receptor-operated Ca^{2+} channels. These aspects of Ca^{2+}_i homeostasis were temperature dependent and could be correlated with the proliferation/differentiation status of the cells. Stimulation with veratridine, a Na^+ channel activator, γ-aminobutyric acid, glycine and glutamic acid evoked characteristic and complex Ca^{2+} responses that were highly age-dependent and cell subpopulation-specific. These results demonstrate that flow cytometric analysis of Ca^{2+}-sensitive fluorescence signals allows a relatively quick, sensitive and effective characterization of Ca^{2+} homeostasis mechanisms for entire populations of cerebral cortical cells throughout embryonic development.

708.16

POSTNATAL DEVELOPMENT OF HIGH THRESOLD Ca^{2+} CURRENTS IN RAT HIPPOCAMPAL CA1 NEURONS. C.E.Niesen*, O.T. Jones, L.R. Mills and P.L. Carlen, Playfair Neuroscience Unit, Toronto Hosp. Research Inst., Univ. of Toronto-NCE Program, Toronto, ON. CANADA M5T 2S8

Striking developmental changes occur in the early postnatal mammalian CNS, including the genesis of many ionic currents. We studied the ontogeny of voltage-dependent Ca^{2+} currents (VDCC) in hippocampal slices from 3 day to 3 week old rats, using the whole-cell patch technique.

The high threshold L-type current was evoked from holding potentials (V_H)= -25 to -35 mV. The peak amplitude of this current "grew" as follows: 3-4 days postnatal (dpn), approx. 100 pA; 7-8 dpn, 250 pA; end of the second week, 1000 pA; end of the third week, 0.9-1.4 nA. Bath perfusion of 2-3 μm nimodipine blocked 60% of this current in the mature animal, while in the newborn of 1 week, < 30% of the L-type current is blocked. In 2 week old CA1 neurons, near adult levels of blockade were achieved by nimodipine.

A small, high thresold (HT) Ca^{2+} current was elicited only variably by large depolarizing steps from V_H= -75 mV in the 4-5 dpn CA1 neuron. In CA1 neurons at 1 week of age, the amplitude was 300-500 pA and the HT current could be evoked consistently. In 2 week old cells, the peak amplitude of this current was 1800 pA and tripled again by the end of the third week. Droplet application of 10 μm ω-conotoxin had little effect on the HT current at 1 week, blocked 20% at 2 weeks and decreased the HT current in 3 week old cells by 30-35% (adult levels).

Both L- and N-type VDCCs showed marked postnatal development, though at different rates. The L-type current tripled its amplitude during the second week, while the N-type current emerged during the second and third weeks postnatal. This confirms imaging studies with fluorescent ω-conotoxin on CA1 neurons that showed a similar, time-dependent genesis of the N-type VDCC, coincident with its spread from soma to dendrites (see L. Mills et al., Soc. for Neurosc. Abst., 1993).

708.17

REGIONAL AND SUBCELLULAR DISTRIBUTION OF N-TYPE Ca^{2+} CHANNELS IN LIVE DEVELOPING RAT HIPPOCAMPUS L.R. Mills*, C.E. Niesen, O.T. Jones, A.P. So and P.L.Carlen. Playfair Neurosc. Unit, MRC group "Nerve Cell and Synapses", Univ. of Toronto, Toronto, ON. CANADA M5T 2S8

Influx of Ca^{2+} through neuronal voltage-dependent channels (VDCC) plays a critical role in multiple cellular functions. Using fluorescein-tagged ω-conotoxin (Fl-ω-CgTx) we examined the spatial distribution of N-type VDCCs in the developing rat hippocampus. In the adult, N-type VDCCs are present on the soma, dendrites and dendritic spines of neurons in areas CA1-3 and dentate gyrus (DG). In newborn live hippocampal slices, detectable N-type VDCCs were restricted to somata of neurons in area CA3; areas CA1-2 and DG showed only scattered Fl-ω-CgTx positive cell bodies. By 4 days postnatal (dpn), cells were labelled in areas CA1 -CA2. During this period, Fl-ω-CgTx positive cells were apparent in other regions of the developing hippocampus, most notably above layers CA1-3 and in the hippocampal fissure. Such cells appeared to be migrating. Areas adjacent to the hippocampus (entorhinal cortex, developing fimbria), the stria terminalis and neocortex also contained Fl-ω-CgTx labelled cells. By 1 week, labelled soma were present in all hippocampal regions. CA3 neurons showed labelling of proximal dendrites extending out 20-40 microns. By 12 dpn, further labelling of dendrites in CA3 and along initial segments of dendrites in CA1 and DG was observed.

Lucifer Yellow fills in 4 day old CA1 neurons revealed apical dendritic arborization extending 300 microns into the stratum radiatum. By 12 dpn, the dendritic tree was enlarged but spinous processes were absent. These results demonstrate the regional and time-dependent ontogeny of N-type VDCCs in the rat hippocampus. In CA1 neurons, N-type Ca^{2+} channels are not present at birth, appear on the soma during the first week of life and are exported to already formed dendritic processes over the second-third week of life.

708.18

CARDIAC CELLS CONTROL $[Ca^{2+}]_i$ AND TRANSMITTER RELEASE IN CULTURED SYMPATHETIC NEURONS. D.A. Przywara*, S.V. Bhave, T.D. Wakade, V. Mashalkar & A.R. Wakade. Dept. of Pharmacology, Wayne State University, Detroit, MI 48201.

Ca^{2+}-dependent changes in growth, survival and transmitter phenotype of developing sympathetic neurons (SN) are controlled by factors released by other cells. Here we show that postsynaptic effector cells control Ca^{2+} entry and [^{3}H]norepinephrine ([^{3}H]NE) release properties of developing SN. SN cultured alone exhibited maximal elevation of $[Ca^{2+}]_i$ and [^{3}H]NE release during low frequency stimulation. Co-culturing SN with cardiac cells reduced stimulated [^{3}H]NE release, $[Ca^{2+}]_i$, and voltage-clamped Ca^{2+}-current by 3 to 5 fold. Co-cultured SN had a positive frequency-[^{3}H]NE release response, characteristic of mature sympathetic neuroeffector organs. This trophic maturation was produced within 24 hrs by sympathetic neuroeffector cells, but not by conditioned medium or other cell types. [^{3}H]NE release was correlated most closely with total $[Ca^{2+}]_i$ during the stimulation period rather than with peak $[Ca^{2+}]_i$. Our studies show that SN supported by only NGF under optimal culture conditions exhibit abnormal Ca^{2+} homeostasis and transmitter release. The abnormalities are corrected by including physiological targets in culture.

OTHER FACTORS AND TROPHIC AGENTS I

709.1

DIFFERENTIAL REGULATION OF ASTROCYTE-DERIVED GROWTH MODULATORY PROTEOGLYCANS AND THEIR INTRINSIC PROPERTIES ARE DETERMINANTS OF REGENERATIVE NEURITE GROWTH IN AN ASTROCYTE MILIEU IN VITRO. M. Guo, K. Dow*, R. Kisilevsky and R. Riopelle. Apps Research Centre of Kingston General Hospital, Queen's University, Kingston, CAN. K7L 2V7.

Mammalian Type 1 astrocytes *in vitro* synthesize and release large quantities of proteoglycans, and heparan sulfate proteoglycans account for virtually all of the substrate-associated neurite growth-promoting activity of astrocyte-conditioned medium. Chromatographic procedures have facilitated characterization of two astrocyte PG's released to conditioned medium. A 510 kDa heparan sulfate proteoglycan (HSPG) and a 58 kDa chondroitin/dermatan sulfate hybrid PG (CS/DSPG) with similar charge densities accounted for 17% of the total released PG's. The two PG's were differentially regulated by growth phases of the astrocytes. In conditioned medium of confluent, non-proliferating cultures the molar ratio of the HSPG to the CS/DSPG was approximately 1:10. However, in subconfluent cultures where astrocytes continued to proliferate, virtually no 510 kDa HSPG was detected.

Both PG's displayed neurite growth-promoting properties when immobilized on a poly-D-lysine substrate, but on a molar basis the 510 kDa HSPG had some 40,000-fold higher specific neurite-promoting activity. By virtue of its higher avidity of binding to the poly-D-lysine substrate, the CS/DSPG inhibited the neurite-promoting activity of the HSPG by displacing it from the substrate.

Supported by the Medical Research Council of Canada

709.2

Characterization and expression of insulin-like growth factor system components during optic nerve regeneration. C. M. Hall* and J. Höstner. Dept. of Anatomy and Cell Biology. University of Göteborg. S-413 90 Göteborg. Sweden

Components of the insulin like growth factor (IGF) system are produced by both neurons and glial cells in the CNS. In order to investigate the role played by IGF-I during nerve regeneration we have characterized IGF-I membrane receptors and soluble IGF binding proteins expressed in the goldfish visual system. Affinity cross-linking showed that two IGF-I receptor subunits of mw. 110 and 120 kD are synthesized in goldfish nervous tissue. Competition binding assays showed that retinal membranes contained IGF-I binding sites that displayed equal high affinity for IGF-I and IGF-II while having more than 1000-fold lower affinity for insulin. The soluble fractions of goldfish retina and brain contained at least three different IGF-binding proteins of apparent mw. 22, 27 and 31 kD. Following crush of the optic nerve, goldfish retinal ganglion cells undergo functional regeneration. Expression of IGF-I binding sites was investigated during optic nerve regeneration using 125I-IGF-I binding to fresh cryosections of regenerating retina followed by autoradiography. In the normal eye there was a prominent binding of IGF-I to all retinal layers. During the phase of axonal regrowth there was a markedly increased expression of IGF-I binding sites in the inner plexiform, retinal ganglion cell and optic nerve fiber layers. The time-course of increased IGF-I binding corresponds to the time during which the optic nerve displays accelerated growth in response to a second lesion-the conditioning lesion effect. These results support a role for IGF-I as a neurotrophic factor.

709.3

CLONING OF A NOVEL NEURONAL G PROTEIN-COUPLED RECEPTOR WITH HOMOLOGY TO THE LDL-RECEPTOR. C.P. Tensen* [1,2], E.R. van Kesteren[2], R.J. Planta [2], E.Vreugdenhil[2] and H. van Heerikhuizen[2]. [1]Department of Biology, [2]Department of Biochemistry and Molecular Biology, Vrije Universiteit, De Boelelaan 1083, 1081 HV Amsterdam, The Netherlands.

In our search for G protein-coupled receptors (GPRs), we have isolated and characterized a cDNA from the central nervous system (CNS) of the mollusc, *Lymnaea stagnalis*, encoding a protein of 1115 amino acids with close sequence similarity to two different types of receptor proteins. Following a twenty residue long signal peptide, the N-terminal part of the protein contains two types of internal repeated sequences. The first type of repeat displays a high sequence similarity with the binding domains of the low density lipoprotein (LDL)-receptor, a characteristic thusfar only found in the LDL receptor-related protein (LRP) and glycoprotein GP 330. The second repeat and the C-terminal part of this receptor are homologous to specific regions of a set of GPRs, the mammalian glycoprotein hormone receptors. In situ hybridization experiments revealed that the mRNA encoding this receptor (designated GRL101) is predominantly located in a cluster of 15-20 neurons within the CNS. The structural resemblance of the N-terminal part of GRL101 with the binding domains of the LDL receptor and its specific expression in a small number of neurons, suggest a role for lipoprotein-like molecules in neuronal G protein-mediated signal transduction. Alternatively, GRL101 might be a receptor for ligands similar to those for LRP. This receptor was originally proposed to have a role in lipoprotein metabolism; recently it has become clear that it is a multiple ligand receptor that can recognize and internalize additional non-related ligands *i.e.* uPA-PAI-1 complexes. Effects of uPA-PAI-1 and a related protein, tPA, on neuronal outgrowth and plasticity were recently reported and we therefore hypothesize that GRL101 might be the first member of a novel class of GPRs that directly transduce signals carried by large extracellular (lipo)protein-(complexe)s, into neuronal events mediated by G-proteins.

709.4

MODAFINIL - A POTENTIAL NEUROPROTECTIVE AGENT IN THE MOUSE 1-METHYL-4-PHENYL-1,2,3,6-TETRAHYDRO-PYRIDINE (MPTP) MODEL OF PARKINSON'S DISEASE (PD). [1]A.M. Janson*, [1]K. Fuxe, [2]M. Goldstein and [3]L.F. Agnati. [1]Dept of Histology and Neurobiology, Karolinska Institutet, Box 60400, S-104 01 Stockholm, Sweden; [2]Dept of Psychiatry, New York Univ. Med. Center, New York, NY 10016; [3]Dept of Human Physiology, Univ. of Modena, Via Campi 287, I-41100 Modena, Italy.

The vigilance promoting drug Modafinil (diphenyl-methyl-sulfinyl-2-acetamide) is demonstrated to protect against MPTP (40 mg/kg sc) toxicity in the nigrostriatal dopamine (DA) system of the black mouse.

A single dose of Modafinil (30 mg/kg ip) at different time intervals before and after MPTP significantly counteracted the MPTP-induced depletion of neostriatal DA as analyzed 14 days later. Modafinil also significantly counteracted the paradoxical MPTP-induced increase in locomotion and motility as analyzed after two weeks.

Chronic administration of Modafinil (30 mg/kg ip daily for 14 days) led to a more pronounced counteraction of the MPTP-induced decrease in striatal DA and also attenuated the MPTP-induced increase in DA turnover. After a similar chronic treatment with Modafinil (10-100 mg/kg) the disappearance of tyrosine hydroxylase immunoreactive neurons in the substantia nigra appeared to be dose-dependently counteracted.

The mechanism of action has not yet been fully established, but it seems to be different from monoamine oxidase B inhibition or blockade of the DA reuptake transporter. The neuroprotective action of Modafinil could lead to new pharmacological strategies in PD.

709.5

ROLE OF A FREE RADICAL SCAVENGER (EGb 761) IN THE MOUSE VISUAL SYSTEM PLASTICITY. P. Clairambault[1], R. De Guevara[1], G. Pinganaud[1], M.T. Droy-Lefaix[2] and Y. Christen[2]*. Lab. Neuroembyrology, University Paris VII ; IPSEN Research Lab., Paris, France.

To analyse the role of a free radical scavenger (EGb 761, IPSEN, France) on the nervous system plasticity, we studied the mouse dorso-lateral geniculate nucleus (dLGN) at 30 days post-natal (PN 30) using the autoradiographic technique after 3H proline injection (Amersham, 6 µCi in 6 µl) in one eye. Morphological and quantitative data were gained at PN 30 in control animals and in unilaterally enucleated at birth. Concurrently, two similar groups were treated with EGb 761 (100 mg/kg, per os, 30 days). A comparative quantitative volumetric analysis between the 4 groups was undertaken either in the dLGN or in the geniculate visual neuropil.

After deafferentation, a significant decrease of the dLGN volume and a significant increase of the visual neuropil was observed on the side ipsilateral to the injected eye, only in the mice treated with EGb 761 suggesting a role of EGb 761 on the plasticity of the retino-geniculated connections.

709.6

THE EFFECTS OF α-MSH and ACTH 4-10 ON MUSCLE FIBER TYPE COMPOSITION OF FAST MUSCLE FOLLOWING NEONATAL SCIATIC CRUSH INJURY. **L.A. Zuccarelli* and F.L. Strand. Department of Biology and Center for Neural Science, New York University, New York, New York 10003.**

Adrenocorticotropic hormone (ACTH) has been shown to enhance the regeneration of peripheral nerve and accelerate the maturation of the developing neuromuscular system. These peptides also have potent positive effects on regeneration during development. Peptide-treated reinnervated developing motor endplates exhibit the size and complexity of interior nerve branching at the nmj comparable to uncrushed neonatal endplates. Here we report on the effects of fragments ACTH 4-10 and α-MSH on the maturation and reinnervation of the extensor digitorum longus (EDL) muscle of 2-day-old rodents which undergo sciatic nerve crush. At 60 days postnatal, the EDL muscles are excised and their weights recorded. The muscles are prepared for staining with myofibrillar ATPase at both alkaline and acid preincubation conditions. Muscle fiber cross-sections are analyzed for fiber type, distribution of fiber type groups and cell density.

The results show that the development of the EDL muscle is accelerated by peptide treatment even in the face of trauma. Neither muscle fiber type composition by percentage nor the distribution of fiber types within the muscle is altered significantly by either denervation or peptide treatment. There is some indication that fiber type grouping is occurring in α-MSH- treated muscle; these muscles also exhibit more complete reinnervation. Muscle weight comparisons and cell density measurements show that peptide-treated muscles exhibit less atrophy than saline counterparts. These results continue to support the efficacious role of ACTH/MSH peptides in PNS regeneration.

709.7

REGULATION OF FGF-2 AND HIGH-AFFINITY FGF-RECEPTOR IN SPINAL GANGLIA AND PERIPHERAL NERVE AFTER LESION. Grothe,C.* and Janet,T. Institute of Anatomy, University of Freiburg, D-7800 Freiburg and Dept. of Anatomy and Cell Biology, University of Heidelberg, D-6900 Heidelberg, F.R.G.

The regulation of basic fibroblast growth factor (FGF-2) and FGF-receptor (R) and their mRNAs in rat dorsal root ganglia (DRG) and sciatic nerve was studied after different lesion protocols.

Sciatic nerve ligation reveals neither a distal nor a proximal accumulation of FGF-2-immunoreactivity (IR). Sciatic nerve crush leads to a decrease of FGF-2- and FGFR-IR in the distal stump; also the proliferating Schwann cells display no FGF-2 or FGFR staining. In the proximal stump no change of the staining pattern compared to control rats is found. Sciatic nerve axotomy results in an increase of the portion of FGF-2- and FGFR-IR DRG neurons. This result could suggest that i) FGF-2-ir neurons are selectively protected from axotomy-induced death or ii) an additional population of neurons is induced to express FGF-2 IR after axotomy.

Northern blot analysis shows a time-dependent regulation of FGFR-1 (*flg*) mRNA in the distal and proximal stump after nerve crush.

These regulatory changes in FGF-2 and FGFR expression suggest that the factor is involved during de-/regeneration.

(Supported by DFG Gr 857/4-2)

709.8

IMMUNOCYTOCHEMICAL LOCALIZATION OF ACTH 4-10 IN THE ADULT RAT BRAIN AFTER 6-OHDA LESIONING OF THE SUBSTANTIA NIGRA. F.J. Antonawich*, S.J. Lee and F.L. Strand. Department of Biology and Center for Neural Science, New York University, New York, N.Y. 10003

Adrenocorticotropic hormone (ACTH 1-39) is a naturally secreted neuropeptide possessing both neurotrophic (amino acids 1-10) and corticotropic properties (amino acids 11-39). Administration of its neurotrophic fragments, and their synthetic analogs, has demonstrated potent effects on the central and peripheral nervous systems. Following 6-OHDA lesions of the nigrostriatal system, Org 2766 (an ACTH 4-9 analog) provides rapid neurotrophic actions by both accelerating compensatory mechanisms necessary for functional recovery and promoting cell survival by providing neuronal protection (Antonawich et al., 1993, Peptides, submitted).

Prior studies using antisera to ACTH 1-39 and ACTH 11-24 have demonstrated immunoreactivity (Ir) in various nuclei of the circumventricular organs. Recent work by Lee et al., 1992 (Society for Neuroscience Abstract #121.2) using an antiserum against the neurotrophic ACTH 4-10 fragment demonstrated Ir in the cerebral cortex, striatum, medial septal area, hippocampus, periventricular area and arcuate nucleus of neonatal brains, while in the adult brains, Ir was restricted to fibers of the arcuate and medial septal area.

Adult male Sprague Dawley rats were lesioned unilaterally with 6-hydroxydopamine (8µg/4µl) infused into the striatum. Immunocytochemical localization of anti-ACTH 4-10 was performed 6 days following surgery. Ir was evident in the injured nigrostriatal system with a more pronounced reactivity on the lesioned side of the brain, where both the substantia nigra and the caudate nucleus of the striatum demonstrated positive staining. Therefore, it appears that the neurotrophic fragment of ACTH reappears in adults following injury to the nigrostriatal system.

709.9

CHANGES IN EXPRESSION OF BASIC FGF INDUCED BY SPINAL CORD ISCHEMIA. D.A. Shackelford[1], D.A. Otero[1], J. Hogg[2], A. Baird[2], A.L. Miller[1]* and J.A. Zivin[1]. [1]Dept. of Neurosciences, UCSD, La Jolla, CA 92093-0624 and [2] Whittier Institute, 9894 Genesee, La Jolla, CA 92037.

Ischemic injury to the brain causes an increase in bFGF and other trophic factors that can help and/or hinder functional neurological recovery. We investigated ischemia-induced changes in the endogenous expression of bFGF mRNA and protein in the rabbit spinal cord ischemia model. Northern analysis of RNA isolated from spinal cords of rabbits subjected to ischemia showed no change in the steady state level of bFGF RNA induced by 0 to 60 min of ischemia. However, an increase in bFGF RNA was seen after 18 h of reperfusion following a 60 min ischemic episode which results in permanent paraplegia in essentially all rabbits. Current studies aim to determine the time course of induction of bFGF RNA, and the cell type producing it in response to reversible versus irreversible ischemia. The protein levels of bFGF in the rabbit spinal cord homogenates were analyzed by immunoblotting. In the particulate fraction, three forms of M_r = 26k, 23k, and 18k were observed. In the cytosolic fraction mainly two forms of M_r = 24k and 18k were detected. The 23-26k Da forms are probably the result of amino-terminal extensions due to the use of alternative initiation codons as reported in other species. Spinal cord ischemia induced the transient appearance of new species of all three FGF forms with decreased electrophoretic mobility resulting in six bands of M_r = 27k, 26k, 24k, 23k, 19k, and 18k (i.e., three apparent doublets). This was observed after 3-5 min of ischemia and by 15 min the new forms were no longer detected in all animals. The slower migrating forms were also detected in homogenates from animals reperfused for 18-96 h. The contribution of phosphorylation or other modification of bFGF to the shift in mobility is being investigated.

709.10

PERMANENT RESCUE OF AXOTOMIZED CLARKE'S NUCLEUS NEURONS REQUIRES MORE THAN TARGET-DERIVED NEUROTROPHIC FACTORS. B.T. Himes* and A. Tessler, Dept. of Anatomy and Neurobiology, The Medical College of PA and VA Medical Center, Philadelphia PA.

The 40% loss of Clarke's nucleus (CN) neurons that follows axotomy in neonatal rats has been attributed to loss of target derived neurotrophic factors. Axotomized CN neurons are rescued for at least 2 months by transplants of embryonic CNS (spinal cord, cerebellum and neocortex) which contain the mRNA for the neurotrophic factor neurotrophin-3 (NT-3), but not by embryonic striatum, which does not contain mRNA for NT-3. At least one peripheral organ that contains mRNA for NT-3, the renal cortex from late embryonic to early postnatal donors, also rescues axotomized CN neurons. To determine whether this rescue is permanent (4 months or longer), P2 rats received grafts of E14 spinal cord, E15 cerebellum or E18-P2 renal cortex into a T8 spinal cord hemisection cavity, and CN neurons were counted at L1 2 months or 4-6 months postoperatively. The transplants survived at all time points examined and integrated with the host spinal cord. At 2 months grafts of spinal cord, cerebellum or renal cortex prevented CN neuron death; at 4-6 months injured CN neurons were maintained only by grafts of spinal cord and cerebellum. Provision of a normal target-derived neurotrophic factor appears to be adequate for temporary survival of axotomized neurons. Grafts of appropriate CNS target tissues are required for permanent rescue of axotomized CN neurons. Supported by NIH grant NS24707 and the VA Medical Research Service.

709.11

CYTOKINE GENE EXPRESSION FOLLOWING TWO MODELS OF BRAIN INJURY. R.M. Klein, R. Choudhuri, J. B. Meara, S. De, V. H. Gattone II*, and N.E.J. Berman. Department of Anatomy and Cell Biology, University of Kansas Medical Center, Kansas City, KS 66160-7400

Cytokines such as tumor necrosis factor alpha (TNF-α) are involved in cellular responses to injury. Microglia, the resident macrophages of the brain, are activated and produce cytokines in response to stimulation by bacterial lipopolysaccharides (LPS). We studied expression of TNF-α and microglial activation in two models of brain injury. To provide a model of injury alone, we injected 5 µl PBS directly into the cerebral cortex of adult CD-1 mice. To provide a model of brain injury in which microglia are activated, we injected LPS directly into the cerebral cortex. Microglial activation was visualized by Fc receptor expression using nonspecific attachment of IgG. LPS injections activated ramified microglia throughout the brain, while PBS injections only activated ameboid microglia in the vicinity of the damage. We studied mRNA levels for TNF-α by Northern blotting, and expression of TNF-α protein by ELISA, bioassay and immunohistochemistry. Following injection of LPS, TNF-α message levels in the brain increased at 2 hr, decreased by 4 hr, but increased again significantly 4 days later, and remained elevated for up to 7 days. TNF-α protein was not detectable in uninjured brain by ELISA or bioassay, but increased after both forms of injury. *In situ* hybridization and immunohistochemical studies indicated that the predominant source of TNF-α mRNA and protein is injured neurons rather than microglia in both injury models. Supported by MH38399 and HD02528.

709.12

UP-REGULATION OF BASIC FIBROBLAST GROWTH FACTOR (bFGF) AND ITS RECEPTOR mRNA EXPRESSION IN RAT BRAIN FOLLOWING TRANSIENT FOREBRAIN ISCHEMIA. K. Takami[1]*, Y. Kiyota[2], M. Iwane[3], M. Miyamoto[2], R. Tsukuda[1], K. Igarashi[3], A. Shino[1], A. Wanaka[4], S. Shiosaka[5], M. Tohyama[4] [1]Drug Safety Res. Lab, Pharmaceu. Dev. Div., [2]Res. Lab. I, Pharmaceu. Res. Div. and [3]Res. Lab. II, Discovery Res. Div., Takeda Chemical Ind. Ltd., Osaka 569, Japan, [4]Dept. of Anat. and Neurosci., Osaka Univ. Med. Sch., Osaka 565, Japan. [5]Dept. of Cellular and Structural Biology, Advanced Inst. of Sci. and Technol., Nara, Nara 630-01, Japan.

We have previously shown that transient forebrain ischemia in rats leads to an early and strong induction of basic fibroblast growth factor (bFGF) synthesis in astrocytes in the injured brain regions. In this study, in order to clarify the targets and roles of such raised endogenous bFGF levels, the mRNA expression of its receptors (*flg* and *bek*) in the hippocampus following transient forebrain ischemia in rats was investigated using an *in situ* hybridization technique. The ischemia induced an increase in the number of *flg* mRNA-positive cells in the hippocampal CA1 subfield, where delayed neuronal death occurred, from an early stage (24 h after ischemia) and this increase was still evident in this area 30 days after. The time-course of the appearance and distribution pattern of *flg* mRNA-positive cells in the CA1 subfield were quite similar to those of bFGF mRNA-positive cells. On the other hand, *in situ* hybridization for *bek* mRNA showed only slight and transient (observed 72 h and 5 days after ischemia) increases in the number of mRNA-positive cells in the CA1 subfield following ischemia. These changes were less marked than those of the *flg* mRNA-positive cells. The use of *in situ* hybridization and glial fibrillary acidic protein immunohistochemistry in combination demonstrated that the cells in the CA1 subfield that exhibited ischemia-induced *flg* or *bek* mRNA expression were astrocytes. These data indicate that transient forebrain ischemia induced up-regulation of FGF receptor expression accompanied by increased bFGF expression in astrocytes and suggest that the increased astrocytic bFGF levels in injured brain regions act on the astrocytes via autocrine systems and are involved in the development and maintenance of astrocytosis.

709.13

EXPRESSION OF hNT3 IN RAT FIBROBLASTS: IMPLICATIONS FOR INTRACEREBRAL GRAFTING. M.C. Senut[1], N.H. Liou[1], S.T. Suhr[1], H.K. Raymon[1], K.R. Jones[2], L.F. Reichardt[2]*, and F.H. Gage[1]. 1. UCSD, La Jolla, CA 92093; 2. UCSF, San Francisco, CA 94143.

Neurotrophin 3 (NT3) has been shown to promote the survival of noradrenergic locus coeruleus neurons as well as other cell types. Long term goals are to assess the biological effects of NT3-producing fibroblasts in normal and lesioned rat brains. Initial studies sought to optimize conditions for NT3 mRNA expression in rat fibroblasts such as 1) promoter and enhancer, 2) culture medium, 3) cell passage number. We designed six different retroviral vectors by inserting the cDNA coding for human NT3 (hNT3) behind either a cytomegalovirus (CMV) or a Moloney murine leukemia virus long term repeat (MLV-LTR) promoter. The effects of upstream tissue-specific enhancers were also evaluated. Primary skin fibroblasts obtained from Fischer 344 rats were infected with the different retroviral vectors expressing hNT3 and a selectable neomycin resistance gene. Uninfected fibroblasts or fibroblasts infected with β-galactosidase were used as controls. NT3 mRNA expression was assessed at different confluent states using PCR and Northern blot analysis. All vectors acheived NT3 mRNA expression in rat fibroblasts, although at various levels. Fibroblasts carrying CMV promoter demonstrated NT3 mRNA levels 2 to 3 fold higher than those carrying MLV-LTR promoter. Fibroblasts with CMV and MLV-LTR promoters expressing hNT3 were selected to be transplanted in different rat brain regions. Preliminary results show that implanted cells survived well after grafting. We are currently investigating the levels of produced and secreted NT3 in the infected fibroblasts *in vivo* and *in vitro* . (Supported by NATO grant 58C91FR and by NIH grant TWO4813).

709.14

GM1 PRODUCES MODERATE ATTENUATION OF PERSISTENT DEFICITS IN HEBB-WILLIAMS MAZE PERFORMANCE AFTER UNILATERAL ENTORHINAL CORTEX LESION. M. Glasier*, M. Goncalves, L. Janis and D. Stein. Brain Research Laboratory, I.A.B., Rutgers University, Newark, NJ 07102.

Transient deficits have been reported after unilateral entorhinal cortex (EC) lesion. To determine whether there is a more persistent deficit, adult male rats with sham (S) or electrolytic lesion (L) of the left entorhinal cortex were examined in a Hebb-Williams maze. In addition, the effect of GM1 on potential deficits was assessed. Rats received GM1 (30 mg/kg, i.p.) or saline (SAL) injections for 14 days after surgery. Starting on day 49 post-surgery, rats were tested on the Hebb-Williams Maze. Ten practice problems were followed by 12 testing problems, each problem incorporating 6 immediate retrials. Two problems/day were given, with 6 hours between problems. ANOVAs revealed that L+SAL rats were significantly (p's <.05) impaired (vs shams) on both total errors and on repeat errors over the 12 testing problems. L+GM1 did not differ significantly from shams or L+SAL. However, L+GM1 were better than L+SAL rats in total errors on 11 out of 12 testing problems (p's < .01, binomial and chi-square analyses), indicating that GM1 may be potentially useful as therapy after this lesion.

Supported by FIDIA Research Laboratories

709.15

SINGLE INTRACEREBRAL INJECTIONS OF NGF IMPROVE BEHAVIORAL PERFORMANCE FOLLOWING DAMAGE TO THE MEDIAL SEPTUM IN RATS. L.S. Janis*, G. Martins, M.M. Glasier, D.G. Stein. Brain Research Laboratory, Institute of Animal Behavior, Rutgers University, Newark, New Jersey, 07102.

Nerve Growth Factor (NGF) has been shown to reduce lesion-induced behavioral deficits by protecting damaged cholinergic neurons in the basal forebrain from degeneration following traumatic injury. Most studies reporting beneficial results have used models of repeated or continuous infusion of NGF in the brain. However, the effects of a single intracerebral NGF injection into rats with brain injury has not been as frequently studied and has produced inconsistent results. The present study examined the effects of a single intracerebral injection of NGF into rats given electrolytic lesions of the medial septum. Rats, initially trained on a win-shift radial maze task, were given the following surgeries: sham, medial septal lesion (MSL), MSL + NGF, or MSL + vehicle, and subsequently retested on the maze. Rats treated with NGF relearned the task in fewer trials and committed less errors than rats with medial septal lesions. In order to determine whether the NGF was working to restore previously learned spatial abilities the type of learning strategy used was also assessed. Preliminary evidence indicates that while a single intracerebral injection of NGF can improve behavioral performance, it is not doing so by restoring previously learned spatial strategies.

Supported by GenRe Corp. and Sigma Xi student research grant.

709.16

AUTOREGULATION OF TRANSFORMING GROWTH FACTOR-BETA 1 mRNA IN THE BRAIN BY INTRAVENTRICULAR INFUSION IN ADULT MALE RATS. C.S. Young-Chan, N.J. Laping, T.E. Morgan, C.J. Huang*, C.E. Finch, N.R. Nichols. Andrus Gerontology Center, Department of Biological Sciences University of Southern California, Los Angeles, CA 90089-0191

Transforming growth factor-Beta 1 (TGF-β1) mRNA increases in the hippocampus in response to deafferenting and neurotoxic lesions, and decreases in the hippocampus in response to glucocorticoid treatment (Morgan et al. 1993 Exp Neurol (in press), Nichols et al. 1991 J Neurosci Res). By intraventricular infusion, TGF-β1 induces hippocampal expression of prominent astrocytic and neuronal markers that are responsive to lesions and injury in the brain (Laping et al. submitted).

We show here that intraventricular infusion of TGF-β1 induces its own mRNA in the hippocampus ipsilateral to the infusion site in adult male Fisher 344 rats. Northern blot hybridization analysis of hippocampal total RNA showed a dose-dependent increase of TGF-β1 mRNA, over a range of 100 - 2000 ng of infused TGF-β1, twenty-four hours post-infusion. We observed no significant change in the mRNA level in the hippocampus contralateral to the infusion site. Apolipoprotein-E (APO-E) mRNA was also examined and shown unaffected by the infused TGF-β1. In-situ hybridization did not show distinct cell patterns of TGF-β1 expression after infusion.

Autoregulation of TGF-β1 occurs in vitro in various cell types. Our observation supports an autoregulatory mechanism for TGF-β1 in the brain. (Supported by AG-07909 to CEF, AG-05528 to NJL, AG-05589 to TEM)

709.17

TRANSFORMING GROWTH FACTOR (TGF)-β1 REGULATES THE mRNA OF COMPLEMENT PROTEINS IN THE YOUNG ADULT MALE RAT. T.E. Morgan*, N.J. Laping, and C.E. Finch. Andrus Gerontology Center, Department of Biological Sciences, University of Southern California, Los Angeles, CA 90089-0191.

TGF-β1 is a multifunctional peptide that plays an important role in peripheral wound healing. Recently, numerous laboratories have found TGF-β1 in the damaged and diseased brain. This study reports on the on-going studies investigating potential roles for TGF-β1 in the adult rat brain (Morgan et al., 1993 Exp. Neurol. in press). Previously we showed that TGF-β1 infused into the lateral ventricle of male F344 rats caused an increase in select astrocytic and neuronal cytoskeletal mRNAs (Laping et al., 1991 Soc. Neurosci. Abstr. 17:754; Morgan et al., 1992 Soc. Neurosci. Abstr. 18:1296). Because of TGF-β1's important role as an inflammatory- and immuno-regulator, we examined the mRNA levels for certain complement proteins in the rat brain after TGF-β1 infusion. Twenty-four hours after infusion of vehicle into the lateral ventricle of the rat brain, C1q mRNA levels increased nearly 2-fold in cortical areas adjacent to the infusion site and in the hippocampus as compared to intact controls. TGF-β1 (500 ng) infused into the lateral ventricle caused C1q mRNA levels to return to intact levels. The mRNA for rat brain sulfated glycoprotein-2 (SGP-2), which is the rat equivalent of the human plasma protein, complement-lysis inhibitor (CLI), also increased in these same areas in response to vehicle-infusion; however, TGF-β1 infusion further elevated SGP-2 mRNA levels. Thus, TGF-β1 may serve in a protective role against complement-mediated cell lysis in the adult brain by decreasing complement proteins and increasing complement inhibitors. Supported by NRSA AG-05589 (TEM) and AG-07909 (CEF).

709.18

TRANSFORMING GROWTH FACTOR (TGF)-β1 AND PERFORANT PATH TRANSECTION INCREASE GLIAL FIBRILLARY ACIDIC PROTEIN (GFAP) TRANSCRIPTION. N.J. Laping*, T.E. Morgan, I.R. Rozovsky, C.E. Finch. Andrus Gerontology Center, Department of Biological Sciences, University of Southern California, Los Angeles CA, 90089-0191.

To evaluate the role of transcription in increases of GFAP mRNA after lesion or TGF-β1, we examined GFAP transcription by nuclear run-on assay. Three GFAP probes were used for this analysis: a cDNA probe which began in exon 1 through the poly-A tail (Nichols et al., 1990); a probe for the entire intron I; and a probe for 600 bp of the 3' end of intron VIII. Perforant path transection increased nascent transcripts in the hippocampus, as detected by all GFAP probes 16 and 24 hours later. However, GFAP transcripts detected by intron VIII increased earlier, 2-3 fold by 4 hours. The signal for intron VIII was less than that for intron I or the full length cDNA probe at all times examined, which suggests that fewer active polymerases are between introns I and VIII. Apolipoprotein-E (APO-E) transcription rates did not change at 4, 16, or 24 hours after lesion.

To extend the in vivo effect of TGF-β1 on GFAP mRNA and to identify if TGF-β1 acts directly on astrocytes, primary astrocyte cultures were treated for 2 hours with 5 ng/ml TGF-β1 (from porcine platelets). GFAP run-on transcripts were increased 3-fold by TGF-β1, whereas those for APO-E were not. Again, the signals detected by the GFAP intron VIII probe were about 3 fold less than the cDNA or intron I probe; even so, the fold changes induced by TGF-β1 were the same for all GFAP probes. These results suggest that TGF-β1 and lesion-induced elevations of GFAP mRNA are due to transcriptional activation. (Supported by AG-07909 and J.D. and C.T. MacArthur Foundation to CEF and AG-05528 NRSA to NJL).

OTHER FACTORS AND TROPHIC AGENTS II

710.1

SCHWANN CELL PROLIFERATION AND INTERCELLULAR COUPLING ARE COORDINATELY REGULATED. K. J. Chandross*, M. Chanson, D. C. Spray and J. A. Kessler. Department of Neuroscience, Albert Einstein College of Medicine, Bx, NY 10461.

Following peripheral nerve injury, Schwann cells undergo a series of cellular alterations which assist in the regenerative process. Some of these changes are stimulated by the release of cytokines and mitogenic factors. Purified rat sciatic nerve Schwann cell cultures were therefore utilized to study phenotypic changes induced by tumor necrosis factor alpha (TNFα), a cytokine released after nerve injury, or forskolin with bovine pituitary extract (F-BPE), a combination known for its mitogenic effects. Treatment with TNFα inhibited proliferation, and reduced both gap junctional conductance (from 0.48 ± 0.09 nS to 0.09 ± 0.05 nS) and dye coupling between cells. However, treatment with TNFα did not alter cell morphology or expression of low affinity nerve growth factor receptor (L-NGFR). By contrast, treatment with F-BPE significantly enhanced Schwann cell proliferation, junctional conductance (1.0 ± 0.2 nS) and dye coupling. In addition, F-BPE altered cell morphology and reduced expression of L-NGFR. These data indicate that factors released after nerve injury induce a series of phenotypic changes in Schwann cells, including the coordinated regulation of proliferation and intercellular communication. Such mechanisms may underlie phasic Schwann cell responses which orchestrate recovery from nerve injury.

710.2

THE DIFFERENTIATION OF SCHWANN CELLS CULTURED ON MONOLAYERS DERIVED FROM AXOLEMMAL-ENRICHED FRACTIONS. R.O. Calderon, B. Maggio, T.J. Neuberger, and G.H. De Vries.* Dept. of Biochemistry & Molecular Biophysics, MCV/VCU Richmond, VA 23298-0614.

We have previously shown that axolemmal membrane forms a stable and reproducible monomolecular layer which can be transferred to cover slips providing a substratum on which Schwann cells (SC) are able to proliferate (J. Neurosci. Res. 34: 206, 1993). We now show that SC grown on these monolayers can respond by upregulating the major glycoprotein of peripheral myelin (P0) and galactocerebroside (GC). Primary Schwann cells were cultured on axolemmal monolayers and the content of DNA, P0 and GC was evaluated using of Hoechst dye as a marker for DNA, a rhodamine secondary antibody as a marker for P0, and a FITC labelled secondary antibody as a marker for GC. SC showed an approximate two-fold increase in P0 content when cultured on axolemmal membrane which had been spread at high lateral surface pressure (33 mN/m) or low lateral pressure (13 mM/m). SC grown axolemmal monolayers spread at 33 mN/m showed the majority of the increased P0 on the surface of the cell, while SC grown on axolemma spread at 13 mN/m showed increased P0 mostly in the cytoplasmic compartment of the cell. GC content was increased 2-3 fold when SC were grown on axolemmal monolayers spread at either condition of lateral surface pressure. However, SC grown on axolemmal monolayers spread at 33 mN/m showed the majority of the increased GC in the cytoplasmic compartment while SC grown on axolemmal monolayers spread at 13 mN/m showed the majority of the increased GC on the external surface. We conclude that lateral surface pressure of the axolemma could be an important factor but in determining the subsequent subcellular distribution of the molecules which increase during differentiation. (Supported by NS15408 and NS10821).

710.3

EPIDERMAL GROWTH FACTOR IS NOT TROPHIC FOR HYPOTHALAMIC NEURONS AND ASTROCYTES E.J. Morris* and H.M. Geller Department of Pharmacology, UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ 08854

Previous studies have demonstrated that in central nervous system cultures, epidermal growth factor (EGF) is an important neurotrophic and mitogenic agent. Furthermore, EGF binding sites have been localized to rat hypothalamus (Chabot et al, Soc Neurosci Abstr. 1988;14:1073). Therefore, we have investigated the functional role of EGF on developing and mature hypothalamic cultures. EGF did not enhance the survival of embryonic day 17 (E17) hypothalamic neurons in microwell cultures plated on a poly-L-lysine or a confluent astrocyte monolayer substrate. In contrast to the strong, dose-dependent, mitogenic effect demonstrated by basic fibroblast growth factor (bFGF), EGF did not enhance proliferation of E17 hypothalamic astrocytes. Also, EGF did not enhance the proliferation of mature (maintained 1 month *in vitro*) hypothalamic astrocytes. However, EGF, as well as bFGF, induced a morphological change in mature hypothalamic astrocytes from a characteristic polygonal to a stellate phenotype. These data demonstrate that the EGF receptor in the hypothalamus does not mediate trophic activity or glial proliferation *in vitro*, but may involve other phenotypic changes in astrocytes. Supported by NIH R01 NS24168.

710.4

INTERACTION OF BASIC FIBROBLAST GROWTH FACTOR AND HEPARIN AND THEIR EFFECT ON ASTROCYTIC TENASCIN PRODUCTION S. Meiners, M. Marone, J.L. Rittenhouse, and H.M. Geller*, Dept. of Pharmacology, UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ 08876

We investigated the interaction of basic fibroblast growth factor (bFGF) and heparin on the expression of the extracellular matrix protein tenascin in cultured rat cerebral cortical astrocytes. Cultures of purified protoplasmic astrocytes were established from the cerebral cortex of neonatal rats and subcultured into tissue culture flasks for Western blotting or onto glass coverslips coated with poly-L-lysine for immunocytochemistry. Basic FGF was added to the cultures at concentrations of 0.1, 1, 2.5, 5, or 10 ng/ml. Levels of tenascin were evaluated by Western blotting techniques and by immunofluorescence analysis of living cells. Basic FGF increased tenascin production in a dose-dependent manner, with the most significant effect seen at 5 ng/ml. Basic FGF treatment also caused a concurrent change in astrocyte morphology from flat and protoplasmic to fibrous. Tenascin production was increased approximately 5-fold in serum-containing medium but only two-fold in serum-free medium. When heparin was included along with bFGF in serum-free medium, tenascin expression was further enhanced. Heparin had a smaller effect in serum-containing medium, suggesting that serum may contain heparin or heparin-like molecules with a similar ability to potentiate the effects of bFGF, thereby limiting the actions of additional heparin. Alternatively, serum may upregulate heparan sulfate proteoglycans on the surface of astrocytes, which may then present bFGF to its high affinity receptor. Previous work in this laboratory demonstrated that neuronal adhesion was reduced on bFGF-treated cultures (Petroski *et al.*, 1991); these results, interpreted in light of the current study, suggest that a bFGF-mediated induction of astrocytic tenascin may be inhibitory to neuronal growth. It is our hypothesis that the action of bFGF during injury may invoke the induction of tenascin on astrocytes, thereby reducing regeneration in the central nervous system.

710.5

INFLUENCE OF GRANULE CELLS ON THE SURVIVAL AND DIFFERENTIATION OF PURKINJE CELLS IN DISSOCIATED CEREBELLAR CULTURES. M.E. Dunn* and E. Mugnaini. Lab. of Neuromorphology, The University of Connecticut, Storrs, CT 06269-4154.

The cerebellar Purkinje cell (PC) is an excellent model for the study of neuronal development because of its stereotypical features and well known intercellular relationships. Deafferentation studies *in vivo* have suggested that the parallel fibers, which are the axons of the cerebellar granule cells (GCs), influence the PC by unknown mechanisms. We have studied PCs grown in dissociated cultures from the E14-E16 mouse cerebellar anlagen. These cultures contain a relatively small density of granule cells. We have analyzed the effects of the addition to the PC cultures of GCs derived from the postnatal cerebellum. Antibody to the calcium binding protein calbindin was used to identify the PCs. We report that the addition of GCs promotes the survival and differentiation of the dissociated PCs. Specifically, the GCs enhance the number of viable PCs, as well as the extent of the PC dendritic arbor and the degree of its dendritic branching after 3 weeks in vitro. Moreover, these effects tend to become more pronounced with increasing numbers of GCs. Ultrastructural examination demonstrates the presence of synaptic contacts between the PC dendrites and the GC axons. Since glutamate exerts trophic influences on receptive neurons in certain models, we are currently investigating the effect(s) of glutamate on the dissociated PCs to determine if this is the factor responsible for the influence of the glutamatergic GCs. Because glutamate has a trophic effect on dissociated GCs, we must further distinguish between direct effects of glutamate on PCs and indirect effects on PCs via the enhancement of GC survival.
Supported by PHS grant NS-09904.

710.6

K252A INDUCES TYROSINE PHOSPHORYLATION AND DIFFERENTIATION IN SY5Y CELLS INDEPENDENTLY OF PROTEIN KINASE-C INHIBITION. Anna M. Maroney, M. Elizabeth Forbes, Marcie A. Glicksman, Lorraine Lipfert, Nicola Neff, Robert Siman, John M. Farah*, and Craig A. Dionne Cephalon Inc., 145 Brandywine Pkwy., West Chester, PA 19380

K252a, an indolocarbazole derivative initially described as a protein kinase C inhibitor, has been shown to promote cholinergic activity in rat spinal cord cultures and survival in chick dorsal root ganglion (Glicksman, *et al.*, J. Neurochem., in press, 1993; Borasio, Neurosci. lett. 108:207-212, 1990). To determine the mechanism by which K252a behaves as a neurotrophic factor we examined the effects of this molecule in a human neuroblastoma cell line, SH-SY5Y. In this cell line, K252a induces neurite outgrowth in a dose-dependent manner. One mechanism by which polypeptide neurotrophic factors transmit their signal is via tyrosine phosphorylation of a variety of substrates. Similarly, K252a induced tyrosine phosphorylation of high molecular weight membrane-associated proteins in the range of 125-180 kDa in a dose and time dependent manner. Tyrosine phosphorylation of several other known proteins has also been demonstrated. These phosphorylation events are independent of protein kinase C (PKC) inhibition since down regulation of PKC by long term incubation with phorbol esters does not block the effect of K252a. In addition, the PKC inhibitors H7 and calphostin do not induce similar tyrosine phosphorylation in the SY5Y cells. K252a induces phosphorylation in responsive neuronal cells such as LAN-5 and SK-N-MC cells but does not induce neurite outgrowth or tyrosine phosphorylation in PC12 cells. We have thus identified an early K252a-induced biochemical event which is reminiscent of the early events triggered by polypeptide growth factors.The survival-promoting effects of K252a in primary cultures suggest that K252a may be a promising agent with therapeutic application to spinal cord injury. The efficacy of K252a may be mediated by activating tyrosine phosphorylation of second messengers that are similarly modified by other growth factors.

710.7

OSTEOGENIC PROTEIN-1 REGULATES L1 AND N-CAM GENE EXPRESSION IN NEURAL CELLS. G. Perides*, G. Hu, D. C. Rueger, M. E. Charness. Depts Pathology, Neurology (Neuroscience) Harvard Medical School, VA Medical Center, West Roxbury, MA 02132; Creative Biomolecules, Hopkinton MA 01748.

Little is known about the regulation of the immunoglobulin cell adhesion molecules (IgCAM) N-CAM and L1. Osteogenic protein-1 (OP-1) is a member of the TGF-ß superfamily that is expressed in the nervous system. We recently showed that human recombinant osteogenic protein-1 (hOP-1) strongly induces the aggregation of dividing neuroblastoma x glioma hybrid NG108-15 cells, in part by inducing the major isoforms of N-CAM (Perides *et al. 1992*, Proc. Natl. Acad. Sci. USA, 89, 10326). Here we show that hOP-1 also induces L1 expression in NG108-15 cells without changing the levels of N-cadherin, neurofilament 200, thy-1, tau, and $G\alpha_S$. The increased adhesiveness of hOP-1-treated NG108-15 cells was inhibited in part by Fab fragments of an anti-L1 polyclonal antiserum. The morphoregulatory and IgCAM-inducing effects of hOP-1 were unassociated with changes in cell proliferation and were not reproduced by cellular differentiation, TGF-ß, phorbol esters, or forskolin. L1 and N-CAM expression first increased 12 to 18 hours after hOP-1 treatment, reached a maximum after 2-3 days, persisted for up to 5 days, and returned to control levels 3 days after hOP-1 withdrawal. The increases in IgCAM protein levels were preceded by large increases in the abundance of L1 and N-CAM mRNAs. Actinomycin D prevented the induction by hOP-1 of L1 and N-CAM mRNAs, suggesting that hOP-1 regulates IgCAM gene transcription. hOP-1 is the first described growth factor that regulates both N-CAM and L1 gene expression.
Supported by AA06662, NS13034, Department of Veterans Administration.

710.8

CHARACTERIZATION, TRANSFECTION, AND TRANSPLANTATION OF MITOGEN-EXPANDED SECONDARY O-2A GLIAL PROGENITOR CELLS. R.D. McKinnon*, RW.Johnson Med.School, UMDNJ, Piscataway NJ08854.

The ability to expand and manipulate primary oligodendrocyte type-2 astrocyte (O-2A) glial progenitor cells *in vitro*, then transplant altered cells for analysis *in vivo*, was examined. Secondary rat cortical O-2A progenitor cells, expanded from primary cultures with mitogens from neuroblastoma B104 conditioned media (CM), responded to recombinant growth factors similar to both primary (non-passaged) O-2A cells and to an O-2A cell line, Central Glial-4 (CG-4) cells. The three populations showed: (i) continued self renewal in media supplemented with either basic Fibroblast Growth Factor (bFGF) or B104-CM, (ii) differentiation in absence of these factors; (iii) induction of a bipolar (motile) morphology in response to either Platelet-Derived Growth Factor (PDGF, AA-homodimeric form) or to B104-CM; (iv) production of Transforming Growth Factor-β (TGF-β), (v) TGFβ-mediated inhibition of mitogen-driven proliferation (PDGF, B104-CM, to a lesser extent bFGF); and (vi) production of a non-TGFβ inhibitor of proliferation, as determined by its activity on TGFβ-resistant cells. Efficient transient DNA transfection was achieved using the 'calcium technique', and infection with retroviral vectors encoding reporter genes (alkaline phosphatase, β-galactosidase) and selectable markers ($G418^R$) allowed the isolation of 'marked' O-2A progenitor cells. The migration and differentiation of marked cells after transplantation into neonatal brain confirmed the utility of B104-CM for preserving the phenotype of O-2A progenitors during their expansion and manipulation *in vitro*. Supported by a Pilot Research Award from the US Nat. Multiple Sclerosis Society.

710.9

NADPH-d REACTIVITY IN ADULT AND METAMORPHOSING XENOPUS LAEVIS SPINAL CORD. M.J. Crowe*, T.J. Brown, J.C. Bresnahan, M.S. Beattie. Neuroscience Program, Dept. of Cell Biology, Neurobiology, and Anatomy, The Ohio State Univ., Columbus, OH 43210

The histochemical NADPH diaphorase (NADPH-d) reaction has long been used to describe distinct populations of neurons in the CNS of several species. Recent evidence has shown that NADPH-d is actually a neuronal nitric oxide synthase (NOS). We examined the spinal cords of adult and metamorphosing (stage 51-62) *Xenopus laevis* (XL) using this procedure. In metamorphosing XL, NADPH-d reactive cells were detected throughout the spinal cord. Large primary motoneurons (MNs) in the medial ventral horn were individually labelled as well as groups of secondary MNs in the lateral motor columns (LMCs) which are present only in the lumbar and cervical enlargements. An accumulation of smaller labelled neurons were seen in the intermediate grey area between the ventral and dorsal horns. In adult XL spinal cords, no MNs or LMCs were stained, although we did observe reactive cells in the intermediate grey. In mammalian species NOS is a potent vasodilator, however we observed no reactivity in blood vessels of either adult or metamorphosing XL. All neurons in the dorsal root ganglia (DRGs) of both adult and metamorphosing XL were stained. This is in contrast with what we have seen in the rat, where only a subpopulation of small-to-medium diameter cells were labelled. The lack of NADPH-d reactivity in adult XL MNs compared to that observed in metamorphosing XL suggests that NOS may be related to processes involved in cellular differentiation in XL spinal cord. Supported by NS07291 and NS10165.

710.10

CDF/LIF-DEFICIENT MICE DISPLAY AN ALTERED COMPLEMENT OF NEURONAL PHENOTYPES IN THE CNS. P. H. Patterson*[1], L. Bugga[1], and C. L. Stewart[2]. [1]Biology Division, Caltech., Pasadena, CA 91125, [2]Roche Institute, Roche Research Center, Nutley, NJ 07110.

The cholinergic differentiation factor (CDF; also known as leukemia inhibitory factor, LIF) is a pleiotropic cytokine that can regulate the survival and gene expression in a variety of peripheral neurons in culture. To investigate the role of this cytokine further, we are examining the brains of mice in which this gene was deleted by homologous recombination. The mutants do not display obvious behavioral abnormalities nor do their brains express gross morphological defects. To search for differences at the cellular level, we are comparing sections from wild type and CDF/LIF-deficient brains after staining for a variety of neuronal differentiation markers. One clear difference is that staining for the neuronal calcium-binding proteins calbindin and parvalbumin is deficient in the visual cortex of the homozygous mutants. This is intriguing because we find that rat visual cortex has particularly high levels of both CDF/LIF and CDF/LIF receptor mRNAs (Banner and Patterson, Soc. Neurosci. Abstr., '93). It is not yet clear if the difference in staining is due to a loss of this subset of neurons or to a phenotypic switch. Dorsal root ganglia from the mutants also display a difference in staining for these calcium binding proteins (and are CDF/LIFR$^+$). This evidence supports a role for CDF/LIF in the regulation of phenotype and/or survival of selected populations of CNS neurons *in vivo*.

710.11

NORADRENERGIC TRANSMISSION INFLUENCES SWEAT GLAND CHOLINERGIC DIFFERENTIATION FACTOR PRODUCTION. Beth A. Habecker* and Story C. Landis. Dept. of Neurosciences, Case Western Reserve Univ. Cleveland, OH 44106

During normal development, the sympathetic innervation of rat sweat glands changes from a noradrenergic to cholinergic phenotype in response to soluble factors released from the glands. Sweat gland cells cultured from postnatal day 7-10 rats do not secrete cholinergic inducing activity, but gland cells co-cultured with sympathetic neurons induce choline acetyltransferase (ChAT) activity in the neurons. We find that extracts from cultured sweat gland cells do not induce ChAT activity in cultured sympathetic neurons, indicating that synthesis of sweat gland differentiation factor is induced by co-culture with neurons. In addition, conditioned medium from sweat gland/ sensory neuron co-cultures does not induce ChAT activity, raising the possibility that noradrenergic transmission mediates the neuronal induction of a differentiation factor in gland cells. PCR and Southern analyses indicate that α1 adrenergic receptors are present in footpads during the first two weeks of postnatal development, but their expression drops by day 21, corresponding with decreased levels of catecholamines in the sweat gland innervation. In contrast, α2 and β2 receptors are present in footpads from early development through adulthood. All these receptor subtypes are expressed in cultured sweat gland cells. Treatment of sweat gland/sympathetic neuron co-cultures with the β antagonists (-)propranolol or (-)alprenolol significantly blocks the induction of ChAT activity in the co-cultures, while the α antagonists prazosin (α1) and yohimbine (α2) inhibit ChAT induction to a lesser extent. Further, conditioned medium from propranolol treated co-cultures does not induce ChAT activity in neuron cultures, suggesting that adrenergic blockade is directly affecting sweat gland cells rather than neurons. These results suggest noradrenergic transmission stimulates production of a sweat gland differentiation factor which in turn alters neuron phenotype.

710.12

INTERFERON-γ (IFN-γ) TREATMENT OF CORTICAL ASTROCYTES INDUCES A FACTOR THAT PROMOTES CHOLINERGIC DIFFERENTIATION OF BASAL FOREBRAIN. G.M. Jonakait and L. Ni*. Dept. Biol.Sci., Rutgers Univ., Newark, N.J. 07102

We have shown previously that IFN-γ promotes cholinergic differentiation in cultured embryonic cells derived from the septal nucleus and adjacent basal forebrain (SN/BF; Jonakait & Ni, 1992).

Since this population of cells is well known for its responsiveness to both nerve growth factor (NGF) and fibroblast growth factor (FGF), we have sought to determine 1) whether the effect of IFN-γ is mediated by a soluble intermediate and 2) whether that intermediate is NGF or basic FGF. Since antibodies against NGF and FGF do not alter the action of IFN on cultured cholinergic neurons, these are probably not important intermediates in the IFN induction of ChAT.

However, an IFN-induced, glial-derived intermediate does exist. This molecule(s), derived from medium conditioned by IFN-treated astrocyte cultures (IFN-CM), raises ChAT activity in E16 SN/BF cultures as much as 15-fold. Its activity is not neutralized by antibodies against IFN. It is very potent, retaining activity at dilutions as low as 10%. Since bFGF, even at doses as high as 100 ng/ml, cannot mimic the action of IFN-CM and since antibodies against FGF do not neutralize its action, the active factor is probably not FGF.

Supported by the Charles and Johanna Busch Bequest.

710.13

ChAT-INDUCING BIOACTIVITY FROM EARLY POSTNATAL RAT HINDQUARTERS IS PRIMARILY CNTF. S. L. Meyer*, E. Knight, Jr., M. J. Hansbury, D. M. Lang, and N. T. Neff. Cephalon, Inc., West Chester, PA 19380.

Extracts prepared from the hindquarters of 14-day-old rats contain factors that enhance ChAT activity in both E14 rat spinal cord cultures and IMR-32 neuroblastoma cells. Our primary goal was to purify and characterize the ChAT-inducing activity in these extracts, using IMR-32 cells as the assay cell. A second goal was to determine if the activity was due to known ChAT-inducing factors, particularly CNTF. CNTF enhances ChAT activity in IMR-32 cells and E14 rat spinal cord cultures at concentrations < 1 ng/ml. The ChAT-inducing activity was partially-purified 3,000-fold from a crude extract of rat hindquarters using ammonium sulfate precipitation followed by chromatography on DEAE-Sepharose, SP-Sephadex, Affi-gel blue and heparin Sepharose. During these studies, a potent neutralizing rabbit antibody was made to recombinant rat CNTF. This highly-specific antibody neutralized greater than 90% of the 3,000-fold purified ChAT-inducing activity assayed on IMR-32 cells. Furthermore, 90% of the ChAT-inducing activity in the crude extract prepared from rat hindquarters was neutralized by CNTF antibody. In addition, immunoblot analysis of the 3,000-fold purified fraction using the CNTF antibody revealed sufficient CNTF to account for all the ChAT-inducing activity. It is concluded that the majority of the ChAT-inducing activity in extracts of rat hindquarters as measured on IMR-32 cells is due to CNTF. Given the potency of CNTF and the ubiquitous contribution of peripheral nerve to postnatal muscle, muscle-derived ChAT-inducing factors would have to be isolated from either embryonic muscle or cultured muscle cells.

710.14

DIFFERENTIAL EFFECTS OF PLATELET-DERIVED GROWTH FACTOR-AA AND -BB ON DOPAMINE NEURONS IN VIVO MMJ Giacobini*, S Almström, K Funa[1], L Olson, Dept. of Histology & Neurobiology, Karolinska Institute, Stockholm, and [1]Ludwig Institute for Cancer Research, Biomedical Center, Uppsala, Sweden

Trophic effects of platelet-derived growth factor-AA and -BB (PDGF-AA, PDGF-BB) were followed in developing rat embryonic day 14 ventral mesencephalic grafts under chronic intermittent treatments with either PDGF-AA or PDGF-BB in the anterior eye chamber of adult rat hosts. Pieces to be grafted were incubated in either 100 ng PDGF-AA/ml buffer, 100 ng PDGF-BB/ml buffer or the buffer vehicle alone and 5 µl of similar solutions were injected intraocularly on day 5, 10 and 15 postgrafting. Host animals were sympathetically denervated 2 weeks prior to grafting enabling evaluation of catecholaminergic fiber outgrowth onto the host iris in whole-mount preparations by use of the Falck-Hillarp technique. Morphological studies of PDGF effects on grafted brain tissue were carried out using markers for tyrosine hydroxylase- and glial fibrillary acidic protein- immunoreactivity. Growth of grafts was followed by repeated observations directly through the cornea of the host using a stereomicroscope. This revealed that there was no apparent effect on volume increase of mesencephalic grafts after the different treatments. However, PDGF-AA significantly enhanced dopaminergic fiber outgrowth from mesencephalic grafts when compared to both PDGF-BB and controls, without an accompanying rise in the number of tyrosine hydroxylase-positive neurons or astroglial elements. In contrast, a significantly greater number of tyrosine hyroxylase-positive neurons were seen in grafts treated with PDGF-BB but without an accompanying increase in outgrowth of fibers. These findings suggest that PDGF-AA may enhance formation of dopamine nerve fibers *in vivo* whereas PDGF-BB may be a maintenance factor for tyrosine hydroxylase-positive cells of the ventral mesencephalon.

710.15

TRANSFORMING GROWTH FACTOR ALPHA GENE EXPRESSION AND THYROID HORMONE REGULATION IN DEVELOPING BRAIN. K.R. Huff* and Q.-X. Yuan. Harbor/UCLA Med Ctr, Dept of Pediatrics. Torrance, CA 90509.

Transforming Growth Factor alpha (TGFa) is a member of the EGF family of growth factors and is stimulatory of many nontransformed cell types. It may also be a ligand for the EGF receptor. EGF mimics and may mediate precocious maturational effects of thyroid hormone (T4) treatment in tissues outside the brain. We have examined TGFa expression and the effects of T4 treatment in the rat, an animal with relative immaturity of brain development and T4 levels at birth.

Neonatal rats were treated days 1-6 of life with T4 or vehicle control. They were sacrificed at various ages for later brain part RNA isolation. TGFa RNA (mRNA) was detected by polymerase chain reaction amplification of cDNA synthesized by reverse transcriptase. The appropriate mRNA was identified by base pair number and positive control. TGFa mRNA was made undetectable by T4 treatment at day 1 in cerebral cortex and brain stem and day 3 in cerebellum. This result correlates with T4 lowering by 60% TGFa protein by RIA to adult values at this age.

We conclude a maturational effect of T4 on TGFa at the gene transcriptional level.

710.16

EXPRESSION OF MEMBERS OF THE NEU (ARIA) LIGAND FAMILY IN CHICK AND RAT CENTRAL NERVOUS SYSTEM

Y. Kuo*, X. Yang, and L. Role. Dept. Anat. & Cell Biol. in the Ctr. Neurobiol & Behav, Columbia Univ, P&S, 630 W 168th St, NY,NY 10032.

Innervation of muscle by motor neurons is accompanied by increased transcription of the subunits that comprise the muscle type nicotinic AChRs. AChR induction is apparently mediated by a soluble factor synthesized and released from motoneurons. Fischbach and colleagues (Cell 72, 5:801-805) have recently reported the cloning of an AChR inducing activity called ARIA which is a member of a family of growth factors homologous to the neu ligand heregulin that includes the glial growth factors and rat Neu differentiation factor. We are interested in the regulation of neurotransmitter receptor expression in cholinoceptive neurons induced by presynaptic input. Our previous studies demonstrate that preganglionic neurons, somatic motor neurons, or media conditioned by either of these cell types increases the magnitude of ACh evoked currents and the number of new surface AChRs in sympathetic neurons. To test the hypothesis that homologs of ARIA can increase neuronal nAChR gene transcription we must identify those homologs and test them for nAChR inducing activity in neurons. PCR screening of rat spinal cord cDNA using oligos from conserved regions of members of this family revealed a fragment whose combination of exons suggest it is a member of the neu family with strongest homology to human heregulin β1. The fragment demonstrates 97% sequence identity in both the Ig-like and the EGF domains. Likewise, screening of a chick cDNA library with this probe at high stringency yielded several related clones. Experiments to express and test for functional effects of these heregulin/ARIA homologs are underway. (Supported by NS 29071)

710.17

EXPRESSION OF THE MYELIN TRANSCRIPTION FACTOR I IN DEVELOPING RAT NERVOUS SYSTEM. M.A.R. Dent, R. Mirsky, K.R. Jessen and L. Hudson, SPON: Brain Research Association. Department of Anatomy, UCL, Gower Street, London WC1E 6BT and NIMDS, Bethesda, Maryland 20892.

Myelin transcription factor I (MyTI) is a novel zinc finger protein that binds to a previously defined cis element of the human PLP promoter. We have studied the expression of MyTI in the rat nervous system by in situ hybridization. Strong expression was observed in the dorsal root ganglia and the spinal cord at embryonic day (E) 13, 14, 16, and 18. At E18 it appeared absent from Schwann cells in peripheral nerves. In the adult spinal cord MyTI is observed mainly in the neurons but there is also some expression in the cells of the white matter. In the brain low levels of MyTI are expressed at E13 and highest levels are observed in the cortical plate by E18. There is also some expression of MyTI in the adult brain.

710.18

DIFFERENTIAL EFFECTS OF LEAD ACETATE ON DEVELOPMENTALLY-REGULATED BRAIN GENE EXPRESSION N.H. Zawia* T. Schmitt and *G. J. Harry, Environmental Immunology & Neurobiology, NIEHS, P.O. Box 12233, RTP, NC 27709.

Lead is a neurotoxicant that is known to produce behavioral, biochemical and structural abnormalities in brain development. Exposure to high levels of lead can produce hypomyelination in the neocortex and fine structural changes in the developing hippocampus. Development and growth are marked by precise differential gene regulation. The objective of these studies was to examine whether stage-specific developmental gene expression was perturbed by lactational exposure to low levels of lead acetate (0.2 % in the drinking water of the dam begun after parturition). Hippocampal and neocortical tissue was obtained from Long Evans hooded rats on postnatal days (PND): 5, 10, 15, 20. No changes in either brain structure and weight or animal body weights were observed in the pups following such exposure to lead. Total RNA was isolated and probed for myelin basic protein (MBP), growth associated protein (GAP-43) and actin gene expression by Northern analysis. A transient repression of hippocampal GAP-43 gene expression was observed on PND 5,10 and 15 with a return to above normal levels by PND 20. Levels of neocortical MBP gene expression peaked prematurely on PND 10 in lead-exposed animals while expression of the actin gene appeared unaltered by exposure to lead. These data suggest that low levels of lead may selectively influence developmental gene expression and may therefore contribute to lead-induced neuropathology.

710.19

SPECIFIC ROLES FOR RETINOIDS IN DEVELOPING AND ADULT BRAIN FUNCTION SUGGESTED BY THE TEMPORAL AND REGIONAL DISTRIBUTIONS OF THE CELLULAR RETINOID-BINDING PROTEINS CRBP I AND CRABP I AS REVEALED BY IMMUNOHISTOCHEMISTRY. Rolf Zetterström, Lars Olson*, András Simon[1], MaiBritt Giacobini, Ulf Eriksson[1]. Department of Histology & Neurobiology, Karolinska Institute and [1]Ludwig Institute for Cancer Research, Stockholm Branch, Stockholm, Sweden.

In cells, retinol (vitamin A) and its active metabolite retinoic acid are bound to cellular retinol-binding protein (CRBP) and cellular retinoic acid-binding protein (CRABP) respectively. We have used immunohistochemistry to localize the possible presence of the two retinoid-binding proteins in the central nervous system. We find a widespread, yet distinct, presence of these two binding proteins in the adult rat central nervous system. Most of the immunoreactivity is neuronal, including somata and processes. CRBP-immunoreactivity (IR) is also found in the walls of cerebral blood vessels, the meninges, the choroid plexus, certain ependymal cells, tanocytes, and certain other glial elements. The CRBP- and CRABP-IR patterns appear to be almost exclusively non-overlapping. Very strong CRBP-IR is found in the dendritic layers of the hippocampal formation and dentate gyrus. CRBP-IR is also present in layer 5 cortical pyramidal neurons. Many subcortical areas, most notably in the hypothalamus and amygdala areas contain networks of varicose CRBP-IR nerve fibers. The medial amygdaloid nucleus contains strongly CRBP-positive neurons. CRABP-IR is more restricted in the adult brain. Strong CRABP-IR is however found in a population of neurons scattered throughout striatum. The developing CNS contains many more CRBP- and CRABP-IR structures than the adult CNS. Striatum of the newborn rat is strongly CRBP-IR in a typical patch/matrix manner, while at the same time containing evenly distributed CRABP-IR neurons. The remarkably selective patterns of CRBP- and CRABP-immunoreactivity suggest that retinol and retinoic acid have important roles in normal brain function. The high levels of CRBP-IR found in hippocampus suggests that one such role might relate to brain plasticity.

710.20

EPIGENETIC IMPRINTING OF ANTI-BRAIN IMMUNITY: POSSIBLE SIGNIFICANCE IN PSYCHONEUROLOGY. A.B. Poletaev*, O.P. Selifanova. Chernobyl-Test Ctr. 10 Rimskogo-Korsakova St., 127577, Moscow, Russia.

Relationship between the presence of antibodies to brain specific proteins of S-100 family and brain development was studied in animal models and in the clinic.

Pregnant Wistar rats were injected with polyclonal antibodies to S-100 protein. Control group received equivalent amount of antibodies to bovine serum albumin. An elevated level of anti S-100 immunoreactivity was detected by ELISA in the serum of 1 month old offsprings of group treated with antibodies to S-100 compared to the control group. Ultrastructural analysis of newborn offsprings brain revealed the delay of glial cell maturation. However no signs of delay were detected in the control group.

Occurrence of antibodies to S-100 proteins was examined in the blood of children with various psychoneurological diseases and their parents. Large deviation in the serum anti S-100 immunoreactivity were detected in more than 80% of children with heavy cerebral palsy or oligophrenia and in their clinically healthy mothers, but not in their fathers.

The obtained data indicate the possibility for inborn "imprinting" of specific autoimmune reactions which may be important in the development of certain forms of diseases in the nervous system.

710.21

CHARACTERIZATION OF PROTEINS IMMUNOLOGICALLY SIMILAR TO CELLULAR RETINOIC ACID AND CELLULAR RETINOL BINDING PROTEINS (CRABP AND CRBP) IN THE CNS OF *MANDUCA SEXTA* AND *DROSOPHILA MELANOGASTER*. D. P. Muehleisen[1], R. S. Gray[1], A. L. Westbrook[1], Z. F. Wang[1], F. Chytil[2], D. E. Ong[2], and W. E. Bollenbacher[1]*, [1]Department of Biology, University of North Carolina at Chapel Hill, Chapel Hill, NC, 27599-3280, [2]Department of Biochemistry, Vanderbilt University, Nashville, TN, 27232.

The highly conserved vertebrate CRABP and CRBP are believed to be cytoplasmic proteins that specifically bind retinoic acid (RA) and retinol, respectively. While their functions are not understood, they are thought to have roles in development by determining RA gradients and in modulating RA's action. Affinity purified rabbit anti-rat CRABP and rabbit anti-human CRBP were used to identify CRABP and CRBP immunoreactive embryonic and post-embryonic CNS neurons in *Manduca sexta*, and post-embryonic neurons in *Drosophila melanogaster*. Genomic southern blots of *Drosophila* and *Manduca* DNA hybridize with a fragment of a bovine CRABP cDNA and rat CRBP cDNA, revealing both insects have genes with high sequence similarity to the vertebrate CRABP and CRBP genes. The organization of the CRBP and CRABP neurons and their functions are being investigated.

710.22

POSSIBLE ROLE OF CGRP IN THE DIFFERENTIATION OF VASCULAR SMOOTH MUSCLE CELLS. J.-L. Connat*, V. Schnüriger, A. Thiévent, A. L. Quiquerez. Lab. d'Anatomie et Physiologie Comparées, Univ. de Genève, 30 quai E. Ansermet, 1211 GENEVE 4, Switzerland.

We studied the differentiation of the wall of the rat hepatic portal vein and possible correlations with the occurence of CGRP innervation. Electron microscopy, as well as immunocytochemistry for marqueurs of smooth muscle cell (SMC) differentiation (desmin and α-smooth actin) demonstrated a differentiation of the muscular layers when CGRP innervation occured at their vicinity. In order to check if this temporal correlation have a physiological meaning, we cultured SMC from rat thoracic aorta and added 10-7M CGRP in the culture medium. We examined the effects on cell proliferation and on expression of α-smooth actin 2,4,6,8 and 15 days after seeding. We noted that 2 differents cases were available.(1) Certain cells which proliferated slowly had their growth rate positively influenced by CGRP, even in presence of 10% FCS in the medium. The quantity of α-smooth actin expressed by these cells was not influenced by the peptide. (2) Some other cells proliferated more rapidly, and their growth rate in 10% FCS medium was inhibited by CGRP. In certain cases, presumably in cells with highest growth rate, CGRP reduced significantly the amount of α-smooth actin expressed by the cells. We also noted an effect of the peptide on the level of mRNA for actin. We tested in the same conditions the effect of CGRP on cultured fibroblasts from the vascular adventitia. No effect was observed in the proliferation tests, neither with cells cultured with 1% FCS, nor with 10% FCS. It is the first clear demonstration of the action of a neuropeptide on the state of differentiation of a tissue, namely the vascular smooth muscle. This suggest that the autonomic nervous system could play a role in the maintenance of tissues integrity.

710.23

EXPRESSION AND CHARACTERIZATION OF HUMAN GROWTH/DIFFERENTIATION FACTOR 1. M. Hendricks, C. Lynch, B. Happel, C. Hoban, C. Kirk, R. Sklar, L. I. Mortin*, I. Isaacs, M. S. Chen and D. Gwynne. Cambridge NeuroScience, Inc., Cambridge, MA 02139

Growth/differentiation factor-1 (GDF-1) is a novel member of the transforming growth factor-β (TGF-β) superfamily and is expressed almost exclusively in the nervous system (Lee, Proc. Natl. Acad. Sci. USA 88: 4250-4254, 1991). In the present study we further resolve the expression of GDF-1 to specific cells of the nervous system and express recombinant human GDF-1 (rhGDF-1) for protein characterization and identification of biological activities.

Immunohistochemical staining of rat hippocampal cultures in vitro with GDF-1 polyclonal antisera showed immunoreactivity specifically with neurons. Ongoing immunostaining of rat brain and spinal cord sections coupled with in situ hybridization studies will further elucidate specific patterns of expression in the nervous system.

Expression of rhGDF-1 in Chinese hamster ovary (CHO) cells demonstrates that it is a secreted protein, as predicted by the presence of an N-terminal signal peptide. Western blot analysis indicates that rhGDF-1 is processed in a manner consistent with cleavage at a RPRR cleavage recognition site to give a 15 kD mature fragment and a 28 kD pro fragment. Native GDF-1 in a mouse brain extract shows a similar processing pattern. Testing of purified rhGDF-1 in various in vitro bioassays is in progress and will help to elucidate the role of this factor in the nervous system.

OTHER FACTORS AND TROPHIC AGENTS III

711.1

EFFECT OF TETRAHYDROBIOPTERIN ON THE PROLIFERATION AND DNA SYNTHESIS OF PC12 CELLS. P.Z. Anastasiadis*[1], J.C. States[3], D.M. Kuhn[2], and R.A. Levine[1,2,4]. [1]William T. Gossett Neurology Labs, Henry Ford Hosp., Detroit, [2]Cellular & Clinical Neurobiology Prgm, Psychiatry, and [3]Center for Mol. Biology, Wayne State Univ., Detroit, MI, [4]Vet. Admin. Med. Center, Allen Park, MI.

Tetrahydrobiopterin (6R-BH_4; cofactor for tyrosine and tryptophan hydroxylases, in catecholamine and serotonin synthesis) is required for the proliferation of cultured murine erythroleukemia cells. BH_4 effects on DNA synthesis and proliferation of cultured rat pheochromocytoma (PC12) cells were tested. BH_4 inhibited (dose-dependently) PC12 cell proliferation; at high concentrations (500 μM) BH_4 was cytotoxic. BH_4 also decreased tritiated thymidine incorporation in PC12 cell DNA; only 6R-BH_4 was active (oxidized pterins and 6S-BH_4 were not). Interferon-γ (IFN-γ) inhibits DNA synthesis in PC12 cells and induces BH_4 biosynthesis; its effects on DNA synthesis were tested under conditions modulating intracellular BH_4. Inhibition of DNA synthesis was additive when cells were incubated for 24 hours with IFN-γ (250-2000 U/ml) and sepiapterin (200 μM; converted to BH_4 inside the cells). N-acetyl-serotonin (NAS), an inhibitor of BH_4 biosynthesis, partially reversed the sepiapterin-induced effect but did not block the IFN-γ-induced inhibition of DNA synthesis. These results suggest that BH_4 may regulate the rate of DNA synthesis in PC12 cells. The IFN-γ- and BH_4-induced effects on PC12 cell DNA synthesis may not be mediated by the same mechanism. Further studies focus on the mechanism by which BH_4 affects DNA synthesis in PC12 cells.

711.2

GROWTH FACTOR REGULATION OF NEURONAL NA CHANNEL EXPRESSION IN PC12 CELLS OVEREXPRESSING RECEPTORS FOR NGF, BDNF, AND PDGF. R.A. Maue* AND G. R. Fanger. Depts of Physiology and Biochemistry, Dartmouth Medical School, Hanover, NH 03755-3833.

The biological activity of growth factors acting through receptor tyrosine kinases can differ dramatically, resulting in growth and proliferation, or cessation of cell division and differentiation. These diverse effects appear to be detemined by both the cellular environment in which the receptors are expressed and the properties of the receptor tyrosine kinase. To investigate the specificity of different receptor tyrosine kinases with regard to neuronal differentiation, we have analyzed sodium (Na) channel expression in rat pheochromocytoma (PC12) sublines stably overexpressing *trk* (6-24, 6-15; Hempstead et al., Neuron 9(1992):883), overexpressing *trk*B (PC12/*trk*B, Ip et al., Neuron 10(1993):137), and overexpressing the β-PDGF receptor (PC-PDGFR-102, PC-PDGFR-111; Heasley and Johnson, Mol. Biol. Cell 3(1992):545). This allows the actions of a number of receptor tyrosine kinases, including those for EGF and bFGF that are already expressed in PC12 cells, to be compared in the same neuronal cell environment. Northern blot analysis of total RNA from control and NGF-treated cells revealed 3- to 5-fold increases in the steady state level of Na channel mRNA in both PC12 and the *trk*-overexpressing 6-24 and 6-15 cells. While there were no dramatic differences in the time course or extent of induction in the 6-24 and 6-15 cells when compared to the parental PC12 cells, basal levels of Na channel mRNA in the 6-24 and 6-15 cells appeared to be elevated, remniscent ofperipherin gene expression in these cells. Whole-cell patch clamp analysis of functional Na channel expression in untreated and PDGF-treated PC-PDGFR-111 cells revealed 3- to 4-fold increases in Na current density, comparable to that occuring in the parental PC12 cells, and to that occurring in the PC-PDGFR-111 cells treated with NGF. The results of studies like these will help define the degree specificity that exists among the receptor tyrosine kinases, provide a basis for identifying neuronal specific aspects of the intracellular signalling associated with their activation, and will provide the basis for mutational studies designed to investigate the mechanisms responsible for promotion of a neuronal phenotype by tyrosine kinases. Supported by NIH NS28767.

711.3

TROPHIC INFLUENCES OF MELANOTROPE CELLS AND MELANOCORTINS FOR DOPAMINERGIC NEURONS IN CULTURES OF RAT HYPOTHALAMUS. F. René[1], J.M. Félix[1], S. Varon[2] and J.C. Louis*[2]. I.P.B.C., Louis Pasteur Univ., 67000 Strasbourg, France[1] and Department of Biology 0601, University of California, San Diego, La Jolla, CA 92093[2].

The melanotrope cells of the intermediate lobe of the pituitary (IL) are selectively innervated by dopaminergic (DA) neurons of the rostral periventricular hypothalamus. We tested the hypothesis that IL melanotropes influence the development of DA neurons. We cultured rat IL cells together with neonatal rat hypothalamus and assessed their influence on tyrosine hydroxylase (TH) expression and on the morphology of DA neurons. We found that the number of TH-positive neurons after 7 days *in vitro* was significantly higher (~2-fold increase) in IL-hypothalamus co-cultures, as compared with hypothalamus cultured alone. Moreover, in IL-containing cultures, the TH-positive neurons displayed a more elaborate neuritic morphology. This was particularly evident for the DA neurons that were in direct contact with IL cells. We next tested whether peptides of the melanocortin family were able to influence the maturation of DA neurons in hypothalamic cultures devoid of IL cells. We found that the number and the maturation of TH-positive cells were enhanced by diacetyl-αMSH, monoacetyl-αMSH and N-acetyl(Cys$^{4-10}$,D-Phe^{7})-αMSH4-13, but not by desacetyl-αMSH, ACTH1-13 and ACTH1-24. These findings indicate that the development and maturation of DA hypothalamic neurons can be modulated by their innervation target cells and suggest that this trophic influence is, at least partly, attributable to αMSH peptides, with the most acetylated forms being the most active.

Support: NINCDS NS-16349, DRET n°90/093 and MRT (France).

711.4

ROLE OF OLIGODENDROCYTES AND ASTROCYTES IN MEDIATING EPIDERMAL GROWTH FACTOR (EGF)'S EFFECTS ON FOREBRAIN MEDIAL SEPTAL CHOLINERGIC NEURONS IN VITRO. I.E. Mazzoni and R.L. Kenigsberg, Centre de Recherche Pediatrique, Hopital Ste-Justine, Montreal, Quebec, Canada. H3T 1C5.

We have recently demonstrated that EGF decreases the activity of the enzyme choline acetyltransferase (ChAT) in fetal cholinergic neurons from the rat medial septum. This effect is indirectly mediated via the glial cells (Kenigsberg et al., Neuroscience 50:85-97, 1992). In our accompanying abstract, we show that microglial cells are not implicated in the EGF-induced decrease in ChAT activity. In this study we now investigate whether the oligodendrocytes (oligos) and/or the astrocytes (astros) may be implicated. Consequently, we proceeded to eliminate the oligos in our cell cultures by complement lysis using anti-galactocerebroside antibodies. Following this treatment, we monitored EGF's effects on the cholinergic neurons on these oligo-free cultures to find no significant difference in ChAT activity when compared to their appropriate EGF-treated controls. As our results to date suggest that neither the microglia nor the oligos are implicated in EGF's effects on cholinergic cell expression, we proceeded to examine the role of astros. Pure astro cultures were prepared from the medial septal region. Conditioned media (CM) from control or EGF-treated pure astro cultures were collected. The addition of EGF to astros induced their transient (48 hour) proliferation, followed by a period of apparent quiescence. We collected fractions of CM immediately following (fraction 1) and after (fraction 2) active proliferation. The effects of CM from EGF and untreated astro cultures were then tested on pure (99%) neuronal cultures from the medial septum. The addition of fraction 1 obtained EGF-treated astros did not significantly affect the activity of ChAT in pure neuronal cultures. However, fraction 2 collected from EGF-treated astros significantly decreased the activity of ChAT (40% from control levels) in pure neuronal septal cultures. The EGF-induced cholinergic neuromodulatory activity present in fraction 2 appears to have a MW>10 kD, to be heat sensitive and labile to repeated freezing and thawing. This data suggest that astros are capable of producing a soluble factor(s) that can affect forebrain cholinergic cell expression. Release of this factor(s) appears to be controled by EGF. The characterization and identification of this astro-derived cholinergic neuromodulatory activity whose expression is controlled by EGF is currently under investigation in our laboratory.

711.5

ROLE OF MICROGLIAL CELLS IN EPIDERMAL GROWTH FACTOR'S (EGF) INDIRECT EFFECTS ON CHOLINERGIC CELL DIFFERENTIATION IN THE MEDIAL SEPTUM. R.L.Kenigsberg and I.E.Mazzoni. Centre de Recherche Pediatrique, Hopital Ste-Justine, Montreal, Quebec, Canada. H3T 1C5.

We have recently demonstrated that EGF decreases the activity of choline acetyltransferase (ChAT) in cholinergic neurons from the fetal rat medial septal area in culture. EGF's effects are mediated indirectly via glial cells present in our cultures (Kenigsberg et al., Neuroscience 50:85-97, 1992). We are now furthering our investigations to determine 1) which glial cell type mediates EGF's indirect action on the cholinergic neurons, and 2) what is the cell source and nature of this glial-derived cholinergic neuromodulatory activity whose expression is controlled by EGF. In this abstract we present some of the results we have obtained in reference to the first question. To assess the importance of the microglia in mediating EGF's effects on the cholinergic neurons, we have attempted to eliminate the microglia from our cultures by using the lysosomotrophic agent L-leucine methyl ester (LME). A 2 hour treatment with 5 mM LME on culture days 2, 3, 4, 5 or 6 did not affect the EGF-induced decrease in ChAT activity. This single-day treatment, however, was only effective in reducing 30-40 % of microglia in EGF-treated cultures. A two-day treatment of the septal cells with LME did not modify EGF's effects on ChAT but could reduce the number of microglia in EGF-treated cultures to those normally found in control sister cultures. As complete elimination of the microglia could not be attained with this treatment, in another group of experiments, various amounts of pure microglial cells were added to our septal cultures. When concentrations of exogenous microglia representing 0.5-9 % of the total septal cells were added to EGF-treated cultures, we could not detect any further decrease in ChAT activity. Interestingly however, the EGF effects on cholinergic cell expression were partially antagonized by the addition of these exogenous microglia. Furthermore, the exogenous microglia increased ChAT activity in control cultures as well. As microglia may be a source of nerve growth factor (NGF), we then examined this possibility. We found that the addition of immunoneutralizing anti NGF antibodies could not block the increase in ChAT activity observed in untreated cultures supplemented with exogenous microglial cells. Therefore, this study shows that microglial cells are not implicated in the EGF-induced decrease in ChAT activity in cholinergic neurons from embryonic septal cell cultures nor do they represent a direct or indirect source of NGF in our system.

711.6

CONSTITUTIVE AND REGULATED EXPRESSION OF MACROPHAGE INFLAMMATORY PROTEIN - 1α (MIP-1) IN PRIMARY CEREBELLAR CULTURES. C.B. McCullum, R.E. Smith[#], R.M. Strieter[+], R.I.Hume,* S.L. Kunkel[#] Department of Biology, [#]Department of Pathology, [+]Department of Internal Medicine Pulmonary Division, University of Michigan, Ann Arbor MI 48109

The cerebellar cortex is an excellent model for the study of cellular interactions because it contains only glial cells and five types of neurons. Inflammatory cytokines have been well characterized as mediators of cell-cell interactions in the immune system but the effect of these molecules in the nervous system is poorly defined. Macrophage inflammatory protein - 1α (MIP-1) is a chemotactic cytokine with diverse regulatory functions. We hypothesized that MIP-1 might play a role in normal neuronal and glial interactions in the cerebellum. To begin to test this hypothesis, we made primary cultures of postnatal day one through nine rat cerebella. Using a sensitive and specific ELISA, we detected constitutive expression of MIP-1 in untreated cultures. Furthermore, cultures treated for 24 hours with 0.1 through 10 ng/ml of IL-1 receptor antagonist protein (IRAP) demonstrated an increase in MIP-1 expression. These experiments are consistent with a role for MIP-1 in signalling in the rat cerebellum.

711.7

RECOMBINANT HUMAN OSTEOGENIC PROTEIN-1 (OP-1) INCREASES ADRENERGIC CELL NUMBER IN AVIAN NEURAL CREST CULTURES. R.G. Wehby*, J. E. Varley, and G.D. Maxwell. Neuroscience Program and Dept. of Anatomy, University of Connecticut Health Center, Farmington, CT 06030.

OP-1 is a member of the TGF β superfamily of proteins (Özkaynak et al. 1990 EMBO J 9:2085). Although originally identified by its ability to induce bone formation in vivo, there are indications that OP-1 and other family members may be present in and act on several tissues (Lyons et al. 1991 Trends in Genetics 7: 408). We have tested the effect of recombinant human OP-1 (Creative BioMolecules, Inc.) on quail trunk neural crest cultures. The number of adrenergic cells which were present after 7 days in vitro was increased in a dose-dependent manner when OP-1 (0-500 ng/ml) was added to medium containing 15% horse serum and 10% chick embryo extract. At 50 ng/ml OP-1 there was an 8-fold increase in adrenergic cell number, and a 175-fold increase at 500 ng/ml. In contrast, both melanocyte and total cell number were unaffected over this range of OP-1 concentrations. Vehicle, consisting of 60% ethanol and 0.025% TFA, showed no effect. We also tested recombinant human TGF β-1 at 50 ng/ml and, in contrast to OP-1, found a decrease in adrenergic cell number relative to controls. In the presence of TGF β-1 total cell number was unaffected, but melanocyte cell number was decreased to about half the value seen in controls. These findings indicate that OP-1 is a potent and specific stimulator of the adrenergic phenotype in avian trunk neural crest cultures. This work was supported by NIH grant NS16115 to GDM and NIH Training Grant 5 T32 DC00025 to RGW.

711.8

PLEIOTROPHIN (PTN) INDUCES PROCESS FORMATION IN O-2A PROGENITOR CELLS. H.-J. Yeh,* I. Silos-Santiago, Maria A. Gurrieri, W. D. Snider, and T. F. Deuel. Dept. of Medicine, Biochemistry, and Neurology, Washington University and the Jewish Hospital of St. Louis, MO 63110.

Pleiotrophin (PTN) is a heparin-binding protein that functions as a weak mitogen and as a neurite outgrowth promoting activity *in vitro*. Because the Ptn gene is expressed in glial cells and perhaps in ependymal cells as well as in neurons during mouse development *in vivo*, we tested PTN with primary cultures of glial progenitors of E15 rat spinal cord and neonatal rat brain to determine if PTN might have a more general influence on process outgrowth in the developing central nervous system. The cells in culture display a typical bipolar or early multipolar morphology and are recognized by the O4 antibody and thus are similar to oligodendrocyte/type-2 astrocyte (O-2A) progenitor derived from the optic nerve. PTN strikingly enhances process outgrowth of glial progenitors in a dose and time-dependent manner. Process formation is readily demonstrated at 500 pM PTN in 2 days. After 2 days with 100 ng/ml and 200 ng/ml PTN, exuberant process outgrowth and extensive branching are observed. A specific monoclonal anti-PTN antibody completely blocks the influence of PTN. In contrast to PTN, cells treated with the platelet-derived growth factor (PDGF) A-chain (50 ng/ml) for 2 days developed only a few long processes that were without branching and were only minimally different from control cells that are grown in basic media with 5 ng/ml (bFGF). Remarkably, cells exposed to PTN (100 ng/ml) and PDGF A (50 ng/ml) together for 2 days were not different from cells treated with PDGF A (50 ng/ml) alone. These results suggest that the expression of PTN in the central nervous system may have a unique role in glial differentiation and that PDGF A may serve to delay this process during development.

711.9

REGULATION OF EXPRESSION OF FGF-2 AND ITS HIGH AFFINITY RECEPTOR (FLG) IN GLIOMA CELLS BY CELL DENSITY, FGF-2, NGF, TGFs AND GDFIF. R. Westermann, C. Grothe & T. Janet- Dept. Anatomy & Cell Biology, W-3550 Marburg, Germany.

Rat C6 glioma cells synthesize FGF-2 mRNA and protein when cultured at low density and in the presence of serum. Under non-proliferative conditions, i.e. high cell density or in serum-free medium, FGF-2 synthesis drastically decreases. This decrease appears to be induced by a soluble glioma-derived FGF-inhibitory factor (GDFIF). TGFbetas overcome the GDFIF-effects and stabilize high FGF-2 mRNA expression also at high cell density, while FGF-2 and NGF have no effects. From these data we conclude that FGF-2 may act as an autocrine mitogen regulated at the transcriptional level. Binding studies of ^{125}I-FGF-2 on C6 cells suggest the presence of both, low and high affinity FGF receptors (FGFR). Using antibodies against FGFRs, a flg-like immunoreactive protein could be detected on the surface and in the cytoplasm of the cells. Therefore, flg may account, at least partially, for high affinity FGF-binding. Like the FGF-2 protein, flg protein is highly expressed in low density cultures and nearly absent in confluent cultures. Surprisingly, the strong flg mRNA synthesis is not influenced by cell density or growth factors. In contrast to FGF-2, flg expression appears to be negatively regulated at the translational level by GDFIF.

711.10

EXPRESSION OF TRANSFORMING GROWTH FACTOR-ß IN C6 GLIOMA CELLS TRANSFECTED WITH CONNEXIN43 CDNA. Ian J. MacPhee[1], Nelson K.S. Khoo[1], Daniel J. MacPhee[2], Victor K.M. Han[3]* and Christian C.G. Naus[1]. Departments of Anatomy[1] and Zoology[2], University of Western Ontario, and Department of Paediatrics[3], Lawson Research Institute, London, Ontario, Canada, N6A 5C1.

When C6 glioma cells are transfected with the cDNA for the gap junction protein connexin43, there is a marked decrease in cell proliferation. Furthermore, when conditioned medium from C6 cells transfected with the connexin43 cDNA is placed on normal C6 cells, the result is a decrease in proliferation. This strongly indicates that a soluble growth factor may be produced by the transfected cells. Since previous studies have shown that transforming growth factor-ß (TGF-ß) is a potent inhibitor of cell proliferation in a variety of cell types, an investigation into the expression of this growth factor was initiated. Utilizing a monoclonal antibody to TGF-ß, immunocytochemistry revealed strong immunoreactivity of TGF-ß protein in the extracellular matrix and in the cytoplasm of both the normal C6 cells and the high expressing connexin43 clone, with the greater amount identified in the latter cells. Northern blot analysis of cytoplasmic RNA showed an elevation of TGF-ß mRNA level in the C6 cells that had been transfected with connexin-43 cDNA. In addition, a mink lung epithelium bioassay revealed that TGF-ß was produced by both normal C6 and transfected cells, in both the active and latent forms of the protein. This suggests that an increase in intercellular coupling may directly or indirectly upregulate the expression of TGF-ß as a means of controlling the proliferation of these tumor cells. Supported by the Medical Research Council of Canada.

711.11

THE EFFECT OF SODIUM PHENYL BUTYRATE ON HUMAN GLIOMA (U373) and MEDULLOBLASTOMA (D324) CELL CULTURES. H.S. Singer*, E. Harley, T.H. Moran, A. Costello, and J. Troncoso. Depts Neurology, Psychiatry, and Pediatrics, Johns Hopkins Univ School of Medicine, Baltimore, MD 21287-8811

Sodium butyrate and associated compounds have been shown to inhibit neoplastic cell growth and induce cytodifferentiation. The mechanism(s) for this activity are being investigated using human glioma (U373) and medulloblastoma (D324) cell cultures. Incubation with sodium phenyl butyrate (NaPB), 2.5 to 10 mM, produces incremental reductions in growth rate (cell count and BUdR incorporation) and morphological changes. At 10 mM concentrations, cell counts are reduced in U373 and D324 lines by 85% and 94%, respectively, and cells assume elongated irregular shapes with increased processes. Changes are reversible when cells are allowed to recover in the absence of NaPB. The effects on cell growth were not significantly affected by the addition of basic fibroblast and transforming growth factors. In U373, NaPB induced choline acetyltransferase activity (ten-fold increase), elevated the concentration of dopamine, and enhanced [^{3}H]-glutamate binding. NaPB reduced adenylate cyclase activity under basal conditions and following stimulation with GTPγS and forskolin in both U373 and D324 cultures. The effects of butyrate on β-tubulin, tau, and GFAP are under investigation. These results show that NaPB induces a marked growth inhibition associated with morphological and biochemical differentiation in human glioma and medulloblastoma cell lines.

711.12

GANGLIOSIDES SELECTIVELY ALTER THE DISTRIBUTION OF LOW MOLECULAR WEIGHT MAP-2 IN NEURO-2A NEUROBLASTOMA CELLS. L-J. Wang, G. Yorke* and F. Roisen. Dept. of Anatomical Sciences and Neurobiology, University of Louisville, Sch. of Medicine, Louisville, KY 40292.

Our previous studies on Neuro-2a cells demonstrated that GM1: (a) enhances MT-dependent neuritogenesis; (b) increases the number of MTs per neurite; and (c) alters the distribution of some MT-associated proteins (MAPs). GM1 has been reported to increase tubulin mRNA. One mechanism underlying the ganglioside-mediated neuritogenesis might be MT specific. Evidence is accumulating which demonstrates that MAPs are likely intraneuronal regulators of development, morphology and plasticity. Since four major gangliosides are found in neuronal membranes, this study determined if GM1, GD1a, GD1b, GT1b or neuraminidase alter the distribution of MAP-2. Neuro-2a cells were cultured with or without ganglioside (150 ug/ml) or neuraminidase (1 unit/ml) for 24 hr prior to immunolocalization with antibodies to: (a) high and low molecular weight MAP-2 (mAb); (b) high molecular weight MAP(mAb) and (c) tubulin (mAb and pAb). Treatment with GM1, neuraminidase or GT1b produced a redistribution of MAP-2 (low molecular weight) from perinuclear cytoplasm to the distal neurites. In contrast, GD1a and GD1b, two ganglioside species with less neuritogenic potential, did not alter the intracellular distribution of MAP-2. EM post-embedding immunogold localization demonstrated that GM1 enhanced the MT cytoskeleton. Western blot analysis revealed that tubulin, but not the low molecular weight MAP-2, increased after GM1 exposure. This study demonstrated that GM1 and GT1b or conversion of endogenous gangliosides to GM1 redistributes the low molecular weight MAP-2 which alters Neuro-2a morphology. Gangliosides may not only stimulate neuritogenesis but also influence the neurite's axonal or dendritic fate. Supported by Alliant Community Trust Fund, Louisville, Kentucky.

711.13

VASOACTIVE INTESTINAL PEPTIDE: A GROWTH PROMOTOR IN NEUROBLASTOMA CELLS. Y. Wollman, G. Lilling*, M.N. Goldstein, M. Fridkin D.E. Brenneman and I. Gozes. Department of Chemical Pathology, Sackler School of Medicine, Tel Aviv University, Tel Aviv, Israel; Tel Aviv Medical Center, Department of Nephrology, Tel Aviv, Israel; Department of Anatomy and Neurobiology, Barnes School of Medicine, Washington University, St. Louis MO, USA; Department of Organic Chemistry, Weizmann Institute of Science, Rehovot, Israel; LDN,NICHD,NIH, Bethesda, MD, USA.

Vasoactive intestinal peptide (VIP) is a neuromodulator and secretagogue for neuronal survival factors (Gozes and Brenneman, J. Mol. Neurosci. 4:1, 1993). Moreover, VIP has been suggested to have growth-stimulating properties on early postimplantation embryos (Gressens et al., Nature 362:155,1993) and to act as a mitogenic factor for embryonic neurons in the sympathetic nervous system (Pincus et al., Nature 343:564, 1990). We now show that VIP had mitogenic activity on a human neuroblastoma cell line (NMB), as measured by cell number and thymidine incorporation. This mitogenic activity was dose dependent and was decreased with culture maturation. Northern blot analysis revealed VIP mRNA transcripts in this neuroblastoma suggesting an autocrine growth factor role for VIP.

711.14

FURTHER CHARACTERIZATION OF COLLAGEN POTENTIATED NEURITE PROMOTING FACTOR. D.E.Coyle*. Department of Anesthesia, University of Cincinnati College of Medicine., Cincinnati, OH 45267-0531.

As previously reported, this laboratory has identified a neurite promoting factor which is potentiated by the presence of collagen and produced by C6 glioma cells which have been adapted to serum free growth conditions. In the present study, CPNPF was examined to provide evidence that it is not an already characterized neurotrophic molecule. At its present state of purity, CPNPF appears to only support neurite outgrowth of PC12 cells for a short period of time (4 days) without the presence of a neurotrophic factor. The activity of this molecule is not dependent on the presence of intact chondroitin or heparin sulfate proteoglycan. This factor is not blocked by neutralizing antibodies directed against NGF, β-FGF, TGFβ2, TGFβ1.2, TGFβ3, TGFβ5, IL-1β, IL6, or EGF. Western blot analysis of CPNPF for the presence of fibronectin or laminin using polyclonal antisera resulted in no protein bands staining for these molecules. This result would indicate that neither the intact forms of laminin or fibronectin, nor fragments which may have resulted from proteolytic products or the production of truncated versions of these molecules by C6 cells is responsible for the neurite outgrowth activity of CPNPF. Incubation of CPNPF with mouse type IV collagen results in no loss of neurite outgrowth activity. Preincubation of CPNPF with the rat type I collagen substrate present in the assay well followed by washing to remove the soluble CPNPF results in no neurite outgrowth compared with control. These two results support the conclusion that CPNPF does not bind to collagen in order to affect its neurite promoting effect. CPNPF by indirect evidence does not appear to be glial derived protease nexin-I since it cannot be isolated by the procedure of Guenther et al. (EMBO J 4: 1963, 1985) and does not induce neurite outgrowth from mouse neuroblastoma 2a cells. From this evidence it appears that serum free growth conditions in the presence of collagen may have induced the production of a potentially new NPF from C6 glioma cells.

711.15

JUN, FOS, KROX AND CREB PROTEIN EXPRESSION IS DIFFERENT IN ADULT DORSAL ROOT GANGLION NEURONS STUDIED IN VIVO AND IN VITRO. T. Herdegen*[1], R. Bravo[2], S.G. Waxman, and J.D. Kocsis. Dept. of Neurol., Yale Univ. Sch. Med. and VAMC, New Haven, CT 06510; II. Institute of Physiol., Univ. Heidelberg[1], 6900 Heidelberg, Germany; Bristol-Myers Squibb Pharm. Res. Inst[2], Princeton, NJ, 08543.

We compared immunoreactivity (IR) for c-Jun, JunB, JunD, c-Fos, Krox-20 and Krox-24 transcription factors in adult rat DRG neurons in culture and in vivo. In vivo lumbar DRG of the untreated rats show a selective basal expression of c-Jun and JunD which is much increased following axotomy by sciatic nerve cut whereas JunB, Fos and Krox proteins remained absent or unchanged (Herdegen et al., Mol. Brain Res. 14: 155-165). For in vitro studies, lumbar DRG were dissociated and plated on glass coverslips and maintained in culture. Expression of proteins was assessed by specific antibodies and visualized by immunocytochemistry, and double-labelling with neurofilament antibody proved the neuronal presence. Within 24 hrs after plating there was intense nuclear labelling of all Jun, Fos and Krox proteins in virtually all neurons. This was also true for Krox-20 which showed an exclusive cytoplamic labelling in vivo. After one week, the IR decreased in large diameter neurons but fairly persisted in small and medium diameter neurons. The decrease was more pronounced for c-Fos, JunB and Krox proteins compared to c-Jun. Nuclear staining was also present for CREB protein. These data demonstrate that DRG can express a variety of transcription factors in vitro which are strictly controlled in vivo even following axotomy. Dissociation of DRG neurons for culture may remove this control indicating that DRG neurons might generate a different protein synthesis pattern as compared to axotomized DRG in vivo.

Supported by the NIH, the VA, Boehringer-Ingelheim Funds and DFG.

711.16

CYTOKINE TREATMENT CAUSES ALTERATIONS IN THE CALCIUM RESPONSES OF CULTURED CEREBELLAR GRANULE NEURONS J. Holliday*, J. Curry, S. Lee, K. Parsons and D.L. Gruol. Dept. of Neuropharmacology, The Scripps Research Inst., La Jolla, CA.

Elevated cytokine levels are associated with several human diseases (including Down syndrome and HIV infection) that result in developmental abnormalities. However, their possible role in producing abnormal development is unknown. Some cytokines are known to directly activate neuronal growth factor receptors in the CNS and are thus likely to affect CNS development. We have studied the effects of cytokine treatment upon neuronal development and function to assess the role of cytokine elevation in producing abnormal CNS function.

Cerebellar granule neurons were chosen for experimentation since they can be grown in relative isolation from other cell types that may also respond to applied cytokines. Their calcium responses to both depolarization and glutamate receptor stimulation (NMDA-type) has been well characterized during the first week of development in culture. Cells were stimulated with 1 sec. applications of either 150 mM KCl or 100 μM glutamate (in the absence of extracellular Mg2+). Intracellular calcium concentration was estimated using fura-2 imaging techniques.

Treatment of granule neurons with 40 units/ml of Il-1β on the first day in culture and supplemented again on the fourth day caused significant increases in the glutamate response but not in the depolarization response or resting level measured at the end of the first week in culture. Other cytokines, such as TNF and Il-6 produced similar results. Chronic cytokine treatment may affect the rate of neuron maturation or may directly affect responses to stimuli.

Supported by #MH47680 and PAF/AmFAR.

711.17

CYTOKINE INDUCED STIMULATION OF mRNA ENCODING THE RECEPTOR FOR SUBSTANCE P IN SYMPATHETIC NEURONS. W.H. Ludlam*, K.E. McCarson[$], J.E. Krause[$], and J.A. Kessler. Depts. of Neuroscience and Neurology, Albert Einstein College of Medicine, Bronx, NY 10461, and [$]Dept. of Anatomy and Neurobiology, Washington University School of Medicine, St. Louis, MO 63110.

The mechanisms which orchestrate the temporo-spacial responses of neurons and non-neuronal cells after neural injury are not well understood. In sympathetic neurons, levels of the neuropeptide substance P (SP) are increased in response to injury, as is the receptor for substance P (SPR) in non-neuronal cells. After injury, soluble cytokines are released into the cellular milieu, and some of these factors, such as leukemia inhibitory factor (LIF), have been shown to increase neuronal levels of SP. We investigated the effects of LIF on the regulation of SPR in cultured sympathetic neurons of the neonatal rat superior cervical ganglion (SCG). In untreated SCG cultures, the level of SPR mRNA was nearly undetectable (0.022 ± 0.008 pg SPR mRNA/ug total RNA) using a solution hybridization/ nuclease protection assay. By contrast, in the presence of LIF (5 ng/ml, 14 days treatment), the SPR mRNA level rose 9.4 fold to 0.207 ± 0.047 pg/ug. SCG SPR mRNA were quantitated by comparing sample levels to a standard curve of different amounts of sense cRNA for the SPR. Conversely, levels of muscarinic receptor mRNA were decreased in LIF treated SCG cultures showing the specificity of the SPR mRNA increase. We are currently investigating cytokine induced regulation of SPR in non-neuronal cell types. These findings indicate that cytokines like LIF may play a role in the coordination of SP and SPR levels in the cells of neural tissue after injury.

711.18

AGENTS STIMULATING cAMP FORMATION ENHANCE INTERLEUKIN 6 PRODUCTION BY RAT CORTICAL ASTROCYTES Grimaldi M., Meucci O., Ventra C., Scorziello A. °G. Pozzoli, °P.L. Navarra and Schettini G.* Sez.. Farmacol., Dip. Scienza Comunicazioni Umane, Univ. di Napoli, Napoli; °Ist. di Farmacol. Univ. del Sacro Cuore, Roma, ITALY.

Within the central nervous system (CNS) interleukin-6 (IL-6) is produced by astrocytes and microglia and induces neuronal differentiation and survival. The production of IL-6 by astrocytes in response to cytokines such as IL-1 or TNF or to LPS has been established. The IL-6 gene contains a multiple regulatory element region (MRE) including a cAMP responsive ones. Here we describe the effects of both forskolin (FSK), a direct activator of the cathalytic sub unit of adenylate cyclase (AC) enzyme and Vasoactive Intestinal Peptide (VIP), a neurotransmitter with neurotrophic actions, known to stimulate AC activity, on IL-6 production by cortical astrocytes, assessed by means of 7TD1 bioassay. FSK stimulated IL-6 production in a dose dependent manner with plateau reached at 1 μM concentration. Similarly, VIP stimulated, over a 24h incubation, in a dose dependent manner, IL-6 production. The effect of VIP was not affected by blocking protein kinase (PK)-C with calphostin, but was strongly reduced by blocking PK-A by using KT-5720, a specific PK-A blocker. These results suggest that the effect of VIP is achieved by activating the low affinity VIP receptor. The effect of FSK was also reduced by KT-5720. In addition the stimulatory effect of VIP and FSK was mimicked by cAMP analogues 8-Br-cAMP and dibutirryl-cAMP. Both these agents increased IL-6 production by astrocytes in a dose dependent manner being dibutirryl-cAMP more effective. FSK was able to increase, in a dose dependent manner, prostaglandins (PGs) E_2 and $F_{2\alpha}$ release by astrocytes an effect not induced by VIP. In presence of 100 nM indomethacin the stimulatory effect of FSK on IL-6 production persisted, while PGs stimulation was blocked. We conclude that IL-6 release by cortical astrocytes is not only regulated by inflammation- infection-related substances such as IL-1 or LPS, but also under the thight control of cAMP-PK-A transducing system.

711.19

The responses of the human leptomeningeal cells for thrombin, EGF, TGF-ß, IL-6, aFGF, PDGF *in vitro*. O. Motohashi, M. Suzuki*, A. Nishino, K. Umezawa, N. Shida, T. Yoshimoto. Div. of Neurosurg., Inst. of Brain Diseases, Tohoku Univ. Sch. of Med., Sendai, Japan 980.

Some disorders of the central nervous system result in the arachnoiditis. Thickening and adhesion of leptomeningeal membranes are reported in the disease processes or trauma and other types of meningitis. Normal pressure hydrocephalus is often recognized after subarachnoidal hemorrhage. Histopathological findings in such disease have been said to be subarachnoidal fibrosis, that is, proliferation of cells and fibers. We have first succeeded in culture of the human leptomeningeal (LM) cells in serum-free media supplemented with insulin, transferrin and dexamethasone. Using this culture system, we investigated the responses of the LM cells to several growth factors, the level of which in the cerebrospinal fluid were elevated in the patient of subarachnoidal hemorrhage. Human LM cells at confluence in the serum-free condition were trypsinized, diluted with the serum containing medium to the density of 7 x 10^3 - 2 x 10^4 cells/ml and seeded in the 24-well multiplates pretreated with poly-l-lysin. 24 hours later, the medium was changed into the appropriate medium for experiment. The cells were counted triplicately every 2 or 3 day with Coulter Counter. In summary, our findings can be stated as follows: (1) We examined the proliferative responses of the LM cells to thrombin, EGF, TGF-ß, IL6, aFGF and PDGF in vitro. (2) Thrombin might promote the LM cell proliferation dose-dependently. (3) TGF-ß might enhance the proliferative effect of thrombin on the LM cells. (4) IL6 and PDGF have a little proliferative effect on the LM cells. (5) EGF and aFGF might have a considerable effect on the LM cell proliferation. These findings suggest that thrombin and TGF-ß, elevated in CSF following SAH, may cause subarachnoidal fibrosis and subsequent hydrocephalus.

711.20

AUTOCRINE AND ENDOCRINE ROLES OF IGF-II SECRETION BY THE CHOROID PLEXUS. C. Nilsson* and S. Gammeltoft. Wallenberg Laboratory, Div. of Molecular Neurobiology, Univ. of Lund, Box 7031, S-220 07 Lund, Sweden.

Studies by in situ hybridization have shown that the choroid plexus (CP) and meninges are the main sites of insulin-like growth factor-II (IGF-II) mRNA expression in the adult mammalian CNS. We have developed primary cultures of CP epithelial cells from rat and sheep to investigate the role of IGF-II in this tissue. Conditioned medium contained similar amounts of both IGF-II and IGF-binding protein-2, as shown by Western immunoblot and gel filtration, demonstrating that IGF-II mRNA is translated and secreted as mature peptide by CP epithelium. Radioreceptor assay gave an approx. amount of IGF-II of 30 and 0.8 nM in rat CP conditioned medium and CSF, respectively. Comparison with data on CP weight and CSF volume and formation shows that the CP could provide both the CSF and brain with IGF-II. The possibility of an autocrine role for IGF-II in the CP epithelium was studied by determining the number of cell divisions during two days in serumfree medium, as measured by immunohistochemical detection of bromodeoxyuridin incorporated into nuclei. Monoclonal antisera to rat IGF-II decreased the number of positive nuclei by 38% ($p<0.01$), while IGF-I and fibroblast growth factor both stimulated mitosis ($p<0.001$). Growth hormone, and the neuropeptides VIP and PACAP had no effect.

In conclusion, IGF-II could have both endocrine and autocrine roles in the CP, although the continuous formation of CSF by the CP would appear to favour an endocrine role.

712.1

IDENTIFICATION AND CHARACTERIZATION OF STEROID REGULATED GENES IN THE NERVOUS SYSTEM OF *MANDUCA SEXTA* M. Mészáros and D. B. Morton*. Arizona Research Laboratories Division of Neurobiology, Univ. of Arizona, Tucson, AZ, 85721

During metamorphosis the nervous system of insects undergoes dramatic changes that are regulated primarily by the ecdysterids. 24 hr prior to the pupal molt in *Manduca sexta* steroid titers are relatively high and they fall to very low levels at 4 hr before the molt. Several physiological events accompanying metamorphosis have been shown to require the rise and then a subsequent decline of ecdysteroid levels. These events also require the expression of a new set of genes. We used subtractive hybridization to isolate the genes that are expressed 4 hr prior to the pupal molt in the nervous system of *Manduca*. These genes are expected to play a role in either ecdysis behavior or in the development of pupal/adult structures. As ecdysteroid levels peak and subsequently fall prior to the molt, these genes are expected to be turned on at very low ecdysone titers after the exposure to high steroid levels. We have isolated six cDNA clones that represent genes that are developmentally regulated prior to metamorphosis in the nervous system. Using *in situ* hybridization we have shown that three of them are expressed in neurons. Currently we are sequencing these clones, trying to identify the neurons that express the genes and looking for possible roles of these cells in metamophosis.

712.2

CHANGES IN OXYTOCIN PROHORMONE PROCESSING IN HYPOTHALAMIC CULTURES. M. Morris*, L.J. Sim, and M.O. Lively. Depts of Physiology/Pharmacology and Biochemistry, Bowman Gray School of Medicine of Wake Forest University, Winston-Salem, NC 27157

There are developmental changes in oxytocin (OT) prohormone processing, with evidence for the presence of C-terminal extended OT (OT-X) in fetal brain and plasma (Altstein and Gainer, 1988, Morris et al., 1992). A hypothalamic culture model was used to further examine OT cellular development and neurosecretion. Dispersed paraventricular (PVN) cultures from 1-2 day old rats were stained for oxytocin neurophysin (NP-OT) and vasopressin neurophysin (NP-VP). Secretion studies were also performed with measurement of media and tissue levels of OT peptides under control and KCL stimulation. The predominant neurophysin cell type in the PVN cultures was NP-OT with very few cells positive for NP-VP. The use of different OT antisera, one specific for amidated OT and another which crossreacts with OT-X, revealed almost no labeling with the amidated specific antisera. Measurement of media and tissue OT supported this finding. HPLC separation with radioimmunoassay showed that the secreted form was OT-X, with a single peptide peak. The tissue contained both OT-X and OT; however, the levels of OT-X were greater (2:1 ratio of OT-X:OT). Stimulation of the cultures with KCL caused a 2-fold increase in the tissue levels of OT and OTX, while media peptides were marginally increased. These results show that cultured hypothalamic oxytocin neurons show alterations in peptide processing which is similar to that observed in the developing animal.

Supported by Pharmaceutical Manufacturing Association and North Carolina Biotechnology

712.3

SYNENKEPHALIN (PROENKEPHALIN 1-70) PROCESSING IN EMBRYONIC RAT BRAIN. M.I. Rodriguez Vida, M.C. Kleid, A. Ase, S. Finkielman, V.E. Nahmod and O.Vindrola*[1]. Inst. Invest. Med. Fac. Med. Univ. Buenos Aires (1427) Argentina. [1] LSU Sch. of Med. Rheumatology. New Orleans. LA 70112.

Synenkephalin is produced and secreted as an intact molecule or as a part of precursors in adult brain and adrenal medulla respectively. However it is cleaved to low molecular weight peptides in proliferating immune cells. Considering that proenkephalin is expressed in embryonic rat brain (ERB) during cell proliferation, we studied the processing of synenkephalin in ERB (E18) and in adult rat brain (ARB). IR-synenkephalin was measured by RIA using a C-terminally-directed antiserum. ARB contained the highest concentration of IR-synenkephalin (ERB: 1361+100; ARB: 2612+264) (results in fmol/mg protein, n=5). Gel filtration chromatography (Sephadex G-50) showed that in ARB most of IR-synenkephalin eluted in the position of the authentic peptide (8.0 kDa), although partial processing to 4.5 and 3.0 kDa peptides was observed. In ERB, synenkephalin was fully cleaved to a 1 kDa peptide, which was further characterized by affinity chromatography and HPLC. IR-Met-enkephalin (after digestion with trypsin and carboxypeptidase B) corresponded mainly to non-processed or partially processed products in ERB, but these were cleaved to free met-enkephalin in ARB. These results show that synenkephalin was cleaved to low molecular weight peptides only in ERB, suggesting an involvement of this molecule in nervous cell proliferation.

712.4

EARLY ONTOGENY OF MELANOTROPIN BINDING SITES IN RAT BRAIN AND SYMPATHETIC NERVOUS SYSTEM. W. Lichtensteiger*, B. Hanimann, M. Schlumpf, W. Siegrist* and A. Eberle*. Inst. of Pharmacology, Univ. of Zürich, CH-8006 Zürich, and *Research Dept., Univ. Hospital, CH-4031 Basel, Switzerland.

Developmental changes in nervous system after manipulation of α-melanotropin (α-MSH) levels suggest a role of melanotropins in ontogeny. In order to provide a basis for a detailed analysis of such effects, we studied the development of binding sites for the analog [^{125}I]Nle^4,D-Phe^7-α-MSH (^{125}I-NDP). Scatchard analysis of ^{125}I-NDP binding to diencephalic membranes revealed a single binding site with Kd = 0.37 nM. For in vitro autoradiography, cryostat sections of fetuses and offspring from time-pregnant Long Evans rats were incubated with 0.4 nM ^{125}I-NDP, non-specific binding was assessed in every second section by coincubation with 200 nM cold NDP. In early fetal life, significant levels of specific binding are detected, at *gestational day (GD) 14* (GD 1 = 24 hr after mating) in the sympathetic chain and in the dorsal meso-diencephalic junction, at *GD 15* in spinal cord, inf. olive, solitary tract nuc., spinal trigeminal tract nuc., and ventral mesencephalon; at *GD 16* i.a., in post. hypothalamus, at *GD 17* in cerebellum and brainstem reticular nuclei. Perinatally, a conspicuous receptor development occurs in caudate-putamen, nuc. accumbens and olfactory tubercle where, from GD 17, receptor density rises to high levels between *GD 20/22* and *postnatal day (PN) 4*, and subsequently decreases towards adult levels. This period is also characterized by the transient presence of binding sites in certain cortical areas, i.a., parietal cortex layers III/IV and hippocampus at *PN 8*. Mediobasal hypothalamic binding sites increase slowly during postnatal life. The different patterns may be linked with the type of endogenous ligand, neuron system, and receptor subtype; binding characteristics are being studied. The early presence of relatively high densities of receptors in distinct locations, marked density variations, and transient expression in regions virtually devoid of receptors in adulthood (e.g., neocortex and cerebellum) lends further support to the idea of a role in developmental processes.

712.5

CHARACTERIZATION OF GALANIN-LIKE IMMUNOREACTIVITY IN THE PRENATAL BRAZILIAN OPOSSUM BRAIN. J. K. Elmquist*, J. Iqbal, L. R. Ross, J. J. Swanson, and C. D. Jacobson. Department of Veterinary Anatomy and Neuroscience Program, Iowa State University, Ames, IA 50011.

Previously our laboratory has demonstrated that galanin-like immunoreactivity (GAL-IR) and GAL receptors are present as early as 1 day postnatal (PN) in the brain of the Brazilian opossum, *Monodelphis domestica*. *Monodelphis* is a small marsupial whose young are born in an extremely immature state with a protracted postnatal period of neurogenesis. We have hypothesized that the presence of GAL-IR and GAL receptors at this early stage of CNS development indicates that GAL may be playing a distinct developmental role in the CNS. Although *Monodelphis* is born in a very immature state GAL-IR was robustly expressed at birth. To further characterize a developmental role for GAL we have looked at the ontogeny and distribution of GAL-IR in the prenatal opossum. We have found GAL-IR as early as embryonic day 11 (E11) in various structures in the forming embryo. Specifically, GAL-IR was observed in the forming trigeminal ganglia and nerve, dorsal root ganglia, spinal cord, sympathetic chain, and presumed vagal fibers in the thorax and abdomen. Additionally, cell bodies containing GAL-IR were observed in the forming diencephalon. This early ontogeny of GAL-IR further indicates that GAL may play an important formative role of the mammalian nervous system. In addition we have compared the distribution of various other neuropeptides and neurotransmitters to that of GAL. At E11, 5-HT and tyrosine hydroxylase immunoreactivity is detectable in the CNS. The distribution is distinct, however from that of GAL-IR. As early as 1PN, vasopressin-like immunoreactivity (AVP-IR) is present in the forming hypothalamus. Adjacent tissue sections indicate that GAL-IR and AVP-IR may be colocalized in a subset of GAL-IR cells. The use of specific GAL antagonists may indicate potential roles for GAL.

712.6

ORNITHINE DECARBOXYLASE ACTIVITIES IN RESPONSE TO IN VITRO HYPOXIA AND OXIDATIVE STRESS: A NOVEL USE OF BRAIN SLICE PREPARATIONS. S. Packianathan, J. Zhao, C.D. Cain and L.D. Longo*; Div. of Perinatal Biology, Depts. of Physiology, Ob/Gyn, and Biochemistry, School of Medicine, Loma Linda Univ., and J.L. Pettis VA Hospital, Loma Linda CA 92350.

Background. Recently, we have demonstrated in fetal rats that acute hypoxia results in significantly elevated ODC activity, polyamine concentrations, and ODC mRNA (*PNAS 90:692-696, 1993*). To study and evaluate this enzyme response, as well as to avoid maternal confounding effects, we have developed an *in vitro* fetal or neonatal brain slice preparation. We have also used this procedure to study the effects of oxidative stress, *i.e.*, free radicals on the slices **Methods.** 500μm thick brain slices from 18 to 21 gestational day rat fetuses and 3 to 5 day old pups were allowed to equilibrate in artificial CSF continously bubbled with 95% O_2/5% CO_2, for 1 h prior to beginning hypoxic exposures. Slices were made hypoxic by replacing the gas with 40%, 21%, or 10% O_2, all with 5% CO_2 and balance N_2. **Results.** In brain slices ODC activity increased significantly to peak between 3 and 4 h after initiation of hypoxia. The increase varied inversely with the O_2 concentration. For instance, in slices from 4 day old pups, after 4 h of 40% O_2, ODC activity was elevated 2-fold above control, 3-fold at 21%, and 6-fold at 10% O_2. Cell viability at the end of the 4 h hypoxic exposure was assessed by ODC activity increase to fetal bovine serum (FBS) stimulation. 0, 3, 5, and 10% FBS was added at the beginning of the hypoxic exposure. At 4 h, the slices showed a serum-dose dependent increase in ODC activity at up to 5-fold, indicating their ability to respond to external mitogenic stimuli even under the hypoxic exposure. In response to 0.3% H_2O_2, slice ODC activity increased two to three-fold at 1 h, and showed a H_2O_2 dose response up to 0.5% before it decreased. **Conclusions.** 1) The fetal brain *in vivo* hypoxic-induced ODC increase can be replicated in a well established *in vitro* system. 2) The low but stable baseline ODC activity suggests that the cells are in a quiescent state. 3) The slice preparation may be a useful tool to study the mechanisms of free radical mediated cellular effects. 4) Apart from electrophysiological evidence, ODC activity may be an additional marker for brain slice viability. (Supported by USPHS grant HD 03807 to LDL)

712.7

THYROID HORMONES ALTER THE TIME COURSE OF CHOLINEACETYLTRANSFERASE (CHAT) EXPRESSION DURING POSTNATAL DEVELOPMENT IN THE FOREBRAIN CHOLINERGIC SYSTEM: A NONRADIOACTIVE IN-SITU HYBRIDIZATION OF CHAT mRMA. J.D. Oh*, A. Rohgani, R. Edwards, and L.L. Butcher, Laboratory of Chemical Neuroanatomy, Dept. of Psychology, University of California, Los Angeles, CA 90024-1563, U.S.A.

In the present experiment, we demonstrate that thyroid hormone manipulation can modify the time course of ChAT expression during the postnatal development of the cholinergic forebrain. Rat pups were made hyperthyroid by admini- stering 1 mg/kg triiodothyronin (T3) i.p. daily. Other groups were made hypothyroid by providing 0.3 % propylthiouracil (PTU) in the diet of the dams. These groups were then compared with the control group who received an isovolumetric injection saline (0.9%) i.p. daily. The pups were perfused at 1,2,3, and 4 postnatal weeks and the tissue sections were stained for ChAT mRNA using a nonradioactive in-situ hybridization technique. As we predicted, thyoid deficient rat pups ($198^{+}25.20$) showed markedly fewer ChAT mRNA-positive cells at postnatal 1 week than the controls ($318^{+}23.71$) and eventually reached the adult level at postnatal week 4. On the other hand, T3- treated pups ($307^{+}43.30$) showed an equivalent number of ChAT mRNA stained neurons as the control at postnatal 1 week . However, whereas the controls reached the adult level of ChAT mRNA neurons at postnatal week 2 and remained at this level until the postnatal 4 week, ChAT mRNA-positive cells progressively decreased in the hyperthyroid group from postnatal week 2 to week 4. At postnatal week 4, the ChAT mRNA cell number of hyperthyroid pups declined down to 70 % of the adult level ($p<0.05$).

NUTRITIONAL AND PRENATAL FACTORS

713.1

UNDERNUTRITION DURING EARLY LIFE DOES NOT AFFECT THE NUMBER OF NEURONS IN THE RAT CEREBRAL CORTEX. K.S. Bedi*, Department of Anatomical Sciences, University of Queensland, Australia, 4072.

Undernutrition during early life is known to cause deficits and distortions of brain structure. However, it remains uncertain whether or not this includes a diminution of the total numbers of neurons. Recent advances in stereological techniques have made it possible to obtain unbiased estimates of total numbers of cells in well-defined biological structures. Rats were undernourished from the 16 day of gestation to 30 postnatal days of age by standardised procedures. These and well-fed control rats were anaesthetised and killed at 70 days of age by intracardiac perfusion with a modified Karnovsky fixative. The left cerebral hemisphere from each animal was embedded in Paraplast and serially sectioned. The sections were analysed by the Cavalieri principle to obtain the total cortical volume, and by the 'disector' method to estimate the numerical density of neurons in the cortex. These values were later used to compute estimates of the total number of cortical neurons for each animal. Well-fed control rats had an average of 26.9 million cortical neurons whilst the perviously undernourished animals had 24.8 million. The difference between these two groups was not statistically significant. It was concluded that undernutrition of rats during early postnatal life does not affect the total numbers of neurons in the cerebral cortex.

713.2

FELINE MATERNAL TAURINE DEFICIENCY: EFFECT ON VISUAL CORTEX OF OFFSPRING. H. Imaki*, Y. Xu, P. Lu and J. A. Sturman. Institute for Basic Research in Developmental Disabilities, Staten Island, NY 10314.

Cats are dependent on an adequate dietary source of taurine, without which they become taurine-deficient. This condition has an adverse effect on reproduction resulting in excessive fetal wastage and low-birth-weight live kittens, and abnormal ontogeny of the CNS. We have previously reported quantitative morphometric and immunohistochemical evidence of abnormalities in newborn visual cortex and now report similar studies of 8-week-old kittens. In contrast to newborn kittens, there were no differences in thickness of individual laminae nor in total thickness despite a persisting four-fold smaller taurine concentration and a 9% smaller total brain weight. There were no differences in number of neurons under $1mm^2$ of surface or in numerical density of neurons. There were more astrocytes in all laminae of taurine-deficient kittens, reaching statistical significance in layers II-III.3 and IV-B.1, and fewer oligodendrocytes in layers IV-B.1 and V-2. Using antibodies raised against amino acids linked to BSA with glutaraldehyde, we examined the localization of taurine, glutamate and GABA in these kittens. There was little difference between groups except for an increased number of GABA-positive cells in taurine-deficient kittens. Thus gross morphological differences are smaller at 8 weeks than at birth, perhaps because only 40% of live-born taurine-deficient kittens survive to 8 weeks, preselecting less affected kittens.

Supported by NIH grant HD-16634.

713.3

NEONATAL MONOSODIUM GLUTAMATE (MSG) DECREASES VULNERABILITY TO THE WEIGHT-LOSS SYNDROME PRODUCED IN ADULT RATS BY VOLUNTARY EXERCISE AND FOOD RESTRICTION. T.S. Rieg*, B.A. Woynicz, K.F. Fulton, S.N. Downing, & P.F. Aravich. Eastern Virginia Medical School, Norfolk, VA 23501; VA Medical Center, Hampton, VA 23667; Hampton University, Hampton, VA 23667.

Monosodium Glutamate (l-glutamic acid) is a neurotoxin when administered neonatally to rats. The arcuate hypothalamic nucleus, the primary source of brain beta-endorphin (BE), is among those areas damaged. A reduction in brain BE has been correlated with anorexia nervosa (AN). MSG also increases relative adiposity and an increase in relative adiposity reduces susceptibility to an animal model of AN. This experiment determined if neonatal MSG alters susceptibility to an animal model of AN produced by exercise (22.5 hr/day running wheel access) and food restriction (1.5 hr/day food access). If a reduction in brain BE causes the syndrome, MSG should increase vulnerability to it, if increased relative adiposity protects against the syndrome, MSG should reduce vulnerability. It was found in male rats, that MSG (4.0 mg/kg, sc, odd pnd 1-9) increased the number of days necessary to reach a 25% weight-loss criterion despite the fact that MSG rats were lighter at the onset and ate less compared to saline controls. There was no effect on wheel running. Finally, MSG exaggerated the effects of the syndrome on relative adrenal weight, but did not alter relative thymus weight and actually attenuated its effects on relative spleen weight. It is concluded that the weight-loss syndrome is affected by increased adiposity rather than reduced BE.

713.4

EFFECT OF PRENATAL ALCOHOL EXPOSURE ON DEVELOPMENT IN THE SUPERFICIAL LAMINA OF THE RAT SUPERIOR COLLICULUS. **K.A. Wall and D.E. Phillips*. Department. of Biology and WAMI Medical Education Program, Montana State University, Bozeman, MT 59717.**

Developmental alcohol exposures have been shown to cause deficits in visually oriented activities in humans and to cause structural changes in the rat optic nerve, thus changes in other areas of the visual system might be expected. Pregnant rats were fed a liquid diet containing either ethanol (6.7% v/v) or a maltose-dextrin caloric supplement. Control and ethanol exposed pups were fostered to surrogate dams after birth until sacrifice or weaning at 20 days. Brain tissues from male pups were removed at 15 and 35 days postnatal and processed for either plastic embedding or for Golgi-Cox impregnation. Standard areas of the superficial lamina (SL) were defined in 2μm plastic sections of the superior colliculus (SC) and the neurons and glial cells were counted. Golgi-Cox stained SC tissue was embedded in celloidin and 120μm sections were cut and mounted for camera lucida drawings of type II ganglion cells. There was a slight decrease in the mean number of neurons per unit area and a slight increase in the mean number of glial cells per unit area at 15 days postnatal. Neither change was statistically significant. There were significant decreases in the number of dendritic branches per neuron and the numbers of branches at specific distances from the cell soma. Dendritic changes were most apparent at 15 days postnatal (reduced 25-43% for both parameters) and were still reduced at 35 days postnatal but to a lesser degree (8-20% for both parameters). These results indicate that a prenatal exposure to ethanol can cause dramatic reductions of type II ganglion cell dendritic branches in the infant rat SC that persist, but to a lesser degree, in adolescent animals. (Supported by NIAAA AA7042 and NIAAA AA05331 Predoctoral Fellowship)

713.5

REM-SLEEP DEPRIVATION IN NORMAL AND MALNOURISHED RATS AT 30 DAYS OF AGE. L. Cintra*, P. Durán, L. Granados, and A. Galván. Inst. Invest. Bioméd. UNAM, México, D.F. 04510.

In previous studies we reported significant decreases of REM sleep (30 and 60 days) after total sleep deprivation during the lights-on phase in malnourished rats. The objective of the present study was to evaluate the effects of protein malnutrition induced by a 6% casein diet, instituted before mating and continued during gestation and into postnatal life, using an inverted dark-light cycle on the sleep-waking cycle before and after REM sleep deprivation. A base-line-recording day was followed by one day of REM sleep deprivation by the platform technique in control and malnourished rats. This was followed by three recovery days in normal (25% casein diet) and malnourished rats of 30 days of age. We found significant decreases of waking and significant increases in SWS in malnourished rats on day 1 during the activity phase and also a significant increase of REM sleep on days 4 and 5 of the rest phase (lights-on). These data reveal a significant delay in the maturation of SWS and REM sleep in malnourished rats and in mechanisms that regulates REM sleep recovery. (Supported by DGAPA IN 202891-IIB-UNAM)

713.6

EFFECT OF PRENATAL PROTEIN MALNUTRITION AND POSTNATAL NUTRITIONAL REHABILITATION ON SPINE DENSITY OF CA1 HIPPOCAMPAL PYRAMIDAL CELLS IN RATS. A.Aguilar*, S. Díaz-Cintra, A.Galván, L. Cintra, T. Kemper and P.J. Morgane. Inst. Invest. Bioméd., UNAM, México,D.F. 04510. Neurol. Unit, Boston City Hosp., Boston MA, 02118 & Worces. Found. Exp. Biol., Shrewsbury MA, 01545.

Prenatal malnutrition produces significant reductions in the dendritic spines of hippocampal CA1 pyramidal cells in *strata radiatum and lacunosum moleculare*. In this study, we studied 15, 30, 90 and 220-day-old rats. 288 pyramidal cells were examined using an imaging system (BIOCOM). We measured the number of spines in three 50 micron segments of perforant path (PP), Schaffer collateral (SF) and commissural fiber input systems, as well as in a 50 micron segment in the largest basal and apical dendrites. We found significant reductions ($p<0.05$) in spine density in all segments studied in all four ages studied Prenatal malnutrition produces severe alterations in the basal and apical dendrites where both PP and SF synapse in 30 and 220-days-old rats. (Supported by DGAPA IN202891, IN204892, NIH HD-22539-04, HD-23338-03).

713.7

PRENATAL MALNUTRITION AND POSTNATAL NUTRITIONAL REHABILITATION ON MOSSY FIBERS AREA OF DENTATE GYRUS OF THE RAT. A. Galván*, L. Granados, S. Díaz-Cintra, L. Cintra, A. Aguilar, T. Kemper, and P.J. Morgane. UISI-INP, SSA, C.P. 14410, and IBM, UNAM. A.P. 70228, México D.F. Neurol. Unit, Boston MA, 02118 & Worces. Found. Exp. Biol. Shrews. MA, 01545 USA.

Prenatal malnutrition produces long-term effects on the spine density in granule cells of the dentate gyrus in the hippocampal formation of rats at 220 days of age. In the present study, we analyzed this effect on the area of mossy fibers from dentate granule cells stained with Timm's technique. 64 male Sprague-Dawley rats were divided into two groups control and rehabilitated, consisting of 15, 30, 90 and 220-days-olds. The mossy fiber area was studied in five frontal levels and analyzed using an imaging digital system (BIOCOM). Results showed a significant decrease ($p<0.05$) in the area of mossy fibers in all levels studied at 220 days of age. At 90-day-old rats showed a significant decrease ($p<0.05$) at level 1, 15 day-old shows a reduction at levels 3-4. These findings indicate a severe long-term effect of prenatal malnutrition on mossy fibers of dentate granule cells. (Supported by DGAPA IN 202891, NIH GRANTS HD-22539-04 & HD-23338-03)

713.8

EFFECTS OF PRENATAL PROTEIN MALNUTRITION ON THE ONTOGENY OF HIPPOCAMPAL LTP. P.J. Morgane*†, R.J. Austin-LaFrance‡, K.S. Abu-Hasaballah‡ and J.D. Bronzino‡. †The Worcester Foundation for Experimental Biology, Shrewsbury, MA 01545, ‡Trinty College, Dept. of Engineering and Computer Science, Hartford, CT 06106.

Prenatally protein malnourished rats (designated 6/25 to indicate pre- and postnatal protein content of diet) were tested as freely-behaving preparations at 15, 30, and 90 days of age for their ability to establish and maintain LTP of the perforant path/dentate granule cell synapse. Tetanization in control (25/25) animals resulted in significant enhancement of both the population EPSP slope and population spike amplitude (PSA) component of the dentate field potential at all ages. This enhancement decayed earliest in adult animals (5-18 hrs. after tetanization), 18-24 hrs. in 30 day olds and was still rising in 15-day old animals at 96 hrs. Adult 6/25 animals showed initial potentiation of the EPSP slope with a corresponding decline in PSA. PSA showed minor enhancement between 3-5 hrs. but this enhancement remained significantly below control levels and decayed to baseline earlier than in controls. 30-day old 6/25 animals had a high variability of response to tetanization. A portion of these animals were similar to the 90-day 6/25 animals while nearly 50% showed declines in PSA which remained below baseline out to 96 hrs. 15-day old 6/25 animals were found to initially potentiate both EPSP slope and PSA measures in a manner similar to controls, however this enhancement decayed to baseline levels within 48 hrs. Results indicate that fetal protein malnutrition impacts both the establishment and maintenance of LTP in a manner that appears to impair the translation of enhanced synaptic activity to enhanced cellular discharge. This may reflect the existence of a tetanization-induced rise in the level of GABAergic inhibitory modulation on the granule cell population of prenatally protein malnourished animals which is not significantly ameliorated by dietary rehabilitation. Supported by NIH-NICHHD Grant # P01-HD-22539

713.9

FETAL ALCOHOL EXPOSURE (FAE) ENHANCES MORPHINE BUT NOT NON-OPIOID STRESS-INDUCED ANALGESIA. M.L. Pilat and A.N. Taylor. Departments of Psychology and Anat./ Cell Biol., BRI, University of California; and Brentwood Div., West LA VAMC, Los Angeles, CA 90024.

It has been demonstrated that maternal ethanol consumption (36% ethanol-derived calories during the last two weeks of gestation) produces alterations in opioid-mediated behaviors in the offspring. In order to further explore the impact of FAE on the opioid system, adult female Sprague-Dawley FAE, pair-fed, and control rats were tested for their tail-flick latency every 30 min after a 3.5 min cold water swim (CWS) and then, a week later, after 5mg/kg morphine. This swim paradigm has been shown to produce a nonopioid analgesia (i.e. not blocked by naltrexone). No differences between the prenatal treatment groups were observed after CWS. At 2hrs after morphine adminstration the FAE group showed significantly more analgesia ($p<.01$) than both the pair-fed and controls, confirming our previous reports. At 60 and 90 min there was a significant effect of treatment due to the FAE and control rats showing more analgesia than the pair-feds. These data suggest that FAE and pair-feeding have long lasting and opposite effects on responsiveness to morphine. Currently we are attempting to determine whether these alterations in the opioid system are due to the prenatal treatments themselves or to the stress response of the dam to these diet manipulations.
(Supported by NIH-HD07228, UCLA Psychoneuroimmunology Program, and VA Medical Research Service.)

713.10

THE EFFECTS OF PRE AND POSTNATAL EXPOSURE TO AMPHETAMINE, FENCAMFAMINE AND COCAINE ON MATERNAL BEHAVIOR AND DEVELOPMENT IN RAT PUPS. W. Klipec*, D. Lawler, S. Catlin, C. Wahling and K. Illig. Department of Psychology, Drake University, Des Moines, IA 50311

Fencamfamine (FCF) is a stimulant with a mechanism of action and behavioral effects similar to both amphetamine (AMPH) and cocaine (COC). Previous research in our laboratory has demonstrated that compared to AMPH and COC, FCF produces transient impairment of neonatal reflexes and development during the first 21 postnatal days in pups reared by dams who received daily 5.6 mg/kg doses of these drugs from mating through 21 postnatal days. The present study extends previous research to investigate growth, development and neonatal reflexes following exposure to 10 mg/kg doses of AMPH, COC and FCF compared to no injection (NI) and saline (SAL) controls. Of the eight AMPH dams to carry litters to full term, five died during labor and two cannibalized their litters. For the remaining AMPH dam, injections were discontinued for two days prior to delivery, which was normal, and gradually increased to 10 mg/kg per day after delivery. Body weight for SAL and COC pups was significantly lower than NI controls from Day 14 through 8-weeks, and FCF pups weighed less than SAL controls from Day 21 through 8-weeks. Incisors erupted by Day 10 for FCF and COC pups compared to Day 14 for both NI and SAL pups. Compared to NI controls, both SAL and FCF pups showed delayed eye opening (Day 18 or later). Unlike previous studies, no consistent effects between groups were found for righting reflex, positive geotaxis or open field activity. All AMPH and FCF, but not COC dams exhibited intense focused sterotypies and disrupted maternal behaviors for at least 4-hours after injections. While the results clearly indicate that FCF has teratogenic potential demonstrable beyond the stress induced effects for the SAL pups, the pattern of results, in the context of prior research, raises questions about the degree to which the effects of FCF are due to prenatal actions or postnatal disruption of maternal behavior.

713.11

THE EFFECTS OF EARLY MALNUTRITION FOLLOWED BY NUTRITIONAL REHABILITATION ON CENTRAL CONDUCTION VELOCITY AND CORTICAL EXCITABILITY IN RATS.

G.J. Quirk*, W.R. Mejía, H. Hesse and H. Su. Dept. of Physiology, National Autonomous University of Honduras Medical School (UNAH), Tegucigalpa, Honduras.

A recent survey has indicated that over 50% of Honduran children under the age of five suffer from some degree of malnutrition. To further understand the effects of such malnutrition on the developing CNS, malnourishment was produced in rat pups from birth by restricting lactating dams to half of their normal consumption of standard laboratory chow. Following weaning (day 21) rats were housed individually and had full access to food and water. Reductions in body weight of 40% at day 21 were reduced to 20% by day 70. Total brain weight was reduced 10% relative to controls ($p < .01$). Close to day 75, male and female rats were anesthetized with urethane and the response to surface electrical stimulation of motor cortex was recorded "killed-end" from the pyramidal tract at C1. Following a single shock, direct (D-wave) and indirect (I-wave, synaptic) activation of corticospinal neurons were observed (Stewart et al. Brain Res. 508:341). Malnourishment reduced the conduction velocity of the fastest PT fibers 24% relative to controls (11 controls: 21m/s; 14 malnourished: 16m/s, $p<.001$). In addition, the size of the D-wave in malnourished rats was significantly reduced throughout a range of stimulus intensities by an average of 40%. No significant difference was observed for the I-wave. Slowed conduction velocity could be due to decreased myelination observed in rehabilitated rats (Sima et al, Acta Neuropath. 42:15). Supported by a Fulbright Grant to GJQ.

713.12

Chronic *in utero* Microdialysis in the Ovine Fetal Brain: Effect of Maternal Hemorrhage on Extracellular Glutamate

D.H. Penning*, D.H. Chestnut, B. Atkins, P. Chatterjee, J. Hrdy & D. Poduska. Dept. of Anesthesia, U. of Iowa, Iowa City, Iowa, 52242.

Hypoxic release of glutamate may produce neurotoxicity in the developing brain. Maternal hemorrhage can produce fetal hypoxemia and acidosis. We hypothesized that maternal hemorrhage may lead to increased extracellular glutamate in the fetal brain. This was tested in 6 chronically instrumented fetal sheep (gestational age 118-125 days, term = 147) Under general anesthesia, fetal and maternal vascular catheters and an electromagnetic flow probe on the maternal uterine artery were placed. A microdialysis probe was placed in the frontal cortex of the fetus. The animals were allowed to recover from surgery for 5 days. On the day of the experiments the probe was perfused with artificial CSF @ 1μL/min. Perfusate was collected and analyzed for glutamate by HPLC. Maternal hemorrhage (5 mL/kg maternal wt.) was repeated until the fetal pH < 7.00 then the blood was re-transfused.

Experimental Period (n=6)	Peak Glutamate % Basal (SE)	Fetal pO2 mmHg (SE)	Fetal pH (SE)	Uterine Blood Flow % Basal (SE)
Control	100 (12)	19.7 (1.2)	7.34 (.01)	100 (1.5)
Hemorrhage	303 (31)	12.8 (1.3)	6.95 (.01)	11 (2)
Recovery (1 Hr)	104 (16)	22.2 (1.7)	7.09 (1.9)	87.3 (1.9)

Maternal hemorrhage in the unanesthetized ewe, producing fetal hypoxemia and acidemia, resulted in an increase in fetal brain extracellular glutamate concentration. Such glutamate release may be involved in the mechanism of fetal brain injury, (e.g., cerebral palsy), resulting from *in utero* hypoxia. (Supported by R.J.Carver Foundation)

713.13

EFFECTS OF ETHANOL ON GLUTAMATE CONCENTRATION IN OVINE FETAL CEREBRAL CORTEX, DETERMINED BY MICRODIALYSIS.

J.D. Reynolds, D.H. Penning, B. Atkins, J. Hrdy, D. Poduska, D.H. Chestnut & J.F. Brien*. Dept. of Pharm. & Tox., Queen's U., Kingston, Canada & Dept. of Anesthesia, U. of Iowa, Iowa 52242.

The cerebral cortex is a target site of ethanol (E) teratogenesis. L-Glutamate (GLU) is a neuroactive amino acid. For normal cerebral cortical development optimal [GLU] is required. We tested the hypothesis that E changes [GLU] in the fetal cerebral cortex. This study was conducted in the instrumented fetal sheep at 118 to 125 days of gestation (term=147 days) with a microdialysis probe in the frontal cortex. The effects of maternal IV infusion, over 5h, of 2 g E/kg body weight (n=3) or 4 g E/kg (n=3), as four equally divided doses, on fetal extracellular [GLU] were determined. [GLU] in the microdialysate and fetal blood [E] (BEC) were determined by established methods. Neither E dose produced fetal lethality during the experiment.

		Dose 1	Dose 2	Dose 3	Dose 4
2 g E/kg	[GLU] % Basal*	66.82 (31.12)	75.60 (31.37)	79.11 (25.93)	55.72 (12.65)
(SD)	Fetal BEC mg/ml	0.79 (0.04)	1.44 (0.16)	2.30 (0.30)	3.27 (0.47)
4 g E/kg	[GLU] % Basal*	180.06 (130.81)	483.38 (573.99)	203.89 (113.86)	123.14 (93.36)
(SD)	Fetal BEC mg/ml	1.53 (0.43)	2.77 (0.74)	3.98 (1.23)	5.40 (0.89)

* Basal [GLU] in microdialysate = 173 +/- 66 (SD) ng/ml

The data demonstrate that: E produces dose-dependent changes in the extracellular fluid [GLU] in the fetal cerebral cortex with a multi-fold increase in [GLU] for 4 g E/kg; and the effects are not solely dependent on the fetal BEC. (Supported by R.J. Carver Foundation & MRC of Canada)

713.14

STIMULATION OF AXONAL ELONGATION BY ALCOHOL IS REVERSED BY PROTEIN KINASE C INHIBITOR.

M. Thompson and M.I. Johnson.* Dept. of Pediatr., Fan Kane Neurol. Res. Lab., Steele Mem. Childr. Res. Cntr., Ariz. Hlth. Sci. Cntr., Tucson, AZ 85724-0001.

Although fetal alcohol exposure may be the most common cause of mental retardation, little is known about the specific mechanisms by which alcohol alters central nervous system development. In an in vitro model of neuronal differentiation, ethanol stimulates the elongation of axons extending from explants of perinatal rat sympathetic ganglia. Because protein kinase C (PKC) is part of one pathway that may be affected by ethanol, we investigated the effect of a PKC inhibitor on alcohol-stimulated axonal growth. Sphingosine in concentrations from 50 nM to 1 μM showed a dose-dependent depression in axonal growth after 5 days in culture (control, 2392 μm ± 62; 50 nM, 2112 ± 76; 100 nM, 1972 ± 88; 500 nM, 1162 ± 57; 1 μM, 861 ± 43; μm ± SEM; $p < 0.02$ to < 0.0001). Removal of the sphingosine resulted in recovery of axonal extent to control levels at day 7 for both 50 and 100 nM sphingosine and partial recovery for cultures treated with 500 and 1000 nM. We also studied whether inhibition of alcohol-stimulated axonal elongation by sphingosine could be reversed by the phorbol ester, PDBu. At concentrations of 25 and 50 nM, PDBu reversed the effects of the 200 nM sphingosine to control values. Other PKC inhibitors acting at both the regulatory and the catalytic site of PKC are under investigation. Studies suggest that one mechanism by which alcohol may act is through a pathway involving PKC, possibly in phosphorylation of proteins important in axonal elongation. (Supported by ADC 82-2689 and USPHS-NIH R01 AA08950).

713.15

EFFECTS OF HYPOXIA ON THE DEVELOPING BRAIN AT MID AND LATE GESTATION IN FETAL SHEEP.

S. Rees*[1], Y. Just, S. Hooper, M. Wlodek & R. Harding. [1] Department of Anatomy & Cell Biology, University of Melbourne, Parkville, Victoria 3052, Australia and Department of Physiology, Monash University, Clayton, Victoria 3168, Australia.

Recent evidence supports the view that prenatal factors, such as hypoxia, may be important antecedents of non-progressive neurological impairment. Little is known about the effects of hypoxia on the structure of the developing brain, particularly in the early preterm fetus. In this study on fetal sheep we have examined the effects of hypoxia (12hr) on the morphology of the brain at 85-95 days (n=11) and 135-140 days (n=3) of gestation (term=146d). In anaesthetised animals, an externally adjustable vascular clamp was placed on the maternal common internal iliac artery and vascular catheters inserted in the fetus. Five days after surgery the clamp was adjusted to reduce uterine blood flow until a 50% reduction in fetal arterial oxygen content had been achieved. One week after hypoxia, the fetal brains were perfused and tissue from the cerebral cortex, underlying white matter and hippocampus examined with light and electron microscopy. At 85-95 days, in all hypoxic animals there was evidence of neuronal degeneration predominantly in the hippocampal pyramidal cell and germinal layers but also in the cerebral cortex, particularly layers 4-6. The width of stratum oriens (hippocampus) was also significantly reduced in hypoxic animals compared with age-matched non-hypoxic control animals (n=11). There was also evidence of lesions in the cortical white matter adjacent to the lateral ventricles. By comparison the effects of hypoxia at 135-140 days were much less pronounced. Degeneration was not observed in the white matter or hippocampus and there was only sparse degeneration in the cortex. Thus in early fetal life, the brain appears to be more vulnerable to the effects of acute hypoxia than it is in late gestation when compensatory circulatory mechanisms have begun to operate.

713.16

THE DEVELOPMENTAL PROFILE OF PKC ISOFORMS IN THE RAT BRAIN IS ALTERED BY GESTATIONAL EXPOSURE TO METHYL MERCURY.

N. Haykal-Coates[1], E.S. Goldey[2], D.W. Herr*[2], H.A. Tilson[2], S. Barone Jr.[1] [1]ManTech Environmental Tech. Inc., [2]NTD/HERL, US EPA, RTP, NC 27711.

Protein kinase C (PKC) mediated phosphorylation has been implicated in neuronal growth and differentiation (Turner *et al., Proc. Natl. Acad. Sci. USA*, 1984). Using immunohistochemistry, we examined the effects of gestational exposure to the neurotoxicant, methyl mercury (CH_3Hg) on the developmental profile of immunoreactivity (IR) for the calcium-dependent, α and β, and calcium-independent, ε, PKC isoforms. Long-Evans dams were dosed on gestational days 6-15 (po) with 0, 0.1, 1, or 2 mg/kg CH_3Hg dissolved in saline. Pups were sacrificed and perfused with buffered paraformaldehyde on postnatal days (PND) 1, 4, 10, 21, 41, and 85. The brains were sectioned sagittally, stained immunohistochemically, and examined throughout the medial to lateral extent of the brain. The greatest density of immunoreactivity of neural cell bodies was seen in the olfactory bulb, hippocampus, inferior colliculus, pons, cerebral, pyriform, and cerebellar cortex, whereas axonal staining was prominent in the brainstem, internal capsule, corpus callosum, anterior commissure, fornix and olfactory tract. In controls, the IR for each isoform was highest at the earliest time-points examined (PND1-4), decreasing dramatically by PND10, and decreasing further after PND21. In all regions examined in the neonate, the locations of IR for isoforms α and ε were similar. In contrast to the neonatal α and ε, there was a different pattern for β IR in the thalamus, neocortex and olfactory bulbs. The highest dose of CH_3Hg produced a persistent increase in regional α IR, a decrease in ε IR at early time points (PND1-4), and no apparent change on β IR. These changes in PKC IR were not related to overt pathology at any of the time points examined. The present results characterize the cellular and regional ontogeny of 3 PKC isoenzymes, and suggests that developmental exposure to CH_3Hg can alter that ontogeny.

713.17

A DEVELOPMENTAL PROFILE OF TRK IMMUNOREACTIVITY IN THE RAT BRAIN IS AFFECTED BY GESTATIONAL EXPOSURE TO METHYL MERCURY. S. Barone Jr.[1*], N. Haykal-Coates[1], E.S. Goldey[2], H.A. Tilson[2]. [1]ManTech Environmental Tech. Inc., [2]NTD/HERL, US EPA, RTP, NC 27711.

The high affinity nerve growth factor receptor, trk has been implicated in neuronal growth and differentiation. In this study we examined the effects of gestational exposure to the developmental neurotoxicant methyl mercury on the developmental profile of immunoreactivity (IR) for this receptor. Long-Evans dams were dosed on gestational days 6-15 (po) with 0, 0.1, 1, or 2 mg/kg methyl mercury dissolved in saline. Pups were sacrificed and perfused with buffered paraformaldehyde on postnatal days (PND) 1, 4, 10, and 21. The brains were sectioned sagittally, stained immunohistochemically, and examined throughout the medial to lateral extent of the brain. The greatest density of immunoreactivity of neural cell bodies was seen in the olfactory bulb, hippocampus, septum, striatum, nucleus basalis, inferior colliculus, pons, cerebral, and cerebellar cortex, and axonal staining was prominent in the brainstem, neocortex, hippocampus, cerebellum, and olfactory tract. In general the regional pattern of this IR was transient except in the olfactory bulb, hippocampus, cerebellum. In controls, trk IR appeared to peak at PND4, decreasing dramatically on PND10 and decreasing further after PND21. An adult-like pattern was apparent on PND21 with trk IR in the hippocampus and cerebellum. Methyl mercury produced a dose-related decrease which was apparent at PND1, PND10, and PND21 but not at PND4. This decrease in trk IR was not related to overt pathology at the time points examined. The present results characterize the cellular and regional ontogeny of the tyrosine kinase, trk and suggest that developmental exposure to methyl mercury can alter the ontogeny of this trophic factor receptor.

713.18

EFFECT OF PRENATAL MALNUTRITION AND POSTNATAL NUTRITIONAL REHABILITATION ON SPINE DENSITY OF CA3 HIPPOCAMPAL PYRAMIDAL CELLS IN RATS. **M. García-Ruiz*, L. Parra, Corkidi, G., L. Cintra and S. Díaz-Cintra. Inst. de Invest. Biomédicas, UNAM, MEXICO, D.F. 04510.**

The effects of prenatal malnutrition (6% casein diet) and postnatal rehabilitation (25% casein diet) were studied on synaptic spine density of CA3 hippocampal pyramidal cells in rats of 4 ages (15, 30, 90 and 220 days old). A total of 288 CA3 cells impregnated with rapid-Golgi were selected for morphometric analysis and the density of spines were counted (using a calibrated ocular reticle and 63x planapocromatic oil objective) in six 50μm segments arising from a proximal largest apical dendrite (3 segments) and middle, terminal and basal segments of the largest dendrite. Results showed that prenatal malnutrition produced significant decreases ($p<0.05$) in spine density. However, the only middle and terminal segments showed deficits at all ages studied. These segments represent the sites where Schaffer recurrents and perforant pathway synapse, respectively. The findings indicate that: a) the reductions on synaptic spine density due to prenatal malnutrition are not compensated by postnatal nutritional rehabilitation, b) since the reductions were found across four ages prenatal malnutrition produces a long-term effect on CA3 pyramidal cells and c) the synaptic changes found in the middle and terminal segments represent deficits in the functional integrity of field CA3. Supported by DGAPA IN202891 AND IN2044892

713.19

DEVELOPMENTAL EFFECTS OF VITAMIN B-6 DEFICIENCY ON THE GABAa RECEPTOR IN THE RAT BRAIN: AN AUTORADIOGRAPHIC STUDY. J. Pilachowski and T.R. Guilarte*. The Johns Hopkins University School of Hygiene and Public Health, Baltimore, MD.

Vitamin B-6 deficiency (B6D) during development is known to result in spontaneous seizures. Seizure activity in B6D experimental animals and human infants is believed to be associated with reduced brain levels of the inhibitory neurotransmitter, GABA. There is a lack of data on the effects of B6D on the postnatal development of the GABAa receptor in the rat brain and its potential role in the seizure susceptibility of these animals.

In the present study, [^{3}H]-muscimol autoradiography was used to determine the effect of B6D on the postnatal development of the postsynaptic GABAa receptor. The results indicate that in normal rats there is an increase in [^{3}H]-muscimol binding in all brain regions analyzed from postnatal day 14 (PN14) to PN28, with a subsequent decrease in the level of binding from PN28 to PN56. Vitamin B-6 deficiency resulted in a marked increase in [^{3}H]-muscimol binding in the granular layer of the cerebellum at PN14 and in cortical and subcortical brain regions at PN28. Deficient rats had a significantly lower level of [^{3}H]-muscimol binding in the cerebral cortex, corpus striatum, and hippocampus at PN56 relative to controls. These results indicate that marginal intakes of dietary vitamin B-6 during development results in marked changes in GABAa receptor binding and may be associated with the increased seizure susceptibility of these animals. (Grant # HD20939).

MOLECULAR AND PHARMACOLOGICAL CORRELATES OF DEVELOPMENT IV

714.1

Expression of a mitogen-inducible cyclooxygenase in brain neurons: regulation by synaptic activity and glucocorticoids W.E. Kaufmann*, K. Yamagata, K. J. Andreasson, C. A. Barnes, and P. F. Worley. Depts. of Neuroscience and Neurology, Johns Hopkins University, Baltimore, MD 21205 and ARL, Div. of Neural systems, Memory and Aging, University of Arizona, Tucson, AZ 85724.

The first step in prostaglandin synthesis involves enzymatic oxidation of arachidonic acid that is catalyzed by endoperoxidase (PGG/H) synthase, also referred to as cyclooxygenase. We have cloned an inducible form of this enzyme from rat brain that is nearly identical to a murine, mitogen-inducible cyclooxygenase identified from fibroblasts (Kujubu et al., 1991). Our studies indicate that this gene, termed COX-2, is expressed throughout the forebrain in discrete populations of neurons and is enriched in the cortex and hippocampus. Neuronal expression is rapidly and transiently induced by seizure or NMDA dependent synaptic activity. No expression is detected in glia or vascular endothelial cells. Basal expression of COX-2 appears to be regulated by natural activity in the developing and adult brain. Both basal and induced expression of COX-2 are inhibited by glucocorticoids, consistent with COX-2 regulation in peripheral tissues. Our studies indicate that COX-2 synthesis may be important in regulating prostaglandin signaling in brain. The marked inducibility in neurons by synaptic stimuli suggests a role in activity-dependent plasticity. Supported by EY08900, EY09374, HD009219, and AG09219.

714.2

DEVELOPMENTAL EXPRESSION OF $GABA_A$/BZ RECEPTOR SUBUNIT mRNAs IN THE MOUSE INFERIOR OLIVARY NUCLEUS. C.-C. Chang*, S. Gullapalli, A. Rotter and A. Frostholm. Department of Pharmacology, The Ohio State University, Columbus, OH 43210.

Inferior olivary neurons receive GABAergic inputs and express $GABA_A$/BZ receptors. In a previous study we have reported (Frostholm et al., 1992) that [^{3}H]muscimol binding sites and $\beta 3$ subunit mRNA are high at birth, but are considerably down-regulated during postnatal week two; $\alpha 1$, $\beta 1$, $\beta 2$, $\gamma 2$ and δ mRNAs are absent, or present at low levels. In the present experiments, we have examined the developmental expression of additional α and γ subunit mRNAs in olivary neurons, which were visualized by in situ hybridization using subunit specific riboprobes. Unlike the $\beta 3$ signal, $\alpha 4$ mRNA was present at moderate intensity at birth and remained stable into adulthood. However, the $\gamma 1$ mRNA signal was completely absent at birth, only becoming detectable between postnatal day (P)7 and P9. Between P9 and P12, the hybridization signal increased rapidly, reaching adult levels at approximately the end of postnatal week three. Since POU domain transcription factors have been implicated in cell specific gene expression, we have also examined the developmental expression of Brn-3 mRNA, which is known to be present in the adult inferior olive. A strong signal was present in olivary neurons throughout their embryonic and early postnatal development; reduced expression was observed in older mice. These findings suggest that the subunit composition of the $GABA_A$/BZ receptor in inferior olivary neurons changes during development, and raise the possibility that Brn-3 may play a role in subunit expression in these cells.

714.3

PROTEIN KINASE C IN THE GOLDFISH RETINOTECTAL SYSTEM: NORMAL AND CHANGES IN REGENERATION. John T. Schmidt*, Dept. of Biol. Sci., SUNY Albany NY 12222.

The regenerating retinotectal projection undergoes an activity-driven sharpening of its retinotopic precision. This sharpening is blocked by NMDA receptor blockers, is associated with an increased capacity for long-term potentiation (LTP), and involves the elimination of errant (nonretinotopic) branches of optic axons. Protein kinase C (PKC) is often involved in synaptic plasticity, including induction of LTP. Inhibiting or activating PKC in the regenerating projection (either intracranially or intraocularly) prevents retinotopic refinement. In this study we examined 1) what neurons in this system contain PKC, and 2) whether PKC activity levels increase during regeneration?

Sections from retina, optic nerve and tectum were immunostained with an antibody against pan PKC (UBI). Staining in retina was similar to that reported in other vertebrates. The antibody stained a subset of bipolar cells and of amacrine cells. Staining in photoreceptors was very light to nonexistent. Most if not all ganglion cell somas stained darkly, although there was much less staining in the optic fiber layer. In optic nerve, there was moderate staining, darker in glial septa. In tectum, there was intense staining of a subset of type XIV cells, both the somas at the top of the periventricular layer and thick dendrites ascending to branch in and below stratum opticum. These cells are very similar to ones previously reported to stain for choline acetyltransferase. Staining was also somewhat darker in SO and in retinal termination bands just below.

PKC activity was measured using a substrate peptide assay (Amersham). Activity was linear with the amount of homogenate, and was eliminated with deletion of lipids or calcium. Levels in tectum were 2X and 4X higher than in retina and nerve respectively. At weekly intervals after the right nerve was crushed, PKC levels were measured in right versus left retina, tectum and nerve. Retinal levels were not significantly different except for a small increase at 3 weeks. Tectal levels decline to 85% of control in weeks 3-5. Nerve levels, however, increased by about 80% in weeks 2,3 and 4. This may indicate either an upregulation in ganglion cells (masked in whole retina by the other cells' contribution), or upregulation in reactive glial cells. Supported by NIH grant EY-03736.

714.4

THE D_2 AGONIST QUINPIROLE MODULATES SPONTANEOUS DOPAMINE RELEASE FROM THE NEOSTRIATUM OF DEVELOPING AND ADULT RATS. S.L. Andersen* and R.A. Gazzara. Center for Developmental Psychobiology, Dept. of Psychology, Binghamton Univ. (SUNY), Binghamton, NY 13902-6000.

Results from previous studies in our laboratory examining the effects of dopamine (DA) agonists and antagonists on K^+-evoked DA release suggest that DA autoreceptor function is apparent by postnatal day 5 and varies as a function of age. In this study we investigated the effects of local administration of the full D_2 agonist quinpirole (QUIN) on spontaneous DA release since high K^+ is more effective in releasing DA in adults relative to younger rats and may have influenced the results.

Extracellular levels of DA in the neostriatum of urethane-anesthetized rats 5, 10-11, 15-16, 21-22 days of age, and adults were determined every 15 min by in vivo microdialysis coupled with HPLC-EC. Increasing concentrations of QUIN (0.01, 0.1, 1, 10, and 100 μM) were perfused successively into the neostriatum via the microdialysis probe. QUIN attenuated extracellular levels of DA (as a percentage of pre-drug baseline) in all ages (5 days, 50%; other ages, 35-40%), which is comparable to the effect of QUIN on K^+-evoked DA release.

In addition, the results of this study will be compared with the effects of locally perfusing the partial D_2 agonist (+)3-PPP into the neostriatum of developing and adult rats.

714.5

LOCAL PERFUSION OF (-)SULPIRIDE VIA MICRODIALYSIS HAS AGE-DEPENDENT EFFECTS ON SPONTANEOUS DA RELEASE IN THE NEOSTRIATUM OF DEVELOPING AND ADULT RATS. R.A. Gazzara* and S.L. Andersen. Center for Developmental Psychobiology, Dept. of Psychology, Binghamton Univ. (SUNY), Binghamton, NY 13902-6000.

A within-subject dose-response analysis of the effects of the D_2 antagonist (-)sulpiride (SULP) was conducted in urethane-anesthetized rat pups 5, 10-11, 15-16, and 21-22 days of age, and adult rats. SULP (0.1, 1, 10, and 100 μM) was administered locally via a microdialysis probe into the neostriatum. Samples were collected every 15 min and extracellular levels of dopamine (DA) and 3,4-dihydroxyphenylacetic acid (DOPAC) were measured by HPLC-EC.

Levels of DA and DOPAC increased following local perfusion of SULP in a dose-dependent manner in all age groups. The maximum effect of SULP on DA levels (as a percentage of the pre-drug spontaneous baseline levels) was greatest in the three youngest age groups (250%) and lower in both the 21-22 day group (190%) and the adult group (135%). Extracellular levels of DOPAC increased at all ages, however, the percentage increase did not differ as a function of age (160%).

These results are consistent with our previous studies that suggest that the D_2 autoreceptor in the neostriatum is functional but not mature by postnatal day 5 in the rat.

714.6

INITIAL CHARACTERIZATION OF PERCOLL-ENRICHED POPULATIONS OF EMBRYONIC RAT HIPPOCAMPAL CELLS. S.V. Smith, A.E. Schaffner, D. Maric, I. Maric, T.G. Smith, Jr.* and J.L. Barker, Laboratory of Neurophysiology, NINDS, NIH, Bethesda, MD 20892

We have used a multi-step discontinuous gradient of Percoll to isolate subpopulations of embryonic (E) hippocampal cells according to their buoyant density after papain dissociation. At least four distinct subpopulations of cells could be routinely isolated at E18-22. The relationship between buoyant density and cell cycle status was evaluated using ip injections of BrdU into mothers prior to sacrifice, followed by Percoll fractionation, then quantitative flow cytometric analysis of anti-BrdU immunofluorescence and propidium iodide staining. Preliminary results indicate that cells of higher density were preferentially in DNA synthesis while cells of lower density were preferentially post-mitotic. Immunocytochemical studies revealed that a number of cells in all four subpopulations expressed neuronal or glial antigens. Analysis of fractionated cells using a voltage-sensitive dye and flow cytometry revealed that the majority of cells in each fraction were well polarized, primarily according to K^+-dependent mechanisms. Stimulation of cells with ligands to probe for electrical and chemical forms of excitability revealed that subpopulations of cells in each fraction exhibited both forms of excitability. These results indicate that fractionation of embryonic cells according to their buoyant density is an effective strategy to enrich for pre- and post-mitotic cells for quantitative analysis of anitgen expression and excitability in entire populations of embryonic cells. Further characterization of the cellular properties expressed in progenitor and differentiating neurons and glia will complement this initial characterization.

714.7

CORTICAL CELLS FROM E17 RAT EMBRYOS CONTAIN A NICOTINE-DISPLACEABLE ALPHA-BUNGAROTOXIN BINDING SITE. J.J. Jensen and F.M. Leslie*, Department of Pharmacology, U.C. Irvine, Irvine, CA 92717

The neuronal alpha-bungarotoxin (BTX) site is a pharmacologically distinct nicotinic receptor subtype that exhibits a unique, transient developmental binding pattern in rat sensory cortex. Although the physiological significance of these BTX sites remain unknown, it has been proposed that they may play a role in growth and development, and in controlling the intracellular free calcium levels in target cells. In order to identify cortical cell types which express this binding site, we have examined the binding of [125-I] BTX to fetal rat cortical cells in primary culture. Cortical cells from E17 rat embryos displayed a nicotine displaceable BTX binding site at 3 to 16 days in culture. When treated with 5-fluorodeoxyuracil on 1 to 3DIV to inhibit glia proliferation, there was an increase in nicotine-displaceable BTX binding in the resulting neuronal enriched cultures. A similar increase in BTX binding was also observed in control cultures at 8DIV when conditioned for 4 hours with media from the 5 fluorodeoxyuracil-treated cultures. Pure glia cells, obtained by plating cortical cells on 24-well plates not coated with polylysine, followed by daily media changes, showed a low level of BTX binding when compared to the mixed neuronal-glia cultures, and this binding was not nicotine-displaceable. Furthermore, cortical cells plated onto a pure established glia layer exhibited fewer nicotine displaceable BTX binding sites when compared to cortical cells plated onto polylysine alone. These results suggest that nicotinic BTX binding proteins are expressed on cortical neurons and not on cortical glia cells, but that this site may be regulated by glia.
Supported by PHS grant #NS 30109.

714.8

NGF-INDUCED DIFFERENTIATION OF PC12 CELLS STIMULATES ACCUMULATION AND SECRETION OF A GP2-LIKE PROTEIN. S.Koshlukova J.Roth*, and J.Aletta.Dept. Pharmacol.,SUNY at Buffalo, Buffalo, NY 14214.

Glycoprotein 2 (GP2) is a major protein of zymogen granule membranes (ZGM) from cells of exocrine pancreas. Although it is known to possess a GPI anchor, a soluble form has been described as well. In efforts to examine the potential biological role(s) of this membrane constituent we have begun to examine several cell lines which possess distinctive secretory pathways. The present study has used the PC12 cell line. A rabbit antiserum against GP2 isolated from pancreatic juice specifically cross-reacts with a PC12 cell protein of 70 kDa on Western blots. NGF-treatment of the cells for 1-2 weeks resulted in an increased immunoreactivity. Using this GP2-specific reagent, we found that the 70 kDa protein co-localized with CA-containing vesicles obtained by differential centrifugation. Attempts to identify a GPI anchor with the use of PI-specific PLC have not been successful. It is interesting, however, that an anti-CRD antibody (which is known to recognize a carbohydrate moiety of the GPI anchor only after it is cleaved from the membrane) unexpectedly cross-reacts with the 70 kDa protein in the vesicle membrane fraction. The GP2-like molecule is also detectable in the culture medium. The apparent release, measured at 6, 12 and 24 hours was greater at each time point from cells treated with NGF for 15 days than from non-treated PC12 cells. In conclusion we report here that a GP2-like protein is (1) present in PC12 cells, (2) enriched in a crude vesicle membrane fraction and (3) can be released from cells in greater amounts after NGF-treatment. We conclude that this molecule may be developmentally regulated in neurons as well as other specialized secretory cells. Supported in part by NIH grant AM-28029 NIDDKD

714.9

NEONATAL 6-OHDA LESIONS SELECTIVELY ALTER THE EXPRESSION OF D1 RECEPTORS AND D1 mRNA IN THE STRIATUM OF ADULT RATS.

P.A. Frohna*, B.S. Neal-Beliveau, and J.N. Joyce, Depts. Psychiatry and Pharmacology, Univ. of Pennsylvania Medical School, Philadelphia, PA, 19104.

Neonatal 6-OHDA lesions have allowed us to study the role of dopamine in the developmental regulation of DA receptors in the striatum. Recently (Neal and Joyce, *Synapse* 11:35, 1992), we reported a decrease in D1 DA receptors in the dorsomedial (DM), dorsolateral (DL), and ventromedial (VM) quadrants of the rostral caudate-putamen (CPu) of adult rats lesioned on postnatal day 1 (P1). In the caudal CPu only the DM quadrant continued to show a significant decrease. D2 DA receptors were unchanged in all regions of the striatum examined. To further investigate the role of DA on the development of the DA receptor systems in the striatum, we performed *in-situ* hybridization histochemistry with [35S]-labeled oligonucleotide probes for the D1 and D2 receptors in adult rats given P1 6-OHDA or saline injections.

In the rostral striatum, there was a trend towards an increase in D1 mRNA in all regions that was significant ($p<.05$) in the DM (+29%) and VM (+28%) CPu, and the core region of the nucleus accumbens (+24%). No change in D1 mRNA levels was found in the caudal striatum. With opposite changes in D1 mRNA and receptor density observed, we autoradiographically measured D1 receptors in the SNpr to determine if there was an increase in receptor transport to striatonigral terminal fields. D1 binding, measured with [3H]SCH 23390, was similar between the two groups in the rostral SNpr. In the caudal SNpr there was a significant decrease in the lesioned group (721 fmol/mg prot) compared to the controls (830 fmol/mg prot; $p<.01$). Therefore, an increase in D1 mRNA in the striatum does not lead to an increase in D1 receptors in the terminal regions of the striatonigral neurons. Like D2 receptors, there was no change in D2 mRNA levels in any region of the striatum of the lesioned animals compared to controls.

These findings further suggest that the D1 DA receptor is developmentally dependent on DA and may require DA for normal transcriptional and translational control. **Funded by R29 MH 43852.**

714.10

EXPRESSION AND FUNCTIONAL MODULATION OF INWARD-RECTIFYING AND CALCIUM-ACTIVATED POTASSIUM CONDUCTANCES IN DIFFERENTIATING SKELETAL MUSCLE.

S. J. Wieland*[1] and Q-h. Gong[1,2]. Depts. of Anatomy[1] and Anesthesiology[2], Hahnemann University, Philadelphia, PA, 19102

The mouse myoblast cell line C2C12 can be controllably induced to undergo fusion into differentiated, non-dividing myotubes in vitro. Among the changes in functional gene expression accompanying differentiation is the induction of an inward-rectifying potassium conductance. The mean inward current of myoballs derived from fused myotubes 7 days after induction of fusion was 1.4 nA at -140 mV. This was approximately 10-fold greater than in dividing myoblasts. The inward rectifier was subject to rapid metabolic control. Exposure of the cytoplasm to 25 μM GTP-γ-S during whole-cell recording produced inhibition of the conductance by 75% within 20 min., while untreated cells were unchanged over this time period. Elevation of intracellular free Ca^{2+} or of intracellular IP_3 also inhibited inward rectifying currents. In contrast to to the inward rectifier, a second potassium conductance which was activated by intracellular Ca^{2+} and apamin-blockable was detectable in both myoblasts and in differentiated myotubes. The sensitivity of this conductance to Ca^{2+} activation was strongly enhanced by the β-adrenergic agonist isoproterenol. Thus two potassium channels which modulate the electrical excitability of skeletal muscle, a calcium-activated, apamin-sensitive channel, and an inward-rectifying channel, are subject to separate control of expression and are separately modulated by cellular messengers.

714.11

LOCAL CEREBRAL PROTEIN SYNTHESIS IN THE RAT DURING NORMAL DEVELOPMENT.

Y. Sun*, G. E. Deibler, J. Jehle, J. Macedonia, I. Dumont, T. Dang, and C. Beebe Smith. LCM, NIMH, Bethesda, MD 20892.

Regional rates of cerebral protein synthesis ($lCPS_{leu}$) were determined with the autoradiographic L-[1-^{14}C]leucine method (Smith et al.,*PNAS* 85:9341, 1988) in 10 (n=7), 14 (n=6), 21 (n=6), 35 (n=6), and 60 (n=6) day- old rats. λ, a constant in the equation for $lCPS_{leu}$ which corrects for recycling, was determined in whole brain at each age (Sun et al., Soc. Neurosci. Abstr. 18:421, 1992) and age-specific values were used in the calculation of $lCPS_{leu}$ in all 43 brain regions. Studies were carried out on freely moving rats maintained at normal body temperature throughout the study. Values for $lCPS_{leu}$ ranged from 5.5 to 31.6 nmol/g/min at 10 days and from 3.8 to 20.8 nmol/g/min at 60 days (adult). In brain as a whole and in most areas of cortex $lCPS_{leu}$ were highest at 10 days gradually decreasing with age. By 60 days of age values were about 60% of those determined at 10 days. There were several noteworthy exceptions to this tendency. In the paraventricular and supraoptic nuclei, $lCPS_{leu}$ *increased* during the course of development reaching peak values in adults. $lCPS_{leu}$ in dorsomedial hypothalamic nucleus, subthalamic nucleus and pineal gland remained relatively constant at all ages examined. In white matter regions, peaks of $lCPS_{leu}$ were reached in the cerebellum and cerebrum at 14 and 21 days, respectively, approximating the times of myelination. The decreases we found in whole brain protein synthesis with developmental age are in agreement with the work of (Dunlop et al. *J. Neurochem.* 29:939, 1977). Application of the [1-^{14}C]leucine method in our study corrects for the effects of recycling and increases the anatomical resolution to the level of individual nuclei and cell layers.

714.12

NEUROCHEMICAL AND BEHAVIORAL ALTERATIONS IN ADULT RATS TREATED THROUGHOUT DEVELOPMENT WITH MAO INHIBITORS.

X. Zhang*, C. Clarke and P.M. Whitaker-Azmitia. Dept. of Psychiatry, SUNY, Stony Brook, New York 11794.

Monoamine oxidase is a crucial enzyme in regulating the levels of the monoamine neurotransmitters dopamine and serotonin, both of which function in the immature brain as developmental signals. A number of human disease states are characterized by low levels of MAO, including Norrie's Disease, attention deficit disorders and alcoholism. As well, some drugs of abuse and environmental toxins decrease the activity of the enzyme. The current study was undertaken to determine what effect lowered MAO activity might have on brain development.

Pregnant Sprague-Dawley rats were treated once daily throughout pregnancy with 3 mg/kg IP of each of clorgyline and deprenyl and the pups treated until sacrifice. The pups were behaviorally tested up to PD 30 and assayed for serotonin and dopamine terminal density using the appropriate transporter labels. MAO-I exposed pups showed significantly increased open field activity at PD 16 ($p<.03$) and PD 30 ($p<.03$) and deficits in passive avoidance at PD 23 ($p<.01$) but no changes in a measure of anxiety or spontaneous alternation. In addition to these behavioral measures, the pups were highly stereotypic and had spontaneous seizures. There were no significant changes in dopamine innervation of caudate or cortex, but serotonin density was greatly reduced in cortex (<10% control, $p<.01$) and greatly increased in caudate (220%, p.01).

714.13

EFFECTS OF PRENATAL COCAINE TREATMENT ON DEVELOPMENT OF ASTROGLIAL CELLS IN RAT PUPS.

J. Rubinstein*, H. Akbari, P.M. Whitaker-Azmitia and E.C. Azmitia. Dept. of Psychiatry, SUNY, Stony Brook, New York 11794 and Dept. of Biology, New York University, New York, New York 10003.

In previous studies, we have found that prenatal treatment with cocaine, significantly alters the growth of the serotonergic neuronal system as well as the expression of the $5\text{-}HT_{1a}$ receptor. Since the serotonin system and astroglial cells are highly interdependent during development, we proposed that cocaine would also cause developmental abnormalities of astroglial cells.

Pregnant Sprague Dawley rats were treated with 40 mg/kg cocaine from gestational day 10 through birth. At PD 3 and 6 pups were perfused with 20 ml of ice cold 4% paraformaldehyde in phosphate buffer (pH 7.2). The brains were sectioned at 50 micron and stained for GFAP (Sigma Immunochemicals, polyclonal, final dilution 1/2000); and S-100 (Chemicon Inc., monoclonal, final dilution 1/500).

In the cocaine-treated pups, astroglial cells were sparse in cortex and hippocampus and only lightly stained for S-100 at postnatal day 3. In addition, the subventricular zone, which supplies most of the cortical astrocytes, contained far more astroglial cells than controls - possibly cells which should have migrated out by day 3. By day six, radial glial cells were still evident in the cocaine-exposed pups, while the control pups had very few remaining radial glial cells.

714.14

FETAL AND MATERNAL DISTRIBUTION OF COCAINE FOLLOWING IV INJECTION IN RATS.

J.T. Bell*, S. Milstein, A. Borella and P.M. Whitaker-Azmitia. Dept. of Psychiatry, SUNY, Stony Brook, NY, 11794.

Although many reports have appeared suggesting that cocaine is behaviorally teratogenic in humans and rats, little work has been done comparing maternal and fetal levels of the drug. Moreover, much of the animal research has been criticized on the hypothetical question of whether or not all fetuses receive equal amounts of the drug. The present study was undertaken to answer these questions.

Pregnant Sprague-Dawley rats (GD17) were injected into the jugular vein with 80 μl ^{3}H-cocaine (NEN 25Ci/mmole) and sacrificed five minutes following the injection by decapitation. Tissues from mother and fetuses were collected and sonicated in 500 μl distilled water. Scintiverse was added to the samples and agitated until an emulsion formed and the samples were counted. The final results were expressed as fmoles/gm tissue.

Maternal distribution was hippocampus=caudate=lungs > heart=brainstem > eyes=liver > placenta. Fetal brain concentrations were approximately 1/7 of maternal (2492 fmoles/gm vs. 15,915 fmoles/mg for hippocampus). There were no significant differences in tissue concentration of the radiolabel among the fetuses. Fetal tissues are currently being analysed autoradiographically.

714.15

WATER DIFFUSIVITY SHOWS SIGNIFICANT MATURATIONAL CHANGES IN RAT BRAIN. T. Nakada*, H. Igarashi, H. Matsuzawa and I. L. Kwee Neurochem. Res. Lab., VANCSC, Martinez, CA 94553 and Dept. of Neurology, Univ. of Calif., Davis, CA 95616.

Diffusivity of water molecules, the main solvent of biological multicomponent solutions (cytosol), is significantly affected by solvent composition. The maturational process of brain in various mammalian species including rats and humans involves changes in the composition of the cytosolic macromolecules, micromolecules as well as the size of the extracellular space. Macromolecular biopolymers (proteins and lipids), which form large solvational spheres (shells), affect water diffusivity by macroscopic populational changes in water molecules in shells of differential entropy, whereas charged micromolecules (Na^+, K^+, free amino acids) primarily contribute by affecting entropy of adjacent water molecules behaving as dipoles (rather than as subject for hydrogen bonds). Accordingly, we investigated maturational changes in water diffusivity in rat brain. In spite of a significant maturational increase in biopolymers (which increase the water population with low diffusivity), diffusivity of rat pup brain water showed an exponential increase with age. The finding strongly indicates that brain water diffusivity is more significantly affected by changes in cytosolic micromolecular levels and size of the extracellular space.

714.16

DEVELOPMENTAL DIFFERENCES IN EFFECTS OF $GABA_A$ AGONISTS IN THE RAT SUBSTANTIA NIGRA: PRO- AND ANTICONVULSANT ACTIONS. D.S. Garant*, E.F. Sperber, and S.L. Moshé. Lab. of Developmental Epilepsy, Albert Einstein College of Med., Bronx, NY 10461.

GABA transmission in the rat substantia nigra (SN) protects against experimental flurothyl seizures. SN infusions of GVG, a metabolic GABA agonist, are anticonvulsant in both adult and 16-day-old rat pups; similar infusions of bicuculline are proconvulsant in both age groups. These data indicate that $GABA_A$ receptor-mediated GABA transmission in SN contributes to seizure control in both mature and immature rats. Infusions of the $GABA_A$ receptor agonists muscimol and THIP yield biphasic dose-response curves in adults: intermediate doses are anticonvulsant like GVG, but high doses are proconvulsant similar to bicuculline. In pups, only proconvulsant effects of muscimol and THIP are observed, suggesting that in both mature and immature rats, high SN concentrations of these drugs are antagonistic at $GABA_A$ receptors. In the immature SN, therefore, these agonists lack the anticonvulsant efficacy that GABA possesses at $GABA_A$ receptors, a profound developmental difference which may contribute to the increased susceptibility of the immature brain to generalized seizures.

714.17

EFFECTS OF COCAINE ON NEURONS CONTAINING TYROSINE HYDROXYLASE (TH) IN FETAL RHESUS MONKEY. O.K. Rönnekleiv*, and B.R. Naylor. Oregon Regional primate Research Center, Beaverton, OR 97006, and Dept. of Physiology, OHSU, Portland, OR 97201-3098.

Pregnant monkeys were treated with cocaine 3mg/kg I.M. or saline, four times a day from day 18 of pregnancy until days 39/40 or 60. Plasma levels of cocaine were ≈ 200 ng/ml in both mother and fetus 50 min after the last dose. Body weight, crown-rump length or head circumference were not different between the two groups of fetuses. The brains from days 39/40 and day 60 fetuses were examined using immunocytochemistry and *in situ* hybridization. The early appearance of TH neurons were not different between cocaine (N=2) and control fetuses (N=2). In both groups TH neurons were present in the mesencephalic flexure and the dorsal hypothalamus by day 39, and fiber projections to the striatum by day 40. Also, in the day 60 fetuses (N=8), TH neurons were distributed similarly in both groups. However, the TH mRNA content as measured by quantitative *in situ* hybridization, was reduced in the substantia nigra and ventral tegmental area in the cocaine treated fetuses ($p < .05$; N=4). These findings suggest that fetal cocaine exposure does not affect the ontogeny of TH neurons, but, alters the expression of TH mRNA in fetal neurons perhaps by activation of D-2 autoreceptors. (Supported by DA07165).

714.18

REPEATED EXPOSURE OF RAT PUPS TO ISOLATION DECREASES ULTRASONIC VOCALIZATION RATES: REVERSAL WITH NALTREXONE. G. A. Goodwin*, V. A. Molina and L. P. Spear. Center for Developmental Psychobiology and Dept. of Psychology, SUNY-Binghamton, Binghamton, NY 13902-6000.

Young rat pups are dependent on the dam for their survival, thus isolation of the neonatal rat pup from the dam presents the young organism with a variety of stressors. Rat pups appear to be very sensitive to isolation as they will almost immediately emit ultrasonic vocalizations when placed in a novel environment with an ambient temperature below 35°C. The question examined in this study concerns the ability of the young rat pup to modify its response to isolation following repeated exposure to that isolation. Adult animals repeatedly exposed to the same aversive situation exhibit an opiate-dependent attenuation in several responses to that stressor. Because isolation of neonatal rat pups can elicit an endogenous opiate release (Spear, Enters, Aswad, & Louzan, 1985), the effect of opiate receptor blockade on the response to repeated isolation was also determined. In the first experiment, pups were seen to decrease vocalization rates following repeated isolations; however, administration of naloxone prior to the final isolation did not significantly reverse this effect. Altering the context in an attempt to dishabituate animals also failed to reverse the decreased vocalization rate. In experiment 2, naltrexone given following the first isolation attenuated the decrease in vocalization rates seen across repeated isolations. These results suggest that the development of this attenuated response to isolation stress in young rat pups is opiate-mediated but that once established, its expression may not be dependent on endogenous opiate release.

714.19

QUANTITATIVE STUDIES OF CORRELATIONS, DNA CONTENTS (CELL NUMBERS) AND PROTEIN CONTENTS OF CEREBRAL CORTEX AND CEREBELLA OF ADOLESCENT RATS. S. Zamenhof.* Dept. of Microbiology and Immunology, and Brain Research Institute, UCLA School of Medicine, Los Angeles, CA 90024.

Previous studies (*Physiol. Behav.* 23:945, 1979) have revealed significant correlations between behavioral performances on one side, and cerebral DNA contents (total cell numbers), cerebellar DNA contents, or protein contents on the other side, in 30 days old rats (age sufficient for behavioral tests and for cerebellar studies). It is likely that higher levels (non-genetic) of these contents are indeed of importance for improvement of behavioral performance. In the present work (30 d. rats) we first determined that these contents themselves are significantly correlated: (DNA cerebral cortex - protein cerebral cortex $r = +0.502$ ($p < 0.05$); DNA cerebellum-protein cerebellum $r = +0.496$ ($p < 0.005$) (but in contrast: cerebral cortex protein - cerebellar protein $r = -0.19$, not significant). Subsequently, following previous studies with neonates (*Life Sci.* 18:1391, 1976), we determined the percentage of rats with *one* parameter (DNA *or* protein) and rats with *both* these parameters higher than the mean +1 standard deviation:

Parameter	Cerebral	Cerebellar	Both in the same rat
a. DNA	14%	25%	5.6%
b. Protein	14%	11%	5.6%
c. DNA *and* Prot.	2.8%	5.6%	<1%

Animals *a* and *b* occur as frequent as predictable from Normal Distribution Curve (15.9%); *c* are statistically more rare (expected 2 to 3% for two noncorrelated parameters). Conceivably, for the improvement of behavioral performance, rare occurrences (genetic or non-genetic) of exceptional values of *many* brain parameters *in the same* animal are required.

715.1

REGIONAL AND AGE-RELATED DIFFERENCES AMONG GLIAL CULTURES IN BETA AMYLOID INDUCED REACTIVE GLIOSIS. A. Höke*, D.R. Canning and J. Silver, Department of Neuroscience, Case Western Reserve University, Cleveland, OH 44106-4975.

Immobilized beta amyloid peptide (βAP) induces increased reactivity in cortical glial cultures and deposition of an axon growth inhibiting matrix containing chondroitin sulfate proteoglycan (CSPG). Expression of CSPG is also seen in the gliotic zone associated with dystrophic neurites around senile plaques in Alzheimer's disease (AD) (Canning et al, Soc Neurosci Abs 1993). In AD, although diffuse deposition of beta amyloid peptide is seen throughout the central nervous system, hallmarks of the disease, dystrophic neurites and senile plaques are mostly confined to hippocampus and cerebral cortices. This region specific pathology could be attributed to differences in glial reactivity in different parts of the CNS. To test this hypothesis, we investigated the expression of CSPG by glial cultures isolated from rat cortex, cerebellum and spinal cord. When these cells were exposed to immobilized βAP *in vitro* and stained with CS-56 (anti-CSPG antibody), cerebral cortical cultures showed high levels of CSPG deposition on βAP after two weeks. However, spinal cord cultures showed minimal levels of CS-56 immunoreactivity and cerebellar cultures fell in between. This reactivity required maturation of the glial cells *in vitro*. If P0 glial cells were exposed to immobilized βAP, they did not deposit CSPG onto the βAP/laminin matrix. They required a maturation period of at least two weeks, before they became capable of depositing CSPG onto βAP/laminin matrix. These findings suggest that glia are regionally heterogenous in their reactive response to βAP. This difference in the interaction between ß-AP deposits and surrounding glia may have a role in the region specific pathogenesis of Alzheimer's disease.

715.2

BLOCKING OF AXON OUTGROWTH BY REACTIVE GLIOSIS INDUCED WITH ß-AMYLOID PEPTIDES. D. R. Canning*, R. J. McKeon, D. A. DeWitt, G. Perry, J. R. Wujek, R. C. A. Frederickson & J. Silver. *Department of Neurosciences, Case Western Reserve Univ. Sch of Med., 10900 Euclid Ave., Cleveland, OH 44106-4975.*

Most axons within the adult vertebrate CNS fail to regenerate following injury and neurodegenerative disease. It has previously been shown that local reactive gliosis at the site of experimentally induced lesions in the rat cortex is accompanied by the deposition of growth inhibitory chondroitin sulfate proteoglycan(s) (CSPG), suggesting that specific molecular components of the deposition may be involved in limiting the growth of regenerating axons in the CNS. However, what triggers the conversion of glial cells to a functionally reactive state leading to this localized deposition is unknown. Here, we show that peptides of the ß-amyloid molecule when presented as an insoluble substrate can induce cortical glial cells *in vitro* and *in vivo* to locally deposit CSPG containing matrices. *In vitro*, the CSPG containing matrix blocks the usual ability of the peptide to allow cortical neurons to adhere and grow to a laminin/ß-amyloid peptide substrate.Since the pathological lesions in the brains of patients with Alzheimer's disease (AD) are characterized by dense deposits of ß-amyloid protein, the peptide may be inducing reactive gliosis during AD pathogenesis. We suggest that an additional effect of ß-amyloid in the AD brain, which compounds the direct effects of the peptide on neurons, is mediated by the stimulation of astroglia to become reactive and deposit growth inhibitory molecules within the neuropil similar to glial scar formation following trauma. Such a process may impair neuronal process survival and regeneration leading to neurite retraction and/or dystrophy around senile plaques in AD. This idea is supported by our findings of CSPG associated with reactive astrocytes around senile plaques in human AD tissue.

715.3

CYTOKINES, INFLAMMATORY CELLS AND ASTROGLIOSIS. V. Balasingam, I. Turley, and V.W. Yong*. Montreal Neurological Institute, McGill University, Montreal, Quebec, Canada H3A 2B4.

Reactive astrogliosis is a characteristic response of astrocytes to injury of the adult CNS. To assess the hypothesis that cytokines from inflammatory mononuclear cells that accumulate around lesion sites have a role in modulating astrogliosis, we took the advantage of the neonatal system in which astrogliosis is reported to be minimal following injury and in which the immune system is relatively immature compared to adult animals. Unexpectedly, a nitrocellulose-membrane implant (NC-implant) into the cortex of P3 mice resulted in a tremendous astrogliotic response (GFAP IR = $1016 \pm 37 \times 10^3 \mu m^2$). In contrast, a neonatal stab wound produced limited astroglial response (GFAP IR = $30 \pm 2 \times 10^3 \mu m^2$) when compared to the adult stab wound (GFAP IR = $496 \pm 22 \times 10^3 \mu m^2$). Utilising the neonatal stab wound model, cytokines were micro-injected into the wound site at the time of injury. All cytokines tested resulted in a significantly increased astrogliosis (e.g., GFAP IR for γ-IFN treated animals = $634 \pm 54 \times 10^3 \mu m^2$). The NC-implant injury paradigm is currently being utilized as a suppository element to administer neurtralising antibodies to cytokines in an attempt to curtail the extent of astrogliosis in both neonatal and adult animals. In addition, we are examining whether there is a spatial and temporal correlation of a particular inflammatory cell type with astrogliosis. We conclude that neonatal astrocytes can become reactive if an adequate injury stimulus is presented, and that the release of cytokines by inflammatory mononuclear cells that accumulate around lesion sites may be a mechanism that contributes to the production of gliosis.

715.4

CHARACTERIZATION OF AXON-GROWTH PERMISSIVE AND INHIBITORY ASTROCYTE CELL LINES. J. Fok-Seang, J.H. Rogers, A.R. Johnson, R.J. Keynes* and J.W. Fawcett. Physiological Laboratory and Dept. of Anatomy, Downing St., Cambridge CB2 3EG, UK.

Several cell lines were generated by infecting newborn rat cortical astrocyte cultures with virus containing either the neu oncogene or the temperature-sensitive mutant form of the SV40T oncogene. Over 60 cell lines were found to be GFAP-immunoreactive, of which 3 containing the SV40 oncogene (T34-2, T27A1 and T27A2) and one containing the neu oncogene (Neu-7) were further characterized.

When newborn rat DRG neurons were grown on monolayers or in 3-dimensional cultures derived from these cell lines, T34-2, T27A1 and T27A2 were found to be permissive for axonal outgrowth to varying degrees, whereas the Neu-7 cell line appeared to be relatively non-permissive. Since all 4 astrocyte cell lines expressed the axon growth-promoting molecules laminin and N-cadherin, the failure of axons to grow on Neu-7 cells may be due to presence of an inhibitor. Neu-7 cells, however, were not immunoreactive for tenascin or chondroitin sulphate proteoglycan, molecules which have been implicated in inhibition, and furthermore, did not show growth cone collapse activity.

Taken together, our findings suggest that Neu-7 is an inhibitory astrocyte cell line and that the inhibitory molecule(s) appears to be different from the major classes of known axon outgrowth inhibitors.

715.5

NEURITE GROWTH INHIBITORS (NI) OF MAMMALIAN CNS MYELIN ARE PRESENT IN XENOPUS SPINAL CORD BUT NOT IN THE OPTIC NERVE. D. Lang*, B. Rubin+, M.E. Schwab+, C.A.O. Stuermer, U. Konstanz, D-7750 Konstanz, +Inst. of Brain Res., U. Zürich, CH-8029 Zürich

While mammalian CNS myelin carries neurite growth inhibiting proteins (NI) (Schwab and Caroni, 1988) which impede axonal regeneration, fish do not (Bastmeyer et al., 1991). Here we demonstrate the distribution of NI in pre- and postmetamorphic amphibians, Xenopus (Anurans), and the neotenic axolotl (Urodeles) using the antibody (AB) IN1. Cryosections were fixed and exposed to IN1 and secondary HRP coupled AB. IN1 staining was compared to the distribution of myelin markers GalC, MBP, PLP.

1. Adult Xenopus spinal cord exhibited IN1 staining in all myelinated tracts but IN1 was absent from optic nerve and tectum. 2. Adult Axolotl showed myelin markers in spinal cord and optic nerve but no IN1 staining. 3. Xenopus tadpoles were free of IN1 and myelin markers. They achieved myelin markers during metamorphosis but IN1 only in the spinal cord, and not in the optic nerve.

Axons regenerate successfully in both spinal cord and optic nerve of Axolotl and Xenopus tadpoles, in adult Xenopus, however, only in optic nerve and not in spinal cord. Thus, the presence of NI in amphibian CNS myelin correlates with the loss of axonal regeneration, and its absence with successful axonal regrowth.

715.6

INVESTIGATION OF THE NEUROPROTECTIVE EFFECTS OF HSP72 USING TRANSGENIC AND PRIMARY CELL CULTURE TRANSFECTION TECHNIQUES

J.B.Uney*,J.N.C.Kew,,S.Pickering,,K.Staley,,M.H.Johnson &M.V.Sofroniew.

Department of Anatomy, University of Cambridge &[1]MRC Laboratory of Molecular Biology. Cambridge. U.K.

In studies using neuronal tissues a great deal of correlative but indirect evidence has been found which suggests that prior expression of hsp72 may be protective against excitotoxicity, free radical damage and ischaemic stress. Recent technical advances allow gene transfer into post-mitotic neuronal cells. In this study we have used the techniques of transgenesis and primary culture transfection to investigate the neuroprotective effects of constitutive overexpression of hsp72.

Transgenic animals have been generated and been shown to express mRNA encoding the human inducible form of hsp72. The rhombotin gene promoter was used to drive expression and this has previously been shown to direct expression to the hippocampal CA1 and CA2 but not CA3 neurons in the adult mouse brain. We are currently utilising this precise expression pattern to assess the neuroprotective effects of hsp72 in the hippocampus by stereotaxically injecting NMDA into the hippocampus of the transgenic animals and comparing the excitotoxin-induced damage of the CA1 and CA2 regions of the hippocampus with the CA3 region. Further to these experiments we have also shown that transfection of dorsal root ganglian primary cultures with an EF-1 alpha-hsp72 expression vector protects neurones and glia against exposure to heat stress.

These data support the hypothesis that overexpression of hsp72 enhances the survival of neurones following stress and suggests that the induction of a stress response in the CNS may provide an alternative form of treatment for CNS diseases.

715.7

GROWTH OF SEVERED AXONS FROM MOTOR CORTEX SUGGESTED BY ELEVATED GROWTH-ASSOCIATED EPITOPES. A.P. Foerster*. Dept. of Biomedical Science, McMaster Univ., Hamilton, ONT L8N 3Z5.

Injections of Fluoro-Ruby (FR), in adult rat medial prefrontal cortex, labeled axons confined to the medial part of the cerebral peduncle (CP). This was severed at bregma -3.5 mm with an implanted 1.5-2 mm wire cutting device [Foerster (1982) J Comp Neurol 210:335]. Immediately postlesion, severed FR-labeled axons faced the cut. At 8 and 20 d some labeled axons had become reoriented to pass towards, and to curve around, the ends of the lesion. At 20 d FR-labeled axons were found in at least 3 mm of the distal CP. These indications of postlesion growth were supported by the appearance at 8 d of FITC-tagged immunoreactivity (IR) (i) with a synaptophysin antibody (G-86, gift of R. Jahn) that labels growing neurites [Phelan and Gordon-Weeks (1992) Eur J Neurosci 4:1180] and (ii) with an antibody for a protein that binds a neurite-promoting site of laminin (LBP) [Kleinman et al. (1991) Arch Biochem Biophys 290:320; gift of H. Kleinman]. IR for these was elevated in labeled axons still facing the lesion, and in some which bypassed it. LBP-IR appeared at 20 d in astrocytes near the lesion with processes parallel to the line of incision, some of which were paralleled by reoriented FR-labeled axons. This suggests that such astrocytes may be involved in guiding regenerated axons around a lesion.

715.8

SCHWANN CELLS PRODUCE IL-6 IN RESPONSE TO NEURONAL COCULTURE. L. M. Bolin*, A. N. Verity, J. Silver, J. Abrams and E.M. Shooter. Department of Neurobiology, Stanford University School of Medicine, Stanford, CA 94305, DNAX Research Institute, Palo Alto, CA 94304.

During peripheral nerve regeneration Schwann cells support and provide neurotrophins to regrowing axons. We have used an in vitro paradigm to examine the induction of Schwann cell trophic activities in response to neurons in coculture. We have determined that the pleotrophic cytokine, IL-6 is a component in conditioned media from Schwann cell - neuronal cocultures. The presence of IL-6 was confirmed by a neuronal differentiation assay in rat PC12 cells and a survival assay with mouse myeloid B9 cells which are IL-6 dependent. A monoclonal antibody that neutralizes IL-6 activity in the B9 assay neutralizes the activity in coculture conditioned medium. An antibody against rat IL-6 reduces PC12 neurite extension in response to coculture conditioned medium. An induction of IL-6 message is seen in total cellular RNA isolated from Schwann cells grown in coculture which is not present in Schwann cells grown alone or in the neurons that are cocultured. Expression of the IL-6 receptor, gp80, is being investigated in both cell types. The immortalized Schwann cell clone used in this study arose spontaneously in a primary culture of adult rat sciatic nerve Schwann cells isolated 24 hr after injury. Since these Schwann cells are transformed during their response to nerve injury, their production of IL-6 may be a contributing factor in successful regeneration.

Supported by an NIH Neonatal and Developmental Biology post-doctoral training grant (HD-07249).

715.9

ACETYL-L-CARNITINE RESTORES CHOLINE ACETYLTRANSFERASE ACTIVITY IN THE HIPPOCAMPUS OF ADULT RATS AFTER PARTIAL FIMBRIA-FORNIX TRANSECTION P. Piovesan, G. Quatrini, L. Pacifici, M.T. Ramacci, G. Taglialatela, L. Angelucci[1], M. Alderdice*[2] Inst. for Res. on Senescence Sigma Tau, 00040 Pomezia, Italy; [1]Inst. of Pharmacology II, School of Medicine, "La Sapienza" Univ. of Rome, Italy; [2] Sigma Tau Inc., Gaithersburg, Maryland 20878.

Partial transection of the septo-hippocampal pathway (fimbria-fornix) in the rat results in an impairment of cholinergic activity in the hippocampus (HIP) similar to that occurring during aging. In the present study we used cholineacetyltransferase (ChAT) and acetylcholinesterase (AChE) activities, and nerve growth factor (NGF) levels as markers of the central cholinergic function following fimbria-fornix lesion. Three-month old Fischer 344 rats bearing a unilateral partial transection of the fimbria-fornix were treated for 1 week prior and 4 weeks following the lesion with Acetyl-L-Carnitine (ALCAR; 150 mg/kg/day in drinking water), a substance known to ameliorate some morphological and functional disturbances in the aged central nervous system. Fimbria-fornix transection significantly reduced ChAT and AChE activities in the HIP ipsilateral to the lesion as compared to the contralateral one. No changes were observed in the cortex. ALCAR treatment restored ChAT activity to normal values in the HIP ipsilateral to the lesion, while AChE levels were lower as in lesioned untreated animals. Lastly, NGF levels in the septum were not affected by the lesion nor by ALCAR. Given the importance of NGF in the re-establishment of hippocampal cholinergic activity after fimbria-fornix transection, these data suggest that ALCAR may facilitate sprouting of surviving cholinergic fibers by enhancing their trophic response to hippocampal NGF.

715.10

GROWTH-ASSOCIATED PROTEINS IN THE SEA LAMPREY. L.D. Margolin* and N.M. Colannino. Cambridge NeuroScience, Inc., Cambridge, MA 02139.

The lamprey spinal cord has been the focus of many studies concerned with the mechanisms of CNS regeneration. Various methods of analysis using higher vertebrates have shown that specific proteins are expressed in response to CNS injury. These proteins, called growth-associated proteins (GAP), are thought to be involved in development, plasticity and regeneration, although their specific role in each process is yet to be determined. Our efforts lie in the elucidation of regenerative processes which lead to full functional recovery in the lamprey by addressing questions pertaining to the presence and role of GAPs.

Sea lampreys (*Petromyzon marinus*) were anesthetized with 0.5g/L Tricaine (Sigma). The brain was exposed and 1-10 µCi of L-[^{35}S] methionine-cysteine (NEN) was pressure injected into the floor of the fourth ventricle, followed in two hours by a systemic injection of cold methionine-cysteine (Sigma). After incubation periods of 6 hours to one week, brain and spinal cord were removed and prepared for standard one-dimensional SDS-PAGE and autoradiographic analysis.

Preliminary studies have shown that radiolabeled amino acids injected into the fourth ventricle of an intact lamprey were transported anterogradely into the spinal cord. One dimensional PAGE showed the presence of many proteins, several of which have incorporated the radiolabel. Subsequent studies will include specimens that are in various stages of functional recovery following complete spinal cord transection.

Supported by NIH grant #1R43NS31073-01

715.11

UPREGULATION OF NITRIC OXIDE SYNTHASE (NOS) IN THE NEUROHYPOPHYSEAL SYSTEM FOLLOWING HYPOPHYSECTOMY. D.E. Scott* and W. Wu, Department of Anatomy & Neurobiology, Eastern Virginia Medical School, Norfolk, VA 23501.

Sprague-Dawley rats were used in the experiment. Animals were hypophysectomized and were divided into two groups with 12 animals in each group. Animals in the first group received daily IP injections of nitroarginine, an inhibitor of NOS. Those in the second received nothing. Following hypophysectomy rats survived 1, 2, and four weeks; 2 rats from both nitroarginine treated and non-treated groups for each survival period were prepared for NADPH diaphorase histochemistry. Another 2 rats were prepared for scanning electron microscopy (SEM). Four normal intact rats were used as controls, 2 for histochemistry and 2 for SEM. Stain intensity with NOS histochemistry of supraoptic and paraventricular (SON/PVN) neurons and their regenerating neurites in controls increased dramatically by 2 weeks and decreased to normal levels by 4 weeks following regeneration. Unlike control rats, relatively few neurites were observed on the floor of the third cerebral ventricle in nitroarginine inhibited rats following hypophysectomy. These data support the hypothesis that NO produced by NOS is directly related to the process of neurohypophyseal regeneration and may play a pivotal role in retargeting and directing regeneration of SON and PVN axons into predictable as well as novel neuroanatomical domains such as the third cerebral ventricle following the trauma of hypophysectomy.

715.12

THE EFFECT OF THE INCREASED ENDOGENOUS BETA-ENDORPHIN (BE) RELEASE INTO THE BLOOD ON THE REGENERATION OF DAMAGED STOMACH MUCOUS (SM)

V.Lebedev* S.Rychkova, V.Alexandrova, A.Savchenko

Lab. of Electroanalgesia, Pavlov Institute of Physiology, St.Petersburg 199034, Russia

It is wellknown that some natural or synthetic opioid peptides applied exogenously accelerate the regeneration of damaged SM. In present study the influence of uninvasive transcranial electrostimulation (TES) with special regime selectively activated brain opioid structures was investigated in rats on the development and repair of cold-restrained-, ethanol- and cystamine-induced SM ulcers. TES sessions elicited the encrease of BE blood level from 9.18±3.17 up to 29.96±2.65 pmol/l and significantly decreased the ulcer numbers and ulcer severity in each experimental model. The TES effects was more pronounced in comparison with ones of i.m.injections of synthetic (D-Ala^2,Arg^6) Leuenkephalin (DAALE) and were reversed by naloxone. Combined effect of TES and DAALE was usually considerably lesser than separate one of each. The encephalinase inhibitor D-Leucine (nonpassed through BBB in rats) substantially potentiated TES healing effects. Conclusion was made that encreased BE release into the blood elicited by TES effectively accelerates the repair of damaged SM. These experimental data were confirmed by high efficacy of usage of TES with this regime for treatment of gastric and duodenum ulcers in patients.

715.13

cDNA LIBRARIES FROM IDENTIFIED LEECH NEURONS. S.A. Korneev, S.E. Blackshaw*, J.A. Davies. Institute of Genetics, and *Department of Cell Biology, University of Glasgow, Glasgow G11 5JS, Scotland, U.K.

Our aim is to investigate synapse establishment and nerve regeneration by isolating molecules specific to regenerating neurons and to different classes of neurons in the medicinal leech *Hirudo medicinalis*. The strategy we have adopted is to use a PCR-based cloning technique to enable us to construct cDNA libraries from dissected, identified neurons. We have optimised the various steps of the procedure by producing a cDNA library from the equivalent of a single leech ganglion which consists of about 400 neurons. The library contained more than 10^6 recombinants and the average size of the inserts was approximately 600 bp. We have sequenced 6 randomly picked clones and 4 clones containing repetitive sequences and found that the library was remarkably free from cloned primer artefacts and ribosomal RNAs. The results obtained have prompted us to use the same approach for constructing a cDNA library from a few (10-15) serotonergic Retzius cells, the most prominent in the leech ganglion. By employing modifications of the reverse transcription reaction and the PCR protocol we have managed to create a cDNA library of about 10^6 recombinants, of average insert size, 700 bp.

TRANSPLANTATION VI

716.1

BALLS AND BRAINS: I. IMMUNOLOGICAL PRIVILEGE FOR CNS GRAFTS. B.J. Baker and R.D. Broadwell*. Div. Neurosurg. and Neuropath., Univ. Maryland Sch. Med., Balto. 21201.

The privilege of the CNS with respect to transplantation may be due to absence of dendritic cells and lymphatic drainage and existence of a blood-brain barrier. Despite reported immunological privilege, the testis has dendritic-like cells, lymphatic drainage, permeable vessels. Syngeneic grafts were transplanted to the testis or CNS in inbred PVG rats. Allogeneic grafts were prepared between PVG and PVG(AO) rats differing at major histocompatibility (MHC) loci and between the PVG and AO rats differing at MHC and minor HC loci. With monoclonal antibodies CNS grafts were deemed surviving if they expressed uniform Thy 1 staining, low MHC class I expression, few MHC class II+ cells, and negligible CD4+/CD8+ cells. Syngeneic grafts to the adult host CNS/testis appeared healthy/viable for 30d. All allogeneic grafts within the CNS parenchyma of adult hosts survived well. CNS allograft viability in the testis at 30d was variable. Vigorous graft rejection occurred between PVG and AO rats and less so between donor PVG and host PVG(AO) rats; graft survival without demonstrable rejection was seen between donor PVG(AO) and host PVG rats. The data suggest immune privilege of the testis is less demonstrable than that of brain. Survival of some allografts in the testis may be due to immunosuppressant factors. Existence of similar factors within the CNS may represent an additional consideration in defining CNS immune privilege.

716.2

BALLS AND BRAINS. II: BLOOD VESSELS IN CNS GRAFTS. R.D. Broadwell, B.J. Baker, and M. Fiandaca*. Div. Neurosurg. and Neuropath., Univ. Maryland Sch. Med., Balto. 21201.

Graft survivability is dependent upon the graft developing a vascular network supplied with host blood. We have analyzed by immunohistochemistry and electron microscopy the origins and morphological characteristics of vessels supplying fetal CNS grafts placed into brains, testes, or beneath the kidney capsule of adult host rats. Syngeneic/allogeneic CNS grafts were performed with donor/host combinations between the inbred PVG and PVG(AO) rats. Monoclonal antibodies used were: OX-27 for MHC class I expression by PVG endothelial cells; OX-26 for the transferrin receptor on blood-brain barrier endothelia; OX-18 for general MHC class I; and OX-7 for identification of healthy CNS neurons. HRP was delivered *iv* to ascertain a graft BBB to blood-borne protein. All grafts to host CNS were vascularized predominantly with host vessels and possessed a BBB with interendothelial tight junctions. Syngeneic grafts to the kidney capsule were supplied with OX-26+/BBB vessels, whereas allogeneic grafts failed to survive. Syngeneic/allogeneic grafts to the testis survived and exhibited OX-26+/BBB vessels; donor vessels predominated within these allogeneic grafts. Host testis MHC+ vessels were evident within CNS grafts and appeared OX-26 negative. The proportions of donor vs. host vessels in CNS grafts, regardless of their MHC expression and permeability characteristics, may depend on host site and/or genetic disparity between donor/host. NIH/NINDS grant #NS18030.

716.3

SURVIVAL OF HUMAN NERVE GRAFTS IN NUDE RATS. N. A. Azzam*, A. A. Zalewski, M. C. Dalakas~ and R. N. Azzam. Lab. of Neural Control and Medical Neurology Branch~, NINDS, NIH, Bethesda, MD 20892.

Nerves from rats can be cryopreserved and stored by a technique that allows the Schwann, vascular and perineurial cells in them to survive after transplantation (J. Comp. Neurol. 331:134-147, 1993). In order to extend the cryopreservation procedure to human nerves, we first needed to find a recipient that would accept human nerves. Accordingly, we investigated whether fresh human nerves would survive in congenitally immunodeficient nude rats. Pieces of human sural nerve were placed into tissue culture medium and, within an hour, transplanted into nude rats. Some nude rats received free grafts (i.e., grafts laid alongside intact peroneal nerves) whereas other rats had their peroneal nerve cut and anastomosed to one end of the human nerve. The grafts were examined 4 and 10 weeks postoperatively by light and electron microscopy. None of the grafts were rejected. A similar pattern of Wallerian degeneration was found in 4-week-old free and anastomosed grafts. At that time, Schwann cells were present inside basement membrane tubes, a few of which contained remnants of degenerating myelin. The perineurium and a moderate number of endoneurial blood vessels also survived. In addition, thinly myelinated axons were present in the proximal portion of an anastomosed graft. At 10 weeks, the number of Schwann cells in free grafts diminished, but overall the tissue was typical of degenerating nerve. In contrast, anastomosed nerve contained numerous myelinated and unmyelinated axons throughout its length. The endothelium of endoneurial blood vessels that was present appeared normal as did the perineurium. These results demonstrate that the cellular components of human nerve grafts can survive in nude rats and that human Schwann cells can myelinate rat axons. This nude rat model may be useful for examining a variety of *in vivo* aspects of human nerve neurobiology.

716.4

REJECTION OF NERVE ALLOGRAFTS AFTER STOPPING CYCLOSPORIN-A IMMUNOSUPPRESSION. A. A. Zalewski*, N. A. Azzam and R. N. Azzam. Lab. of Neural Control, NINDS, NIH, Bethesda, MD 20892.

After immunosuppressive therapy with Cyclosporin-A (Cy-A) is stopped, nerve allograft rejection occurs and host axons that had regenerated into the graft degenerate despite the fact that the axons are not foreign tissue. The present experiment was performed to correlate immune events and tissue loss in nerve allografts after terminating Cy-A treatment. Nerve grafts (4 cm long) were taken from American Cancer Institute (ACI) rats and joined to the peroneal nerve of Fischer (FR) rats that received Cy-A (10 mg/kg, intraperitoneally). After one week, Cy-A was stopped and the allografts were examined 2-6 weeks postoperatively by light and electron microscopy. No immune reaction nor destruction of allogeneic perineurial, vascular or Schwann cells was found in 2- or 3-week-old grafts. These grafts underwent Wallerian degeneration and were invaded proximally by regenerating, thinly myelinated, host axons. At 4 weeks, the perineurium of each graft became infiltrated and was destroyed by mononuclear cells. Many endoneurial blood vessels of these grafts were occluded and their endothelial cells were missing or degenerating. Despite the immune reaction, Schwann cells remained and myelinated many of the host axons that grew 3 cm into the grafts. However, at 6 weeks, most allogeneic Schwann cells were absent from all grafts, and no host axons were found. Some of the basement membrane tubes which formerly housed allogeneic Schwann cells were occupied by macrophages or were empty and fragmented. There was also evidence (i.e., masses of condensed DNA) that perineurial and Schwann cells were killed by apoptosis. These results demonstrate that there is a sequential rejection of tissue components in nerve allografts and that host axonal degeneration is related to adverse immune or metabolic effects of allogeneic Schwann cells.

716.5

PROBLEMS IN MEASURING VISUAL ACUITY IN ALBINO RATS. Susan E. Maier* & Ian Steele Russell. Texas A&M University Health Science Center, College Station, TX, 77843-1114.

Adult albino rats exposed to continuous light for 30 days show a complete loss of photoreceptors (rods) in the retina. This preparation provides a useful model for retinal transplantation research, yet the evidence regarding the extent of visual impairment following such visual loss is equivocal. To develop a behavioural procedure for testing hypotheses about visual loss, we began by generating psychophysical curves for brightness and line orientation in intact albino rats. It was evident early in testing that the rats were using nonvisual cues to solve the task, thus we double eyepatched these animals and retested them. The results show that covering the eyes had little effect on line orientation scores. We next examined the role of other cues, and preliminary data suggests that rats use various other cues including odor and vibrissa. Our data serve as a caution to researchers using albino rats that these animals are adept at solving a visual task using nonvisual cues.

716.6

TRANSPLANTATION OF DISSOCIATED RETINAL GANGLION CELLS INTO THE SUPERIOR COLLICULUS OF POSTNATAL RATS. J.J. Miguel-Hidalgo* and L.M. Chalupa. Dept. Psychology and Center for Neuroscience, Univ. California, Davis, CA 95616.

To examine the influence of extra-retinal factors upon the morphological differentiation and survival of retinal ganglion cells (RGCs), we injected suspensions of dissociated RGCs, obtained from postnatal rats, into the superior colliculus of littermates. A 2% solution of fluorogold (FG) was injected into the retinorecipient centers of 2 to 7 days old rats to allow unequivocal identification of RGCs. Two days later the retinas of these rats were dissected, cells were enzymatically dissociated, and the resulting suspensions injected with a micropipette into the superior colliculus (SC) of littermates. After a variable survival period these animals were killed with an overdose of pentobarbital, the brains were fixed by perfusion with 2% paraformaldehyde, cryoprotected in a 30% sucrose solution overnight and sectioned with a vibratome at a thickness of 100 µm. Examination of these sections revealed that many FG-labeled ganglion cells survived for as long as three weeks. Furthermore, in some cases these neurons exhibited extensive processes demonstrating that the SC was capable of supporting the survival and growth of retinal ganglion cells. A clear laminar specificity was evident, in that, the highest proportion of surviving RGCs was found in the superficial laminae of the SC, with only a few such cells observed in the deeper layers. We are now in the process of assessing how the differentiation of RGCs surviving in the SC compares to that within the intact retina. (Supported by NEI EY03993. J.J. M.-H. is a Fogarty International Center postdoctoral fellow).

716.7

RETINA REPAIR BY TRANSPLANTATION IN ORGAN-CULTURED CHICK EYES

W. Halfter*, Dept. of Neurobiology; University of Pittsburgh

Embryonic chick and quail eyes (E3 to E5) were organ-cultured for 1 or 2 days. Prior to culture, pieces of retina were removed. The wounds were closed by orthotopic and heterotopic transplants of retinal or brain tissue of different ages and by collagen gels. The integration of retina or brain transplants into the host retina was consistently very good, with a perfect alignment of graft and host tissue at the inner retinal surface. Transplants without basal lamina, such as tectal transplants stripped of their pial membrane, or retinal transplants facing with their ventricular surface the vitreous body, were covered with a host-derived new basal lamina. When mesenchymal tissue was grafted into the retina, no inner limiting membrane was formed, and the grafts were not integrated into the host retina. Collagen gels were only integrated when the gels were soaked with extract from retinal basal lamina. Ingrowth of optic axons into the collagen gels was observed. Our results show that wounded retinal tissue can be repaired by implantation of CNS tissue and collagen gels into the lesion site.

716.8

TRANSPLANT-MEDIATED PUPILLARY LIGHT REFLEX: EFFECT OF UNILATERAL LESIONS OF THE OLIVARY PRETECTAL NUCLEUS

M. J. Young*, S.J.O. Whiteley and R.D. Lund Dept. of Anatomy, University of Cambridge, Cambridge CB2 3DY UK

Embryonic retinae transplanted to the surface of the midbrain of neonatal rat hosts innervate visual targets in the host brain, and when stimulated, are capable of eliciting a pupillary light reflex (PLR) in the host eye. Previous work has shown that unilateral lesions of the olivary pretectal nucleus (OPN) result in a decrease in the efficacy of the PLR in rats, but do not abolish either the direct or consensual PLR. Here we have extended this work to examine the effect of unilateral OPN lesions on a transplant-mediated PLR.

Retinae from embryonic day 14 Long-Evans (LE) rats were transplanted to neonatal LE rats. The right eye was removed at the time of transplantation. Four-six weeks after transplantation a fiber optic light guide (4x10mm) was cemented over the transplant to allow for repeated stimulation of the grafted retinae over time. Pupillometric analysis was performed on these animals to characterise the transplant-mediated PLR under a range a light intensities. Latency, amplitude, and the rate of pupilloconstriction were analyzed. After establishing baseline conditions, one group of animals received a unilateral OPN lesion. The right OPN was lesioned, thereby eliminating the crossed input from the host eye, as well as the transplant projection to that OPN. A control group received sham lesions. The animals were allowed to recover for one week, and were then tested again.

Unilateral OPN lesions result in an increase in the efficacy of the transplant-mediated PLR, while the host direct PLR is substantially reduced. Lesions in animals that did not possess a transplant-mediated PLR showed far less of an effect on the direct PLR. These results suggest a dynamic interaction between the transplant and the host, possibly occurring across the midline, such that when the crossed retinal input is removed, the efficacy of the transplant projection (largely to the left OPN) is substantially enhanced.

Supported by grants from the NIH, MRC, and the Cambridge Foundation

716.9

INFERIOR COLLICULUS LESIONS AND GRAFTS: DEFICITS AND RECOVERY OF SOUND LOCATING ABILITY. M.C. Zrull* and J. R. Coleman. Depts. of Psychology, Appalachian State Univ., Boone, NC 28608, Univ. of South Carolina, Columbia, SC 29208.

The ability to detect sound in the horizontal plane is impaired by bilateral damage to the inferior colliculus (IC). Recovery from spatial hearing deficits is promoted by IC primordia grafts; rats with grafts after lesions detect sounds at random spatial loci more accurately than conspecifics with IC lesions only (Zrull et al., 1991). We continued to examine behavior affected by IC lesions and tectal grafts in a sound localization task which is more complex than a spatial detection task.

Hooded rats (N=15) were trained to suppress licking when the second pulse of a noise burst pair (125 ms ea., 150 ms IBI, 50 dB SPL) came from a different location than the first pulse. After baseline sessions, anesthetized rats received IC lesions (N=5, LO), lesions plus E18 tectal tissue grafts (N=5, LG), or sham surgery (N=5, SH). By 35 days post-surgey, localization performance ratios (LRs) show LG rats (.63±.03) localize sound between levels of SH rats (.72±.03) and LO rats (.50±.03), $F(16, 65)=1.80$, $p<.05$. LG rats never achieve LRs of SH controls; however, SH and LG rats exhibit similar task-behavior patterns relative to rats with lesions only. Analysis of the data via item response methods allowed estimation of measurement models yielding quality of localization behavior indices. Models indicate grafts may promote recovery after IC lesions by sparing the orderliness of sound localization behavior (LG, .6 and SH, .9 *vs* LO, .1). Analysis of brain sections show similar lesion sites in LG and LO rats, and some evidence of viable grafts in LG rats. Relative to LO rats, soma and processes of dorsal lemniscal neurons are spared in grafted animals.

716.10

RECOVERY OF SPATIAL MEMORY FUNCTION IN FORNIX-LESIONED RATS WITH INTRA-RETROSPLENIAL AND/OR INTRA-HIPPOCAMPAL TRANSPLANTS Y.J. Li*, W.C. Low Departments of *Neurosurgery and Physiology, and Program in Neuroscience, University of Minnesota Medical School, Minneapolis, MN 55455

The retrosplenial cortex has been implicated in spatial learning and memory function. Earlier studies in our laboratory and others have demonstrated that fornix lesions destroy cholinergic inputs to the retrosplenial cortex and the hippocampus while cholinergic grafts derived from the fetal septal nucleus can reinnervate both areas in a pattern similar to that of normal afferents. In the present study we investigated the functional effects of such a reinnervation in the RSC alone, in the hippocampus alone, or a combined reinnervation in both areas. Two months after surgery the fornix-lesioned (FX) animals, fornix-lesioned animals with intra-retrosplenial grafts (RSC), fornix-lesioned animals with intra-hippocampal grafts (HIP), fornix-lesioned animals with combined RSC and hippocampal grafts (COM), and normal control (NC) animals, were tested for their performance in spatial navigation using Morris water maze. In the spatial navigation test the NC, FX, RSC, HIP, and COM groups had 9±0.9, 4.8±0.8, 6.6±1.0, 7.6±0.7, and 9.1±1.2 platform crossings (mean±SEM) respectively. Statistical analysis with Mann-Whitney U test indicated that: 1) RSC performance was significantly better than FX ($p=0.015$); 2) HIP performance was also significantly better than FX ($p=0.0015$); 3) there was no significantly difference between RSC and HIP groups; 4) COM performance was significantly better than RSC ($p=0.023$) but did not differ from HIP ($p=0.236$). These results demonstrate that cholinergic intra-retrosplenial graft can ameliorate spatial learning and memory impairment induced by fimbria-fornix lesion. In addition, animals with combined grafts performed better than those with RSC graft alone. (This was supported in part by PHS grant NS-24464).

716.11

AMELIORATION OF BEHAVIORAL DEFICITS IN RATS WITH NUCLEUS BASALIS MAGNOCELLULARIS (NBM) LESIONS 1.5 MONTHS FOLLOWING GRAFTS OF CHROMAFFIN CELLS, BUT NOT KIDNEY CELLS, TO THE CEREBRAL CORTEX. E.S. Yuzda*, Z.C. Koty and S.A. Welner. Douglas Hospital Research Centre, Department of Psychiatry, McGill University, Montreal, Quebec, Canada H4H 1R3.

Chromaffin cell (CC) grafts to the cerebral cortex of NBM-lesioned rats have been shown to ameliorate lesion-induced deficits in performance of a task involving spatial memory at 3 months post-grafting. To test if these behavioral effects are related to the cell type grafted and not to the grafting procedure itself and whether behavioral effects occur at a time point earlier than 3 months, the effects of kidney cell (KC) grafts at 1.5 months post graft were tested. Thus, T-maze pre-trained rats were lesioned bilaterally in the NBM with 0.12M quisqualic acid and, two weeks after lesioning, were administered either no graft, KC grafts or CC grafts. Rats were retested on the T-maze 1.5 months post-graft. Results showed that, at this earlier time point, CC grafted rats also showed a clear improvement in T-maze behavior, performing as well as control animals. However, rats receiving control grafts of KC performed no better than lesioned animals. Lesions were verified by evaluating acetylcholinesterase staining in the area of the NBM; all lesioned groups, regardless of graft type or no graft, showed equally poor staining in the NBM. These results indicate that amelioration of behavioral deficits via CC grafts to the cerebral cortex are evident as early as 1.5 months post-grafting and do not occur with a control graft. Since we have previously evidenced CC grafts to contain basic fibroblast growth factor, a known neuroprotectant, at 1.5 months post-grafting (Yuzda et al., Soc. Neurosci. #473.2, 1992), it is interesting to speculate on the involvement of this growth factor in the effects of CC grafts to the cerebral cortex. (MRCC)

716.12

CHRONIC D-2 RECEPTOR ACTIVATION ENHANCES BOTH THE K^+-INDUCED ACTIVATION OF TYROSINE-HYDROXYLASE AND THE RELEASE OF DOPAMINE FROM CULTURED FETAL RAT DOPAMINERGIC NEURONS

F.L. Van Muiswinkel, B. Drukarch[1], W.J.A.J. Smeets*, C.A.M. Jongenelen[1], H.W.M. Steinbusch and J.C. Stoof[1]. Depts of Pharmacology, Neurology[1], and Anatomy*, Faculty of Medicine, Free University Amsterdam, The Netherlands.

In Parkinsonian patients, previously subjected to neuronal grafting therapy, the survival and functional status of dopaminergic grafts might be impaired by the concurrent pharmacotherapy with L-DOPA and/or dopamine (DA) D-2 receptor agonists. Therefore, we studied the effects of chronic DA D-2 receptor activation on cultured fetal rat mesencephalic DA neurons using the activity of Tyrosine Hydroxylase (TH) and the uptake/release of [^{3}H]DA as neurochemical parameters. Though the uptake capacity for [^{3}H]DA (~survival) remained unaltered in cultures previously treated for 12 consecutive days with 1μM of the selective DA D-2 receptor agonist LY171555, the 20 mM K^+-evoked release of [^{3}H]DA was significantly increased in pretreated cultures by approximately 35% (A). In our cultures, TH activity (as determined by the release of ^3H_2O from ^{3}H-[3,5]tyrosine) appeared to be tetrahydrobiopterin-, Fe^{2+}, and temperature sensitive but could not be inhibited by an acute challenge with 1μM LY171555. However, incubation with 56 mM K^+ for 3 min increased TH activity in a Ca^{2+}-dependent manner. Whilst the basal activity of TH remained unaltered upon chronic agonist treatment for 12 days, the K^+-induced activation of TH was significantly higher in pretreated cultures (179% vs 150% in control) (B). Since both phenomena (A/B) are Ca^{2+}-dependent, it is tempting to speculate that these changes in excitability reflect alterations in the Ca^{2+} homeostasis. Thus, a change in the functional status of fetal DA neurons has occurred, due to the chronic inhibitory effect of DA D-2 autoreceptor activation.

AGING PROCESSES III

717.1

FOOD RESTRICTION DELAYS THE AGE-RELATED INCREASE IN HYPOTHALAMIC GLIAL FIBRILLARY ACIDIC PROTEIN (GFAP) MRNA IN F344 RATS. N.R. Nichols*[1], C.E. Finch[1] and J.F. Nelson[2]. [1]Andrus Gerontology Center and Dept. of Biological Sciences, Univ. of Southern California, Los Angeles CA 90089-0191, [2]Dept. of Physiology, Univ. of Texas Health Science Center, San Antonio TX 78284-7756.

Astrogliosis during brain injury, disease and advancing age is characterized by increased GFAP, the intermediate filament protein of astrocytes. We have shown that GFAP mRNA also increases at later ages in rodents and humans (Nichols et al., Neurobiol. Aging, in press). Chronic food restriction (FR) in rodents delays the onset and incidence of disease, retards many aging processes and extends life span. Therefore, if GFAP mRNA expression is a marker of age-related processes, its pattern might be altered by FR. Male F344 rats were FR to 60% of ad libitum (AL) levels beginning at 6 wk of age or fed AL and were killed in the AM (lights on: 0400 h) at 3, 6, 13 (AL only), 18, 24-25, and 33 (FR only) mo of age. Total RNA was extracted by a guanidinium cesium chloride method from frozen hypothalamic (HY) tissue of individual rats. GFAP mRNA prevalence was determined by RNA blot hybridization using a ^{32}P-cRNA probe synthesized from a nearly full-length rat GFAP cDNA. HY GFAP mRNA increased 3-fold at 24-25 mo in AL rats ($p<0.0001$) compared with 3 and 6 mo groups. There were no differences in HY GFAP mRNA between AL and FR rats from 3 to 18 mo. HY GFAP mRNA increased 2-fold at 24/25 ($p<0.05$) and 3-fold at 33 mo ($p<0.0001$). At 24-25 mo of age HY GFAP mRNA was 50% higher in AL than FR rats ($p<0.01$). Therefore, FR delays the increase in magnitude of GFAP expression during aging, indicating that GFAP mRNA is a biomarker of brain aging and/or central effects of age-related disease. Supported by AG 07909 (CEF) and AG 01188 (JFN).

717.2

AGE, SEX, LIPOFUSCIN AND LIGHT DAMAGE IN THE QUAIL RETINA. K.V. Fite*and L. Bengston, University of Massachusetts, Neuroscience & Behavior Program, Amherst,MA, 01003.

Accumulation of the age related pigment, lipofuscin, in the retinal pigment epithelium (RPE) was investigated in the Japanese quail at 4 and 12 months of age. Quail were exposed to 18 hours of 3200 lux, broad-spectrum, white light at 4 months of age. Half were given an identical exposure, again, at 12 months of age. Six weeks after exposure, RPE lipofuscin was quantitatively assessed as well as histopathological evaluation of photoreceptor outer segments, outer nuclear layer, and counts of rod and cone densities for all conditions compared to controls.

Major increases in lipofuscin were observed in females vs. males at 12 months of age (controls).In the double-light-exposure condition, females showed even greater lipofuscin accumulation. Histopathology effects upon outer segments and outer nuclear layer were greater in males than in females. A major (70%) loss of rods occurred only in females. A small, nonsignificant reduction in cones (12-15%) occurred in both sexes. An inverse correlation occurred between lipofuscin and rod density across age, sex and experimental condition ($r=-.78$), suggesting that destruction of rods by light damage contributes directly to increased RPE lipofuscin. Thus, age, sex and light exposure history appear to be critical variables influencing the amount of lipofuscin in the RPE. (Supported by grant EY0 7370).

717.3

EFFECTS OF SWIMMING EXERCISE ON AGE-RELATED CHANGES IN MOTONEURONS AND PERIPHERAL NERVES IN THE RAT. K. HASHIZUME AND K. KANDA*. Departments of Kinesiology and Central Nervous System, Tokyo Metropolitan Institute of Gerontology, Itabashi-ku, Tokyo 173, Japan.

Our previous studies demonstrated that both number and soma cross-sectional area of the rat medial gastrocnemius (MG) motoneurons decreased with advanced age. The number of myelinated fibers in the MG nerve also declined, but fuscicular areas and axon diameters were increased in the aged animals. In order to study effects of long-term physical exercise on these age-related changes, male Fischer rats aged 17 months were divided into two groups, exercised and non-exercised ones. Rats of exercised group (n=15) were subjected to swim (30min, 3 days / wk) for 10 months until they were sacrificed at age of 27 months. Non-exercised, control rats (n=11) were raised under usual laboratory conditions. After the exercise period, the MG motoneurons were labeled by retrograde transport of horseradish peroxidase. Transverse semithin sections of the MG nerve were also stained with toluidine blue. The mean number of MG motoneurons in the exercised rats was significantly larger than that in the control rats. Correspondingly, fewer myelinated fibers (including both efferents and afferents) were found in the MG nerve of the control rats compared to the exercised rats, although the difference was not significant. We did not see the atrophy of motoneuronal somata in the exercised rats. The mean fuscicular areas and axon diameters in the exercised rats were significantly smaller than those in the control rats. These findings suggest that long-term, moderate endurance exercise retards the progressive changes in motoneurons and peripheral nerves in aging rats.

717.4

MAST CELL ACTIVATION AND PROLIFERATION, AND OCCURRENCE OF HIGH ENDOTHELIAL VENULES IN AGING PERIPHERAL NERVE. M. G. Nunzi* and M. G. Fiori. FIDIA Res. Labs., Abano Terme and Dept. of Orthopedics, Univ. of Brescia, Italy.

In rats, age-related axonal atrophy and loss was reported to begin at 13-14 mos in the most distal parts of peripheral nerves; these phenomena are followed by vacuole formation, segmental demyelination/remyelination and proliferation of mast cells (MC): the latter has been thought to be associated with either Wallerian degeneration or demyelination. We studied the sciatic nerves of 27-mo-old rats and found that there was a 5-fold increase in the overall MC number as compared to adult (8-mo-old) rats. MC were distributed unevenly, with a much higher percentage in the endoneurial than epineurial compartment; about 40% of endoneurial MC were degranulating. Some MC were noticed encased in macrophages engulfing degenerated axons. More noteworthy was the quantitative morphological association between MC and high endothelial venules (HEV), specialized vessels subserving the passage of inflammatory cells into the tissues. The occurrence of HEV had been already described in experimental allergic neuritis, also characterized by an increase in degranulated MC. We suggest that the MC invading aged peripheral nerves are derived from both proliferation of a resident population and transluminal passage mediated by HEV. By virtue of the release of histamine and several cytokines, MC may contribute to either edema and segmental demyelination or regenerative process.

717.5

Aging-Dependent Increase in Very Long Calcium Tail Currents in Rat Hippocampal Slice CA1 Neurons. P.W. Landfield*, O. Thibault, L.W. Campbell and E.M. Blalock, Dept. Pharmacol., Univ. Kentucky College of Medicine, Lexington, KY, 40536.

In previous current clamp studies, we have described an aging-dependent increase in the duration and amplitude of Ca^{2+}-dependent afterhyperpolarizations (AHPs) and Ca^{2+} action potentials (spikes). Under voltage clamp conditions, we have also described very long (500-2000 ms) Ca^{2+} tail currents in these same slice neurons. Elsewhere in this meeting (Thibault *et al*) we present evidence that single L-type Ca^{2+} channel openings following repolarization appear to account for long-lasting whole cell Ca^{2+} tail currents. One possibility for the aging effect on the AHP and the Ca^{2+} spike, therefore, is that aging may increase the probability of repolarization openings and, thereby, increase Ca^{2+} entry following command depolarizations.

To test this hypothesis, we recorded voltage-sensitive Ca^{2+} currents from both young (3-5 months) and aged (25-27 months) rat CA1 hippocampal neurons in the *in vitro* slice preparation. Using sharp electrode voltage clamp, the Cs^{+}-, TTX- and TEA-treated neurons did not show significant effects of age in passive membrane properties (ie: input resistance). However, with aging, we found a statistically significant increase in post-depolarization tail currents following a 200 ms depolarization step to -30 mV. Thus, these high voltage activated, dihydropyridine-sensitive aftercurrents appear to be enhanced in aged brain neurons, which likely accounts for the effect of aging on the AHPs under more physiological conditions. Further, larger tail currents could substantially increase overall Ca^{2+} influx into aged neurons, and contribute to Ca^{2+}-mediated cytotoxicity. (Supported by AG10836, AG04542 and Miles, Inc.).

717.6

INTRACORTICAL DISTRIBUTION OF SEROTONERGIC AND CATECHOLAMINERGIC TERMINALS IN THE AGED RATS. K. Iwata*[1,2], K. Kitajima[2], Y. Tsuboi[1], J. Yagi[1], K. Kanda[3], N. Okado[4] and R. Sumino[1,2], Depts. Physiol[1]. and Pathophysiol[2]., Sch. Dent., Nihon Univ., Tokyo 101, Dept. Central nervous system[3], Tokyo Metropolitan Institute of Gerontology, Tokyo 173, Dept. Anat., Inst. Basic Med. Sci., Univ. Tsukuba[4], Ibaraki 305.

Serotonergic and noradrenergic terminal distributions in the cerebral cortex of the aged (28-31 months) and young (3-9 months) rats were studied with immunohistochemical techniques. The animals were anesthetized with sodium pentobarbital (50 mg/kg, i.p.) and perfused with 4% paraformaldehyde in PB. Frontal serial sections were cut from the frontal (Fr), somatosensory (So), occipital (Oc) and visual (Vi) cortices and processed with ABC immunohistochemistry that employed polyclonal antisera to serotonine (rabbit anti 5-HT) and dopamine β-hydroxylase (rabbit anti DBH). A total of 25,408 5-HT LI and 30,088 DBH LI terminals in the cerebral cortex were drawn and counted. Distribution of 5-HT like immunoreactive (5-HT LI) and DBH like immunoreactive (DBH LI) terminals was dense in superficial (I-II) and sparse in deeper (III, IV, V and VI) laminae of both young and aged animals. The number of 5-HT LI fibers and terminals was decreased in the superficial laminae of Fr, So and Oc cortices, which was less extent in Vi cortex of the aged rats. In the deeper laminae alteration of density of 5-HT LI fibers and terminals was not found. On the other hand, DBH LI fibers and terminals were slightly decreased in the superficial laminae of Fr, So and Oc cortices, but not in the deeper laminae. However, the number of them in Vi cortex was decreased in both superficial and deep laminae of the aged rats. These findings suggest that serotonergic fibers and terminals in the superficial laminae (Fr, Oc and So cortices) and those of noradrenergic ones (Vi cortex) would be mainly involved in degeneration process of the cerebral cortex.

717.7

FOOD RESTRICTION-INDUCED HYPERADRENOCORTICISM IS ASSOCIATED WITH REDUCED PLASMA AND ADENOHYPOPHYSEAL, ACTH AND UNCHANGED ADENOHYPOPHYSEAL POMC RNA. E.S. Han[1], N. Levin[2], N. Bengani[2], J. Roberts[2], Y. Suh[1], K. Karelus[1], and J.F. Nelson*[1]. [1]Dept. of Physiology, U. of Texas Health Science Center, San Antonio, TX 78284-7756., [2]Mt. Sinai Medical Center, NY, NY.

Chronic food restriction (FR), which retards many aging processes and extends life span, potentiates the diurnal elevation of plasma corticosterone (B) in rats (J Gerontol 46:B171-9, 1991). Neither the physiological significance nor the cause of increased B in FR rats is known. We therefore investigated whether FR alters plasma levels of ACTH or anterior pituitary (AP) content of ACTH, POMC mRNA or its precursors, since these molecules both regulate and can be suppressed by B. Measurements were made in 6-7 mo.-old male Fischer 344 rats that had been fed ad libitum (AL) or FR (60% of AL calories) since 6 wk of age. Tissue and plasma were collected at 0500h and 1500h (12h:12h L:D; L on at 0530h). POMC mRNA in total RNA and POMC primary transcript, processing intermediate and mature mRNA in nuclear RNA were measured by slot blot and RNase protection assays. ACTH in plasma and in AP homogenates were measured by RIA. AP content of POMC mRNA from total RNA, and AP contents of POMC primary transcript, processing intermediate, and mature mRNA from nuclear RNA did not differ in FR and AL rats. By contrast, AP ACTH content was 52% lower and plasma ACTH was 21% lower in FR than in AL rats ($P<0.01$). Reduced ACTH in plasma and AP of FR rats is consistent with enhanced negative feedback by the elevated B of the FR state. The absence of increased plasma ACTH in the FR rats indicates that factors other than increased immunoreactive ACTH underlie the FR-induced elevation of plasma B (e.g., altered ACTH bioactivity, adrenal sensitivity to ACTH or B metabolism). The absence of suppressed AP POMC mRNA and its precursors indicates that altered translational efficiency, post-translational processing or turnover of ACTH underlies the reduced AP ACTH content of the FR rat.

717.8

AGE-DEPENDENT REGULATION OF PHOSPHOINOSITIDE TRANSDUCTION SYSTEM IN THE RAT SPINAL CORD. O.J. Igwe*, Division of Pharmacology, Schools of Pharmacy & Medicine, UM-KC, Kansas City, MO 64108

Many processes associated with intracellular calcium homeostasis have been reported to change with the aging process. To determine whether the intracellular calcium-mobilizing second messenger, inositol 1,4,5-trisphosphate [Ins(1,4,5)P_3] and its receptor [Ins(1,4,5)P_3R] displays age-dependent regulation, Ins(1,4,5)P_3 contents and [^{3}H]Ins(1,4,5)P_3 binding site density were quantified in the spinal cords of young (3 months old), adult (12 months old) and senescent (25-months old) male Fischer 344 rats. Spinal cord contents of Ins(1,4,5)P_3 were significantly increased ($p \leq 0.01$) in the 25-month old rats compared to the 3- and 12-month old animals. The density of Ins(1,4,5)P_3R in particulate membranes derived from the 25-month old rats was significantly reduced ($p \leq 0.01$) but with a significant increase ($p \leq 0.04$) in binding affinity, compared with the 3- and 12- month old animals. No significant differences were observed in spinal cord Ins(1,4,5)P_3 accumulation and Ins(1,4,5)P_3R density in young and adult rats. A significant change in Ins(1,4,5)P_3R affinity suggests an age-dependent modification of Ins(1,4,5)P_3R protein in spinal cord neurons. The present data indicate that the spinal cord contents of Ins(1,4,5)P_3 increase with age, but with decreased efficacy and number of Ins (1,4,5)P_3-activatable Ca^{2+} channels in the spinal cord of senescent rats. These age-related changes may contribute to the attenuated responsiveness of spinal cord neurons by phosphoinositide-coupled receptors during the aging process. Supported by SEP-Marion Merrell Dow Foundation and USPHS grant AR 41606.

717.9

AXON DEMYELINATION AND STAINING PATTERN FOR NEUROPEPTIDES IN THE AGED RAT SPINAL CORD

B. Ulfhake (1)*, T. Hökfelt (2), H. Johnson (1), S. Kullberg (1) and X. Zhang (2), Departments of (1)Anatomy, and (2)Histology and Neurobiology, Karolinska Institutet, S-104 01 Stockholm, Sweden.

With increasing age gait and postural mechanisms become impaired. In part this may be due to demyelination and/or dystrophy of axons in afferent pathways to the motor system. In this report we present data on the occurrence of demyelination in the spinal cord white matter and the staining pattern in the dorsal horn for calcitonin gene-related peptide (CGRP), galanin (GAL), galanin message associated peptide (GMAP), neuropeptide Y (NPY), somatostatin (SOM) and substance P (SP) in the 30 months old Sprague Dawley rat.

Demyelination was studied by quantifying myelin bodies (MB) in Marchi stained sections of the spinal cord. Immunohistochemistry was used to study the distribution of neuropeptides in the dorsal horn.

Numerous MBs were encountered in all parts of the white matter. However, large local variations in the density of MBs were eveident. For example, the medial dorsal column pathway disclosed a very much higher density of MBs, and there was also a clear rostro-caudal gradient with a higher density at cervical spinal cord levels compared to lumbar levels for this pathway. Thus, it seems that not only are different projecting pathways affected differently but also that the distal regions of the long projecting axons are more extensively engaged compared to the more proximal parts.

The staining pattern in the dorsal horn for CGRP, GAL, GMAP, SOM and SP was rather similar for aged and young adult rats, while NPY-LI seems to have increased in the aged rat.

717.10

MOTONEURONS IN THE LUMBAR SPINAL CORD OF THE AGED RAT CONTAIN INCREASED LEVELS OF CGRP-LIKE IMMUNOREACTIVITY AND mRNAs FOR α-CGRP AND GAP-43.

H. Johnson (1)*, T. Hökfelt (2) and B. Ulfhake (1), Departments of (1)Anatomy and (2)Histology and Neurobiology, Karolinska Institutet, S-104 01 Stockholm, Sweden.

With immunohistochemistry and *in situ* hybridisation the content of CGRP-LI and mRNAs for the α-subtype of calcitonin gene-related peptide (CGRP) and the 43 kD growth associated protein (GAP-43) was studied in lumbar motoneurons (MNs) of 30 months old Sprague Dawley rats and compared with young adult rats. To quantify CGRP-LI we used photometry of 'optical' tissue-sections images recorded with a confocal microscope after removal of autofluorescence contribution. Quantification of the mRNA messages was accomplished by optical density (OD) readings of silver-grains over cell body profiles.

CGRP: Compared to young adult rats a significantly smaller number of neurons in the lateral motor nucleus stained positively for CGRP in the aged rat, while the number of positive neurons in the medial motor nucleus was similar in the two age groups. The content of CGRP-LI in positive aged rat MNs was, however, significantly higher ($p<0.001$). The OD of α-CGRP mRNA in aged rat MNs exceeded the level in young adult rat by about 100% ($p<0.001$).

GAP-43 mRNA: The OD readings for GAP-43 mRNA was 3 times higher in aged rat MNs compared to young adult MNs ($p<0.001$).

The possible mechanism(s) underlying these changes is discussed in relation to age-related changes in the muscles and afferent input to the MNs.

717.11

ALTERATIONS IN TARGET INNERVATION & COLLATERAL SPROUTING IN THE AGING SYMPATHETIC NERVOUS SYSTEM. George A. Kuchel* Mt. Sinai, New York and Montreal General Hospital Research Institute, 1650 Cedar, Montreal, Quebec, Canada H3G 1A4

The ability to sustain appropriate target innervation and to undergo collateral sprouting following losses of related neural inputs may favor the maintenance of normal function. Tyrosine hydroxylase immunohistochemistry (TH IHC) was used in the rat pineal gland for quantitative morphological measurements of changes in sympathetic fiber innervation density and in homotypic collateral neuronal sprouting with aging. Rhodamine-coupled IHC was specific for sympathetic fibers, with the fluorescent image captured by a SIT camera and analyzed with an image analysis system. This target tissue receives bilateral and overlapping sympathetic innervation from the two superior cervical ganglia. Young (4 m. old) rats underwent a unilateral sympathetic denervation of the pineal gland and 1 day later exhibited an approximately 50% decrease in the area fraction represented by TH immunoreactive profiles in this target tissue ($p<.05$). Ten days after this lesion, the density of TH immunoreactivity increased to over 80% of control values ($p<.05$). In aged (25 m. old) animals, endogenous fluorescence produced by the presence of lipofuscin was subtracted from the captured image, revealing a more than 50% decrease in innervation density to this target tissue in aging. The density of TH immunoreactive profiles decreased by approximately one half in aged animals lesioned 1 day earlier ($p<.05$). However, 10 days after a unilateral denervation it was still approximately one-half of that obtained in control aged rats($p<.05$), providing morphologic support for a failure in collateral sprouting in this system in aging.

717.12

AGE-RELATED INCREASE IN AXONAL TORPEDOES IN CEREBELLAR PURKINJE CELLS OF TWO NORMAL MOUSE STRAINS. J. Bäurle and U. Grüsser-Cornehls*, (SPON: European Neuroscience Association), Dept. Physiology, Freie Universität Berlin, Arnimallee 22, 1000 Berlin 33, Germany.

Spindle-shaped axonal varicosities, also termed spheroids or torpedoes, are described to appear in relatively small numbers in various regions of the brain in the course of numerous neuropathological diseases but are not forcibly related to these. Torpedoes in larger amounts particularly in cerebellar Purkinje cell axons are found during the postnatal development of rats and in different cerebellar-affected mutants *(hpc, lc, nd, nr, tg^{la})*, all of them showing massive Purkinje cell degeneration. In addition neurotoxic agents or neoplasms can also lead to axonal torpedoes. Therefore these swellings are considered to represent a relatively unspecific sign of axonal degeneration, namely in 'dying-back-neuropathies' and in myelin deficiencies.

The present study systematically investigated the occurrence of torpedoes in two different mouse strains (B6CBA and C57BL/6J) during aging (up to 32 months) using Calbindin D-28k immunocytochemistry. The results show that the number of torpedoes in Purkinje cell axons increases almost linearly from a basic level of about 0.1% in 2-4-month-old mice up to 13.7% in 32-month-old animals. It is therefore suggested that torpedoes in the cerebellum of these mouse strains are age-related. While axons and terminals of affected cells undergo degeneration, the somata and dendrites are in most instances preserved and exhibit normal morphology. As a consequence, a graded denervation of cerebellar and vestibular nuclei with age is hypothesized. Therefore even in the absence of age-related Purkinje cell degeneration, which is still a controversial point, a normal number of Purkinje cells does not guarantee for a quantitatively normal cerebello-nuclear projection.

717.13

AGE-DEPENDENT CHANGES IN GLUTAMATE AND ASPARTATE CONCENTRATIONS IN THE NON-HUMAN PRIMATE AND RODENT BRAIN. P. Chan*, D.A. Di Monte, M.S. Sandy, L.E. DeLanney, I. Irwin and J.W. Langston. California Parkinson's Foundation, San Jose, CA 95128.

Excitatory amino acids have been proposed to play an important role in age-related neurodegenerative disorders. In this study, the concentrations of the excitatory amino acids glutamate and aspartate were determined in the frontal cortex, ventral mesencephalon, striatum, hippocampus and cerebellar cortex of 2 month old (n=10), 18 month old (n=10), and 29 month old (n=6) C57BL/6 mice, and in the substantia nigra, caudate, putamen, globus pallidus, accumbens and hippocampus of young (n=6, age=3 years old), mature (n=6, age=9 years old) and old (n=6, age= >16 years old) squirrel monkeys. Glutamate was significantly decreased in 18 month old mice as compared to 2 month old mice in all areas except frontal cortex. In 29 month old mice, further decreases were observed in the ventral mesencephalon and striatum, but not in the cerebellar cortex or hippocampus. Aspartate was significantly decreased in the frontal cortex, ventral mesencephalon, striatum, and hippocampus of 18 month old vs 2 month old mice; in the oldest mouse group, a further decrease was found in the ventral mesencephalon, striatum, and hippocampus. In addition, a decrease in aspartate concentrations occurred in the cerebellar cortex in 29 month old vs 18 month old mice. In mature vs young monkeys, a significant decrease in glutamate was found in the putamen and accumbens, but no further loss was measured in the oldest animals. Aspartate levels declined with age in the substantia nigra, putamen and accumbens, reaching statistical significance in old vs young monkeys. These data indicate that decreases in excitatory amino acids occur in the ageing brain, possibly as a consequence of changes in metabolism and/or biodisposition. These changes may ultimately affect excitotoxicity.

717.14

REDUCED ADRENOCORTICOID RESPONSE TO CORTICOTROPIN SECRETAGOGUES IN THE AGED RAT. G. Cigliana, S. Scaccianoce, R. Nicolai, L.A.A. Muscolo and L. Angelucci*. Institute of Pharmacology II, "La Sapienza" Univ., 00185 Rome, Italy.

The aging process, as well as some pathological conditions (depression, dementia, Alzheimer's disease, etc.), has been shown to have a profound impact on the normal functioning of the hippocampus-hypothalamo-pituitary-adrenocortical axis system. Aged rats have been reported to have increased basal levels of circulating corticosterone (B), an impaired ability to recover from their hypothalamo-pituitary-adrenocortical stress response, an augmented plasma B response to stress (although with some stress and strain dependency) and a reduced susceptibility to dexamethasone suppression test. These alterations might arise from a reduced hippocampal negative feedback control, as suggested by the age-dependent loss of hippocampal adrenocorticoid receptors. Among the hypothalamic factors endowed with corticotropin secretagogue activity, CRF and vasopressin (AVP) are considered as the major physiological mediators of hypothalamic control of ACTH secretion. Thus, we have investigated the effect produced by the aging process on the dynamics of the response to CRF and AVP, and on the adrenocortical response to a cold stress (4 °C for 90 min). Freely-moving jugular catheterized male Sprague-Dawley rats (3- and 24-month-old) have been injected with CRF (0.01, 0.05 and 0.5 µg/kg iv), or AVP (0.05, 0.1 and 1.0 µg/kg iv) or CRF and AVP (0.05 and 0.1 µg/kg iv, respectively). Blood samples for hormones determinations (B and ACTH) have been collected 60 min before and 15, 30 and 90 min after injection. The results have: a) confirmed the increased basal levels of B in aged rats; b) indicated that with aging the response to corticotropin secretagogues is dampened but prolonged; and c) shown that the cold stress response of the 24-month-old rat was, at least with regard to the peak, not different from that of 3-month-old. (Supported by CNR grant 91.004.32.40).

717.15

AGE-RELATED ALTERED CALCIUM REGULATION IN SYNAPTOSOMES AND ACUTELY DISSOCIATED BRAIN CELLS: PROMINENT EFFECTS IN HIPPOCAMPUS. J. Satrústegui, A. Martínez-Serrano, J. Chowen*, R. Pereira and M. Villalba. Dept.Biología Molecular. Centro de Biología Molecular. C.S.I.C.-Universidad Autónoma de Madrid. 28049-Madrid. SPAIN.

An altered regulation of neuronal cytosolic calcium levels has been suggested to play a role in the pathogenesis of neurodegeneration and Alzheimer's disease. However, evidence for an age-dependent alteration in cytosolic calcium regulation is at present controversial. Whereas we have observed a varying but consistent age dependent increase in $[Ca^{2+}]_i$ in whole rat brain synaptosomes using a variety of detectors (Martínez et al.(1988) Neurosci. Lett 26, 1089-1092; Martínez-Serrano et al.(1992) J. Biol. Chem. 267: 4672-4679), other groups failed to confirm this using cerebrocortical preparations (Canzoniero, et al. (1992) Biochem. Biophys. Acta. 1107, 175-178. Farrar, et al. (1989) Neurosci. Lett. 100, 319-325; Giovanelli & Pepeu (1989) J. Neurochem. 53, 392-398). The present work was aimed at studying whether changes in synaptosomal calcium homeostasis are uniform throughout the brain or affect specific brain regions. A second question addressed in this work is whether the effect of ageing on calcium homeostasis is restricted to the nerve terminal or a more general process affecting also cell bodies. In order to study these questions, we have used synaptosomes and a preparation of acutely dissociated brain cells (Villalba et al.(1992) Brain Res. 570: 347-453) obtained from different brain regions of the adult rat to study in parallel the variations in calcium homeostasis in synaptosomes and dissociated neurons from 3- and 30-month old rats. The results indicate that mean resting $[Ca^{2+}]_i$ obtained at rest and after high K depolarization were unchanged in cerebral cortex synaptosomes but increased in hippocampal synaptosomes at 30 months of age. On the other hand, $[Ca^{2+}]_i$ levels increased with age in brain cells from both cerebral cortex and hippocampus. Since the altered regulation of $[Ca^{2+}]_i$ appears to affect predominantly cell bodies and synaptosomes derived from hippocampus, this can explain the discrepancies obtained earlier. An altered calcium regulation in cell bodies and synaptic terminals in the hippocampus may be involved in the development memory impairments in old age.

717.16

MORPHOLOGICAL CHANGES AT NEUROMUSCULAR JUNCTION INDUCED BY EXERCISE DURING AGING. M.A. Fahim *, and A.S.A. Mohamed. Fac. of Med., UAE Univ., PO Box 17666, United Arab Emirates.

To evaluate exercise effect on morphological age changes, we studied the relationship between endplate parameters and muscle fiber diameter.

Male C57BL/6J mice aged 12, 18 and 24 months at sacrifice, were treadmill-exercised for 2 months for 1 h/day at velocities up to 30 m/min. The area (A) perimeter (P), nerve longitudinal extent length (E) and fiber diameter (FD) of camera lucida drawings of zinc iodide osmium-stained nerve terminals were analyzed. Non-linear regression and multivariate analysis were used to study the inter-relationships among the mentioned parameters under aging and exercise conditions.

Mathematically, any non-linear relationship can be represented successfully by polynomial representation. Application of such generalized method of analysis showed encouraging results which cannot be seen by using linear regression analysis.

At 12 months E was strongly correlated with FD, then deteriorated during aging, and exercise did not affect it. At all ages A was not correlated with FD. Exercise increased such correlation, especially at 18 months. Including the linear multivariate analysis among FD and endplate parameters shows only the influence of exercise, especially at age 18 months ($p < 0.008$). Ratio of E/FD was strongly correlated with endplate parameters, especially at age 12 months and improved with exercise ($r^2=0.721$) .

The success of finding a relationship between endplate and FD with high correlation represents the ability to track the possible changes of these parameters or, to predict their new states. Estimating these relationships with low correlation implies either the stochastic variations of these parameters or non-linear multivariate analysis should be considered.

718.1

GENOMIC STRUCTURE OF DARPP-32 AND ARPP-21, MARKERS OF A DIFFERENTIATED STRIATAL PHENOTYPE. M.E. Ehrlich*[1], S. Blau[1], L. Daly[1], N. Kane[1], A. Fienberg[2], and G. Teitelman[3], New York University Medical Center[1], The Rockefeller University[2], and SUNY Health Sci. Ctr., Brooklyn[3].

DARPP-32 and ARPP-21 are dopamine- and cyclic-AMP regulated phosphoproteins which are highly enriched in the dopaminoceptive medium-size spiny neurons of the striatum, thus defining a specific phenotype. Both genes are first transcribed post-migration, and the mRNA levels are not altered by surgical or pharmacologic manipulations of the dopaminergic pathway. This coordinated expression implies the existence of common transcriptional regulatory elements. The structures of the murine DARPP-32 and ARPP-21 genes have been determined. Transcription is initiated at multiples sites in each gene, and there are no TATA or CAAT boxes. Both genes contain multiple introns, but alternatively spliced products have not been identified. Constructs containing the presumptive promoters direct expression of the transgene to the striatum and other areas of endogenous gene expression in DARPP-32 and ARPP-21-*lacZ* mice.

718.2

WARNING! MOLECULAR INFORMATION FROM TETRAPLOID ANIMALS MUST BE EVALUATED WITH CAUTION. Carl Risinger* and Dan Larhammar. Dept of Medical Genetics, Box 589, Uppsala University, S-751 23 Uppsala, Sweden.

Gene duplication followed by mutations is a common mode of gene evolution and has probably given rise to the many gene families in higher animals. An extreme type of gene duplication is genome doubling, *i.e.* tetraploidization. The tetraploid animals *Xenopus laevis*, goldfish, carp, trout and salmons are extensively used in scientific research and have recently been subjected to molecular cloning studies. Although the tetraploid condition has been known for decades, it is rarely acknowledged by molecular biologists. We have studied SNAP-25 in goldfish, *Carrasius auratus*. SNAP-25 (synaptosome-associated protein of 25 kDa) is a neuron-specific protein recently shown to be involved in synaptic vesicle docking or fusion.

Isolation and sequencing of six clones from a goldfish retina cDNA library revealed that all six clones are distinct at the nucleotide level. They correspond to two loci, SNAP-A and SNAP-B, that arose by gene duplication early in bony fish evolution prior to tetraploidization. The three SNAP-A clones have identical amino acids and are alleles of the same locus. The SNAP-B clones correspond to two loci and their divergence agrees with tetraploidization approximately 20 million years ago. This also agrees with studies of several sequences from the closely related carp, *Cyprinus carpio*, which suggest that tetraploidy arose 15-20 million years ago (Larhammar and Risinger, submitted). Surprisingly, several of the SNAP-25 clones have frame-shift mutations. Furthermore, studies of amino acid sequences for goldfish SNAP-25 and carp somatotropin show that replacements seem to accumulate predominantly in one of the duplicated loci.

These findings emphasize the importance of sequencing multiple clones from tetraploid animals in order to make sure that functionally relevant loci are beeing studied.

718.3

SEPARATING GENES INDUCING NEUROPATHY AND SEIZURES IN TREMBLER[J] MICE. M.E.Shy, G. Smith, L.D. Siracusa, S.S. Scherer, G.Alexander*, A.Buchberg. Thomas Jefferson Univ.Phila,PA

Trembler[J] (Tr[J]) is an autosomal semi-dominant mutation resulting in mice which have seizures and a severe demyelinating peripheral neuropathy. The neuropathy is associated with a point mutation in exon 1 of the Schwann cell myelin gene Pmp-22. Pmp-22 mRNA is also expressed at lower levels by unidentified cells in the CNS. The relationship between Pmp-22 expression and seizures is unknown. We have observed frequent clinical seizures in offspring from Tr[J] mice crossed with Mus spretus mice and few seizures in Tr[J] mice on a pure C57BL/6J background. To begin to identify genes inducing seizures, C57BL/6J-Tr[J]/+ mice were crossed with C57BL/6J +/+ or M. spretus +/+ mice. The F1 mice were examined for seizures and for neuropathy. Tr[J] animals were identified by PCR with genomic DNA. M. spretus Tr[J]/+ mice had morphological evidence of neuropathy and significant seizure activity detectable by EEG. C57BL/6J-Tr[J]/+ animals had neuropathy but no EEG abnormalities. Therefore, gene(s) in M. spretus interact with -22 or a closely linked gene, inducing or exacerbating epileptic activity. Backcrossing the F1 mice to C57BL/6J will permit identification of the gene(s) responsible for the seizures.

718.4

DISTRIBUTION OF NEURONAL AND GLIAL mRNAs WITHIN NEURAL CELL BODIES AND PROCESSES. C.F. Landry*, J.B. Watson, V.W. Handley and A.T. Campagnoni, MRRC, UCLA School of Medicine, Los Angeles, California, 90024.

We have found that neuronal and glial mRNAs can be classified based on their selective distribution within neural cell bodies and processes. Using *in situ* hybridization with digoxigenin-labeled cRNA probes, we identified four types of neural mRNA distributions. These included: 1) mRNA species that were confined to the cell soma, i.e. mRNAs encoding the glial proteins, proteolipid protein and 2'3'-cyclic nucleotide-3'-phosphodiesterase and the neuronal enzymes, neuron-specific enolase and glutamate decarboxylase; 2) mRNA species that were found primarily in perinuclear cytoplasm but that were also observed in cell processes, i.e. those encoding the protein kinase C substrates, RC3 and GAP-43, which were identified either in the proximal (GAP-43) or proximal and distal (RC3) dendritic processes of neurons; 3) mRNA species that were abundant within the cell soma but that were also found throughout cellular processes, i.e. myelin basic protein mRNA, which was localized to the cell soma and myelin sheaths of oligodendrocytes; 4) mRNA species that were localized primarily to cell processes, i.e. MAP2 mRNA, which deeply stained dendritic fields but only lightly stained neuronal cell bodies. The distribution patterns of these mRNAs are likely to reflect the mechanisms by which the protein products of these molecules are targeted within neurons and glia. (Supported by NS23022, NS23322, HD25831 and RG2233A1 from the NMSS.)

718.5

PCR-BASED DIFFERENTIAL SCREENING TO IDENTIFY REGIONAL BRAIN SPECIFIC cDNAS. A. Dopazo, M.G. Erlander* and J.G. Sutcliffe. Dept. Molecular Biology, The Scripps Research Institute, La Jolla, CA 92037.

We are systematically surveying the expression pattern of rat mRNAs in a side-by-side comparison of cDNAs from different tissues, brain regions and physiological/behavioral paradigms using a method in which subsets of mRNAs are amplified via the polymerase chain reaction (PCR) using selected primers and picogram quantities of cDNA. For each pair of primers, 50-100 different bands ranging from 100-500 nucleotides are observed on sequencing gels and the band pattern is reproducible in duplicate runs of the same tissue and is completely changed when either primer is changed. For a given primer pair, 50-60% of the bands corresponding to amplified cDNAs derived from the brain are not detected in the liver cDNA samples. A lower percentage (1-3%) of the bands (cDNAs) differ among the cDNAs of different brain regions. Initial experiments demonstrated that known mRNAs of low to moderate prevalence give rise to bands with the expected tissue distribution using the appropiate primer pairs. So far, surveying rat hypothalamus, cortex, cerebellum and liver cDNAs has yielded the nucleotide sequences of thirty brain specific cDNAs whose heterogeneous anatomical expression pattern within the brain confirm the distribution indicated by the initial PCR surveying. By using a systematic set of primers, it should be possible to survey the expression of 10-20,000 mRNAs for a given paradigm, isolate cDNAs for those with interesting distributions and determine nucleotide sequences.

718.6

METHODS FOR ENHANCING TRANSGENE EXPRESSION IN PRIMARY RAT FIBROBLASTS. N.H. Liou, L.J. Fisher,* J. Ray, S.T. Suhr, S. Thode and F.H. Gage. Department of Neurosciences, UCSD, La Jolla, CA 92093-0627.

The Moloney murine leukemia retrovirus (MLV) is an effective vehicle for delivering transgenes to a variety of cell types. However, there is evidence that the MLV-long terminal repeat (MLV-LTR) promoter/enhancer may not sustain transgene expression after infected cells are implanted into a host animal. We are exploring several strategies for increasing transgene activity within engineered cells after grafting, including (1) developing alternate promoter/enhancer configurations for maintaining gene expression and (2) directly manipulating LTR activity. For the former approach, we have constructed vectors that express transgenes from either DNA viral (Cytomegalovirus (CMV) immediate-early promoter and the SV40 promoter) or non-viral (collagen and dihydrofolate reductase) promoters. In some constructs, cell-specific enhancers have also been inserted upstream of the promoters to potentially increase gene expression. Finally, vectors have been constructed with a variety of transgenes (sizes ranging from 0.5 kb to 3.1 kb) to assess differences in gene expression that may be linked to the transgenes. Results to date indicate that the CMV promotor sustains transgene activity within engineered fibroblasts for at least 6 months post-confluence in vitro. These results are consistent with work on muscle cells that suggests the CMV promoter is active for up to 6 months after intracerebral grafting. While the housekeeping promoters do not appear to be as strong as the viral promoters, they may be more effective in achieving long-term expression of the transgenes. Cells containing the various constructs are currently being characterized both in vitro and in vivo. In addition to developing new expression vectors, we have also explored the potential for regulating LTR activity directly through endogenous glucocorticoid response elements (GRE). In vitro, application of dexamethasone to fibroblasts that express a transgene from the LTR promoter has been found to induce a marked increase in transgene mRNA levels, protein levels and protein activity. This latter approach may offer a direct method for regulating transgene activity within engineered cells after grafting. Work supported by NIH AG10435.

718.7

INTRACELLULAR MICROINJECTION OF DNA INTO HIPPOCAMPAL PYRAMIDAL CELLS. K.E. Miller, D.J. Wigston*, K.A. Shirley and H. Joshi. Program in Neuroscience, Dept. of Physiology, and Dept. of Anatomy & Cell Biology, Emory University School of Medicine, Atlanta, GA 30322.

Hippocampal pyramidal cells are widely used to study long term potentiation and the development of neuronal morphology. However, molecular dissection of these phenomena is limited owing to the lack of procedures to introduce exogenous gene constructs into the cells. Conventional methods of DNA-mediated transfection in dividing cells, such as calcium phosphate/DNA precipitation and electroporation have not been successful because of toxicity or damage to cells, and the use of retroviral vectors is not possible in non-mitotic cells such as neurons. Because of these difficulties we examined the possibility of direct microinjection of plasmids into cultured neurons. We cultured hippocampal pyramidal neurons from embryonic day 17 mice at low density along with astrocytes plated on separate coverslips using the method of Banker and Goslin (In: Culturing Nerve Cells, Banker and Goslin Eds.; MIT Press). Four days after plating, neurons were microinjected with plasmids encoding for β-galactosidase driven by the Rous sarcoma virus (RSV) promoter, and allowed to incubate for 18 hours. Expression of the *lac Z* gene was then detected with 5-bromo-4-chloro-3-indolyl-β-D-galactoside (X-Gal), yielding a blue precipitate. In our first set of experiments in which we injected 183 cells, we found 5 cells that expressed exogenous β-galactosidase. However, since many of the injected cells died, an estimate of the success rate based on these numbers would be overly conservative. Intracellular microinjection of DNA constructs might therefore be a useful technique for dissecting the function of proteins specific to the nervous system, and it offers the advantages of single cell specificity and extremely high level, long term, production of RNAs and proteins.

718.8

GENERATION OF CHIMERIC PROMOTERS FOR EXPRESSING FOREIGN GENES FROM HERPES SIMPLEX VIRUS VECTORS

X. Wu*1, M. Cynader2, and F. Tufaro1. Departments of Microbiology and Immunology1 and Ophthalmology2, University of British Columbia, Vancouver, B.C. Canada

We are developing a whole genome Herpes simplex virus vector to express foreign genes from chimeric promoters. Herpes simplex virus exhibits two modes of gene expression in its natural human host: the lytic phase and the latent phase. During the latent phase, viral gene expression is largely repressed, and there is abundant expression in neurons of a single transcription unit, the latency associated transcript (LAT). To test whether the LAT promoter can be regulated by other cis-acting elements, we have generated chimeric promoter constructs to test the interactions of several regulatory elements: the CMV major I/E enhancer, and the SCG10 neuronal silencer element (NRSE).

In transient assays in Vero cells, the NRSE down regulated the LAT promoter by 10% with one NRSE, and by 30% with two elements compared to control constructs. However, the CMV enhancer stimulated LAT promoter activity by at least two fold regardless of the presence of a single NRSE element. Surprisingly, when head-to-tail dimers of the silencer element was placed upstream from the CMV enhancer-LAT promoter elements, there was a ten-fold increase in transcription, indicating that we have uncovered a previously unidentified stimulatory activity of neuron specific silencer elements. It may be that the neuronal silencer dimer and CMV enhancer will be useful for upregulating a normally weak LAT promoter, thereby allowing for high expression in neurons infected with recombinant herpes vectors.

718.9

DEFECTIVE HERPES SIMPLEX VIRUS VECTORS FOR THE STUDY OF PROMOTER AND GENE FUNCTION IN THE NERVOUS SYSTEM. K.C. New*[1], R.L. Martuza[2], K. Gale[3] and S.D. Rabkin[1,2]. Dept. of Microbiology[1], Neurosurgery[2] and Pharmacology[3], Georgetown University Medical Center, Washington, D.C. 20007

Defective herpes simplex virus (HSV) vectors have previously been used by us and others to express foreign marker genes such as ß-galactosidase in differentiated neurons both *in vitro* and *in vivo* (Mol. Cell. Neurosci. 2:320-330, 1991). This work was hindered by limited efficiency of expression for variable lengths of time. We have focused on improving the generation of defective HSV vectors and achieving greater efficiency of expression. Defective HSV genomes containing two transcription units, with two marker enzymes, ß-galactosidase and human placental alkaline phosphatase, are being used to study promoter function in neuronal cell cultures and in the CNS *in vivo*. Multiple transcription units offer an easy method of comparing expression from different promoters. We have demonstrated that defective HSV vectors containing multiple transcription units lead to co-expression of both products in individual cells, although many cells express only one detectable marker. Once promoter activity is characterized by this system, the promoter may be used to drive expression of physiologically significant genes so that direct determinations may be made on their function in the CNS. Many animal models of CNS disorders, such as epilepsy, are amenable to manipulation by appropriately expressed genes. The large size available for DNA insertion is a major advantage of the defective HSV vector system over other viral vectors.

718.10

EXPRESSION OF E. COLI ß-GALACTOSIDASE IN LOW-DENSITY CULTURES OF HIPPOCAMPAL NEURONS. S. Kaech, J.A. Drazba* and E. Ralston. Lab. of Neurobiology, NINDS, NIH, Bethesda MD 20892.

To study the establishment of axonal-dendritic polarity in low-density cultures of rat hippocampal neurons, a simple technique allowing expression of any foreign gene would be advantageous. We have assessed the feasibility of standard DNA transfection techniques, using the E. Coli enzyme ß-galactosidase (ß-gal) as a marker. We could not observe expression of ß-gal after calcium precipitation or electroporation but we obtained 40-200 transfected cells per 35mm dish with the commercial transfection reagents Transfectam® and DOTAP. Best results were obtained when transfection immediately followed the trituration step in the neuron isolation protocol. The transfection efficiency was similar whether expression of ß-gal was driven by a globin promoter or by a neuron-specific enolase promoter. It was also similar when cytoplasmic ß-gal was compared to ß-gal targeted to the nucleus. We verified that the small fraction of cells transfected (<1%) was mostly neuronal, by double-staining for ß-gal and for either neuron (MAP2)- or glia (GFAP)-specific markers. Transfected neurons expressing high levels of ß-gal were polar and developed normally for at least 7 days. ß-gal was present in all cell processes, including the entire length of the axon. Thus DNA transfection can be used to introduce foreign genes into post-mitotic polarizing neurons.

718.11

INSTANT NESTED / SECOND PCR or DIRECT GEL PCR. C.Waibel and H.M.Valivullah.* Biology Dept., Georgetown University, Washington D.C. 20057-1028.

Polymerase Chain Reaction (PCR) has become an important and indispensable method in neurobiology. A common problem that a research or a diagnostic laboratory encounters in a PCR reaction is unexpected PCR products. Unexpected amplified products are often observed in a PCR, especially when optimizing the conditions of the PCR to a particular set of primers and template. To verify the authenticity of the amplified product an investigator uses several methods. Sequencing the amplified product is an ideal but costly way to confirm if the amplified sequence is authentic. Often a pair of primers (nested primers) that are specific for the amplified fragment of DNA, is used to do a second or nested PCR. This is a quick way to confirm the authenticity of a PCR product from the first reaction. It is not very likely the primers in the first and second PCR will hybridize falsely to give the expected fragment length in both cases. In this study we describe a quick and easy method to perform the second or nested PCR after the first PCR. In this 'direct gel method' the gel was cut out and placed into the second PCR directly. TAE buffer over TBE buffer is preferred for electrophoresis and LMP, HGT, GTG and NuSieve 3:1 agaroses are preferred in this order mentioned. Rinsed gel pieces gave a little better results than non-rinsed gel pieces. Using this method, it is not necessary to elute and/or precipitate DNA from the gel from the first PCR to do a second PCR.

718.12

CONSTITUTIVE AND HYPERTHERMIA-INDUCIBLE HEAT SHOCK PROTEINS IN THE RABBIT BRAIN. P. Manzerra*, S.J. Rush and I.R. Brown. Dept of Zoology, Univ of Toronto, Scarborough Campus, West Hill, Ontario, Canada, M1C 1A4

Two dimensional western blotting facilitated the resolution of constitutively expressed hsc70 protein from stress-inducible forms. High levels of hsc70 were observed in the control rabbit brain. The inducible hsp70 protein showed peak levels in the nervous system 5 to 10 hours after hyperthermic treatment. Antibodies specific to inducible hsp70 were utilized for immunocytochemistry and detected the appearance of abundant levels of hsp70 in astrocytes and oligodendrocytes in hyperthermic animals. Antibodies which detect the constitutive hsc70 revealed high levels of this protein in neuronal cell populations. In Purkinje neurons for example, hsc70 was detected at abundant levels in both the neuronal cell bodies and in dendritic processes radiating into the molecular layer of the cerebellum. The pattern of hsc and hsp70 protein in the control and hyperthermic rabbit brain supports the distribution of constitutive and stress-inducible mRNA transcripts of the hsp70 multigene family which is observed using in situ hybridization and Northern blotting. However, to date we have not been able to detect the inducible hsp70 protein in Purkinje neurons which show a delayed induction of hsp70 mRNA compared to the rapid response seen in glial cells. The high hsc70 protein level in these neurons may dampen hsp70 levels.

718.13

EXPRESSION OF HEAT SHOCK GENES IN THE RABBIT NERVOUS SYSTEM. J.A. Foster*, S.J. Rush and I.R. Brown. Dept. of Zoology, Univ of Toronto, Scarborough Campus, West Hill, Ontario, Canada, M1C 1A4

To aid in the identification of the types of neural cells which demonstrate expression of constitutive (hsc) or hyperthermia-inducible (hsp) members of the hsp70 multigene family, neuronal and glial-specific riboprobes have been utlilized. Neuron-specific enolase was employed as a neuronal marker while proteolipid protein was used for oligodendrocyte identification. In the control rabbit brain, hsc70 mRNA was observed at abundant levels in neurons of all cerebellar layers- basket and stellate neurons, Purkinje neurons and deep cerebellar neurons. A strong induction of hsp70 mRNA was noted in 1 hr hyperthermic animals in oligodendrocytes of deep white matter fibre tracts and of the molecular layer. In the brain stem, hsc70 mRNA was localized to neuronal-enriched regions including precerebellar nuclei and was not detected in glial-rich fibre tracts. After heat shock, a strong induction of hsp70 mRNA was noted in oligodendrocytes in the brain stem including afferent and efferent fibre tracts of the cerebellum. The high level of constitutive hsc70 expression in neuronal populations may exert a feedback mechanism which dampens hsp70 induction in these neurons after heat shock. The present investigations were carried out using both radioactive and non-radioactive in situ hybridization procedures.

718.14

Constitutive levels of the immediate early gene zif268 mRNA in skeletal muscle cells are regulated by membrane depolarization and intracellular calcium. S.Abu-Shakra,* A.J.Cole,[1] R.N.Adams,[2] and D.B.Drachman[2]. Depts. of Neurology, Wayne St. Univ.,* Detroit MI 48201, Massachussetts Gen. Hosp.,[1] Boston MA 02114, and Johns Hopkins Univ.,[2] Baltimore MD 21205.

Neural synaptic activity is known to regulate several properties of skeletal muscle cells, including acetycholine receptor synthesis and distribution, ionic channels, contractile protein composition, and enzymatic profile. One of the earliest genomic responses to neural activity in skeletal muscle is immediate early gene (IEG) induction. We previously showed that the IEG zif268, which encodes a transcription factor, is rapidly and transiently expressed in skeletal muscle in response to neural stimulation (Abu-Shakra et al., Molec. Brain Res., in press). Basal levels of skeletal muscle zif268 mRNA in vivo are low, and therefore hard to study. In the C_2 skeletal muscle cell line, which forms fused branched myotubes resembling mature noninnervated muscle cells, zif268 mRNA levels are easily detectable. We have used northern analysis to study the regulation of constitutive zif268 expression in these cells. Treatment with tetrodotoxin (10μM), a Na^+ channel blocker, for 6 hours decreased zif268 mRNA levels by 28% ± 6% (n=5, $p<0.05$), while a 3 hour treatment failed to decrease the basal level (n=8). Treatment with PN200-110 (1μM), an L-type voltage sensitive calcium channel blocker, for 3 hours (n=7) and 6 hours (n=5), failed to decrease basal zif268 mRNA levels. Treatment with Ryanodine (30μM), which blocks Ca^{+2} efflux from sarcoplasmic reticulum stores, decreased zif268 levels by 18% ± 5% (n=4, $p<0.05$) after only 3 hours. These findings suggest that membrane excitability and intracellular Ca^{+2} levels play a role in constitutive zif268 gene expression, while Ca^{+2} influx through the L-type Ca^{+2} channels is not needed to maintain these basal levels.

718.15

CHOLINE (Ch) AND HEMICHOLINIUM-3 (HC-3) PROTECT AGAINST AF64A-INDUCED CHANGES IN N-*MYC* EXPRESSION IN THE HUMAN NEUROBLASTOMA CELL LINE (LA-N-2). L.R. Santiago[1], R. A. Kroes[2], L.C. Erickson[1,2] and I. Hanin[1]*. Depts. [1]Pharmacology and [2]Medicine (Section Hematology/Oncology), Loyola University Chicago Stritch School of Medicine, Maywood, IL 60153, USA.

AF64A (ethylcholine aziridinium) reduces choline acetyltransferase (ChAT) activity and steady state expression of the N-*myc* gene in LA-N-2 (Santiago *et al.*, 1992). To assess the specificity of AF64A's action, we studied the effect of Ch and HC-3 on steady state expression of the N-*myc* gene in LA-N-2, following exposure to 0, 50, 100 and 250 μM AF64A, using the S1 nuclease protection assay. HC-3: The highest dose of AF64A (250 μM) at t=3h produced a maximal (53%) reduction in steady state levels of N-*myc* message compared to control. Reductions of only 42, 32 and 8% of N-*myc* message levels were observed at 250 μM AF64A at 3h, in the presence of 10^{-5}, 10^{-4} and 10^{-3} M HC-3, respectively. Ch: In these experiments, 250 μM AF64A produced a 63% decrease in N-*myc* message levels at 3h. Reductions of 52, 37 and 9% of N-*myc* mRNA levels were observed at this dose of AF64A, in the presence of 10^{-5}, 10^{-4} and 10^{-3} M Ch at the same time point, respectively. Thus, a reduction of N-*myc* message induced by AF64A was significantly attenuated by agents that utilize (Ch), or inhibit (HC-3), the high affinity Ch transport system (HAChT). These data provide additional evidence for AF64A's cholinospecificity, by nature of its dependence on the HAChT system for accessibility to its target of neurotoxic action. We are currently examining the possibility that AF64A also causes changes in the steady state expression of the human ChAT gene, and that Ch and HC-3 can have a modulatory action on AF64A-induced changes in ChAT expression in LA-N-2. Supported by the Earl Bane Foundation Research Trust.

718.16

CHRONIC ELECTROCONVULSIVE SEIZURES (ECS) INDUCE A LONG-LASTING AP1 COMPLEX CONTAINING NOVEL FOS-RELATED PROTEINS. B.T. Hope*, M.B. Kelz, R.S. Duman, and E.J. Nestler. Laboratory of Molecular Psychiatry, Yale Univ. Sch. of Med., New Haven, CT 06508.

To better understand changes in gene expression induced by chronic ECS, we are studying ECS regulation of c-Fos and related proteins and their AP1 DNA binding activity. Acute ECS increases AP1 binding activity in cerebral cortex and hippocampus, which is associated with the induction of c-Fos and several related proteins. These changes return to control levels within 18 hours. In contrast, chronic ECS leads to increases in AP1 binding activity which remain at peak acute levels for 2 days following the last chronic ECS, and are 50% of peak acute levels at 7 days. Supershift experiments (using an antibody from M. Iadarola, NIH) show that this chronic AP1 binding activity is formed, in part, by Fos-related antigens (Fra's). Western blots with this antibody show that acute and chronic ECS induce largely different Fra's: chronic ECS is associated with negligible levels of c-Fos and most other Fra's induced acutely, but high levels of several bands, including a doublet of 37-39 kD, which are not induced significantly by acute ECS. These apparently novel Fra's may account for the chronic AP1 binding activity. The acute and chronic AP1 binding activities show different affinities for probes containing a variety of AP1-like DNA sequences, indicating possible functional differences between these complexes.

These results provide a mechanism by which certain genes that contain AP1 sites can be selectively regulated by chronic (vs acute) ECS. As we have obtained similar results in the striatum and nucleus accumbens following chronic vs acute cocaine treatment, induction of novel AP1 binding proteins may represent a general mechanism involved in the long-term regulation of brain function.

718.17

INDUCTION OF NUCLEAR FACTOR-κB IN RAT BRAIN FOLLOWING SEIZURE ACTIVITY. A.V. Prasad, N. Mohan, B. Chadrasekar, W. H. Pilcher and S. A. Joseph*. Div. of Neurosurgery, Univ. of Rochester Med. Ctr., Rochester, NY 14642.

The DNA-binding protein NF-κB is a pleiotropic transcription factor involved in the transcriptional regulation of several specific late-response genes. We have observed and demonstrated for the first time that NF-κB expression was induced in tetrazole-treated rats. The transcription factor NF-κB was assayed in brains from male Sprague-Dawly rats (230-250g) treated (s.c) with 1,5-pentamethylene tetrazole at a fixed dose of 85 mg/kg (LD_{50}). Clonic-tonic seizure activity was observed 15 min post drug administration, lasting for a few minutes. Whole brains obtained at various time intervals (1-120h) post seizure acitivity were homogenized, and nuclear extracts were prepared. Electromobility shift assays were carried out using the NF-κB binding protein detection system (Promega, Madison, WI). The NF-κB subunits p50 and p65 were assayed by the Western blotting method in the same samples using respective purified rabbit polyclonal antibodies. The results of the assay emphasize that the transcriptional regulation has a role in generalized seizure activity and could lead to transcription of specific late-response genes.

718.18

ENHANCEMENT OF HSP70-RELATED mRNA IN HIPPOCAMPUS OF ECS TREATED-RATS. F.Passarelli*, B.Angeletti, F.Orzi, D.Orrù and E.D'Ambrosio.*Dept. of Neurosci., University "La Sapienza", I[a] Neuro, V.le dell'Università and Istituto Medicina Sperimentale, C.N.R., (Rome)Italy.

The expression of heat shock proteins is largely increased by several physical and chemical stressors.The aim of the study is to evaluate the effect of electroconvulsive shock on the expression of HSP70-related mRNA of rat brain. Rats weighing 250-300 g were divided into 3 groups: first group received ECS once a day for 7 days; a second group received a single ECS; a third group of unshocked rats served as control. The animals were then sacrificed at various time intervals from treatments and the brain processed for *in situ* hybridization. Two oligonucleotides synthesized according to published rat sequence specific respectively for constitutive hsc73 and inducible hsp70 were used as [^{35}S]dATP labelled probe. The corresponding sense oligos were also utilized as hybridization control. The results shows that the mRNA expression was strongly increased mainly in the hippocampal granule cell layer of the hilus of the dentate gyrus following the treatments.The enhanced signal was detected 2hr after last seizures and persisted during 24 hr and returned to the basal values after 7 days. There is no difference between the single and repeated ECS demonstrating that the effect is observed in response to the last seizures themselves rather that the treatments *"per se"*.

718.19

EFFECTS OF CHLORPROMAZINE ON THE INDUCTIONS OF c-*fos* AND *jun* B BY ELECTROCONVULSIVE SHOCKS IN RAT BRAIN. H. Y. Jung[1], Y. M. Ahn[1], U. G. Kang[1], J. B. Park[2], Y. S. Kim[1]*. Departments of Psychiatry[1] and Biochemistry[2], Seoul National University College of Medicine, Seoul, Korea 110-460.

From studies with cultured cell, chlorpromazine(CPZ) has been reported to block the induction of c-*fos* by cell depolarization. However haloperidol, antipsychotics like CPZ was known to induce c-*fos* in striatum and other rat brain regions. So we examined whether CPZ itself elicited the induction of immediate early genes and blocked these genes induced by ECS *in vivo*.

Intraperitoneal injection of CPZ induced c-*fos* and *jun* B in striatum but not in cerebellum. And the induction in striatum was found to be relatively dose-dependent. When we treated rats by ECS 30 min after pretreatment with 10mg/kg of CPZ, which was clinically effective dosage, inductions of c-*fos* and *jun* B by ECS were observed in striatum and other brain regions. Above results indicated that CPZ induced c-*fos* and *jun* B and could not completely block the induction of these genes by ECS *in vivo*.

718.20

DETECTION OF CHYMOTRYPSIN mRNA EXPRESSION IN THE BRAIN BY RIBONUCLEASE PROTECTION ASSAY. X. C. WANG, K. I. STRAUSS, A. M. LATIES* & D. M. JACOBOWITZ, Lab. of Clin. Sci., NIMH, Bethesda, MD 20892.

Chymotrypsin is synthesized, stored and secreted in exocrine pancreas for intestinal protein digestion. Pancreatic RNA from rat was isolated in the presence of guanidine thiocyanate. Polyadenylated RNA was isolated from total rat pancreatic RNA by binding to oligo(dT)-cellulose. A 5'-primer (27-mer), including an upstream EcoR I site, and a 3'-primer (28-mer), including a downstream Hind III site, were designed to amplify a portion of cDNA (about 500 bp) from rat pancreatic mRNA using the PCR. The sequence of the fragment was confirmed to be a portion overlapping exon-6 and exon-7 of rat chymotrypsin B gene. It was subcloned into the pGEM-4Z vector and used as a template for in vitro transcription of an antisense riboprobe. Using the micropunch RNase protection procedure for quantitation of mRNA in discrete brain regions we have detected chymotrypsin mRNA in rat brain. Regions such as hippocampus, striatum, thalamus and cortex contain chymotrypsin mRNA. Future work will be directed towards determining the presence in the brain of chymotrypsin in normal and neurodegenerative diseases (e.g. Alzheimer's).

PRESYNAPTIC MECHANISMS V

719.1

ERRORS IN ESTIMATING APPARENT DIFFUSION COEFFICIENT FROM RATIO OF BOUND TO UNBOUND Ca^{2+} *J.L. Winslow (*,1,2), M.P. Charlton (2)* (1)Biomedical Engineering, (2)Physiology Dept. U. of Toronto, Toronto, Ont. M5S 1A4.

Diffusion of Ca^{2+} in cytoplasm can be slowed by binding to non-diffusable sites and characterized by the apparent diffusion coefficient $D_c^a = D_c/(1+\beta)$, where $\beta =$ [bound Ca^{2+}]/[unbound Ca^{2+}]. D_c^a has been used in place of D_c in the diffusion equation $\frac{\partial C}{\partial t} = D_c \frac{\partial^2 C}{\partial x^2}$. Biologically, D_c is measured and β estimated to obtain D_c^a. **This is only correct if the immobile buffer does not saturate and β is constant.** We numerically solved the *simultaneous* reaction-diffusion equations for diffusable Ca^{2+}, non-diffusable buffer and product, A,Q, over a long cylinder with initial $[Ca^{2+}]_i = 0.1\mu M$ and used the program as a computational preparation The simulations start with an injection of Ca^{2+} in the cylinder's center. (1) For no buffer ($A=Q=0$), we found the best-fit of the appropriate analytical solution to the numerically computed time-response to obtain $\overline{D}_c$, as would be done in biological experiments. This agrees with the specified D_c in the computational preparation. (2) Repeat step 1, but now $A>0$ and Q is the equilibrium value, to obtain another value of $\overline{D}_c$, which should agree with D_c^a, but does not, *because $\beta = Q/C$ varies by 10^4 depending on location and time.* **To avoid errors in diffusion estimates based on constant β, simultaneous equations must be used. Measurements and models which use D_c^a must be interpreted critically.**

719.2

POISSON-PROCESS ELECTRICAL STIMULATION: CIRCUIT AND AXONAL RESPONSES. K. Moradmand, T.N. Hangartner+, and M.D. Goldfinger*. Dept. Physiology & Biophysics and +Dept. Biomedical & Human Factors Engineering, Wright State University, Dayton, OH 45435.

This work addresses the transformation of Poisson process electrical stimulation by GI primary afferent axons. To generate a Poisson process, amplified resistor noise was sampled by a Schmitt-trigger which drove a monostable multivibrator to elicit pulses (0.1 msec/+4.2V). The pulse mean frequency was modulated by the noise envelope's amplitude. Both steady-state (Interevent Interval Distribution, IID) and real-time (Expectation Density, ED) stochastic estimators were used to characterize the temporally-stable output. The IID contained sequentially: a short (0.1 msec) dead-time (DT), a sharp rise, and a single exponential fall-off. The ED contained sequentially: a short DT and a sharp rise to a maintained noisy plateau which corresponded to the mean rate.

Poisson process electrical stimulation was applied percutaneously to the receptive field of a GI primary afferent axon in the Na-Pentobarbital anesthetized cat. The elicited trains of propagated impulses were recorded extracellularly within the cuneate fasciculus. For a given mean stimulus frequency, several stimulus amplitudes were tested. The temporally-stable axonal impulse train contained departures from the stimulus train. The impulse ED contained sequentially: a longer DT (which decreased with higher stimulus amplitude and/or mean frequency); an early peak, and a noisy plateau. The impulse ED peak delay was approximately equal to the doublet interval found separately. By filtering all doublet intervals from the axonal impulse train, the high-probability ED peak was eliminated. Thus, Poisson process stimulation elicited a Poisson-like impulse pattern which included short-interval conditioning via doublet repetitive firing.

Supported NIH-NCRR, Amer. Heart Ass'n., & Wright State University School of Medicine and College of Science and Mathematics.

719.3

AN INCREASE IN SPATIAL VARIANCE IN THE PROBABILITY OF NEUROSECRETION IS ASSOCIATED WITH DRUG-INDUCED INHIBITION OF MITOCHONDRIA. S. D. Provan* and M. D. Miyamoto. Dept. of Pharmacology, East Tenn. State Univ., Johnson City, TN 37614.

Transmitter release from nerve terminals depends on an increase in $[Ca^{2+}]_i$, which normally occurs by influx of Ca^{2+} through voltage-sensitive channels. Increases in $[Ca^{2+}]_i$ may also arise from release of Ca^{2+} sequestered by mitochondria. This would be expected to produce a variation in the $[Ca^{2+}]_i$ at individual transmitter release sites, due to differing distances from the source of Ca^{2+}. This in turn should increase both mean probability of release (p) and spatial variance in p ($var_s\ p$). We wished to see whether Hg^{2+}, methyl Hg (MeHg) and flufenamic acid (Flu), agents known to inhibit mitochondria, produced increases in p and $var_s\ p$. Miniature endplate potentials (mepps) were recorded from isolated frog cutaneous pectoris, and unbiased estimates of m (no. of quanta released), n (no. of functional release sites), p, and $var_s\ p$ made (*Am. J. Physiol.* 264: 1051-1060, 1993). $[K^+]$ was raised to 10 mM to increase mepp frequency, and data recorded after 10 min equilibration. $HgCl_2$ (1 μM) produced increases in m, n, p and $var_s\ p$, all of which peaked after 15 min and returned to control values (N = 6 continuous recordings). Similar findings of an increase, peak and decline in m, n, p and $var_s\ p$ were obtained with 100 μM MeHg (N = 6). This effect was not abolished by 3 μM tetrodotoxin and 1.8 mM Co^{2+}, which suggested that the effect was due to permeation of MeHg through the membrane rather than through voltage-sensitive Na and Ca channels. Finally, an increase, peak effect and decline in m, n, p and $var_s\ p$ was found with 200 μM Flu (N = 6). Previous studies have shown that increases in m, n and p can occur *without* increases in $var_s\ p$, using agents *not* associated with mitochondrial inhibition (K^+, Ca^{2+}, or linopirdine). The present results indicate that changes in $var_s\ p$ might be used as a real-time indicator of drug-induced release of Ca^{2+} from mitochondria. Whether similar effects occur with drug-induced inhibition of smooth endoplasmic reticulum remains to be determined. (Supported by NIH NS22457).

719.4

INCREASE OF TONIC AND EVOKED PRESYNAPTIC INHIBITION BY A GABA-T BLOCKER. **H. Golan and Y. Grossman*. Unit of Physiology, Faculty of Health sciences, Ben-Gurion Univ., Beer-Sheva 84105, Israel.**

We have previously shown that the GABA transaminase (GABA-T) blocker ethanolamine-O-sulfate (EOS), selectively increases presynaptic inhibition. In order to examine the mechanism underlying this phenomenon, action potentials (AP) and presynaptic inhibitory potentials (PIP) were recorded intracellularly at the secondary branches of the excitatory axon in the opener muscle of the crayfish walking leg. EOS (5-10 mM) had no effect on axonal R_m (n=3), while causing up to a 7 mV depolarization in 4 of 7 axons tested and up to 14 mV reduction in the AP amplitude. AP width was also increased, reflecting an increase of local tonic conductance in remote presynaptic sites. Applied 0.2 mM GABA generated similar effects. These findings were correlated with a 20% reduction in the excitatory transmission. Evoked inhibition was tested by delivering either a single or a short train of stimuli to the inhibitory axon 1-15 ms before evoking excitor AP. EOS enhanced the PIP amplitude (X3), but reduced the AP by only 2-5%. Considering the changes in electrotonic spread due to increased distal conductance, the predominant effect of the GABA-T blocker would appear to enhance presynaptic tonic, and evoked, GABA release,

719.5

SODIUM NITROPRUSSIDE INHIBITS NEUROTRANSMITTER RELEASE AT THE NEUROMUSCULAR JUNCTION OF THE FROG. Clark A. Lindgren* and Melissa V. Laird. Department of Biology, Grinnell College, Grinnell, IA 50112.

Using intracellular recording, end plate potentials (EPPs) were measured at the neuromuscular junction on the sartorius muscle of the frog, *Rana pipiens pipiens*. Sodium Nitroprusside (SNP), a compound which decomposes in the presence of light to Nitric Oxide (NO) and other miscellaneous by-products, was applied at concentrations ranging from 0.01 μM to 1.0 mM. EPPs were reduced by an average of 57% within minutes of application of SNP. This reduction of EPPs was shown through quantal analysis (i.e. under condition of lowered extracellular calcium and elevated magnesium) to result from an inhibition of neurotransmitter release from the motor nerve endings. This inhibition was long-term; reversal of the inhibition was observed only at the lower SNP concentrations. The inhibition was also stimulus dependent. EPPs were reduced almost immediately when SNP was applied while stimulating the motor nerve; however, depression was delayed (approximately 5 minutes) if SNP was applied in the absence of nerve stimulation. In summary, these results suggest that NO inhibits the release of neurotransmitter from motor nerve endings of the frog. (Supported by NIH Grant NS-29520 and a grant from the Howard Hughes Medical Institute to Grinnell College.)

719.6

ARACHIDONIC ACID CONTRIBUTES TO STRIATAL SYNAPTIC DEPRESSION BY REDUCING PRE-SYNAPTIC ACTION POTENTIALS. D.D. Fraser*, K. Hoehn, S. Weiss and B.A. MacVicar. Neuroscience Research Group, University of Calgary, Alberta, Canada. T2N 1N4.

In the striatum, both arachidonic acid (AA) release and induction of synaptic depression require co-activation of D_1 and D_2 receptors, suggesting the phenomena may be linked. We reported previously that AA depresses the peak Na^+ current in striatal neurons and shifts the inactivation curve to hyperpolarized potentials. Since depression of the pre-synaptic spike could result in a reduction of transmitter release we have now investigated the actions of AA on Na^+-dependent action potentials and spontaneous synaptic currents in striatal cultures. In agreement with our voltage-clamp data, action potentials evoked from the resting membrane potential (-60 mV) were depressed significantly by 10 μM AA (36%; n=10). At a holding potential of -80 mV, single action potentials were minimally depressed (15%, n=10), whereas repetitive bursting was reduced dramatically (n=8). The spike threshold and input resistance were unaffected by AA. In voltage-clamp mode, spontaneous synaptic currents that were blocked by 1 μM TTX or 200 μM Cd^{2+} were depressed by 10 μM AA (n=5). The frequency and amplitude of synaptic currents were reduced by 51% and 36%, respectively. These observations are consistent with the hypothesis that AA-induced synaptic depression involves a reduction of the pre-synaptic Na^+-dependent action potential.
Supported by the MRC (Canada) and Ciba Geigy.

719.7

PRESYNAPTIC DEPRESSION OF GLUTAMATERGIC SYNAPTIC TRANSMISSION BY HYPOGLYCEMIA.
M. W. Fleck*[1,2], A. M. Palmer[2,3], and G. Barrionuevo[1,2]. *Departments of [1]Behavioral Neuroscience, [2]Psychiatry, and [3]Pharmacology; University of Pittsburgh, Pittsburgh, PA 15260.*

We examined the effects of hypoglycemia on glutamatergic synaptic transmission in area CA1 of rat hippocampal slices. Decreasing glucose from 10 mM to 0.2 mM depressed the calcium-dependent potassium-evoked release of glutamate by ~ 60% and depressed Schaffer collateral-evoked population excitatory postsynaptic potentials (pEPSP) and population spikes (PS) to less than 10% of their initial amplitudes. The hypoglycemic depression was complete within 1 hr at room temperature. The apparent EC_{50} for glucose was ~ 0.6 mM. Maximal pEPSP amplitudes but not maximal PS amplitudes were significantly reduced by hypoglycemia. In addition, paired-pulse facilitations of the pEPSP and PS were enhanced from 10% to 60% and from 100% to 300%, respectively. Paired-pulse facilitation was enhanced across a 32-fold range of stimulus intensities and was most pronounced at inter-stimulus intervals less than 100 msec. The enhancement was not attenuated by the GABA-$_A$ receptor antagonists picrotoxin or bicuculline, nor by the NMDA receptor antagonist D-APV. Whole-cell recordings revealed that hypoglycemia decreased the amplitudes of evoked EPSCs and the frequency but not amplitude of spontaneous EPSCs in pyramidal cells of area CA1. Thus, hypoglycemia depresses excitatory synaptic transmission in area CA1 of the hippocampus by modulation of the release mechanism. Supported by NS24288, and an NIMH Predoctoral fellowship.

719.8

GLUCOSE-DEPLETION INHIBITS SYNAPTIC TRANSMISSION IN THE CENTRAL NERVOUS SYSTEM OF THE RAT. T. Akasu*, S. Shoji and H. Hasuo. Dept. Physiol., Kurume Univ. Sch. Med., Kurume 830, Japan.

To study the role of glucose on synaptic transmission in the central nervous system, intracellular recordings were made from neurons in the dorsolateral septal nucleus, in vitro. Lowering the concentration of extracellular glucose resulted in a concentration-dependent hyperpolarization associated with a decreased input resistance. The spontaneous action potential was inhibited during the hyperpolarization. EPSP, IPSP and LHP evoked by stimulations of fimbria/fornix pathway were inhibited by depletion of extracellular glucose (0-2 mM). Substitution of 2-deoxy-D-glucose (11 mM) for glucose mimicked the effects of glucose-depletion. Mannoheptulose (10 mM) and dinitrophenol (50 μM) produced the hyperpolarization and the inhibition of postsynaptic potentials even in the presence of 11 mM glucose. Sulfonylureas, glibenclamide (10 μM) and tolbutamide (1 mM), did not antagonize the effects of glucose-depletion. Depolarizing response produced by direct application of glutamate and hyperpolarization produced by either muscimol or baclofen were almost unchanged, when glucose was reduced to 1-2 mM. These results indicate that intracellular glucose metabolism regulates the function of septal neurons not only by changing the resting membrane potential, but also by presynaptically affecting synaptic transmission from the hippocampal formation to the septum nucleus.

719.9

METHYLMERCURY INCREASES THE INTRATERMINAL CONCENTRATION OF AN ENDOGENOUS HEAVY METAL MF Denny* and WD Atchison. Dept. Pharmacol. Toxicol., Neurosci. Prgm. and Inst. Environ. Toxicol., Mich. State Univ., E. Lansing, MI, 48824.

Methylmercury (MeHg) is a neurotoxic metal known to elevate intracellular Ca^{2+} concentration upon acute exposure. We have shown previously that MeHg also causes an immediate elevation in the free intracellular concentration of an endogenous polyvalent cation within isolated nerve terminals. We postulated that this cation may be Zn^{2+}. Zn^{2+} elevates fura-2 fluorescence intensity at all excitation wavelengths including the Ca^{2+}-insensitive wavelength of 360 nm; MeHg itself does not interact with fura-2. Addition of MeHg to fura-2 loaded synaptosomes causes a shift in excitation spectra similar to that of Zn^{2+}. Zn^{2+} also quenches quin2 fluorescence intensity at all excitation wavelengths. As such, elevations in intracellular Zn^{2+} concentration should quench quin2 fluorescence. Addition of MeHg to synaptosomes loaded with quin2 decreases intensity. The MeHg-induced alterations in both fura-2 and quin2 fluorescence intensity were reversed by the subsequent addition of the cell-permeant heavy metal chelator TPEN. These results using fluorescent indicators suggest that Zn^{2+} is the endogenous polyvalent cation responsible for these changes in intensity. Positive identification as well as quantification will require using ^{19}F-NMR. The intrasynaptosomal source of the cation(s) is unknown. Synaptic vesicles are known to contain Zn^{2+}, and MeHg has been shown previously to alter vesicular release of several transmitters. Perhaps MeHg causes release of Zn^{2+} associated with synaptic vesicles. Preincubation of fura-2 loaded synaptosomes with agents known to cause mobilization of vesicles (K^+ depolarization, 0.1 μM veratridine or 6 nM α-latrotoxin) did not affect the elevations in fluorescence intensity induced by MeHg. Thus, either the metal is not mobilized upon release of synaptic vesicles or the vesicles are not the source of the cation(s). Supported by NIH grant ES03299. MFD is the recipient of a student fellowship from the Hazelton Co.

720.1

FREQUENCY-DEPENDENT INHIBITION OF ELECTRICALLY-EVOKED ^{3}H-NOREPINEPHRINE (^{3}H-NE) RELEASE FROM RAT CORTICAL SLICES BY ω-CONOTOXIN MVIIA (ω-CTM) AND ω-CONOTOXIN GVIA (ω-CTG).

S. Wurster and D.J. Dooley*. Dept. of Neuroscience, Parke-Davis. Pharmaceutical Research Division, Warner-Lambert Co., Ann Arbor, MI 48106.

The extent of inhibition of K^+-evoked ^{3}H-NE release from rat brain slices by the N-type channel blocker ω-CTG has recently been shown to decrease with increasing [K^+] (Biochem. Pharmacol. 45 (1993) 165). This could indicate a voltage dependence in the inhibitory action of ω-CTG, or a decreasing relative contribution of Ca^{2+} influx through N-type Ca^{2+} channels when noradrenergic nerve endings are depolarized by tetantic K^+-depolarizations for extended periods of time. Electrically-evoked ^{3}H-NE release is devoid of such long lasting membrane depolarization and matches the physiologically action potential-induced neurotransmitter release more closely . In the present study we have evaluated the effects of varying the frequency of electrical field stimulations on the inhibition of ^{3}H-NE release caused by ω-CTM and ω-CTG. Rat neocortical slices preloaded with ^{3}H-NE were superfused continuously and stimulated with 90 pulses ranging in frequencies from 0.3-100 Hz. ω-CTM or ω-CTG were added to the superfusion buffer 40 min before the stimulation. Evoked release was inhibited by the reversible blocker ω-CTM and the essentially irreversible blocker ω-CTG at all stimulation frequencies. The maximal inhibition, however, decreased with increasing frequency: 80% (0.3 Hz), 75% (3 Hz), 60% (30 Hz) and 40% (100 Hz). In contrast, the relative IC$_{50}$'s (2-4 nM) of ω-CTM were independent of stimulation frequency. Taken together these results seem to indicate an increasing contribution of non-N-type Ca^{2+} channels to ^{3}H-NE release from rat neocortical slices with increasing stimulation frequency. Alternatively, this phenomenon may reflect the failing ability of Ca^{2+} extrusion systems to decrease free intracellular [Ca^{2+}] during high frequency stimulations back to resting levels before the nerve endings are depolarized by the next pulse.

720.2

Snail and Spider Toxins Block Action Potential-Induced Ca^{2+} Influx in Rat Dorsal Root Ganglion Neurons.

T. M. Piser[1]*, R.A.Lampe[2], R.A.Keith[2], S.A. Thayer[1], [1]Department of Pharmacology, University of Minnesota Medical School, Minneapolis MN, 55455. [2]Department of Pharmacology, ICI Americas Inc., Wilmington, DE 19897.

Action potential-mediated transient increases in intracellular free calcium concentration were elicited by field potential stimulation of rat dorsal root ganglion neurons and measured by indo-1-based microfluorimetry. ω-Conotoxin (100 nM) and nitrendipine (1 μM), blockers of high-threshold voltage-gated Ca^{2+} channels, inhibited action potential-mediated Ca^{2+} influx by 79 ± 2% (n=14) and 13 ± 3% (n=10), respectively. ω-Grammotoxin (267 nM), a novel peptide toxin purified from the venom of the tarantula spider, *Grammostola spatulata*, blocked action potential-mediated Ca^{2+} influx as effectively as ω-conotoxin (76 ± 4%, n=6). In contrast to block by ω-conotoxin, the block produced by ω-grammotoxin was reversible. Preliminary patch clamp experiments indicate that ω-conotoxin and ω-grammotoxin block in common a component of whole cell Ca^{2+} current . These results are consistent with the conclusion that ω-grammotoxin is a novel inhibitor of N-type voltage-gated Ca^{2+} channels.

720.3

PHARMACOLOGY OF ION CHANNELS INVOLVED IN HIGH-K^+-EVOKED [3H]-GLUTAMATE RELEASE.

S. Katragadda*, J. B. Fischer, A. G. Knapp, K. G. Pratt, S. M. Goldin. Cambridge NeuroScience, One Kendall Sq., Bldg. 700, Cambridge, MA 02139.

High K^+-evoked [3H]-glutamate release from rat brain nerve terminals can be resolved into three different components using a novel rapid superfusion technique. A) A *Ca-independent* component that is due to the reversal of glutamate transporter that lasts as long as depolarization is continued. This component can be specifically blocked by glutamate uptake blocker D,L threo-β-hydroxy aspartate. B) A *transient Ca-dependent* component that decays with a τ of < 0.2 sec and C) a *persistent Ca-dependent* component that decays in a few seconds. We are interested in understanding the nature of ion channels that are involved in Ca-dependent glutamate release. Tetrodotoxin (up to 30 μM) did not show any effect on any component of K^+-evoked glutamate release. This indicates that Na channels do not contribute to Ca-dep. release under our experimental conditions. ω-Aga IVA blocked 65% of Ca-dep. glutamate release indicating that most of the Ca-dependent release is governed by 'P-type' Ca channels, confirming earlier reports. ω-Cg toxin GVIA and ω-Cm toxin MVIIA exhibited only marginal block at saturating concentrations (< 20 %) suggesting that 'N-type' channels do not play major role in Ca-dependent glutamate release. Therefore among the Ca channels, P-type channels are primarily involved in this process whereas N-type channels play only a minor role.

720.4

EFFECTS OF CALCIUM CHANNEL BLOCKERS ON SEIZURE ACTIVITY IN CULTURED RAT HIPPOCAMPAL NEURONS.

S.S. Lin*, R.E. Numann, and R.L. Simon Departments of CNS and CVMD, Wyeth-Ayerst Research, CN 8000 Princeton, NJ 08543.

There are a variety of voltage dependent calcium channels found in neurons. Using specific blockers for each of the different channels, many investigators have implicated a role for the N-type calcium channel in the release of neurotransmitters. However, Horne and Kemp (Br. J. Pharmacol. 1991) found that omega-conotoxin GVIA, a specific blocker of N-type channels, did not inhibit the paroxysmal depolarization shifts (PDSs) that are characteristic of epileptic seizure activity. We have tested specific blockers of different calcium channels to determine which are involved in the generation of PDSs in a cultured rat hippocampal model of epilepsy (Furshpan and Potter, Neuron, 1989). Omega-conotoxin GVIA had little or no effects on PDS induced currents. By contrast, omega-agatoxin, a specific blocker of P-type channels (Mintz et al, Neuron, 1992) reduces PDS currents by 70-90%. Surprisingly, nimodipine, which blocks L-type channels, was the most effective antagonist, inhibiting almost all of the PDS induced currents.

720.5

WITHDRAWN

720.6

PHARMACOLOGICAL CHARACTERIZATION OF THE ENDOGENOUS CALCIUM AND BETA SUBUNIT-ENHANCED CALCIUM CURRENT IN XENOPUS OOCYTES.

J. D. Mills*, A. Kondo, M. E. Adams & E. Perez-Reyes. Depts. Entomology & Neuroscience, University of California, Riverside, CA 92521 & Loyola University Medical Center, Maywood, IL 60153.

The *Xenopus* oocyte, widely utilized for expression of heterologous ion channels, expresses an endogenous calcium-dependent chloride current, $I_{Cl(Ca)}$, which is involved in the fertilization process. We found that this chloride current is 75-85% blocked by crude *Agelenopsis aperta* spider venom (n=4). Both the polyamine (1:2500 dil.; n=3) and peptide fractions (1:1250 dil.; n=3) from the venom blocked a similar percentage. ω-Aga-IIIA, a toxin isolated from the venom, inhibited 80% of $I_{Cl(Ca)}$ (200 nM, n=30). Elevation of $[Ca]_o$ from 5 - 40 mM increased the IC_{50} value from 12 - 69 nM, and maximum block decreased from 80% to 55%. $I_{Cl(Ca)}$ was insensitive to nifedipine (n=2), ω-conotoxin GVIA (n=40) and ω-Aga-IVA (n=20). Attempts to characterize the endogenous Ca channels directly were hampered by extremely low values of I_{Ba} in most oocytes. However, in a small percentage of oocytes expressing large I_{Ba}, we observed a potent blocking action by ω-Aga-IIIA (n=10), but no effect of dihydropyridines (n=3) or ω-Aga-IVA (n=3). Effects of ω-conotoxin GVIA (0.3-5 μM) were variable between oocyte batches. In some batches, up to 68% block was observed, while no significant block was observed in others.

Injection of cloned β2 and β4 subunits enhanced the endogenous current by 11- and 3-fold, respectively. ω-Agatoxins affected the β-enhanced currents in a similar manner as the endogenous currents. ω-Aga-IVA had no effect on β-enhanced $I_{Cl(Ca)}$ showing that the channel was not P-type. β-enhanced barium currents were up to 60% blocked and β-enhanced chloride currents up to 85% blocked by 200 nM ω-Aga-IIIA . (Supported by NIH grants NS24472 and HL46702).

720.7

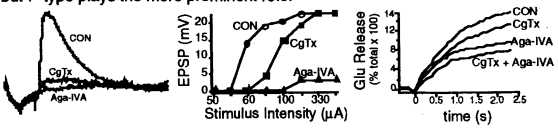
INHIBITION OF NEURONAL VOLTAGE-SENSITIVE CALCIUM CHANNEL (VSCC) RESPONSES: EFFECTS OF SYNTHETIC ω-Aga-IVA AND SYNTHETIC ω-GRAMMOTOXIN SIA. R. A. Lampe*, T. J. Mangano P. A. DeFeo, M. B. Horn, and R. A. Keith. Dept. of Pharmacology, Zeneca Pharmaceuticals Group., Wilmington, DE 19897.

ω-Aga-IVA and ω-grammotoxin SIA (ω-GsTx) are naturally occurring spider venom peptides that have been isolated from *Agelenopsis aperta* and *Grammostola spatulata*, respectively. ω-Aga-IVA is reported to be a selective inhibitor of P-type VSCC (Mintz *et al.*, Nature, 355:827, 1992) whereas ω-GsTx inhibits P- and N-type VSCC responses, but does not displace ^{125}I-ω-conotoxin GVIA in binding studies (Lampe *et al.*, J. Mol. Pharmacol., in press). Studies using purified preparations are subject to the possibility that trace impurities may contribute to the observed pharmacology. In an attempt to address this possibility, synthetic samples of ω-Aga-IVA and ω-GsTx were obtained and tested in functional assays of neuronal VSCC activity. Synthetic ω-Aga-IVA (commercially available from Peptides International) and ω-GsTx (custom synthesis by Bachem, California) were tested against K^+-evoked synaptosomal (rat brain) $^{45}Ca^{++}$ influx and release of ^{3}H-D-aspartate from rat hippocampal slices. ω-Aga-IVA and ω-GsTx caused a concentration-dependent inhibition of synaptosomal $^{45}Ca^{++}$ influx with IC_{50} values of approximately 80 nM and 50 nM, respectively. These potency values are similar to those previously reported for the natural peptides, with the exception that synthetic ω-GsTx is approximately 3 times more potent than the natural peptide. At 200 nM, both peptides inhibited ^{3}H-D-aspartate release by approximately 50%, again agreeing well with values previously reported for the natural peptides. The results suggest that trace impurities were not substantially contributing to the pharmacology of the purified preparations.

720.8

MULTIPLE CALCIUM CHANNEL TYPES REGULATE GLUTAMATE RELEASE IN THE HIPPOCAMPUS. J.L. Luebke*, K. Dunlap, and T.J. Turner Physiology & Neuroscience, Tufts University School of Medicine, Boston, MA 02111

We have employed peptide toxins that specifically block N-type (ω-CgTx) or P-type (ω-Aga-IVA) calcium channels to determine which channel types control evoked synaptic transmission between Schaffer collaterals and CA1 pyramidal neurons in rat hippocampal slices. In parallel experiments, rapid superfusion techniques allowed a direct measure of calcium-dependent [^{3}H]-glutamate release from hippocampal synaptosomes. ω-Aga-IVA (100 nM) and ω-CgTx (1 μM) both reduced the amplitude of glutamatergic excitatory postsynaptic potentials in CA1 neurons. ω-Aga-IVA was much more efficacious than ω-CgTx, however, producing 100% block in ~15 min. (as compared to 75% block by ω-CgTx in ~30 min.) for a given stimulus amplitude. Furthermore, the inhibitory effects of both toxins could be overcome through an increase in stimulus intensity (presumably by recruiting additional synaptic inputs), but much smaller increases were required to overcome the effects of ω-CgTx. These electrophysiological results were corroborated by the biochemical studies of [^{3}H]-glutamate release. When synaptosomes were stimulated with 30 mM KCl, 100 nM ω-Aga-IVA inhibited [^{3}H]-glutamate release by 40%, compared to the 16% inhibition produced by 1 μM ω-CgTx. Taken together, these data suggest that both P-type and N-type calcium channels trigger glutamate release from hippocampal neurons, but P-type plays the more prominent role.



720.9

BLOCK OF INSECT NEURONAL CALCIUM CHANNELS BY PEPTIDE SPIDER TOXINS. E.A. Ertel, M.M. Smith, T.A. Bale, V.A. Warren & C.J. Cohen* Merck Res Labs, Rahway, NJ. 07065.

Previous voltage-clamp studies of insect neuronal Ca channels were hampered by preparations with small currents and poor voltage control. Using freshly dispersed neurons from locust (*Schistocerca americana*) thoracic ganglia or cockroach (*Periplaneta americana*) A6 ganglia, we are able to record robust well-controlled Ba currents. Locust Ca channels have kinetics similar to those of high-voltage-activated Ca channels in mammalian neurons, but they activate at more negative voltages (≈-50 mV). We have isolated peptide toxins from the spider *Agelenopsis aperta* by HPLC. We used insecticidal activity against blowfly larvae as the bioassay for ω-Aga-IA and inhibition of binding of ^{125}I-ω-conotoxin GVIA to rat brain synaptosomal membranes for ω-Aga-IIB and ω-Aga-IIIA. Identification was confirmed by N-terminal sequence analysis. We observe potent Ca channel block by all 3 toxins, in contrast to earlier studies with housefly neuromuscular junction, where only toxin types I and II are inhibitory (Bindokas, J. Neurophysiol, 66:590). Percent block by each toxin varies widely among cells, but each toxin-sensitive component is generally affected with IC_{50}≈ 20 nM. These results indicate neuronal heterogeneity and multiple types of Ca channels. We also evaluated Ca channel block by ω-Aga-IA in guinea pig atrial myocytes and find no effect of 100 nM toxin on L- or T-type Ca channels. This contrasts with earlier reports using rat DRG neurons [Scott, Mol Pharm. 38:711]. ω-Aga-IIB and ω-Aga-IIIA inhibit binding of ^{125}I-ω-conotoxin GVIA to rat brain synaptosomal membranes (IC_{50}≈ 20 pM), but 100 nM ω-Aga-IA does not. This suggests that N-type Ca channels are also not blocked by ω-Aga-IA.

720.10

PHARMACOLOGICAL CHARACTERIZATION OF THE P-TYPE CALCIUM CHANNEL IN ISOLATED RAT NEOCORTICAL NEURONS. A.M.Brown, P.C. Schwindt* and W.E.Crill Dept. of Physiology & Biophysics, University of Washington School of Medicine, Seattle, WA 98195.

High voltage activated (HVA) calcium currents were recorded in pyramidal neurons acutely isolated from sensorimotor cortex of 7 - 20 day old rats using the whole cell patch clamp configuration. Application of 100 nM and 200 nM ω-Aga-IVA reduced the HVA current by 31.5%. The reduction of the HVA current by ω-Aga-IVA was less when 10 μM nifedipine was added first. ω-Aga-IVA did not affect calcium tail currents lengthened by Bay K 8644 suggesting that ω-Aga-IVA does not inhibit the dihydropyridine-sensitive HVA component. The reduction of the HVA current by 10 μM ωCgTx was not affected by the order of its application. A dilution of 1:1000 FTX reduced the HVA current by 81.4% and occluded the inhibitory effects of ω-Aga-IVA. We conclude that about 30% of the HVA current in acutely isolated rat neocortical neurons is specifically inhibited by ω-Aga-IVA and is carried by P channels. ωCgTx specifically inhibits the N channel component but nifedipine at 10 μM partially inhibits the ω-Aga-IVA sensitive component. FTX is not a specific inhibitor of the P channel in these neurons. Supported by ONR N0014-90-5-1627, NS 16792, and W.M.Keck Foundation.

720.11

SMALL *PLECTREURYS* TOXINS. W.D. Branton* and Zhou Yi. Department of Physiology, University of Minnesota, Minneapolis MN 55455.

The venom of the spider *Plectruerys tristes* contains a family of peptide calcium channel blockers with members specific for insect and vertebrate calcium channels. Both insect and vertebrate toxins are present in at least two apparent molecular weight (MW) ranges. The most abundant toxins have a MW of about 5-6 kDa. Previously, we structurally characterized one of the insect toxins (PLTXII) in the 5-6 kDa range. PLTXII is a 44 amino acid peptide with a unique O-Palmitoyl Threonine amide as the final residue. All of the peptide calcium channel blockers in the venom probably share a similar C-terminal structure. The lipid is apparently a requirement for activity, and we have speculated that the unique structure may be associated with a unique mechanism of action. Although synthesis or expression of toxins like PLTXII are potentially feasible, toxins with smaller peptide components would be easier to synthesize. To this end we are investigating toxins with apparent MW of 2.5 kDa or smaller. We have identified both insect and vertebrate selective activities and have partially purified an insect toxin. These toxins are likely to be fatty acylated like PLTXII. They elute even later than PLTXII on C18 reverse phase, as would be expected of a toxin similar to PLTXII but with a smaller hydrophilic peptide component; and the small toxins (like PLTXII and others in the higher MW range) shift to a much more hydrophilic retention time after saponification to remove ester linked lipids. The apparent size of 2.5 kDa suggests that the peptide component is approximately 20 amino acids. If this estimate proves accurate, it may be possible to efficiently synthesize the structure once it is established.

720.12

MULTIPLE SUBTYPES OF VOLTAGE-GATED CALCIUM CURRENTS IN NEURONS OF THE CHICK EDINGER WESTPHAL NUCLEUS. Z. Lucaj and J.T. Fujii* Dept. of Anatomy and Cell Biology, Wayne State U. Sch. of Med., Detroit, MI 48201.

Calyciform terminals, formed by axons projecting from the neurons of the Edinger Westphal (EW) nucleus and terminating in the chick ciliary ganglion, express N-type calcium channels (Stanley and Goping et al.,1991). Whole cell voltage clamp techniques were used to record from the cell bodies of EW neurons in slices obtained from embryonic day 16 chicks. Depolarizing step commands of 10mV from a holding potential of -70mV were used to study the voltage-current relationship of calcium currents. A voltage step from a prepulse of -40mV to 0mV was used to isolate high-voltage activated (HVA) from low-voltage activated (LVA) calcium currents. These commands indicated the presence of both LVA and HVA calcium currents.

Pharmacological agents that were bath applied yielded the following results: (i) 10mM nickel chloride caused a reversible decrease in the amplitude of the calcium current, (ii) cadmium chloride (100μM) produced a reversible decrease in the amplitude of the calcium current, and (iii) omega-conotoxin (2μM) caused an irreversible decrease in the amplitude of the calcium current. There was also an omega-conotoxin resistant component of the whole cell current. This data suggests that currents similar to N-type and T-type calcium currents are expressed in EW neurons of E16 chicks. This work was supported by NSF BNS-8719391, NEI R01EY09768 and an award from The Michigan Eye Bank and Transplantation Center.

720.13

CHARACTERIZATION OF L-TYPE CALCIUM CHANNELS IN MUSSEL OOCYTE PLASMA MEMBRANES. S. Krantic*, M. Tomkowiak and P. Guerrier. Lab. of Mol. and Cell. Biol., UMR 49 CNRS-ENS, Lyon 69000, France.

Membrane depolarization induced by an excess KCl (53mM) triggers an intracellular Ca^{2+} surge and activates metaphase I-arrested oocytes of the mussel (*Mytilus galloprovincialis*). This response is inhibited by the dihidropyridine (DHP) analog, nitrendipine, thus suggesting the involvement of L-type voltage-gated calcium channels in KCl-dependent oocyte activation. We therefore performed binding assay on semi-purified membrane preparations of metaphase-arrested oocytes, using a radioactive DHP analog, [^{3}H]-PN 200-110. Saturation data suggest the existence of a single class of DHP binding sites with an apparent affinity of 1.82±0.38 μM and a maximal binding capacity of 998±115 pmol/mg protein. Only DHP-related compounds could inhibit [^{3}H]-PN 200-110 specific binding with following order of potencies: nitrendipine > PN 200-110 > fluspirilene > nifedipine = bepridil > flunarizine = diltiazem. These data point to the existence of an original form of L-type calcium channels in mussel oocyte plasma membranes.

720.14

DISTRIBUTION OF N-TYPE VOLTAGE-SENSITIVE CALCIUM CHANNELS IN DEVELOPING AND ADULT RAT BRAIN. B.D. Shivers*, J.M. Hopkins and P.D. Doyle. Neuroscience Pharmacology, Parke-Davis Pharmaceutical Research Division, Warner-Lambert Co., Ann Arbor, MI 48105.

Using the iodinated snail neurotoxin ω-Conotoxin MVIIA and the method of *in vitro* autoradiography, we localized N-type voltage-sensitive calcium channels (VSCCs) in developing and adult rat brain. In adult brain, N-type VSCCs were most abundant in olfactory bulb glomeruli, olfactory tubercle, cerebral cortex, caudate-putamen, CA1 hippocampal region, lateral septum and substantia gelatinosa in the spinal cord. N-type VSCCs were moderately abundant in the hypothalamus, superior colliculus, cerebellar molecular layer, midbrain, substantia nigra reticulata, medial geniculate and posterior lobe of the pituitary. A low density of N-type VSCCs was observed in the adrenal medulla. Removal of retinal projections eliminated most of the N-type VSCCs in the contralateral superior colliculus. N-type VSCCs were more widely expressed in perinatal than adult brain. These results suggest that N-type VSCCs are distributed throughout the neuroaxis existing on some neurons presynaptically where they regulate neurotransmitter release. In developing brain, the N-type VSCC may participate in activity-dependent changes in synaptic connectivity.

720.15

DIFFERENT ROLES OF N- AND L-TYPE CALCIUM CHANNELS IN DEPOLARIZATION-INDUCED CALCIUM TRANSIENTS IN CA3 PYRAMIDAL NEURONS. E.M. Elliott[1]*, A.T. Malouf[2], and W.A. Catterall[1]. Depts. of [1]Pharmacology and [2]Neurological Surgery, University of Washington, Seattle, WA 98195.

N- and L-type calcium channels have a complementary distribution in hippocampal pyramidal neurons: N-type are predominantly dendritic and presynaptic while L-type are primarily somatic (Westenbroek et al., *Nature* (1990) 347, 281; *Neuron* (1992) 9, 1099). The roles of each of these channels in mediating the somatic calcium increase in response to depolarization may also differ. Individual CA3 neurons in cultured hippocampal slices were intracellularly injected with fura-2 and stimulated with a 20 Hz pulse train for 10 sec via an electrode placed at the soma or 200 μm along the apical dendrite. With somal stimulation, antagonists of L channels (1 μM nimodipine or isradipine) inhibited the somatic calcium increase by 56% while an antagonist of N channels (5 μM ω-conotoxin GVIA) had no effect. In contrast, with dendritic stimulation, both N and L channel antagonists inhibited the stimulus-induced somatic calcium increase by 35% and 47% respectively. Inhibitors of glutamate-mediated synaptic transmission via NMDA and non-NMDA receptors (100 μM AP-5 and 20 μM CNQX, respectively) inhibit the somatic calcium increase by 37% following dendritic but not direct somatic stimulation. L channel antagonists inhibited the somatic calcium transient remaining after AP-5 and CNQX by 40% while ω-conotoxin had only small effects. Our results indicate that L-type calcium channels play an essential role in somatic calcium transients in response to either direct somal or indirect dendritic stimulation consistent with their preferential localization in cell bodies, while N-type calcium channels only participate in calcium transients in response to dendritic stimulation consistent with their preferential localization in dendrites and presynaptic terminals.

720.16

INHIBITION OF CALCIUM CURRENTS BY ZINC: PREFERENTIAL EFFECTS ON LOW VOLTAGE-ACTIVATED T CURRENTS AND ALLEVIATION OF ACTIONS BY ALBUMIN. S. Huck*, I. Steffan, M. Razavi and S. Boehm. Departments of Neuropharmacology and Analytical Chemistry, University of Vienna, A-1090 Vienna, Austria.

Interference of zinc with various ligand- and voltage-gated ion channels has attracted considerable attention in the recent past. We now report effects of zinc on the low voltage-activated (LVA) component which significantly exceeded the inhibition of high voltage-activated (HVA) Ca^{2+} currents. The effects of zinc were attenuated by albumin in a concentration-dependent manner.

Whole cell Ca^{2+} currents were recorded in embryonic chick sensory neurons using the patch clamp technique. LVA Ca^{2+} currents were dissected from HVA components by step depolarizations to -30mV from a holding potential of -80mV and/or pretreatment of cells with ω-conotoxin GVIA. Zinc, applied in concentrations ranging from 0.8μM to 800μM readily and reversibly inhibited the LVA current with an IC_{50} of 30μM. HVA components, induced by test pulses to +20mV, were inhibited by only 7% at a concentration of 50μM Zn^{2+}.

Effects of 100μM Zn^{2+} on LVA Ca^{2+} currents were antagonized by bovine serum albumin (BSA) in a concentration-dependent manner. These experiments were conducted in order to reevaluate our previous observation that BSA selectively enhanced the LVA Ca^{2+} component (Huck et al., Soc. Neurosci. Abstr. Vol. 17, p.1158, 1991) and after having detected Zn^{2+} as a major contaminant in our previous perfusion system.

The attenuation of Zn^{2+} effects is explained by its strong binding to albumin. We have measured free Zn^{++} using inductively-coupled plasma atomic emission spectrometry (ICP-AES) in a quasi steady-state ultrafiltration assay with a Centriprep 30 (Amicon, M_r-cutoff 30kDa) concentrator. In the presence of 2mg/ml BSA, Zn^{2+} binding showed little saturation with concentrations ranging from 6.25μM to 200μM. High concentrations of Zn^{2+} (≥1mM) caused apparent denaturation of BSA. More than 95% were bound when 50μM zinc were incubated with 2mg/ml BSA.

Since Zn^{2+} is released at synaptic sites in the hippocampus, the effects on LVA Ca^{2+} currents may add to its range of physiological actions previously described. The binding of zinc (and other divalent cations) by albumin must be taken into consideration when albumin effects are observed in *in vitro* assay systems.

720.17

THE EFFECTS OF VERAPAMIL AND EMOPAMIL QUATERNARY AMMONIUM SALTS ON EVOKED NEURONAL CALCIUM INFLUX AND ^{3}H-D-ASPARTATE RELEASE. R. A. Keith*, T. J. Mangano, P. A. DeFeo, G. E. Ernst and E. J. Warawa. Depts. of Pharmacology and Medicinal Chemistry, Zeneca Pharmaceuticals Group., Wilmington, DE 19897.

Verapamil and emopamil are structurally related calcium channel/5-HT_2 receptor antagonists that differ in their ability to provide neuroprotection in experimental studies. The quaternary ammonium salts (QAS) of these compounds were prepared and tested in assays of neuronal calcium channel function to determine whether the compounds act at intra- or extracellular sites. The compounds were tested in K^+-evoked: 1) rat brain synaptosomal $^{45}Ca^{++}$ influx, 2) release of ^{3}H-D-aspartate from rat hippocampal brain slices, and 3) increase in intracellular calcium in neocortical neurons in primary culture. Verapamil, emopamil and the emopamil QAS caused concentration-dependent and comparable (IC_{50} values ~ 30-40 uM) inhibition of synaptosomal $^{45}Ca^{++}$ influx and ^{3}H-D-aspartate release. The verapamil QAS was considerably less active in these assays (IC_{50} > 100 uM). The evoked increase of intracellular calcium in neocortical neurons was inhibited with the following rank order of potency (approximate IC_{50} value, uM): emopamil (5) > verapamil (15) > emopamil QAS (30) > verapamil QAS (>100). The results suggest that verapamil and emopamil inhibit nerve terminal calcium channel function (synaptosomal $^{45}Ca^{++}$ influx and ^{3}H-D-aspartate release) by acting at distinct intracellular and extracellular sites, respectively. Verapamil may inhibit cell body calcium influx (evoked increase of intracellular calcium in neocortical neurons) by acting predominantly at intracellular sites, whereas emopamil may act at both intra- and extracellular sites.

720.18

DUAL Na^+/Ca^{2+} CHANNEL BLOCKERS INHIBIT ^{3}H-NOREPINEPHRINE (^{3}H-NE) RELEASE EVOKED BY K^+-DEPOLARIZATION BUT NOT BY ELECTRICAL FIELD STIMULATION IN RAT NEOCORTICAL SLICES. J.J. Geer*, S. Wurster and D.J. Dooley. Dept. of Neuroscience, Parke-Davis Pharmaceutical Research, Division of Warner-Lambert Co., Ann Arbor, MI 48106.

Both Na^+ and Ca^{2+} channels are important for synaptic neurotransmitter release. Na^+ channels propagate action potentials which activate voltage-sensitive Ca^{2+} channels in nerve endings, triggering exocytosis. In vitro, neurotransmitter release can be evoked by several methods. Electrical field stimulation closely mimics action potentials in time resolution and channel involvement. K^+-depolarization clamps the membrane potential for an extended period of time and only partially depends on Na^+ channels. Using both stimulation protocols, we examined the effects of several lipophilic dual Na^+/Ca^{2+} channel blockers on ^{3}H-NE release. Rat neocortical slices preloaded with ^{3}H-NE were stimulated either electrically with 90 pulses at 3 Hz or by increasing [K^+] in the superfusion buffer from 3 mM to 25 mM for 2 min. The superfusion buffer contained the NE re-uptake blocker (+)-oxaprotiline (1μM) and the α_2-antagonist idazoxan (1μM) to prevent autoinhibition. The amount of ^{3}H-NE released by each stimulus was comparable, averaging 12% of tissue tritium. Lidoflazine (10μM), R 58735 (10μM), RS 87476 (3μM), and flunarizine (10μM), added to the superfusion buffer 30 min before the stimulation, did not affect electrically-evoked ^{3}H-NE release, but inhibited K^+-stimulated release by 45-65%. Furthermore, inhibition of K^+-evoked ^{3}H-NE release was concentration dependent.

A possible explanation for the stimulus-dependent effects of these drugs on ^{3}H-NE release could be that they preferentially affect a Ca^{2+} channel state that is accessible only during the long-lasting membrane depolarization of a K^+-stimulation but not during the shorter lifetimes of channel states during an electrical stimulation.

720.19

MULTIPLE COMPONENTS OF VOLTAGE-GATED CALCIUM CHANNEL CURRENTS IN ACUTELY ISOLATED GRANULE CELLS FROM THE RAT HIPPOCAMPUS. F.Viana* and B. Hille. Univ. of Washington, Dept. of Physiology & Biophysics, Seattle, WA 98195.

In the hippocampus, induction of some forms of LTP is calcium-dependent but independent of NMDA receptor activation suggesting a possible role for voltage-gated Ca^{2+} channels in the modulation of synaptic transmission. We used the whole-cell configuration of the patch-clamp technique to investigate properties of calcium channels in the soma and proximal dendrites of acutely isolated rat granule cells. From -90 mV, depolarizations to -30 mV revealed a small (<100 pA) inward current that inactivated rapidly and almost completely within 100 ms. From the same potential, steps to 0 mV activated a larger (200-1000 pA) inward current. This high-voltage activated (HVA) current peaked near -10 mV (5 mM Ba^{2+}) and had transient and sustained components with different steady-state inactivation properties. All of the HVA current was highly sensitive to Cd^{2+} (50% block at ~ 1μM), compared with ~ 20 μM for the LVA current. The dihydropyridine Ca^{2+} channel agonist (+)-S-202-791 enhanced the peak HVA current and slowed the inward tail current at -40 mV. These effects were readily reversed by application of nifedipine (NIF), implicating L-type channels. 10 μM NIF inhibited the peak current by 32±4% (mean±SE). Application of the N-type channel blocker ω-conotoxin GVIA (CTX) caused a further 15±1% reduction of the barium current. In addition, a large fraction (~50%) of the HVA current was blocked irreversibly by application of 500 nM synthetic ω-agatoxin IVA (AGA), suggesting the presence of P-type channels. The combined application of NIF, AGA and CTX spared ~20% of the peak HVA current. In contrast, the low-threshold current was insensitive to all three agents.

In summary, we present evidence for multiple pharmacological components of transient and sustained barium currents in granule cells. (Supported by USPHS grant #NS08174 and the W.M.Keck and McKnight Foundations.)

720.20

EFFECTS OF Ca^{2+} CHANNEL BLOCKERS ON THE K^+- or 4-AMINOPYRIDINE EVOKED $|Ca^{2+}|_i$ INCREASE AND DOPAMINE RELEASE IN RAT STRIATUM SYNAPTOSOMES. C.M. Carvalho, C.B. Duarte, I.L. Ferreira and A.P. Carvalho*. Center for Neurosciences of Coimbra, Dep. of Zoology, Univ. of Coimbra, 3049 Coimbra Codex, Portugal

Depolarization of striatum synaptosomes with 20 mM KCl transiently increases the $|Ca^{2+}|_i$ by about 300 nM, and evokes the release of 18.8±1.4% of the total $|^3H|$dopamine ($|^3H|DA$). 4-aminopyridine (4-AP) at 1 mM rises the $|Ca^{2+}|_i$ by about 200 nM and releases 20.4±3.4% of the total $|^3H|DA$. Nitrendipine (1 μM) decreases the change in $|Ca^{2+}|_i$ evoked by K^+ and 4-AP by, respectively, 7% and 13%, whereas it decreases $|^3H|DA$ release by 20% and 23%, respectively. w-Conotoxin GVIA (w-CgTx) at 0.5 μM more effectively blocked the effect of 4-AP on the $|Ca^{2+}|_i$ than on the release of $|^3H|DA$. Neomycin (0.35 mM) blocked by, respectively, 32% and 50% the $|Ca^{2+}|_i$ increase stimulated by K^+ or 4-AP depolarization. Similarly, neomycin inhibits potently the release of $|^3H|DA$ evoked by the two agents. Neomycin and w-CgTx together further inhibited the effects of K^+ and 4-AP on the $|Ca^{2+}|_i$ and $|^3H|DA$ release. Our results indicate that striatal synaptosomes are endowed with w-CgTx-sensitive (N-type) and nitrendipine-sensitive (L-type) calcium channels, both coupled to the release of $|^3H|DA$. The neomycin-sensitive and w-CgTx- and nitrendipine-insensitive channels may be P-type channels (Supported by INIC and JNICT).

CALCIUM CHANNEL PHARMACOLOGY AND MODULATION IV

721.1

BIOLOGICAL CHARACTERIZATION OF A P-TYPE CALCIUM CHANNEL BLOCKER, ω-Aga-K, FROM *AGELENOPSIS APERTA* SPIDER VENOM. L.D. Hirning*[1], A.L. Mueller[1], N. Alasti[1], L.D. Artman[1], E.G. Delmar[1], E.E. Nemeth[1], T.N. Parks[2], W.R. Gray[2], B.C. Albensi[2] and H. Jackson[1]. [1]NPS Pharmaceuticals, 420 Chipeta Way, Salt Lake City, UT 84108 and [2]University of Utah, Salt Lake City, UT 84132.

The venom of the funnel-web spider *Agelenopsis aperta* contains a number of peptides which block voltage-sensitive Ca^{2+} channels (VSCC) (*Ann. Rev. Neurosci.* 12:405, 1989). One such peptide was shown previously to block synaptic transmission in the chick cochlear nucleus by a presynaptic mechanism involving VSCC blockade (*Soc. Nsci. Abstr.* 12:730, 1986). The amino acid sequence of this peptide, ω-Agatoxin-K (ω-Aga-K), was determined in 1986 (48 amino acid residues, 4 disulfide bonds, 5.3 kD) and disclosed in U.S. Patent #5,122,596. Recently, ω-Aga-K was identified independently by M. Adams as ω-Aga-IVB.

P-type Ca^{2+} channel current recorded in cerebellar Purkinje cells is blocked potently (IC_{50} = 30 nM) by ω-Aga-K. N-type and L-type VSCC in a variety of cell types (N1E-115 cells, cerebellar granule neurons, sympathetic neurons) are less sensitive to ω-Aga-K. ω-Aga-K blocks DHP/ω-CgTx-resistant cerebellar Ca^{2+} spikes, but is less effective against hippocampal Ca^{2+} spikes. ω-Aga-K is a more efficaceous blocker of glutamatergic EPSPs (83% block, IC_{50} = 252 nM) than monosynaptic GABAergic IPSPs (46% block, IC_{50} = 199 nM) in rat hippocampal slices; ω-CgTx displays the reverse efficacy. ω-Aga-K inhibits the Ca^{2+}-dependent release of tritiated neurotransmitters, being more effective than ω-CgTx at blocking the release of DA from the striatum (55% block, IC_{50} ~ 100 nM), and GABA from the striatum (100% block, IC_{50} ~ 100 nM) or hippocampus (45% block, IC_{50} ~ 100 nM). The release of ACh from either striatum or hippocampus is relatively unaffected by ω-Aga-K. These data together identify ω-Aga-K as a unique probe with which to identify and characterize VSCC in the mammalian CNS.

721.2

TWO NEW *AGELENOPSIS* SPIDER PEPTIDE P-TYPE CALCIUM CHANNEL BLOCKERS ARE FOLDING ISOMERS. A.H. Ganong*, N.A. Saccomano, L.D. Hirning†, A.L. Mueller†, C.J. Siok, D. Phillips, S.D. Heck, M.K. Ahlijanian, and R. A. Volkmann. Pfizer Inc, Central Research Division, Groton, CT 06340 and †NPS Pharmaceuticals, Salt Lake City, UT 84108.

The venom from the funnel web spider *Agelenopsis aperta* has been a rich source of neuroactive peptides, including ω-Aga-IVA which is a selective P-channel blocker (Mintz *et al.*, *Nature*, 1992, **355**, 827; Hirning *et al.*, *Neurosci. Abstr.*, 1992, **18**, 970). We report here two new P-channel antagonist peptides from *Agelenopsis* venom. These 48 amino acid peptides, termed ω-Aga-K and ω-Aga-K2, have identical amino acid sequences, but are chromatographically distinct on RP-HPLC. Their primary structure (including 8 cysteines) is similar to that of ω-Aga-IVA. The current data suggest that ω-Aga-K and ω-Aga-K2 can interconvert, and, therefore, may differ in disulfide bonding pattern.

ω-Aga-K and ω-Aga-K2 are potent (IC_{50} < 100 nM) and fully efficacious blockers of P-channels as evidenced by their inhibition of barium currents in whole-cell recordings from rat Purkinje neurons. These peptides do not block ω-conotoxin GVIA-sensitive barium currents in IMR32 cells. ω-Aga-K does not block fast Na^+ currents or TEA-sensitive K^+ currents recorded in IMR32 cells at 10 μM.

Both peptides inhibit ω-conotoxin GVIA/nifedipine-resistant $^{45}Ca^{2+}$ uptake into rat brain synaptosomes and show efficacious inhibition of synaptic transmission of the Schaffer-CA1 synapse in rat hippocampal slices. These new peptides will be useful in defining the functional role of P-channels in central neurons. (see Hirning *et al.*, this volume.)

721.3

PRODUCTION AND BIOLOGICAL CHARACTERIZATION OF SYNTHETIC ω-AgaIVA. M.K. Ahlijanian*, G. Andrews, B. Guarino, N.A. Saccomano, L.D.Hirning#, A.L. Mueller#, N. Alasti# C.J. Siok, M.J. Welch, A.H. Ganong, R.A. Volkmann. Pfizer Inc.,Central Research Division, Groton, CT 06340 and #NPS Pharmaceuticals, Salt Lake City, UT 84108.

ω-AgaIVA, a 48-amino acid peptide toxin isolated from the spider *Agelenopsis aperta*, selectively blocks P-type voltage-dependent calcium channels. Using solid phase methods, we synthesized ωAga-IVA and characterized its calcium channel antagonist activity in a variety of functional assays. In rat cerebellar Purkinje cells, synthetic (s) ωAga-IVA inhibits whole cell calcium current by greater than 90% at a concentration of 100 nM. The IC50 is 5 nM and relief of block was voltage-dependent. In rat brain synaptosomes, sωAga-IVA inhibits up to 85% of potassium-stimulated ^{45}Ca flux with an IC50 of 30 nM. In the latter two assays, ω-conotoxin GVIA (CgTx) (1 μM) and nimodipine (10 μM) are only marginally effective. sωAga-IVA inhibits calcium-dependent ^3H-GABA release from rat striatal and hippocampal slices. sωAga-IVA (3 μM) also completely blocks Schaeffer-CA1 field potentials. CgTx maximally blocks this response by 39% at a concentration of 3 μM. In no experiment could the actions of sωAga-IVA be distinguished from those of native ωAga-IVA. The biological activity of sωAga-IVA unequivocally identifies this molecule as a selective antagonist of P-type calcium channels. sωAga-IVA will serve as an important tool for probing the function of P-type calcium channels.

721.4

P-LIKE Ca^{2+} CHANNELS IN HUMAN CEREBELLUM AND SMALL CELL LUNG CARCINOMA BIND ω-CONOTOXIN-MVIIC AND ARE IMMUNOPRECIPITABLE BY LAMBERT-EATON MYASTHENIC SYNDROME (LES) IgGs THAT REACT HIGHLY WITH N-TYPE Ca^{2+} CHANNELS. VA Lennon, T Kryzer, GE Griesmann, NA Pinsky, G Miljanich†, J Ramachandran†, EH Lambert§*. Mayo Clinic, Rochester, MN 55905; †Neurex Corp., Menlo Park, CA 94025; §University of Minnesota, Minneapolis, MN 55455.

RNA transcripts encoding a human neuronal class A (P-like) voltage-gated Ca^{2+} channel (VGCC) were recently identified in a small cell lung carcinoma (SCLC) cell line (*Mayo Clin Proc* 67:1150, 1992). This suggested that a tumor-associated P-like VGCC might be the antigen that induces certain autoimmune paraneoplastic neurologic disorders in patients with SCLC. We therefore investigated in 3 SCLC lines (2 from patients with neurologic autoimmunity, 1 without) the effect of the synthetic P-like VGCC antagonist ω-CTx-MVIIC (SNX-230;*Neuron* 9:69, 1992) on depolarization-dependent $^{45}Ca^{2+}$ influx. All 3 were inhibited, to a maximum of 73±4.5% (mean±SE) compared with 23±4.7% for ω-CTx-GVIA (Peninsula Labs, at $1x10^{-4}$M). The IC50 for MVIIC inhibition of Ca^{2+} influx was ~$1x10^{-7}$M. A specific binding site for ^{125}I-labelled MVIIC was found in digitonin-solubilized membranes of SCLC (~2 fmol/mg protein) and cerebellum (~1300 fmol/mg). Solubilized cerebellar MVIIC receptors (Kd ~300 pM) were immunoprecipitated by IgG from 0 of 7 patients with SCLC-related autoimmune CNS disorders and 0 of 5 normal subjects, but were precipitated by IgG in 3 of 4 LES patients' sera that were highly reactive with GVIA receptors. The data suggest that neuronal P-like and N-type VGCC may be related antigenically. (Supported by *NCI* grant *CA-37343*).

721.5

CALCIUM CHANNEL SENSITIVITY TO *AGELENOPSIS APERTA* VENOM MEASURED BY STOPPED-FLOW FLUORIMETRY IN RAT BRAIN SYNAPTOSOMES. M.M.Thomas* and S.M.J.Dunn Dept. of Pharmacology, U. of Alberta, Edmonton, Canada T6G 2H7

The sensitivity of voltage-dependent calcium channels to crude venom and isolated venom components from the funnel web spider, *Agelenopsis aperta*, has been measured in synaptosomal preparations from rat brain. Synaptosomes were loaded with the calcium chelating dye, Fura 2-AM and depolarization-dependent changes in intra-synaptosomal free calcium concentration were measured in stopped-flow experiments by following the fluorescence quench of the entrapped dye. Rapid mixing with a high potassium ($30mM_{final}$) buffer resulted in an increase in intrasynaptosomal calcium concentration. Crude spider venom blocked 50% of the observed response. The dose dependency was apparently biphasic with IC_{50} values of 100ng/mL and 16μg/mL. FTX, a polyamine fraction from the venom effectively blocked 40% of the quench (IC_{50}=4nL/mL) and synthetic FTX blocked the response at millimolar concentrations. ω-Aga IVA, a polypeptide toxin purified from *A. aperta* blocked the effect at micromolar concentrations. In the same preparations, dihydropyridine antagonists and ω-conotoxin GVIA had little effect on the observed responses. Calcium channels in mammmalian nerve terminals are thus sensitive to spider venom components but not other calcium channel ligands. Supported by AHFMR and Toupin Foundation.

721.6

FTX SELECTIVELY BLOCKS P-TYPE CALCIUM CURRENT IN HIPPOCAMPAL AND OLIVARY NEURONS.

A. Mantridi,* S. Charpak, B. Cherksey, M. Sugimori and R. Llinas. Department of Physiology & Biophysics, New York University Medical Center 10016 N.Y.

Funnel web spider toxin (FTX) blocks the high-threshold calcium spikes in adult cerebellar Purkinje cells by acting on P-type calcium channels (Llinas et al. PNAS 86, 1689,1989). These channels cannot be distinguished from N- and L-types channels on the basis of their single-channel conductances (Usowicz et al. Neuron 9, 1185, 1992). Since FTX could in principle interact with more than one channel subtype, we addressed the issue of its selectivity in inferior olive neurons and in hippocampal dentate gyrus granule cells, by performing whole-cell recordings in adult guinea pig brain slices.Olivary neurons exhibit a low-voltage transient T-current and a slowly inactivating high-voltage current that underlie, respectively, the low-threshold and high-threshold calcium spikes. Purified FTX did not affect the former while it almost entirely blocked the latter. L- and N-type channel blockers (nifedipine 20 μM, ω-conotoxin GVIA 4 μM) were ineffective. In contrast, the P-type current contributed in the range of 40% of the high-voltage current in hippocampal granule neurons, the remaining being blocked by ω-conotoxin (~ 15%) and nifedipine (~ 40%). Our results suggest that P-channels are differently distributed in the CNS and that they can be selectively discriminated on the basis of their sensitivity to FTX.

721.7

FTX-INDUCED P-TYPE CALCIUM CHANNEL BLOCKADE (P-CCB) INHIBITS SYNAPTIC TRANSMISSION IN THE CANINE STELLATE GANGLION (SG). HK Schedewie, N Boban and JP Kampine.* Department of Anesthesia, Medical College of Wisconsin, Milwaukee, WI 53226

We have previously shown that N-type calcium channel blockade (N-CCB) inhibits synaptic transmission in the SG by about 50%, whereas L-type CCB had insignificant effects. The present study was designed to investigate the *in vitro* effects on synaptic transmission of FTX spider toxin (courtesy Dr. Llinas), the prototype of a new class of potent CCB that inhibit high-threshold voltage-gated P-channel calcium conductance. Six SG were isolated from adult mongrel dogs, desheathed and superfused with Krebs' buffer at pH 7.4 and 37°C. Compound action potentials (CAP) were generated by supramaximal stimulation of the preganglionic T_3-ramus and were recorded from the ventral *ansa subclaviae*. The percent CAP change from control was measured after SG superfusion for 1 hour, each, with dilutions of 2, 4 and 6 x 10^{-5} of partially purified FTX. Superfusate Ca^{2+} concentrations were then doubled from 1.25 to 2.5 mM and finally SG were exposed to dexmedetomidine (DMT) and atipamezole (ATI) to assess the effect of combined P- and N-type CCB. Results: 1. P-CCB caused profound inhibition of synaptic transmission in the SG in a dose-dependent fashion ($p < 0.01$); 2. Increasing extracellular calcium concentrations were only partially able to overcome P-CCB; 3. DMT-induced N-CCB was additive with P-CCB ($p < 0.01$); 4. ATI failed to reverse the effects of DMT, suggesting possible interference by FTX at the N-type calcium channel. Conclusion: P-type channel calcium transport seems to play an essential role in the process of synaptic transmission in the peripheral sympathetic nervous system. Mode and site of action appear to be distinct from N-channel calcium conductance.

721.8

N Channel Inhibitory Activity of the Polyamine Funnel Web Spider Toxin, FTX, synthetic FTX and Spermine. M. G. Hamilton, R. Frew and P. M. Lundy* Pharmacology and Therapeutics, Defence Research Establishment Suffield, Box 4000, Medicine Hat, AB T1A 8K6

The P channel inhibitor isolated from the funnel-web spider *Agelenopsis aperta*, known as funnel-web toxin (FTX), its synthetic analogue sFTX, and their structural analogue spermine were examined for effects on K^+ stimulated Ca^{2+} influx and ωCgTx binding. In chicken brain synaptosomes, which contain exclusively N type VSCCs, FTX (1 μL/mL), sFTX (1-5 mM) and spermine (1-5 mM) all caused concentration dependant inhibition of K^+ stimulated Ca^{2+} influx. The N channel blocker ωCgTx (1 μM) reduced influx by 97%, while Cd^{2+}, very dilute *Hololena curta* venom and a partially purified fraction from this venom, all also markedly reduced K^+ stimulated Ca^{2+} influx. In comparable studies using rat brain synaptosomes, spermine and sFTX reduced Ca^{2+} influx in a concentration related fashion while FTX (1μL/ml) totally abolished influx. Maximal inhibition of influx by ωCgTx was 25% while *Hololena curta* venom (1:2,500 dilution) and Cd^{2+} (50uM) reduced influx by >80%. Spermine and sFTX both inhibited [^{125}I]ωCgTx binding to rat and chicken synaptosomal membranes. These results strongly suggest that sFTX and FTX in addition to their blocking activity at the P channel, have major inhibitory effects on pharmacologically defined N type Ca^{2+} channels.

721.9

PHARMACOLOGICAL PROPERTIES AND LOCALIZATION OF CALCIUM CHANNEL TYPES IN THE CEREBELLAR CORTEX. P. A. Doroshenko, A. Woppmann, G. Miljanich, G.J. Augustine*. Dept. Neurobiol., Duke Univ. Medical Center, NC 27710 and Neurex Corp., Menlo Park, CA 94025.

We have used whole-cell patch clamp recordings from Purkinje cells in thin slices of cerebellum to examine the ability of a variety of synthetic peptide toxins to block Ca channels. We first screened the activity of three peptide toxins: (1) SNX-111, (MVIIA) which blocks N-type Ca channels with higher affinity than the "classical" conotoxin (GVIA); (2) SNX-230 (MVIIC), which blocks a class of Ca channels that are not sensitive to blockers of N- or L-type Ca channels (Neuron 9: 69, 1992); and (3) SNX-260, an analog of SNX-230 which exhibits higher affinity and specificity for SNX-230-sensitive channels. Excitatory PSCs evoked by stimulation of either climbing fiber (CF) or parallel fiber (PF) inputs, as well as Purkinje cell Ca currents, were blocked by SNX-230 and SNX-260 (1-10 μM), but not SNX-111. Interestingly, inhibitory PSCs spontaneously produced by electrical activity of interneurons were not eliminated by any of these compounds. We next used confocal laser-scanning microscopy to visualize the binding of a biotinylated derivative of SNX-260 associated with streptavidin-Texas Red. Punctate high-affinity toxin labeling was found throughout the molecular layer and may correspond to the presynaptic terminals of the parallel fibers. Other high-affinity sites were found in the granule cell layer, perhaps staining presynaptic terminals that innervate granule cells. Lower affinity binding sites were visualized on Purkinje cell dendrites, where SNX-260-sensitive Ca channels are known to reside, as well as on the cell bodies of granule cells. We conclude that the Purkinje cell Ca channels cannot as yet be distinguished from those of the PF or CF presynaptic terminals. However, the excitatory and inhibitory presynaptic terminals of the cerebellar cortex clearly possess different types of voltage-gated Ca channels. This presumably imparts different regulatory properties on transmission at the two types of synapses. Partially supported by a HFSP grant to GJA.

721.10

RADIOAUTOGRAPHIC COMPARISON OF THE DISTRIBUTION OF HIGH AND LOW AFFINITY BINDING SITES FOR [^{125}I]ω-CONOTOXIN GVIA IN RAT BRAIN: INFLUENCE OF CALCIUM. S. Vandaele*(1) and T. Reader(2), (1)Unité de neurocytologie moléculaire, Département de pathologie, and (2)Département de physiologie, Université de Montréal, Montréal, Québec, Canada.

The N-type calcium channel ligand [^{125}I]ω-Conotoxin GVIA (*ω–CgTx) was used at low (30 pM) and high concentrations (300-500 pM) to compare in rat brain the distribution of high and low affinity binding sites (apparent K_d in the 15 pM and 500 pM range respectively). Sections were incubated with *ω-CgTx (2000 Ci/mmol) according to Maeda et al. (*Brain Res.. 489:21*, 1989) in the absence or in the presence of Ca^{++}. Without Ca^{++}, the high affinity binding sites appeared uniformly distributed throughout brain, except for higher densities in the granular and pyramidal layers of hippocampus, the layer II of piriform cortex, the layer IV of cerebral cortex, and the granular layer of cerebellum. The low affinity binding sites were similarly distributed, except that the molecular layer of cerebellum was now more densely labeled than the granular layer. The presence of 30mM Ca^{++} greatly diminished *ω-CgTx binding in most brain regions, and lead to identical distribution patterns for low and high affinity sites. Some regions were more densely labeled than others. The highest density of *ω-CgTx binding sites was found in cerebral cortex, basal ganglia, neuropil layers of hippocampus, substantia nigra, and the molecular layer of cerebellum, while lower densities of *ω-CgTx binding sites were found in the other brain regions. Almost no labeling was found in densely cellular layers, such as the granular and pyramidal layers of hippocampus, the layer II of piriform cortex, the Purkinje cell layer of cerebellum. These results are consistent with the existence of several families of ω-CgTx binding sites in the central nervous system, some of which may be characterized by their sensitivity to Ca^{++}. If associated to presynaptic calcium channels, these Ca^{++}-sensitive *ω-CgTx binding sites could be preferentially located on inhibitory terminals contacting nerve cell bodies. *Supported by the Savoy Foundation (S.V.)*

721.11

N-TYPE (CLASS B) NEURONAL Ca^{2+} CHANNELS EXPRESSED IN SMALL CELL LUNG CARCINOMAS OF PATIENTS WITH AND WITHOUT LAMBERT-EATON MYASTHENIC SYNDROME (LES). M Oguro-Okano, GE Griesmann, T Kryzer, ED Wieben†, VA Lennon*. Depts. of Immunology, Neurology, Laboratory Medicine & Pathology, and †Biochemistry & Molecular Biology, Mayo Clinic, Rochester, MN 55905.

We previously reported cloning from a prototypic small cell lung carcinoma (SCLC) line two cDNA fragments encoding a P-type (class A) voltage-gated Ca^{2+} channel (VGCC) and a neuroendocrine form (class D) of L-type VGCC (*Mayo Clin Proc* 67:1150, 1992). By reverse transcription of RNA and PCR amplification of cDNA, we have now identified transcripts for the ω-conotoxin-GVIA-sensitive class B VGCC in all of 9 SCLC cell lines, 4 derived from patients with the paraneoplastic neuromuscular disorder LES, and 5 from patients without LES. Oligonucleotide primers encompassing the class B α_1 subunit sequence encoding domain II, S2 through S5, yielded a PCR product of 488 base pairs. Sequencing of several clones revealed a cDNA fragment predicted to encode a VGCC of N-type. No PCR product was obtained using RNA from a colonic carcinoma line derived from a patient with LES, nor using RNA from a human B lymphoma. A protein in digitonin-solubilized SCLC membranes bound ^{125}I-ω-CgTx-GVIA with high affinity (Kd = 3 pM), and was immunoprecipitable by LES IgG. Production of pathogenic autoantibodies that impair the stimulated release of neurotransmitter in peripheral cholinergic synapses presumably reflects a genetically determined immune response initiated by an immunogenic form of class B VGCC expressed in SCLC. (Supported by *National Cancer Institute* grant *CA-37343*).

721.12

PHARMACOLOGICAL PROPERTIES OF LOW-VOLTAGE-ACTIVATED CA CHANNELS IN UNDIFFERENTIATED HUMAN RETINOBLASTOMA CELLS. A. Juhasz, L.W. Haynes* and S. Barnes, Neuroscience Research Group, University of Calgary, Alberta, Canada T2N 4N1.

Undifferentiated human retinoblastoma cells (Y-79 and WERI) produce oscillations and action potentials under current clamp due to transient Ca channel activation. These channels appear similar to other T-type channels on the basis of gating kinetics and single channel conductance (Barnes & Haynes, *Brain Res.* 598:19-22), but they have been considered unique due to both a reported requirement for Ca to gate and a relativley high sodium/calcium permeability ratio (Gomez et al., *Invest. Ophthalmol. Vis Sci.* 29:243, 1988). The aim of the present work was to characterize these channels pharmacologically to allow further comparison with existing Ca channel descriptions.

In contrast to the previous study (which used TMA), equimolar replacement of sodium with N-methyl-D-glucamine in the bathing medium had no effect on the magnitude of macroscopic barium current. Furthermore, in the presence of 10 mM barium, addition of extracellular calcium at 0, 0.1, 1 and 5 mM had no effect on channel gating, but did reduce the transient current in a manner consistent with the mole fraction effect. Comparative blocking efficacies of cadmium ($K_{½}$ = 110 ± 26 μM, n=5, SE) and nickel ($K_{½}$ = 75 ± 14 μM, n=6) are in the range expected for T-type Ca channels. Nifedipine (10 μM) had almost no effect on the transient barium current, but completely (n=3) or partially (n=1) blocked it at 100 μM. *w*-conotoxin GVIA (3 μM) had no effect, ruling out characterization as N-type, but the failure of ethosuximide (5 mM) to block and the weak block (5%) by amiloride (100 μM) suggest that this retinoblastoma Ca channel falls loosely within a T-type classification.

Supported by the Medical Research Council of Canada and the Alberta Heritage Foundation for Medical Research.

721.13

ω-Conotoxin-Sensitive and Insensitive Calcium Channel Subtypes in the Soma and Nerve Terminals of a Mammalian Central Neuron. T.E. Fisher* and C.W. Bourque. *Centre for Research in Neuroscience, Montreal General Hospital and McGill University, Montreal, Canada, H3G 1A4.*

Calcium channel subtypes are likely to be selectively distributed within the neuron in order to execute specific functions. We have therefore undertaken a comparative study of calcium channels in isolated somata and nerve endings of the magnocellular neurosecretory cells (MNCs) of the rat supraoptic nucleus. Standard whole-cell patch clamp techniques have shown previously that while only the somata express a "T-like" calcium current (τ≈30 ms), both preparations express a more slowly inactivating calcium current component(s) (τ≈200 ms). In the present work we have used the "N-type" specific blocker ω-conotoxin GVIA (ω-CgTX) to further characterize this slowly inactivating current in the two preparations. In the terminals, ω-CgTX blocks over 80% of the total inactivating current with a IC 50 of about 50 nM. In the somata however, μM concentrations of ω-CgTX block only about 35% of the slowly inactivating current. These data suggest that there is a distinct component of slowly inactivating calcium current that is insensitive to block by ω-CgTX and that is expressed predominantly in the somata of the MNCs.

Supported by FCAR, the Heart and Stroke Foundation, and the MRC of Canada.

721.14

CHARACTERIZATION OF ω-CONOTOXIN RECEPTOR / N-TYPE CALCIUM CHANNEL IN IMR32 AND PC12 CELLS H. Liu, D. R. Witcher, M. Pragnell, J. D. Coulter*, K. P. Campbell HHMI, Neuroscience Program and Dept. of Physiology and Biophysics, University of Iowa College of Medicine, Iowa City, IA 52242

In order to characterize calcium channels in human neuroblastoma IMR32 cells and rat pheochromocytoma PC12 cells, various antibodies were tested for their ability to recognize ω-conotoxin (ω-CgTX) receptor / N-type calcium channel from these two cell lines. Both cell lines express the ω-CgTX receptor (Kd=0.8 +/- 0.2 nM, Bmax ~500 fmol/mg for PC12 and Bmax=240 +/- 70 fmol/mg for IMR32) as shown by ^{125}-I ω-CgTX binding. A monoclonal antibody $VD2_1$ which is directed against a common region on all β subunits and polyclonal antibody sheep-46 which recognizes the rabbit brain ω-CgTX receptor complex, immuno-precipitate ω-CgTX receptor from both cell lines as well as from rabbit brain. This suggests that N-type calcium channel has epitopes in human and rat which are similar to that of rabbit brain. Polyclonal antibodies affinity-purified from a rabbit brain β C-terminal fusion protein which is specific to the rabbit brain β subunit of ω-CgTX receptor, also immunoprecipitates ω-CgTX receptor from these two cell lines as well as from the rabbit brain, suggesting that the receptor in all three species may be the same β gene product in the ω-CgTX receptor. Polyclonal antibodies affinity-purified from fusion proteins, one recognizing rabbit brain α_{1B} specific loop region between the second and third repeats of α_1 subunit, the second recognizing α_{1B} specific C-terminal region, immunoprecipitate ω-CgTX receptor from these two cell lines as well as from rabbit brain, suggesting that ω-CgTX receptor in all three species consist of an α_{1B} subunit as well as a β subunit. We are currently studying biosynthesis, phosphorylation regulation and subunit composition of calcium channels in these cell lines.

721.15

PHARMACOLOGY OF WHOLE-CELL CALCIUM CURRENTS IN HIPPOCAMPAL DENTATE GRANULE NEURONS. *L.S. Eliot* and D. Johnston*, Div. Neuroscience, Baylor Coll. Medicine, Houston, TX 77030

Hippocampal granule cells contain at least 3 different types of voltage-gated Ca^{2+} channels (Fisher et al., *J. Neurophys.* 64:91), raising the possibility that distinct channel types underlie different Ca^{2+}-dependent functions of the neuron, particularly, synaptic transmission from their mossy fiber terminals. As a first step in testing this hypothesis, we are identifying antagonists for the various channel types using whole-cell voltage clamp in acutely dissociated granule cells. Whole-cell Ba^{2+} currents activate around −30 mV, peak at +5 to +10 mV, reverse around +50 mV, and are completely blocked by 200 μM Cd^{2+} when elicited either with step or ramp (2.2 mV/ms) commands from −70 mV. The dihydropyridine antagonist, nimodipine (5 μM), blocks just under 40% of peak Ba^{2+} current whether elicited by steps (39.0 ± 3.1%, n=5) or ramps (38.1% ± 3.9%, n=11). Nimodipine blocks in a dose as low as as 100 nM (20.3 ± 0.3%, n=3) and saturates between 2 μM and 5 μM, with 10 μM blocking 39.5 ± 1.4% of peak current (n=3). The snail toxin, ω-conotoxin GVIA (CgTX, 3-6 μM), blocks 22.7 ± 2.7% of peak current (n=7). CgTX is effective at lower doses, but 3 μM acts rapidly and appears saturating since subsequent application of 6 μM produces no additional block. Applied together, saturating doses of nimodipine and CgTX block 58.9 ± 1.1% of peak current (n=5), indicating that the two antagonists do not occlude each other and that they act specifically in granule cells at L-type and N-type channels, respectively. Both blockers inhibit larger fractions of total current from −40 mV holding potentials, suggesting that the remaining, nimodipine- and CgTX-resistant current is inactivated at depolarized potentials. We are currently investigating whether components of this residual current are blocked by putative T-channel blockers and by the P-channel blocker, ω-agatoxin IVA. (NS09255, MH44754, and MH48432.)

721.16

AUTORADIOGRAPHIC LOCALIZATION OF [^{125}I]ω-CONOTOXIN GVIA BINDING SITES IN HUMAN HIPPOCAMPUS AND CEREBELLUM. B.C. Albensi[1*], K.T. Ryujin[2,3], J.M. McIntosh[4,5], S.R. Naisbitt[5], B.M. Olivera[5] and F. Filloux[2,3,4]. Neurosci. Prog.[1] and Depts. of Neurol.[2], Pediatr.[3], Psychiat.[4], and Biol.[5], Univ. Utah, Salt Lake City, UT 84132.

Only limited studies of ω-conotoxin GVIA (ω-CgTx) binding to human nervous tissue have been performed to date. We describe the labeling of human hippocampus and cerebellum with [^{125}I]ω-CgTx in order to characterize the distribution of N-type Ca++ channels. A pattern similar to that present in rodent brain is described. Human tissue was obtained at autopsy from three individuals dying of non-neurological causes. Slide-mounted tissue sections (10μM) of hippocampal formation and lateral cerebellar hemisphere were labeled with 200 pM [^{125}I]ω-CgTx for association/dissociation and "mapping" experiments, and with varying concentrations (5 pM to 5 nM) for saturation analysis. Autoradiograms were quantitated using image analysis.The binding of [^{125}I]ω-CgTx to human brain is primarily irreversible and saturable under these conditions with an extimated K_D of 910 pM and B_{max} of 11.5 fmoles/mg tissue. The distribution of binding sites is heterogeneous. Densest binding within hippocampus is seen within the hilus of the dentate gyrus (2.65 fm/mg tiss), the pyramidal layer (2.45 fm/mg tiss CA1) and in the adjacent str. radiatum (1.87 fm/mg tiss CA3). Within the cerebellum, binding is denser in the molecular layer (2.17 fm/mg tiss) than in the granule cell layer (1.54 fm/mg tiss). Binding within white matter tracks is lowest overall. These findings suggest that ω-CgTx may serve as a valuable tool for future studies of N-type Ca++ channels in human brain.

721.17

SPECIFIC LOCALIZATION OF OMEGA-CONOPEPTIDE SNX-230 (MVIIC) BINDING SITES IN THE MOUSE NEUROMUSCULAR JUNCTION. Y.Sugiura*, C-P.Ko, A.Woppmann and G.Miljanich. Dept. Biol.Sci., Univ.Southern California, Los Angeles, CA90089. NEUREX Corp., Menlo Park, CA 94025.

SNX-230 (MVIIC) and its analog, SNX-260, inhibit transmitter release at the mouse neuromuscular junctions (NMJ) by blocking non-N type voltage-sensitive Ca^{++} channels (see abstract by Bowersox, et al). The present study aimed to examine if SNX-260 can be used as a specific probe for the localization of voltage-sensitive Ca^{++} channels in the mammalian NMJ. Mouse diaphragm muscles were incubated with biotinylated SNX-260 (3μM) which, like native SNX-260, blocked transmitter release. The muscles were then stained with FITC-ultra-avidin and double-labeled with rhodamine conjugated α-bungarotoxin (BTX). The fluorescence microscopic observations revealed that SNX-260 recognized NMJs which were identified with BTX staining. Control muscles pretreated with native SNX-260 and followed by the above procedures showed no labeling at the NMJ. The structure labeled by SNX-260 was thinner than and outlined by acetylcholine receptor clusters. In addition, spots of labeling with SNX-260, which might be indicative of active zones, could sometimes be seen. SNX-260 also stained some nerve fibers leading to the NMJ. The labeling with SNX-260 disappeared after muscles were treated enzymatically to remove the nerve terminal. Furthermore, SNX-260 labeling was not observed in denervated muscles. These findings suggest that SNX-260 binding sites are located in the nerve terminal and are consistent with the physiological data that SNX-260 blocks voltage-sensitive Ca^{++} channels in the mammalian NMJ. (Supported in part by NIH grant NS 30051 to CPK)

721.18

ω-AGATOXIN-IVA ABOLISHES EXCITATORY AND INHIBITORY SYNAPTIC TRANSMISSION BETWEEN CEREBELLAR NEURONS IN TISSUE CULTURE. W. Raabe*, R.D. Randall and M.E. Adams. Depts. Neurology and Physiology, VA Med Ctr. and U. of Minnesota, Minneapolis, MN 55417, Depts. Entomology and Neuroscience, UC, Riverside, CA 92307.

The characteristics of the calcium channel responsible for transmitter release from excitatory and inhibitory cerebellar neurons were investigated in cerebellar neurons grown in primary tissue culture. Whole cell patch voltage clamp recordings were obtained from large ($\geq$15 μm $\varnothing$) cerebellar neurons presumed to be Purkinje cells. Excitatory postsynaptic currents (EPSCs) were elicited by stimulation of a nearby granule cell ($\leq$8 μm $\varnothing$) with an unpolished patch pipette. Inhibitory postsynaptic currents (IPSCs) were elicited by stimulation of medium size and large cerebellar neurons ($\geq$8 μm $\varnothing$), basket, stellate, Golgi or Purkinje cells.

Addition of Cd^{2+} (20-50 μm) to the extracellular solution abolished evoked but not spontaneous miniature EPSCs and IPSCs. This demonstrated the Ca^{2+} dependence of evoked EPSCs and IPSCs. ω-Agatoxin-IVA (100 nM solution containing 0.1 mg/ml cytochrome C) abolished evoked EPSCs and IPSCs 10-35 min after application by perfusion or direct addition to the bath. Spontaneous miniature EPSCs were not affected by ω-Agatoxin-IVA. Large depolarizations applied through the stimulus electrode could partially restore evoked EPSCs and IPSCs. These observations indicate that the presynaptic Ca^{2+} channel responsible for excitatory transmitter release from cerebellar granule cells and inhibitory transmitter release from basket, stellate, Golgi or Purkinje cells is a high voltage activated Ca^{2+} channel of the P-type.

(Supported by a grant from the Department of Veterans Affairs.)

721.19

INTERACTION OF PRESYNAPTIC CALCIUM CHANNELS WITH SYNAPTOTAGMIN AND SYNTAXIN. M.J. Seagar*, A. Yoshida, C. Leveque, N. Martin-Moutot, O. El Far, P. David, B. Lang, J. Newsom-Davis and M. Takahashi. INSERM U374, Faculté de Médecine Nord, 13916 Marseille 20, France; Mitsubishi Kasei Institute of Life Sciences, Tokyo, Japan; Institute of Molecular Medicine, John Radcliffe Hospital, Headington, Oxford, UK.

The synaptic vesicle protein synaptotagmin (p65) associates with w-conotoxin GVIA (wCgTx) sensitive calcium channels and is thought to be a target for autoantibodies from patients with Lambert-Eaton myasthenic syndrome (LEMS), an autoimmune disease which affects neurotransmitter release. The specificity of LEMS IgG has been examined using proteolytic cleavage and a panel of sequence-directed anti-synaptotagmin antibodies.

The synaptotagmin/calcium channel interaction involves syntaxin (HPC-1), a 35kDa synaptotagmin binding protein localized in the presynaptic plasma membrane. Monoclonal antibodies directed against syntaxin immunoprecipitate approximately 50% of CHAPS solubilized wCgTx receptors and immunoaffinity methods are being used to identify calcium channel associated proteins which may play a role in docking synaptic vesicles near sites of calcium entry prior to exocytosis.

721.20

INHIBITORY EFFFECT OF INTRACELLULAR Mg^{2+} ON ω–CONOTOXIN-SENSITIVE Ca^{2+} CHANNEL CURRENTS. H.A. Pearson* and A.C. Dolphin. Dept. Pharmacology, Royal Free Hospital Sch. of Med., London NW3 2PF, U.K.

We have previously observed that the presence of functional ω-conotoxin-sensitive Ca^{2+} channel currents (I_{Ba}) in cerebellar granule neurones is dependent on the solution used to fill pipettes during whole-cell patch-clamp experiments(1). This has led to the present study in which we have investigated the effect of free intracellular magnesium ion concentration ($[Mg^{2+}]_i$) on I_{Ba} in these cells.

I_{Ba} was recorded from the soma of cerebellar granule neurones (-80mV holding potential) using internal solutions containing varying concentrations of free Mg^{2+} and Mg-bound ATP (Mg.ATP). Values for $[Mg^{2+}]_i$ and $[Mg.ATP]_i$ in each solution were calculated from the concentrations of $MgCl_2$ and K_2ATP added to the solution, using a computer program. In the presence of a constant $[Mg^{2+}]_i$, increasing $[Mg.ATP]_i$ from 0.3mM to 2.0mM was found to increase I_{Ba} from -102±11pA (n=12) to -195±20pA (n=9). This change was not associated with an increase in any particular component of I_{Ba} since the proportion of current inhibited by ω-conotoxin (ω-CgTX) was unchanged (29.3±5.7% at 0.3mM , n=5, and 39.0±11.3% at 2.0mM Mg.ATP, n=4). In contrast to this, increasing $[Mg^{2+}]_i$ in the presence of constant $[Mg.ATP]_i$ led to a decline in I_{Ba} from -123±11pA (n=12, 0.13mM $[Mg^{2+}]_i$) to 66.8±8.4pA (n=9, 1.0mM $[Mg^{2+}]_i$). This decline in current was associated with a loss of ω-CgTX sensitivity (from 26.0±5.1% inhibition at 0.13mM $[Mg^{2+}]_i$ to 7.1±2.4% at 1.0mM $[Mg^{2+}]_i$, n=8 and 4 respectively). Inhibition of I_{Ba} by Mg^{2+} had no effect on the inactivation kinetics of the current, or on the proportion of currrent that inactivated during the activating voltage step.

These data suggest that internal Mg^{2+} selectively inhibits ω-CgTX-sensitive Ca^{2+} channel currents in these cells. Preliminary evidence suggests that a rapid block of the open channel is not the mechanism of action.

REF: (1) Pearson, H.A. and Dolphin, A.C (1993). *J. Physiol.* **459**, 244P

ION CHANNEL MODULATION AND REGULATION IV

722.1

ACTIVITY-DEPENDENT CHANGES IN VOLTAGE-SENSITIVE CALCIUM CURRENTS. S.J. Hong* and G.A. Lnenicka. Dept. of Biol. Sci., State Univ. of New York, Albany, NY 12222.

Previous experiments demonstrated that Ca^{2+} influx produced a long-term reduction in initial transmitter release from the motor terminals of a crayfish abdominal phasic motoneuron, F3 (Hong & Lnenicka, *Brain Res.* 605:121-127, 1992). To investigate possible mechanisms by which Ca^{2+} influx produces this synaptic change, we have examined the effect of Ca^{2+} influx on voltage-activated Ca^{2+} channels using two-electrode voltage-clamp techniques. To measure the Ca^{2+} current in the cell body of F3, Na^+ and K^+ currents were blocked with 1 μM TTX, 50 mM TEA, 1 mM 4-AP, and Cs^+. The Ca^{2+} current (I_{Ca}) was activated at approximately -50 mV and peaked near 0 mV. I_{Ca} was blocked by 10 mM Co^{2+} or 6 mM Mn^{2+}. To examine the effects of Ca^{2+} influx on the voltage-activated Ca^{2+} conductance, the cell was conditioned with 50 msec voltage steps to 0 mV delivered at 1 Hz for 10 min. I_{Ca} declined dramatically during conditioning. After conditioning I_{Ca} slowly recovered and reached an asymptote. One hour after conditioning, there was a 6% (n=3) decrease in peak I_{Ca} when the cell was conditioned in normal Ca^{2+} saline (13.5 mM), whereas there was a 26% (n=3) decrease when conditioned in higher Ca^{2+} (27 mM). To verify that Ca^{2+} influx was responsible for the reduction in I_{Ca}, we performed double-pulse experiments in normal Ca^{2+} and $0Ca^{2+}+Ba^{2+}$ saline. The conditioning pulse (P1) was varied, whereas the subsequent test pulse (P2) was set to 0 mV. The amplitude of I_{Ca} during P2 was inversely correlated to Ca^{2+} entry during P1. The peak I_{Ba} during P2 depressed significantly less than I_{Ca} at corresponding voltage steps of P1. These results suggest that long-term Ca^{2+}-dependent inactivation of Ca^{2+} influx may play a role in activity-dependent changes in transmitter release. (Supported by NSF grant IBN-9121757.)

722.2

P CHANNELS ARE RESPONSIBLE FOR SPIKE DEPENDENT RELEASE, BUT SUSTAINED RELEASE INVOLVED OTHER VARIETIES OF CALCIUM CHANNELS. G.R. Gonzalez Burgos, F.I. Biali, B.D. Cherksey, M. Sugimori, R. Llinás and O.D. Uchitel*. Fac. de Medicina Univ. Bs As Paraguay, 2155 Buenos Aires (11121) Argentina and Dept. of Physiology/Biophysics, NYU Medical Center, NY, NY 10016.

Transmitter release at the nerve terminals is mediated by the influx of Ca^{2+} through voltage-dependent calcium channels (VDCC). Many types of VDCC have been found in neurons (T,L,N and P) but uncertainty remains about which ones are involved in neuronal excitation-secretion coupling. We have studied the effect of VDCC blockers on the release of tritium labelled acetylcholine (3H-ACh) and on synaptic transmission in the rat superior cervical ganglion (SCG). Potassium-evoked 3H-ACh release was markedly reduced in the absence of Ca^{2+} or the presence of Cd^{2+}. The release was also inhibited by the toxin purified from the funnel-web spider venom (FTX, a P-type channel blocker) and by w-conotoxin (w-CgTx, a N-type channel blocker), but was unaffected by the L-type channel blocker nitrendipine. In contrast, postsynaptic action potentials recorded in the postganglionic nerve and triggered by preganglionic stimulation were blocked by FTX, but not affected by w-CgTx or nitrendipine. Thus, P-type calcium channels play a dominant role in synaptic transmission at the SCG, but other not well defined types of VDCC mediate transmitter release evoked by K^+ stimulation. Support by CONICET, Argentina, NIH-NINCDS13742, NIH-NIAAG09480.

722.3

INHIBITORY COUPLING OF μ-OPIOID RECEPTORS TO P-TYPE CALCIUM CHANNELS IN RAT SENSORY NEURONS. K.I. Rusin* and H.C. Moises. Dept. of Physiology, Univ. of Michigan, Ann Arbor, MI 48109.

We previously showed that μ-opioid receptor activation decreases omega-CgTx-sensitive (N-type) high threshold calcium current in rat DRG neurons. In addition, we found that in many neurons, μ-agonists reduce a fraction of omega-CgTx-resistant current even in the presence of saturating concentrations of dihydropyridines. To characterize channel types that might contribute the latter current component we recorded whole cell barium currents through calcium channels in DRG neurons acutely dissociated from 19-30 day old rats. The effects of application of the μ-selective agonist PLO17 (0.3-3 μM) were examined on high threshold current components, isolated on the basis of their voltage dependency of inactivation and sensitivity to omega-CgTx (N-type), nifedipine (L-type) and Aga IVA (P-type). PLO17 reduced N- and P-type high threshold current components, whereas a nifedipine-sensitive, sustained (L-type) component was unaffected. However, in one third of the cells PLO17 continued to inhibit a slowly inactivating component even in the presence of saturating concentrations of all three calcium channel antagonists. These data suggest that μ-opioid receptors are negatively coupled to at least three types of high threshold calcium channels in DRG neurons, including an omega-CgTx-sensitive (N-type) channel, an Aga IVA-sensitive (P-type) channel and a channel that is insensitive to omega-CgTx, Aga IVA and nifedipine (presumably O-type). (Supported by DA03365 to H.C.M.)

722.4

ANANDAMIDE, AN ENDOGENOUS CANNABINOID, INHIBITS CALCIUM CURRENTS (I_{Ca}) AS A PARTIAL AGONIST IN N18 NEUROBLASTOMA CELLS. K. Mackie*, W.A. Devane†, and B. Hille, Physiology and Biophysics, University ofWashington, Seattle, WA 98194 and †NIMH/LCB, Bethesda, MD 20892.

Anandamide (arachidonyl ethanolamide) (AND) has been identified as an endogenous ligand of cannabinoid receptors on the basis of its ability to displace ^{3}H-synthetic cannabinoid in a binding assay. Previously we showed that synthetic cannabinoid agonists inhibit N-type I_{Ca} via a PTX-sensitive mechanism in NG108-15 cells. Here we used N18 cells and whole-cell voltage clamp to show that AND also potently inhibits N-type I_{Ca} in a PTX-sensitive fashion. As for the cannabinomimetic amino-alkylindole, WIN 55,212-2, inhibition by AND is voltage-dependent and NEM-sensitive. However, AND is less efficacious than either WIN 55,212-2 or the non-classical cannabinoid, CP 55,940. Indeed, AND appears to act as a partial agonist at cannabinoid receptors. Application of WIN 55,212-2 always caused further inhibition of I_{Ca} in cells exposed to a maximally effective concentration of AND, and application of AND caused a partial recovery of I_{Ca} in cells exposed to a maximally effective concentration of WIN 55,212-2. The AND analogs, dihomo-γ-linolenyl ethanolamide and arachidonyl propanolamide, both of which bind to the cannabinoid receptor and inhibit adenylyl cyclase, inhibited I_{Ca} in a fashion similiar to AND. This partial agonist property of AND in I_{Ca} inhibition suggests, that while it inhibits N-type I_{Ca}, its actions as a neuromodulator in the intact animal may be more complex than would be inferred by extrapolating the results of *in vivo* studies with (-) Δ^9-THC or potent synthetic cannabinoids. (Supported by NS01588, NS08174 and the McKnight and W.M. Keck Foundations.)

722.5

ETHANOL INHIBITION OF CA CHANNELS IS CONSISTENT WITH THE INTERACTION OF A SINGLE DRUG MOLECULE WITH A SINGLE TARGET SITE. X. Wang, J. Lemos, G. Wang, S.N. Treistman*, Dept. of Pharmacol., Univ. Mass. Med. Sch., Worcester, MA 01655 and Worcester Fdn. Exper. Biol., Shrewsbury, MA 01545.

Ingestion of ethanol results in a decreased level of plasma vasopressin, which appears to be caused by inhibition of arginine vasopressin (AVP) release from the neurohypophysis. Ethanol, at concentrations below that constituting legal intoxication, blocks dihydropyridine-sensitive 'L-type' Ca^{2+} channels in isolated nerve terminals of the rat neurohypophysis. Ethanol reduced the channel open probability in a concentration-dependent manner. To allow finer resolution of channel openings and to better characterize the mechanisms of ethanol action, Bay K 8644 was used to prolong the openings of L-type Ca^{2+} channels. In the presence of this dihydropyridine (DHP), the reduction of the channel open probability could be determined to be due primarily, although not completely, to a shortening of the open duration of this L-channel. Channel conductance was unaffected by ethanol, even at high concentrations. These results are consistent with previous macroscopic data indicating that calcium channels in these peptidergic terminals are targets for ethanol action, and indicate that ethanol acts directly on the gating characteristics of the L-type channel. The results of a Hill plot analysis of ethanol's effects on the channel open probability, as well as analysis of the distributions of open and closed times are consistent with the interaction of a single drug molecule with a single target site, following first order kinetics. (Supported by NIH grant AA08003).

722.6

GALANIN DECREASES BARIUM CURRENTS IN DISSOCIATED MUDPUPPY PARASYMPATHETIC NEURONS. L.A. Merriam* and R.L. Parsons. Department of Anatomy and Neurobiology, University of Vermont, Burlington, VT 05405

Barium currents (I_{Ba}) were recorded from neurons dissociated from the mudpuppy cardiac ganglion using the perforated patch technique. The extracellular solution contained in mM:110 NMGCl, 10 TEA, 5 HEPES, $3X10^{-7}$M TTX, 3.6 $BaCl_2$. In addition to nystatin (1720 units/ml), the patch solution contained in mM: 90 Cs-aspartate, 20 CsCl, 5 $MgCl_2$, 10 HEPES. With cells held at -90 mV, inward currents were first seen with steps to ~-20 mV and peak currents occurred at +10 mV. ω-conotoxin (1 μM) decreased I_{Ba} by 85% whereas 10 μM nifedipine decreased I_{Ba} by 10-15% suggesting that most of the current was due to the activation of N-type channels. With bath application, galanin produced a concentration-dependent (10^{-10}-10^{-6}M), slowly developing, longlasting decrease in I_{Ba} (maximum decrease ~60%). Galanin decreased I_{Ba} after treatment with 10 μM nifedipine, but not after exposure to 1 μM ω-conotoxin indicating that galanin preferentially inhibited N-type channels. Galanin reduced the peak current without producing a consistent shift in the I-V relationship. Depolarizing steps to +70 or +100 mV did not reverse the galanin-induced decrease in I_{Ba}. The results suggest that galanin produces a decrease in I_{Ba} in mudpuppy parasympathetic neurons that does not appear to be voltage-dependent. Supported by NIH grant NS 23978.

722.7

A BIPHASIC DOPAMINERGIC MODULATION OF THE HIGH VOLTAGE-ACTIVATED Ca^{2+} CURRENT (HVA-I_{Ca}) OF IDENTIFIED SNAIL NEURONS. J. Golowash, J. Pastor, and D. Paupardin-Tritsch*. Lab. Neurobiology, Ecole Normale Supérieure, 75005 Paris, France.

Identified neurons of the snail *Helix aspersa*, either *in situ* or disssociated from the ganglia, were respectively voltage-clamped with two microelectrodes or using the whole cell patch clamp configuration. Dopamine (DA) induced a biphasic modulation of HVA-I_{Ca} consisting of an initial fast decrease of HVA-I_{Ca} followed by a slower enhancement of this current. DA reduced HVA-I_{Ca} by 33.9 ± 18.8% in the *in situ* neurons (n=22) and by 38.0 ± 11.6% in the dissociated cells (n=26). The augmentation of HVA-I_{Ca} was significantly larger in the *in situ* neurons than in the dissociated ones (25.6 + 20.8% increase versus 8.4 + 3.5%). In addition, the DA enhancing effect showed a marked desensitization in the *in situ* cells which did not appear in the dissociated ones.

None of the classical intracellular mechanisms (PKA, PKG, PKC, arachidonic acid, intracellular Ca^{2+}, Ser-Thr phosphatases) appear to be involved in any of the two sucessive DA-induced modulations of HVA-I_{Ca}. Intracellular application of the protomer A of *Bordetella pertussis* depressed in parallel and with the same intensity both the decrease and the enhancement of HVA-I_{Ca} by DA. As previously shown for other identified snail neurons by Harris-Warrick et al. (Neuron, 1988, 1, 27-32), the inhibitory action of DA very likely involves the α subunit of a G_o protein, immunologicaly related to mammalian brain α_o subunit. A possible role of the βγ subunits of the snail G_o in the enhancement of HVA-I_{Ca} by DA will be discussed.

722.8

MODULATION OF CALCIUM CURRENTS IN INSULIN-SECRETING CELLS BY STIMULANTS OF THE CYCLIC AMP PATHWAY. J.A.Love*, C. Owyang, and D.C. Dawson. Departments of Internal Medicine and Physiology, University of Michigan Medical Center, Ann Arbor, MI 48109

Stimulation of adenylate cyclase and elevation of intracellular cyclic AMP levels potentiate glucose-stimulated Ca^{++} influx and insulin secretion by pancreatic ß cells. The mechanisms of this effect remain to be defined. We have previously demonstrated that high-threshold, voltage-dependent Ca^{++} currents in insulin-secreting HIT-T15 cells are modulated by other second messengers (Neurosci. Abstr 18:798, 1992). We used stimulants of cyclic AMP production to determine whether this second messenger system also modulated Ca^{++} currents. Acute exposure to the adenylate cyclase activator forskolin (5-10 μM; 5-15 minutes) resulted in an increased fractional open-time (.026 vs. .054) of single Ca^{++} channel currents from cell-attached patches in 2/3 cells tested. Exposure to forskolin (5-10μM) in the presence of the phosphodiesterase inhibitor IBMX (100μM) resulted in an increase in fractional open-time (.037 vs. .095) in 5/8 patches. Finally, the membrane permeable cyclic AMP analog 8-bromo-cyclic AMP (2mM) increased fractional open-time (.048 vs. 092) in 2/4 patches. These results suggest that voltage-dependent Ca^{++} channels are one site where the cyclic AMP pathway can act to elevate intracellular Ca^{++} and potentiate insulin secretion. Supported by the NIH.

722.9

CELL $[Ca^{++}]_i$ REGULATION AND THE ACTIVATION OF A CALCIUM-ACTIVATED CURRENT. L.D. Partridge*. Dept. of Physiol., Univ. of New Mexico, Albuquerque, NM 87131.

Intracellular Ca^{++} is an important messenger in a number of cellular processes including the gating of ion channels. Activity of calcium-activated non-selective (CAN) channels underlies neuronal burst firing, neurosecretory activity, and perhaps excitotoxicity.

Our fura-2 measurements in *Helix* neurons indicate that $[Ca]_i$ decays with a $\tau \approx 5$ sec following intracellular Ca^{++} injection. CAN currents measured under similar conditions relax with a $\tau \approx 2$ sec. In modelling studies, we have shown that CAN currents are activated by Ca^{++} in a very local sub-membrane domain. Our inside-out patch records indicate that CAN channels do not inactivate in the presence of cytoplasmic Ca^{++}. Current through CAN channels should thus be a good indicator of sub-membrane Ca^{++} activity.

In this study I assessed the role of cellular Ca^{++} sequestering mechanisms in regulating the pool of Ca^{++} available to activate CAN channels. Burster neurons from *Helix aspersa* were single electrode voltage clamped and quantitative pressure injections of Ca^{++} were used to activate CAN currents. Thapsigargin, ruthenium red, and NaN_3 were used to block respectively uptake by ER and mitochondria, and all ATP-coupled Ca^{++} transport. There was no significant change in CAN current decay constant (τ=2.10 s.d.=1.56) with any of these treatments. Thapsigargin caused about a 10 fold reduction (from about 5 nA/fmol Ca^{++}) in the activation of CAN current. Neither Ca-ATPases in the plasma membrane and ER nor Ca-H exchange in mitochondria are major factors in reducing Ca^{++} that activates CAN channels. Maintained increased sub-membrane $[Ca^{++}]$ following block of ER uptake has an inhibitory effect on CAN channel activation.

722.10

CA^{2+} INFLUX THROUGH L-TYPE, BUT NOT N-TYPE, CA^{2+} CHANNELS ACTIVATES CA^{2+}-DEPENDENT K^+ CURRENT IN ACUTELY ISOLATED CHICK CILIARY GANGLION NEURONS. M. E. Wisgirda* and S. E. Dryer, Program in Neuroscience, Florida State University, Tallahassee, FL 32306.

Neurons of the chick ciliary ganglion are known to express Ca^{2+}-activated K^+ currents ($I_{K(Ca)}$) and two types of Ca^{2+} currents, the so-called "N-type" that is sensitive to blockade by omega-conotoxin, and the "L-type" that is sensitive to blockade by dihydropyridines. The interactions between $I_{K(Ca)}$ and the two Ca^{2+} channel subtypes were examined in ciliary ganglion neurons obtained from E11-E13 chick embryos. Standard whole-cell and perforated patch recording methods were used. Application of omega-conotoxin, either in the bath saline (2 μM) or by pressure ejection onto the cell (20 μM), produced a substantial blockade of Ca^{2+} current but had little or no effect on $I_{K(Ca)}$. By contrast, bath application of 10 μM nifedipine produced a substantial blockade of $I_{K(Ca)}$. The L-type Ca^{2+} channel agonist S(-) Bay K 8644 caused an increase in $I_{K(Ca)}$. These results suggest that L-type Ca^{2+} channels are the sole source of Ca^{2+} for activation of Ca^{2+}-dependent K^+ current in ciliary ganglion neurons. Supported by NIH Grant NS-27013.

722.11

MODULATION OF CALCIUM-DEPENDENT POTASSIUM CHANNELS BY CALMODULIN. A. J. Boileau* and C. Kung. Neuroscience Training Program and Dept. of Genetics, University of Wisconsin, Madison, WI 53706.

Paramecium tetraurelia is a model system for the study of ion channels and their modulation in governing a behaviorally relevant action potential. Our laboratory has several behavioral mutants, many of which have point mutations in the calmodulin gene. One set of such "cam" mutants shows defects in macroscopic calcium-dependent potassium currents in whole-cell voltage clamp.

The present study shows that the connection between calmodulin and the channel may be direct. Calmodulin (CaM) application to excised inside-out patches of *Paramecium* membranes containing a previously described 72 pS gK(Ca) causes an increase in Po and the number of opening events. Either *Paramecium* CaM or bovine CaM modulate the channel, but troponin C application has no effect. The effect is calcium-dependent and can be seen at 200nM CaM.

722.12

WHOLE-CELL RECORDING OF THE SLOW CALCIUM-DEPENDENT I_{AHP} IN RAT HIPPOCAMPAL CA1 NEURONS P. Watson*, L. Zhang, J.L. Weiner, T.A. Valiante, A. Velumian, S.S. Jahromi, S. Schertzer, P. Pennefather, P.L. Carlen, Playfair Neurosci. Unit, MRC group "Nerve Cell and Synapse", Bloorview Epilepsy Prog., Univ. of Toronto, Canada M5T 2S8.

In mammalian brain neurons, a train of action potentials is followed by a slow afterhyperpolarization (AHP) lasting seconds, which is generated by a K^+ current (I_{AHP}) highly sensitive to intracellular Ca^{2+}. In the past decade, the I_{AHP} has been extensively studied using sharp electrodes. However, it has not been well demonstrated in the whole-cell recording mode, suggesting that internal dialysis with conventional whole-cell recordings may interfere with normal intracellular Ca^{2+} homeostasis.

To address this issue, we studied the slow I_{AHP} current in mature rat hippocampal neurons in brain slices using patch pipette solutions containing 2 mM K-ATP and different potassium salts including KMethylsulfate, KCl, KGluconate, KGlutamate or KCitrate. Only KMethylsulfate-loaded neurons showed stable AHP (I_{AHP}) and strong firing adaptation as observed with sharp electrodes. In neurons dialysed with other potassium salts, the AHP (or I_{AHP}) was small and unstable. The magnitudes of the AHP in neurons dialysed with different potassium salts were ranked as follows: KMethylsulfate > KCl > KGluconate > KGlutamate > KCitrate. Likewise, sustained voltage-activated Ca^{2+}-currents were stable in neurons dialysed with cesium methanesulfonate, but not with cesium gluconate or cesium chloride. The mechanisms underlying the interactions between internally applied anions and cytoplasmic Ca^{2+} handling are under investigation. We suggest that the KMethylsulfate-containing internal medium is favourable for whole-cell recording of ionic currents sensitive to, or modulated by intracellular Ca^{2+}.

722.13

NOREPINEPHRINE, BUT NOT ACETYLCHOLINE, INHIBITS I_{AHP} IN RAT HIPPOCAMPAL SLICES BY ACTIVATING PROTEIN KINASE A. R. Nouranifar, T. Wong, E. M. Landau, and R. D. Blitzer*, Depts. of Psychiatry and Pharmacology, Bronx VAMC and Mt. Sinai Medical Center, New York, NY 10029.

The coupling of transmitter receptors to the Ca2+-dependent K+ current, IAHP, was studied in the whole cell configuration in CA1 pyramidal cells. The time-course, voltage-dependence, and pharmacological characteristics of the current were similar to those obtained using sharp electrodes. It was inhibited by acetylcholine, norepinephrine, and by tetraethylammonium only at a high concentration (10 mM, but not 1 mM). The effect of norepinephrine on IAHP was enhanced in the presence of baclofen, as seen with sharp electrode recording (Andrade, Neuron 10:83, 1993). Rundown of IAHP was greatly delayed by inclusion of an ATP-regenerating system in the pipet solution, permitting long-term (>2 h) recordings of agonist-sensitive IAHP. Prior dialysis of cells with the protein kinase A inhibitor Rp-cAMPS blocked the effect of norepinephrine on IAHP, but did not alter the effect of acetylcholine. The sensitivity of the noradrenergic effect to Rp-cAMPS confirms and extends work implicating cAMP in the effect of norepinephrine (Madison & Nicoll, J. Physiol. 372:245, 1986). The block of IAHP by acetylcholine does not proceed through protein kinase A, and the transduction of the cholinergic effect remains unknown. (Supported by NARSAD and a VA Merit grant)

722.14

POSSIBLE MODULATION BY PKC OF THE *trans*-ACPD INHIBITION OF THE SLOW I_{AHP} IN DENTATE GRANULE NEURONS OF THE HIPPOCAMPUS M. A. Abdul-Ghani*, T. A. Valiante, P. S. Pennefather, P. L. Carlen. MRC Nerve Cell and Synapse Group, Playfair Neuroscience Unit, University of Toronto, Canada.

Glutamate is the major excitatory transmitter in the brain, acting through ionotropic and metabotropic receptors. Activation of metabotropic glutamate receptors has been shown to inhibit several calcium and potassium currents in hippocampal neurons. Using the whole cell configuration of the patch clamp technique, we have studied the mechanism underlying the inhibition of the post spike-train afterhyperpolarization current (I_{AHP}) by mGluR receptor activation in dentate granule neurons in the hippocampal slice preparation. *Trans*-ACPD (10 μM) completely inhibited I_{AHP}. After 15 min dialysis of the cell with GDP-β-S (1.2 mM), trans-ACPD still inhibited I_{AHP} while the serotonin inhibition of I_{AHP} was completly blocked. Furthurmore, unlike serotonin, the inhibitory effect of trans-ACPD on I_{AHP} was observed when the slices were preincubated for three hours with two non-specific kinase inhibitors, either H-7 (300 μM), or staurosporine (1 μM). However, application of the the phorbol ester PMA (5 μM) (which by itself reduced I_{AHP} by approximately 30%) slightly reduced the inhibition of I_{AHP} by activation of the mGluR. Our results show that the transduction mechanism that mediates the inhibition of the AHP by activation the mGluR is different from that which mediates the regulation of the AHP by other neurotransmitters.

Acknowledgement: T.A.V. is supported by an MRC Studentship and M.A.G. is an MRC fellow.

722.15

SEROTONIN INHIBITS THE SLOW AHP IN DENTATE GRANULE NEURONS VIA A G-PROTEIN MEDIATED MECHANISM. P. L. Carlen, T. A. Valiante, M. A. Abdul-Ghani, P. S. Pennefather, S. S. Jahromi*. MRC Nerve Cell and Synapse Group, Playfair Neuroscience Unit, University of Toronto, Canada.

The post-spike train afterhyperpolarization current (I_{AHP}) is an intrinsic inhibitory ionic mechanism that contributes to neuronal excitability. Serotonin has been shown to block I_{AHP} through the 5-HT_4 receptor subtype, however little is known about the signal transduction pathway responsible for this effect. To elucidate this transduction pathway we have used the whole cell configuration of the patch clamp technique to record from dentate granule neurons in the hippocampal slice preparation. Intracellular dialysis with 1.2 mM GDP-β-S for 15 minutes blocked the inhibitory effect of 5-HT (10 μM) on I_{AHP} indicating mediation through G-proteins. Preincubation of the brain slice with a potent non-specific protein kinase inhibitor, staurosporin (1μM for three hours) profoundly reduced the inhibition of I_{AHP} by 5-HT. These results indicate that 5-HT inhibits the AHP through protein kinase(s) linked to serotonin receptors via G-proteins.

Acknowledgement: T.A.V. is an MRC student and, M.A.G. is an MRC fellow.

722.16

cAMP-DEPENDENT PROTEIN KINASE (PKA) MEDIATES THE MODULATION OF THE HIPPOCAMPAL Ca^{2+}-ACTIVATED K^+ CURRENT I_{AHP} AND ACCOMMODATION BY NOREPINEPHRINE, SEROTONIN AND HISTAMINE, BUT NOT BY ACETYL CHOLINE OR GLUTAMATE.

P.Pedarzani* & J.F.Storm, *Inst. of Neurophysiol., Univ. of Oslo, Norway.*

The slow Ca^{2+}-activated K^+ current I_{AHP} underlying accommodation in hippocampal pyramidal neurons is regulated by multiple transmitters. It has been postulated, but not proven that protein phosphorylation underlies some of these effects (Nicoll, Science 241, 545-551, 1988). By intracellular application of kinase- and phosphatase inhibitors and catalytic subunit of protein kinase A (PKA-C), we have now obtained compelling evidence that PKA mediates the suppression of I_{AHP} and accommodation by three transmitters.

Whole-cell recordings were obtained from 125 CA1 pyramidal cells in slices from young rats. I_{AHP} was seen as a slow tail current following depolarizing steps which elicited Ca^{2+} influx. I_{AHP} was reversibly suppressed by norepinephrine (NE), isoproterenol, serotonin (5-HT), histamine (HA), carbachol, muscarine, the metabotropic glutamate receptor (mGluR) agonist t-ACPD, or a cAMP analog (CPT-cAMP). When the selective PKA-inhibitor Rp-cAMPS was applied intracellularly, it blocked the effects on I_{AHP} by isoproterenol, HA, 5-HT, and CPT-cAMP but not by carbachol or t-ACPD. The pseudo-substrate PKA peptide inhibitor Walsh PKI also prevented suppression of I_{AHP} by isoproterenol or CPT-cAMP, while the protein phosphatase inhibitor microcystin or PKA-C both caused run-down of I_{AHP} in the absence of applied agonists. Rp-cAMPS also blocked the effects on spike accommodation by NE, 5-HT or HA , but not by carbachol. These results indicate that protein kinase A mediates the effects of NE, 5-HT or HA on I_{AHP} and accommodation.[Thanks to S.Døskeland for PKA-C and A.Czernik, A.Nairn & P.Greengard for PKI.]

722.17

INTERNAL PERFUSION OF RAT HIPPOCAMPAL NEURONES IN BRAIN SLICES: EFFECTS ON NEURONAL EXCITABILITY. A.Velumian*, L.Zhang, P.Pennefather, M.P.Charlton, P.L.Carlen. Playfair Neurosci. Unit, MRC Nerve Cell and Synapse Group, Department of Physiology, Bloorview Epilepsy Program, Addiction Research Foundation, University of Toronto, Toronto Ont., Canada M5T 2S8

We have developed a simple method of multiple and fast replacements of solutions in the patch pipette tip and thereby inside the recorded cell, which allows the study of various aspects of internal modulation of neuronal membrane ion currents in brain slices. This method allows more than 10 effective replacements of different internal solutions within one hour during recording from a single neuron. The blockade of voltage-activated K^+ channels by replacement of internal 150 mM K^+ by 150 mM Cs^+ ions occurred within 2-3 minutes, including the one minute "flow latency" due to solution flow through the perfusing tube. The recovery on return to 150 mM K^+ occurred at nearly the same speed. $GABA_A$ receptor-mediated IPSCs were reversed due to replacement of internal gluconate by chloride ions within 1.5-2 minutes. Internally perfused Cs^+ and TEA^+ resulted in the blockade of K^+ channels and the appearance of a plateau on the falling phase of the action potential. The changing main anions in the internal solution, including gluconate, methylsulphate and chloride, produced sharp changes in the spike adaptation pattern and in the slow afterhyperpolarization following depolarizing pulse-evoked spike train. The effects of Ca buffers with different K_ds on spike adaptation, AHPs and underlying currents are being studied.

ION CHANNELS: CELL FUNCTION

723.1

NEURON, A COMPUTER PROGRAM SPECIALIZED FOR SIMULATING NERVE FUNCTION. Michael Hines & John W. Moore*, Dept. Neurobiology, Duke Univ. Med. Cntr. Durham NC 27710

NEURON was designed to solve two especially difficult kinds of problems: 1) complex membrane properties involving many ion-specific channels and ion accumulation and 2) where cable properties of cells play an important role, possibly including extracellular potentials close to the membrane.

NEURON provides **continuous cable sections** which may be connected together to form any type of branched cable and which are endowed with properties which may vary continuously with position along the section. This innovation allows one to keep the physical properties of the neuron entirely separate from the numerical issue of size of spatial compartments.

User defined channels may be entered conveniently by expressing models in terms of kinetic schemes and sets of simultaneous equations. Fast and efficient computation is carried out by compiling these model descriptions and using an implicit integration method optimized for branched structures.

We will demonstrate the great flexibility which results from using an object oriented interpreter to setup the physical properties of the cables, control the simulation, and plot the results. Furthermore, the interpreter provides **a highly convenient graphical interface** for setting parameters, managing either voltage clamp or current injection, and graphing variables as a function of time and position.

723.2

ACTIVITY-DEPENDENT CONDUCTANCES PRODUCE NONUNIFORM CURRENT DISTRIBUTIONS IN MODEL NEURONS. M. S. Siegel, E. Marder*, and L. F. Abbott. Departments of Biology and Physics and Center for Complex Systems, Brandeis Univ., Waltham, MA 02254.

We study biophysically realistic model neurons in which membrane current maximal conductances are slowly varying dynamic variables regulated by intracellular calcium. This allows us to model activity-dependent effects arising from processes that develop and modify membrane channels. Starting with no conductances (a passive cell), the multi-compartmental model neurons develop a heterogeneous and realistic distribution of membrane conductances: strong inward currents in the soma tapering to a constant intermediate level along the axon and weak inward and strong outward currents in the dendrite. Thus an initially passive and electrically homogeneous cell differentiates into several active substructures: an axon hillock/soma region capable of initiating action potentials, a mature axon capable of sustaining action potentials and a dendritic arbor that adapts locally to synaptic input. Different dendritic regions can specialize to define separate "computational units," forming "hot" and "cold" spots in the active dendritic membrane The morphology of the neuron and the intracellular calcium concentration determine the final spatial distribution of currents. Supported by MH46742.

723.3

A QUANTITATIVE MODEL OF CILIARY NEURONS OF THE CHICK CILIARY GANGLION. *Richard Bertram & Stuart E. Dryer*[*1] *Supercomputer Computations Research Institute and Program in Neuroscience[1], Florida State University, Tallahassee, FL 32306.*

The chick ciliary ganglion contains two populations of neurons. One of these, the ciliary neurons, receive dual electrical-chemical synaptic inputs from preganglionic fibers, and innervate striated muscle in the iris and ciliary body. Ciliary neurons are unusual amongst autonomic neurons in being able to maintain *sustained* firing rates of greater than 200 Hz during injection of depolarizing current pulses. We have developed a mathematical model of these cells that includes the following ionic currents, with amplitudes and kinetic parameters based on voltage-clamp data from these neurons: a rapidly-inactivating Na^+ current (I_{Na}); a sustained high-threshold Ca^{2+} current (I_{Ca}); a slowly-inactivating delayed rectifier (I_{DR}); a transient rapidly-inactivating K^+ current (I_A); a mixed-cationic inward rectifier activated by hyperpolarization (I_{IR}); a large voltage-dependent Ca^{2+}-activated K^+ current ($I_{K(Ca)}$); a smaller voltage-independent Ca^{2+}-activated K^+ current (I_{AHP}); and an ohmic leak (I_L). The model also includes first-order mechanisms for the influx and elimination of cytoplasmic free Ca^{2+}. The model exhibits sustained firing rates of 250 Hz during injection of depolarizing current pulses, with very little change in interspike interval during the course of the train. The model also predicts a gradual decrease in spike amplitude during the course of the train. Each of these are features observed in current-clamp recordings from ciliary neurons. Supported by NS-27013.

723.4

COMPUTATIONAL ANALYSIS OF THE SENSITIVITY OF NEURONAL BEHAVIOR TO CHANNEL DENSITY DISTRIBUTIONS IN HIPPOCAMPAL CA3 NEURONS. R.M. Eichler West*, L.R. Petzold, and G.L. Wilcox, Army High Performance Computing Research Center, Grad. Program in Neuroscience, Depts. of Computer Science and Pharmacology, U of Minnesota, Minneapolis, MN, 55415.

Previous computational models of single neurons have focused primarily on the passive electrical properties of branched dendritic tree structures. Evidence from patch clamp recordings and immunohistochemical studies suggest that voltage-gated channels are more prevalent in the soma and dendrites than had been previously suspected and that these channels mediate non-linear behavior. This experimental evidence suggests an explanation for the observed shortcomings of the passive models. The present study applied combinatorical analysis to simulations of multi-compartment neural models incorporating six types of voltage-activated channels.

Determining non-linear signaling behavior of realistic neural models using finite-element representations requires knowledge of the probable density of active and passive conductances on the cell surface that is not completely described in the experimental literature. Exploration of all possible combinations would be computationally intractable. We have explored several classes of channel density distributions in an attempt to match simulation data with experimental findings. We have superimposed these distributions on morphometric data (generated by the Eutectic Neuron Tracing System) from hippocampal CA3 neurons using six classes of channels with appropriate kinetics adapted from Traub. We performed the calculations on a CM-5 (Thinking Machines Corporation) using the integration package DASPK and displayed the results on an IRIS VGX workstation. We analyzed the electrical and ionic responses of the soma and dendritic compartments to intracellular current injections. The analysis examined the sensitivity of observed behavior to random noise and to dendritic morphological resolution. The results indicate that inclusion of voltage-gated conductances in neuronal simulations strongly affects the behavior of the model. (RMEW and use of the CM5 supported by a contract between the ARO and the U of MN for the AHPCRC; contract #DAAL02-89-C-0038. GLW partially supported by NIH K02-D4-00145.)

723.5

ACTIVITY-RELATED CALCIUM DYNAMICS IN LAMPREY MOTONEURONS AS REVEALED BY VIDEO-RATE CONFOCAL FLUORESCENCE MICROSCOPY. B.J. Bacskai[1,2], P. Wallén[3], V. Lev-Ram[2], S. Grillner[3], and R.Y. Tsien[1,2*]. [1]Howard Hughes Medical Institute and [2]Dept. of Pharm., Univ. of Cal. San Diego, La Jolla, CA 92093, [3]Nobel Institute for Neurophysiology, Karolinska Institute, Stockholm, Sweden

Calcium influx plays a key role in various mechanisms regulating neuronal activity in the segmental network for locomotion in the lamprey. A Ca-dependent K-conductance underlies the late afterhyperpolarization and thereby regulates the firing behavior of motoneurons and interneurons, including the termination of the burst during locomotor activity. Similarly, during endogenous pacemaker-like oscillations induced by NMDA-receptor activation, calcium entry gives rise to the hyperpolarizing phase, also through activation of Ca-dependent K-channels. However, the localization and time course of calcium fluxes in neurons of the locomotor network have not been previously investigated. Therefore, measurements of $[Ca^{2+}]_i$ in motoneurons of the isolated spinal cord of *Ichthyomyzon unicuspis* were obtained with a high-speed laser scanning confocal microscope. Intracellular iontophoresis of the calcium indicator fluo-3 allowed visualization of free Ca^{2+} changes in cell bodies (as deep as 150 µm into the preparation), dendrites, and the axon at up to video frame rates (30/s) with high spatial resolution. Extracellular stimulation of descending reticulospinal axons gave rise to a rapid and localized $[Ca^{2+}]_i$ increase in ventromedial, distal dendrites, concomitant with the compound EPSP. Intracellular current injections to elicit single or trains of action potentials produced rapid increases in $[Ca^{2+}]_i$ throughout the cell, with the smallest changes observed in the cell body, possibly due to its small surface to volume ratio. The increase in calcium persisted for a few seconds after single spikes and tens of seconds after trains of 50 spikes. Fluo-3/AM loading revealed fast, dynamic changes in $[Ca^{2+}]_i$ in motoneuron axons near the ventral roots during NMDA-induced fictive swimming, suggesting the presence of a calcium component in the action potential. Antidromic stimulation of ventral roots while observing $[Ca^{2+}]_i$ in motoneuron axons indicated that the calcium increase required external Ca^{2+}, was dihydropyridine and verapamil-insensitive, and could be blocked by nickel.

723.6

NON-LINEAR PROPAGATION OF AGONIST-INDUCED CALCIUM WAVES IN ASTROGLIA. S. Yagodin, L.A. Holtzclaw, and J. T. Russell*, LCMN, NICHD.

In rat cortical astrocytes, neurotransmitters such as norepinephrine and glutamate induce intracellular calcium signals by release from cellular stores following IP_3 formation. We have studied these agonist-induced calcium signals with high temporal (0.25s) and spatial (0.8 - 5 µm) resolutions. Calcium signals consistently originated from a single locus within an astrocyte, and this locus remained invariant for subsequent challenges with agonists that are coupled to IP_3. In these loci the resting Ca^{2+} concentration is elevated with respect to the rest of the cytoplasm. From the locus of origin, wave propagation is achieved by a process of diffusion and regenerative Ca^{2+} release in multiple cellular loci provoked by the advancing wave front; in this way, wave propagation is non-linear and saltatory. The regenerative loci appear as discrete focal areas of 7 µm to 15 µm in diameter, have different Ca^{2+} activation thresholds, and possess intrinsic oscillatory properties capable of initiating local Ca^{2+} waves which collide and annihilate. The data show that multiple sites exist within astroglia with different thresholds of activation for calcium release in a hierarchical manner, such that the low threshold centers initiate waves, and the higher threshold centers provide for regenerative amplification. In this way, since diffusion of IP_3 in the cytoplasm is rapid, wave propagation could proceed with IP_3 channel openings alone. The spatio-temporal organization of Ca^{2+} waves in astrocytes could provide a cellular mechanism for transduction of the strength of agonist action to discrete cytoplasmic sites as well as to the cell nucleus.

723.7

Ca^{2+} HOMEOSTASIS IN CULTURED RAT MYENTERIC NEURONS: THE EFFECTS OF CHRONIC DEPOLARIZATION ON $[Ca^{2+}]_i$ TRANSIENT DECAY RATES. D.J. Fickbohm*[1] and A.L. Willard[1,2]. Neurobiology Curriculum[1] and Dept. of Physiology[2], Univ. of North Carolina, Chapel Hill, N.C. 27599.

Chronic depolarization affects the development of rat myenteric neurons in cell culture. Growth in depolarizing medium containing 25 mM K^+ decreases the density of voltage-dependent I_{Ca}'s and causes sustained elevation of $[Ca^{2+}]_i$ (Franklin et al., J. Neurosci., 1992). The decreased I_{Ca} density (i.e., decreased Ca^{2+} influx) in chronically depolarized neurons leads to a decrease in AP-evoked $[Ca^{2+}]_i$ transient amplitudes (Fickbohm & Willard, Neurosci. Abstr., 1992). Here we report that $[Ca^{2+}]_i$ transients decay faster in chronically depolarized neurons than in control neurons. These findings support the hypothesis that Ca^{2+} homeostasic mechanisms are enhanced in chronically depolarized neurons.

Myenteric neurons dissociated from intestines of 2-3 d old rat pups were grown in cell culture for 3-10 d in medium containing control (5 mM) or elevated (25 mM) KCl. $[Ca^{2+}]_i$ was monitored in fura-2-loaded cells, using a video-based system. Neurons were stimulated with a pair of Pt wires. Brief trains of pulses (1-16 pulses) elicited $[Ca^{2+}]_i$ transients with a rapid rise (<3 s to peak) and a slower decay (τ, range = 2.1-73.9 s). The decay rates were faster for a chronically depolarized population than for a control population at almost every $[Ca^{2+}]_i$ transient amplitude. The mean τ of $[Ca^{2+}]_i$ transients in chronically depolarized neurons is significantly lower than in control cells (14.3 s vs 18.0 s). Interestingly, the maximum decay rates occur at the same $[Ca^{2+}]_i$ transient amplitude (≈12 nM) for both populations, indicating that the relationship between decay rate and amplitude is unchanged. In conclusion, chronically depolarized myenteric neurons restore $[Ca^{2+}]_i$ to basal levels faster than do control cells. This suggests that the Ca^{2+} handling abilities of rat myenteric neurons are upregulated by chronic depolarization. Supported by NIH grant NS24362.

723.8

DISRUPTION OF CALCIUM HOMEOSTASIS LEADS TO CALCIUM INFLUX IN NEURONAL CELLS. Chris Mathes*# and Stuart H. Thompson#, #Hopkins Marine Station, Stanford University, Pacific Grove, CA 93950 and *Neurosciences Ph.D. Program, BRI, UCLA, LA, CA 90024.

Muscarinic receptor agonists (1 mM carbachol; 1 min.), EGTA/AM (10 µM; 30 min. load), and thapsigargin (1 µM; 15 min.) disrupt calcium homeostasis in N1E-115 mouse neuroblastoma cells. In nystatin perforated-patch voltage clamp experiments, these manipulations activate a voltage-independent calcium current that is reduced by bath application of $Mn^{++}$and Ba^{++}. During the calcium current, addition of Mn^{++} or Ba^{++} causes a biphasic response: an initial block followed by an increase of inward current. The blockable component is the calcium current turned-on by carbachol, EGTA/AM, and thapsigargin. The Mn^{++} and Ba^{++} influx component occurs through hyperpolarization activated non-selective cation channels (I_H). Carbachol, EGTA/AM and thapsigargin activate the same calcium current, but they differentially affect calcium stores. Using fura-2/AM to monitor calcium concentration changes in single cells, we observe that intracellular stores are emptied by thapsigargin, but not by muscarinic receptor activation or EGTA/AM loading. These results suggest that global changes in calcium homeostasis, including store depletion, lead to voltage-independent calcium influx. (supported by NIH NS14519 to SHT and NIH MH10425 to CM)

723.9

SPECIFIC Ca CHANNEL-SECRETION COUPLING IN ADRENAL CHROMAFFIN CELLS. C. R. Artalejo+ and M. E. Adams*, +Dept. Neurobiology & Physiology, Northwestern Univ., Evanston, IL 60208 *Dept. Entomology & Neuroscience, Univ. of California, Riverside, CA 92521

The chromaffin cells of the adrenal medulla secrete catecholamines and several neuropeptide hormones in response to neuronal stimulation. Secretion is a Ca^{2+}-dependent process that is triggered by the entry of Ca^{2+} through voltage-gated Ca channels in the plasma membrane. We have found that chromaffin cells contain three distinct types of Ca currents carried by "non classical" N-type channels, P-type Ca channels and a novel class of L-type channel known as "facilitation" Ca channels. These channels can be distinguished by their sensitivity to three selective toxins: ω-conotoxin-GVIA (N-type), ω-agatoxin-IVA (P-type) and dihydropyridines (L-type). Together these channels comprise > 99% of the total Ca current present in these cells.

In the present work we have analyzed the contribution of different Ca channel types to the secretory process using capacitance measurements as an assay of catecholamine release. Although all three Ca channel types can contribute to secretion, the coupling between facilitation Ca channels and exocytosis is far more efficient than for the other two channels. That is, for the same magnitude of Ca current mediated by each of the three channels, more secretion (5 fold larger) is elicited when Ca enters through the facilitation pathway. Thus it appears likely that the facilitation Ca channel is most closely coupled to exocytotic sites. These results support the hypothesis that these channels may be the underlying regulatory system in the "fight or flight" response.

723.10

Characterization of Sodium-Dependent Oscillations Induced by Calcium Channel Blockers in Leech Neurons. J.D. Angstadt*, A.M. Saran, and D.S. Wolfe. Dept. of Biology, Siena College, Loudonville, NY 12211.

Superfusion of leech ganglia with Co^{2+} or Ni^{2+} saline (Ca^{2+} replaced with an equimolar concentration of Co^{2+} or Ni^{2+}, respectively) evokes synchronized, Na^+-dependent membrane potential oscillations (Angstadt and Friesen, 1991, J. Neurophysiol. 66:1858-1872). Our working hypothesis is that the depolarizing segment of these oscillations is generated by Na^+ influx through non-inactivating Na^+ channels and that repolarization depends on activation of an electrogenic Na^+/K^+ pump. Based on this hypothesis, we predicted that other effective Ca^{2+} channel blockers would also evoke oscillations. To test this prediction, we superfused isolated leech ganglia with La^{3+} and Cd^{2+} salines. Consistent with our hypothesis, both salines evoked slow membrane potential oscillations in Retzius cells. However, evoked oscillations occurred only transiently in most preparations, with rhythmic activity ceasing after a 3-6 min. This is similar to oscillations induced by Mn^{2+} saline, but in contrast to oscillations evoked by Co^{2+} saline, which persist for hours. The explanation for the variable stability of oscillations evoked by different Ca^{2+} channel blockers is not yet clear.

Previous studies showed that the Na^+/K^+ pump inhibitor ouabain disrupts oscillations by causing prolonged depolarization (Angstadt and Friesen, 1991). To assess further the role of the Na^+/K^+ pump in the repolarizing segment of oscillations, we superfused ganglia with K^+-free Co^{2+} saline (K^+ replaced with an equimolar concentration of Na^+). Removal of extracellular K^+ also would be expected to suppress pump activity. As expected, K^+-free saline increased the duration of oscillations by prolonging the duration of the depolarizing plateaus, providing further evidence that the Na^+/K^+ pump contributes to repolarization of the oscillations.

723.11

A MECHANISM FOR ECTOPIC BURSTS OF IMPULSES IN CENTRAL DEMYELINATED AXONS. R. Kapoor[1], P.A. Felts[2] and K.J. Smith[2*]. [1]Inst. Neurol., Queen Sq., London, UK, and [2]Depts. of Neurol. and Anatomy, UMDS, Guy 's Campus, London, UK.

The mechanisms by which ectopic discharges and positive symptoms are produced in diseases of myelinated axons remain poorly understood. We have recently shown (Soc. Neurosci. 41.1, 1992) that bursts of impulses can be induced in normal myelinated fibres by a raised periaxonal, internodal $[K^+]$: the locally elevated extracellular $[K^+]$ results in inward, and thereby excitatory, K^+ currents. When recorded periaxonally the K^+ currents produce prolonged negative voltage shifts and bursts of impulses. Using *in vitro* techniques we have now obtained intraxonal recordings of ectopic bursts of impulses occurring spontaneously in rat dorsal column axons passing through experimental demyelinating lesions. In some recordings, judged to be at or near the site of generation of the bursts, the discharges were associated with periodic, slow depolarisations of the axonal membrane. Bursting could be induced in demyelinated axons by high frequency stimulation, consistent with a rise in $[K^+]_o$. Upon movement of the recording micropipette from an intraxonal to a periaxonal position, the depolarisations underlying the bursts changed to negative shifts similar in appearance to those described above in normal fibres with high periaxonal $[K^+]$. In addition, periodic hyperpolarization and conduction block occurred during tetanic stimulation, suggesting activity of the Na^+-K^+ ATPase. We suggest that ectopic discharges in demyelinated axons may result from an elevated $[K^+]_o$ (arising either as a result of K^+ accumulation during periods of intense axonal firing, and/or because of impaired K^+ buffering), and that the discharges may be interrupted periodically by hyperpolarisation resulting from Na^+-K^+ ATPase activity.

723.12

SPONTANEOUS "BURST" AND "REGULAR" FIRING IN DISSOCIATED NEURONS FROM RAT NEOCORTEX

E.Guatteo, A.Bacci, S. Franceschetti, G. Avanzini and E.Wanke (SPON: European Neuroscience Association) Dip. di Fisiologia e Biochimica Generali and Istituto Neurologico "C. Besta", Milano I-20133, Italy.

Intracellular recording in the in vitro slice preparation and whole-cell, patch-clamp recording of acutely dissociated neurons from the rat (postnatal days 14-16) sensimotor cortex were combined to study the pattern of firing. The classical "regular firing" and "intrinsically bursting" behaviour was found to be present in both preparations suggesting the idea that intrinsic membrane properties underly the excitable properties. Dissociated neurons showed resting potentials around -65 mV and very high input resistance R_I (1 GΩ, n=9). The high fluctuations of the resting potential in the region -60/-80 mV caused frequently spontaneous firing. In 5 out of 9 neurons spontaneous repetitive firing was observed for more than 30-40 min. Various shapes of the action potentials (AP) were observed: brief (1-2 ms) and high (110 mV) APs, spikes with shoulder, broad and small spikes. Reversible pharmacological treatments with TTX, QX-314, Cd^{2+}, Ni^{2+}, DHPs and omega-CgTx suggested the cooperation of various Na^+ conductances and T, N, L and P Ca^{2+} channels in the excitable behaviour even around the resting potential region. Voltage clamp analysis of the ionic conductances are in progress also on neurons from older animals in order to study the ion channels ontogenesis in the neocortex.

723.13

SYNAPTIC ACTIVATION TRIGGERS SODIUM SPIKES IN THE APICAL DENDRITES OF LAYER V CORTICAL PYRAMIDAL CELLS

W.G. Regehr*[1], J. Kehoe[2], P. Ascher[2], C.M. Armstrong[1], [1]Dept of Physiology, University of Pennsylvania, Philadelphia, PA 19104; [2]Ecole Normale Supérieure, Paris, France.

We have recorded from layer V pyramidal cells in thin slices of rat motor cortex. The soma was voltage-clamped by means of a low resistance patch pipette. Depolarizations were induced in distant, unclamped regions of the neuron by activating synapses far out on the apical dendrite. This caused small, slowly rising synaptic currents as recorded at the soma. The synaptic current in the unclamped regions sometimes elicited regenerative responses, which were seen at the soma as a complex, multicomponent current spike. The later components of the spike complex could be blocked by hyperpolarization, leaving only the synaptic current and the first component. Local application of TTX to the soma and basal dendrites eliminated the later spike components but spared the first spike, which apparently was synaptically triggered far out in the apical dendrite. Extracellular stimulation sometimes caused a short latency response that we identified as a directly stimulated dendritic sodium spike. Step depolarizations applied to the soma also caused multiple TTX-sensitive spikes, which merged into a single spike for large depolarizing steps. TTX applied to the apical dendrite significantly reduced the amplitude of the merged spike. The major conclusion from our work is that sodium action potentials are generated in the apical dendrite in response to synaptic input.

723.14

A PERSISTENT SODIUM CURRENT UNDERLYING SUBTHRESHOLD MEMBRANE POTENTIAL OSCILLATIONS IN MESOPONTINE CHOLINERGIC NEURONS *IN VITRO*.

C.S. Leonard* & Prashanth Kumar A.K., Center for Neural Science, New York University, 6 Wash. Pl., NY, NY 10003.

Brainstem cholinergic neurons are believed to play a pivotal role in triggering rapid eye movement sleep through a sustained increase in their action potential firing rate. The repetitive firing of these cells is controlled, in part, by an intrinsic, subthreshold membrane potential oscillation that is TTX sensitive (Leonard & Llinás, *Soc. Neurosci. Abst* 15: 1144, 1989). Here we report that a persistent Na current which activates at membrane potentials negative to the inactivating Na current, contributes to these membrane potential oscillations. Current and voltage clamp experiments were conducted in guinea pig brainslices of the laterodorsal tegmental nucleus (LDT) using both intracellular and whole-cell recording methods. Intrinsic oscillations were observed at membrane potentials between -60mV and spike threshold. In this range, brief depolarizing current pulses produced plateau potentials that were enhanced in Ringer containing 0.1mM Ca, Cd (0.4mM),TEA (15mM) and Cs (2mM) and were blocked by TTX (0.5μM). Steady-state I-V curves indicated the presence of TTX-sensitive inward rectification and the subtraction currents from before and after TTX revealed a distinct inward current. This current fully activated within 20mS and showed no inactivation for the duration of the voltage steps (>100mS). Since this current actives at membrane potentials positive to -65mV, it contributes to the TTX-sensitive plateau potentials and provides depolarizing drive for the membrane potential oscillations as well as enhancing subthreshold synaptic input. Supported by NS27881.

723.15

PLASTICITY OF SODIUM AND POTASSIUM CHANNELS IN RAT BLADDER AFFERENT NEURONS FOLLOWING SPINAL CORD INJURY OR PARTIAL URETHRAL OBSTRUCTION. Naoki Yoshimura* and William C. de Groat. Dept. of Pharmacology, Univ. of Pittsburgh, Pittsburgh, PA 15261.

Spinal cord injury (SCI) or partial urethral obstruction (UO) in rats enhance the spinal micturition reflex pathway and induce hypertrophy of the urinary bladder (UB) and bladder afferent neurons (B-AN) in dorsal root ganglia. This study examined the functional changes in B-AN following SCI and UO. Whole-cell patch-clamp recordings were performed on acutely dissociated B-AN labelled by axonal transport of a fluorescent dye (Fast Blue) injected into the bladder wall. Transection of the spinal cord (T_8-T_9) or partial ligation of the urethra performed 4 and 6 weeks, respectively, prior to the experiment resulted in 4- and 7-fold increases in UB weight and hypertrophy of B-AN. After SCI, the following functional changes were observed: (1) a reduction ($p<0.01$) in thresholds for action potential and Na^+ current activation from -20.4 ± 0.9 (mean ± s.e., n=20) and -26.5 ± 1.7 mV (n=15) to -27.9 ± 0.9 (n=25) and -38.9 ± 1.1 mV (n=11), respectively. (2) an increase ($p<0.01$) in TTX-sensitive Na^+ current densities from 17.9 ± 9.2 (n=13) to 80.6 ± 16.1 pA/pF (n=11) in association with a decrease ($p<0.05$) in TTX-resistant Na^+ current densities from 60.1 ± 5.5 (n=13) to 32.1 ± 9.5 pA/pF (n=11). (3) a shift ($p<0.05$) toward negative membrane potentials in the voltage dependence of steady-state inactivation of A-type K^+ current: 1/2 maximal inactivation voltages changed from -79 ± 3.6 (n=9) to -89 ± 4.9 mV (n=11). However, after UO, these properties of B-AN did not differ significantly from those in normal rats except for a moderate increase ($p<0.05$) in TTX-sensitive Na^+ current densities to 51.2 ± 10.3 pA/pF (n=16). These results indicate that SCI in comparison to UO produces more prominent functional changes in B-AN, although both conditions produce similar hypertrophy of UB and B-AN. This suggests that changes within the spinal cord as well as in the hypertrophied bladder muscle may be required to induce functional plasticity in B-AN following SCI.

723.16

DIVERSITY OF Na AND Ca CURRENTS IN CHOLINERGIC NUCLEUS BASALIS NEURONS. R. Klink*, M.P. Faure, A. R. KayΥ and A. Alonso. Dept. Neurology & Neurosurgery, McGill University, MNI, Montreal, Canada H3A 2B4; and ΥDept. of Biol. Sci., Univ of Iowa, Iowa City, IA 52242.

Magnocellular basal forebrain (BF) cholinergic neurons are characterized electrophysiologically by transient outward rectification and prominent low-threshold rhythmic bursting. In order to investigate the voltage-gated ionic currents underlying this rhythmicity, neurons from the guinea pig substantia innominata and magnocellular preoptic nucleus were acutely dissociated. Choline acetyl transferasase immunostaining demonstrated that 75% of the dissociated neurons and all those with a large rounded soma with 2-3 dendritic processes were cholinergic. Moreover, only the cholinergic cells bound and internalized fluoro-neurotensin and hence could be used as a marker. Whole-cell patch-clamp recordings demonstrated in all magnocellular cholinergic neurons a transient outward K current and at least two components in both the Ca and Na currents. With 500ms step depolarizations from a V_h of -90mV a transient (T-type) Ca current activated at a threshold of ~ -60 mV and inactivated rather slowly with a τ of ~90ms at -50 mV. A second, persistent Ca-current component activated at a threshold of ~45mV. With respect to the Na currents, inactivation kinetics analysis revealed the existence of two distinct components. At -50mV the Na-current decayed as a single exponential with a τ of ~30ms while positive to -30mV both a fast and slowly decaying component were required to obtain a good fit. While the T-type Ca-current is the main current responsible for the bursting behavior, the low-threshold slowly inactivating Na-current can also provide pacemaker drive and may be involved in sustaining the oscillatory behavior of the BF cholinergic neurons.

Supported by grants from the MRC (to AA) and NIH (to ARK)

723.17

IONIC CURRENT IN NEURONS ACUTELY DISSOCIATED FROM ADULT COCKROACH'S PROTOCEREBRUM

X.-S. Zhang[1], J. Inouchi[1], J. Chen[1], T. Inoue[1] and M. Ichinose*[2]. [1]Lab. of Neurophysiology, Nat. Ins. Seri. and Entom. Sci., Tsukuba, Ibaraki 305 and [2]Dept. of Physiology, Shimane Medical Univ., Izumo 693, Japan

Neurons were acutely dissociated from the protocerebrum of adult cockroach (*Periplaneta americana*) by both enzymatic and mechanical isolation. Collagenase 1mg/mL and hyaluronidase 1mg/mL were used for enzyme treatment. Isolated neurons with neurites survived for more than 4 hr after the isolation. The electrical properties of the dissociated neurons with a diameters in the range of 10-15 μm were studied with whole-cell patch clamp recording. Voltage dependent currents could be separated into two ionic components: an inward calcium current and an outward potassium current. The calcium current was recorded in the external saline without Ba^{2+} and was blocked by Cd^{2+}. Maximum activation occurred near 0 mV. The magnitude of potassium current increased with depolarization and decreased by TEA and 4-AP. No sodium current was recorded. The result demonstrated that acutely dissociated protocerebrum neurons from adult cockroach with neurites can be used for electrophysiological and pharmacological studies on ionic channel current.

Supported by SCF in the basic research core system from STA, Japan.

723.18

DIABETES MELLITUS IS ASSOCIATED WITH ALTERED CALCIUM CURRENTS BUT NOT POTASSIUM CURRNTS IN CAPSAICIN-SENSITIVE PRIMARY AFFERENT NEURONS. J. Wiley*, K. Hall, D. Mann. Dept. of Internal Medicine, University of Michigan, Ann Arbor, MI. 48109

Little is known about abnormalities in neural function that underlie diabetic autonomic neuropathy. We examined the possibility that DM is associated with functional changes in voltage-activated calcium and potassium currents present in capsaicin-sensitive dorsal root ganglion (DRG) neurons. Wistar bio-bred (BB) rats were used in these studies: i) adult male non-diabetic controls (C) and matched diabetics (D), DM duration 12-32 wks treated with insulin to maintain serum glucose between 350-450 mg%. These animals demonstrate histopathological criteria for diabetic peripheral neuropathy. Currents were recorded using the whole-cell variation of the patch clamp technique. I_{Ca} were recorded in media that blocked Na^+ and K^+ currents. I_K were recorded separately in the presence of tetrodotoxin. Capsaicin-sensitive DRG's demonstrated several I_{Ca} and I_K current components that differed in their voltage ranges for activation, inactivation and responses to nifedipine (NF; antagonizes L-type calcium currents evoked from a holding potential (V_h)= -40mV at clamp potential (V_c)= +10mV, ω-conotoxin (ω-CTx; antagonizes N-type calcium currents evoked from V_h= -80mV at V_c= +10mV), cadmium (Cd, 0.5mM; blocks I_{Ca}), TEA (1mM; inhibits $I_{K(Ca)}$) and charybdotoxin (Cbtx, 20nM; antagonizes $I_{K(Ca)}$). In C the mean peak I_{Ca} evoked from -40mV was 3.42±.28 nA and from -80mV was 4.30±.31 nA. The D group demonstrated a significant increase in the peak of the I_{Ca} evoked from -40 mV (5.85±.35 nA) and from -80 mV (7.89±.50 nA) ($p<.001$). The C and DM groups did not show any shift in the peak of the I_{Ca} current-votage relation or the voltage-dependence of steady-state inactivation. In C, NF(10 μM) reduced I_{Ca} evoked from -40mV by 32% and ω-CTx(1 μM) reduced I_{Ca} evoked from -80mV by 28% supporting the presence of additional (NF- and ω-CTx-insensitive) I_{Ca} in DRGs. The effects of NF and ω-CTx were similar in the C and D groups. Outward currents were evoked from V_h= -80mV at V_c= +20mV. The mean peak amplitude of outward currents in C were 17.5±.95 and in D 16.1±1.1 nA ($p=.31$). The peak of the outward currents were reduced similarly in C and D by Cd, TEA and Cbtx. In conclusion, DM was associated with an increase in high voltage-activated I_{Ca} in DRG neurons but outward I_K currents were not affected. Altered regulation of $[Ca]_i$ reportedly contributes to cellular injury and the increase in I_{Ca} may, thereby, participate in the mechanisms that underlie DM autonomic neuropathy.

723.19

ELECTROPHYSIOLOGICAL AND IMMUNOLOGICAL EFFECTS OF LEUKOTRIENE B_4 COMPARED TO LIPOPOLYSACCHARIDES ON CULTURED ASTROCYTES.

H. Köller, W. Däubener#, M. Siebler, K. Pilz#.
Spon: European Neuroscience Association
Dept. of Neurology and #Dept. of Microbiology, Heinrich - Heine University, Moorenstraße 5, D 4000 Düsseldorf, Germany

After stimulation with immunologically active substances like Leukotriene B_4 (LTB_4) and lipopolysaccharides (LPS) electrophysiological properties of cultured astrocytes from new-born rats were tested in order to investigate a coupling between immunological activation and putative functional impair-ment. Both substances lead to a marked depolarization which in case of LTB_4 (1μM) was mediated by a reduction of K^+ conductance. In contrast, LPS (5μg/ml) seemed to stimulate a Na^+ dependent uptake system and by this way induce a depolarization. Dexamethasone (1μM) inhibited the depolarization induced by LTB_4 in contrast to that induced by LPS. As a marker for immunological activation the production of IL-6 was tested after stimulating the astrocytes either by LTB_4 or LPS. LTB_4 in contrast to LPS did not induced a marked production of IL-6.

Our results indicate that both substances lead to a marked alteration of electrophysiological properties by specific intracellular mechanisms and the depolarization is not a stimulus for the production of IL-6. The marked depolarization, which e.g. impairs the glial capacity for maintaining ional homeostasis, may contribute to neuronal and glial dysfunction during inflammatory CNS diseases.

(Supported by the Deutsche Forschungsgemeinschaft, SFB 194, B7 / B8).

724.1

DIFFERENTIAL DOPAMINERGIC REGULATION OF STRIATAL AND HIPPOCAMPAL ACETYLCHOLINE RELEASE IN VIVO. A. Imperato*, M.C. Obinu, L. Dazzi and G.L. Gessa. Department of Neuroscience Bernard B. Brodie, University of Cagliari, via Porcell, 4 09124 Italy.

The existence of dopaminergic afferents to the main cholinergic brain areas has been well established, suggesting a possible functional interaction between these two systems. However, the mechanism through which this interaction takes place *in vivo* is still under investigation.

We have studied by means of brain microdialysis and by the use of selective D_1 and D_2 dopaminergic drugs, the dopaminergic control over the cholinergic transmission in the striatum, where cholinergic neurons appear to be interneurons, and in the hippocampus, which is site of termination of cholinergic neurons originating mainly from the septum. SKF 38393 (10 mg/kg i.p.) increased (50%), while LY 171555 (0.2 mg/kg i.p.) decreased (30%) acetylcholine release in the striatum.
In contrast, both compounds enhanced acetylcholine release in the hippocampus (82 and 95%, respectively), and also caused an additive effect when injected together (182%). Moreover, cocaine (10 mg/kg) and amphetamine (2 mg/kg) induced a much greater enhancement of acetylcholine release in the hippocampus compared to the striatum.

These results suggest that dopaminergic mechanisms regulating acetylcholine release in the hippocampus are different from those in the striatum. Moreover, while D_1 and D_2 receptors appear to exert an opposite control on striatal acetylcholine release, they only show a facilitatory and synergistic effect on hippocampal acetylcholine release.

724.2

GLUCOSE ATTENUATES MORPHINE-INDUCED DECREASES IN HIPPOCAMPAL ACETYLCHOLINE OUTPUT. M.E. Ragozzino*, G.L. Wenk[1] & P.E. Gold. Dept. Psychology, U. Virginia, Charlottesville, VA 22903 and [1]Div. Neural Systems, Memory and Aging, U. Arizona, Tucson, AZ 85721.

Glucose attenuates the effects of both systemic and intraseptal morphine injections on memory and other measures. One hypothesis is that the morphine-induced behavioral impairments may be the result, in part, of a decrease in acetylcholine (ACh) release which can be reversed by administration of glucose. In the present experiment, we employed *in vivo* microdialysis procedures in freely moving rats to measure ACh release in the hippocampus after morphine and glucose injections. Dialysate samples were collected every 12 min. From 20-40 min after morphine (IP, 10 mg/kg) injections, ACh output was reduced by 30% compared to baseline levels. Concomitant treatment with glucose (100 mg/kg) blocked this effect, with ACh output remaining at baseline. Glucose and saline injections alone produced a transient increase in ACh output evident only during the first 12 min after injection, apparently in response to handling during injection. Thus, glucose attenuates morphine-induced decreases in ACh release in the hippocampus under pharmacological conditions similar to those employed in studies in which glucose attenuated morphine-induced behavioral deficits. These findings are consistent with the view that regulation of acetylcholine release contributes to pharmacological regulation of memory and other measures. [Supported by NSF BNS90-12239, NSF BNS 89-14941, NIA AG07648, ONR N0001489-J-1216, and NIMH 5-T-32-MH18411].

724.3

RECEPTOR-MEDIATED EFFECTS OF INTERLEUKIN-2 ON HIPPOCAMPAL ACETYLCHOLINE RELEASE. D.Seto* & R.Quirion. Douglas Hospital Research Centre & Dept of Pharmacology & Therapeutics, McGill University, Montréal, Québec H4H 1R3

Previous studies have suggested: 1) the presence of IL-2 immunoreactivity in brain (Luber-Narod & Rogers 1988; Araujo et al., 1989; Lapchak et al., 1991); 2) the existence of one of the highest densities of [125I]-IL-2 binding sites in the hippocampus, a region of enriched ChAT immunoreactivity (Araujo et al., 1989); 3) that IL-2 and IL-2R immunoreactivities are present in, respectively injured rat brain and Alzheimer-lesioned brains (Nieto-Sampedro & Chandry 1987; Luber-Narod & Rogers 1988); 4) that IL-2 plays a possible modulatory role in acetylcholine (ACh) release from the hippocampus (Araujo et al., 1989); 5) that chemically lesioned hippocampi exhibit more [125I]-IL-2 binding sites and are more sensitive to IL-2 inhibition of ACh release (Araujo et al., 1989). Since it is believed that deficits in cholinergic transmission underlie some of the cognitive hallmarks of Alzheimer's disease and given the observations that point to the normal and diseased hippocampus as a target for IL-2 modulation, it is of importance to define the spectrum of effects of IL-2 on cholinergic neurotransmission in the hippocampus. Recently, using *in vitro* brain-slice superfusion, we established that IL-2 modulates K(+)-evoked hippocampal ACh release in a biphasic dose-dependent (f*M*-potentiation; n*M*-inhibition), time-dependent, stimulation-dependent manner and displays a reversible profile (Hanisch et al., J Neurosci., in press). To test whether these effects are receptor-mediated, the anti-TAC antibody against the IL-2 receptor α subunit was used. Our results suggest that the inhibition by IL-2 of ACh release requires binding to low-affinity receptor subunit, demonstrating the specificity and the type of IL-2 receptor involved. Furthermore, profiles of IL-2 effects on ACh release were similar using either K(+) or veratridine as depolarising agents. It thus provides further evidence for the relevance of IL-2 as a modulator of ACh release in the hippocampus. (Supported by FRSQ & MRC)

724.4

ENDOGENOUS SEROTONIN FACILITATES ACETYLCHOLINE RELEASE *IN VIVO* FROM RAT DORSAL HIPPOCAMPUS VIA 5-HT-3 RECEPTORS. S.Consolo*, G. Russi, M.Zambelli and R.Bertorelli. Istituto di Ricerche Farmacologiche "Mario Negri", 20157 Milan, Italy.

The role of serotonin (5-HT) in regulating *in vivo* acetylcholine (ACh) release from the rat dorsal hippocampus was investigated using the microdialysis technique. The two 5-HT releasers d-fenfluramine and its active metabolite d-norfenfluramine or the 5-HT uptake inhibitor citalopram facilitated *in vivo* ACh output from dorsal hippocampi. The cholinergic effects of d-norfenfluramine (7.5 mg/kg, i.p.) and citalopram (10 μM, applied by reverse dialysis) were completely prevented by a 14-day chemical lesion of the raphe nuclei. The increase in ACh output induced by d-norfenfluramine (5 mg/kg, i.p.) was antagonized by the 5-HT-3 antagonists tropisetron (0.5 mg/kg, i.p.) and DAU 6215 (60 μg/kg, i.p.) but not by the mixed 5-HT-1 and 5-HT-2 antagonist metergoline (2 mg/kg, s.c.).The selective 5-HT-3 receptor agonist, 2-methyl-5HT (10 μM), applied by reverse dialysis, induced a rise in ACh release; this effect was abolished by concurrent infusion of tropisetron (10 μM). Thus, the overall control exerted by endogenous 5-HT on hippocampal ACh release *in vivo* is primarily facilitatory in nature.

724.5

SEROTONIN-3 RECEPTORS CONTROL INHIBITION OF ACETYLCHOLINE RELEASE FROM FRONTAL CORTEX AND FACILITATION FROM DORSAL HIPPOCAMPUS: A MICRODIALYSIS STUDY WITH 2-METHYLSEROTONIN AND DAU 6215. H. Ladinsky*[1], F. Borsini[1], R. Cortes[2], J. M. Palacios[2], S. Giorgi[3], G. Russi[3] and S. Consolo[3]. [1]Boehringer Ingelheim Italia, Milan 20139, [2]Consejo Superior Investigaciones Cientificas, Barcelona 08034 Spain and [3]Istituto di Ricerche Farmacologiche "Mario Negri" Milan 20157 Italy.

We investigated whether 5-HT_3 receptors modulate in vivo ACh release from rat frontal cortex (FC) and dorsal hippocampus (DH), ACh-rich brain areas showing significant amounts of specific 5-HT_3 binding sites. In FC, the selective 5-HT_3 agonist 2-methyl-5-HT (125-1000 μg, i.c.v.) reduced ACh output in a dose-dependent manner. The inhibitory effect was slow in onset (reaching its nadir of 40% at 120 min), and was long lasting (>240 min). By contrast, in DH, 2-methyl-5-HT raised ACh output, giving a bell shaped dose response curve. The facilitatory effect (25% and 50%; 125 and 250 μg, i.c.v.) was rapid in onset and transient, while 500 μg and 1000 μg, i.c.v. doses were ineffective. The potent, selective 5-HT_3 antagonist DAU 6215 (30 μg/kg, i.p.) blocked the effects of 2-methyl-5-HT. Autoradiographic analysis of the regional localization of [^{3}H]DAU 6215 showed concentrations of these binding sites in the external layers of the FC, and in the hippocampus. Thus, the data show that 5-HT_3 receptors oppositely control ACh output from FC and DH and suggest differences in neuronal circuitry involved.

724.6

EFFECTS OF ACUTE AND CHRONIC MORPHINE ON EXTRACELLULAR ACETYLCHOLINE IN THE NUCLEUS ACCUMBENS AND PREFRONTAL CORTEX OF FREELY MOVING RATS. P. Rada*, G.P. Mark and B.G. Hoebel. Dept of Psychology, Princeton University, Princeton, NJ 08544.

We have previously reported a decrease in extracellular acetylcholine (ACh) in the nucleus accumbens (NAC) during acute morphine treatment; after 7 daily injection, this decrease in ACh was no longer apparent, and a dose of naloxone caused a large increase in ACh[1]. In the present microdialysis study basal recovery of ACh was significantly and chronically lowered by an escalating dose of morphine (from 20 raised to 80 mg/kg in 1 wk). In the NAC, ACh decreased from 600 ± 60 fmol in naive rats down to 370 ± 75 fmol in morphine dependent rats ($T=2.36$; $Df=33$; $p<.05$). No basal chronic effect was observed in the PFC. Superimposed on these basal levels, acute morphine (20 mg/kg; ip) significantly increased extracellular ACh levels in the PFC ($F(1,8)=3.7$; $p<.001$) while decreasing it in the NAC. Following chronic exposure, morphine (80 mg/kg; ip) still produced an increase in ACh in the PFC ($F(1,8)=5.6$; $p<.001$) and a decrease in the NAC ($F(1,8)=8.5$; $p<.001$). Naloxone (5 mg/kg; ip) caused an exaggerated increase in ACh in both PFC and NAC. These results confirm and extend evidence that cholinergic systems are involved in opiate dependence and withdrawal. Supported by USPHS grant 30697.

1. Rada et al. *Brain Research* **561:** 354-356; 1991.

724.7

INCREASES IN STRIATAL ACETYLCHOLINE BY SKF-38393 ARE MEDIATED THROUGH D1 DOPAMINE RECEPTORS IN STRIATUM AND NOT THE FRONTAL CORTEX. A. Zocchi* and A. Pert BPB, NIMH, Bethesda MD 20892

There is considerable evidence to suggest that D1 and D2 dopamine receptor agonists exert differential effects on striatal cholinergic function. D2 receptor agonists appear to inhibit striatal acetylcholine (ACh) release while D1 agonists increase release. While D2 receptor agonists seem to modulate ACh release through D2 receptors located directly on the cholinergic interneurons, the mechanism underlying the actions of D1 receptor agonists on cholinergic function remain controversial. It has been proposed by some that the D1 receptor effects are mediated through striatal actions, while others have suggested that they are determined indirectly through the frontal cortex. The experiments reported here represent a further attempt to resolve this controversy. It was found that focal applications of the inactive and active enantiomers of SKF-38393 (A D1 dopamine receptor agonist) to the rat striatum via reverse dialysis increased extracellular ACh in a stereoselective manner. Infusions of SKF-38393 into the frontal cortex, on the other hand, were ineffective in altering striatal ACh. Furthermore, partial hemisections caudal to the frontal cortex did not alter the ability of systemically administered SKF-38393 to increase striatal ACh. Taken together, these results suggest that at least some of the effects of D1 receptor agonists on striatal cholinergic function are mediated through actions in the striatum and not the frontal cortex.

724.8

NICOTINIC RECEPTOR AGONISTS INCREASE ACETYLCHOLINE AND NOREPINEPHRINE LEVELS IN RAT CORTEX: A MICRODIALYSIS STUDY. K.L. Summers* and E. Giacobini. Dept. Pharmacology, Southern Illinois University School of Medicine, Springfield, IL 62794-9230.

Patients suffering from Alzheimer's disease (AD) may benefit from treatment with nicotinic agents as nicotine has been shown to improve memory. Transverse microdialysis was employed to compare the effects of several nicotinic receptor agonists on the simultaneous release of acetylcholine (ACh), norepinephrine (NE), dopamine (DA), and serotonin (5-HT) in rat cortex in vivo. No cholinesterase inhibitor (ChEI) was added to the perfusate. L-nicotine (3.6 μmol/kg, s.c.) significantly increased ACh levels (100%) in the dialysate for 2 hrs and significantly increased NE levels (85%) for 1 hr. D-nicotine (3.6 μmol/kg, s.c.) increased ACh and NE (30%) for 1 hr, indicating stereoselectivity. Metanicotine (3.6 μmol/kg, s.c.) elevated ACh (110%) and NE (80%) for less than 2 hrs. Anabaseine (3.6 μmol/kg, s.c.) appears to be more potent than nicotine at elevating levels of both ACh (150%) and NE (100%) while fluoronicotine (3.6 μmol/kg, s.c.) appears to be less potent at elevating ACh (70%) and NE (20%). No significant effects of these compounds have been observed on DA or 5-HT levels. (Supported by R.J. Reynolds Tobacco Co.)

724.9

BOTULINUM TOXIN A ENHANCED PROTEIN PHOSPHORYLATION AND INHIBITION OF ACETYLCHOLINE RELEASE. G.H. Sterling*, K.E. Asermely, M.R. McCafferty and J.J. O'Neill. Dept.of Pharmacology, Temple Univ. Sch. of Med., Phila., PA 19140

Considerable evidence supports the role of phosphorylation of neuron specific proteins, e.g., synapsin and synaptophysin, in regulation of neurotransmission. In this study concerning factors regulating presynaptic cholinergic function, the effect of Botulinum toxin A (BoNT A), a potent inhibitor of ACh release, on protein phosphorylation was examined. Synaptosomes from rat cerebral cortex were preincubated with BoNT A (0-100 nM) followed by 45 mins with ^{32}P-orthophosphate. Proteins were separated by SDS-PAGE gels with radioactivity identified by autoradiography and liquid scintillation counting. BoNT A increased, in a dose dependent manner, the steady state phosphorylation of several neuron specific proteins: e.g., the vesicle specific 38 kDa protein, synaptophysin, identified by Western blot; a 43 kDa protein (possibly B50), thought to be associated with neurotransmitter release; an unidentified 28 kDa component and a doublet of 80-86 kDa, resembling the synapsins which depend on cAMP dependent kinase for phosphorylation. 4-Aminopyridine, known to reverse BoNT-induced inhibition of ACh release, restored steady state phosphorylation toward control levels. These effects of BoNT A on protein phosphorylation may provide important insights into presynaptic factors regulating cholinergic neurotransmission and lead to sites for drug intervention in BoNT-induced toxicity.

724.10

EFFECTS OF PHYSOSTIGMINE AND SOME NITRIC OXIDE-CYCLIC GMP RELATED COMPOUNDS ON MUSCARINIC RECEPTOR-MEDIATED AUTOINHIBITION OF HIPPOCAMPAL ACETYLCHOLINE RELEASE. T. Suzuki and K. Kawashima*, Department of Pharmacology, Kyoritsu College of Pharmacy, Tokyo 105, JAPAN.

We have investigated the effects of 1) the cholinesterase inhibitor physostigmine and 2) drugs which are known to change intracellular cGMP levels, on the autoinhibition of acetylcholine release from rat hippocampal slices. Autoinhibition was triggered by submaximal electrical stimulation in both the absence and presence of physostigmine. The results obtained indicate that an unusual increase in the extracellular acetylcholine content, such as that induced by cholinesterase inhibition, is not essential for autoinhibition triggering. Dibutyryl cGMP reduced significantly the stimulation-evoked acetylcholine release in the presence, but not in the absence, of atropine. Neither sodium nitroprusside nor glyceryl trinitrate exerted a dibutyryl cGMP-like effect. N^G-Nitro-L-arginine did not lessen the autoinhibition. These results indicate that an increase in the intracellular cGMP level reduces acetylcholine release, and that the muscarinic receptor stimulation-nitric oxide synthesis-(soluble) guanylyl cyclase activation pathway is not involved in the cholinergic autoinhibition processes.

724.11

EFFECT OF NITRIC OXIDE ON ACETYLCHOLINE RELEASE IN THE STRIATUM: A MICRODIALYSIS STUDY. R. Guevara-Guzman*†, K.M. Kendrick, R. Senaris and P.C. Emson. Dept Neurobiology, AFRC Babraham Institute, Babraham, Cambridge, U.K., †Dept Physiology, Faculty of Medicine, Natl. Univ. Mexico. Mexico 04510, D.F.

Nitric oxide (NO) has recently been shown to function as a neuronal messenger in the brain. Furthermore it has been reported that activation of NMDA receptors leads to the formation and release of NO. The aim of this paper was to study the effect of different NO releasers on acetylcholine (ACh) release *in vivo*. The experiments were conducted in male wistar rats anaesthetized with urethane. Microdialysis probes (CM-12, 3 mm membrane length, CMA microdialysis) were placed in the striatum. Ringer (pH 6.0) containing 10 μM neostigmine was pumped through the probes at 1.5 μl/min using a CMA 100 pump. A liquid switch was used to introduce different substances into the system. Sampling was at 15 min intervals and ACh was measured using HPLC with electrochemical detection. NMDA (50μM) stimulated ACh release and this was blocked by the NMDA receptor antagonist APV. The NO releasers, sodium nitroprusside (SNP) isosorbide (ISO), S-nitroacetyl penicillamine (SNAP) and S-nitrosoglutathione (SNOG) all increased ACh release at 10^{-3}. Potassium ferrocyanide, penicillin and glutathione, which do not produce NO, did not alter ACh release. When NO gas was bubbled through buffered Ringer to provide a pure NO stimulus via the microdialysis probe, the results showed that NO administered in this manner also stimulated ACH release. This NO effect was Ca++ dependent and could be blocked by the NO scavenger haemoglobin or by the NO synthase inhibitor, nitroarginine. The effect of NO was not mediated via the NMDA receptor since SNAP still increase ACh release in the presence of APV concentrations that blocked its release in response to NMDA. The cGMP agonist, 8-bromo-cyclic GMP and dibutyrl-cyclic GMP at 10^{-3} also increased ACh release suggesting that the effect of NO on ACh release might be mediated via cGMP. This data proved that NO may play a significant role in ACh release in the striatum.

724.12

EFFECTS OF CONCUSSIVE BRAIN INJURY ON NEUROTRANSMITTER MONOAMINES AND ACETYLCHOLINE IN THE RAT BRAIN. K.Shima, S.Okuyama*[1], Y.Imagawa[1] and S.Ogawa[1]. Dept. of Neurosurgery, National Defense Medical College, Tokorozawa, 359; [1]Dept. of Pharmacology, Research Center, Taisho Pharmaceutical Co. LTD., Omiya, 330

Previous studies have demonstrated a transient release of acetylcholine (ACh) after moderate fluid-percussion injury. The objective of the present study is to investigate whether there is a similar release of ACh in the rat brain after closed head injury (CHI) and to determine whether there are accompanying changes in other aminergic neurotransmitters following CHI. Male Wistar rats were divided into a CHI group and a sham control group. CHI was induced by dropping a 400 g weight from 70 cm on the vertex. The tissue concentrations of the neurotransmitter monoamines dopamine (DA), norepinephrine (NE), serotonine (5-HT), and acetylcholine (ACh) and of their major metabolites (dihydroxyphenyl acetic acid (DOPAC), homovanillic acid (HVA) and 5-hydroxyindole acetic acid (5-HIAA)) were measured using HPLC in 9 brain areas. Measurements were determined at 15 min, 30 min, 2 h, 4h and 24 h after CHI. Compared to sham controls, the concentrations of DA, NE, 5-HT and ACh up to 30 min after CHI were decreased or unchanged in the many brain areas, whereas DOPAC and HVA levels were significantly increased in the amygdala, pons and cerebellum. 5-HIAA level was increased in the amygdala, hippocampus and cortices 2-24 h after CHI. At 24 h after CHI ACh level was significantly ($p<0.001$) increased in 7 of 9 areas. These results demonstrate that concussive brain injury in the intact skull model, as well as fluid- percussion injury through a vertical craniectomy, causes a transient increase in cholinergic neuronal activity in the pontine area. Moreover, monoaminergic neuronal derangements may be related to the pathophysiology of concussive CHI.

724.13

EXTRACELLULAR RELEASE OF ACETHYLCHOLINE, NORADRENALINE AND SEROTONIN IN THE CEREBRAL CORTEX INCREASES IN RESPONSE TO WALKING IN RATS.

M. Kurosawa*, Y. Sakiyama and A. Sato. Department of Autonomic Nervous System, Tokyo Metropolitan Institute of Gerontology, 35-2 Sakaecho, Itabashi-ku, Tokyo-173, Japan.

Cerebral cortical blood flow (CBF) increases during exercise. We have demonstrated that CBF increases via cholinergic nerve fibers originating in the nucleus basalis of Meynert in rats. In the present experiments, we examined whether release of acethylcholine (ACh) in the cerebral cortex increased during walking in rats. Release of noradrenaline (NA) and serotonin (5-HT) in the cerebral cortex which contribute to the vasoconstirictive regulation of the CBF was also investigated. Extracellular release of ACh, NA and 5-HT in the parietal lobe of the cerebral cortex was measured using a combination of microdialysis and high-performance liquid chromatography. Walking at the speed of 2.3 m/min for 5 min produced significant increases in ACh, NA and 5-HT release in the cerebral cortical extracellular space. The mean increase in ACh release (205 % of control) was much greater than that of NA (131 %) or 5-HT (133 %). It is suggested that the vasodilative effect of ACh on CBF during walking is more influential than vasoconstrictive effect of NA and 5-HT, and that the increased release of ACh in the cerebral cortex may contribute at least partly to the increase in CBF during walking.

724.14

HALOTHANE ANESTHESIA DIMINISHES ACETYLCHOLINE (ACh) RELEASE IN THE MEDIAL PONTINE RETICULAR FORMATION (mPRF).

J. C. Keifer*, L. Becker, R. Lydic
Department of Anesthesia, Pennsylvania State University, College of Medicine, Hershey, Pa 17033.

Anesthesia and sleep are distinctly different, but both states have features in common including: 1) altered EEG activity, 2) eye movements, 3) hypotonia, and 4) altered regulation of breathing. Since the mPRF is a cholinoceptive brainstem area known to be involved in REM sleep generation, we are testing the hypothesis that ACh release in the mPRF is altered during exposure to inhalational anesthesia. Cats were implanted with electrodes for measuring cortical EEG, EOG, and EMG. Following induction of anesthesia with 1.2% Halothane (measured by Raman Spectroscopy), a microdialysis probe was inserted into the mPRF. Microdialysis samples were collected at 10 minute intervals for measuring ACh. Replicate samples were collected at 1.2, 0.6 and 0% expired halothane. Analysis of variance for anesthetic effect on ACh release was significant ($F=18.155$, $p< .001$). ACh release was significantly reduced following exposure to 1.2% (-33%) and 0.6% (-31%) halothane when compared to the awake state. These observations are consistent with the hypothesis that mPRF levels of ACh contribute to generating some of the physiologic traits comprising the anesthetic state induced by halothane.

Support: Department of Anesthesia, FAER Grant (JCK), HL-40881(RL).

724.15

PHORBOL DIACETATE INCREASES TRANSMITTER RELEASE AND REVERSES THE EFFECT OF HALOTHANE AT THE NEUROMUSCULAR JUNCTION:

M. Sokoll*, S-I Lee, S. Kamath, T.Gerhold,
Department of Anesthesia, University of Iowa College of Medicine, Iowa City, IA 52242.

Previously we have demonstrated the ability of halothane (HAL) to decrease transmitter release from the motor nerve terminal. In this study we investigated the ability of phorbol-diacetate (PDA) to reverse this effect of HAL.

METHODS: We studied the effect of PDA (0.25, 0.5 and 1.0 μM) alone and combined with HAL (1.0%) on the miniature endplate current (MEPC) and endplate current (EPC) of the cut rat diaphragm preparation. The diaphragm was rapidly removed from a halothane anesthetized rat and mounted in a plexiglass bath. The tissue was perfused with control, PDA or PDA-HAL containing solutions sequentially. Transmembrane currents were recorded using the two microelectrode voltage clamp. MEPC's were analysed for amplitude and time constant of decay. EPC's were elicited at 0.4 and 40 Hz and analysed for amplitude and quantum content (m) (direct method).

RESULTS AND DISCUSSION: Addition of halothane 1% caused a 15% decrease in MEPC amplitude which was not altered by PDA. Halothane produced a 32% decrease in m of EPC #1 and a 40% decrease in m of the plateau phase of the tetanic train. These results indicate that PDA has the ability to reverse the halothane induced decrease of m of the indirectly stimulated motor nerve terminal and that probability of release and mobilization of transmitter are both involved.

724.16

NEUROMUSCULAR TRANSMISSION AND MUSCARINIC ACETYLCHOLINE RECEPTORS.

R.J. Storella* and T.S. Ackerman. Dept. of Anesthesiology, Hahnemann Univ., Philadelphia, PA 19102.

Measurements of Ca++ currents from motor nerve endings and tritiated acetylcholine (ACh) release indicate that muscarinic antagonists can alter ACh release at the neuromuscular junction. We investigated the effect of atropine on train-of-four (TOF) fade, which is used clinically to monitor neuromuscular transmission. TOF fade is due to a decrease in ACh release caused by d-tubocurarine (dTC). Isometric twitch tension (TT) was measured from a standard mouse phrenic nerve-diaphragm preparation at 37°C. TOF stimulation consisted of 4 pulses at 2 Hz every 11.5 seconds. Preparations were exposed to 0.75 μM dTC during a control period and then treated by adding either saline or 20 μM atropine (n=4 for each treatment). Block (first TT in the TOF to pre-dTC TT) and fade (fourth to first TT in the TOF train) were determined for both the control and treatments periods. There were no differences in the effect of treatment (expressed as percent of the control period) between the saline and atropine groups for either block (92.5±14 vs. 92.5±9.6) or fade (119±49.8 vs. 102±30). Additionally, for both treatment groups, there were no changes in the amount of fade between control and treated periods (paired t-tests). Thus, we could not find support for the implication from other studies that atropine might alter ACh release (and thus TT) under these clinically relevant conditions.

Supported by NIH NS28165.

ACETYLCHOLINE RECEPTORS: MUSCARINIC

725.1

CHOLINERGIC REGULATION OF INTRACELLULAR CALCIUM LEVELS IN AN IMMORTALIZED HIPPOCAMPAL CELL LINE.

J. P. Sullivan*, T. Giordano, L. M. Monteggia, M. Downen[1], B.H. Wainer[1], and S. P. Arneric. Neuroscience Research, Pharmaceutical Products Division, Abbott Laboratories, Abbott Park, Il, 60064 and [1]Dept. of Pathology, Albert Einstein University, New York, NY 10461

HN33 cells, derived via somatic cell fusion of hippocampal cells from postnatal day 21 mice to N18TG2 neuroblastoma cells exhibit morphological and cytoskeletal features which are typical of their neuronal parents but which are not expressed by the N18TG2 cells (Lee et al., J. Neurosci., 10; 1779, 1990). Furthermore, these cells display electrophysiological properties typical of primary hippocampal neurons when differentiated with retinoic acid. HN33 cells have not, however, been extensively studied with respect to neurotransmitter-associated properties. In the present study, the cholinergic regulation of intracellular calcium levels in undifferentiated and differentiated HN33 cells was investigated.

In the presence of physiological (1 mM) extracellular Ca^{2+}, carbachol (ED_{max} = 500 μM) and acetylcholine (ED_{max} = 50 μM) caused a dose-related increase in intracellular Ca^{2+} levels in fura-2-loaded undifferentiated (n=3) and differentiated HN33 cells(n=3). The elevation of $[Ca^{2+}]_i$ was biphasic, consisting of a transient initial peak followed by a decline to a plateau that was 40% higher than the basal level. EC_{50} values for the peak and plateau carbachol-induced response in undifferentiated cells were 5 μM and 3 μM, respectively. (-) Nicotine (10-500 μM) had no effect on calcium levels. The M3 muscarinic receptor antagonist, 4-DAMP, was a more potent inhibitor (IC_{50} = 4.5±1.2 nM, n=3) of carbachol-induced increases in Ca^{2+} levels than pirenzepine (IC_{50} = 180±16 nM, n=3).

These results demonstrate the existence of mAChRs in both differentiated and undifferentiated HN33 cells that are coupled to changes in intracellular levels of calcium via activation of M3 receptors.

725.2

EFFECTS OF METAL CATIONS ON MUSCARINIC RECEPTOR MEDIATED CHANGES IN FREE INTRACELLULAR CALCIUM IN HUMAN SH-SY5Y NEUROBLASTOMA CELLS.

J. Naarala, M. Tuomala and K. Savolainen*, Natl. Publ. Hlth. Inst., Dept. Toxicol., Kuopio, Finland.

The effects of aluminum (Al), magnesium (Mg), potassium (K), zinc (Zn), cupric (Cu), lead (Pb) and sodium (Na) acetate on free intracellular calcium ($[Ca^{2+}]_i$) were studied in human SH-SY5Y neuroblastoma cells. The cells were first incubated for 60 min with each one of the metal acetates at a concentration of 30 μM and then the cells were stimulated with 10 μM of acetylcholine (ACh), and the changes in $[Ca^{2+}]_i$ measured, applying a fluorometric method using a fluorescent calcium probe fura-2. All metal acetates inhibited the ACh-induced elevations of $[Ca^{2+}]_i$ in human SH-SY5Y neuroblastoma cells. Zinc was the most potent one of the acetates causing a 90 % attenuation in ACh-induced elevation of $[Ca^{2+}]_i$. The suspected neurotoxin, aluminum, produced a 40 % reduction, as did also the cupric acetate. These results shed new light on these metallic neurotoxins, because calcium in excess is also neurotoxic. Pb, Mg, and Na acetates produced similar effects with a 20–30 % reduction in the ACh-induced elevation of $[Ca^{2+}]_i$. The potassium acetate had the most striking and unexpected effect on $[Ca^{2+}]_i$. The inhibition of ACh-induced $[Ca^{2+}]_i$ elevation by K-acetate was 50 % at low doses even though it alone, by depolarizing the neurones, remarkably elevates $[Ca^{2+}]_i$ at high doses. Thus, K-acetate may have opposite effects on $[Ca^{2+}]_i$ at low and high doses, respectively. The effects of metal cations on $[Ca^{2+}]_i$ may be due to modification of the binding of ACh to the muscarinic receptors in neuroblastoma cells. Supported by The Academy of Finland.

725.3

COUPLING OF m2 AND m4 MUSCARINIC RECEPTOR SUBTYPES TO Ca^{2+}-DEPENDENT K^+ CHANNELS IN TRANSFORMED NL308 NEUROBLASTOMA X FIBROBLAST HYBRID CELLS. M. Noda[1], M. Katayama[2], D. A. Brown[3], J. Robbins[3], S. Marsh[3], N. Ishizaka[1], K. Fukuda[2], N. Hoshi[1], S. Yokoyama[1], and H. Higashida[1]*. [1]Kanazawa Univ. Sch. Med., Kanazawa 920, Japan, [2] Kyoto Univ. Facul. Med., Kyoto 606, Japan and [3]Univ. College London, London WC1E 6BT, UK.

The muscarinic acetylcholine receptor (mAChR) subtype (m1-m4)-specific DNAs were transfected in NL308 neuroblastoma-fibroblast hybrid cells and clones expressing each of the individual mAChR subtypes m1, m2, m3 and m4 were obtained. ACh increased phosphoinositide (PI) turnover in m1- and m3-transformed cells, but little or no production of it was detected in m2- and m4-transformed cells. In cells expressing m1- and m3-subtypes, ACh produced an initial outward K^+ current, followed by an inward current due to the actvation of Ca^{2+}-dependent cationic channels. No M-current inhibition evoked by ACh was recorded in m1- and m3-transformed NL308 cells, because they are M-current deficient mutant cells. In m2- and m4-transformed cells, only the initial K^+ current was detected. The outward currents were associated with a rise in intracellular Ca^{2+} as measured with Fura-2 or Indo-1, and were inhibited by chelating intracellular Ca^{2+} with external BAPTA-AM, or by external charybdotoxin or Ba^{2+}, hence they were attributed to the activation of a Ca^{2+}-dependent K^+ current. However, the outward current produced in m2- and m4-transformed cells was blocked by pretreatment with 5 ng/ml *Pertussis* toxin (PTX), whereas that in m1- and m3-transformed cells was not. These results indicate that m2- and m4-receptors in transformed NL308 cells couple to a PTX-sensitive G protein which is capable of mobilizing intracellular Ca^{2+} and activates $I_{K(Ca)}$, whereas m1- and m3-receptors activate a similar process through a different, PTX-insensitive G-protein.

725.4

CARBACHOL - PHORBOL ESTER INTERACTIONS AT MUSCARINIC RECEPTORS IN RABBIT STRIATUM.

L. X. Cubeddu *, I. S. Hoffmann and R. K. Talmaciu. Dept.of Pharmacology, School of Pharmacy, Central University of Venezuela, Caracas, Venezuela.

In rabbit striatal slices, both 4-beta-12,13-dibutyrate phorbol-ester (PDBu) (EC50%= 82±12 nM) and carbachol (EC50%= 2.3±0.35 uM) enhanced the release of dopamine (DA) evoked by electrical stimulation. No additivity was observed when slices were treated simultaneously with 0.1 uM PDBu and 10 uM carbachol. Pretreatment with PDBu (0.01-0.1 uM) abolished carbachol-induced facilitation of DA release and pretreatment with carbachol (3-100 uM) antagonized the enhancement in DA release produced by PDBU. The effect of carbachol was blocked by atropine (0.1 uM) but not by hexamethonium (10 uM); whereas, the enhancement in the evoked-release of DA produced by PDBu was not modified by either antagonist. If receptors are blocked with atropine (0.1 uM), carbachol failed to antagonize PDBu-induced facilitation of DA release. PDBu, but not carbachol, antagonized the inhibition of ACh release induced by DA D2-receptor and by MAChR agonists on ACh release.

This is the first report to indicate that activation of MAChR by an agonist prevents the effects of an active phorbol-ester. We suggest that activation of MAChR (probably M1) enhances DA release possibly through activation of PKC. It also appears that the PKC linked to MAChR (activated both by carbachol and PDBu) mediates increases in DA release; whereas the activation of the "other" PKCs (activated by PDBu) exerts no effect on DA release, but may have other actions, for example inducing receptor internalization in ACh neurons.

Support: CDCH F.07-10-2084.89, UCV, and Fundacion Jose Maria Vargas.

725.5

PERTUSSIS TOXIN BLOCKS M_2 MUSCARINIC RECEPTOR-MEDIATED CONTRACTIONS OF THE GUINEA PIG ILEUM WITHOUT INHIBITING M_3-MEDIATED-CONTRACTIONS. E. A. Thomas* and F. J. Ehlert. Department of Pharmacology, University of California at Irvine, College of Medicine, Irvine, CA 92717.

We have previously demonstrated, by pharmacological characterization with AF-DX 116, that contractions of the guinea pig ileum can be elicited by M_2 muscarinic receptors when measured in the presence of histamine and forskolin or isoproterenol. (Thomas et al. Mol. Pharmacol. in press). We have further characterized the M_2 nature of the contractile response by examining the effects of pertussis toxin on oxotremorine-M (oxo-M) induced contractions. Under basal conditions, pertussis toxin treatment (18 hr; 32 C°) had no effect on the EC_{50} value of the concentration-response curve to oxo-M, an M_3-mediated event. In contrast, when ilea were pre-contracted with histamine (1 μM) and then relaxed back to baseline with forskolin (2 μM) before oxo-M induced contractions were measured, pertussis toxin shifted the concentration-effect curve to oxo-M by a 6.1-fold increase in EC_{50}. Ilea were then incubated with 4-DAMP mustard (40 nM; 1 hr) in the presence of AF-DX 116 (1 μM) to selectively inactivate the M_3 muscarinic receptors, and contractions to oxo-M were repeated in the presence of histamine (1 μM) and forskolin (1 μM). Under these conditions, pertussis toxin blocked the concentration-effect curve to oxo-M by a 30-fold increase in the EC_{50} value. In addition, 4-DAMP mustard treatment caused only a 4.6-fold increase in the EC_{50} value for oxo-M in the control tissues, and a 22-fold increase in EC_{50} in the pertussis toxin-treated tissues. These results demonstrate the importance of M_2 muscarinic receptors in the contraction of the guinea pig ileum. *Supported by N.I.H. Grants NS 26511 & 30882.*

725.6

AN ASSAY FOR m4 RECEPTORS IN RAT STRIATAL MEMBRANES USING DOPAMINE-FORSKOLIN STIMULATION OF ADENYLATE CYCLASE AND cAMP DETERMINATION BY AUTOMATED ELISA. Kris Eckols and Neil DeLapp*, Lilly Research Laboratories, Eli Lilly & Co., Indianapolis, IN 46285

The m_2 and m_4 subtypes of muscarinic receptors are negatively coupled to adenylate cyclase. In rat striatum, m_4 receptors are present at considerably higher density than m_2 receptors as shown by analysis of mRNA (Brann *et. al.*, J. Neurosci. 8: 4646, 1988), binding studies (Potter *et. al.*, Life Sci. 52: 433, 1993), and immunoprecipitation of receptor proteins (Wolfe *et. al.*, Mol. Pharm. in press, 1993). Analysis of mRNA has shown a heavy co-localization of m_4 and dopamine D_1 receptors (Bloch *et. al.*, J. Neurosci. 12: 2591, 1992). Adenylate cyclase was assayed by a modification of the method of Olianas and Onali (Mol. Pharm. 23: 393, 1983) using a combination of 100 μM dopamine and 50 nM forskolin(D+F) to stimulate enzyme activity. cAMP was determined with a commercial ELISA (Cayman Chemical) using a BioMek workstation with side loading arm to automate plate preparation, addition of samples, and plate development.

The D+F combination stimulated adenylate cyclase activity 3.5 - 5.5 fold above basal. Stimulated activity was inhibited 45-55% and 40-50% by 1 mM carbachol and 1 mM oxotremorine M respectively. By comparing the response due to carbachol, SCH23390 (a D_1 antagonist), and a combination of the two, it was estimated that 60% of the carbachol effect on D+F stimulated activity results from inhibition of dopamine-stimulated cyclase. Agonist potencies in the assay were oxotremorine M = McNA343 > pilocarpine > carbachol > arecoline > bethanecol. McNA343 and pilocarpine were partial agonists. Rank order of antagonist potencies was 4DAMP > atropine > methoctramine > pirenzipine > AFDX116. Agonists and antagonists potencies were similar to those reported by Olianas and Onali for inhibition of striatal membrane basal activity. Inhibition of D+F stimulated activity was greater than that reported by Onali *et. al.* for inhibition of basal activity, and addition of dopamine to the assay may reduce the contribution of m2 receptors due to co-localization of D_1 and m_4. The use of a commercially available cAMP ELISA has allowed partial automation of the assay thereby increasing productivity and eliminating the necessity for use of radioisotopes.

725.7

CHARACTERIZATION OF THE MUSCARINIC RESPONSE OF WT3 CELLS TO CARBACHOL USING THE CYTOSENSOR™ MICROPHYSIOMETER. **K. De Moor, S. Pitchford, M.A. Hirst, & B.S. Glaeser*, Molecular Devices Corporation, Menlo Park, CA 94025.**

The Cytosensor Microphysiometer has been used to monitor the responses of M1 muscarinic receptors transfected into CHO cells (WT3). This instrument directly measures changes in cellular acidification rates by monitoring the extracellular acidification of a low-buffered F12 medium bathing the cells. We have investigated serum starvation, exposure/washout times and medium modifications to determine the effect on receptor-mediated activation of WT3 cells by the agonist, carbachol. Cells were exposed for 30 seconds to increasing concentrations of carbachol (0.01-100μM) followed by a 30-minute washout. Prior to carbachol exposure, WT3 cells typically required 2-3 hours to reach stable baseline acidification rates. This occurred both with non-starved or 18-hour serum-starved cells. This equilibration time was greatly reduced, however, by the addition of 1% serum to the low-buffered F12 medium. WT3 cells responded to carbachol treatment with a transient increase in acidification rates. These responses increased in a dose-dependent fashion (EC_{50}~2μM). Rapidly repeated stimulation with carbachol (<15 minutes between doses) resulted in attenuated responses and a shift in the dose response curve. Serum starvation resulted in a reduction in the peak response (increase above basal acidification rate) but no alteration in the EC_{50} value. In other studies, atropine inhibited the carbachol response.

725.8

Effects of c'AMP on Muscarinic Receptor-Mediated IP_1 and IP_3 Accumulation in the Rat Parotid Gland. E. H. Gerstin, Jr* and F. J. Ehlert, Department of Pharmacology, College of Medicine, University of California, Irvine, CA 92717.

We have demonstrated that the adenylate cyclase activator, forskolin (75 μM) can dampen the accumulation of inositol monophosphates (IP_1) elicited by the muscarinic agonists oxotremorine-M (OXO-M) and pilocarpine (PILO) in a slice preparation of the rat parotid gland. (*Soc. Neurosci. Abs.* 18:481.17, 1992) The active second messenger, inositol trisphosphate (IP_3), is similarly affected by forskolin. OXO-M (10 μM) increased production of IP_3 3.1-fold in the absence of lithium. Forskolin (75 μM) dampened this response, decreasing the IP_3 accumulation to 2.1-fold ($P > 0.02$). In the presence of Li^+ (10 mM), the OXO-M effect was 7.8-fold, and was reduced to 2.6-fold by the addition of forskolin (75 μM). The reductions in IP_3 correlate with those seen in measurement of IP_1, indicating that the measured effects of forskolin on IP_1 accumulation accurately reflect effects on IP_3 production. To investigate whether receptors coupled to activation of adenylate cyclase had similar effects on the accumulation of inositol monophosphates, we first determined the abilities of various agonists to increase c'AMP levels. Isoproterenol (0.1 mM), 2-chloroadenosine (0.1 mM), and prostaglandin E_2 (10 μM) all demonstrated ability to elevate c'AMP levels in the parotid slice prep, by 24-, 4-, and 3-fold, respectively, in the presence of IBMX (1 mM). Collectively, our data indicate that β-adrenergic, adenosine A_2, and PGE_2 receptors may dampen the phosphoinositide hydrolysis response to muscarinic agonists in the rat parotid gland. Studies are underway to test this hypothesis. *Supported by NIH Grants NS 26511 and 30882.*

725.9

MUSCARINIC RECEPTORS STIMULATE c'AMP ACCUMULATION IN THE RAT PERIPHERAL LUNG.

E. Esqueda, E. H. Gerstin Jr., and F. J. Ehlert*. Department of Pharmacology, College of Medicine, University of California, Irvine, Ca 92717.

Muscarinic receptors in the rat peripheral lung were investigated using radioligand binding and second messenger assays. When measured by the displacement of [^{3}H]N-methylscopalamine binding, the competition curve of the M_1 selective antagonist pirenzepine was consistent with a two-site model. The proportion of high affinity sites was 9%, and the observed K_D's of the high and low affinity sites were .069 and 1.4 μM, respectively. Adenylyl cyclase activity was then tested in a slice preparation by assaying for c'AMP accumulation in the presence of the muscarinic agonist oxotremorine M (OXO-M) and forskolin (FSK). Cyclic AMP levels were increased 1.6 fold over basal by OXO-M (10 μM) alone, and 4.3 fold by FSK (37 μM) alone. When added together, OXO-M potentiated FSK and caused a 9.6 fold increase in c'AMP levels. This response was inhibited by pirenzepine with an observed K_I value of 0.8 μM, indicating that a muscarinic receptor with low affinity for pirenzepine is causing the accumulation of c'AMP. In preliminary experiments, prostaglandin E_2 (10 μM) alone stimulated the accumulation of c'AMP by 1.5 fold; with the addition of OXO-M (10 μM), the accumulation was potentiated by ~20%, to 1.9 fold. The potentiation of FSK by OXO-M was unaffected by treatment with tetrodotoxin, confirming that it was not elicited by release of neurotransmitters. *Supported by NIH Grants NS 26511 and 30882.*

725.10

COMPARATIVE ALTERATIONS OF CORTICAL CHOLINERGIC MARKERS FOLLOWING LONG TERM BILATERAL LESIONS OF THE SUBSTANTIA INNOMINATA IN THE RAT.

D. Cécyre*, I. Aubert, J.-C. Martel and R. Quirion, Douglas Hosp. Res. Ctr and McGill Univ., Depts of Psychiatry and Neurology & Neurosurgery, Montreal, QC, Canada H4H 1R3.

In rats, lesions of the substantia innominata (SI), an area enriched with cholinergic perikarya, have been shown to produce cholinergic deficits in the cortex. However, most of these studies were performed following short term SI lesions. In this study, we were particularly interested in the effect of SI lesions combined with aging. Therefore, bilateral ibotenic acid (two infusions of 0.5 ul each; 5 mg/ml) lesions of the SI and sham operations were performed in 2 months old rats which were sacrificed only at 21 months. Brain sections were then processed for either [^{3}H]pirenzepine (5 nM) M1 sites, [^{3}H]AF-DX 384 (0.2 nM) putative M2 sites, [^{3}H]*N*-methylcarbamylcholine (20 nM) nicotinic sites and [^{3}H]hemicholinium-3 (10 nM) high affinity choline reuptake site (HACU) receptor autoradiography. Cortical choline acetyltransferase (ChAT) activity was also measured. The density of HACU binding sites was significantly reduced (45%) in the frontal and parietal cortices of long term SI lesioned rats. No changes in HACU binding was observed in the occipital and entorhinal cortices. ChAT activity was decreased (20%) in the cortex (all cortices included) of lesioned rats. The density of other cholinergic markers was not decreased in cortical areas of lesioned rats.

Supported by the Alzheimer Society of Canada and MRCC.

725.11

NEUROTROPHIC-LIKE EFFECTS MEDIATED BY AF102B, AN M1-SELECTIVE MUSCARINIC AGONIST, IN PC12M1 TRANSFECTED CELLS.

D. Gurwitz*, R. Haring, R. Pinkas-Kramarski#, R. Stein# and A. Fisher. Israel Inst. Biol. Res., Ness-Ziona 70450, and #Dept. Biochem,, Tel-Aviv University, Tel-Aviv 69978, Israel.

Rat pheochromocytoma (PC12) cells stably transfected with the cloned rat m1 muscarinic acetylcholine receptor (PC12M1 cells) were shown to undergo phenotypic change upon treatment with oxotremorine (Pinkas-Kramarski *et. al.*, J. Neurochem. 59:2158, 1992). This was manifested by neurite outgrowth, which synergized with NGF-mediated neurites. We now report that similar neurotrophic-like effects are mediated by AF102B, an M1-selective muscarinic agonist, and a candidate Alzheimer's disease (AD) drug (Fisher *et. al.*, JPET 257:392, 1991). However, unlike the neurotrophic-like effects of non-selective muscarinic agonists such as oxotremorine or carbachol, which were evident in the absence of NGF, AF102B (up to 100 μM) did not induce neurite outgrowth by itself, but synergized strongly (ED_{50} = 5 μM) with 2 nM NGF, which by itself mediated only a mild response. This synergized response was not observed in non-transfected PC12 cells, and was completely blocked in PC12M1 cells by 10 μM atropine, indicating involvement of m1 receptors. In addition, atropine retracted neurites which were previously extended by co-incubation of PC12M1 cells with AF102B and NGF. AF102B (100 μM) mediated a robust (> 10-fold) stimulation of phosphoinositide hydrolysis in PC12M1 cells. Neurites extended by a combined treatment with NGF and AF102B were stable for long periods in culture (> 10 days), indicating that the signaling mechanism(s) responsible for maintenance of neurites were not desensitizing in the continued presence of AF102B. Since neuronal cell-death in AD probably involves decreased production and/or availability of neurotrophines, which in turn compromises survival of cholinergic neurons, our observations imply that AF102B may, under certain conditions, compensate for such deficiencies and thus may constitute a novel treatment for AD. *Supported by Snow Brand, Japan.*

725.12

AF150(S) AND AF151(S): NEW M1 AGONISTS MEDIATE m1-SELECTIVE SIGNALING, NEUROTROPHIC-LIKE EFFECTS AND RESTORE AF64A-COGNITIVE DEFICITS IN RATS

A.Fisher*, E. Heldman, D. Gurwitz, R. Haring, H. Meshulam, R. Brandeis, M. Sapir, D. Marcianno, D. Barak, Z. Vogel# and Y. Karton. Israel Institute for Biological Research, Ness-Ziona, & # The Weizmann Institute, Rehovot, ISRAEL.

Using computer-assisted molecular modeling we designed two new rigid thiazoline-piperidine analogs, AF150(S) and AF151(S) [US. Pat. Appl., April, 1990]. These compounds are selective and efficacious agonists for M1AChRs in rat cortex (CT) vs cerebellum (CER) {binding of ^{3}H-pirenzepine ± GppNHp (Gp) vs ^{3}H-QNB, respectively; e.g., for AF150(S) in CT: $K_{H\text{-}Gp}$= 0.39 μM (42%), $K_{L\text{-}Gp}$= 14 μM; K_{L+Gp}= 8.9 μM (100%); in CER: K_L= 22 μM}. In CHO cells stably transfected with cloned m1 muscarinic receptor (m1AChR), AF150(S) and AF151(S) are full agonists in elevating $[Ca^{2+}]_i$, but partial (30-50% vs. carbachol, CCh) and full agonists, respectively, in stimulating phosphoinositides hydrolysis (in CHO and PC12M1 cells). In CHO cells transfected with m1AChR, these compounds are inactive when compared with CCh in elevating cAMP levels. In epithelial cells derived from human salivary gland rich in m3AChR, AF150(S) is an antagonist to CCh, whilst AF151(S) is a partial agonist in elevating $[Ca^{2+}]_i$. Both compounds synergized (EC_{50}~ 1μM) with NGF (2 nM) in mediating neurotrophic-like effects in PC12M1 cells, being about 5-folds more potent than AF102B, an M1 selective agonist. In AF64A (3nmole/2μl/side, icv)-treated rats, AF150(S) (1 mg/kg, po) restored cognitive impairments in a passive avoidance test, in Morris water maze and in a radial-arm maze (0.5, 1 mg/kg, po), without producing adverse effects up to 100 folds higher doses. Due to their profile at m1AChRs and activation of only distinct G-protein subset(s), their neurotrophic-like activity *in vitro*, and beneficial effects *in vivo*, such agonists can be unique candidates for the treatment of Alzheimer's disease.

725.13

M-1 MUSCARINIC RECEPTOR MEDIATED FACILITATION OF ACETYLCHOLINE RELEASE IN THE RAT URINARY BLADDER BUT NOT IN THE HEART.

G.T. Somogyi, M. Tanowitz and W.C. de Groat, Department of Pharmacology, University of Pittsburgh, Pittsburgh, PA 15261.

The contribution of presynaptic muscarinic receptors to frequency dependent facilitation of ^{3}H-acetylcholine (ACh) release was studied in isolated muscle strips from the body of the urinary bladder (UB) and cardiac atrial tissue of the rat. In strips prelabelled with ^{3}H-choline electrical field stimulation increased ^{3}H-ACh outflow during superfusion. The quantity of ^{3}H-ACh release was influenced by the pattern and duration of stimulation. Continuous stimulation (CS) with trains of 20-100 shocks released ten times larger amounts of ACh than the same number of shocks presented as short trains (10 shocks per train) with 5 sec inter-train intervals (IS). The volley output of ^{3}H-ACh release during CS was facilitated as the number of shocks was increased between 5 and 70. ACh release decreased with longer train durations (100-360 shocks). Facilitation was not detected with IS using 20 - 360 shocks. The facilitation of transmitter release was antagonized completely by the administration of atropine (1 μM) or pirenzepine (0.05 μM) a selective M1 antagonist. Administration of the anticholinesterase agent, eserine (5 μM), markedly facilitated ACh release induced by CS and IS. Atropine blocked the facilitation. Release of ACh from atrial strips did not exhibit CS-induced facilitation. Eserine decreased IS and CS-evoked ACh release in the atrium. It is concluded that continuous stimulation of postganglionic cholinergic nerves in the rat urinary bladder leads to activation of M1 muscarinic facilitatory presynaptic receptors which enhance the release of ACh. These receptors were not detected in the atrium. Presynaptic facilitation may be an important mechanism for modulating neural input to bladder during micturition. Supported by NSF Grant BNS-890-8934.

725.14

MUSCARINIC ACETYLCHOLINE RECEPTORS IN URINARY BLADDER.

T.A. Cawley, Jr.,* P. Wang, M.R. Ruggieri, and G.R. Luthin. Departments of Pharmacology and Urology, Temple University School of Medicine and Department of Physiology & Biophysics and Institute for Neuroscience, Hahnemann University, Philadelphia, PA 19102.

In rat myocardium the m2 subtype of muscarinic acetylcholine receptor (mAChR) inhibits contractility, while in urinary bladder mAChR activation produces a contractile response. To investigate the possible differences in mAChRs that mediate these opposite contractile responses, subtype levels were determined in the rat bladder. Receptors were labelled using [^{3}H]QNB, solubilized, and immunoprecipitated using subtype-specific anti-peptide and anti-fusion protein antisera. The m2 subtype of mAChR was found to comprise ~ 90% of the total mAChRs in bladder, the remainder being the m3 mAChR subtype. Using immunocytochemistry (see Shickley, et al. accompanying abstract), the m2 mAChRs were localized to cells discrete from those labelled using anti-m3 mAChR antisera. When activated by carbachol, the m2 mAChR and G proteins could be solubilized and co-precipitated using anti-mAChR or anti-G protein antisera. Thus, it is likely that mAChR-effector coupling differences seen in bladder and heart are not due to differences in mAChR subtype or to differences in mAChR-G protein interactions but may be reflective of the type of G protein activated. Supported by NIH DK43333.

725.15

STABLE ASSOCIATION OF m3 MUSCARINIC RECEPTORS AND G PROTEINS. P. Wang and G.R. Luthin.* Department of Physiology & Biophysics and Institute for Neuroscience, Hahnemann University, Philadelphia, PA 19102.

Rat parotid gland contains predominantly the m3 subtype of muscarinic acetylcholine receptor (mAChR). We studied interactions of this mAChR with guanine nucleotide binding proteins (G proteins). When carbachol was used to inhibit [^{3}H]NMS binding to parotid membranes, only low-affinity binding was observed, and this was not altered in the presence of GTP or GTP analogues. Direct labelling of parotid membranes by [^{3}H]oxotremorine-M was also not possible, in contrast to m2 mAChR containing tissues. In spite of the lack of high affinity agonist binding to this tissue, we could co-purify the m3 mAChR with G proteins following agonist labelling, solubilization, and immunoprecipitation. An interaction with Gi but not Go was established using this protocol. Together, these results suggest that a stable association of the m3 receptor with G proteins requires the presence of agonist, but does not require high affinity agonist binding. **Supported by NIH NS23006.**

725.16

IDENTIFICATION AND CHARACTERIZATION OF A HIGH-AFFINITY PERIPHERAL-TYPE BENZODIAZEPINE RECEPTOR IN RABBIT URINARY BLADDER SMOOTH MUSCLE. M.R. Ruggieri,* E.J. Uhlman and R.J. Smyth. Departments of Pharmacology & Urology, Temple University School of Medicine, Philadelphia PA 19140.

The existence of the peripheral-type benzodiazepine receptor (PBR) has been demonstrated in several types of smooth muscle as well as in steroidogenic tissues. The present study utilized radioligand binding and in-vitro contractility experiments to identify and characterize a PBR in rabbit urinary bladder smooth muscle. [^{3}H]PK11195 bound to bladder membranes with high-affinity and density (K_d = 310 pM, B_{max} = 6.4 pmol/mg protein) indicating the presence of a PBR, whereas [^{3}H]flunitrazepam bound with moderate affinity and density (K_d = 30 nM, B_{max} = 372 fmol/mg protein). There was no significant difference in [^{3}H]PK11195 or [^{3}H]flunitrazepam binding between bladder base and dome. The rank order potency of various benzodiazepines and isoquinoline carboxamides in displacing the binding of [^{3}H]PK11195 was Ro5-4864 > diazepam = flunitrazepam >> Ro15-1788 = clonazepam. No significant binding of [^{3}H]Ro15-1788 was detected in bladder base or dome, denoting the absence of a central-type benzodiazepine receptor. Ro5-4864 and PK11195 inhibited nerve-evoked contractions in a concentration-dependent manner (IC_{50} = 42 μM and 56 μM, respectively). Carbachol- and KCl-induced contractions were also inhibited by Ro5-4864 and PK11195. KCl-induced contractions were inhibited to a greater extent than carbachol-induced or field-stimulated contractions with all the drugs tested. Both Ro5-4864 and PK11195 significantly increased the ED_{50} for calcium-induced contractions following a cholinergic stimulus compared to control. This data demonstrates the presence of a PBR in urinary bladder smooth muscle, capable of altering contractility in-vitro through modulation of calcium activity. **This study was supported by National Institutes of Health grants DK43333 and DK40579 to MRR.**

725.17

PROSTATE GLAND CONTAINS HIGH LEVELS OF THE m1 SUBTYPE OF MUSCARINIC RECEPTOR. R.J. Smyth,* M.R. Ruggieri, M.D. Colton, P.Wang, T. Shickley and G.R. Luthin. Department of Physiology & Biophysics and Institute for Neuroscience, Hahnemann University and Departments of Pharmacology, Anatomy & Cell Biology and Urology, Temple University School of Medicine, Philadelphia, PA 19140.

The prostate glandular epithelium secretes and the capsular smooth muscle contracts in response to activation by cholinergic neurons. Muscarinic acetylcholine receptor (mAChR) subtypes were analyzed in adenoma specimens from the human prostate gland, to determine the subtypes present in prostate that might be responsible for the secretory component of the muscarinic response. Immunohistochemical studies with antibodies to the m1 mAChR demonstrated immunoreactivity on the secretory epithelium of the prostate. Contractile studies demonstrated that the adenoma tissue did not contract in response to carbachol stimulation whereas the prostatic capsule did. Pharmacological studies were performed using selective drugs to inhibit [^{3}H]QNB binding to membrane homogenate. Pirenzepine and hexahydrosiladifenidol, both selective for the m1 mAChR, were more potent than methoctramine (m2 selective) in binding studies. Immunoprecipitation was performed on [^{3}H]QNB labelled mAChRs following solubilization from prostate membranes, using anti-peptide or anti-fusion protein antibodies. The m1 subtype of mAChR comprised > 80% of the total mAChRs, with m2 and m3 subtypes each ~ 5% of the total. No m4 or m5 mAChRs could be found in this tissue using the precipitation assay. The human prostate adenoma thus becomes the first mammalian tissue shown to contain predominately the m1 subtype of mAChR, and the first tissue shown to contain significant levels of this subtype outside of the brain. This should allow examination of the role of the m1 mAChR in excitation-secretion coupling. **Supported by NIH DK43333 and NS23006.**

ACETYLCHOLINE RECEPTORS: MUTAGENESIS OF MUSCARINIC AND NICOTINIC RECEPTORS

726.1

MUTATION OF CONSERVED ASPARTATE AND ARGININE RESIDUES IN TRANSMEMBRANE HELIX 3 OF THE MUSCARINIC ACETYLCHOLINE RECEPTORS. E.C.Hulme, K.M.Page, P.Jones, C.A.M Curtis and R.Morris*. MRC National Institute for Medical Research, Mill Hill, London, NW7 1AA, UK.

We have studied the contribution of conserved Asp and Arg residues in transmembrane helix 3 of the m1 and m2 muscarinic receptors to agonist binding and receptor activation. To do this, we have used conservative mutations (Asp105Glu, rm1 mAChR; Asp103Glu, hm2 mAChR; Arg123Lys, rm1 mAChR) which perturb, but do not destroy, receptor function and ligand binding. The results are consistent with the hypothesis that the positively-charged headgroups of muscarinic agonists dock with a region of the receptor which is relatively remote from the conserved Asp. The establishment of a tight ionic bond with the Asp carboxylate group is coordinated with the formation of productive interactions between the receptor and the ligand side-chain. This may be contingent on the induction of a conformational change in the receptor. The side-chain of Arg123 may participate in an intramolecular bond, which constrains the agonist-induced conformational change. We are investigating the hypothesis that this bond may be broken as a result of agonist binding, allowing the Arg side-chain to participate in binding and activation of the GTP binding protein.

726.2

MUTATION OF ASPARTATE 103 TO ASPARAGINE OR GLUTAMATE IN HUMAN MUSCARINIC RECEPTOR SUBTYPE Hm2: EFFECT OF SUBTYPE SELECTIVITY VERSUS pK_i ON RECEPTOR BINDING. C.J. Spencer,* R.D. Schwarz, D.W. Moreland, A.J. Thomas and H. Tecle. Parke-Davis Pharm. Res. Div. of Warner-Lambert Co., Ann Arbor, MI 48105

Previous work in our laboratory has shown that mutation of Asp 103 to Asn (D103N) in the Hm2 subtype transiently expressed in COS-7 cells decreased the affinity of ^{3}H-NMS for the receptor by 29 fold. This mutation preserves the relative size of the target residue but eliminates the negative charge of the amino acid. Examination of a small group of reference muscarinic agonists and antagonists suggested that this mutation could be a tool to refine the selection of compound specificity for m2 vs. m1. Those compounds displaying m2 selectivity showed a more dramatic reduction of affinity in the mutant compared to wild type (WT) than did those with m1 selectivity. These studies were expanded to ask whether the pK_i of a compound or its subtype selectivity in m1 vs. m2 WT receptors correlated with the affinity change seen with the mutation. Results from additional reference agonists and antagonists showed there was little relationship between pK_i and the affinity shift; however, the selectivity for m2 did parallel the shift. Examination of six novel muscarinic agonists with no appreciable subtype selectivity also showed a minimal relationship between pK_i and the change in affinity between WT and D103N. These results suggest that the affinity shifts observed in the D103N mutated Hm2 receptor are a consequence of more than the charge of the ligand in the environment of the receptor. These studies will be extended to the mutation of Asp 103 to Glu.

726.3

SITE-DIRECTED MUTAGENESIS OF ASPARTATE 103 IN THE Hm2 MUSCARINIC RECEPTOR: EFFECT ON RECEPTOR BINDING OF 1-AZABICYCLO[2.2.1]HEPTAN-3-ONE OXIMES. R.D. Schwarz*, C.J. Spencer, J. Jaen, T. Mirzadegan, D. Moreland, H. Tecle, A. Thomas, and R. Davis, Parke-Davis Pharmaceutical Res., Div. of Warner-Lambert Co., Ann Arbor, MI.

Like other G-protein coupled receptors, key aspartate residues appear to be involved in ligand binding at muscarinic receptors. In our laboratory, mutation of asp 103 to asn (D103N) in Hm2 receptors produced a 29-fold reduction in affinity for [^{3}H]-NMS while mutation to ala resulted in a loss of specific binding. Using the D103N mutant, selected muscarinic agonists and antagonists were examined for changes in affinity compared to wild type receptors. Subtype selectivity (m1 vs. m2) appeared to correlate better with affinity changes rather than pKa values; the more m2, the greater the loss in affinity (Spencer, et al., this meeting). Recently, Tecle, et al. (Life Sci. 52:505, 1993) described a novel series of 1-azabicyclo[2.2.1]heptan-3-one oxime agonists with increased length and bulk relative to traditional muscarinic ligands. In the present study, compounds from this group were studied for changes in affinity between D103N mutants and wild type receptors. While some compounds showed no change, others showed up to 40-fold decreases in affinity. The relationships between structural modifications and affinity changes observed in the binding experiments will be discussed.

726.4

THE STRUCTURE OF THE LIGAND BINDING DOMAINS OF A MUSCARINIC RECEPTOR INVESTIGATED BY RANDOM-SATURATION MUTAGENESIS. Tracy A Spalding*, David Hill-Eubanks and Mark R Brann. Molecular Neuropharmacology, Department of Psychiatry and Vermont Cancer Center, University of Vermont, Burlington, VT 05405.

Ligand binding to the muscarinic receptor involves the third, fifth and sixth transmembrane domains, (TM 3, 5 and 6). To define the amino acid residues involved in ligand binding, TM3 and TM5 were subjected to random saturation mutagenesis which was carried out using PCR in conjuction with a randomly mutated primer. Recombinant receptors were amplified in E. coli to produce a library of 3000 mutants, which was transfected into NIH 3T3 cells and screened for functional receptors using 0.1mM carbachol. Since m5 muscarinic activity transforms NIH 3T3 cells, cells expressing active receptors grow into foci. Around 5-15% of the recombinant receptors formed foci, and mRNA purified from these has been sequenced by RT-PCR. Using random-saturation mutagenesis, every possible substitution of every amino acid in each transmembrane domain of the receptor can be tested to show whether or not that mutation is tolerated. It is also possible to screen for constitutively-activating mutations. The pattern of tolerated and activating mutations should provide information on the residues involved in the binding of carbachol to the receptor, the residues which play key roles in the activating mechanism of the receptor, and the orientation of the seven transmembrane helices.

726.5

EFFECTS OF SITE-DIRECTED MUTAGENESIS OF A HIGHLY CONSERVED ARGININE RESIDUE ON THE FUNCTION OF THE M1 MUSCARINIC RECEPTOR. S.Z. Zhu, S.Z. Wang, J. Hu and E.E. El-Fakahany*. Div. of Neuroscience Research in Psychiatry, University of Minnesota Medical School, Minneapolis, MN 55455.

Point mutation of the arginine residue at position 123 of the m1 muscarinic receptor sequence into asparagine (Asn^{123}) was induced and the mutant gene was stably transfected into Chinese hamster ovary cells. This residue is conserved in all G-protein-coupled receptors in an invariant fashion at the beginning of the second cytoplasmic loop. This mutation almost abolished agonist-induced increase in phosphoinositide hydrolysis, even though wild and mutant receptors were expressed at similar numbers. There was no significant effect on antagonist binding. In marked contrast, while the binding of carbachol to wild receptors exhibited high and low-affinity components, this binding in Asn^{123} mutant cells was to a single low-affinity state. There was also a disappearance of agonist-induced enhancement of the specific binding of [^{35}S]GTP-gamma-S in membranes. Taken together, our data suggest that this highly conserved arginine residue plays an important role in coupling of m1 muscarinic receptors to G-proteins.

726.6

SITE DIRECTED MUTAGENESIS IN THE CHANNEL DOMAIN M2 OF THE NEURONAL NICOTINIC ACETYLCHOLINE RECEPTOR REVEALS A FUNCTIONAL STRATIFICATION. D. Bertrand*, J.-L. Galzi, A. Devillers-Thiéry, S. Bertrand and J.-P.Changeux Dpt of Physiology, CMU, 1211 Geneva, Switzerland

Investigation at the amino acid level of the functional properties of the residue lining the ionic pore was done in the chick α7 receptor. We found that the wild type receptor displays a rather high selectivity for calcium, with a pCa/pNa of about 10, that can be abolished by single point mutations at two distinct sites within M2. The first site is the intermediate anionic ring, where substitution of glutamates by alanines abolishes the calcium permeability without significantly affecting the other physiological properties of the receptor. The second site is composed of two adjacent leucine rings (positions 254 and 255), where replacement by threonines or asparagines reduces calcium permeability by at least 1000 fold. These mutations also cause significant modifications of response time courses, apparent agonist sensitivity and Hill numbers of agonist dose-resonse relationships. Together with previous findings (Devillers-Thiéry et al., 1992; Galzi et al. 1992) these mutagenesis experiments reveal a functional stratification of the channel domain M2.

726.7

SINGLE AA SUBSTITUTIONS IN i3 OF THE PORCINE MUSCARINIC RECEPTOR ALTER G-PROTEIN COUPLING CHARACTERISTICS. D.A. Bulsec* and M.I. Schimerlik, Biochemistry and Biophysics, Oregon State University, Corvallis, OR 97331.

The N-terminal region of the third intracellular loop (i3) plays an important role in G-protein coupling of the muscarinic acetylcholine receptor. Site directed mutagenesis was used to introduce single amino acid substitutions in i3 of the porcine atrial muscarinic receptor (pm2) followed by stable expression in Chinese hamster ovary (CHO) cells. Substitution of Ala with Glu at amino acid position 212 (A212E) or Lys with Ala in position 214 (K214A) resulted in binding characteristics similar to wildtype. The K_d for ^{3}H-QNB binding is 21±5 pM for wildtype receptor while A212E and K214A have dissociation constants of 40±11 pM and 26±2 pM respectively. Agonist competition binding experiments show that both possess high affinity binding sites for carbachol and oxo-M. Stimulation of phosphatidyl inositol (PI) metabolism for A212E and K214A resembled wildtype receptor for carbachol, acetylcholine and pilocarpine in both fold stimulation and EC_{50}, while oxo-M appeared normal for K214A. In A212E, oxo-M does not promote coupling to PI metabolism and has about half the maximal percent inhibition of adenylyl cyclase. The coupling of K214A to PI metabolism is similar to wildtype but appears to be mediated by a different G-protein. In CHO cells, wildtype receptor is coupled to both inhibition of adenylyl cyclase and stimulation of PI metabolism by a PTX sensitive G-protein(s). K214A on the other hand couples to PI metabolism through a G-protein with lowered PTX sensitivity. 100 ng/ml PTX failed to inhibit PI metabolism in K214A while wildtype receptor was completely uncoupled. Although 1 μg/ml PTX did reduce the PI response seen in K214A, it failed to completely abolish it. K214A inhibits adenylyl cyclase with reduced efficacy with PTX sensitivity similar to wildtype. Thus, single amino acid substitutions superficially result in an unchanged receptor but more detailed examination indicates that the G-protein coupling properties are altered. Oxo-M appears to become a partial agonist for the mutant A212E, while K214A appears to couple to PI metabolism either through a different G-protein or through both PTX sensitive and PTX insensitive G-proteins. *(Supported by NIH grant # HL23632 to M.I.S.)*

726.8

STRUCTURE/FUNCTION OF MUSCARINIC RECEPTOR/G-PROTEIN COUPLING **Molecular Neuropharmacology Division, Department of Psychiatry and Vermont Comprehensive Cancer Center, Univ. of Vermont, Burlington, VT 05405.** E. S. Burstein*, D. H. Eubanks, H. B. Jørgensen, & M. R. Brann.

The N- and C-terminal regions of the third cytoplasmic loop of several G protein coupled receptors play pivotal roles in G-protein coupling. To define structural requirements for receptor/G-protein coupling, we subjected these regions of the m5 muscarinic receptor to random-saturation mutagenesis, and identified mutant receptors that retain function. The positions and frequency of conservative versus nonconservative substitutions within N-i3 displayed periodicity, providing compelling evidence for an α-helix. All the amino acids on one side of the helix only tolerate certain highly conserved substitutions, while all the amino acids on the opposite face tolerate multiple nonconservative substitutions. The conserved face is comprised of hydrophobic residues surrounding an invariant arginine. A library of receptors mutated in C-i3 was constructed, and is being similarly analyzed.

726.9

IDENTIFICATION OF AN INTRACELLULAR TYROSINE RESIDUE CRITICAL FOR MUSCARINIC RECEPTOR-MEDIATED STIMULATION OF PHOSPHATIDYL INOSITOL HYDROLYSIS. K. Blüml*, E. Mutschler# and J. Wess. Natl. Inst. of Diabetes and Digestive and Kidney Diseases, Lab. of Bioorganic Chemistry, Bethesda, MD 20892, and #Dept. of Pharmacology, Univ. of Frankfurt, Germany.

Several lines of evidence suggest that the N-terminal portion of the third cytoplasmic loop (i3) of muscarinic (m1-m5) and other G protein-coupled receptors is of pivotal importance for G protein recognition and activation. It has been shown (Wess et al., Mol. Pharmacol. 38, 517, 1990) that replacement of the first 16 amino acids of the i3 loop of the rat m3 muscarinic receptor (primary biochemical response: stimulation of PI hydrolysis) with the corresponding segment of the human m2 receptor (primary biochemical response: inhibition of adenylyl cyclase) resulted in a mutant receptor that was no longer able to activate the PI pathway. Based on this observation, the present study was designed to identify specific amino acids within the N-terminal portion of the i3 region of the m3 receptor (rat) which are required for efficient G protein coupling. Functional analysis of several chimeric m2/m3 muscarinic receptors (transiently expressed in COS-7) showed that the first 4 amino acids of the i3 domain of the m3 receptor (Arg252-Ile253-Tyr254-Lys255) are of primary importance for receptor-mediated stimulation of PI hydrolysis. Further mutational analysis of this 4-amino-acid segment by single amino acid substitutions demonstrated that Tyr254 is essential for efficient activation of the PI pathway. This Tyr residue (or its aromatic character) is conserved among most biogenic amine and glycoprotein hormone receptors, suggesting that it may also play an important functional role in other G protein-coupled receptors.

EXCITATORY AMINO ACIDS: EXCITOTOXICITY VII

727.1

GLUTAMATE-INDUCED INTRACELLULAR pH CHANGES IN HIPPOCAMPAL NEURONS DEMONSTRATE ALTERED ENERGY METABOLISM RESULTING FROM Ca^{2+} AND Na^+ LOADS. G. J. Wang*, R. D. Randall and S. A. Thayer. Dept. of Pharmacology, Univ. of Minnesota Medical School, Minneapolis, MN 55455.

Glutamate(Glu)-evoked increases in the free intracellular H^+ concentration, $[H^+]i$, in single rat hippocampal neurons grown in primary culture were studied with carboxy SNARF-based dual emission microfluorimetry. The response was blocked by the NMDA receptor antagonist CGS19755 and required extracellular Ca^{2+}; Ba^{2+} substituted for Ca^{2+}. Pretreatment with 2-deoxy-D-glucose completely blocked the Glu-induced $[H^+]i$ increase but did not block Glu-induced Ca^{2+} influx. We hypothesize that the Glu-induced Ca^{2+} load uncouples mitochondrial respiration. Consistent with this hypothesis, the mitochondrial uncoupler FCCP mimicked and occluded the Glu-induced $[H^+]i$ increase. Maximally effective concentrations of FCCP (1 μM) not only blocked the Glu-induced $[H^+]i$ increase but also reversed the effect of Glu on $[H^+]i$. Thus, following treatment with FCCP the same Glu challenge that produced a $[H^+]i$ increase now decreased the FCCP-induced $[H^+]i$ elevation. The Glu-induced $[H^+]i$ decrease did not require extracellular Ca^{2+}, was blocked by 100 μM CNQX, required extracellular Na^+, and was mimicked by veratridine. Arachidonic acid (10 μM) uncoupled mitochondria and produced pH changes similar to those elicited by FCCP. Thus, depending on the state of the mitochondria Glu produced either a Ca^{2+} dependent acidification or a Na^+ dependent alkalinization. We postulate that exposure to both an uncoupling agent, such as arachidonic acid, and a large Na^+ load shuts down glucose metabolism.

727.2

NMDA INDUCES A RAPID, CONCENTRATION AND Ca^{2+} DEPENDENT ACIDOSIS IN CULTURED FETAL RAT HIPPOCAMPAL NEURONS. R.P. Irwin*, S-Z. Lin, R.T. Long and S.M. Paul. Section on Molecular Pharmacology, Clinical Neuroscience Branch, National Institute of Mental Health, NIH, Bethesda, Maryland 20892

Using the pH sensitive fluorescent indicator 2',7'-bis-(2-carboxyethyl)-5-(and-6)-carboxyfluorescein (BCECF) and the Ca^{2+} sensitive indicator fura 2 we studied the ability of NMDA to alter intracellular pH (pH_i) and intracellular calcium $[Ca^{2+}]_i$ in fetal rat hippocampal neurons and glia. NMDA (2.5-250 μM) applied for 60 sec to hippocampal neurons resulted in a rapid, usually reversible (with recovery of pH_i taking several minutes) and concentration-dependent reduction in pH_i with an EC_{50} of 39 μM and E_{max} (ΔpH) of -0.53. By contrast, glial cells showed little or no change in pH_i following exposure to NMDA. The NMDA-receptor induced intracellular acidification of hippocampal neurons was blocked by the NMDA receptor antagonist [3-[(±)-2-carboxypiperazine-4-yl]-propyl-1-phosphonic acid (CPP). Removal of extracellular Ca^{2+} eliminated both the NMDA-induced elevation in $[Ca^{2+}]_i$ and the reduction in pH_i suggesting that the NMDA-induced acidification is mediated by Ca^{2+} influx. Increasing extracellular pH from 7.4 to 8.0 (thereby reducing extracellular $[H^+]$ by ~75%) did not significantly attenuate the NMDA-induced reduction in pH_i suggesting an intracellular source of H^+. Finally, exposure of neurons to neurotoxic concentrations of NMDA for more prolonged periods of time (≥10 min) resulted in a more prolonged reduction in pH_i. We postulate that the NMDA-induced reduction in pH_i may mediate some of the actions of glutamate. For example, a prolonged reduction in pH_i induced by excitotoxins may result in an inhibition of important metabolic enzymes (e.g. phosphofructokinase) leading to ATP depletion and cell death. The ability (or inability) of neurons to rectify pH_i following exposure to NMDA/glutamate may therefore determine their susceptibility to excitotoxins.

727.3

MK-801 INDUCES EXTENSIVE NEURONAL NECROSIS IN POSTERIOR CINGULATE/RETROSPLENIAL CORTICES. D. F. Wozniak, M. McEwen, M. A. Sesma*, J. W. Olney, A. S. Fix. Washington University, St. Louis, MO 63110 and Eli Lilly & Co, Greenfield IN 46140.

We have previously shown that phencyclidine (PCP), or various PCP receptor ligands (PRL), cause pathomorphological changes in pyramidal neurons of the rat cerebral cortex. A single systemic (sc or ip) injection of dizocilpine (MK-801), the most selective and potent PRL known, causes this neurotoxic reaction to occur in rats, with females being more sensitive (ED_{50} = 0.18 mg/kg, sc) than males (ED_{50} = 0.32 mg/kg, sc). The neurotoxic reaction is restricted to pyramidal neurons in layers III and IV of the posterior cingulate/retrosplenial (PC/RS) cortices, and consists of vacuole formation and dissolution of mitochondria. Although we initially considered this reaction reversible, Allen and Iversen (Science, 1990, 247: 221) reported that MK-801, at a high dose (5 mg/kg, ip), killed a small number of cingulate neurons (5 necrotic neurons per section) in adult male rats. In the present study, we treated adult female rats (5 - 7 months old) with a very high dose of MK-801 (10 mg/kg, ip) and counted the number of necrotic neurons in hematoxylin and eosin-stained sections of PC/RS cortices 4 days later. Since preliminary data indicated that the acute vacuole reaction induced by MK-801 was less severe in the rostral and more severe in the caudal portions of the PC/RS cortical area, we counted necrotic neurons at both rostral and caudal levels. At a rostral level (1.6 to 3.5 mm posterior to bregma), the number of necrotic neurons ranged from 29 to 107 per section and at a more caudal level (6.1 to 8.3 mm posterior to bregma) the counts ranged from 64 to 191 necrotic neurons per section. It appears from these findings that the neuron-necrotizing effects of MK-801 can involve a much larger number of neurons than was previously described. Supported by RSA MH 38894 (JWO), AG 05681 (DFW/JWO).

727.4

RETINAL TOXICITY INDUCED BY D,L-2-AMINO-3-PHOSPHONOPROPIONIC ACID (D,L-AP3) IN THE RAT: A LIGHT AND ELECTRON MICROSCOPIC EVALUATION. A.S. Fix*, J.W. Horn, J.A. Johnson, C.A. Johnson, R.L.Hall, and J.P. Tizzano. Toxicology Research Laboratories, Lilly Research Laboratories, Eli Lilly and Co., Greenfield, IN 46140.

Metabotropic glutamate receptors are thought to have a role in the development and plasticity of the nervous system. D,L-AP3, a phosphono-substituted analog of aspartic acid, has mixed agonist/antagonist activity at metabotropic glutamate receptors. After treatment of neonatal rats, D,L-AP3 produced neurobehavioral deficits (Tizzano et al., NS Abstr., 1990, 1991) and severe atrophy of the retina and optic nerve (Fix et al., NS Abstr., 1992). The present study examined the evolving retinal lesion induced by D,L-AP3. Neonatal rats were treated intraperitoneally with vehicle or 400 mg/kg/day D,L-AP3 on postnatal days (PND) 3 through 6. On PND 5, 7, 10, 15, and 20, retinas were examined by light and electron microscopy. At PND 5 and 7, cells injured by D,L-AP3 were evident in two developing retinal layers; the middle of the outer neuroblastic layer and the more differentiated inner nuclear layer. Injured cells had swollen, pale cytoplasm or remained as dense, pyknotic debris. Retinal damage progressed at PND 10 and 15, since more cells were injured and necrosis of additional retinal layers was observed. By 20 PND, retinal atrophy was severe, although ganglion cells remained. This study indicates that affecting metabotropic glutamate receptors during postnatal development can severely alter normal retinal development in the rat.

727.5

EFFECT OF AGE, SEX, AND STRAIN OF RAT ON MK(+)801-INDUCED BEHAVIORAL RESPONSES AND RETROSPLENIAL CORTICAL NEURONAL NECROSIS. L.L. Truex*, E. Gomez, C.A. Johnson, R.A. Smith, K.A. Wightman, and A.S. Fix. Toxicology Research Laboratories, Lilly Research Laboratories, A Division of Eli Lilly and Company, Greenfield, IN 46140.

Early investigations in our laboratory suggested that after single doses of MK(+)801, behavioral responses and retrosplenial neuronal necrosis might vary with age, sex, and strain of rat. To examine this, male and female (70±4 or 127±2 days of age) Fischer 344 (F344) and Sprague Dawley (SD) rats were given a single subcutaneous dose of 0, 0.5, 1.0 or 5.0 mg/kg MK(+)801. The severity of behavioral responses and the degree of neuronal necrosis in the retrosplenial cortex were determined. Within 30 minutes of treatment, all rats given 5.0 mg/kg MK(+)801 developed ataxia, twitching, and lateral recumbency; rats given lower doses exhibited the same behavioral responses but to a lesser degree and duration. Differences in the character of behavioral responses were not noted when comparing age, sex and strain. However, male rats recovered within 24 hours but female rats were still moderate to severely ataxic 2 days after treatment. Also, ataxia was more severe for all older rats in the 5.0 mg/kg group. In female rats, necrosis of retrosplenial neurons occurred in all treatment groups and was extensive in the 5.0 mg/kg group. However in male rats, limited necrosis was detected only in the 5.0 mg/kg group. Although there was a suggestion of increased neuronal necrosis in SD rats, obvious differences were not evident when comparing strain and age. These behavioral and histologic data indicate that female F344 and SD rats are more sensitive than male rats to the effects of MK(+)801.

727.6

GLUTAMATE ENHANCES ZINC TOXICITY IN XENOPUS OOCYTES EXPRESSING EXCITATORY AMINO ACID RECEPTORS. J.M. Nave* and J.D. Connor Department of Pharmacology, The Pennsylvania State University College of Medicine, Hershey, PA 17033

Glutamate receptor agonists and zinc are known to be neurotoxic independently of each other. We have recently shown that exogenous zinc administration enhances kainic acid-mediated CA1 and CA3 pyramidal cell toxicity in vivo (Brain Res., 604: 298-303 (1993)). We wanted to test whether the reverse is true: i.e. can excitatory amino acid receptor activation enhance cytotoxicity caused by zinc. The effects of glutamate on toxicity produced by zinc were studied using the Xenopus oocyte expression system. Oocytes were injected with total (cerebrum) rat brain poly(A)$^+$ mRNA or distilled water. Zinc (50 mM-400 mM) toxicity in incubated oocytes before and after glutamate was measured two ways: by persistent depolarization of the membrane potential and by dye (trypan blue) exclusion. In oocytes injected with mRNA, subtoxic doses of glutamate (1mM) significantly enhanced the toxicity caused by zinc compared to water-injected controls. Moreover, toxic responses elicited by zinc alone and zinc with glutamate were significantly augmented when calcium was depleted from the medium. These findings (in concordance with previous in vivo findings from our laboratory) suggest that glutamate and zinc may act synergistically to cause cytotoxicity and that calcium may protect cells from the toxic effects of zinc.

727.7

DISSOCIATION OF SEIZURES AND CA1 HIPPOCAMPAL DAMAGE INDUCED BY THE CYANIDE METABOLITE 2-IMINOTHIAZOLIDINE-4-CARBOXYLIC ACID (2-ICA). R.S. Bitner, G.E. Isom and G.K.W. Yim* Department of Pharmacology and Toxicology, Purdue University, West Lafayette, IN 47907.

The cyanide metabolite 2-ICA produces MK-801 and CNQX-sensitive seizures in mice and selective CA1 hippocampal damage following 7-day icv infusion at a subconvulsive dose in rats. In the present study, seizure activity was observed in all mice receiving a single i.c.v. injection of 2-ICA (3.2 umol). When sacrificed 5 days later, 7 out of 10 mice displayed CA1 hippocampal damage. As expected, 2-ICA induced seizures were seen only in 3 out of 10 mice pretreated with MK-801 (1 mg/kg i.p.). However, CA1 damage was seen in 60 % of these animals. Consistent with our earlier findings in rats, these studies in mice indicate that 2-ICA-induced CA1 hippocampal damage is independent of seizure activity. Moreover, the inability of MK-801 to prevent 2-ICA damage suggests that NMDA receptor activation may not be required for 2-ICA neurotoxicity. (Supported in part by NIH grants ES4140 & RR0586.)

727.8

ONTOGENY OF IBOTENIC ACID LESION IN RAT BRAIN REVEALED BY COMBINED MRI AND HISTOLOGY. N. Ben-Horin[1]*, R. Schul[1], S. Hazvi[1], P. Bendel[2] and Y. Dudai[1]. Depts. of [1]Neurobiology and [2]Chemical Physics, Weizmann Institute, Rehovot, Israel.

We have analyzed the ontogeny of a neurotoxic lesion in rat brain. Rats were each unilaterally microinjected with ibotenic acid at 5 sites aiming at the piriform cortex (PC). The brains were imaged at 8 time points during 60 days post-lesioning, using *in vivo* magnetic resonance imaging (MRI). The MR spin-echo, T_2-weighted images were taken at 4.7 T. Following MRI at each time point, the brain of some of the animals was sectioned and stained for cytoarchitecture (Nissl), myeloarchitecture (modified Heidenhain) and cytochrome oxidase. The MR images taken 2 days post-lesioning revealed a high intensity signal, indicating edema, in extended areas in and around the PC and around the injection needle penetration sites in the neocortex. Similar but smaller affected areas were detected by the histological stainings. All the techniques revealed additional damage, including increased size of the contralateral ventricle relative to the ipsilateral one, and deformation of structures lining the edematous area in the neocortex (e.g. corpus callosum and hippocampus). With time, a dramatic decrease was detected in the size of the affected area surrounding the PC. The appearance of the other affected areas essentially returned to normal. Each of the techniques gave unique information about the lesion, with MRI being the most reliable in determining the extent of edema and structural deformation. Our data emphasize the need to take into account the post-lesion interval and the multiple and often distributed consequences of the lesion. The data also indicate that ca. 2 weeks elapse before transient acute effects of the trauma and the lesion subside. (N.B.H. is a recipient of a Stone Post-Doctoral Fellowship).

727.9

NMDA RECEPTOR ACTIVATION SELECTIVELY DESTABILIZES LIGATIN mRNA IN HIPPOCAMPAL NEURONS. D.M. Panchision, C.M. Gerwin, R.J. DeLorenzo*, and E.R. Jakoi. Department of Neurology, Medical College of Virginia, Richmond, VA 23298.

Acute activation of N-methyl-D-aspartate (NMDA) receptors in hippocampal neurons results in the down-regulation of ligatin, a receptor for phosphoglycoproteins present on dendritic and somal surfaces (Jakoi et al., Brain Res 582:282-290, 1992). This change in ligatin protein is accompanied by a selective reduction (40-80%) in steady state levels of mRNA coding for ligatin, whereas mRNAs encoding neuron-specific enolase and glucose-6-phosphate dehydrogenase are not decreased. Using nuclear run-on assays, *in situ* hybridization, and transcriptional inhibition studies with α-amanitin, we found that the NMDA receptor-mediated effect on cytosolic ligatin mRNAs occurs post-transcriptionally via an accelerated degradation of the RNA transcripts. Furthermore, steady state levels of ligatin mRNAs are altered by inhibition of protein synthesis in either the presence or absence of NMDA receptor activation, suggesting that ligatin mRNA stability is coupled to translation. The data support the hypothesis that modulation of mRNA stability is an early consequence of NMDA receptor activation leading to long-term changes in neuronal function.

This work was supported by AHA-William Randolph Hearst award 92009590 (ERJ), and by NIH grant NS25630 and Jacob Javits award NS23350 (RJD).

727.10

ROLE OF PROTO-ONCOGENE *c-fos* ACTIVATION IN GLUTAMATE-MEDIATED NEUROTOXICITY. J.R. Dave*, M.A. DeCoster, E. Knight, T. Dang, B.P. Doctor, F.C. Tortella and H.S. Ved. Division of Neuropsychiatry and Biochemistry, Walter Reed Army Institute of Research, Waashington, DC 20307-5100.

Earlier *in vivo* and *in vitro* studies have reported that activation of the glutamate/NMDA receptor complex results in stimulation of *c-fos* oncogene. However, the effect of glutamate-mediated *c-fos* activation in various anatomical regions of the brain and the functional importance of *c-fos* activation are poorly defined. The objectives of the present study were to determine if 1) glutamate produced differential effects in neuronal cultures derived from cerebral cortex, hippocampus and cerebellum and 2) treatment with *c-fos* antisense (AS) oligonucleotide prevented glutamate toxicity. Primary cultures enriched in neurons dissociated from embryonic rat cerebral cortex, hippocampus and cerebellum were treated in a serum-free media with either vehicle or glutamate (100 μM) for 15, 30 or 60 min and *c-fos* mRNA levels were determined. Treatment of cells with glutamate produced a transient increase in *c-fos* mRNA levels. The increase in *c-fos* mRNA was highest in neuronal cells obtained from cerebellum and lowest in those from cerebral cortex; hippocampus exhibited intermediate stimulation. Similar changes in glutamate-induced calcium flux, as measured by fluo-3, were observed in cells obtained from these brain regions. Addition of 5 μM *c-fos* AS oligonucleotide along with 100 μM glutamate produced a minimal (5-15%) neuroprotective effect in neurons obtained from cerebellum, an intermediate (40-50%) effect in hippocampus and a maximal (60-70%) effect in cortex. These results demonstrate that glutamate has differential specificity for anatomical regions of the brain and suggest a causative role of *c-fos* proto-oncogene in glutamate-mediated neuronal toxicity.

727.11

REDUCTION OF GLUTAMATE-LIKE IMMUNOREACTIVITY IN THE LOCUS COERULEUS FOLLOWING REPETITIVE INJECTIONS OF β-AMYLOID PEPTIDE INTO THE HIPPOCAMPUS OF THE RAT. Z. Wang, R.-H. Liu, V.K. Reddy and C.D. Barnes*. Department of VCAPP, Washington State University, Pullman, WA 99164-6520.

The neurotoxic effect of β-amyloid protein (β-AP) has been reported to be associated with excitotoxicity of glutamate (Glu). Recently, the presence of Glu in the locus coeruleus (LC) has been demonstrated in the mouse, rat and cat. In this study we injected β-AP (25-35 fragments) into the rat hippocampus to examine its effect on Glu- and tyrosine hydroxylase (TH)-like immunoreactive (LI) neurons in the LC. β-AP injections were made repetitively once every five days (two or three injections; 3 nmol in 2 μl of distilled water). Fluorescent microspheres (either alone or with β-AP) were also injected into the hippocampus in some rats to show the Glu- and TH-LI coeruleo-hippocampal neurons. Control experiments were conducted by injecting the vehicle (distilled water) into the hippocampus.

The results from this study revealed a significant cell loss in the hippocampus at the site of β-AP injection as well as a reduction of Glu- and TH-LI neurons in the LC. Furthermore, in β-AP/microsphere injected animals, only 34% and 50% of hippocampal projection neurons contained Glu and TH respectively, compared to 93% and 92% in animals that received microspheres alone. The reduction of Glu- and TH-like immunoreactivity was more prominent in the dorsal division of the LC, correlating with the predominant concentration of coeruleo-hippocampal neurons in this area. These results suggest that β-AP not only has a neurotoxic effect on hippocampal cells but also on noradrenergic cells whose axons project to this area. In addition, β-AP is known to cause severe damage to noradrenergic terminals (Emre, M. et al., *Neurobiology of Aging*, 13:553, 1992) and this in turn may lead to increased firing of LC cells resulting in depletion of Glu.

727.12

GP120, AN HIV-1 DERIVED PEPTIDE, SELECTIVELY AUGMENTS NMDA-MEDIATED HIPPOCAMPAL INJURY IN PERINATAL RODENTS. Faye S Silverstein, John DE Barks*, Rong Sun University of Michigan, Ann Arbor, MI 48109

Recent data suggest that gp120, a glycoprotein secreted by HIV infected macrophages, is neurotoxic, and that toxicity is mediated, in part, by activation of NMDA-type excitatory amino acid (EAA) receptors. Since susceptibility to NMDA-mediated injury peaks in immature rodents, we examined the neurotoxicity of gp120 alone, and co-injected with NMDA in 7 day old (P7) rats using stereotaxic coordinates targeted to right dorsal hippocampus (HIP) or striatum. Our previous studies suggested that HIP neuropil was most susceptible and that loss of tissue mass was a sensitive index of injury. Injury was assessed on P12, by histopathology and estimates of bilateral HIP volumes (from serial cross-sectional areas). GP120 elicited subtle, dose-dependent HIP injury (1 ng, n=3, no injury; 50 ng, n=7, subtle cell loss close to injection site, 7/7, mild HIP atrophy, 1/7; 200 ng, n=5, focal cell loss 4/5, mild HIP atrophy, 3/5). Co-injection of 50 ng gp120 with 5 nmol NMDA (n=22) produced much greater HIP atrophy than 5 nmol NMDA alone (n=23) (gp120+NMDA, -43±3.3%, NMDA, -21±2.6%, $p<0.001$, t-test). In striatum, gp120 did not influence the severity of NMDA-mediated injury. In contrast, co-injection of 50 ng gp120 with 25 or 50 nmol quisqualate did not increase HIP injury. Thus, gp120 increased EAA-induced neuropathology in a region and agonist-specific fashion. These data provide strong support for the hypothesis that locally secreted gp120 may potentiate the neurotoxicity of endogenous EAA neurotransmitters, acting at NMDA-type receptors, in HIV-infected brain.

727.13

D-ASPARTATE EFFLUX FROM ASTROCYTES OCCURS DURING GLUCOSE BUT NOT OXYGEN DEPRIVATION AND IS PREVENTED BY THREO-B-HYDROXY-ASPARTIC ACID. M.C. Longuemare* and R.A. Swanson, Dept. of Neurology, Univ. of California and VAMC, San Francisco, CA 94121.

Controversy exists as to whether astrocytes are a major source of excitatory amino acid release during cerebral ischemia. To address this issue, primary rat cortical astrocytes were preloaded with [^{3}H]-D-aspartate (D-ASP) and subjected to substrate deprivation. Glycolytic blockade caused an 85% reduction in intracellular D-ASP ($[D\text{-}ASP]_I$) in one hour. Preloading the cells with the transport inhibitor threo-B-hydroxy-aspartic acid (TBHA) completely prevented this efflux, suggesting reversal of uptake as the route of efflux. To investigate the mechanism of uptake reversal, D-ASP-loaded astrocytes were depolarized (4mM Na, 142mM K) in the presence of ouabain. Depolarization produced an 80% reduction in $[D\text{-}ASP]_I$ which was largely prevented by preloading cells with TBHA. These results support the hypothesis that glucose deprivation causes disruption of ion homeostasis and reversal of glutamate uptake. In contrast to glycolytic inhibition, blockade of oxidative metabolism with sodium azide failed to induce efflux of D-ASP. These results suggest that continued glycolytic metabolism is an important determinant of whether excitatory amino acids will be released from astrocytes during ischemia.

727.14

MK-801 ATTENUATES INCOMPLETE BUT NOT COMPLETE INFARCTION IN PHOTOTHROMBOTIC DISTAL MIDDLE CEREBRAL ARTERY OCCLUSION (MCAO) IN RATS. H.Yao, C.G.Markgraf, W.D.Dietrich, R.Prado, B.D.Watson and M.D.Ginsberg*. Cerebral Vascular Research Center, Department of Neurology, University of Miami School of Medicine, Miami, FL 33101

Despite its published success, the non-competitive NMDA antagonist, MK-801, failed to reduce infarct volume in a stroke model of Sprague-Dawley rats (Yao et al., Stroke 1993). In this study, we studied the effects of MK-801 on incomplete (selective neuronal injury) as well as complete infarction in the same model mentioned above, using Wistar rats. MK-801 (1mg/kg, i.p.), administered 30 min before distal MCAO, failed to affect the volume of complete infarction (n=5-6, mean±S.D., 95±16 mm3 and 92±14 mm3 in untreated and treated groups, respectively). By contrast, the volume of incomplete infarction was reduced by 44% with MK-801 treatment. Nonetheless, the potentially treatable zone of incomplete infarction, which consisted of 7% of total injury volume (complete+incomplete infarct), was small. Thus, therapeutic efficacy with MK-801 would be small in this model of thrombotic distal MCAO.

727.15

NEUROBEHAVIORAL EFFECTS OF PERINATAL INTRACRANIAL ADMINISTRATION OF EXCITOTOXINS. B.A. Pappas*, K. Hewitt, J.N. Armstrong, W. McCleod and D.C. McIntyre. Dept. of Psychology, Carleton Univ., Ottawa, Canada K1S 5B6.

The neonatal rat brain is most sensitive to the glutamate receptor agonist N-methyl-d-aspartate (NMDA) at around 7 days of age (McDonald and Johnston, Brain Res. Rev., 1990). We have observed that medial septal injections of NMDA but not ibotenic (IBO) or quisqualic (QUIS) acid at this age severely impairs behavior and causes marked ventricular hypertrophy and periventricular tissue loss. The brain damage was evident ten days after injection and persisted into adulthood. The NMDA-injected rats also developed spontaneous recurrent seizures. NMDA and IBO but not QUIS caused transient reductions of hippocampal and cortical choline acetyltransferase activity. IBO caused minimal ventricular hypertrophy ten days later but substantial hypertrophy in adulthood. The neural and behavioral effects of NMDA were prevented by systemic administration of MK-801 thirty minutes beforehand. Administration of MK-801 thirty minutes after afforded lesser but significant protection.

727.16

QUANTITATIVE EVALUATION OF THE PROTECTIVE EFFECTS OF GLUTAMATE RECEPTOR ANTAGONISTS IN A PHOTOTHROMBOTIC MODEL OF RETINAL ISCHEMIA. F. Moroni Jr., G. Lombardi, S. Faussone-Pellegrini and F. Moroni*. Depts. Pharmacology, Ophthalmology and Histology, Univ. of Florence, 50134 Firenze, Italy.

We previously described a quantitative "in vivo" model to study ischemic neuronal damage in the rat retina. Neuronal damage was quantified by measuring the activity of the marker enzymes choline acetyl-transferase and glutamic acid decarboxylase (*Vision Res.*, 1993, in press). Retinal ischemia was induced by injecting rats i.v. with the photosensitive dye rose bengal (80 mg/kg) and by exposing one of their eyes to cold light filtered at 560 nm. This results in profound damage of retinal tissue. The inner nuclear and the inner plexiform layers had a diffused swelling and numerous neurons had the chromatin densely packed forming clumps distributed all along nuclear contours. Blood vessels were enlarged and had thrombotic material. With the aim of investigating whether excitatory amino acid antagonists were able to reduce this neuronal damage we intravitreally administered to the rats, immediately after the photochemical reaction, the following compounds: 1) aminophosphonovaleric acid; MK801 (competitive and non competitive NMDA antagonists); 2) NBQX and GYKY 52466 (competitive and non competitive AMPA antagonists); 3) 7-Cl-thiokynurenic acid (glycine antagonist and free radical scavenger) and 4) thiokynurenic acid (nonselective glutamate antagonist and radical scavenger). A significant degree of protection was obtained when large doses of these antagonists (50-200 nmoles) were injected intravitreally. However, a maximum degree of protection (a reduction of 80% of the damage found in saline injected eyes) was obtained when both AMPA and NMDA receptor antagonists were simultaneously injected. This was also obtained by administering 20-50 nmoles of thiokynurenic acid. In conclusion, antagonism of both AMPA and NMDA receptors results in better protection than selective antagonism of only one receptor type.

727.17

INTRACELLULAR CALCIUM DEPOSIT FORMATION AFTER EXCITOTOXIC LESIONS OF THE RAT GLOBUS PALLIDUS. N. Mahy[1*], J Rivero[3,5], F. Bernal[1], M. Lorente[2], R. Sainz[1], N. Andres, X. LLovet[3], K. Fuxe[1,4] and A. Prats[2]. U. Biochemistry[1] and Dept. Anatomy[2], Sch.. Medicine, Scient. Tech Serv.[3] Univ. Barcelona, Spain, Dept Histol. Neurobiol. Karolinska Inst. Stockholm, Sweden[4] and CONICET, Argentina[5].

Microinjections of ibotenic acid (25 mM) into the globus pallidus of adult male Sprague-Dawley rats led to the progressive appearance of intracellular calcium deposits related to a progressive neurodegenerative process. In the present study electron microprobe showed the existence of two different groups of deposits, one of which contained phosphorus and the other one silicon and aluminium. A concomitant rise in potassium was associated with the lesion. Transmission electron microscopy revealed a strong astro and microglial activation and the presence of thin fibrous material in the calcium deposits linked with mitochondria and other intracellular organelles. The pattern of distribution of these deposits excludes their possible capillary origin. In summary, our data show the diversity of intracellular calcium deposits and establish a close relationship between their progressive formation and the neurodegenerative processes. (Supported by FISss 90E0593-2E; N.A is a fellow of CIRIT)

727.18

MODULATION OF GLUCOCORTICOID RECEPTOR BINDING IN PRIMARY CULTURES OF DISPERSED HIPPOCAMPAL CELLS FOLLOWING NMDA TREATMENT. D. O'Donnell*, R. Alonso, J. Diorio, and M.J. Meaney, Develop. Neuroendocrinology Lab., Douglas Hosp. Res. Ctr; Depts. of Psychiatry and Neurology & Neurosurgery, McGill Univ., Montreal, Canada H4H 1R3.

Glucocorticoids have been shown to exacerbate neuronal cell loss in cases of excitatory amino acid mediated insults. The present study examined the effects of NMDA treatment on glucocorticoid receptor (GR) binding capacity in a model of delayed excitotoxicity in primary cultures of dispersed hippocampal cells. Two hours following a 10 min. exposure to a lethal dose of NMDA (500 μM), a 30-40% decrease in GR binding capacity was detected in comparison to vehicle-treated cultures. Examination of cell viability indicated no cell loss at this early time point. Western blot analysis from total cell culture homogenates revealed no change in the 97 kDa immunoreactive GR protein thus ruling out degradation of the receptor as a likely cause for the decrease in GR binding. Elevation of intracellular calcium (Ca^{2+}) is the hallmark NMDA neurotoxicity. *In vitro* manipulations of Ca^{2+} concentrations in cell culture homogenates successfully mimicked the NMDA-induced decrease in GR binding capacity. These findings suggest that during the initial stages of NMDA toxicity, which are associated with a massive influx of Ca^{2+}, effects of glucocorticoids may be dampened. This study strengthens the hypothesis that intracellular Ca^{2+} may act as a physiological modulator of GC hormone action at the receptor level.

727.19

GLUTAMATE-INDUCED ENERGETIC STRESS IN HIPPOCAMPAL SLICES. D.H. Pennington, A. J. Douglas, W.D. Lust and T.S. Whittingham*. Department of Neurosurgery, Case Western Reserve University, Cleveland, OH 44106.

We previously reported that exposure of hippocampal slices to 1 mM glutamate for 30 minutes produced a marked decrease in ATP and PCr, and elevation of tissue lactate. This metabolic stress did not appear to be mediated by NMDA receptor stimulation or enhanced glutamate uptake. We have now investigated the possible roles of the kainate, AMPA and metabotropic receptors in triggering these alterations. Transverse hippocampal slices from male mongolian gerbils were equilibrated for 2 hr before exposure to artificial cerebrospinal fluid (ACSF) containing an experimental condition. Slices were frozen after 30 min, assayed for ATP and PCr, and metabolite values expressed as nmoles per mg protein.

1 mM glutamate produced a 13% fall in ATP (from 10.21 to 8.88; $p < 0.05$) and a 30% drop in PCr (from 19.59 to 13.79; $p < 0.05$). Kainate exposure produced a dose-dependent fall in both ATP and PCr, with 10 μM producing a maximal effect (ATP fell to 8.99 and PCr to 13.87). Another kainate agonist, 1 μM domoic acid, produced a similar fall in ATP and PCr. AMPA also caused a substantial fall in the high energy phosphates, but was maximally effective at 100μM (ATP = 5.65; PCr = 10.11). However, two other AMPA agonists, quisqualate and aniracetam, were relatively ineffective. Finally, the metabotropic agonist ACPD (1 μM) was the most potent agonist tested, dropping ATP to 4.95 and PCr to 8.34. The agonist experiments suggest that all three glutamate receptor subtypes which were tested could produce the metabolic effects. Three receptor antagonists were tested at concentrations of 100 μM. CNQX blocked the kainate- and AMPA- and ACPD-induced metabolic stress. The kainate antagonist GAMS also blocked the metabolic effects of kainate, AMPA and ACPD. The metabotropic antagonist, AP3, was effective in blocking the ACPD effects. Finally, CNQX was the only antagonist which prevented the metabolic changes during exposure to 1 mM glutamate. The results suggest there are multiple mechanisms by which glutamate can elicit an energetic stress in vitro, involving kainate, AMPA and metabotropic receptor subtypes, but not involving the NMDA receptor. CNQX appears to be the only compound capable of blocking all aspects of the metabolic stress.

727.20

COMPARISON OF NON-CHOLINERGIC NEUROTRANSMITTER EFFECTS FOLLOWING INTRACRANIAL INFUSIONS OF COLCHICINE AND IBOTENIC ACID INTO THE BASAL FOREBRAIN. L.W. Shaughnessy*[1], S. Barone Jr.[2], W.R. Mundy[3], D.W. Herr[3] & H.A. Tilson[3]. [1]Curriculum in Neurobiology, UNC, Chapel Hill [2] ManTech Environmental Tech. Inc. [3] NTD/HERL RTP, NC 27711

Intracranial infusion of various toxicants has been used to experimentally produce an animal model of basal forebrain neurodegeneration. The goal of the present experiment was to make direct comparisons of the effects of ibotenic acid and colchicine infusions on several non-cholinergic neurotransmitter systems. Bilateral infusions of either vehicle (0.5µl 0.1 M phosphate buffer, pH 7.4/site) colchicine (3.0µg/0.5µl/site) or ibotenic acid (6.0µg/0.5µl/site) were made in the nucleus basalis magnocellularis (NBM) of male Long-Evans rats. Four weeks post-lesion, behavioral assessments were made, and one week later, the rats were sacrificed for neurochemistry or histochemistry. Previous data showed that basal forebrain cholinergic and cognitive effects were similar following infusion of either neurotoxicant (Shaughnessy *et al., Soc. Neurosci Abst.* 1992). Analysis of several non-cholinergic measures revealed differences between the two neurotoxicants. Both neurotoxicants produced decrements in glutamic acid decarboxylase (GAD) staining in regions surrounding the NBM as determined by computerized image analysis. GAD immunoreactivity was significantly decreased in the reticular nucleus of the thalamus and the globus pallidus following ibotenic acid (41%) and colchicine infusions (21%), with a greater decrease in both area and density of stain for ibotenic acid. Catecholamine and indoleamine levels were measured in frontal and parietal cortex, striatum, and hippocampus using HPLC. No effects were observed in cortical or hippocampal catechol- or indoleamines. However, colchicine infusion decreased striatal dopamine and 3,4-dihydroxyphenylacetic acid (DOPAC) 21 and 24%, respectively, while ibotenic acid infusion had no effect on neurotransmitter levels. These results are discussed in relation to the cholinergic hypothesis of cognitive function.

EXCITATORY AMINO ACIDS: EXCITOTOXICITY VIII

728.1

NMDA-RECEPTOR MEDIATED NEUROTOXICITY IN CULTURED CEREBELLAR NEURONS IS ENHANCED AT LOW TEMPERATURES. A. Novelli* and A. Marini. Dept. Functional Biol., Biochem., Oviedo Univ., Sch. of Med., 33006 Oviedo, Spain; and Dept. of Neurology, Veterans Medical Center, Washington D.C. 20422, USA.

We have previously shown that 24 h exposure of cultured cerebellar neurons to glutamate (GLU) into the growth medium at 37ºC, produces a concentration-dependent neurotoxicity, which is entirely blocked by NMDA-receptor antagonists (Novelli et al. Brain Res. 1992, 577, 41), and that GLU neurotoxicity increases with days in culture (DIC), being negligible at 5 DIC (Fernández et al. 1993, Mech. Neur. Injury and Degen., in press). We now report that the concentration of GLU producing signs of excitotoxicity such as darkening and swelling of the cell bodies in virtually all the neurons at 5-6 DIC, was reduced from >1000 µM to ~20 µM by lowering the incubation temperature to 25ºC for 30 min before and 60 min after the addition of GLU to the growth medium. 24 h after returning the culture to 37ºC, 50% of the neurons were dead following exposure to 25 µM GLU. Maximum toxicity by GLU (500 µM) was limited to ~65% of the neurons. GLU (1000 µM) failed to produce any neurotoxicity in cultures continously kept at 37ºC. At 24 DIC, the concentration of GLU producing 50% neurotoxicity (NTC50) shifted from ~15 µM at 37ºC to ~5µM at 25ºC, while 90% neurotoxicity was observed at ~25 µM at 37ºC, and ~10 µM at 25ºC. The neurotoxic effect of GLU was blocked by the NMDA receptor antagonist MK-801 (1µM) at any DIC. The neurotoxicity elicited via stimulation of the ionotropic NON-NMDA receptor by domoate (DOM), was not significantly enhanced by temperature changes in 5-6 DIC cultures. At 24 DIC, a modest shift in DOM NTC50 was observed (~5 µM at 25ºC vs. ~7.5 µM at 37ºC). Nevertheless, such shift was abolished in the presence of MK-801, indicating a role for DOM-induced release of endogenous GLU in the enhancement of neurotoxicity. Our results suggest that cerebellar neurons at early DIC already possess NMDA receptors that may mediate neurotoxicity, although they appear to be protected by other mechanisms which are temporarely impared by short exposures to lower than physiological temperatures. Research supported by CICYT, SAL 91-0613.

728.2

COMPUTER SIMULATION OF N-METHYL D-ASPARTATE-RECEPTOR-DEPENDENT CHANGES IN A MAMMALIAN SPINAL NEURON. P. Saxena, R. Goldstein†, T. Marczynski*, L. Isaac Dept. Pharmacol. and Computer Ctr†., Univ. of Ill. at Chicago, 60612.

Previously, we showed that dynorphin-induced neuron toxicity was mediated by N-methyl-D-aspartate (NMDA) sites in rat spinal cord. To study this excitotoxic process we developed a computer neuron model to simulate membrane effects of NMDA-receptor activation. This is a compartmental model based on Hodgkin-Huxley type equations. Parameter values are within the range typically observed in mammalian neurons. We included voltage-dependent Mg^{2+} blockade of NMDA-associated ion channels and the kinetics of Ca^{2+} influx through these channels. Synaptic input may be placed at varying distances from the axon and the model is designed to minimize computing time.

The output of our model is in harmony with observations of potential changes following application of NMDA to neurons. Also, it responds to simulated changes in extracellular Na^+ and K^+ in a manner characteristic of neurons. We are currently working to incorporate our neuron model into a larger simulation of a spinal reflex pathway. Our goal is to use the model to explore mechanisms of dynorphin-induced neuron toxicity.
Supported by NIH NS-30295.

728.3

NMDA ANTAGONISTS AND MPP$^+$-RELATED DOPAMINERGIC TOXICITY IN MESENCEPHALIC CULTURES. W.J. Nicklas, E. Derr-Yellin and G.D. Zeevalk. Dept. Neurology, UMDNJ-Robert Wood Johnson Med. Sch., Piscataway NJ 08854.

Excitatory amino acid (EAA) receptor activation has been implicated in neurodegeneration associated with various modes of metabolic stress. The dopaminergic (DAergic) neurotoxin, MPP$^+$, is thought to cause cell death by mitochondrial inhibition but whether excitotoxicity is involved has been controversial. Rat mesencephalic cultures incubated with MPP$^+$ for 24 h evinced a dose-dependent decrease in DA cell viability as measured by DA uptake immediately after the 24 h incubation or 2 to 3 d later. However, at $< 1\mu$M MPP$^+$ the DA uptake 2 to 3 d after treatment was no longer depressed and was actually increased over controls. At $> 5\mu$M MPP$^+$, there was no recovery or augmentation of uptake. Thus there was a clear dose relationship for irreversible DAergic damage. In all cases, GABA uptake, as a measure of non-specific damage, was unaffected. The decrease in DA uptake observed 2-3 days after treatment, but not the acute decrease at 24 h was prevented by incubation during the 24 h insult period with NMDA antagonists (MK-801 or CGS-19755). The KA/AMPA antagonist, CNQX, had no effect. At present, it is not completely clear whether the NMDA antagonists are directly protecting against MPP$^+$ toxicity or if the antagonists themselves are exerting a trophic-like action on the survivability of stressed neurons.

728.4

DEVELOPMENT OF NEURODEGENERATION INDUCED BY 3-NITROPROPIONIC ACID (3-NPA) INTOXICATION. C.N. Allen*[1,2], J. Marino[1], and C.K. Meshul[3,4]. Cntr for Res on Occup and Environ Toxicol[1], Dept Physiology[2] and Dept Med Psychol[3], OR Hth Sci Univ and V.A. Med Cntr[4], Portland, Oregon 97201.

3-NPA, a naturally occurring neurotoxin, is a suicide inhibitor of succinate dehydrogenase (SDH), a component of the mitochondrial electron transport chain complex II. 3-NPA is proposed to produce neurodegeneration by reducing neuronal energy levels, making neurons more vulnerable to glutamate excitotoxicity. We used a silver impregnation procedure to study the development of neurodegeneration in rats continously receiving 3-NPA via osmotic minipumps. Two days of continuous 3-NPA intoxication (6.7 mg/day) produced a significant loss of weight which continued for up to 7 days. In contrast, rats receiving chronic 0.9% saline had a significant weight gain by 5 days. During the 3-NPA intoxication behavioral signs progressed from a wobbly gait to complete ataxia. The silver staining showed no evidence of degeneration at 1, 2 or 3 days of chronic 3-NPA intoxication. Degenerating fibers were observed within the diencephalon after 5 days with increasing fiber degeneration at 7 days. Removal of the 3-NPA after 5 days caused a reversal of the weight loss but degenerating fibers were still present in the diencephalon at day 10. Severely affected animals showed complete deterioration of the striatum and were ataxic. 3-NPA is a useful tool to study the effects of chronic exposure to metabolic toxins. Supported by NIH grant NS19611 and Dept of Veterans Affairs.

728.5

TGF-β1 ATTENUATES NMDA-INDUCED $[Ca^{2+}]_i$ ELEVATIONS AND NEUROTOXICITY IN CULTURED RAT HIPPOCAMPAL NEURONS J.H.M. Prehn[1]*, C.J. Marcuccilli & R.J. Miller. Dept. Pharmacol. and Physiol. Sciences, Univ. of Chicago, Chicago, IL. 60637.

Transforming growth factor-β1 (TGF-β1), a multifunctional cytokine, is virtually absent in the CNS of humans and other mammalian species. In contrast, TGF-β1 expression increases strongly under a variety of pathophysiological conditions, such as cerebral ischemia, Alzheimer's disease and AIDS neuropathology. Previous studies have demonstrated that TGF-β1 has neuroprotective properties (Prehn et al., 1993), but its mechanism of action is poorly understood. In cultured rat hippocampal neurons we found that bath application of TGF-β1 (3 and 10 ng/ml) did not change the basal free $[Ca^{2+}]_i$ as determined by fura-2 based microfluorimetry. However, perfusion with TGF-β1 (3 ng/ml) for 10 or 30 min was able to reduce the increase in $[Ca^{2+}]_i$ produced by fast application of the glutamate receptor agonist NMDA (30 μM) by 18 and 20 %, respectively. In contrast, kainate (10 μM)-induced $[Ca^{2+}]_i$ elevations were enhanced by 24 - 30 % in approx. 70 % of the neurons perfused with TGF-β1 (3 ng/ml). In the same culture system, TGF-β1 (1 - 10 ng/ml) dose dependently reduced neuronal injury induced by exposure of the cultures to 100 μM NMDA for 20 minutes. Neuronal injury caused by exposure to 100 μM kainate for 18 h was reduced by treatment with 1 ng/ml TGF-β1, but potentiated at higher concentrations of TGF-β1. In further experiments, cultured rat astrocytes were heat stressed for 60 minutes at a temperature of 42.5 °C. Treatment with TGF-β1 (10 ng/ml) led to an increased expression of the 70 kD heat shock protein (HSP70) compared with controls as demonstrated by Western blot analysis and immunocytochemistry. The present study suggests that TGF-β1 acts on both neurons and astrocytes to induce a variety of responses that could modulate neuronal survival.

[1]Supported by the Deutsche Forschungsgemeinschaft.

728.6

ANDROGEN ALTERS NMDA-MEDIATED RESPONSES IN HIPPOCAMPAL CA1 PYRAMIDAL CELLS. W.A. Pouliot, R.J. Handa and S.G. Beck* Departments of Pharmacology, and Cell Biology, Neurobiology and Anatomy, Loyola University Medical Center, Maywood, IL 60153.

Anabolic/androgenic steroid abuse is a silent epidemic among athletes at all levels of competition. The psychological side effects associated with steroid abuse implicate the limbic system. Androgen receptor protein and mRNA are high in the CA1 region of the hippocampus. Our goal was to determine if the androgen 5-α-dihydrotestosterone propionate (5-DHTP) modulates the physiology of CA1 hippocampal pyramidal neurons and/or glutamate receptor mediated responses. Standard intracellular recording techniques in hippocampal slices *in vitro* were used. Male Sprague-Dawley rats, age 6-8 weeks, were either sham operated (SHAM), gonadectomized (GDX), or gonadectomized with two Silastic capsules of 5-DHTP surgically implanted (GDX+DHTP) to generate supraphysiological plasma levels of DHTP. Rats were sacrificed 3 weeks after surgery. Hippocampal slices from GDX+DHTP treated rats were perfused with buffer containing a high concentration of 5-DHTP (10 nM) to mimic the *in vivo* environment. High concentrations of DHTP altered active membrane properties of CA1 pyramidal neurons. The fast afterhyperpolarization was decreased in cells from GDX+DHTP treated rats. To test 5-DHTP's effects on glutamate receptor mediated actions, slices were exposed to 1 μM, 10 μM, and 30 μM concentrations of N-methyl-D-aspartate (NMDA), a synthetic glutamate agonist. In cells from SHAM and GDX treated rats, NMDA led to irreversible depolarization; however the GDX+DHTP cells were protected from the excitotoxicity. We conclude that DHTP altered hippocampal CA1 neuronal membrane properties and protected the pyramidal neuron from NMDA induced excitotoxicity.

Supported by USPHS NS28512, MH00880, and NSF BNS9109226.

728.7

DELAYED IMMEDIATE-EARLY GENE ACTIVATION AND THE EXCITOTOXIC CASCADE Y. Shan*, L.R. Carlock & P.D. Walker, Departments of Anatomy/Cell Biology & Molecular Biology/Genetics, Wayne State University School of Medicine, Detroit, MI 48201.

Excitatory amino acid receptor overstimulation produces a rapid cascade of neuronal death and glial/immune activation. Previously, we had demonstrated that intrastriatal injection of the NMDA agonist, quinolinic acid (QA), triggers an extended phase of immediate-early gene (IEG; c-fos, c-jun, jun-B, zif/268) activation that overlaps with the induction of glial transcripts (GFAP, IL-1B) and the decline of neurotransmitter mRNAs (Shan *et al.*, Soc. Neurosci. Abstr. 18 [1992] 167). Since it is possible that glial IEG activation may contribute to the spread of the excitotoxic cascade, the following experiments represent further characterization of the delayed IEG transcriptional phase.

Adult male rats received unilateral intrastriatal injections of QA followed by sacrifice at acute time periods throughout the first day of the lesion. Northern analysis revealed normal neurotransmitter mRNA levels up to 6 hours following QA injection with a rapid decline thereafter. Interestingly, the delayed phase of IEG activation exhibited peak levels at 6 hours. Additional studies that attempt to determine if the delayed IEG transcriptional phase only occurs in relationship to excitotoxicity will be discussed. (Supported by NS24236 and the WSU Office of Neuroscience Programs)

728.8

DEVELOPMENTAL ALTERATIONS IN THE VULNERABILITY TO NMDA RECEPTOR ACTIVATION DETECTED WITH DIFFUSION-WEIGHTED NUCLEAR MAGNETIC RESONANCE IMAGING. M. van Lookeren Campagne[1], H.B. Verheul[2], K. Nicolay[3] and R. Balázs*(spon:ENA).[1]Neth. Inst. Brain Res., Amsterdam, [2]Dep. Neurosurg., Univ. Hosp. Utrecht, [3]Bijvoet Center for Biomol. Res., Utrecht Univ., The Netherlands.

Contrast in diffusion-weighted images (DwIs) reflects differences in molecular translational movement (Brownian motion) of water molecules. We observed that an increase in signal intensity in DwIs taken from CNS of rat pups after intracerebral injection with NMDA parallels an increase in the intracellular volume fraction, measured with electrical impedance and confirmed by histology. Diffusion-weighted imaging therefore offers a possibility to detect, in vivo, local changes in cell volume as an early consequence of excitotoxic injury. In postnatal day (PND) 7 rat pups, intracerebral injection of NMDA shows a rapid increase in cell volume over a large area, encompassing the ipsilateral dorsal striatum, parietal cortex and the rostral part of the hippocampus. The degree of cell swelling, as well as the number of areas in which cells acutely respond to NMDA, peak between PND4-10 and decline rapidly at older ages. The ontogenetic peak of enhanced vulnerability to NMDA coincides with an enhanced diffusion of injected extracellular tracer molecules in the normal brain, detectable with magnetic resonance imaging techniques. We suggest that developmental changes in diffusion of NMDA together with changes in NMDA receptor properties contribute to the peak in vulnerability of the neonatal brain to intracerebrally injected NMDA.

728.9

HIV COAT PROTEIN GP160 EVOKES POTENTIATION OF NMDA-INDUCED RESPONSE OF CULTURED HUMAN EMBRYONIC NEURONS. P.-M. LLEDO*[1], A. LANISELLE[2], M. TARDIEU[2] & L-D. VINCENT[1]; [1]CNRS, I.A.F., 91198 Gif-sur-Yvette, France; [2]INSERM, Lab. Neurobiol., U. 56, Hôpital Bicêtre, 94275 Le Kremlin-Bicêtre, France.

The envelope protein of the human immunodeficiency virus (gp 120) has been proposed to cause neuronal death in developing murine hippocampal cultures or rat retinal ganglion cells. In HIV-infected individuals, gp120, released after cleavage of gp160 from HIV-infected macrophages or other cells in the brain, has been proposed as the etiology for the pathophysiology of AIDS central nervous system (CNS) disease. In this study, we have used cultured human embryonic cerebral cortical and spinal neurons to test whether gp160 is itself neurotoxic. Neuronal cultures of 10-30 days from 8 to 12-week old foetuses were exposed for various times (1-45 min) to a buffered Locke's solution containing up to 500 pM gp160 (Transgene) without any effect on voltage-gated Ca^{2+} currents or on intracellular free Ca^{2+} concentration ($[Ca^{2+}]_i$). By contrast, the large rises in $[Ca^{2+}]_i$ induced by 50 µM NMDA were potentiated by acute (15 min) exposure to 50-250 pM gp160. This potentiation, which was not mimicked by other glycoproteins such as IgG, provides a mechanism by which HIV infection may cause neuronal death through Ca^{2+}-mediated excitotoxicity induced by NMDA and, therefore, may be relevant to the pathophysiology of HIV-related CNS disease.

(Supported by the C.N.R.S. and I.N.S.E.R.M.)

728.10

CHARACTERIZATION OF THE ACUTE NEUROTOXICITY OF GLUTAMATE IN PRIMARY CULTURES OF CEREBELLAR GRANULE CELLS. F.W. Berman, T.F. Murray*.College of Pharmacy and Toxicology Program, Oregon State University, Corvallis, Oregon, U.S.A.

The objective of the current study was to develop and characterize an excitotoxicity assay in the presence of a normal physiologic milieu. Cerebellar granule neurons derived from 8 day old rat pups were grown in primary culture. Neurotoxicological assays were performed at 12 days in culture in normal physiologic medium at 25°C. Under these conditions, N-methyl-D-aspartate (NMDA) was shown to be non-toxic at concentrations as high as 800µM. The neurotransmitter glutamate was toxic with an EC50 of 94µM. Several non-competitive antagonists at the NMDA receptor were tested against a 300µM glutamate challenge. These compounds afforded neuroprotection with the following rank order of potency: (+)-5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imindhydrogen maleate (MK-801) > 1-[1-(2-thienyl)cyclohexyl]piperidinehydrochloride (TCP) > dextrorphan > ketamine > dextromethorphan. The competitive NMDA receptor antagonist D-2-amino-5-phosphonopentanoic acid (D-AP5), demonstrated neuroprotection as well. The strychnine-insensitive glycine site antagonist, 3-amino-1-hydroxypyrrolidin-2-one (HA-966), was also effective in preventing glutamate neurotoxicity. Interestingly, the quinoxaline derivative 6-cyano-7-nitroquinoxaline-2,3-dione(CNQX), which acts at the AMPA/kainate receptors, also potently prevented neurotoxicity at concentrations less than those expected for significant occupancy of the strychnine-insensitive glycine binding site. These results suggest that glutamate is excitotoxic in the presence of Mg^+ and that this effect is first triggered by an action at the AMPA/kainate receptors, whereby neuronal depolarization via sodium influx effects release of the voltage dependent magnesium blockade of the NMDA receptor ion channel. (Supported by DAO7218.)

728.11

NEURODEGENERATION INDUCED BY ISCHEMIA OR GLYCOLYSIS INHIBITORS IS PREVENTED BY LACTATE INFUSION IN HIPPOCAMPAL SLICES. AM Benz, Y Izumi, CF Zorumski, DB Clifford*, MT Price & JW Olney, Depts of Psychiatry and Neurology, Washington Univ, St Louis, MO

Using rat hippocampal slices, we have shown participation of nitric oxide (NO) in NMDA excitotoxicity, and it has been proposed that NO may play a role in ischemic neurodegeneration. Although the mechanism by which NO damages neurons is not understood, it is known that NO induces ADP-ribosylation which may inhibit glyceraldehyde-3-phosphate dehydrogenase (GAPDH), an enzyme required for glycolysis. As a first step toward investigating the potential role of GAPDH inhibition in excitotoxic phenomena, we have begun examining the effects on rat hippocampal slices of blocking glycolysis by GAPDH inhibitors. Consistent with findings of Schurr et al., blocking glycolysis with the GAPDH inhibitors, iodoacetate (IA) or iodosobenzoate (IB), caused a loss of extracellularly recorded EPSPs, whereas addition of lactate or pyruvate to the medium conserved EPSPs. Histologically, inhibition of glycolysis by IA or IB caused severe degenerative changes in CA1 neurons resembling changes induced by NMDA (edematous swelling and nuclear pyknosis). These changes were prevented by including lactate in the incubation. Exposure of the hippocampal slice to simulated ischemia (O_2/glucose deprivation) also caused severe NMDA-like degeneration which was prevented by inclusion of lactate. These findings suggest that either glycolysis inhibition or simulated ischemia causes neurotoxic changes in CA1 neurons which can be prevented by supplementing with an end product of the glycolytic pathway. Further pursuit of these findings may shed new light on mechanisms underlying the role of NO in NMDA excitotoxicity and on methods of protecting against ischemic neuronal degeneration. Supported by RSA MH 38894 (JWO), RSDA MH00964 (CFZ).

728.12

CLIMBING FIBERS MEDIATE IBOGAINE-INDUCED PURKINJE CELL DEGENERATION. E. O'Hearn and M.E. Molliver*. Johns Hopkins University School of Medicine, Baltimore, MD 21205.

Ibogaine, a plant indolealkylamine proposed for treatment of drug addiction, leads to degeneration of parasagittal bands of Purkinje cells (PKCs) in the vermis. This toxicity is demonstrated by loss of PKC MAP-2, calbindin, and Nissl-stain with positive silver staining of PKC bodies and processes[1]. Markedly activated astrocytes and microglia are co-extensive with the degenerating PKCs[2]. Harmaline, a structurally related indole alkaloid which also induces PKC degeneration[1], is known to excite inferior olivary neurons and causes sustained PKC activation via climbing fibers[3].

In order to determine whether climbing fibers mediate ibogaine-induced PKC degeneration, we lesioned the inferior olive with the neurotoxic regimen of 3-acetylpyridine followed by harmaline and nicotinamide[4]. Ibogaine (100 mg/kg i.p.) administered one week later had a markedly reduced neurotoxic effect on PKCs compared to the loss of PKC MAP-2, calbindin, calcium-calmodulin kinase II, and Nissl-stain seen after ibogaine was given to rats with inferior olives intact. Activation of astrocytes and microglia was also significantly reduced. These results demonstrate that ibogaine-induced PKC degeneration requires an intact olivo-cerebellar projection. We propose that the toxicity is due to excessive release of an excitatory amino acid from climbing fiber axons and that ibogaine-induced PKC degeneration is a new model of experimentally induced endogenous excitotoxicity.

[1]O'Hearn & Molliver. Neuroscience, In Press; [2]O'Hearn et al. Neuroreport 4:299 (1993); [3]Lamarre et al. Brain Res. 32:246 (1971); [4]Llinás et al. Science 190:1230 (1975). (Support: NINDS NS09293 and NIDA 271-90-7408)

728.13

PURKINJE CELL DEGENERATION AFTER IBOGAINE TREATMENT: ULTRASTRUCTURAL EVIDENCE. M.A. Wilson* and M.E. Molliver. Johns Hopkins University School of Medicine, Baltimore, MD 21205

The indole alkaloid ibogaine has been proposed for use in the treatment of drug addiction. However, in rats ibogaine induces glial activation in the cerebellar vermis[1] and signs of Purkinje cell (PC) degeneration including loss of Nissl staining, MAP2 and calbindin, and staining of PC's with the Gallyas reduced silver method for degenerating neurons[2]. We now present ultrastructural evidence that ibogaine treatment produces Purkinje cell degeneration.

In untreated controls, PC's exhibit finely granular chromatin, infoldings of nuclear membrane filled with polysomal ribosomes, prominent rough ER and Golgi apparatus, mitochondria, few lysosomes, and hypolemmal cisternae of smooth ER. Twenty-four hours after ibogaine treatment (100 mg/kg, i.p.) profoundly abnormal PC's are seen in small zones of the vermis; intervening PCs exhibit normal morphology. Most abnormal cells appear to have undergone edematous necrosis, leaving a large void in the PC layer containing vacuolar and granular debris. While fragments of the nuclear membrane may remain, few organelles are identifiable. Other PC's exhibit cytopathologic features of dark cell degeneration: they are extremely electron-dense and have aggregated chromatin, an extremely irregular nuclear membrane, clusters of clear vacuoles, swollen mitochondria and remnants of ER. Extremely dark, abnormal PC dendrites are present in the molecular layer directly above degenerating PC bodies. Further studies will attempt to determine whether these two types of cytopathology reflect distinct neurotoxic mechanisms or different stages in a single pathologic process. [1]O'Hearn et al. '93; [2]O'Hearn & Molliver '93. (Support: NIDA DA04431, 271-90-7408)

728.14

IGF-I confers glutamate-sensitivity to glutamate-resistant cerebellar granule cells. P.Calissano, M.T.Ciotti, D.Mercanti, C.Volonté*, D.Merlo, S.Giannetti, A.Angelini.
Institute of Neurobiology,C.N.R. via C.Marx 00162 Roma,Italy.

We have previously reported that 8 days old rat cerebellar granule cells cultured in vitro in the presence of a neurite outgrowth adhesion complex(NOAC) differentiate but are resistant to the otherwise lethal action of glutamate. NOAC-cultured neurons grown also in the presence of human recombinant IGF-I become fully sensitive to the toxic action of this excitatory aminoacid. The effect of this somatomedin is not mimicked by other growth factors such as NGF, EGF ,PDGF ,b-FGF, a-FGF, alfa-TGF. The glutamate-sensitizing action of IGF-I is concentration dependent(half maximum effect at 2-4 ng/ml), occurs within 1-3 days and is accompanied by the appearance of functionally active, glutamate-operated Ca++ channels and of voltage-gated Na+ and late K+ channels. Removal of IGF-I from the culture medium restores the glutamate resistant phenotype of NOAC-cultured neurons with a t 1/2 of 30 min. We postulate that the constitutive phenotype of cerebellar granule is glutamate-resistant and becomes glutamate-sensitive under the action of IGF-I and possibly of other epigenetic cues.

728.15

THE GANGLIOSIDE GM1 ATTENUATES THE DEVELOPMENT OF HALOPERIDOL-INDUCED ORAL MOVEMENTS IN RATS.
O. A. Andreassen[1] and H. A. Jørgensen[2]. (SPON: European Neuro-science Association). Dept. of Physiology[1] and Psychiatry[2], Univ. of Bergen, N-5009 Bergen, Norway.

Tardive dyskinesia (TD) is a serious side effect of long-term neuroleptic treatment. The mechanism of TD development is still unknown. It has been proposed that TD may be a result of neuroleptic-induced glutamate neurotoxicity in the striatum. To investigate this hypothesis, the effect of the anti-excitotoxic ganglioside GM1 (Fidia, Italy) was studied in a rat model of TD (female Sprague-Dawley rats). Four groups of rats (n = 10) were treated for 14 weeks with combinations of haloperidol decanoate 38 mg/kg IM every fourth week, GM1 20 mg/kg SC daily or placebo (sesam oil and saline). In an initial acute experiment a similar regimen was used, but ordinary haloperidol 1.2 mg/kg was injected IP. Haloperidol-induced behaviour was videotaped and vacuous chewing movements (VCM), an analogue to human TD, were scored.

Haloperidol induced a significant increase in VCM both in the acute and in the chronic experiment. Co-administration of GM1 did not attenuate the acute haloperidol effect. In the chronic experiment the co-administration group showed significant less VCM compared to animals receiving only haloperidol. GM1 administration in the placebo group did not affect the number of VCM.

These results may indicate that neuroleptic-induced excitotoxic neurodegeneration is a mechanism for the development of chronic VCM in rats and possibly for TD in humans.

728.16

EFFECT OF OPIOID AGONISTS ON EXCITOTOXIC DAMAGE TO THE STRIATOPALLIDAL GABAERGIC NEURONS.
K. Jhamandas*, S. Beare, R.J. Boegman. Department of Pharmacology and Toxicology, Queen's University, Kingston, ontario, Canada K7L 3N6.

The enhancement of methionine-enkephalin levels, observed in striatum and globus pallidus post excitotoxin injury to the striatum (Ruzicka and Jhamandas, Brain Res. 536: 227-239, 1990), may represent an adaptive response by which endogenous opioids modulate injury to other neurons. To determine if opioids have this potential, the present study evaluated the ability of μ, δ and κ receptor agonists to influence excitotoxic injury to the striatopallidal gabaergic neurons. Intrastriatal infusion of quinolinic acid (QUIN) (36, 72, 108 and 144 nmol, 1 μl volume) in the rat produced a dose-related depletion of glutamate acid decarboxylase activity (GAD), a biochemical marker for gabaergic neurons, in both the striatum and globus pallidus. This effect of QUIN was fully blocked by kynurenic acid. Co-injection of a kappa receptor agonist, U50,488H (50, 100 150 nmol), with QUIN (108 nmol) partially prevented the depletion of GAD activity. This effect, however, was not shared by DAGO or DPDPE, agonists which activate μ and δ receptors, respectively. The results suggest that certain opioids have the potential to modulate excitotoxin-induced injury to striatopallidal gabaergic neurons.
(Supported by the Medical Research Council of Canada)

728.17

DEXAMETHASONE ENHANCES NMDA RECEPTOR-MEDIATED INJURY IN THE POSTNATAL RAT.
D.E. Supko* and M.V. Johnston. Kennedy Krieger Research Institute and Johns Hopkins University School of Medicine, Baltimore, MD 21205.

Pretreatment with glucocorticoids can worsen brain injury resulting from hypoxia-ischemia or kainic acid injections in adult rats. Conversely, in postnatal rats, glucocorticoid pretreatment affords protection against hypoxic-ischemic brain damage. In the present study, we investigated the effect of a glucocorticoid on excitatory amino acid-induced neurotoxicity in postnatal rats. The synthetic glucocorticoid, dexamethasone (0.7 mg/kg, ip) was administered to 7 day old rats one hour prior to unilateral intrastriatal injection of either NMDA (20 nmol), AMPA (20 nmol) or kainic acid (35 nmol). One week later, damage was assessed both histologically and by measurement of striatal cytochrome oxidase activity. Dexamethasone enhanced NMDA receptor-mediated striatal damage by 56% ($p<0.01$) but had no effect on either AMPA or kainic acid receptor-mediated injury. Pretreatment with the phospholipase A_2 inhibitor, mepacrine (20 m g/kg, ip) or the adrenal mineralocorticoid, aldosterone (5 mg/kg, ip) did not affect NMDA-induced striatal damage. The effect of dexamethasone on NMDA sensitive [^{3}H]glutamate binding was assessed autoradiographically but no differences in binding were apparent in the striatum. Although these results do not suggest a mechanism, they indicate glucocorticoids may enhance neurotoxicity resulting from excessive NMDA receptor activation.

EXCITATORY AMINO ACIDS: EXCITOTOXICITY IX

729.1

Comparison of hippocampal injury caused by endogenous and exogenous glutamate in organotypic cultures.
R. C. Tasker*, J. K. Park and J. J. Vornov Dept. Intensive Care, HSC, Great Ormond St., London, England and Dept. of Neurology The Johns Hopkins School of Medicine, Baltimore, MD 21205

The characteristic regional vulnerability of the hippocampus to ischemic injury can be reproduced in organotypic cultures of hippocampal slices. While injury can be prevented by NMDA receptor antagonists, the regional vulnerability to ischemia or direct glutamate exposure are distinct from the distribution of NMDA receptors. The time course of glutamate and ischemic injury are slower than injury caused by direct exposure to NMDA itself.

We have now examined the injury caused by inhibition of sodium-dependent glutamate uptake to investigate the toxicity of endogenous glutamate. Blockade of uptake elevates extracellular glutamate to toxic concentrations. A 30 min exposure to either of the specific antagonists of glutamate transport,*threo*-3-hydroxyaspartate (THA) or L-*trans*-PDC caused selective neuronal injury. PDC is a more potent inhibitor of uptake and was more toxic than THA. The injury caused by endogenous glutamate was partially blocked by TTX or MK-801, as we previously observed for exogenous glutamate or simulated ischemia. The histologic appearance of glutamate-mediated injury was distinct from that caused by NMDA. Ultrastructurally, glutamate exposure caused a slow, edematous degeneration of dendrites and neuronal cell bodies. While glutamate toxicity is dependent upon the NMDA receptor, other mechanisms seem to create a pathologic process distinct from direct NMDA toxicity.

729.2

TWO COMPONENTS IN NEUROTOXICITY BY THE METABOTROPIC RECEPTOR ANTAGONIST AP3 IN CULTURED CEREBELLAR NEURONS .
M. T. Fernández*, A. Torreblanca and A. Novelli. Dept. Functional Biol., Biochem. and Mol. Biol. Section, Oviedo Univ., Sch. of Med., 33006 Oviedo, Spain.

Metabotropic glutamate receptor (GLURm) activation by glutamate (GLU) or trans-ACPD appears to stimulate phosphatidil inositol hydrolysis, intracellular calcium mobilization, protein kinase C activation, changes in cAMP levels, and excitatory amino acid release. Several authors have recently reported that the GLURm antagonist 2-amino-3-phosphonopropionate (AP3) may lead to neuronal degeneration. Using cerebellar neurons at 15-19 days in culture we have found that exposure to AP3 (50 μM) in the culture growth medium for 24 h did produce 95 % neuronal dead, which was completely prevented by the addition of the NMDA receptor antagonist MK-801 (1μM). Longer exposures (72 h) to AP3, did produced a neurotoxic effect which could not be blocked by MK-801, nor by the combined addition of MK-801 and the ionotropic NON-NMDA antagonist CNQX. Neurotoxicity by AP3 was completely prevented by the addition of GLU (1mM) in the presence of MK-801. Trans-ACPD (1mM), which per se did not produce neurotoxicity, failed to protect from neurotoxicity by AP3. Depolarizing conditions, such as increasing KCl to final 50 mM in the growth medium, significantly reduced AP3 neurotoxicity both at 24 h and 72 hours. The possibility that endogenous GLU release may account for KCl effect appears unlikely since in the absence of MK-801, KCl produced only 30% neurotoxicity, as compared to 95 % neurotoxicity produced by 50 μM GLU, which in the presence of MK-801, was insufficient to antagonize to any extent AP3 neurotoxicity. We suggest that inhibition of tonic GLURm stimulation may affect mechanisms of control of NMDA receptor-mediated neurotoxicity. Other mechanisms, independent of NMDA receptors, involved in neuronal survival appear also to be under the control of GLURm. Both, NMDA-dependent and independent type of mechanisms share a common sensitivity to depolarizing conditions.
Research supported by CICYT, SAL 91-0613.

729.3

POSSIBLE ROLE OF EXCITOTOXICITY-INDUCED INHIBITION OF CAM KINASE II ACTIVITY IN DELAYED NEURONAL DEATH. S. B. Churn*, D. Limbrick, and R. J. DeLorenzo. Dept. of Neurology, Med. Coll. of VA, Richmond, VA 23298.

Excitotoxic activation of glutamate receptors results in a delayed, calcium-dependent neuronal necrosis (J Neurosci 1987;7:39). The same excitotoxic paradigm also results in significant inhibition of CaM kinase II activity in neuronal cultures (Stroke 1993;24:271). The present study was undertaken to characterize the pharmacology and relationship of excitotoxic glutamate receptor activation-induced inhibition of CaM kinase II and delayed neuronal death in primary neuronal cultures. Hippocampal cultures (16 days in culture) from D-2 neonatal rat pups were exposed to glutamate + 10 μM glycine for 10 minutes. After glutamate exposure, the cultures were evaluated for neuronal survival or harvested and studied for CaM kinase II activity under standard conditions. Excitotoxic glutamate exposure resulted in significant inhibition of CaM kinase II activity. Both cell death and inhibition of kinase activity were blocked by co-incubation with 20 μM MK-801, but were not blocked with co-incubation with 200 μM CNQX. In addition, 100 μM NMDA treatment resulted in significant inhibition of CaM kinase II activity and significant cell death. Thus, excitotoxic activation of NMDA receptor channels resulted in both delayed neuronal cell death and significant inhibition of CaM kinase II. Significant inhibition of CaM kinase II activity was observed immediately following excitotoxic glutamate treatment and persisted at all time points measured. In parallel experiments, both cell death and the glutamate-induced inhibition of CaM kinase II were blocked by removal of calcium in the extracellular medium. In addition, the inhibition of kinase activity was not reversible with phosphatase treatment. The rapid decrease in CaM kinase II activity could have significant effects on neuronal calcium/calmodulin-dependent effector systems and may be an important initial molecular change that triggers a cascade of events that culminate in delayed cell death.

729.4

NMDA RECEPTOR STIMULATION BLOCKS NEURONAL VOLUME REGULATION. K. B. Churchwell*, P. A. Rosenberg and K. Strange. Depts. of Anesthesia, Neurology, and Medicine (Div. of Nephrology), Children's Hospital, Boston, MA 02115.

Excitotoxic neuronal injury consists of at least two components: (1) isotonic cell swelling due to salt and water entry, and (2) a delayed phase associated with Ca^{2+} influx. We have been studying isotonic swelling-induced neuronal injury and subsequent recovery using real-time, video microscopy measurements of cell volume. Exposure of neuron-enriched, astrocyte-poor, cortical cultures to 100 μM veratridine (VT) for 10-15 minutes caused a 1.8 ± 0.1 fold increase in neuronal somal volume that persisted for at least 90 min. This volume increase was blocked by bath Na^+ removal or by addition of 5 μM tetrodotoxin, indicating that swelling was due to entry of Na^+ through Na^+ channels. Treatment of cells with VT together with an NMDA antagonist (0.1-10 μM MK801, 5 mM AP5, or 100 μM 7-chlorokynurenate) had no effect on the magnitude of cell swelling. NMDA receptor antagonist-treated cells, however, underwent nearly complete volume recovery within 50-70 min after VT exposure. For example, cells treated with 10 μM MK801 or 100 μM 7-chlorokynurenate had mean relative volumes of 1.06 ± 0.1 and 1.09 ± 0.1, respectively, 90 min after exposure to VT. In contrast, cells treated with the non-NMDA receptor antagonist CNQX (100 μM plus 1 mM glycine) remained swollen following VT exposure (relative volume = 1.9 ± 0.15). Our results demonstrate: (1) cortical neurons possess powerful volume regulatory mechanisms that protect them from swelling induced by increased solute influx, and (2) NMDA receptor stimulation appears to inhibit these mechanisms. These findings suggest that excitotoxic injury may be caused, at least in part, by disruption of neuronal osmoregulatory capabilities. Elucidation of the mechanisms and control of neuronal volume regulation may provide insight into the prevention and treatment of ischemic brain injury. (Supported by NIH grant NS30591 and the American Heart Association).

729.5

INCREASED $[K^+]_o$ AND CELL SWELLING AFFECTS UPTAKE AND RELEASE OF GLUTAMATE AND ASPARTATE BY PRIMARY ASTROCYTE CULTURES

H.K.Kimelberg*, C.Charniga, E.Rutledge And S.K.Goderie
Division of Neurosurgery, and Department of Pharmacology/Toxicology, Albany Medical College, Albany, NY 12208

Astrocytes are considered to be important in the uptake of the excitatory amino acids (EAAs) glutamate and aspartate and thus play an important role in the control of their extracellular EAA levels. Reversal of uptake and swelling-induced release have both been proposed as mechanisms whereby net astrocytic uptake can be reduced. In this study we have examined how high $[K^+]_o$ (to induce both reversal of EAA uptake and slow swelling) and hypotonic media (to induce rapid swelling), affect both uptake and release of [^{3}H]L-glutamate and [^{3}H]D-aspartate in primary astrocyte cultures, prepared from the cerebral cortices of 1 day old rat pups. Uptake of [^{3}H]L-glutamate is reduced both by exposure to hypotonic and high $[K^+]_o$ media. One reason for the hypotonic swelling-induced reduction of uptake is possibly due to increased release, thus short-circuiting the uptake process. Hypotonic swelling-induced release appears to be a mechanism separate from reversal of the uptake system. It occurs to the same extent in cells that have been incubated in Na^+-free media to deplete them of $[Na^+]_i$ as in cells not depleted of Na^+. Part of the high K^+-induced release also appears to be due to swelling. We have found that lowered temperature (37° to 26°C) reduces high K^+, but not hypotonic media-induced release, but has much less effect on uptake of EAAs. This inhibition by temperature of high K^+ medium-induced release of EAAs from astrocytes may contribute to the protection against ischemic damage seen due to lowered temperature. Increased $[K^+]_o$ and astrocytic swelling are known to occur in stroke and trauma and diminished uptake and increased release of EAAs from astrocytes may well contribute to the increased levels of EAAs seen under such conditions. (Supported by NS 23750 and NS 30303).

729.6

AN INHIBITOR OF Na^+-DEPENDENT GLUTAMATE (Glu) UPTAKE, L-*trans*-PYRROLIDINE-2,4-DICARBOXYLATE (L-*trans*-PDC), SLOWS THE CLEARANCE OF Glu AND POTENTIATES Glu TOXICITY *IN VIVO*. A.K. Butler*, S. Djali, B.A. McLaughlin, and M.B. Robinson. Children's Seashore House; Depts of Ped., Pharm., and Neuro., U. PA., Phila., PA, 19104.

Accumulation of extracellular Glu may contribute to the neurotoxicity observed in acute insults to the CNS. Although Glu kills neurons in cell cultures, and this toxicity is attenuated by uptake, there are several conflicting reports regarding the toxicity of Glu *in vivo*. Last year, we reported that co-injection of 5 μmol Glu and 2 μmol of L-*trans*-PDC into the striatum did not cause significant neuronal loss. The goal of the present studies was to determine if L-*trans*-PDC slows the clearance of Glu *in vivo* and potentiates Glu toxicity. 2 μl of Glu (4 μmol) ± L-trans-PDC (4 μmol) was injected into rat striatum (AP -0.6, ML -3.5, DV -5.7 from bregma at 20°) while the extracellular fluid was continuously sampled with a microdialysis probe (CMA 10) 1.5 mm from the injection site (AP +2.6, ML +2.5, DV -5.1 from bregma). Body temperature was maintained between 37° and 38° C. Thionin staining was used to evaluate damage and stereotaxic placement. Several amino acids were measured by HPLC. Although the injection of Glu by itself caused a small increase in Glu (0- to 10-fold, n=6), co-injection with L-*trans*-PDC caused a dramatic increase (approx. 200-fold, n=8). L-*trans*-PDC alone did not alter the extracellular concentration of Glu. Glu or L-*trans*-PDC alone did not cause significant damage at the site of probe placement, but co-injection caused extensive gliosis that extended to the probe. These studies suggest that Glu toxicity is potentiated by co-injection with L-*trans*-PDC. (NS29868, P30-HD-26979)

729.7

LARGE RETINAL GANGLION CELLS ARE MORE SENSITIVE TO GLUTAMATE TOXICITY.

E.B. Dreyer*, S. K. Storm, and S.A. Lipton. Harvard Medical School and Dept. of Neurology, Children's Hospital, Boston, MA, 02115

Previous work in our laboratory (Hahn et al., 1988, PNAS, 85:6556) has demonstrated that glutamate toxicity to retinal ganglion cells is predominately mediated through the NMDA subtype of glutamate receptor. Other work has suggested that glutamate toxicity may play a role in the retinal ganglion cell loss seen in glaucoma (Dreyer et al., 1992, Soc Neurosci Abstr 190.1). In this disease, selective loss is seen of large retinal ganglion cells (Quigley, et al., 1981 Arch Ophth, 99:635). Accordingly, we sought to explore whether glutamate preferentially killed large retinal ganglion cells in culture.

Using a toxicity assay previously described (Hahn et al., 1988, PNAS, 85:6556), postnatal day 7-10 rat retinal ganglion cells were exposed to 0.8 mM Mg^{2+}, and 3 mM Ca^{2+} (the normal vitreal concentration), with or without 100 μM glutamate. After one day in culture, retinal ganglion cells were scored based on viability and size. Glutamate had little or no effect on the viability of retinal ganglion cells smaller than 10 microns. However, this concentration of glutamate was toxic to 79% of the larger retinal ganglion cells ($p < 0.05$, Student's paired t test). This suggests that larger retinal ganglion cells are more sensitive to NMDA-receptor mediated toxicity. If glutamate does indeed play a role in glaucomatous visual loss, our findings may explain in part why larger retinal ganglion cells are lost in this disease.

729.8

NEURONAL CELL DEATH IN ORGANOTYPIC CULTURES OF RAT HIPPOCAMPUS AFTER THE REMOVAL OF BLOCKERS OF GLUTAMATERGIC SYNAPTIC TRANSMISSION. D. M. D. Landis*[1], L.D. Pozzo Miller[2], N.K. Mahanty[2] and J.A. Connor[2], [1]Depts of Neurology and Neurosciences, Case Western Reserve University, Cleveland, OH 44106; [2]Roche Institute of Molecular Biology, Roche Research Center, Nutley, NJ 07110.

The neuronal death that occurs in organotypic cultures of neonatal rat hippocampus during the first 21 days *in vitro* (div) is largely prevented by culture in the presence of agents that reduce glutamatergic synaptic transmission (Pozzo Miller & Landis, Soc. Neurosci. Abstr. 18:440, 1992). In dissociated hippocampal cell cultures, culture in such agents and subsequent removal of them results in seizure-like activity and neuron degeneration (Furshpan & Potter, Neuron 3: 199-207, 1989). We have carried out parallel experiments with organotypic cultures. Starting at 7div, slice cultures were exposed to 10.4mM Mg^{2+}, 3mM kynurenic acid, or 100μM D,L-APV for three weeks (medium was changed every 48 hours). Sequential examination by confocal microscopy of propidium iodide (PI) fluorescence in these "blocked" cultures confirmed the prevention of spontaneous cell death. At 28div, the cultures were placed in normal medium (no glutamate receptor antagonists, 1.07mM Ca^{2+}, 1mM Mg^{2+}), and PI fluorescence was imaged after 6, 24 and 96 hours. Removal of high Mg^{2+} was followed by widespread degeneration of neurons in cell layers CA1, CA3, CA4 and the dentate gyrus, evident at 6 hours after return to normal medium and maximal at 24 hours. Removal of kynurenic acid or of D,L-APV was followed by extensive degeneration of neurons in CA1, CA3 and CA4, detected 24 hours after return to normal medium. These results are additional evidence that glutamate receptors are involved in the spontaneous cell death in these slice cultures.

729.9

MONITORING OF EXTRACELLULAR ASPARTATE AND GLUTAMATE LEVELS IN PATIENTS WITH TRAUMATIC HEAD INJURY USING IN VIVO MICRODIALYSIS. J.J. Woodward,* A.K. Stout, J. Myseros, H. Young, and R. Bullock, Depts. of Neurosurgery and Pharmacology, Medical College of Virginia, Richmond, VA 23298.

Intracerebral microdialysis was used to monitor the levels of the excitatory amino acids glutamate and aspartate in the extracellular fluid of several human subjects who had suffered severe head injury. The amino acids present in the dialysate were derivatized with o-phthaldialdehyde in the presence of thiol, and aspartate and glutamate levels were quantitated using high performance liquid chromatography. Patients with diffusely-damaged brains (as determined by CAT scan) had normal concentrations of aspartate and glutamate in their extracellular fluid (~1 and ~2 μM respectively). Patients with microdialysis probes placed in an area of focally-contused tissue demonstrated a near tenfold increase in glutamate and aspartate levels throughout the monitoring period (eight hours). One patient with diffuse injury but uncontrollable intracranial pressure had glutamate levels almost one hundred times that of patients with diffuse injury and normal intracranial pressure, and the levels remained elevated for several days. These results indicate that focal injury is associated with elevated glutamate and aspartate levels, while diffuse injury is not. However, intracranial pressure may be a more important determinant of extracellular glutamate and aspartate levels. Supported by NIAAA AA08089, DA 07027, and a grant from the Alcoholic Beverage Medical Research Foundation.

729.10

INHIBITION OF SUCCINATE DEHYDROGENASE BY MALONIC ACID PRODUCES AN "EXCITOTOXIC" LESION IN RAT STRIATUM James G. Greene*, Richard H. P. Porter, Robert V. Eller, and J. Timothy Greenamyre, University of Rochester, Rochester, NY 14642

We report that reversible inhibition of succinate dehydrogenase (SDH), an enzyme central to both the tricarboxylic acid (TCA) cycle and the electron transport chain, produces an "excitotoxic" lesion in rat striatum which can be blocked by the NMDA antagonist, MK-801. Male Sprague-Dawley rats received intrastriatal stereotaxic injections of the SDH inhibitor malonic acid (1 or 2 μmoles) in combination with i.p. injections of vehicle or MK-801 (5 mg/kg) 30 minutes before and 210 minutes after malonic acid. Animals were sacrificed 72 hours after surgery, and brains processed for histology, cytochrome oxidase activity, and [^{3}H]MK-801 and [^{3}H]AMPA autoradiography. The higher dose of malonic acid (2 μmoles) produced large lesions that were markedly attenuated by treatment with MK-801 (28.1 ± 3.6 mm^3 vs. 4.7 ± 2.6 mm^3; $p < 0.001$). [^{3}H]MK-801 and [^{3}H]AMPA binding were reduced in the lesions by 60% and 63%, respectively. One micromole of malonic acid produced smaller lesions (9.7 ± 2.2 mm^3) that were completely blocked by MK-801 treatment. The toxic effects of malonic acid were due to specific inhibition of SDH since co-injection of a 3-fold excess of succinate with the malonic acid blocked the striatal lesions. We conclude that inhibition of SDH can lead to NMDA receptor-mediated excitotoxic neuronal death. These results have implications for the pathogenesis and therapy of neurodegenerative diseases. *(NS01487 & T32 GM08427 and Fisons Pharmaceuticals)*

729.11

ADRENALECTOMY ATTENUATES KAINIC ACID-INDUCED SPECTRIN PROTEOLYSIS AND HEAT SHOCK PROTEIN 70 INDUCTION IN HIPPOCAMPUS AND CORTEX. M.T. Lowy*, L. Wittenberg and S. Novotney, Dept. of Psychiatry, Case Western Reserve University, Cleveland, OH 44106

Glucocorticoids have been shown to augment the damaging effects of a variety of neurotoxic insults in the hippocampus and other brain areas. Evidence suggests that the endangering effects of glucocorticoids may be due to augmenting the cascade of events due to excitatory amino acid (EAA) receptor stimulation. A potential mechanism responsible for EAA-induced neuronal damage is activation of calcium-sensitive proteases, such as calpain, which then proteolytically degrades cytoskeleton structural proteins, such as spectrin. The present study was designed to determine if glucocorticoids can regulate the spectrin proteolysis produced by the excitatory amino acid agonist, kainic acid (KA). Rats were adrenalectomized (ADX) or sham operated and 7 days later injected with KA (10 mg/kg). Twenty four hrs later rats were sacrificed and tissues obtained for Western blot analyses of the intact spectrin molecule and the proteolytically derived breakdown products. KA produced an approximate 7-10 fold increase in the 145-155 kDa spectrin breakdown products in the hippocampus and frontal cortex relative to ADX or sham rats injected with vehicle. ADX attenuated the KA-induced increase in breakdown products by 43-80%. Induction of heat shock protein 70 (hsp70) has been suggested to be a sensitive indicator of cellular stress and is induced by neurotoxic insults. KA induced large amounts of hsp70 in both hippocampus and frontal cortex of sham-operated rats which was markedly attenuated (85-95%) by ADX. These results suggest that part of the endangering effects of glucocorticoids on hippocampal and cortical neurons may be due to augmentation of calpain-induced spectrin proteolysis. Supported by a grant from Alzheimer's Disease and Related Disorders Assoc.

729.12

EFFECT OF METHAMPHETAMINE ON DOPAMINE AND GLUTAMATE RELEASE IN RAT STRIATUM AND NUCLEUS ACCUMBENS. T.Abekawa*, T.Ohmori, T.Koyama. Dept. of Psychiatry ,Hokkaido Unv.Sapporo 060 Japan

To investigate a potential role of glutamatergic and dopaminergic neural transmissions in the methamphetamine(MA)neurotoxicity, we examined effects of a high dose of MA (5mg/kg,sc,at 2h intervals,4 injections) on extracellular concentrations of amino acids such as glutamate,glutamine,homoserine,glycine,taurine,alanine and those of monoamines such as DA,DOPAC,HVA and 5-HIAA in nucleus accumbens (NA) and striatum (ST) using in vivo microdialysis. Five days after the microdialysis ,the rats were decapitated and tissue concentrations of monoamines were measured. The toxic dose of MA markedly increased extracellular concentrations of DA, and decreased those of DOPAC,HVA and 5-HIAA in both NA and ST. Magnitude of these changes was not different between NA and ST. Extracellular concentrations of glutamate were increased in ST,but not in NA,while other amino acids showed no changes in both NA and ST. Tissue concentrations of 5-HT and 5-HIAA were decreased to 43-58% of control values in both NA and ST, whereas those of DA,DOPAC and HVA showed 43-54% decrease in ST ,but no changes in NA.These data suggest that the marked increase of DA release was not directly related to MA-induced dopaminergic neurotoxicity. The increase in glutamate release found only in ST may be related to the dopaminergic damage in ST. However,interrelationship between MA-induced serotonergic damage and dopaminergic or glutamate neural transmissions remains to be elucidated.

729.13

EXCITOTOXICITY MAY INVOLVE RECRUITMENT OF OTHER RECEPTORS, NEURONAL LOSS, THEN A GRADED IMMUNE RESPONSE Linda M. Pullan*, Maryann Britt, Pauline G. Dargis, Kathy A. Paschetto, and Robert J. Stumpo. Department of Pharmacology, ZENECA Pharmaceuticals Group, ZENECA, Inc., Wilmington, DE 19897 USA

Excitotoxic damage in focal ischemia can be explored with NMDA or kainate lesions. NMDA or kainate injected into the right striatum of Sprague Dawley rats gave a consistent reduction of choline acetyltransferase activity and a substantial increase in [3H]PK 11195 binding to the macrophage or microglia ω3 benzodiazepine site at 5 days after the agonist injection. The loss of choline acetyltransferase activity after NMDA injections was largely complete after 2 days while the ω3 binding increased over 5 days. Protection of against NMDA lesions by delayed intraperitoneal NMDA competitive antagonist CGP 37849 or by the non-competitive antagonist MK-801 was essentially complete for the loss of choline acetyltransferase but only partial for the increase in ω3 binding. The glycine antagonist (R)-HA-966 was as efficacious as competitive or non-competitive antagonists. The protection against NMDA lesions demonstrated some stereoselectivity; the sedative (S)-enantiomer was not protective of the choline acetyltransferase activity. Interestingly, the non-NMDA antagonist NBQX also offered similar protection against NMDA lesions. The results suggest that excitotoxicity mediated by glutamate receptors involves not only the neuronal loss seen as decreases in choline acetyltransferase activity but also a delayed immune response seen with ω3 binding. The protection of the immune response may be graded even when the neuronal loss is completely protected. Initiation through either the NMDA or kainate/AMPA receptors may recruit both receptor populations.

729.14

PREGNENOLONE SULFATE POTENTIATES NMDA-INDUCED CELL DEATH IN CULTURED NEURONS. C. E. Weaver, JR.*, F. -S. Wu and D. H. Farb. Department of Pharmacology and Experimental Therapeutics, Boston University School of Medicine, Boston, MA 02118.

We have shown previously that the neurosteroid pregnenolone sulfate (PS) enhances the current induced by N-methyl-D-aspartate (NMDA) in voltage-clamped neurons. Here we report that PS potentiates NMDA-induced excitotoxicity in primary cultures of rat hippocampal neurons. Cultures (17-19 days *in vitro*) were exposed to 16 hr treatments of drugs. Assessment of cell survival was performed using the trypan blue exclusion method. NMDA (0.25-100 μM) dose-dependently induced neuronal cell death with an EC_{50} of 14 μM and maximal cell death of 79%. MK-801 (10 μM) completely blocked NMDA-induced neurotoxicity. PS (50-200 μM) when applied together with 12.5 μM NMDA potentiated NMDA-induced neurotoxicity in a dose-dependent manner. In addition, PS (100 μM) reduced the NMDA EC_{50} to 5 μM with little effect on the maximum NMDA-induced cell death, consistent with positive allosteric modulation of the NMDA receptor by PS. PS (100 μM) alone produced little cell death. The immediate precursor of PS, pregnenolone (20 μM), had little or no effect on NMDA-induced cell death. Over stimulation of the NMDA receptor has been proposed to play a role in many neurodegenerative diseases. Our present findings not only confirm our previous observation that PS acts as a positive modulator of the NMDA receptor but also demonstrate that PS may potentiate the physiological consequence of NMDA receptor over activation.

729.15

PLATELET-ACTIVATING FACTOR (PAF) STIMULATES GLUTAMIC ACID RELEASE FROM HIPPOCAMPAL SYNAPTOSOMES. V.L. Marcheselli* and N.G. Bazan. LSU Eye Center and LSU Neuroscience Center, LSU Med. Ctr. Sch. Med., New Orleans, LA 70112

During brain ischemia, platelet-activating factor (PAF) is generated. A massive release of excitatory amino acid neurotransmitter also takes place and may be involved in neuronal damage. The PAF antagonist BN-52021 is neuroprotective in ischemia-reperfusion (Panetta et al., *BBRC* **149**, 580-587, 1987), and competes with a specific PAF receptor of synaptic membranes (Marcheselli et al., *JBC* **265**, 9140-9145, 1990). Moreover, PAF enhances excitatory neurotransmission (Clark et al., *Neuron*, **9**, 1211-1216, 1992). A perfusion cell was used in the present study to show the mechanism of PAF-induced glutamic acid release from [^{3}H]-glutamic-acid-loaded rat hippocampal synaptosomes. PAF, at concentrations of 10 and 100 nM, produced a 25% release from the total [^{3}H]-glutamic acid taken up, which is antagonized by 1 μM BN-52021. 40 mM KCl induced a 30% [^{3}H]-glutamic acid release, which cannot be blocked by the PAF antagonist. Lower PAF concentrations, such as 100 pM or 1 nM, were incapable of inducing glutamic acid release, which is in agreement with binding data on synaptosomal membranes indicating a K_D of 1.2 nM. We conclude that the protection in ischemia reperfusion demonstrated by BN-52021 resides mainly in blocking the control mechanism of glutamic acid release at the presynaptic level. Supported by NIH grant NS23002.

729.16

PROTECTION AGAINST THE TERATOGENICITY OF COCAINE BY CLOZAPINE.
E. Yablonsky-Alter*, T. I. Lidsky, S.P. Banerjee.
City College of New York, Medical School, N.Y. and Inst. of Brain Research, Staten Island, N.Y.

Administration of Cocaine (10 mg/kg, IP) to pregnant mice decreased the striatal dopamine levels by 28%, 34%, 29% and 33% in 3, 7, 11 and 21 day old pups respectively. Co-administration of clozapine (3 mg/kg, IP) prevented the changes in the concentration of striatal dopamine. In contrast, co-administration of haloperidol failed to provide similar protection. Since clozapine blocks the electrophysiological effects of glutamate release on striatal neurons, its antiglutamate actions may contribute in the protection of cocaine induced damages to the striatal dopaminergic neurons. Thus, cocaine induced teratogenicity may involve excitotoxicity.

EXCITATORY AMINO ACIDS: PHARMACOLOGY VI

730.1

L-GLUTAMATE EXCITATION OF VTA DOPAMINE NEURONS IS PREFERENTIALLY MEDIATED BY ACTIVATION OF NMDA RECEPTORS: EXTRA- AND INTRACELLULAR ELECTROPHYSIOLOGY IN BRAIN SLICES. T. Wang* and E. D. French. Dept. Pharmacol., Univ. of Arizona, Tucson, AZ 85724.

The present study assessed the effects of L-glutamate (L-GLU) on the neurophysiology of VTA dopamine neurons in midbrain slices using extra- and intracellular recording methods. L-GLU perfusion at 10-100 μM produced dose-dependent increases in firing rate, with no changes in pattern of firing, while higher concentrations led to a loss of activity reminiscent of depolarization inactivation. These effects were reflected by the pronounced membrane depolarizations observed in intracellular recordings. The effects of low doses ($\leq$30 μM) of L-GLU on firing rate and membrane potential were *completely* antagonized by co-perfusion with the non-competitive NMDA blocker, phencyclidine, or the selective competitive NMDA receptor antagonist, CGS 19755, but not by the selective non-NMDA blocker NBQX. However, at $\geq$300 μM L-GLU's effects could not be completely blocked without the presence of both CGS 19755 and NBQX. These results suggest that in physiological-like conditions that low extracellular levels of glutamate excite dopamine neurons via a preferential activation of NMDA receptors, and that only at higher concentrations of L-GLU are non-NMDA receptors brought into play.

730.2

GLUTAMATE AGONISTS PRODUCE RECEPTOR-SPECIFIC INCREASES IN THE FREQUENCY OF SPONTANEOUS EPILEPTIFORM DISCHARGES (SEDs) IN THE RAT CORTICAL WEDGE. L.J. Robichaud*, R.J. Chang, W.J. Vaalburg, and P.A. Boxer. Neuroscience Pharmacology, Parke-Davis Pharmaceutical Research, Division of Warner-Lambert Co. Ann Arbor, MI 48105.

In the cortical wedge model, SEDs result when extracellular Mg^{++} is removed. Since Mg^{++} blocks the NMDA ion channel in a voltage dependent manner, discharges appear to depend on activation of NMDA receptors. Various types of NMDA antagonists block SEDs; the non-NMDA antagonist NBQX has no effect. NMDA, AMPA, and kainate at $\geq$10 μM caused depolarizations and blocked SEDs. Several drugs including polyamines increase SED frequency, possibly by increasing glutamate release. The present experiments attempted to mimic increased glutamate release by adding low concentrations of glutamate agonists. At concentrations $\leq$10 μM each agonist caused little or no depolarization but increased SED frequency in a sustained (90 min), dose-related manner. For example, 1 and 3 μM NMDA increased SED frequency by 35% and 200%, respectively. Continuous treatment with 1 μM and 10 μM of the competitive NMDA antagonist CPP caused a ~3 and 10-fold respective shift of the concentrations of NMDA increasing or blocking SED frequency. The AMPA and kainate concentration-response curves were right-shifted by NBQX but not by CPP. In summary, low concentrations of glutamate agonists produce sustained receptor-specific increases in SED frequency. Agents that increase glutamate release have the potential to increase SED frequency, although the mechanism of action is not understood.

730.3

AN *IN VITRO* STUDY OF EXCITATORY AMINO ACID (EAA)-MEDIATED SYNAPTIC TRANSMISSION IN THE DIAGONAL BAND OF BROCA.
J.C. Easaw* and J.H. Jhamandas, Dept. of Medicine (Neurology), Univ. of Alberta, Edmonton, Alberta, Canada T6G 2B7.

Anatomical studies suggest that afferents to the diagonal band of Broca (DBB), a basal forebrain region, contain the EAAs glutamate and aspartate. We therefore examined the actions of specific EAA receptor agonists on DBB neurons *in vitro* utilizing the whole cell patch clamp technique. We further assessed a role for EAAs in mediating synaptic transmission within the DBB.

Coronal slices (400μm), including the DBB, were taken from male Sprague Dawley rats, placed in a small recording chamber, and superfused with artificial cerebrospinal fluid. Exogenous application of N-methyl-D-aspartate (NMDA, 10μM), α-amino-3-hydroxy-ethyl-4-isoxazolepropionic acid (AMPA, 10μM), and kainate (5μM) evoked an excitation in 31 DBB neurons (resting membrane potential 69.5$\pm$11.5mV). Bipolar electrodes were placed adjacent to the horizontal limb and single stimuli (200μs duration @ 2Hz, range 10-70 volts) evoked graded excitatory post-synaptic potentials (EPSPs) or currents (EPSCs) in 32 neurons. Voltage and current clamp recordings revealed the following responses: 1) a fast AMPA/kainate receptor-mediated EPSP(C)s, 2) an NMDA-sensitive slower component of the fast EPSP(C)s, and 3) the metabotropic receptor agonist trans-1-amino-cyclopentyl-1,3-dicarboxylate (t-ACPD, 10μM) reduced EPSP(C) amplitude without changing membrane conductance. This action of t-ACPD may be indicative of a presynaptic effect.

These data indicate that both NMDA and non-NMDA receptors mediate excitatory synaptic transmission within the DBB.

Supported by the MRC and the AHFMR.

730.4

NMDA-RECEPTOR ACTIVATION STIMULATES DENDRITIC RELEASE OF DOPAMINE IN THE PARS RETICULATA OF THE RAT BRAIN. M.G. Rosales., G.Flores, S. Hernández and J. Aceves*. Dept. of Physiology, Biophysics and Neurosciences. CINVESTAV-IPN. Apartado Postal 14-740. 07000 México, D.F. México.

As is well known, the dopaminergic cells of the pars compacta of the rat substantia nigra release DA not only from their presynaptic terminals in the striatum but also from their dendrites in the pars reticulata. By microdialysis, we have recently shown (Martinez-Fong et al., Brain Res. 595, 309, 1992) that presynaptic NMDA receptors modulate the release of DA from nigrostriatal terminals in the unanesthetized rat. Here we have explored if the glutamatergic input to the pars reticulata could modulate not only the firing frecuency of the nigral cells (Robledo and Fèger, Brain Res. 518, 47, 1990), but also the release of DA from dopaminergic dendrites. As a first approximation, we have studied if the activation of the NMDA receptors stimulated, as in striatum, the release of DA. The experiments were done in anesthetized (chloral hydrate) rats. A microdyalisis probe was stereotaxically implanted into the right pars reticulata. DA was measured in the perfusate by HPLC with electrochemical detection. N-Methyl-D-Aspartate (NMDA) (1mM) (corrected concentration 100 µM) added to the perfusion medium caused an increment of 130 ± 25% (n=6)in the release of DA. The selective antagonist of NMDA receptors, 2-amino-phosphonovalerate (1 mM), totally blocked the effect of NMDA (n=5). These results suggest that the glutamatergic innervation of the pars reticulata may modulate via NMDA receptors the release of DA from the dendrites projecting from the pars compacta into the pars reticulata.
(Supported by a grant (0586N-9108) from CONACYT of México)

730.5

PROPERTIES OF EXCITATORY POSTSYNAPTIC CURRENTS IN NEONATAL RAT SYMPATHETIC PREGANGLIONIC NEURONES. Krupp, J., Gombos, G.* & Feltz, P.; Laboratoire de Physiologie (URA CNRS 1446); 21, rue René Descartes; 67084 Strasbourg Cedex; France.

The objective of our investigation was to characterize electrically evoked excitatory postsynaptic currents (EPSCs) in sympathetic preganglionic neurones (SPNs) by means of the patch-clamp technique in thin (200-300 μm) spinal cord slices of neonatal rats (P0-P14). SPNs were identified after Lucifer Yellow injection on the basis of morphological criteria. Except for two cases the evoked EPSCs of all SPNs recorded (n=36) had two components. The entire EPSCs reversed at 1.2±4.6 mV (n=12). After blockade of the NMDA component by APV (10-40 μM;n=11), the EPSC had a linear peak I/V relation with a reversal potential of -0.3±3.9 mV (n=9). The kinetics of this component depended critically on the stimulation site: accordingly, the rise time (10-90%) could be as fast as 0.4 ms and the monoexponential decay had a time constant of less than 2 ms. When blocking the non-NMDA-component by CNQX (5 μM;n=10), the peak I/V relation of the EPSC reversed at 2.1±1.3 mV (n=6). It had a region of negative slope conductance at holding potentials more negative than -30 mV. This region of negative slope conductance was abolished in Mg^{2+}-free saline. The rise times (10-90%) for this component ranged from 3.3 to 9.5 ms. At a V_h of +50 mV the time constants of the biexponential decay were γ_1=62.0±14.9 ms and γ_2=401.9±64.9 ms (n=4). Both time constants decreased with hyperpolarization. Our results show, that SPNs receive fast excitatory synaptic inputs using L-glutamate or an analogue to act on glutamate receptors of the NMDA- and non-NMDA-type.

J.K. was supported by a Boehringer Ingelheim Fonds fellowship.

730.6

NMDA ANTAGONISTS CGS19755 AND MK801 DIFFERENTIALLY BLOCK STRIATAL FOS-LIKE IMMUNOREACTIVITY INDUCED BY THE D_1-DOPAMINE AGONIST SKF-38393 IN NEONATAL 6-HYDROXYDOPAMINE LESIONED RATS. Kevin B. Johnson*, James P. O'Callaghan, Robert A. Mueller and George R. Breese. Curriculum in Neurobiology and Brain and Development Research Center, Univ. of North Carolina School of Medicine, Chapel Hill, N.C., 27599

Previous studies from this laboratory have shown that administration of the D_1-dopamine agonist SKF-38393 induces a dose-dependent increase in striatal c-fos-like immunoreactivity in neonatal 6-OHDA-lesioned rats. Repeated, intermittent administration of D_1-dopamine agonists can sensitize neonatally lesioned rats to the behavioral effects of dopaminergic agonists, and this behavioral sensitization has been shown to be blocked by administration of the NMDA antagonists MK801 and CGS-19755. In the present study, we sought to determine the ability of NMDA antagonists, at doses known to block the behavioral sensitization of D_1-dopamine receptor-mediated responses, to block this striatal c-fos response. In neonatally lesioned rats, pretreatment with the competitive NMDA antagonist CGS-19755 (10 mg/kg, i.p.) produced a 71% decrease in the number of fos-positive striatal cells induced by SKF-38383 (1.5 mg/kg, i.p.) alone. In contrast, pretreatment with a high dose of the noncompetitive NMDA antagonist MK801 (1 mg/kg, i.p.) did not reduce the number of striatal fos-positive cells induced by the same dose of SKF-38393. Further studies will explore the nature of these antagonistic effects, and seek to determine whether the differential effects of these two NMDA antagonists may reflect the delineation of regionally-selective NMDA receptor subtypes. Supported by HD-23042 and HD-03110.

730.7

MECHANISM UNDERLYING FACILITATION OF NMDA DEPOLARIZATION BY THAPSIGARGIN (TG), CYCLOPIAZONIC ACID (CPA) AND A23187 AT CORTICAL PYRAMIDAL NEURONS. R.S. Neuman* and S.Rahman, Fac. of Med. Memorial University, St. John's, Nfld. Canada A1B 3V6.

TG, CPA and A23187, agents which raise intracellular Ca^{2+} concentration, are similar to serotonergic, muscarinic and noradrenergic agonists (Rahman and Neuman, Eur. J. Pharmacol. 231(1993) 347) and 1S,3R ACPD (submitted) in that they selectively facilitate NMDA induced depolarization of cortical neurons (Rahman and Neuman, this meeting). This could result from an indirect affect (presynaptic) or a direct (postsynaptic) action. We have tested TG, CPA and A23187 on cortical wedges perfused with a cocktail consisting of 0.1 μM TTX, 1 μM prazosin, 10 nM scopolamine, 10 nM ritanserin, and 50 μM AP3. This cocktail reduced, but did not abolish the facilitation. Moreover, facilitation by these agents was present in Mg^{2+} free medium, which eliminates serotonin and phenylephrine induced facilitation. Nifedipine (1-2 μM) reduced, but did not block the facilitation to TG, CPA and A23187. Finally, 1 μM calmidazolium and 1 μM chlorpromazine, which inhibit calmodulin, did not block the facilitation by these agents, but instead prevented the loss of facilitation which occurs at high concentrations.

In conclusion, we suggest: 1) TG, CPA and A23187 act directly on pyramidal neurons to facilitate the NMDA response; 2) a rise in intracellular Ca^{2+} is responsible for the facilitation and 3) the facilitation does not result from an action on calmodulin or involve alteration in the voltage dependence of the NMDA receptor channel. Supported by the Canadian MRC.

730.8

THAPSIGARGIN (TG), CYCLOPIAZONIC ACID (CPA), AND A23187 FACILITATE NMDA RECEPTOR MEDIATED DEPOLARIZATION OF RAT CORTICAL NEURONS. S. Rahman* and R.S. Neuman, Fac. of Med. Memorial University, St. John's, Nfld. Canada A1B 3V6.

Using grease gap recording from cortical wedges we have shown that activation of 5-HT_2 receptors facilitates NMDA depolarization of pyramidal neurons (Rahman and Neuman, Eur. J. Pharmacol. 231 (1993) 347). Employing similar methods, we now report that agents which raise intracellular Ca^{2+} mimic this facilitation.

TG, an inhibitor of Ca^{2+}-ATPase, applied concurrently with 50 μM NMDA for 2 min, reversibly facilitated the NMDA depolarization. The facilitation increased in magnitude over the range of 10 to 100 nM, but was not significantly different from control at 300 nM. Perfusing wedges with nominally Ca^{2+} free medium or buffering internal Ca^{2+}, using 50 μM BAPTA AM, prevented the TG induced facilitation. These treatments also blocked the 5-HT facilitation. CPA (5 to 30 μM), another Ca^{2+}-ATPase inhibitor, as well as A23187 (0.25 to 3 μM), a Ca^{2+} ionophore, dose-dependently facilitated the NMDA depolarization. Magnitude of the CPA facilitation was similar to that induced by TG, whereas the response to A23187 was significantly larger. When quisqualate and kainate were substituted for NMDA, no enhancement was observed with CPA.

In conclusion, facilitation of the NMDA depolarization induced by TG, CPA and A23187 is similar to that found with 5-HT. The facilitation is selective for NMDA receptor activation and requires both external and internal Ca^{2+}. (see also Neuman and Rahman, this meeting). Supported by the Canadian MRC.

730.9

QUISQUALATE (QA), GLUTAMATE (GLU) AND AMPA BUT NOT ACPD ACTIVATE THE SAME SUBPOPULATION OF NEURONS AND BLOCK KAINATE (KA)-INDUCED ELEVATIONS OF CALCIUM IN PRIMARY TISSUE CULTURE J. D. Sherrod & P. P. McCaslin*, Dept. Pharmacol. & Toxicol., Univ. Miss. Med. Ctr., 2500 N. State Str., Jackson, MS 39216-4505

We examined the ability of GLU to activate different excitatory amino acid (EAA) receptors in cerebellar granule cells in tissue culture using cell imaging with the fluorescent dye, fluo 3. In the presence of 1.2 mM Mg^{2+}, GLU did not activate either the N-methyl-D-aspartate (NMDA) or the KA receptors. However, GLU consistently activated about 15% of the neurons in the presence of Mg^{2+} which was the same population of neurons activated by both QA and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA); the metabotropic GLU receptor agonist, aminocyclopentane-1,3-dicarboxylic acid (ACPD), did not activate these neurons. QA was the most potent compound tested in activating this subpopulation of neurons with 0.5 μM QA consistently elevating intracellular Ca^{2+}. If Mg^{2+} was removed from the medium, GLU resulted in elevations of Ca^{2+} in all the neurons, and this increase was blocked by the NMDA receptor antagonist, AP7, but not by the KA receptor antagonist, GAMS, thus indicating that GLU will only activate the NMDA and QA but not the KA receptors. We tested several endogenous EAAs for their ability to activate the KA receptor; homocysteate, aspartate, cysteate, cysteine sulfinate, homocysteine sulfinate and quinolinate were unable to activate the KA receptor in the presence of Mg^{2+}; a number of amino acids were tested in conjunction with GLU for their ability to activate the KA receptor but none were found to do so. Collectively, these results show that QA, AMPA and GLU activate a subpopulation of cerebellar granule neurons, and that no endogenous amino acid tested was able to activate the KA receptor. Supported by NIDA grant DA 64843.

730.10

PREGNENOLONE SULFATE-MEDIATED INCREASES IN INTRACELLULAR CALCIUM IN CULTURED CHICK CORTICAL NEURONS. J.M. Fahey*, G.A. Pritchard, D.G. Lindquist and L.G. Miller. Department of Pharmacology and Experimental Therapeutics, Tufts University School of Medicine, Boston, MA 02111.

Pregenenolone sulfate has been reported to selectively augment glutamate-induced depolarizations mediated by the N-methyl-D-aspartate (NMDA) subtype of the glutamate receptor. The present study examines the ability of this neuroactive steroid to potentiate NMDA-mediated increases in intracellular calcium of cultured chick cortical neurons using the fluorescent dye Fura2. Pregnenolone sulfate alone elevated intracellular calcium in a dose-dependent manner (1-500 μM). These increases in intracellular calcium were attenuated by 1 μM nimodipine, a voltage-sensitive calcium antagonist, and 50 μM 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), a non-NMDA glutamate receptor antagonist. Dizocilpine, an NMDA receptor antagonist, did not inhibit the elevations in intracellular calcium caused by pregnenolone sulfate. In the presence of NMDA, pregnenolone sulfate increases in intracellular calcium were also inhibited by nimodipine and CNQX but not dizocilpine. These data indicate that the mechanism by which pregnenolone sulfate increases intracellular calcium may be mediated either through non-NMDA receptor subtypes of the glutamate receptor or voltage-gated calcium channels.

730.11

FORSKOLIN INHIBITS GLUTAMATE-INDUCED $[Ca^{2+}]_i$ INCREASES IN CULTURED RAT STRIATAL NEURONS. K.R. Hoyt* and I.J. Reynolds. Department of Pharmacology, Univ. of Pittsburgh School of Medicine, Pittsburgh, PA 15261.

In a single neuron, interactions between neurotransmitter receptors may occur via second messengers. We are studying the modulation of glutamate receptor-mediated increases in $[Ca^{2+}]_i$ by neurotransmitters. Because some neurotransmitters (e.g. dopamine) activate adenylate cyclase, we investigated the effects of cAMP on glutamate responses. Forskolin (5μM) was used to increase $[cAMP]_i$ by activating adenylate cyclase. Glutamate-induced $[Ca^{2+}]_i$ increases were measured in single cultured striatal neurons loaded with fura-2. Glutamate responses were elicited by applying 3 μM glutamate for 20 sec. We found that a 1 min exposure to forskolin significantly inhibited glutamate responses compared to solvent (0.05% DMSO) controls. This forskolin-induced inhibition developed slowly and got progressively greater with time, reaching a limit of about 36 % inhibition within 1 hour of forskolin exposure. For example, the first glutamate response after forskolin was 92 ± 9 % (n=5) of untreated control responses compared to 99 ± 7 % (n=4) in cells treated with DMSO in place of forskolin (mean ± s.d.; $p>0.05$, not significant). By the fifth glutamate response (approximately 35 min) after forskolin the response had decreased to 73 ± 5% of control compared to 88 ± 7 % of control in the DMSO controls ($p<0.05$). However, forskolin did not consistently or significantly inhibit NMDA/glycine, kainate or AMPA responses in similar experiments. The mechanism underlying the selective effect of forskolin on glutamate responses is currently under study.
Supported by NIMH (MH18273).

730.12

CARBAMAZEPINE INHIBITION OF NMDA-STIMULATED CALCIUM INFLUX IN CEREBELLAR GRANULE CELLS.
C. Hough*[1], R.P. Irwin[2], X.-M. Gao[1], M.A. Rogawski[3] and D.-M. Chuang[1]. [1]Biological Psychiatry Branch and [2]Clinical Neuroscience Branch, NIMH, and [3]Neuronal Excitability Section, Epilepsy Research Branch, NINDS, Bethesda, MD 20892

The most common toxic effects of carbamazepine (CBZ) are ataxia, nystagmus and vertigo, which suggests that the drug specifically impairs cerebellar function at high doses. Since the cerebellum expresses unique NMDA receptor subunits, we wondered whether the cerebellar impairment produced by CBZ could be due to a particular sensitivity of cerebellar NMDA receptors to the drug. Here we investigated the effects of CBZ on NMDA receptor responses in cultured rat cerebellar granule cells using fura-2 fluorometry. CBZ blocked Ca^{2+} influx induced by 100 µM NMDA (+10 µM glycine) in a dose-dependent manner. At a concentration of 100 µM, CBZ produced a 27 ± 3% block of the Ca^{2+} signal, whereas the Ca^{2+} responses to 120 mM KCl or 100 µM carbachol were unaffected. In contrast, NMDA responses in cultured hippocampal neurons were less sensitive to the drug. We conclude that this sensitivity of cerebellar neurons to supratherapeutic concentrations of carbamazepine could account for the clinical observation that the major CNS side effects of CBZ are referable to the cerebellum. Moreover, these data may provide insight into the finding (Gao and Chuang, *Neuroscience Lett.* **135**: 159, 1992) that NDMA can completely block delayed toxicity of 100 µM CBZ in cerebellar granule cells.

730.13

GLYCINE SITE INVOLVEMENT IN GLUTATHIONE STIMULATION OF N-METHYL-D-ASPARTATE RECEPTOR-MEDIATED NEURONAL CALCIUM ENTRY. R.D. Trent*, L.A. Randoll and S.W. Leslie. Div. Pharmacol. and Toxicol., Univ. Texas at Austin, Austin, TX 78712.

Glutathione (γ-glutamylcysteinylglycine) stimulates the entry of calcium into dissociated neurons from newborn rats (< 24 hr old). This influx of calcium can be blocked by the N-methyl-D-aspartate (NMDA) receptor antagonists APV, Mg^{++} and MK801. This study investigated the effects of glycine, the NMDA receptor co-agonist, and 5,7-dichlorokynurenic acid (5,7-DCKA), a glycine site antagonist, on glutathione-mediated neuronal calcium entry into fura-2 loaded neurons isolated from newborn rats.

Reduced or oxidized glutathione (GSH or GSSG, 2 mM) was added to a cuvette containing 3.5 x 10^6 fura-2 loaded cells 50 sec after the addition of glycine (0, 1, 10 or 100 µM). Although these concentrations of glycine markedly enhance the response to NMDA in this preparation, there was no effect on glutathione stimulation measured as nM change in intracellular calcium.

In a second set of experiments NMDA, GSH and GSSG concentration response curves were generated in the presence and absence of 5,7-DCKA (185nM and 370nM). The NMDA curves showed significant overall effect for both concentrations of 5,7-DCKA as well as significant NMDA × 5,7-DCKA interaction characteristic of a noncompetitive antagonist. The GSH and GSSG curves, while showing a slight but significant overall effect for 5,7-DCKA, did not show significant glutathione × 5,7-DCKA interaction. This parallel shift in concentration response curves is characteristic of a competitive antagonist.

These experiments provide evidence for involvement of the NMDA receptor glycine site in glutathione stimulation of calcium influx. (Supported by NIAAA grants R01 AA09337 and R37 AA05809)

730.14

N-METHYL-D-ASPARTATE RECEPTOR-LIKE COMPLEX IS COUPLED TO PERTUSSIS TOXIN-SENSITIVE G-PROTEIN AND PHOSPHOLIPASE C IN C6 CELL. H.Shinno, M.Mikuni*, K.Saitoh, S.Yamawaki and K.Takahashi Div. Mental Disorder Res., Natl. Inst. Neurosci.,NCNP,Tokyo, 187 and Dep. Neurol. Psychiat., Hiroshima Univ., Sch. Med.,Hiroshima, 734, Japan.

We have studied pharmacological property of glutamate receptors in C6 glioma cell. Glutamate and N-methyl-D-aspartate(NMDA) but not kainate caused the increase in the intracellular Ca^{2+} concentration ($[Ca^{2+}]i$) and in inositol 1,4,5-trisphosphate (IP_3) level in the absence of extracellular Mg^{2+} and Ca^{2+}. D-APV, HA-966, MK-801 and Mg^{2+} inhibited the increase in $[Ca^{2+}]i$ induced by glutamate. Glutamate-induced increase in $[Ca^{2+}]i$ was abolished by pertussis toxin(PT).

We also found that there was an interaction between NMDA receptor and serotonin-2(5-HT_2) receptor in the presence of Mg^{2+}. Glutamate inhibited 5-HT_2 receptor-mediated intracellular Ca^{2+} mobilization and IP_3 formation in a concentration dependent manner. D-APV, PCP, MK-801 and HA-966 reversed the inhibitory effect, while L-APV did not. The same result was obtained in the absence of extracellular Ca^{2+}. Treatment with PT abolished the inhibitory effect. These pharmacological results suggest that the inhibitory effect is mediated via NMDA receptor-like complex coupled to PT-sensitive G protein and phospholipase C.

730.15

CALCIMYCIN EXHIBITS A PERMISSIVE EFFECT ON NMDA STIMULATION OF PHOSPHATIDYLINOSITOL HYDROLYSIS IN NEONATAL RAT CEREBELLUM J.Li* and S.S.Smith. Anat. Dept, Neuro. Ins, Hahnemann U., Phila.

The calcium ionophore calcimycin (A23187) can enhance NMDA-induced responses (Markram and Segal, 1991), an effect which may be important for development and plasticity. The products of phosphatidylinositol(PI) hydrolysis have also been implicated in developmental processes in the CNS. Therefore, in the present study, the effect of calcimycin on NMDA stimulation of PI turnover was investigated. PI turnover was assessed by using [H^3]-inositol in 160 uM cross chopped slices of cerebellar tissue from rats at postnatal day 7-10. Following a 5 min preincubation of the slices with calcimycin (100 uM) and a 20 min incubation with NMDA (10 uM) or other stimulants, chloroform extraction and anion exchange chromatography were used to isolate total inositol phosphates. Preloading with calcimycin increased NMDA-stimulated PI turnover up to 44% above control levels ($P<0.05$) (The control level represents an elevated basal level in the presence of calcimycin alone). NMDA alone did not alter PI turnover in neonatal cerebellum. Calcimycin potentiation of NMDA- induced PI hydrolysis was not blocked by the nitric oxide synthase blocker, nitroarginine (100uM), suggesting that this gaseous second messenger does not mediate this NMDA-associated effect. In addition, calcimycin failed to significantly elevate levels of quisqualate(10uM)-induced PI turnover. These data suggest that this permissive effect of calcimycin is specific for the NMDA receptor. One of the important conclusions drawn from the above data is that the ability of NMDA receptor activation to stimulate PI turnover is dependent upon an adequate level of $[Ca^{2+}]i$, an effect which may be required for maximal activation of phospholipase C, a Ca^{2+}-dependent enzyme. NMDA-induced activation of PI hydrolysis under these conditions may be important for developmental processes in the cerebellum. (Supported by USAFOSR#F49620-93-1-0136 DEF)

730.16

NMDA RECEPTORS EXPRESSED IN OOCYTES: EFFECTS OF LIGANDS RELATED TO HIV INFECTION Prado de Carvalho L.*, Bochet P, Rossier J. Institut Alfred Fessard, CNRS, 91198 Gif sur Yvette, Cedex, France.

NMDA receptors in Xenopus oocytes injected with in vitro transcripts of cloned subunits (NMDAR1a or 1b + NMDAR1c, gift from Dr. Nakanishi), or with rat cortex mRNA were studied under voltage clamp. In oocytes injected with in vitro transcripts, glutamate with glycine (Glu + Gly) was always a better agonist than NMDA, with higher affinity (10^{-6} M, and 10^{-5} M for NMDA) and higher efficacy (maximal response up to 2 times the maximal response with NMDA). Glu and NMDA responses were voltage dependently blocked by Mg^{++}(EC_{50} ~ 60 µM at −100 mV). Quinolinic acid (QN) had little or no effect (up to mM concentrations, with or without Gly). QN had also little or no effect in mRNA injected oocytes expressing NMDA, AMPA, $Gaba_A$ and serotonin receptors. However, homoquinolinic acid (HQN) with Gly was a potent NMDA agonist, with affinity and efficacy comparable to those of NMDA. Incubation with GP 160 (330 pM, 15 min), had no effect on Glu induced responses. Memantine (gift of Dr. Quack, Merz and Co), a recently described NMDA open channel blocker, inhibits responses induced by Glu, NMDA and HQN. Current-voltage curves show that memantine blockage, unlike that of Mg^{++}, was not voltage dependent. Under these conditions submaximal doses of memantine and Mg^{++} do not seem to interfere with each other. The effect of memantine was reversible, with washout time of 1-2 min. Dose response curves of memantine, tested on saturating concentrations of Glu + Gly, show an EC_{50} of~10^{-7} M.
This work was supported by the Agence Nationale de Recherche sur le SIDA.

730.17

N-ACETYL-L-ASPARTYLGLUTAMATE (NAAG) DOES NOT MODULATE NMDA-STIMULATED [^{3}H]NE RELEASE THROUGH THE GLYCINE RECEPTOR OR VIA THE L- OR N-TYPE VOLTAGE-DEPENDENT CALCIUM CHANNEL (VDCC) . P.S. Puttfarcken* J.S. Handen, J.T. Coyle, and L.L. Werling. Dept. of Psychiatry, Harvard /MGH, Boston, MA 02129, and Dept. of Pharmacology and Program in Neuroscience, The GWU Med. Ctr., Washington, DC 20032.

The release of preloaded radiolabeled norepinephrine ([3H]NE) from slices of rat hippocampus can be stimulated by excitatory amino acids (EAAs) that interact with the N-methyl-D-aspartate (NMDA) receptor. The dipeptide NAAG is colocalized with NE in projections of the locus coeruleus. Under conditions in which the activity of NAALADase, the enzyme responsible for the degradation of NAAG, was inhibited, studies have demonstrated that NAAG serves as a neuromodulator of EAA-stimulated NE release by acting at least partially through the NMDA receptor/channel complex. NAAG (>250μM) directly stimulated release of [^{3}H]NE albeit by a small percent. Additionally, NAAG inhibited the release evoked by NMDA and L-glutamate in a concentration-related manner. However the mechanism by which NAAG exerts this action does not appear to be solely competitive. Experiments were performed to identify potential sites of action for NAAG. Both 7-chlorokynurenate or 1-hydroxy-3-aminopyrrolidone-2 (HA966) decreased [^{3}H]NE release in a concentration-dependent manner. Glycine did not reverse inhibition by NAAG, and the inhibition produced by NAAG in the presence of glycine antagonists was greater than that produced by NAAG alone. Similarly, the addition of NAAG in combination with either the L-type voltage-VDCC blocker nitrendipine, or the N-type channel blocker ω-conotoxin, produced an additional inhibition. These data suggest that NAAG does not elicit its actions through the strychnine-insensitive glycine recognition site nor via the L- or N-type VDCC.

730.18

N-METHYL-D-ASPARTATE RECEPTORS AND SPINAL NOCICEPTIVE PROCESSING M.J.Cumberbatch and P.M.Headley. SPON: Brain Research Association. Dept. of Physiology, University of Bristol, BS8 1TD, England.

NMDA can enhance nociceptive responses in spinal neurones, NMDA antagonists can reduce such responses and NMDA receptor activation may trigger long-term nociceptive processes. However it remains unclear to what extent these changes are effected exclusively by NMDA receptors.

Recordings were made from single dorsal and ventral horn neurones in αchloralose anaesthetized, spinalized rats. Iontophoretic NMDA enhanced responses to noxious heat (146±33%(S.E.M) of control, after subtracting background activity of 25 spikes/s) which returned to control immediately after NMDA ejection ceased. (RS)-alpha-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid (AMPA) and kainate were only slightly less effective (115±8% and 123±20% control respectively). The NMDA antagonist D-2-amino-5-phosphonopentanoate (AP5) reduced not only responses to noxious heat and pinch (69±7%) but also spontaneous activity (43±12%). When this fall in spontaneous was compensated by maintaining a constant background discharge with AMPA, AP5 no longer significantly decreased the responses (87±4%). Equivalent effects were seen with the non-NMDA receptor antagonist 6-cyano-7-nitroquinoxaline dione (CNQX; 56±8% without, versus 85±5% with compensation).

If activation of synaptic NMDA receptors triggers long-term enhancement of nociception, then exogenous NMDA should cause a maintained increase of phasic nociceptive responses. It did not do so. NMDA receptors do however mediate background synaptic input under these conditions, but not phasic thermal or mechanical nociceptive responses. These results show that caution is required in interpreting the role of NMDA receptors in nociception.

This work was supported by the MRC.

EXCITATORY AMINO ACIDS: RECEPTORS X

731.1

MODULATION OF SPERMINE-INDUCED [3H]TCP BINDING BY GUANINE NUCLEOTIDES ON THE NMDA RECEPTOR COMPLEX. T. Yamamoto*[1,2] H. Yamamoto[2] and T. Moroji[2]. [1]Lab. of Molecular Recognition Yokohama City Univ., Yokohama 236, Japan and [2]Dept. of Psychopharmacol., Tokyo Inst. of Psychiatry, Tokyo 156, Japan.

The N-methyl-D-aspartate(NMDA) receptor complex consists of several modulatory site, i.e., strychnine-insensitive glycine-site, high-affinity Mg^{2+}-site(stimulatory), low-affinity Mg^{2+}-site(inhibitory), polyamine-site and phencyclidine(PCP)-site. We have previously demonstrated that the high-affinity Mg^{2+}-site and glycine-site may be coupled to a GTP/GDP binding site using [3H]TCP binding to rat cortical membranes. In this study, we further examined the effect of guanine nucleotides on the spermine-induced [3H]TCP binding to well washed rat cortical membranes comparing with glutamate- and glycine-induced binding. Guanine nucleotides strongly inhibited spermine-induced [3H]TCP binding while showing a lesser effect on Mg^{2+}, glycine- and glutamate-induced binding. GDP was most potent compound. GDP inhibited spermine-induced [3H]TCP binding with an IC_{50} of 11.2 μM. Stimulation of [3H]TCP binding with either glycine or Mg^{2+}, nor glutamate, was also inhibited by GDP(IC_{50}= 181 and 96.3 uM, respectively). Scatchard analysis indicates that the GDP inhibition of spermine-induced [3H]TCP binding results from a decrease in the number of binding site. These results suggest that spermine-site is more closely coupled to GDP/GTP site than the other modulatory sites.

731.2

CHARACTERIZATION OF PHENCYCLIDINE (PCP) BINDING IN MOUSE BRAIN AND SPINAL CORD MEMBRANES. A. A. Larson* and K. Kovacs. Department of Veterinary Pathobiology, University of Minnesota, St Paul, MN 55108, U.S.A.

Our behavioral studies suggest the presence of a PCP site in the mouse spinal cord that is not linked to NMDA activity. To test this hypothesis more directly, we examined PCP binding in mouse brain and spinal cord using [^{3}H]1-[1-(2-thienylcyclohexyl)piperidine] ([^{3}H]TCP). Whole brain and spinal cord tissues each resulted in a two-site model having high (K_dH =5.5 and 9.3 nM respectively) and low (K_dL =155 and 149 nM respectively) affinity binding. Brain homogenates yielded a high density ($B_{max}H$ = 680 fmol/mg protein) of high affinity sites, which constitute a much smaller population ($B_{max}H$ = 231 fmol/mg protein) in the spinal cord. The number of low affinity sites was about the same in both tissues ($B_{max}L$ = 2993 and 2881 fmol/mg protein in the brain and spinal cord). Competition studies with (+)MK-801 clearly resolved two sites with distinct K_i values of 1.4 and 3827 nM in the brain, 1.2 and 3845 nM in the spinal cord. The abilities of haloperidol, DTG and (+)3-PPP to compete with high affinity for [^{3}H]TCP binding in the spinal cord suggest that the low affinity site labeled by [^{3}H]TCP has characteristics of a *sigma* receptor. Inclusion of GBR12909, a dopamine transport inhibitor at concentrations up to 1 μM, had no effect on either high or low affinity [^{3}H]TCP binding. These data suggest that the majority of PCP binding in the spinal cord is not linked to the NMDA receptor complex. *(USPHS grants DA04090, 04190 and 00124).*

731.3

TIME DEPENDENT EFFECTS OF PHENCYCLIDINE ON NMDA RECEPTOR BINDING IN RAT HIPPOCAMPUS M. Gottschalk*, X.-M. Gao, C.A. Tamminga Maryland Psychiatric Research Center, University of Maryland, Baltimore, MD 21228

Phencyclidine (PCP) is a psychotomimetic drug which produces both acute and delayed psychotic effects in humans and psychosis exacerbation in schizophrenia. Hence it has been used in animals as a model of psychosis. We have previously demonstrated that a single administration of PCP in rats produces a dose-responsive increase in NMDA-sensitive [^{3}H]glutamate binding in hippocampal CA_1 24 hours after a dose. In this study, we analyzed the time course of NMDA receptor binding changes following PCP treatment at 3, 6, 12, 24 and 48 hours after a single dose (8.6 mg/kg). Our results show that NMDA receptor binding in CA_1 shows no change at 3 hours; however binding is increased by 21% at 6 hours (p=0.25), and by 24% at 12 hours (p<0.05). The effect reaches its maximum at 24 hours with a 34% increase (p<0.05). By 48 hours, the NMDA binding has returned to control levels.

This report shows that the alterations in NMDA receptor binding after a single PCP administration are time related, extended, and transient. We have previously reported a similar extended time course for changes in [^{14}C]2-deoxyglucose metabolism following PCP treatment (Gao et al. Eur. J. Pharmacol. in press, 1993). These data, taken together with the known clinical time course of PCP-induced psychosis in humans which includes delayed psychotomimetic effects, may have implications for the underlying mechanism of the psychotomimetic action of PCP.

731.4

DELAYED ALTERATIONS IN NMDA RECEPTOR BINDING AFTER MK801 TREATMENT IN RATS X.-M. Gao*, C.A. Tamminga, Maryland Psychiatric Research Center, University of Maryland, Baltimore, MD 21228

We have previously demonstrated that a single dose of PCP in rat produces a dose-related increase in NMDA-sensitive ^{3}H-glutamate binding in hippocampus CA_1 region at 24 hours. Because clinical psychotomimetic effects of PCP can also show this delayed property, this neurochemical finding could be informative about the mechanism of PCP-induced psychosis. MK801 is a non-competitive NMDA receptor antagonist, which acts at the level of the NMDA receptor operated ion channel as an open channel blocker, a site of action shared by PCP. Here, we report the effect of MK801 at 24 hours (1 mg/kg) on NMDA sensitive ^{3}H-glutamate binding, and on ^{3}H-kainate (KA) binding. NMDA and KA glutamate receptors were quantified according to standard technique, using ^{3}H-glutamate and ^{3}H-KA, respectively (Gao et al. Neurosci. Abstr. 18:377, 1992). MK801 produced an increase of 22% in NMDA receptor binding in CA_1 pyramidal cells (MK801 = 211.5 ± 17.6, Control = 173.0 ± 21.2, mean ± SD, n=6,P<0.01), 24 hours after the dose, and also an increase in the same receptor in posterior cingulate cortex and thalamus. MK801 also increased ^{3}H-kainate binding sites in dentate gyrus at 24 hours (MK801=151.3±10.1, Control=134.5±12.4, P<0.05). Thus, MK801 shows the same delayed effect on NMDA receptor binding as previously demonstrates for PCP. This delayed effect may be mediated at the NMDA-PCP receptor on the Schaffer collaterals extending from CA_3 to CA_1. These late NMDA receptor alterations may be related to the psychotomimetic actions of PCP.

731.5

ALTERED EXCITATORY AMINO ACID RECEPTOR EXPRESSION AFTER HYPOTHERMIC CIRCULATORY ARREST IN THE DOG.
M.E. Blue*, J.M. Redmond, K. Zehr, M.S. Lange, M. Gillinov, M.V. Johnston and W.A. Baumgartner. The Kennedy Krieger Institute and Depts. of Neurology and Surgery, The Johns Hopkins Medical Institutions, Baltimore, MD 21205.

Hypothermic circulatory arrest (HCA) is used clinically to provide a bloodless field for repair of congenital heart defects and other surgical procedures. HCA is accompanied by a characteristic pattern of ischemia and neurological injury which resembles that produced by the excitatory amino acid (EAA) transmitter, glutamate. In the present study, we examined the role of glutamate neurotoxicity in a canine model of HCA. Using quantitative receptor autoradiography, ^{3}H-glutamate binding to NMDA receptors and ^{3}H-AMPA binding was characterized in hippocampus 3 days after HCA. Both NMDA and AMPA receptors declined in density after HCA, with the greatest decreases in a zone spanning the cell bodies, basal dendrites and proximal apical dendrites of pyramidal cells in CA1 and CA 3 and in the molecular layer of the dentate gyrus. The decrease in EAA receptors was most marked in these regions, which have enhanced binding site density, whereas zones with fewer EAA receptors were less affected by HCA. The distribution of diminished receptor expression also correlated with the selective pattern of necrosis in the hippocampus and dentate gyrus.

Additional experiments determined whether administration of the EAA antagonists, MK-801 and NBQX and GM1-ganglioside would reverse the decline in EAA receptors after HCA. All three compounds preserved AMPA and NMDA receptors and afforded neuronal protection in regions of the hippocampus and dentate gyrus where receptor density was highest. These results suggest a role for excitotoxicity in HCA and some possible therapeutic approaches for ameliorating the associated neuronal deficits.

731.6

ETHANOL EFFECT ON THE NMDA RECEPTOR COMPLEX: ALTERATION IN BINDING OF THE LIGAND MK-801 IN CORTEX/HIPPOCAMPUS IN VITRO.
K.S. Phillips, J. Gonzalez, P.K. Randall and S.W. Leslie*, Div. of Pharmacol. and Toxicol., The Univ. Texas at Austin, Austin, TX 78712.

Ethanol has been demonstrated to inhibit NMDA receptor-mediated functions. We investigated this phenomenon in more detail by examining ethanol action on MK-801 binding to the internal PCP receptor site as an index of the functional status of the NMDA-associated ionophore.

Membrane preparations of adult rat cortex/hippocampus were assayed according to the method of Javitt and Zukin (PNAS 86:740-744, 1989). Briefly, homogenates (0.2-0.4 mg protein) were incubated with 12 concentrations of [^{3}H]MK-801 from 1.2 - 150 nM , each under conditions of basal, 10 uM glutamate or 10 uM glutamate/30 uM D-serine, and ethanol (0, 50, or 100 mM) for 4 hrs at 37 C. Non-specific binding was determined by displacement with 50 uM PCP. Kd and Bmax were estimated by Scatchard analysis initially with Program Ligand, followed by nonlinear regression with MACSPSS 4.0, coupled to iterative computation of equilibrium free and bound concentrations.

For each assay all concentration curves for the different conditions were fit simultaneously to a two-site model with high and low Kd's shared across curves. These analyses generated estimates of high and low affnity constants (Kd) and receptor densities (Bmax), compatible with a definition of open and closed states of the ion channel, respectively. Ethanol (50 and 100 mM) did not alter the apparent dissociation constants of MK-801 binding; however, it decreased the % high affinity receptors in basal and agonist/(+/-)coagonist conditions. This finding is due, at least in part, to an apparent increase in the low affinity receptor density. Association experiments further explained this conclusion, in that ethanol diminished fast component binding (MK-801 rapid diffusion in open channel state) in the presence of glutamate. Slow component binding, however, was increased by ethanol, suggesting that ethanol does not simply prevent activation of the channel by the agonist. (Supported by NIAAA grants R01 AA09337 and R37 AA05809)

731.7

NMDA AND NON-NMDA RECEPTOR BINDING IN THE BRAIN OF THE NAPLES HIGH AND LOW-EXCITABILITY RATS: AN AUTORADIOGRAPHIC STUDY.
M.P. Pellicano and A.G. Sadile. (SPON: European Brain and Behaviour Society). Dipt. Fisiologia Umana "F. Bottazzi", 2nd Univ. Naples (SUN), Naples, Italy.

The Naples High- (NHE) and Low-Excitability (NLE) rats, selectively bred for high and low activity in a Làt-maze, have been proposed as animal model to study hippocampal functions. The anatomical distribution of NMDA and non-NMDA receptors has been studied in the brain of these rats by quantitative autoradiography using ^{3}H-L-glutamate as ligand. Twenty µm thick cryostat sections were incubated for 45 min at 4 °C with 150nM ^{3}H-L-glutamate (56 Ci/mmol) in 50 mM Tris-HCl buffer (pH 7.2) containing 2.5 mM $CaCl_2$ alone or in presence of 100µM NMDA or 2.5µM quisqualate (QA). Non specific binding was determined in presence of 1mM unlabelled glutamate. The sections were exposed to tritium-sensitive films for 3 weeks at 4 °C. Quantitative analysis revealed (i) higher levels of total binding in NHE than in NRB and NLE-rats in all areas but cerebellum; (ii) a positive correlation in hippocampal CA1 and dentate gyrus between binding sites and activity level with NLE < NRB < NHE; (iii) a higher displacement by QA than NMDA across brain areas (but not cerebellum) in NHE compared to NRB and NLE-rats; (iv) the displacement by NMDA was lower in the hippocampus of NLE than NRB and NHE-rats; (v) a negative correlation between QA binding and activity level with NLE > NRB > NHE in all areas but cerebellum. In the hippocampus, NLE-rats have lower binding sites for both NMDA and QA-receptors, whereas NHE-rats have higher binding sites for QA-receptors. Thus, the hippocampus and neocortex of the NHE and NLE rats appear to be differentially modelled by an unbalance between NMDA and non-NMDA receptors.

Supported by CNR 92.01092.CT04, MURST 40% and 60% grants.

731.8

NE-100, A NOVEL SELECTIVE σ RECEPTOR ANTAGONIST, MODULATES [^{3}H]TCP INTACT CELL BINDING POSSIBLY THROUGH INTRACELLULAR CROSS-TALK BETWEEN σ-1 SITE AND NMDA/PCP RECEPTOR/ION CHANNEL COMPLEX.
H. Yamamoto[1], T. Yamamoto[1], N. Sagi[1], V. Klenerová[1], F. Mizobe*[2], S. Okuyama[2], T. Moroji[1]. [1]Dept. of Psychopharmacol., Tokyo Inst. of Psychiatry, Tokyo 156, [2]Dept. of Pharmacol., Res. Center, Taisho Pharmceutical Co., Ltd., Saitama 330, Japan.

N,N-dipropyl-2-[4-methoxy-3-(2-phenylethoxy)phenyl]-ethylamine monohydrochloride (NE-100), a highly selective σ ligand, is effective in behavioral tests of phencyclidine. More precise pharmacological profile of NE-100 will be also presented by Okuyama et al. at this meeting. NE-100 was tested of an ability to modify muscarinic acetylcholinergic phosphatidylinositol turnover and [^{3}H]TCP binding in primary cultured neuronal cells derived from fetal rat telencephalon. NE-100 inhibited IP_3 production induced by 1mM carbachol, suggesting that NE-100 acts at σ-1 site. Haloperidol, NE-100, DuP 734 and XJ 448 decreased [^{3}H]TCP intact cell binding under physiological condition. The order of potency is NE-100> haloperidol> DuP 734> XJ 448. Considering the finding which NE-100 has no affinity for NMDA/PCP receptor/ion channel complex, inhibitory action of NE-100 on [^{3}H]TCP intact cell binding would be explained by a cross-talk between σ-1 site and NMDA /PCPreceptor/ion channel complex.

731.9

DEVELOPMENTAL CHANGES IN [^{3}H]-KAINATE BINDING IN HUMAN BRAINSTEM SITES VULNERABLE TO PERINATAL HYPOXIA-ISCHEMIA
A Panigrahy*# and HC Kinney#+ Dept of Neurology# and Pathology+, Children's Hospital, Boston MA 02115

While the pathogenesis of CNS neuronal death secondary to hypoxia-ischemia (H-I) is complex and multifactorial, the distribution and ontogeny of excitatory amino acid receptors likely contribute to the regional and developmental patterns of selective vulnerability. In humans the brainstem is more susceptible to H-I in the fetal, perinatal, and early infant periods than in childhood and adulthood, with specific regions of vulnerability in the inferior olive (IO), basis pontis (BP), inferior colliculus (IC), and reticular core. We postulated that the selective vulnerability of these brainstem regions in early development coincides with a transient elevation in binding to the kainate (KA) receptor, a nonNMDA receptor implicated in glutamate-induced neuronal death complicating H-I. To test this idea, we compared the quantitative distribution of [^{3}H]KA binding in multiple nuclei between human fetuses (n=4; 19-25 gestational weeks), young infants (n=4; 1-9 postnatal mos), and "mature" individuals (n=3; 1 child and 2 adults) without neurological disease or H-I. Quantitative receptor autoradiography was used, with 4nM [^{3}H]KA for total binding and 4nM [^{3}H]KA and 100uM KA for nonspecific binding. The highest brainstem binding (>20 fmole/mg tissue) at any age occurred in the fetal IC, fetal and infant BP, and infant IO. Binding in the IC decreased significantly between the fetal and infant groups and was negligible in the mature group. Binding in the IO increased from the fetal to infant period and was significantly lower in the mature group. Binding in the mature BP was significantly lower than the fetal and infant BP. In the reticular core, binding was intermediate (10-20 fmole/mg) in the fetal group, compared to negligible levels in the mature group. In contrast, binding was intermediate in cranial nerve and other nuclei, with no significant differences among the fetal, infant, and mature group. Quench did not fully account for decreased [^{3}H]KA binding. These data support the hypothesis that the vulnerability of certain nuclei in the immature human brainstem to H-I coincides with a developmentally-regulated, transient elevation in KA receptor binding. (Supported by an AOA Medical Student Fellowship and NICHD 20991.)

731.10

ETHANOL SENSITIVITY OF GLUTAMATE RECEPTORS EXPRESSED IN XENOPUS OOCYTES DOES NOT DIFFER AFTER INTERNAL PERFUSION.
S.N. Treistman, H. Bayley*, V. Anantharam, A. Wilson, Dept. Pharmacol., Univ. Mass. Med. Sch., Worcester, MA 01655, and Worcester Fnd. Exp. Biol., Shrewsbury, MA 01545.

We have shown previously that homomeric assemblies of the NMDAR1 subunit expressed in Xenopus oocytes are sensitive to ethanol at pharmalogically relevant concentrations. We wished to assess the importance of cytoplasmic elements for this ethanol response. To perfect the necessary technique, we examined oocytes injected with GluR1 and GluR2. The experimental protocol was modified from protocols previously described (Dascal, et al., J. Neurosci. Meth., 39:29-38, 1991; Taglialatela, et al., Biophys. J., 61:78-82, 1992). Briefly, the oocyte was allowed to seal to the lumen of a fire-polished pipette, and was then pierced with the perfusion pipette, and the inside of the oocyte was perfused with 30 µl of "internal medium" containing K-MeS and K-ATP. The exterior of the oocyte was perfused with control medium containing 10 µM glutamate either with or without ethanol. Glutamate-induced currents in the internally-perfused oocyte were 67±7% of the currents elicited by 2-electrode voltage clamp in the same oocytes. Glutamate current was reduced 32±7% by 100 mM ethanol in the intact oocyte, and 28±2% in the internally-perfused oocyte. Thus, inhibition of the glutamate receptors was likely to result from direct interaction of ethanol with the receptor, and did not require the involvement of cytoplasmic elements. (Supported by NIH grant AA05542).

731.11

IN VITRO ETHANOL EFFECT ON BINDING OF THE NMDA RECEPTOR ANTAGONIST, CGP 39653, DURING POSTNATAL DEVELOPMENT. R. Robichon, P.K. Randall, K.S. Phillips, M. Weaver * and S. W. Leslie, Div. of Pharmacol. and Toxicol., The Univ. Texas at Austin, Austin, TX 78712.

Previous studies have shown that ethanol inhibits NMDA receptor-mediated functions. To investigate further the mechanism of these actions, we examined acute ethanol exposure on properties of antagonist binding at the NMDA receptor site during postnatal (PN) development in the rat.

Membrane preparations of whole brain from animals, ages 7-14-21-28 and 90 days (PN) were assayed according to the method of Sills et al. (Euro.J Pharmacol. 192:19-24, 1991). Briefly, tissue (0.2-0.4 mg protein) was incubated with 12 concentrations of [^{3}H]CGP 39653 from 0.29 - 60 nM and ethanol (0, 24, 48, or 96 mM) for 2 hrs at 4 C. Non-specific binding was determined by displacement with the agonist, 10 uM glutamate. Binding parameters, Kd and Bmax, were estimated by Scatchard analysis using Program Ligand.

The affinity (Kd) of CGP 39653 was found to vary significantly among PN ages with affinity increasing from 7 day to adult. Ethanol exposure (>25 mM) lowered the affinity of antagonist binding in 7-day and 14-day whole brain homogenates. The affinity of 7-14 day tissue remained lower than older ages in the presence of ethanol (25 to 100 mM). Receptor density (Bmax) was lower in 7-day and adult tissue compared to 14 to 28-day whole brain homogenates. Ethanol exposure (> 25 mM) significantly increased Bmax in tissue of all ages, analyzed jointly. This increase was maximal at 25 mM ethanol, and was highest in 21-day and lowest in 7-day whole brain homogenates (25 to 100 mM). These results suggest the hypothesis that ethanol may alter the confirmation of the NMDA receptor site to a more antagonist-conferring state; and that this configuration is synonymous with an inactivated form of the receptor. This response to ethanol appears more sensitive at certain stages of postnatal development than others. (Supported by NIAAA grants R01 AA09337 and R37 AA05809)

731.12

ANTIDEPRESSANT-INDUCED ADAPTATION OF THE NMDA RECEPTOR COMPLEX. R.T. Layer*, G. Nowak, P. Popik, P. Skolnick and I.A. Paul. Lab. Neuroscience, NIH, NIDDK, Bethesda, MD 20892.

Chronic treatment with either antidepressant drugs (tricyclics, MAOIs, and atypicals) or electroconvulsive shock (ECS), reduces the affinity of glycine to inhibit [^{3}H]5,7-DCKA binding to the stychnine-insensitive glycine modulatory site of the NMDA receptor complex (Nowak *et al.*, PNAS, submitted; Paul *et al.*, Eur. J. Pharmacol. - Mol. Pharmacol. Sect., in press). We examined the time course and dose-response relationships after treatment with imipramine (IMI), citalopram (CIT) and ECS. The first significant increases in the IC_{50} of glycine to inhibit [^{3}H]5,7-DCKA binding were observed after treatment for 7 d with ECS; 14 d with IMI and; 10 d with CIT. These effects disappeared by the 10th d after withdrawal from treatment. The E_{MAX} for ECS (350% > control) was twice that of IMI. The ED_{50} values for IMI and CIT were 5 mg/kg, however the E_{MAX} for IMI was twice that of CIT. These characteristics indicate that antidepressant-induced decreases in the affinity of glycine to inhibit [^{3}H]5,7-DCKA binding are: 1) slowly developing (7-14 d) adaptive phenomena and; 2) remarkably persistent after antidepressant withdrawal. Moreover, these data are in good agreement with the clinical differences observed among these treatments. These findings lend further support to the hypothesis that the NMDA receptor complex plays a critical role in the neural adaptation presumed to underlie the therapeutic response to antidepressant treatment.

731.13

ENFLURANE INHIBITION OF GLUTAMATE-STIMULATED [^{3}H]MK-801 BINDING IS ATTENUATED BY SPERMIDINE. D.C. Martin*, R.L. Dennison, and R.S. Aronstam. Departments of Anesthesiology and Pharmacology & Toxicology, Medical College of GA, Augusta, GA, 30912

N-methyl-D-aspartate (NMDA) receptors are a class of glutamate receptors that mediate excitatory transmission in the mammalian nervous system. We have previously demonstrated that the inhibition of glutamate-dependent [^{3}H]MK-801 binding to the NMDA ionophore by the volatile anesthetic, enflurane, is attenuated by the positive NMDA allosteric modulator, glycine. The present study examined the influence of the NMDA receptor allosteric agonist, spermidine, on the disruption of glutamate-dependent [^{3}H]MK-801 binding by enflurane. Membranes for radioligand binding assays were prepared from rat forebrains by homogenization followed by differential centrifugation. Following treatment with Triton X-100 (0.04%), the membranes were extensively washed. Enflurane (0.15-1.0 mM) inhibited glutamate-stimulated [^{3}H]MK-801 binding by as much as 50% with an IC_{50} value of 0.4 mM. The presence of 100 μM spermidine reduced the inhibition of glutamate-dependent [^{3}H]MK-801 binding by enflurane from 50% to 12%. In the presence of 100 μM glutamate, spermidine stimulated glutamate-dependent [^{3}H]MK-801 binding by 150% (10 μM spermidine) and mitigated the disruption of [^{3}H]MK-801 binding by enflurane in a concentration-dependent manner with an EC_{50} of approximately 3 μM. In the absence of glutamate, enflurane had little influence on [^{3}H]MK-801 binding stimulated by spermidine. These results suggest interactions between general volatile anesthetics and polyamine allosteric modulatory sites on NMDA receptors. Supported by GM 46408 and the Medical Research Service of the Veterans Administration.

731.14

CONANTOKINS: PEPTIDE LIGANDS OF THE NMDA RECEPTOR R. A. Myers*, J.-F. Hernandez, J. L. Torres J., Rivier and B. M. Olivera. Dept. of Biology, University of Utah, Salt Lake City, UT 84112 and Clayton Laboratories for Peptide Biology, Salk Institute, La Jolla, CA 92138.

The conantokins are peptides isolated from the venom of fish-hunting marine snails of the genus *Conus*, which target to the N-methyl-D-apartate (NMDA) receptor (Olivera et al. (1990) *Science* 249, 257). These peptides have 17-27 amino acids, at least four of which are the modified amino acid γ-carboxyglutamate, which confers an α-helical conformation onto these peptides (Myers et al. (1990) *J. Toxicol.-Toxin Reviews* 9, 179). Electrophysiological studies with conantokins suggest that these peptides discriminate between different NMDA receptor subtypes (Hammerland et al., (1992) *Eur. J. Pharmacol.* 226, 239; Haack et al., manuscript submitted). Structure/function studies, both with the naturally occurring peptides and the numerous chemically-synthesized analogs, have allowed identification of which amino acids are necessary for activity. Radioiodinated analogs have also been prepared and employed in a crosslinking assay which was used to follow the purification of complexes of >300 kd from whole rat brain membranes. With a purification strategy including affinity chromatography with an immobilized NMDA ligand, SDS-PAGE reveals that the complexes are glycoproteins composed of subunits with M_rs of 100-170 kd; one such protein (160 kd) has been purified to homogeneity. Preliminary evidence suggests that the SDS-PAGE banding pattern varies according to brain tissue type, thus supporting the existence of multiple NMDA receptor subtypes. (Supported by GM48677.)

731.15

EVIDENCE FOR TWO GLUTAMATE, TWO GLYCINE, AND THREE MAGNESIUM/CALCIUM BINDING SITES ON THE NMDA CHANNEL. G. von Euler* and Y. Liu. Dept. of Histology and Neurobiology, Karolinska Institutet., Box 60400, S-10401 Stockholm, Sweden

In order to elucidate the complex effects of glutamate and glycine on the NMDA receptor-coupled ion channel, we have investigated the effects of these amino acids on equilibrium [^{3}H]MK-801 binding in cerebrocortical membrane preparations from male rats, in the presence of various concentrations of Mg^{2+}, Ca^{2+} and H^+.

1: The potency of glutamate and glycine to synergistically increase specific [^{3}H]MK-801 binding was strongly enhanced by low concentrations of Mg^{2+} and Ca^{2+} (<30 μM) and inhibited by H^+.

2: In the presence of Mg^{2+} (>0.1 mM) glutamate and glycine synergistically decreased the affinity of [^{3}H]MK-801 binding. Ca^{2+} and H^+ partly antagonized the decrease, whereas the competitive glutamate and glycine antagonists D-CPP and 7-Cl-KYNA completely reversed the decrease in [^{3}H]MK-801 binding affinity.

3: High concentrations of Mg^{2+}, Ca^{2+}, and H^+ inhibited [^{3}H]MK-801 binding, probably by a direct competition at a common binding site. Thus, acidic conditions counteracted the blocking effect of Mg^{2+}.

Based on these results we have developed a mathematical model which closely mimics experimental data. The model indicates the presence of at least two glutamate, two glycine, and three magnesium/calcium binding sites on the NMDA channel. Our results suggest that glutamate and glycine synergistically decrease the binding affinity of Mg^{2+} to its voltage-dependent site within the channel pore, in addition to the opening of the NMDA channel.

731.16

EFFECT OF MONO-, DI- AND POLYVALENT CATIONS ON NMDA SENSITIVE [^{3}H]CGP-39653 BINDING IN RAT BRAIN MEMBRANES. A. Mukhin*†, E. Kovaleva† and E.D. London†,§,¤ †Neuroimaging and Drug Action Section, Addiction Research Center, NIDA, NIH, Baltimore, MD 21224; §Dept. Pharmacol. Exp. Ther., School of Medicine, University of Maryland, Baltimore, MD 21201; and ¤Dept. Radiology, The Johns Hopkins School of Medicine, Baltimore, MD 21205.

It has been shown previously that divalent cations can enhance the binding of [^{3}H]MK 801 ([^{3}H]dizocilpine) in preparations of well-washed membranes from rat brain. This effect is demonstrable in the presence of low, but not saturating concentrations of L-glutamic acid (GLU). One might suggest that this stimulation is due to an enhancement by divalent cations of the affinity of the NMDA receptor for GLU.

Using [^{3}H]CGP-39653 (CGP) as a radioligand probe for GLU recognition sites on the NMDA receptor, we observed that $MgCl_2$ or $CaCl_2$ (EC_{50} 0.8 mM) can enhance CGP binding up to five-fold in well-washed membranes from rat brain. Monovalent cations (Li^+, Na^+, K^+, and Cs^+), added to incubations as chloride salts, produced similar stimulation of CGP binding, but with lower potencies (2.5-fold stimulation with EC_{50} values about 8 mM). Of the cations studied, the most potent ones were the polyamines spermine and spermidine, which enhanced CGP binding up to six times, with EC_{50} values of 6 μM and 20 μM, respectively. Effects of the cations on CGP binding were not additive, suggesting that all of the cations acted through the same mechanism and the same binding sites.

Our preliminary results indicate that CGP binding sites in well-washed membranes are present in two affinity states, and that cations convert low affinity binding sites to high affinity sites. The findings have implications regarding at least part of the mechanism by which polyamines modulate the function of the NMDA receptor.

732.1

ACUTE INTRAVENOUS INFUSION VS. MULTIPLE INFUSIONS OF FENTANYL DERIVATIVES IN A RAT EEG MODEL.

S. La Marca, R.J. Lozito, M.H. Ossipov, R.W. Dunn, and T. Jerussi*.
Anaquest, Inc., 100 Mountain Ave., Murray Hill, NJ 07974

Administration of anesthetic agents to rats produces a loss of righting (LOR) which is predictive of clinical anesthesia. Following bolus i.v. administration of fentanyl, sufentanil, alfentanil, and remifentanil, the ED_{100} doses for LOR were 0.035, 0.003, 0.05, and 0.020 mg/kg, respectively. For the EEG infusion studies, rats were implanted with jugular catheters and 5 cortical electrodes just below the dura mater. Each agent was infused at a rate of 0.02 ml/min such that each animal received the ED_{100} dose every 60 seconds until LOR was observed and the infusion was stopped. Following acute infusion to LOR, the difference in time from return of the righting reflex (RRR) to baseline EEG for fentanyl, sufentanil, alfentanil, and remifentanil was 30.9, 35.3, 14.9, and 1.3 minutes, respectively. Following a three hour washout period, multiple infusions (three successive infusions to LOR) were administered. Following RRR (after the third LOR) the return to baseline EEG for fentanyl, sufentanil, alfentanil, and remifentanil was 56.1, 57.1, 13.6, and 2.9 minutes, respectively. There were no statistically significant differences between the acute and multiple infusions for the return to baseline EEG for alfentanil and remifentanil, but there was significant increases in time to return to baseline following multiple infusions of fentanyl and sufentanil. These results show that there was no cumulation of alfentanil and remifentanil with respect to EEG effects but cumulation was observed for fentanyl and sufentanil.

732.2

COGNITIVE AND EEG RECOVERY FOLLOWING BOLUS INTRAVENOUS ADMINISTRATION OF ANESTHETIC AGENTS.

R.W. Dunn*, R.J. Lozito, and S. La Marca.
Anaquest, Inc., 100 Mountain Ave., Murray Hill, NJ 07974

Bolus intravenous administration of fentanyl and the fentanyl derivatives, alfentanil, remifentanil, and sufentanil, as well as the intravenous anesthetic agents etomidate and propofol, produce anesthesia in rats as measured by the loss of righting (LOR) with calculated ED_{150} doses (derived from linear regression analysis) determined to be 0.06, 0.09, 0.037, 0.007, 2.51, and 6.12 mg/kg, respectively. Immediately, 15 minutes, and 30 minutes following intravenous administration of the ED_{150} dose of each of these agents and subsequent return of the righting reflex, trained animals were assessed for cognitive recovery in an eight-arm radial arm maze. Performance during each session was scored by visual observation in terms of efficiency of responding (percentage of correct arm entries within 10 minutes). Animals administered fentanyl or sufentanil were unable to successfully complete the maze throughout the testing periods. Animals receiving remifentanil showed cognitive recovery within the first testing interval (immediately following return of righting reflex), while animals receiving alfentanil, etomidate, or propofol showed recovery at the 15 minute testing interval following return of the righting reflex. In a separate experiment, bolus intravenous administration of the ED_{150} dose of these agents was evaluated in an acute EEG model. Rats implanted with 5 frontal cortical electrodes were assessed for the return to baseline control EEG levels following return of righting reflex. Return to baseline EEG levels were 0.3, 2.88, 5.06, 16.25, 31.29, and 43.98 minutes for remifentanil, propofol, alfentanil, etomidate, fentanyl, and sufentanil, respectively. These data show that the return to efficient cognitive functioning corresponds to the return to normal baseline EEG levels.

732.3

ACUTE & CHRONIC NALTREXONE DECREASES THE HYPERACTIVITY OF AUTISM B.H. Herman*, G. Asleson, E. Lukens, I. Borghese, L. Anselmi, R.P. Allen, M.B. Benoit, I. Chatoor, & P. Papero. Med Dev Div, NIDA, NIH,Rockville, MD 20857, Brain Res Cen & Dept Psychiat, Children's Natl Med Cen & Dept Pediatrics, GWUSM, Washington, DC 20010, & Dept Psychiat, JHUSM, Baltimore, MD 21214

Hyperactivity is a common and severe symptom of autism (Asleson et al. Soc Neurosci 17: 1346,1991). Here the effects of the opioid receptor antagonist, naltrexone, were investigated after both acute (1x week) and chronic (3x week) administration (0.5, 1.0, 1.5, 2.0 mg/kg) versus placebo on the motor/attentional behavior of 20 autistic children. Measures included a subjective scale (parents' ratings, Conners' 10 Item Parent Teacher Rating Scale (CPTRS)) and two objective tests (# quadrants entered (Qs), activity monitor (AAM) counts during a playroom test (BRCSPT)). The CPTRS revealed that naltrexone induced highly significant decreases for acute (N=14) and chronic drug (N=12) (p's < 0.001). Acute naltrexone also significantly decreased Qs entered during the BRCSPT (N=20; p<0.05) but this effect was not significant for chronic drug. Results for the AAM measure were inconclusive due to a small N for acute (N=9) and chronic naltrexone (N=7) although for both drug trials there were overall decreases in AAM counts under naltrexone versus placebo (p's > 0.10). These data suggest that hypermobilization of opioid peptides and possibly dopamine (Herman et al. Soc. Neurosci, 18: 657, 1992) underlies the hyperactivity/ inattention of autism (Supported by FDA, NICHD, DuPont, Janusz Korczak Foundation).

732.4

COMPARISON OF CARDIOVASCULAR AND OTHER PHYSICAL SIDE EFFECTS OF ACUTE AND CHRONIC ADMINISTRATION OF NALTREXONE IN AUTISTIC CHILDREN. G.S. Asleson*, B.H. Herman, J.A. Lewis, A. Powell, R. Ruckman, I.F. Borghese, E. Lukens, L.S. Anselmi Med Dev Div, NIDA, NIH, Rockville, MD 20857, Brain Res. Cen., Depts Psychiat. & Cardiology, Children's Natl. Med. Cen. & Dept. Peds., George Washington USM, Washington DC, USA 20010

The physical side effects of acute and chronic naltrexone (0.5,1.0,1.5,2.0 mg/kg) versus placebo (P1,P2) were investigated in 20 autistic children. Measures obtained before and 55 min after placebo/drug included: auscultated heart rate (HR), pulse rate, systolic and diastolic blood pressure (BP), mean arterial blood pressure (MAP) and axillary body temperature (BT). Serum concentrations of the liver enzymes SGOT and SGPT were obtained by venipuncture 60 min after drug. EKG parameters (Axis, PR, QRS, QT, QTc) were obtained 3h after drug/placebo. Acute naltrexone had no significant (NS) effects on any of these measures in comparison with placebo (p's>0.10). Chronic naltrexone had NS effects on pulse, MAP, systolic and diastolic BP, SGOT and SGPT (p's> 0.10). There was a small decrease in AHR (maximum=7bpm) under naltrexone, however this effect was NS (p>0.10). Chronic naltrexone produced a small decrease in axillary body temperature (0.15-0.32°C), but this effect just failed to attain statistical significance (p=0.051). Therefore, these data provide further support for the clinical safety of naltrexone on cardiovascular, BT and liver function in children. Supported by FDA, NICHD, DuPont, Janusz Korczak.

732.5

EFFECTS OF ACUTELY AND CHRONICALLY ADMINISTERED NALTREXONE ON SOCIAL BEHAVIOR AND LANGUAGE OF CHILDREN WITH AUTISM. I.F. Borghese*, B.H. Herman, L.S. Anselmi, G. Asleson, E. Lukens, M.B. Benoit, I. Chatoor, & P. Papero. Med Dev Div, NIDA, NIH, Rockville, MD 20857, Brain Res Cen & Dept Psychiat, Children's Nat Med Cen, Dept Pediatrics, George Washington USM, Washington DC, 20010.

Hypermobilization of brain opioids appear to play a role in autism (Herman, In J.J. Ratey (Ed.), Mental Retardation: Developing Pharmacotherapies, 1991). Here we examined the effects of naltrexone (0.5,1.0,1.5,2.0mg/kg)-acute (1X per week) and chronic (3X per week) administration-versus placebo (P1, P2) in 20 autistic children. Dose-dependent decreases in the severity of autistic symptoms as measured by the Childhood Autism Rating Scale were produced by acute (N=11/20) and chronic (N=18/20) naltrexone (p's<0.0001).

Language was assessed in three playroom tests (BRCSPT) where Ss were evaluated for 10 min. in the presence of a volunteer (V) or mother (M): no toys NT/V, toys T/V, and NT/M. Acute naltrexone significantly decreased nonmeaningful vocalizations (NMV), but it had no significant effects on the frequency of words and talking in the 3 conditions. In contrast, chronic naltrexone did not produce any significant effects on either NMV or words and talking. Sponsored by FDA, NICHD, DuPont, & Janusz Korczak Foundation.

732.6

EVALUATION OF ACUTE VERSUS CHRONIC NALTREXONE ON PAIN SENSITIVITY IN AUTISTIC CHILDREN. E. Lukens, B.H. Herman, G. Asleson, I. Borghese, M. Benoit, I. Chatoor, P. Papero, & A. Arthur-Smith* Med Dev Div, NIDA, NIH, Rockville, MD 20857, Brain Res Cen & Dept Psychiat, Children's Natl Med Cen & Dept Pediatrics, George Washington USM, Washington, DC 20010

Opiate antagonists fail to produce hyperalgesia in normal normotensive nonstressed adults (e.g.,Grevert & Goldstein, Science 199:1093,1978). In contrast, in schizophrenia where brain opioids may be hypermobilized, naltrexone significantly decreases evoked response to pain stimuli (Davis & Buchsbaum, Mod Probl Pharmacopsychiat 17:97, 1991). Here we show that a subgroup of "pain-insensitive" autistic children show significant evidence of a hyperalgesic response to acute but not chronic naltrexone.

The effects of naltrexone (0.5,1.0,1.5,2.0 mg/kg) versus placebo administered acutely (1X per week) and chronically (3 X's week) on the distress responses of autistic children to venipuncture blood draw were investigated. Collapsed across all 20 Ss, both acute and chronic naltrexone failed to significantly effect the number of autistic children demonstrating either distress vocalization (DV) or lacrimation in response to venipuncture. In a subgroup of "pain-insensitive" (baseline, no lacrimation response to venipuncture) autistic children (N=13/20), acutely administered naltrexone significantly increased the % showing lacrimation (p<.05) while similar effects were not obtained with chronic naltrexone. Supported by FDA, NICHD, DuPont and Janusz Korczak Foundation.

732.7

OPIOIDS AND TIMEOUT FROM AVOIDANCE: EFFECTS OF FENTANYL, METHADONE, AND U50,488.

M. Galizio* and C. Ordronneau, Department of Psychology, University of North Carolina at Wilmington, Wilmington, NC 28403

The effects of mu- and kappa-opioid drugs on rat behavior maintained under negative reinforcement schedules were studied. Rats were trained on concurrent schedules in which responses on one lever postponed shock on a free-operant (Sidman) avoidance schedule, and responses on another lever produced brief (2-min) periods of signaled timeout from avoidance on a variable-ratio schedule. Mu-agonists fentanyl (.003-.1 mg/kg) morphine (1-10 mg/kg) and methadone (1-10 mg/kg) all decreased timeout lever responding at doses that increased or had no effect on avoidance. These effects were reversed by naltrexone. However, the kappa-agonist U50,488 (1-20 mg/kg) did not show comparable actions. U50,488 produced variable effects at low doses, and non-selectively decreased responding at higher doses. These findings suggest the possibility that selective reduction of timeout responding is mediated by the mu receptor system.

732.8

CUES ASSOCIATED WITH SOCIAL STRESS INDUCE CFOS ONCOGENE EXPRESSION. C.A.Cohen*, K.A. Miczek, and R.M. Kream. Department of Psychology, Tufts University, Research Building, 490 Boston Ave., Medford, MA 02155, Department of Anesthesiology, Tufts University School of Medicine, 136 Harrison Ave., Boston MA 02111.

Socially stressed animals show marked potentiation of the analgesic effect of morphine, as demonstrated by a shift in the dose response curve to the left, during the social stress experience. Within 24 hours after social confrontation, there is also evidence for emerging tolerance to opiate analgesia, and tolerance continues to develop as a function of time. Previously, we have reported that the threats by an aggressive opponent are sufficient to significantly increase cFos expression one hour after stress in the periaqueductal gray of the brainstem, followed by a significant increase in the density of met-enkephalin stained fibers at three hours. Surprisingly there was also a significant increase in cFos at 18 hours even though the subjects remained undisturbed in their home cage prior to perfusion. This suggests that there were a set of cues previously associated with the stressful encounter that evoked this biochemical change. In the current experiment, an intruder male rat was placed into the home cage of a resident and subsequently exposed to attack and threat. Once the animal showed unambiguous signs of submission, the intruder was placed into a protective cage for one hour while being exposed to the threat of attack. The intruder was then placed into its home cage and perfused at 18 hours or was returned to the resident's cage in the absence of the resident for one hour and then perfused at 18 hours. In comparison with subjects with the same history of stress, the re-exposed intruders showed a significant increase in cFos expression. Studies are now underway to determine whether administration of an opiate antagonist, such as naloxone, can reverse the expression of cFos.

732.9

ANXIOLYTIC EFFECTS AND A PUTATIVE SITE OF ACTION FOR THE KAPPA OPIOID AGONIST U-50,488H. T.H. Privette* and D.M. Terrian. Department of Anatomy and Cell Biology, East Carolina University School of Medicine, Greenville, North Carolina 27858.

Prodynorphin-derived peptides and other kappa(κ)-selective opioid agonists produce a spectrum of physiological and behavioral effects including enhancement of analgesia, a lowered reward threshold, and changes in measures of motivation. Collectively, these and other effects suggest prodynorphin-derived peptides may act to ameliorate the integrated response of an animal to physical and psychological stress. Although anxiety is a prominent psychological component of the stress response, the ability of κ opioid agonists to suppress anxiety-related behaviors has not been examined.

The objective of the present study was to investigate the effects of the κ_1 opioid agonist, U-50,488H, on anxiety-related behavior using the elevated plus-maze. Male Sprague-Dawley rats were injected i.p. 20 minutes before testing with either PBS vehicle or U-50,488H at doses of 0.01, 0.1 and 1.0 mg/kg. Each rat was placed in an open field for 5 minutes to measure spontaneous locomotor activity and then immediately placed on the elevated plus-maze for 5 minutes. Our results showed that: (1) U-50,488H had no sedative effects on spontaneous locomotor activity, (2) U-50,488H dose-dependently reduced measures of anxiety on the elevated plus-maze, (3) intrahippocampal microinjections of the selective κ_1 antagonist nor-binaltorphimine completely reversed the anxiolytic effect of U-50,488H, and (4) i.p. administration of U-50,488H attenuated the recovery of glutamate in the hippocampus by push-pull perfusion.

These data indicate that U-50,488H is endowed with potent anxiolytic properties and suggest that prodynorphin-derived peptides may act as endogenous anxiolytics within the hippocampus, possibly through a mechanism involving the κ_1-mediated inhibition of glutamate release, to reduce this psychological component of stress. Supported by AFOSR 89-0531.

732.10

EFFECTS OF ICV AND IT NALOXONE AND MORPHINE ON THE ACQUISITION AND EXPRESSION OF CONDITIONED HYPOALGESIA. H. Fod* and R.F. Westbrook. School of Psychology, University of New South Wales, Sydney, Australia.

The study examined the involvement of supraspinal and spinal opioid mechanisms in mediating the acquisition and expression of the conditioned hypoalgesia associated with exposure to a heat stressor. The results showed that acquisition of conditioned hypoalgesia was enhanced by an ICV administration of naloxone and attenuated by an ICV administration of morphine. However, acquisition of the hypoalgesia was unaffected by IT administration of either drug. The results also showed that expression of the conditioned hypoalgesia was enhanced by an ICV administration of naloxone, but was unaffected by an IT administration of the opioid antagonist. Thus, these results suggest a role for supraspinal opioids in mediating the acquisition and expression of the conditioned hypoalgesia associated with exposure to a heat stressor.

732.11

INVERSE GENETIC CORRELATION BETWEEN MORPHINE ANALGESIA AND HYPERLOCOMOTION IN MICE SELECTIVELY BRED FOR STRESS-INDUCED ANALGESIA. J.S. Mogil*, M. Shomer, W.-C. Chang, B. Kao, and J.C. Liebeskind. Dept. of Psychology and Brain Research Institute, UCLA, Los Angeles, CA 90024.

Studies using inbred mice, especially the divergent DBA/2 and C57BL/6 strains, have uncovered an inverse genetic relationship between opiate mechanisms of analgesia and reward. Strains exhibiting high analgesia display low morphine-induced hyperlocomotion (MIH), morphine preference, addiction potential, and withdrawal severity, and vice versa. We have previously demonstrated that mice selectively bred for high (HA) and low (LA) swim stress-induced analgesia also display high and low morphine analgesia, respectively, a difference apparently mediated by a single genetic locus. In the present study, 29th generation HA and LA mice, as well as their F_1 hybrids (HAxLA and LAxHA), were tested for MIH in the open field 20 min after morphine (10 mg/kg, i.p.). LA mice displayed significantly more line crosses than HA mice. F_1 hybrids also were significantly more hyperlocomotive than HA mice, and did not differ significantly from LA mice. Thus, the LA phenotype (high locomotion) is fully dominant over the HA phenotype. Preliminary data from our laboratory indicate that LA mice also exhibit a higher conditioned place preference to morphine, supporting the hypothesis that LA mice display up-regulated opiate reward systems simultaneously with down-regulated opiate analgesia systems. Supported by NIH Grant NS07628 and an Unrestricted Pain Research Grant from the Bristol-Myers Squibb Company.

732.12

A SPECIFIC PERIAQUEDUCTAL GRAY REGION MEDIATES INTRASPECIFIC DEFENSIVE REACTIONS EVOKED BY PRECIPITATED MORPHINE WITHDRAWAL. A. Depaulis, D. Eichenlaub, C. Lazarus and R. Bandler* LNBC, Centre de Neurochimie du CNRS, 5 rue Blaise Pascal, 67084 Strasbourg Cedex, France and Department of Anatomy and Histology, University of Sydney, NSW 2006, Australia.

Opiate withdrawal is known to evoke emotional reactions including defensive behavior in rodents. We have shown recently in the cat and the rat that distinct populations of neurons within the midbrain periaqueductal gray matter (PAG) are responsible for the coordination of different defensive strategies. The present study investigated in the rat the kind of defensive reactions evoked by morphine withdrawal and whether PAG neurons are involved in these effects. Rats were made tolerant to morphine by subcutaneous implantations of 75-mg morphine pellets (NIDA, Rockville). Precipitated withdrawal was induced either by systemic injection of naloxone hydrochloride (1 mg/kg, i.p.) or intra-PAG injection of naloxone methyl bromide (2 nmol in 0.2 µl). When tested for 8 min with a non-aggressive partner in a neutral test cage, rats experiencing systemic precipitated morphine withdrawal displayed confrontational defensive reactions (i.e., upright postures, backward movements) and immobility as compared to controls. Similar confrontational defensive reactions were evoked, along with 22-28 kHz ultrasonic vocalizations, by intracerebral injections of naloxone methyl bromide made in the *intermediate third of the lateral PAG* of morphine tolerant rats. No effects were evoked, however, when these injections were made in the *caudal third of the lateral PAG*, an area which has been shown to coordinate flight and avoidance reactions. This study suggests that morphine withdrawal evokes a specific defensive strategy in the rat and indicates that neurons in the intermediate third of the lateral PAG are implicated in these effects.

732.13

MODULATION OF OPIATE TOLERANCE ON SOCIAL BEHAVIORS BY MK-801. G. Bernatzky*, J. Panksepp, E. Nelson and A. Saba Dept. of Zoology, Univ. of Salzburg, Salzburg, Austria and Dept. of Psychology, Bowling Green State University, Bowling Green, OH 43403.

Low doses of opiates have powerful effects on the social behaviors of young animals, including strong reductions in separation distress vocalizations (DVs) and both increases (low dose) and decreases (high dose) in rough-and-tumble play. This work evaluated whether NMDA receptor blockade with MK-801 would inhibit the development of tolerance to opiate effects in these animal models of emotions as reported for models of pain in adult rats (Trujillo & Akil, Science, 1991, 251, 85-87). DVs were measured in young chickens and play in juvenile rats.

Tolerance to opiates was weak in both models. Morphine at 5 to 10 mg/kg exhibited sustained inhibition of both DVs and play for up to 10 days of once daily drug administration. Some opiate tolerance could be demonstrated in both models after 10 days using reduced morphine challenges (e.g., 1 - 2.5 mg/kg). This tolerance was apparently partially blocked by co-administration of 0.2 mg/kg of MK-801, while the mere administration of the NMDA antagonist for the ten day pre-treatment period did not itself modify sensitivity to opiates. MK-801 alone (.05 - .2 mg/kg) induced a weak suppression of DVs, and a powerful inhibitory effect on social play.

These results highlight the fact that opiate tolerance to the emotional effects of morphine is rather weak in young animals, suggesting the presence of endogenous anti-tolerance factor during youth and/or gradual developmental insensitivity to opiates. Some apparent inhibition of the tolerance could be produced by concurrent treatment with MK-801, suggesting the existence of neurochemical controls over opiate tolerance in social control networks of young rats and chickens which resemble those reported for pain in adult rats. However, the possibility that MK-801 induced inhibition of opiate tolerance is merely an artifact of the behavioral and cognitive disrupting effects of MK-801 needs further experimental attention.

732.14

OPIATE REGULATION OF AFFILIATION BETWEEN PRIMATE MOTHERS AND INFANTS. S.E. Shelton* and N.H. Kalin, Dept. of Psychiatry, Univ. of Wisconsin Sch. of Med., Madison, WI 53792-2475

For the primate infant, affiliation with its mother is imperative to survive. Because affiliation is an interactive process between mother and infant, we separately examined the importance of mothers' and infants' opiate systems in regulating affiliative behaviors within the dyad. On different occasions, opiate agonists and antagonists were administered to rhesus mothers and infants immediately preceding reunion. In the first series of experiments infants were administered either morphine or naltrexone. Results demonstrated that morphine (0.1 mg/kg) decreased clinging between infant and mother ($p < .020$). This was associated with significant increases in locomotion and environmental exploration. In contrast, naltrexone (5 mg/kg) increased clinging ($p < .03$) and as a result infants engaged in less locomotion and exploratory behavior. When mothers received morphine or naltrexone similar effects on affiliative behaviors were observed. These results demonstrate, in primates, the importance of opiate systems in regulating the level of affiliation between a mother and her infant. Of particular interest is the demonstration that alterations in opiate systems in either the mother or her infant have similar effects on affiliation. This suggests that complementary affiliative behaviors occurring between a mother and her infant are modulated by parallel changes in brain opiate systems. These findings may have important implications for the development of attachment bonds as well as in understanding conditions associated with abnormal affiliative responses.

732.15

BEHAVIORAL RESPONSES TO AN ARTIFICIAL NIPPLE IN THE DEVELOPING RAT FETUS: EFFECTS OF μ AND κ OPIOID ACTIVITY. S. R. Robinson*, T. C. M. Hoeltzel, and W. P. Smotherman, Laboratory of Perinatal Neuroethology, Center for Developmental Psychobiology, Binghamton University, Binghamton, NY 13902-6000.

Newborn mammals have the capacity to recognize and express specific behavioral responses to the nipples of the lactating mother within hours or minutes of birth. Experimental study of the rat fetus in vivo has confirmed that organized action patterns can be elicited by an artificial nipple fashioned from soft vinyl on the last three days of gestation. Other aspects of neonatal suckling behavior are affected by endogenous opioid activity, but little is known about the influence of opioids on nipple-evoked behavior. In the present study, rat fetuses were prepared for direct observation on E19, E20 or E21 and were pretreated by ip injection of the selective κ opioid agonist U50,488, the μ opioid agonist DAMGO ([D-Ala^2,NMe-Phe^4,Gly^5-ol]-enkephalin), or the isotonic saline vehicle. Behavioral responses to the artificial nipple were videotaped for frame-by-frame analysis of fetal movements. Perioral contact with the nipple elicited a suite of appetitive behavioral responses, including mouthing activity, licking, oral grasping of the nipple, and forelimb treadling. Fetuses also expressed aversive reactions, including facial wiping and head avoidance. U50,488 reduced appetitive responses and increased avoidance of the nipple, resulting in shorter periods of attachment. In contrast, DAMGO promoted certain appetitive responses to the nipple and facilitated longer attachment on E21. In addition to documenting the prenatal ontogeny of this important neonatal behavior, these findings imply a role for endogenous opioids in modulating behavior during the newborn rat's first suckling episode.

This research is supported by Grant HD 28231 to WPS and SRR.

732.16

SUCKLING-INDUCED CHANGES IN RESPONSIVENESS TO THE HYPOALGESIC EFFECT OF MORPHINE. B. C. Woodside*[1] and J. Rochford[2]. [1]Center for Studies in Behavioral Neurobiology and Department of Psychology, Concordia University, [2] Douglas Hospital Research Centre, Department of Psychiatry, McGill University, Montreal.

There is growing evidence that there are fluctuations both in endogenous opiate systems and responsiveness to exogenously administered opiates across reproductive states in rats. In the first study responsiveness to the analgesic effect of morphine was compared among independent groups of rats that were at different stages of lactation, cycling, or ovariectomised . Animals were placed on a hot plate (52º C) prior to (baseline) and 30 minutes after a subcutaneous injection of morphine (5mg/kg) and latency to lick the hindpaw was measured. Dams tested on Day12 or 18 postpartum had shorter paw lick latencies after morphine administration than either the ovariectomised or cycling females. The latencies of dams tested on Day 6 or 24 of lactation did not differ from the ovariectomised or cycling groups. There were nodifferences among the groups on the baseline measure. Subsequent studies showed that both the time course and the dose response curves to morphine-induced hypoalgesia were reduced in dams on day 12 of lactation in comparison to cycling controls. Data obtained in the final study showed that the reduced response to the hypoalgesic effect of morphine in postpartum dams was not dependent on the delivery of milk but was dependent on suckling stimulation. These data add further to the suggestion that reproductive state modulates opiate sensitivity. (Supported by NSERC).

732.17

OPIATE MODULATION OF PALATABILITY BY THE SECOND GUSTATORY RELAY : IMPLICATION OF LATERAL HYPOTHALAMIC NEURONES. S. Moufid-Bellancourt, R. Razafimanalina and L. Velley* Lab. Neurosci. Comportementales et Cognitives, URA CNRS 339, Universite de Bordeaux I, 33405 Talence France.

Lesions of lateral hypothalamic (LH) neurones modify the taste preference of rats for saccharin and also modulate the well-known effects of morphine on palatability (Touzani and Velley, 1990, Pharmacol. Biochem. Behav. 36;585). We later showed, by local injections, that the second gustatory relay station, the medial parabrachial area (PBm) is, concomitantly, one of the central sites where morphine effects on palatability are mediated and also the site of LH-mediation of morphine effects (Moufid-Bellancourt and Velley, 1993, submitted).

Here we attempt to determine if LH lesions modify sensitivity to the different subtypes of opiate receptors present in PBm. We tested, by local injections into the PBm, a μ agonist, DAGO (0.01-0.1μg / 0.2μl) and a κ agonist, DynA 1-13 (0.1-3μg / 0.2 μl). Finally, we tested a mixture of two inactive doses of DAGO and DynA 1-13 (respectively, 0.01 and 0.1 μg).

Results show that: 1) at the highest doses used, μ and κ agonists exert opposite effects on saccharin ingestion: the μ agonist increasing while the κ agonist decreases this measure; 2) LH lesions induce marked changes in reactivity towards these opiate agonists: the μ agonist decreasing global liquid intake while the k agonist selectively decreases water consumption. In sham-lesioned rats, both μ and κ agonists significantly depressed water intake, this effect not being observed in LH lesioned subjects; 3) reactivity to PBm injections of morphine is not significantly modified by previous injections of μ or κ agonists and 4) the profile of the morphine effect is reproduced by combined injection of μ+κ agonists at doses at which neither is active alone.

732.18

EXCITATORY AMINO ACID (EAA) ANTAGONISTS ATTENUATE SEIZURES INDUCED BY ICV MORPHINE-3-GLUCURONIDE (M3G). M.E. Fundytus*, S. Norris and T.J. Coderre, Pain Mechanisms Laboratory, Clinical Research Institute of Montreal and Dept. of Psychology, McGill University, Montreal, Quebec, Canada.

High-dose morphine and low-dose M3G administered intrathecally or intracerebroventricularly (icv) produce a "toxic activation syndrome" and seizure activity. EAA antagonists selective to the NMDA receptor decrease the activation associated with administration of high doses of morphine (Jacquet, Y. and Squires, R.F., *Behav. Brain Res. 31*, 85-88, 1988). Besides the NMDA receptor, there are two other ionotropic EAA receptor subtypes (AMPA and kainate) and a family of metabotropic EAA receptor subtypes. The purpose of the present investigation was to determine whether icv pretreatment with receptor subtype selective EAA antagonists could attenuate seizure activity induced by icv injection of M3G.

Rats were injected icv with one of the following: saline; a competitive NMDA receptor antagonist (CPP); a non-competitive NMDA receptor antagonist (MK-801); an AMPA/kainate receptor antagonist (CNQX); a metabotropic receptor antagonist (L-AP3); or an opioid antagonist (naloxone). Behaviour was observed for a ten minute baseline period, then rats were injected icv with 5 μg M3G and behaviour was observed for an additional 20 minutes. Recorded behaviours included writhing (seizure activity), ambulating, grooming, resting and rearing. Each of the EAA antagonists decreased seizure activity, whereas the opioid antagonist naloxone was without effect. The activity measures (ambulating, grooming, resting and rearing) did not differ between groups, indicating that all rats were equally active.

The present results suggest that activity at EAA receptors may play a significant role in the toxic effects of low doses of the morphine metabolite morphine-3-glucuronide, and perhaps, therefore, of high doses of morphine. These toxic effects do not appear to involve opioid receptors, as the opioid antagonist naloxone was unable to decrease seizure activity in the present study.

732.19

NEUROADAPTATION OF RATS TO U-50,488 (U-50) REVEALED BY I.C.V. ADMINISTRATION OF NOR-BINALTORPHIMINE (norBNI). A. Cowan* and C.W. Murray, Dept. of Pharmacology, Temple Univ. Sch. of Medicine, Philadelphia, PA 19140

When U-50 is given to rats over 5 days by twice-daily peripheral injection, s.c. challenge with one of several opioid antagonists causes a qualitatively distinct behavioral syndrome. Here, we examined the ability of norBNI, a selective antagonist at kappa receptors, to uncover the behavioral consequences of persistent agonism at these sites. NorBNI was given i.c.v. since this antagonist, per se, elicits confounding behavioral effects after s.c. administration (Prog. Clin. Biol. Res. 328: 303, 1990). Male S.D. rats (200-220 g) were each implanted i.c.v. with a cannula. They were then injected s.c. with U-50 (10-40 mg/kg) or saline at 8.30 a.m. and 8.30 p.m. over 5 days. The rats (n=5-8) were housed individually (at 20°C) and, 4 hr after the 9th injection, were challenged i.c.v. with either norBNI (0.3, 1, 10 and 25 nmol) or saline. Increasingly pronounced behavioral syndromes were associated with higher doses of norBNI (1-25 nmol). At 25 nmol, norBNI provoked head and body shakes, yawning, licking penis, scratching and hyperthermia (over 1 hr).

CATECHOLAMINE RECEPTORS: α - AND β -ADRENERGIC

733.1

MULTIPLE ALPHA-ADRENOCEPTOR SUBTYPES: MODULATION OF PALPEBRAL APERTURE AS AN *IN VIVO* MODEL OF α_{1A}-ADRENOCEPTOR-MEDIATED ACTIVITY IN THE RAT. K. Bervoets* and M.J. Millan. I.D.R.S., 7 rue Ampère, 92800 Puteaux - France.

Evidence from binding and cloning studies has demonstrated the existence of multiple subtypes of α_1-adrenoceptor: these have been provisionally termed α_{1A}, α_{1B}, α_{1C} and α_{1D}. Of these, the α_{1A} and the α_{1B} sites are present in the CNS of man and rats. Currently, no simple *in vivo* paradigms of α_1-adrenoceptor-mediated activity are available. Thus, as a potential model for the characterization of actions at α_1-adrenoceptors, we have examined the role of α_1-adrenoceptor subtypes in the modulation of palpebral aperture in rats. Drugs were given s.c., 30 min before verification. Aperture was scored as follows: 4, normal; 5, exophthalmia; 3-2-1-0-, one-quarter, one-half, three-quarters and fully-closed, respectively. The subtype-non-selective α_1-antagonist (ANT), prazosin and the subtype-non selective α_1-agonist (AGO), cirazoline, provoked pronounced ptosis and exophthalmia, respectively. The preferential α_{1A}-AGOs, oxymetazoline and methoxamine (which do not pass the blood-brain-barrier) also elicited exophthalmia. Further, the structurally-diverse, preferential α_{1A}-ANTs, 5-methylurapidil, WB 4101 and benoxathian (of which the latter does not pass the blood-brain-barrier) all elicited ptosis. In distinction, chloroethylclonidine, which irreversibly inactivates α_{1B}- (and α_{1C})-adrenoceptors failed to modify palpebral aperture. Chlorpromazine and spiperone, which show some preference for α_{1B}-adrenoceptors, were only weakly active. In contrast to α_1-adrenoceptor ligands, the selective α_2-AGO, UK 14,304 and the selective α_2-ANT, L 657,743, did not modify palpebral aperture. Further, selective AGOs and ANTs at particular serotonin and dopamine receptor subtypes were also inactive. In conclusion, these data suggest that peripheral α_{1A}-adrenoceptors are involved in the modulation of palpebral aperture in the rat. This parameter offers a simple, rapid and robust model for the characterization of drug actions at α_{1A}-adrenoceptors *in vivo*.

733.2

CLONED RAT HOMOLOG OF THE BOVINE α1C-ADRENERGIC RECEPTOR EXHIBITS AN α1A-LIKE RECEPTOR PHARMACOLOGY. T.M. Laz, C. Forray*, K.E. Smith, P.J.J. Vaysse, P.R. Hartig, C. Gluchowski, T.A. Branchek, and R.L. Weinshank. Synaptic Pharmaceutical Corp., Paramus, NJ 07652.

Oligonucleotide probes based on the bovine α1C-adrenergic receptor sequence were used to screen a rat brain cDNA library at reduced stringency. We isolated a 2.3 kb cDNA whose deduced amino acid sequence contained seven putative transmembrane helices characteristic of G protein-coupled receptors. The rat cDNA exhibited ~88% amino acid identity with the bovine α1C receptor but only ~45% amino acid identity with the rat α1A and α1B adrenergic receptors, suggesting that the rat clone is the species homologue of the bovine α1C receptor. Transient transfection of the cDNA in COS-7 cells resulted in specific and saturable binding of [^{3}H]prazosin (Kd= 0.33±0.01 nM; Bmax= 5pmol/mg prot). In competition binding assays the rank order of potencies for α1-agonists and antagonists was WB-4101 ≥ prazosin ≥ 5-methyl urapidil > indoramin> oxymetazoline (Oxy) ⩾ norepinephrine (NE). Treatment of intact cells with chloroethylclonidine at 10 or 100μM for 30 minutes, inactivated 25% of the [^{3}H]prazosin binding sites. In transiently transfected COS-7 cells the agonists NE, epinephrine, phenylephrine, Oxy, and methoxamine stimulated the formation of [^{3}H]inositol phosphates between 9- and 23-fold. The expressed rat α1 receptor has many of the features of the pharmacologically defined α1A receptor-subtype, yet the pharmacology is distinct from those of the cloned rat α1A/D, rat α1B and bovine α1C receptors.

733.3

α_{2A}-ADRENERGIC RECEPTORS: PRE- OR POST-SYNAPTIC? C-G. Go[1*], C. J. Aoki[1], C. Venkatesan[1] & H. Kurose[2]. [1]Center for Neural Science, New York University, NY, NY 10003 & [2] Duke Univ. Med. Ctr, Durham, NC 27710.

Are all catecholaminergic transmissions synaptic? To address this question, we used a polyclonal rabbit antiserum directed specifically against the third intracellular loop region of the A-subtype α_2-adrenergic receptors (α_{2A}-AR) (Kurose et. al., 1993) to determine whether these receptors are restricted to morphologically identifiable synaptic junctions in adult rat brains. We examined the locus coeruleus (LC), a major noradrenergic cell group, and the neocortex, a major noradrenergic terminal field, since both regions exhibit intense immunoreactivity (ir) for α_{2A}-AR by light microscopy (Aoki et. al., submitted). Electron microscopic analysis of the LC revealed α_{2A}-AR-ir within dendrites, axons and glia. Within axonal terminals, ir was associated with the plasma membrane and small clear vesicles. Within dendrites, ir was predominantly associated with the plasma membrane. Interestingly, ir within axons and dendrites were rarely associated with identifiable synaptic specializations. In the neocortex, labeling was most prevalent within axonal terminals but was also detectable near postsynaptic densities of distal dendrites. Ir within cortical somata was often associated with plasma membranes and mitochondria. Examination of tissue dually labeled for α_{2A}-AR and a catecholamine-synthesizing enzyme showed that: (1) synaptic associations between profiles labeled for the two markers exist but are rare; (2) catecholaminergic profiles rarely exhibit receptor ir; but (3) the two types of labeled profiles are in close proximity to one another. These results demonstrate that a large portion of α_{2A}-AR ir is non-synaptic, thus suggesting that catecholamines mediate their effects through volume transmission. CA is supported by NIH EY08055 (FIRST), NS30944 & NSF RCD9253750 (Presidential Faculty Fellowship).

733.4

THE CLONING AND EXPRESSION OF THE OPOSSUM KIDNEY (OK) CELL LINE ALPHA-2C ADRENERGIC RECEPTOR. H.S. Blaxall*, D.R. Cerutis, N.A. Hass and D.B. Bylund. Dept. of Pharmacol., Univ. Neb. Coll. Med., Omaha, Nebraska 68198-6260.

Pharmacologically four subtypes of alpha-2 adrenergic receptors have been defined: A, B, C, and D. Previous studies from our laboratory have characterized the alpha-2 adrenergic receptor in the OK cell and opossum kidney to be of the alpha-2C subtype. The human homologue of the OK alpha-2C adrenergic receptor is designated C4. We have performed radioligand binding with OK cell membranes and COS-C4 cell membranes. The correlation coefficient between the pKi values was found to be 0.95. To further characterize the OK alpha-2C adrenergic receptor, RT-PCR was performed on RNA isolated from OK cells using oligo- nucleotides to the second and seventh transmembrane regions. 3' and 5' RACE procedures were used to obtain a full length clone OKc2. This was directionally cloned into the expression vector pRc/CMV (Invitrogen) sequenced and transiently expressed in COS-1 cells. The sequence obtained for the OK alpha-2C had only 68% sequence similarity at the amino acid level to C4 whereas C4 and the rat homologue RG10 were 90% similar. Radioligand binding was performed on membranes from these cells using [^{3}H]rauwolscine which bound with high affinity chacteristic of alpha-2C pharmacology. (Supported by NIH grant GM40784).

733.5

ALPHA-AGONIST INTRACELLULARLY MODULATES INHIBITORY AMINO ACID RESPONSES IN RAT SUBSTANTIA NIGRA NEURON. J. Nabekura*, T. Oomura, M. Munakata and N. Akaike. Dept. Neurophysiol., Tohoku Univ. Sch. Med., Sendai 980, Japan

Taurine (Tau) is enriched in substantia nigra (SN). The SN receives the afferent nerve terminals containing Tau from striatum, suggesting that Tau may play a functional role in this region. Recently, we reported that, in SN neuron, Tau elicited Cl^- current by activaing glycine receptor and that an intracellular cAMP modulated the Tau response via the activation of PKA. It is known that there are noradrenaline (NA) receptors in SN, especially in the pars reticulata. The NA receptors couple with various G proteins and regulate the production of cAMP and other intracellular second messenger systems. Here, we studied the effect of NA on Tau-induced current (I_{Tau}) in acutely dissociated rat SN neurons using a nystatin perforated patch clamp technique. NA itself did not elicite any current. When either alpha 1 or 2 adrenoceptor was activated, I_{Tau} increased gradually over 5 min whereas the activation of beta adrenoceptor had no effect on I_{Tau}. The treatment with pertussis toxin blocked the enhancement of I_{Tau} by NA. Either IBMX or forskolin inhibited the facilitatory effect on I_{Tau}. The results suggest that alpha 2 adrenoceptor activates Gi protein, resulting a decrease of intracellular cAMP which reduces an activity of PKA kinase A, and finally disinhibits the I_{Tau}. However, the intracellular mechanism about the facilitatory effect of alpha 1 adrenoceptor on I_{Tau} is unclear at this moment.

733.6

CNS AND PERIPHERAL TISSUE EXPRESSION OF MULTIPLE ALPHA ADRENERGIC RECEPTOR SUBTYPES IN NEWBORN RATS EXPOSED TO MATERNAL COCAINE. S. K. McCune*, M. M. Voigt and J. M. Hill Lab. of Developmental Neurobiology, NICHD, NIH, Bethesda, MD and Dept. of Neonatology, Children's National Medical Center, George Washington Univ. Med. Center, Washington, D.C. 20892.

Catecholamine levels are known to be elevated in the off-spring of mothers exposed to cocaine. We examined the behavioral characteristics of rat pups exposed *in utero* to cocaine and then investigated the expression of multiple alpha adrenergic receptor subtypes by *in situ* hybridization in the brains, hearts, lungs and kidneys of the pups.

Three pregnant rats were treated with daily injections of cocaine from E8-E20 while 2 mothers were injected with saline. Equal numbers of pups from each litter were maintained for behavioral testing while the remainder of the animals were sacrificed at birth. Statistically significant differences between the two groups were observed for righting on day 0 and startle responses.

The expression of multiple alpha adrenergic receptor subtypes (1A, 1B, 1C, 2A and 2C) was studied by *in situ* hybridization in newborn and three week old animals. The alpha-1B receptor subtype is found in the globus pallidus of the normal newborn but not in the 3 week old. There was a statistically significant increase in the expression of the alpha-1B receptor subtype in the globus pallidus of the cocaine treated pups versus the saline treated. Expression in the globus pallidus was not detected in the 3 week old animals of either group. There were no other differences detected in any of the other subtypes in any of the tissues examined.

The globus pallidus is an area responsible for initiating motor behavior and also has connections with the limbic system. The behavioral differences seen between the two groups of animals may be related to the increased adrenergic receptor expression in this area. Transiently expressed receptor subtypes in the perinatal period may play a significant role in pharmacologic responses during the newborn period.

733.7

MECHANISM OF ACTION OF JUDAS GUT EXTRACT ON AORTIC RINGS FROM THE GUINEA PIG. E. Gijón*, M. Lorenzana, L. Cartas, and X. Garcia. Dep. of Physiol and Dep. Pharmacol. Sch. Med. UNAM. Ap. P. 70-250, México, D.F. 04510, MEXICO. FAX (525) 550-2920.

Judas gut (JG) infusion is used in traditional medicine for relieving pain and inflammation. Hydroalcoholic extract of JG studied on male guinea pig vascular smooth muscle prepared for in vitro isometric recording contracts relaxed vascular smooth muscle, contractions were similar to that induced by catecholamines. JG contractions were not calcium dependent or endothelium-dependent (1). Further experiments explored the mechanisms of action of JG extract as an adrenergic agonist. Propranolol did not block JG contraction. Phentolamine partially blocked JG contraction. Zolertine and yohimbine blocked JG contraction in dose dependent form, and prazosine partially blocked JG contraction. These results suggest an alpha-adrenergic synergism, and membrane effects on calcium movement and/or receptor interaction will be explored.

1. Abs. West. Pharmacol. Soc/ASCEP, 1993 p. 87 (119) (Partially supported by CONACyT)

733.8

TSH REGULATES ALPHA 1 ADRENERGIC RECEPTORS WHICH MEDIATE $[Ca^{++}]_i$ RISE IN PC Cl3, VIA A cAMP-DEPENDENT PATHWAY. O. Meucci,* A. Scorziello, ^M.T. Berlingieri, A. Avallone, °A. Fusco M. Grimaldi, C. Ventra, and G. Schettini. Dept. Pharmacology, and ^ Dept. Biol. Patol Cell. Mol., School of Medicine, Univ. of Naples and °Univ. of Catanzaro, Via S. Pansini 5, 80131 Napoli, ITALY.

We studied the effect of TSH on norepinephrine (NE)-induced cytosolic calcium rise in PC Cl3 rat thyroid cells. The cells were grown both in presence (PC 6H) and in absence (PC 5H) of TSH and intracellular calcium levels were measured by fura-2. In PC 6H the cytosolic calcium rise induced by NE was characterized by an early transient spike followed by a second phase of sustained calcium levels, while in PC 5H we observed only a slower monophasic response. The acute addition of TSH to PC 6H caused a modest increase of basal calcium and a 50% enhancement of the calcium rise induced by NE. This latter effect was abolished in a calcium free medium and not observed in PC 5H. Both NE and TSH stimulated inositol phosphates production in PC 6H and by a lesser extent in 5H. The reintroduction of TSH in the culture medium of PC 5H induced the recovery of NE-stimulated intracellular calcium rise similarly to the native PC 6H, an effect abolished by the simultaneous treatment of cells with cycloexymide. Also the treatment of PC 5H with forskolin restored the effect of NE on calcium level., while the treatment with PMA was uneffective. To characterize the α1 adrenergic receptor subtypes mediating the effect of NE in PC Cl3 cells, we used antagonists of α1A and B receptors (WB4101 and CEC respectively). In these experimental conditions we found that 1) CEC caused a marked inhibition of NE-induced calcium increase in PC 6H, while slightly affected NE response in PC 5H; 2) WB4101 at a concentration specific for α1A receptors (0.01μM) reduced the effect of NE in PC 6H and 5H; 3) CEC+WB4101 0.01μM completely abolished the noradrenergic response in both groups of cells. These results show that, in PC Cl3, TSH modulates NE-induced calcium rise through a kinase A-dependent pathway, and that α1B receptors expression are the main target of this regulation. Binding studies with 3H-prazosin confirmed this hypothesis since we observed in PC 5H a lower Bmax than in PC 6H. (Supported by A.I.R.C. 1992 grant to Gennaro Schettini)

733.9

LARGE SCALE PRODUCTION OF MEMBRANE EMBEDDED α_2-RECEPTORS IN MAMMALIAN CELLS. S. Ala-Uotila, M-T. Matikainen, M. Koulu* and M. Jalkanen. Dept. of Pharmacology and Centre for Biotechnology, Univ. of Turku, PO Box 123, SF-20521 Turku, Finland.

We have generated and characterized stable recombinant cell lines of adherent Shionogi 115 mouse mammary tumor cells (S115) expressing the α_2-AR subtypes α_2-C2, α_2-C4 and α_2-C10.

In order to obtain large amounts of homologous material for pharmacological studies and for studies on receptor structure and function, we studied three different cell culture techniques: culture bottles, roller bottles and bioreactor (hollow fiber, 0.8m^2). Nude mice were also injected with S115 tumor cells expressing receptor subtypes α_2-C4 and α_2-C10. Bioreactor was by far the most efficient and least money and time consuming way of producing large quantities of cells expressing α_2-adrenergic receptors.

Scatchard analysis of [^{3}H]rauwolscine binding assayed on the hollow fiber material revealed B_{max} values of 6 and 0.7 pmol/mg protein and K_ds 3 and 4.5nM for the recombinant C2 and C10 receptors. Total amount of protein in the batch varied from 0.8 to 1.4g, but it is possible to optimize this type of production system to reach the level of 2-3g total protein.

733.10

COMPARISON OF RECOMBINANT α_2-C2-ADRENOCEPTORS PRODUCED IN MAMMALIAN, INSECT AND YEAST CELLS. K. Pohjanoksa, A. Marjamäki, S. Ala-Uotila, C. Oker-Blom, D. Sizmann, H. Kurose and M. Scheinin*. Dept. of Pharmacology and Centre for Biotechnology, Univ. of Turku, and Dept. of Biochemistry, Åbo Akademi, SF-20520 Turku; VTT Technical Research Centre of Finland, SF-02151 Espoo, Finland; and Duke University, Durham, NC 27710, USA.

We have compared ligand binding properties of the human α_2-adrenoceptor subtype α_2-C2 in three cell lines expressing recombinant α_2-C2-AR: Shionogi S115 mouse mammary tumour cells, *Spodoptera frugiperda* Sf9 insect cells and *S. cerevisiae* yeast cells. [^{3}H]Rauwolscine binding in cell homogenates was inhibited by prazosin, oxymetazoline, RX821002, chlorpromazine and (-)-noradrenaline with and without the GTP-analogue Gpp(NH)p. The K_i-values of each agent were in close agreement between the different cell lines. This indicates that α_2-C2-AR retains its binding characteristics irrespective of the host cell system. The expected 43 kDa α_2-C2 protein from each cell line was visualized with immunoblotting using a polyclonal α_2-C2-AR-antibody.

733.11

USE OF RECOMBINANT HUMAN α_2-ADRENOCEPTORS TO CHARACTERIZE SUBTYPE SELECTIVITY OF ANTAGONIST BINDING. A. Marjamäki*, K. Luomala, S. Ala-Uotila and M. Scheinin. Dept. of Pharmacology and Centre for Biotechnology, Univ. of Turku, SF-20520 Turku, Finland.

We have generated stably transfected Shionogi S115 mouse mammary tumour cell lines expressing separately the human α_2-AR subtypes α_2-C10, α_2-C2, and α_2-C4. Binding of [^{3}H]-rauwolscine was inhibited by co-incubation of S115 cell homogenates with ten α_2-adrenergic antagonists and oxymetazoline, a weak partial agonist known to discriminate the receptor subtypes. Other useful agents for discrimination of subtypes were prazosin, chlorpromazine, phentolamine and yohimbine. The most sensitive indices for differences between subtypes were Ki ratios chlorpromazine/oxymetazoline, prazosin/oxymetazoline and chlorpromazine/atipamezole. Correlation analysis between our results for human-type receptors and published data for their rat α_2-adrenoceptor homologues demonstrated good general agreement, with some interspecies differences in the affinity of rauwolscine, phentolamine and oxymetazoline.

733.12

CENTRALLY-INDUCED OCULAR HYPOTENSION IN THE RABBIT: INVOLVEMENT OF ALPHA-2 AND IMIDAZOLINE RECEPTOR SUBTYPES. W. R. Campbell*, S. Long, R. Strothers and D. E. Potter. Department of Pharmacology and Toxicology, Morehouse School of Medicine, Atlanta, Georgia 30310-1495.

Glaucoma, a leading cause of blindness particularly in African-Americans, results in irreversible neuronal damage within the optic nerve head. One focus of research efforts is to understand the mechanisms of aqueous humor dynamics and how it relates to pressure within the eye. The purpose of this study was to examine the effects of alpha-2 and imidazoline receptor subtypes on centrally mediated changes in intraocular pressure. New Zealand White rabbits were fitted with indwelling stainless-steel guide cannulae for injections into the CNS. After 5 days recovery the centrally applied (icvt) and topically applied effects of UK-14,304-18 (brimonidine), oxymetazoline, and moxonidine were examined with and without the antagonists rauwolscine, idazoxan, and efaroxan. Efaroxan and idazoxan (3.0 ug vs icvt, 10.0 ug vs topical) were more active in inhibiting the ocular hypotensive actions of oxymetazoline (0.9 ug, icvt; 50.0 & 500 ug, top) and moxonidine (0.3 ug, icvt; 25.0 ug, top), but were ineffective on the actions of UK (0.9 ug, icvt; 50.0 ug, top). Conversely, rauwolscine (3.0 ug, icvt; 10.0 ug top) inhibited the ocular hypotension induced by both icvt and topically applied UK, but was much less active on the actions of OXY and MOX. These data show that ocular hydrodynamics are mediated by sites in the CNS, and that both alpha-2 and imidazoline receptor subtypes play an important role in the central mediation of intraocular pressure. Supported, in part, by EYO6338.

733.13

A TIME-COURSE OF ALTERED THYROID STATES ON THE NORADRENERGIC SYSTEM IN RAT BRAIN. A. Yu*, J. Yang & S. M. Tejani-Butt. Department of Psychiatry, University of Pennsylvania School of Medicine, Philadelphia, PA 19104.

Thyroid hormone (TH) dysfunction has been linked with abnormalities in noradrenergic (NA) neurotransmission. Low TH levels and dysfunction of NA receptors have been postulated in depression. The present study was aimed at investigating the time-course of thyroidectomy (TXT) and T_4 replacement on β_1 and β_2-adrenoceptors (ß-ARs), α_2-adrenoceptors and presynaptic transporter sites for norepinephrine (NET) in the adult rat brain. Using selective radioligands for each of these systems, measurements in discrete brain regions were made by the technique of quantitative autoradiography. Statistical comparisons were made by ANOVA followed by post-hoc analyses using Newman-Keuls test ($p < 0.05$). A significant decrease in ^{125}I-iodopindolol binding to β_1-ARs in the cortex and hippocampus, and to β_2-ARs in the hypothalamus was seen following TXT (7, 14 or 35 days). A decrease in ^{3}H-idazoxan binding to α_2-adrenoceptors was observed selectively in the amygdala, and ^{3}H-nisoxetine binding to NET sites was found to be decreased in the hypothalamus following TXT. T_4 replacement (for 7 or 28 days) caused radioligand binding to recover to control levels in brain regions where alterations were observed after TXT. These results indicate that a neuromodulatory link between TH and the NA system exists in the rat brain, that is both region and receptor specific. (Research funds from USPHS grants MH 44210 and MH 45472).

733.14

HYPOTHYROIDISM-INDUCED SHIFTS IN HIPPOCAMPAL ADRENERGIC RECEPTOR BINDING: IMPLICATIONS TO ISCHEMIA-INDUCED HIPPOCAMPAL DAMAGE. Susan Hemmings Dept. of Physiology and Ashfaq Shuaib*, Sask. Stroke Research Center, Univ. of Saskatchewan, Saskatoon, Canada, S7N 0W0.

Hypothyroidism was induced in male Fischer 344 rats by administration of propylthiouracil (0.05%) for six weeks. Plasma levels of thyroid hormones confirmed hypothyroidism: control rats (CON): Total T_3 - 67.14±3.93 ng/dl, Total T_4 - 3.37±0.14 μg/dl; Hypothyroid rats (HYPO): Total T_3 - 30.54±1.68 ng/dl, Total T_4 - 1.56±0.14 μg/dl. Functional hypothyroidism was demonstrated by: i) a decrease in hepatic plasma membrane α_1-adrenergic receptor binding: CON - 998.10±57.59 fmol/mg; HYPO - 508.50±15.49 fmol/mg; and ii) an increase in hepatic plasma membrane γ-glutamyl-transpeptidase activity: CON - 6.20±0.18 nmol/mg/min; HYPO - 69.37±7.09 nmol/mg/min. Membranes were isolated from hippocampi and specific α_1 and β_2 adrenergic receptor binding determined by [^{3}H] Prazosin and [^{125}I] Iodocyanopindol binding. Hypothyroidism effected a shift in the balance of α_1 and β_2 adrenergic receptor binding: CON - α_1:88.59±10.22 fmol/mg, β_2:1191.28±82.47 fmol/mg; HYPO - α_1: 139.31±20.15 fmol/mg; β_2: 934.32±50.29 fmol/mg. This receptor shift may explain the protective effect of hypothyroidism on ischemia-induced hippocampal damage by favoring inhibitory input and limiting excitotoxic input by catecholamines. Funded: Cosmo. Fndn. Can. (Inc).

733.15

CIS-ACTING ELEMENTS OF THE RAT β1-ADRENERGIC RECEPTOR GENE. R. P. Searles[1*], V. J. Nipper[1], and C. A. Machida[1,2], [1]Div. of Neuroscience, Oregon Regional Primate Research Center, Beaverton, OR 97006, and [2]Dept. of Biochemistry and Molecular Biology, Oregon Health Sciences University, Portland, OR 97201.

Cis-acting components important for basal expression of the rat β1-adrenergic receptor (β1AR) gene have been localized by using a series of deletion mutants of the 5′ flanking sequences to control expression of the reporter gene luciferase in rat C6 glioma cells. Maximum expression of luciferase is achieved using β1AR sequences between -479 and -1 ([-479,-1]) relative to the translation initiation codon. Successive deletions from -479 to positions -369, -329, and -299 reduces expression by 42%, 71%, and 78%, respectively. Expression from [-479,-299] is equivalent to the expression of the deletion mutant [-1252,-479], which lacks the region between -479 and -1. These data suggest that primary determinants of expression are present within the region from -479 to -299. Within this region is an inverted CCAAT box (-359 to -355) positioned similarly to the inverted CCAAT sequence which has been designated to be part of the human β2AR promoter. A mutant which substitutes a 6 base linker for 11 bases of β1AR sequence, including this CCAAT box, expresses only slightly less than the intact [-479,-1] construct. It is therefore unlikely that this inverted CCAAT box is important for expression of the rat β1AR. Deletions from the 3′ end of the region [-479,-1] also affect expression; the construct [-479,-258] expresses 48% less than [-479,-1]. Within the deleted region are an SP1 site and an AP3 site, either of which may be responsible for the decline in activity. Further deletion mutants are being examined to carefully map the region from -479 to -1 for specific sequences important for basal level expression.

733.16

THE 5′ FLANKING REGION OF THE RAT β_3-ADRENERGIC RECEPTOR GENE: DIVERGENCE WITH THE HUMAN GENE AND IMPLICATIONS FOR SPECIES-SPECIFIC GENE EXPRESSION. J.A. Brown[1*] and C.A. Machida[1,2], [1]Div. Neuroscience, Oregon Regional Primate Research Center, Beaverton, OR 97006, and [2]Department of Biochemistry and Molecular Biology, Oregon Health Sciences University, Portland, OR 97201.

β_3-adrenergic receptors (β_3-ARs) were the first "atypical" β-adrenergic receptors to be molecularly cloned. β_3-AR mRNAs exhibit species-specific expression (human vs. rodent) in distinct anatomical regions and appear to be expressed abundantly within rodent adipose tissue, but only at low levels within corresponding human tissues. In order to determine the genetic basis of the differential expression of the rat and human β_3-AR genes, we cloned and sequenced the rat gene and compared the 5′ flanking regions of the two genes to identify potential discriminators in transcriptional regulation. We have found that the rat and human β_3-AR flanking regions are only 58% similar, unlike the close sequence similarity observed between the coding blocks (>90%) and also observed between species for the 5′ flanking regions of other β-AR subtype genes (>90%). In addition, the rat β_3-AR gene lacks the four potential cAMP responsive elements identified within the 5′ flanking region of the human receptor gene. The striking divergence in regulatory sequences between the rat and human β_3-adrenergic receptor genes may potentially explain the differences in species-specific expression and tissue localization of the rat and human receptor mRNAs.

733.17

LACK OF ECS INDUCED BETA RECEPTOR CHANGE IN ANESTHETIZED, VENTILATED RATS. S.K. Brannan*, A.L. Miller, A.M. Biedeger, D.J. Jones. Dept. of Psychiatry and Anesthesiology, The Univ. of Texas Health Science Center at San Antonio, TX 78284.

The purpose of this study is to explore electroconvulsive shock (ECS) induced noradrenergic receptor regulation in anesthetized and ventilated (A&V) rats. Previous studies have established that chronic ECS induces a reduction in beta receptor density in rat cerebral cortex. One limitation of these studies is that they did not control for the effects of oligemia and hypoxia on CNS receptors during ECS. The present study addresses this concern by measuring beta receptor density in rat cerebral cortex according to the following groups: ECS+A&V (A), Sham+A&V (B), ECS alone (C), and Sham alone (D). Anesthesia is induced with methohexital (15mg/kg) via a femoral vein catheter followed by administration of succinylcholine (2mg/kg). Rats are then intubated and kept on 97% O_2. ECS is administered via ear clips (40mA, 150 pulses/sec, 1 millisec/pulse, 1sec duration) on a M-W-F schedule until a total of 6 sessions ECS or Sham is reached. 24 hrs after their last session the rats are sacrificed and their brains dissected out and frozen. Receptor density was determined by scatchard analysis of saturating isotherms of [^{125}I]ICYP (20-600nM). The maximal binding density (Bmax) of group C (122.2 fm/mg protein) was less than that of group D (136.7 fm/mg protein), but there were no significant differences between Groups A and B (132.6 and 132.5 fm/mg protein respectively). There were no significant differences in Kd between the groups. Although cortical beta receptor density is felt to indicate antidepressant response, this may be an epiphenomena. If so, our interpretation of previous experiments will be profoundly altered.

733.18

The Effect of Propranolol on Bone Remodeling in Rats During Simulated Weightlessness. H. W. Burden*, G. T. Price, C. A. Hodson, A. T. Davenport, C. M. Manring, and D. M. Terrian. Dept of Anatomy and Cell Biology and Obstetrics and Gynecology, East Carolina University School of Medicine, Greenville, NC 27858.

Propranolol administration has been reported to significantly affect bone properties in rats (Minkowitz et al., J. Orthop. Res., 9: 869, 1991). In the present study, the effects of this drug on remodeling in the tibia during simulated weightlessness were evaluated. Two groups of adult female Sprague Dawley rats were fitted in a back harness and the animals suspended for 14 days at an angle of approximately 30° head down tilt. One group served as a suspension control and the other group received propranolol (100 μg daily, ip). A gravity control group of rats was allowed to ambulate normally. On day 14, tibias were removed, weighed and subsequently processed for histomorphometric analysis. The areas of metaphyseal trabeculae and osteoid were determined using a point counting method with a Merz grid. There was no difference in tibial weights (mg/100g bw) in the three groups. The area of metaphyseal trabeculae and osteoid was significantly ($p<0.05$) reduced in suspension control animals vs gravity controls. Propranolol significantly ($p<0.05$) reversed the effect of simulated weightlessness on metaphyseal osteoid area but not total trabecular area. These data indicate that propranolol stimulates bone formation in this model of simulated weightlessness. Supported by NASA Grant No. NAGW-2927.

HYPOTHALAMIC-PITUITARY-GONADAL REGULATION: REGULATORY ASPECTS

734.1

GLUCOSE DETECTORS IN THE CAUDAL BRAIN STEM CONTROL ESTROUS CYCLES IN SYRIAN HAMSTERS. J.E. Schneider*, Y. Zhu, J.M. Swann and J.M. Gabriel. Department of Psychology, Lehigh University, Bethlehem, PA 18015 and Department of Biology, Rutgers University, Newark, NJ 07102.

Fertility and ovulatory cycles are inhibited by decreased food intake or increased energy expenditure in a variety of mammals including human beings. In Syrian hamsters, the signals that mediate these effects appear to be related to the availability of oxidizable glucose. For example, estrous cycles are inhibited by treatment with high doses of 2-deoxy-d-glucose (2DG) a drug that inhibits cellular glucose utilization. To examine the role of glucose detectors in the central nervous system, hamsters fed *ad libitum* were treated with a variety of doses of 2DG injected intracerebroventricularly (ICV) every six hours on days 1 and 2 of the estrous cycle. Estrous cycles were inhibited by 75mg/10μl ICV injections of 2DG, but not by intraperitoneal (IP) injections at the same dose. Next, we compared the effects of IP injections of 2DG (1750 mg/kg) in hamsters with or without lesions of the area postrema (AP). Both sham and lesioned hamsters showed at least two consecutive four-day estrous cycles prior to the start of 2DG injections. 2DG treatment blocked estrous cycles in 89% of the hamsters with sham lesions, but failed to block cycles in any of the 10 hamsters with confirmed lesions of the AP. Finally, we examined the effects of 2DG on neuronal activity in the AP using immunocytochemistry for FOS. We observed cells stained for FOS in the AP and nucleus of the solitary tract (NTS) in hamsters perfused three hours after 2DG treatment (1750mg/kg). No FOS staining was observed in the AP/NTS in vehicle-treated hamsters. These results are consistent with the idea that estrous cycles are controlled by glucose detectors located in the AP and/or NTS. Supported by research grants BNS9121056 from NSF (J.E.S.) and HD28467 from NICHD (J.M.S).

734.2

THE FASTING-INDUCED SUPPRESSION OF LH SECRETION IN MALE RHESUS MONKEYS IS DECREASED BY CASTRATION. J.L. Cameron*, D.L. Berger, and C.M. Zeigler. Departments of Behavioral Neuroscience, Psychiatry, and Cell Biology & Physiology, University of Pittsburgh, Pittsburgh, PA 15260.

Brief periods of fasting (i.e., 1-2 days) significantly suppress LH secretion in several species, including rats, monkeys and men. In male rhesus monkeys, we have previously shown that LH secretion is suppressed within the first 4-6 h after the daily meal is missed and becomes progressively more suppressed with continued fasting (fed: 4.57± 0.53 LH pulses/12 h; 1 day fasting: 1.86±0.46 LH pulses/12 h). In a number of nonprimate species, fasting has been shown to be more effective in suppressing LH secretion in gonad-intact versus castrate animals. To address this issue in primates, we examined whether the sensitivity of the gonadal axis to brief periods of fasting in male monkeys is influenced by castration. Five castrate male rhesus monkeys with indwelling venous catheters were maintained on jacket/tether/swivel systems. Blood samples were collected on two days, a day of normal feeding (1 meal at 1100 h), and after 2 days of fasting (samples collected at 7.5 min intervals from 1600-2400 h on each day), for measurement of pulsatile LH secretion. Two days of fasting caused no significant change in mean plasma LH concentrations (fed: 109.2±23.3 ng/ml; fasted: 114.5±30.1 ng/ml) or in LH pulse frequency (fed: 9.2±0.6 pulses/8 h; fasted: 9.4±0.3 pulses/8 h). These results suggest that either the inhibitory signal provided to the gonadal axis by fasting is mediated via a gonadal steroid dependent mechanism, or that the inhibitory signal provided by short-term fasting is not great enough to significantly suppress the elevated central drive to the gonadal axis in the castrate state. Studies examining the effects of fasting on LH secretion in castrate monkeys maintained on steroid hormone replacement regimens are currently being performed to explore these two possibilities.

734.3

AREA POSTREMA LESIONS ABOLISH METABOLIC INHIBITORS-INDUCED SUPPRESSION OF ESTROUS BEHAVIOR IN SYRIAN HAMSTERS. H.-Y. Li* and G. N. Wade. Neuroscience and Behavior Program and Department of Psychology, University of Massachusetts, Amherst, MA 01003.

Previous studies in our laboratory showed that treatment with metabolic inhibitors which block glycolysis (2-deoxy-D-glucose, 2DG) and fatty acid oxidation (methyl palmoxirate, MP) suppresses steroid-induced estrous behavior in ovariectomized Syrian hamsters. Although it is known that the ventromedial hypothalamus (VMH) is involved in facilitation of estrous behavior, very little is known about the pathway(s) conveying visceral information into the VMH and thus leads to changes in lordosis. Since area postrema (AP) is anatomically located for transmission of information related to metabolic fuel availability and it projects to the VMH directly or indirectly via lateral parabrachial nucleus, we investigated the hypothesis that lesions of the AP can prevent the attenuation of estrous behavior caused by metabolic challenges.

Ovariectomized Syrian hamsters were either sham-operated or were given aspiration lesions of the AP. Each group of animals was further divided into two groups that were treated with 2DG (750 mg/kg) + MP (25 mg/kg) or vehicles for 48 h. Estrous behavioral was induced by sequential injections of 5 μg of estradiol benzoate and 200 μg of progesterone. Duration of lordosis (DL) was measured out of 180 sec. Our results showed that there was significant difference in DL between lesioned and sham groups treated with metabolic inhibitors (124 ± 13 *vs.* 76 ± 20), but there was no significant difference between 2DG + MP and vehicle-treated groups in AP-lesioned animals (124 ± 13 *vs.* 118 ± 15). This suggests that the VMH receives at least part of visceral information via the AP; moreover, AP lesions can block the metabolic signal generated by glucoprivation and lipoprivation which suppress estrous behavior in intact animals. (Supported by NS 10873 and MH 00321)

734.4

PREGNANCY-ASSOCIATED OPIOID RESTRAINT OF OXYTOCIN NEURONES INVOLVES A PRESYNAPTIC ACTION ON BRAINSTEM CATECHOLAMINERGIC AFFERENTS. R.J. Bicknell*, T. Onaka, G. Leng, S.M. Luckman & A.J. Douglas†, Department of Neurobiology, AFRC Babraham Institute, Babraham Hall, Cambridge CB2 4AT, U.K. and †Department of Physiology, University Medical School, Edinburgh EH8 9AG, U.K.

Endogenous opioid inputs inhibitory to oxytocin (OXT) neurones become activated during pregnancy in the rat, probably as a consequence of elevated circulating sex steroids. This central opioid action does not involve κ-active opioid peptides co-expressed in the neurohypophysial neurones (J. Neuroendocr., in press). We have shown that morphine, a μ-opioid, inhibits the electrical activity of OXT neurones, decreasing OXT release and suspending parturition. We have examined the cellular site of opioid action following systemic administration of cholecystokinin (CCK) which activates OXT neurones via brainstem catecholaminergic cells (1).

We now report: (i) CCK (20μg/kg) induces expression of Fos-immunoreactivity (Fos-IR) in the supraoptic nuclei (SON) and in 45% of tyrosine hydroxylase (TH)-IR cells in both C2/A2 and C1/A1 regions of the rat brainstem. Morphine (1mg/kg) pretreatment prevents CCK-induced expression of Fos-IR cells in SON, but does not affect the percentage of TH-IR cells expressing Fos-IR in the brainstem, (ii) Electrically-evoked release *in vitro* of [^{3}H] noradrenaline from preloaded SON explants from virgin rats was inhibited 41% by morphine, (iii) Under anaesthesia, the electrical activity of SON OXT neurones was excited by systemic CCK. In virgin rats this response was not influenced by blocking endogenous opioid actions with naloxone (2mg/kg) but in late pregnant rats CCK excitation was enhanced by 260%.

Thus morphine actions to reduce OXT cell activity are likely to involve presynaptic rather than cell body inhibition of brainstem catecholaminergic inputs and pregnancy induces endogenous opioid systems to act on this mechanism.

(1) Luckman, S.M. (1992) J. Neuroendocr. 4, 149-152

734.5

DEVELOPMENT OF METABOLIC RESPONSE IN MALE QUAIL BRAIN DURING SEXUAL MATURATION. R. Teruyama, M. M. Beck*, J.H. Douglas, N.J. Gonzalez and T.M. Brown. Dept. of Animal Sci., Univ. of Nebraska-Lincoln, Lincoln, NE 68583.

The eyes have been strongly implicated as a significant component in the photosexual response of Japanese quail, but the mechanism is not clear. A neural retino-hypothalamic (via SCN) connection has been proposed as one possibility. This study addresses the metabolic response (2-deoxyglucose, 2DG) of male quail during sexual maturation. Quail raised to 10 wk of age from hatching on 8h L:16h D received .0125 mCi of 2DG/100g body wt on day 0 and +3, +6, +9, +12 and +18 days after onset of 16 h L:8h D. Brains were processed for autoradiography; testis wt, cloacal gland size and serum testosterone were used as indicators of reproductive response to photoperiod. Reproductive responses were curvilinear, reading maximum levels at d +18. Heavily labeled nuclei were identified in four discrete neural pathways: thalamofugal, tectofugal, ascending auditory, and efferent vocalization. It is hypothesized that the auditory and vocalization pathways responded to increased crowing as the birds matured, perhaps influenced by increasing T. No accumulation of label was found in SCN at any time. In addition, the hypothalamic nuclei involved in copulatory behavior did not show evidence of metabolic activity, perhaps because males were raised without females. In this study evidence for a metabolic response to photoperiod was not found in SCN, though it should be noted that all birds were sacrificed during the daylight period.

734.6

EVIDENCE FOR A DIRECT PATHWAY FROM THE SCN TO THE GNRH SYSTEM IN THE FEMALE RAT HYPOTHALAMUS EM van der Beek[1], VM Wiegant[2]*, and RM Buijs[3] [1]Dept of Cell Biol; [2] Dept of Med Pharmacol, Med Fac, Utrecht Univ, Utrecht, and Dept of Human & Animal Physiol, Agricult Univ, Wageningen, The Netherlands; [3]Netherlands Inst for Brain Res, Amsterdam, The Netherlands, and Loeb Res Inst, Ottawa Civic Hosp, Ottawa, Canada.

The influence of the suprachiasmatic nucleus (SCN) on the regulation of the reproductive cycle is most evident in the female. Timing of the preovulatory luteinizing hormone (LH) surge is determined by the SCN. Destruction of the SCN leads to a cessation of the ovarian cycle, while implantation of estrogen in ovariectomized rats results in daily LH surges. The anatomical substrate for these effects is not known. Studies involving lesions of the SCN have demonstrated a direct vasoactive intestinal polypeptide (VIP) containing pathway to gonadotropin releasing hormone (GnRH) neurons (VanderBeek et al., J. Neuroendocri. 5:137). To further investigate the existence of a direct connection between the SCN and the GnRH system we have combined tract-tracing using the antrograde tracer Phaseolus Vulgaris Leu-agglutinin (Pha-L) with double labelling immunocytochemistry in LM and EM studies. In addition, VIP input on GnRH neurons was studied at the EM level. Small unilateral Pha-L deposits in the rostral ventrolateral portion of the SCN revealed a partially bilateral projection to the preoptic area, and Pha-L immunoreactive fibers were in close apposition to a number of GnRH neurons. EM studies showed contacts between Pha-L containing fibers and GnRH immunoreactive structures. In addition, synaptic interactions of VIP containing fibers with GnRH neurons were observed. The present study provides substantial evidence for a monosynaptic pathway from the SCN to the GnRH system in the hypothalamus of the female rat. This pathway may be the anatomical substrate for the circadian regulation of the ovarian reproductive cycle.

734.7

EFFECT OF ANTERIOR HYPOTHALAMIC AREA (AHA) LESIONS ON PHOTOPERIOD-INDUCED SHIFTS IN REPRODUCTIVE ACTIVITY OF THE EWE. S.M. Hileman, D.E. Kuehl, G.L. Jackson*. Department of Veterinary Biosciences, University of Illinois, Urbana, IL 61801.

Areas of the brain involved in photoperiodic control of reproduction are not well defined. Our objective was to determine the effect of AHA lesions on responses of the sheep reproductive system to shifts in length of the daily photoperiod. Eleven intact Suffolk ewes received bilateral radiofrequency lesions of the AHA (AHAX) and 5 received sham lesions (SHAM). Ewes were then placed in photoperiod chambers and exposed to alternating periods of long (16L:8D) and short (10L:14D) days of about 90 days for approximately 1 year. Blood samples were collected twice weekly to monitor progesterone and prolactin release and, on one occasion, hourly for 24 h to assess melatonin release. Lesions increased ($P<0.001$) the interval between start of long days and cessation of estrous cycles during both long-day periods but did not affect ($P>0.10$) the interval between start of short days to onset of estrous cycles for either short-day period. Duration of both anestrous periods was shorter ($p<0.001$) for AHAX than for SHAM ewes. No effects of lesions were evident on serum patterns of either prolactin or melatonin. These results suggest that AHA lesions disrupt neuronal pathways mediating the effects of shifts in photoperiod on reproductive activity in this species. Furthermore, these lesions appear to mainly affect response to long-day-induced suppression of reproductive activity.

734.8

EFFECTS OF PROLACTIN ON EXPRESSION OF THE mRNAs ENCODING THE IMMEDIATE EARLY GENES ZIF/268, NUR/77, C-FOS and C-JUN IN THE HYPOTHALAMUS. C.A. Sagrillo* and M. Selmanoff, Department of Physiology, University of Maryland, School of Medicine, Baltimore, MD 21201-1559.

Prolactin (PRL) exerts a short-loop negative feedback effect on hypothalamic neurons which control its secretion from the anterior pituitary gland. The purpose of this study was to identify particular hypothalamic neurons which respond to acute PRL exposure. Quantitative in situ hybridization histochemistry was used to visualize the induction of mRNAs for four different immediate early genes (IEGs): zif/268 (NGF1-A), nur/77 (NGF1-B), c-fos and c-jun. Increasing evidence indicates that excitation of neurons often results in the rapid transcription of IEGs. Three groups of male rats were studied: unmanipulated controls, rats injected sc with 2.4 mg ovine PRL suspended in polyvinylpyrrolidone (PVP) and PVP-injected controls. Animals were decapitated 0, 0.5, 1, 2, 3 or 4 h following the injection. Brains were immediately frozen at -70°C and 12 µm sections were prepared. Sections were hybridized with ^{35}S-dATP-labeled specific oligonucleotide probes 45 to 60 nucleotides in length. In all rats, all probes labelled cells in the cortex (particularly the cingulate and piriform), the hippocampus and the striatum. In the arcuate region, a greater number of cells were labeled with the zif/268 and nur/77 probes in PRL-treated compared with PVP-treated control rats. The labeled neurons are co-extensive with, but are not necessarily the tuberoinfundibular dopaminergic neurons. The data suggest that cells located in the arcuate region may be involved in mediating PRL autofeedback effects. (Supported by NIH grant HD21351).

734.9

SEASONAL PROLACTIN SECRETION IN FEMALE SIBERIAN HAMSTERS IS MODULATED BY VASOACTIVE INTESTINAL PEPTIDE. Andrew M. Snyder,*and Lori L. Badura. University of Connecticut Health Center, Farmington, CT 06032, and Behavioral Neuroscience, University of Connecticut, Storrs, CT 06264.

The role of central VIP in the photoperiodic modulation of basal PRL release was investigated by employing chronic infusion of VIP or a VIP antagonist (AVIP) via microdialysis probes implanted within the hypothalamus. In a first experiment, female hamsters housed under a 16L:8D photoperiod were fitted with probes and received constant daily infusions of 800 ng VIP, 1.2 ug AVIP, or the vehicle, for 7 weeks. For the VIP infusions, animals with probes located in the PVN region showed a significant rise in circulating PRL as early as one week post-surgery. In contrast, neither vehicle infusions, nor VIP infusions located in other neural regions, induced changes in basal PRL. Similarly, AVIP induced a decline in circulating PRL when infused in the PVN region, but not when the probes were located in other neural sites.

The role of VIP in the short-day induced decline in PRL release was investigated in a second experiment. Animals were again fitted with dialysis probes and then transferred to a 10L:14D photoperiod where they received VIP or vehicle infusions for 9 weeks. VIP infused into the PVN region prevented the short-day induced decline in basal PRL that was shown by both the vehicle-treated animals and animals receiving VIP in other neural regions. These findings taken together suggest that hypothalamic VIP may act at the level of the PVN to stimulate anterior pituitary release of prolactin, and that the activity of this peptide may be under photoperiodic control.

734.10

AGE DEPENDENCE ON THE EFFECTS OF LESIONING THE DORSAL RAPHE NUCLEUS (DRN) IN PREPUBERTAL RATS ON FIRST OVULATION. M.E.Ayala and R.Domínguez*. B.R.R.U. FES-Zaragoza UNAM. AP 9-020, México DF

The serotonergic denervation induced by p-chloroamphetamine administration to prepubertal rats, affected first ovulation in an age-dependent way. Because the role of the serotoninergic innervation of the hypothalamus arising from the DRN is controvertial, to analyze if its participation in the regulation of ovulation varies with the age of the animal, the effects of an electrolytic lesion of the DRN at different ages of the juvenile period on ovulation at the first day of vaginal estrus, were studied. The results obtained were:

GROUP	Age of lesion	Ovulation rate	Ova shed
Control	-----	26/34	7.9±0.3
Lesion	21 day	3/7	7.3±0.9
	24 day	6/8	10.0±0.7*
	27 day	8/8	9.6±0.6*
	30 day	0/8*	0*

* $p<0.05$ vs control group.

Present results suggest that the role of the innervation arising from the DRN is inhibitory at the begining of the juvenile period and excitatory at the end. Supported by DGAPA and PUIS, UNAM.

734.11

ENDOCRINE PULSATILITY AND MUSCLE SPASM ACTIVITY IN SPINAL CORD INJURED (SCI) MEN BEFORE AND DURING NALOXONE INFUSION.
N.L. BRACKETT[1,2]*, C.M. LYNNE[1,3], M. WEIZMAN[1], M. AMADOR[1], M. ABAE[4]. The Miami Project to Cure Paralysis[1], Dept. of Neurological Surgery[2], Dept. of Urology[3], Dept. of OB/GYN[4], Univ. of Miami Sch. of Med., Miami, FL 33136.

The present study sought to determine: (1) if the pulse release of luteinizing hormone (LH), follicle-stimulating-hormone (FSH) and cortisol (C) were different in SCI men compared to non-injured men (controls), and (2) if infusion of naloxone would alter the pulse release of LH, FSH and C in either group. SCI (n=3) and control (n=3) subjects were placed in a research wing of Jackson Memorial Hospital, (Miami, FL) for 48 consecutive hours. Following an overnight adaptation period, blood (3 cc) was drawn every 15 minutes for 30 hours. Physiologic saline was infused during hours 2-7 and naloxone (bolus of 8 mg followed by 4 mg/hr) was infused during hours 24-30 of blood draws. Serum was separated and stored (-80^0) for 1-3 months prior to assay. Serum levels of LH, FSH, and C were determined by chemiluminescence assay (Amerlite System, Amersham). Pulse analysis was performed using PULSEFIT.

There was no difference between the two groups in the frequency or amplitude of LH and FSH peaks before naloxone infusion. During naloxone infusion, LH and FSH pulse frequency increased above saline baseline in 2 SCI subjects. Frequency of C pulses in the two groups were similar, but diurnal variation in C was flattened in SCI subjects. During naloxone infusion, an unexpected side effect occurred in SCI subjects. There was an increase in frequency, duration and intensity of muscle spasm activity. Spasms occurred throughout the body, but primarily below the level of injury.

These results indicate that the pulse release of gonadotropins may not be different in SCI men, but that the neuromodulatory role of endogenous opioids may be different in SCI men.

NEUROENDOCRINE REGULATION: OXYTOCIN, VASOPRESSIN, FLUID BALANCE, AND THE PINEAL

735.1

INTRACELLULAR CALCIUM CHANGE OF CELLS IN HYPOTHALAMIC SUPRAOPTIC REGION.
H. Yamashita*, K. Inenaga, L.-N. Cui, T. Nagatomo and H. Tanaka. Dept. of Physiology, Univ. Occupational & Environmental Health, Sch. of Med., Kitakyushu 807, Japan.

Neurotransmitters or neuromodulators modify neural activities in the central nervous system. Accumulating evidence suggests that such modification is accompanied with intracellular calcium change. To know the intracellular calcium change in cells located in supraoptic regions, thin slice preparations from young rats and Fura-2 image technique were used in this experiment. Some cells showed spontaneous change of $[Ca^{2+}]_i$. $[Ca^{2+}]_i$ in the cells was decreased by TTX or TTX/Ca^{2+} free medium. Angiotensin II (AII), endothelin (ET), kainic acid (KA) and NMDA increased $[Ca^{2+}]_i$. The fast component of $[Ca^{2+}]_i$ induced by AII and ET was maintained with TTX/Ca^{2+} free medium but not the slow component. The increase of $[Ca^{2+}]_i$ induced by KA and NMDA was abolished with TTX/Ca^{2+} free medium. These data suggest that the fast component of intracellular calcium increase induced by AII and ET is due to calcium release from the calcium store in cells of hypothalamic supraoptic region.

735.2

ALTERED EXPRESSION OF GLUT1 & GLUT3 GLUCOSE TRANSPORTERS IN THE NEUROHYPOPHYSIS OF DIABETIC RATS.
S.J. Vannucci*, F. Maher, I.A. Simpson. M.S. Hershey Medical Center, Hershey, PA 17033 and NIH/NIDDK/DB, Bethesda, MD 20892

The neurohypophysis (NH) is an extension of the central nervous system which contains axon terminals of vasopressin- and oxytocin-secreting neurons of the hypothalamus and glial-like pituicytes, but lacks a blood-brain barrier. The transport of glucose into these cells is mediated by the glucose transporter proteins, GLUT1 and GLUT3. In rat brain GLUT1 (55kDa) is concentrated in the blood-brain barrier, 45 kDa-GLUT1 is widely distributed in the neuropil; GLUT3 is the major neuronal transporter. Both GLUT3 and 45 kDa-GLUT1 are detected in the NH. In streptozotocin (STZ)-diabetic rats the hypothalamo-NH complex shows anatomic evidence of increased synthetic/secretory activity. The purpose of this study was to determine whether STZ-diabetes alters the expression of NH GLUT1 or GLUT3. Adult male rats were injected i.p with STZ , 65 mg/kg. Blood glucose was elevated (> 500 mg/dl) by 3d after STZ, demonstrating onset of diabetes. Animals were studied daily through 1 week, then at 2 and 4 wks. GLUT1 & GLUT3 were studied in individual NH by Western blot analysis with specific anti-C-terminal antisera; autoradiograms were quantitated by phosphorimage analysis. By day 3, NH GLUT3, per mg protein, increased by 20% and stayed at this level through 4 weeks; NH GLUT1 decreased by 20% at 3d, remained there through 2 wks and was 53% of age-matched control at 4 wks. The decrease in NH GLUT1 is similar to the peripheral cellular response to high glucose. However, the increase in NH GLUT3 (presumably axon terminal) in STZ-diabetes is the first demonstration of an up-regulation of GLUT3 in response to metabolic stress.

735.3

ROLE OF THE NUCLEUS MEDIANUS IN THE RELAXIN-INDUCED SECRETION OF VASOPRESSIN AND OXYTOCIN IN ANAESTHETIZED RATS.
B.J. Geddes and A.J.S. Summerlee*. Dept. of Biomedical Sciences, University of Guelph, Guelph, Ont., Canada N1G 2W1.

Injection of relaxin (iv) causes the release of both vasopressin and oxytocin as well as a rise in systemic arterial blood pressure (Parry, L.J. & Summerlee, A.J.S. (1991); Endocrinology 129: 47). It has been shown that central angiotensin II within the forebrain angiotensin pathway mediates the central effects of relaxin. The present experiments were done to investigate the role of the nucleus medianus in this action. Urethane anaesthetized female (SD) rats received an electrolytic lesion of the nucleus medianus. Blood samples were then taken at five intervals following an iv injection of either relaxin (5μg in 0.01mL) or physiological saline (0.01mL). Plasma vasopressin and oxytocin levels were determined by specific radioimmunoassay and analyzed by ANOVA. Lesion of the nucleus medianus but not control or sham-lesion significantly attenuated ($p<0.05$) the relaxin-induced secretion of both vasopressin and oxytocin. These results implicate the nucleus medianus in the central action of relaxin.

735.4

BRAIN ANGIOTENSIN MECHANISMS MEDIATE SEROTONIN (5HT)-INDUCED STIMULATION OF VASOPRESSIN (VP), BUT NOT OXYTOCIN (OT) SECRETION IN CONSCIOUS MALE RATS.
M.S. Brownfield[1]*, P.A. Rittenhouse[2], L.D. Van de Kar[2], and J.A. Saydoff[1] Departments of Comparative Biosciences, Univ. of Wisconsin Sch. Vet. Med., Madison, WI 53706[1] and Pharmacology, Loyola Univ. Sch. Med., Maywood, IL 60153[2].

We have previously shown that brain 5HT depletion blocks osmotically stimulated VP release and intraventricular (ivt) administration of 5HT to conscious male rats stimulates the secretion of both vasopressin and oxytocin, via a $5HT_{1c/2}$ mechanism. We questioned whether the serotonergic influence on VP cells was direct since immunocytochemical (ICC) studies have reported that 5HT terminals on VP cells are very scarce and on OT cells are only moderate. We hypothesized that the serotoninergic effect on VP was indirect, possibly acting via an intermediate pathway, possibly angiotensinergic in nature.

In a preliminary study we administered rats the angiotensin converting enzyme antagonist enalapril (60 mg/l) for 4 days in their drinking water prior to administration of the serotonin releaser d-fenfluramine (2 mg/kg ip) 30 and 60 minutes before sacrifice. Enalapril administration significantly blocked the VP, but not the OT response to fenfluramine. This suggests that inhibition of angiotensin II formation inhibits the normal VP response to 5HT release via fenfluramine.

We also tested the effect of selective brain angiotensin II receptor blockade by the AT_1 antagonist losartan (10 μg icv.) 30 minutes prior to 5HT stimulated (10 μg icv.) VP and OT secretion in serial blood samples of chronically cannulated conscious rats. Results showed that losartan administration significantly blocked the vasopressin, but not the oxytocin response to serotonin. Correlative ICC for cfos showed overlapping distributions of 5HT and angiotensin activated nuclei.

These studies suggest that 5HT stimulates both VP and OT secretion, and that VP secretion is activated by a brain angiotensinergic intermediate pathway.

735.5

RELEASE OF OXYTOCIN FROM SPINAL CORD SYNAPTOSOMES --- A MODULATORY ROLE BY MITOCHONDRIA. M. Daddona & J. Haldar*. Biological Sciences, St. John's Univ., Jamaica, NY 11439.

Previously we have shown that 56 mM KCl-induced oxytocin (OT) release from spinal cord (SC) synaptosomes (SP) is Ca^{++} dependent but differs in 3,100g (p2) and 12,000g (p3) pellets. Since SP contain mitochondria (Mit), an organelle which stores Ca^{++}, we have investigated if the difference in release pattern with p2 and p3 is related to the presence of Mit. SC of male Sprague Dawley rats was separated into cervical, thoracic and lumbosacral regions. Tissues were homogenized in 270 mM sucrose and p2, p3 pellets were separated. The presence of Mit in p2 & p3 pellets were determined morphologically by electron microscopic (EM) studies and biochemically by N-ethylmaleimide insensitive glycerophosphate acyltransferase (GAT). EM studies showed that in p2, Mit were located inside the SP while in p3 Mit were outside the SP. The GAT content of the p2 pellet was much higher than that of the p3 pellet. To determine whether the presence of Mit reduced SP Ca^{++} concentration by a Ca^{++} uptake process, ruthenium red, a mitochondrial Ca^{++} uptake inhibitor was used. Addition of 3 μM ruthenium red, 1 min prior to the addition of high KCl solution, reversed the OT release pattern in p2 pellet. The results suggest that mitochondria exert a modulatory role in OT release from spinal cord synaptosomes.

735.6

EFFECTS OF THE LESION OF THE NUCLEUS OF THE TRACTUS SOLITARIUS ON LABOR OF RATS. J.G. Ninomiya and I. Zarco de Coronado*. Fac. de Med., UNAM. A. Postal 70-250, C.P. 04510 and Esc. Médico Militar, U.D.E.F.A., México, D.F., México.

It has been described that approximately 85% of the afferent fibers of the vagus nerve have their first central synapses at the nucleus of the tractus solitarius (NTS). This study was made, in order to know about the role of these connections on the integrative function of paraventricular nucleus (NP) during parturition. Unilateral or bilateral DC current 0.5 nAmp during 20 sec was inyected 24 to 48 hrs before parturition, at NTS of anesthetized pregnant rats. Bilateral lesions produced a delayed labor (12-15 hrs) and all the fetuses were born dead. The unilateral lesions of NTS caused a delayed labor (5-30 hrs) and most of the fetuses were born dead. Our results indicate that the connection between the uterus and NTS is part of the complex neuroendocrine circuit involving NP, which plays an important integrative role for parturition.

735.7

IS BASIC FGF A NEUROENDOCRINE MODULATOR OF FLUID BALANCE? A.M. Gonzalez[1], E. Stopa[2], A. Logan[3], and A. Baird*[1]. [1]Dept. Molecular and Cellular Growth Biology, The Whittier Institute for Diabetes and Endocrinology, La Jolla, CA 92037; [2]Dept. Pathology, Rhode Island Hospital, Providence, RI 02903; [3]Wolfson Research Labs, Birmingham, UK B15 2TT.

Although the brain and pituitary are the richest sources of basic FGF, its exact physiological functions in these tissues are still unknown. Its localization in ependyma, subfornical organ, and magnocellular neurons from paraventricular and supraoptic nuclei suggests that it may be involved in water balance. In order to investigate the potential role of pituitary basic FGF as a neuroendocrine modulator of water balance, we studied the presence and distribution of basic FGF, its high affinity receptor (FGFR1), and their corresponding mRNAs in the neurohypophysis of normal rats. Extraction and Western blotting of the normal rat neurohypophysis show the presence of the three expected molecular forms of basic FGF. Similar analysis of this extract for FGFRs reveals proteins of the expected molecular weight (150kDa) as well as an immunoreactive protein of 80kDa. In the normal rat, basic FGF and FGFR localize to both the neurohypophysis and pars distalis, but not in the pars intermedia. In the neurohypophysis, basic FGF is detected in basement membranes, pituicytes and associated with Herring bodies. Pituicytes and Herring bodies also show intense immunostaining for FGFR. As expected from Northern analysis, *in situ* hybridization studies with a cRNA for rat basic FGF show that the pars distalis, pars intermedia and neurohypophysis all have non detectable to very low levels of basic FGF mRNA. In contrast, there is an intense positive signal for FGFR1 mRNA in the neurohypophysis that appears even more intense at the border between the adenohypophysis and neurohypophysis. The cells containing the FGFR mRNA were identified as pituicytes. Preliminary studies of the human pituitary gland reveal a distribution of basic FGF within the neurohypophysis which is similar to that seen in the rat. Classical models of fluid balance are currently being evaluated in order to identify the source of basic FGF (*i.e.*, SON and PVN nuclei) and test its potential role as a neuromodulator of posterior pituitary function. (Supported by NIH DK18811 and AG10682).

735.8

NEUROTENSIN-EVOKED EXCITATION OF RAT MAGNOCELLULAR NEUROSECRETORY CELLS (MNCs) INVOLVES INTRACELLULAR Ca^{++} MOBILIZATION. K. Kirkpatrick* and C.W. Bourque. Centre for Research in Neuroscience, Montreal Gen. Hosp. & McGill University.

We have previously shown that neurotensin (NT) excites rat supraoptic MNCs via postsynaptic receptors (Kirkpatrick and Bourque, soc. Neurosci. Abst., 1991). In this study we examined a possible role for intracellular [Ca^{++}] in NT-evoked responses using superfused rat hypothalamic explants and acutely isolated MNCs. As previously reported, application of NT (0.1-10 μM) during intracellular recordings from MNCs in explants excited 29 of 31 cells tested. In contrast, 4 of 5 MNCs impaled with electrodes containing 0.8M BAPTA failed to respond to NT application, suggesting that Ca^{++} may play a role in transducing NT-evoked excitation.

This possibility was further examined by incubating isolated MNCs in a medium containing 1-5 μM of the [Ca^{++}] indicator Fluo3-AM (45 minutes; 32°C). These cells were later perfused (≈1ml/min) at 30-34° and changes in fluorescence (emmission at 525 nm; excitation at 490) were monitored using a Leitz confocal scanning laser microscope. Bath application of NT (1-10 uM; 1 min) produced increases in cytoplasmic fluorescence in 9 of 12 cells bathed in the presence of Ca^{++} (1 mM). NT-evoked intracellular [Ca^{++}] increases were also observed from 8 of 10 cells perfused with media containing 0 Ca^{++} and 0.5 mM EGTA. These results suggest that the NT-evoked excitation of MNCs involves the mobilization of Ca^{++} from internal stores. *Supported by FCAR and the MRC of Canada.*

735.9

INHIBITORY EFFECTS OF ATRIAL NATRIURETIC FACTOR (ANF) ON SUPRAOPTIC NEURONS ISOLATED FROM THE RAT. Stéphane H.R. Oliet* and Charles W. Bourque. *Centre for Research in Neuroscience, Montreal General Hospital and McGill University, Montreal, P.Q. Canada H3G 1A4.*

ANF-like immunoreactivity has been recently found within brain areas involved in the control of fluid and electrolyte homeostasis including the supraoptic nucleus (SON). In order to investigate possible post-synaptic effects of ANF, we have used a preparation of acutely isolated rat SON neurons devoid of synaptic inputs. Bath-application of ANF (1-20nM) inhibited spontaneous spiking activity recorded in the on-cell mode in 17 of 23 cells tested. Whole-cell voltage-clamp recordings indicated that ANF increased membrane conductance due to the activation of a current reversing at -97±2 mV (n=12). When the extracellular [K^+] was raised (n=6), the reversal potential of the current shifted in strict correspondence with the K^+ equilibrium potential, suggesting that ANF was activating a current highly selective for K^+. Similar effects were evoked in 5 of 7 cells when dibutyryl-cGMP (0.5-1mM) was added to the perfusate.

Our results suggest that endogenously released ANF may modulate the electrical activity of rat supraoptic neurons via the activation of post-synaptic K^+ channels, possibly involving the production of cGMP.

Supported by the MRC and Heart & Stroke Foundation of Canada.

735.10

PASSIVE ELECTRICAL PROPERTIES OF SUPRAOPTIC NUCLEUS (SON) NEURONS OBSERVED WITH WHOLE CELL RECORDINGS *IN VITRO*. W.E. Armstrong*, Dept. of Anat. Neurobiol., Univ. Tenn., Memphis, TN 38163.

The electrical properties of neurons should be estimated more accurately with whole cell recordings by reducing current leak at the electrode-membrane interface and by reducing electrode noise. Whole cell voltage recordings were obtained from intact neurons in the SON of the rat hypothalamo-neurohypophysial explant. With an internal solution containing (in mM) 135 K-gluconate, 5 KCl, 2 $MgCl_2$, 4 KOH, 2 ATP, 0.2 GTP and 10 HEPES, properties commonly observed with sharp electrodes, including transient outward rectification, a prominent afterhyperpolarization, spike-frequency adaptation and in some cells, a depolarizing afterpotential, were observed.

Neurons recorded with the whole cell method exhibited input resistances at least twice that of those seen with sharp electrodes (400-500 vs 200 MΩ). With hyperpolarizing pulses, a linear steady state I/V range from about -60 to - 90 mV was present, but this range was not strictly passive as indicated by asymmetries between the discharging and charging limbs of voltage transients, and between the responses to depolarizing and hyperpolarizing current injection. In addition, a voltage related change in the estimated membrane time constant (τ_0) was present in some neurons in the linear steady state range. The passive range was more narrow and covered only a 5-10 mV region at least 10-15 mV beneath spike threshold. Analysis of voltage transients in this passive region revealed a membrane time constant 2-4 times longer than that seen in sharp electrode recordings, with τ_0 ranging from 25-54 ms across neurons. Taking advantage of the lack of a somatic shunt, an equivalent cylinder analysis of some neurons revealed a dendritic electronic length of ~1.0 calculated from transients exhibiting an equalizing time constant, a value similar to that estimated previously with sharp electrodes (Neurosci. 38: 485, 1990). Transients from other cells were monoexponential. Supported by NIH NS23941 (WEA).

735.11

NEURAL MODULATION OF OXYTOCIN (OT) RELEASE DURING SUCKLING. E.Koehler*, S.Mantz, and J.Summy-Long. Dept.of Pharmacol., MS Hershey Med.Ctr., Penn.State Univ., Hershey, PA, 17033

During lactation new GABAergic synapses form on oxytocinergic neurons which may modulate release of OT by suckling. Moreover, pharmacologic evidence documents that nitric oxide(NO) attenuates release of OT during dehydration (Summy-Long et al, Neurosci.Lett. 1993,in press). We therefore examined in conscious lactating rats whether GABA or NO modulates suckling-induced release of OT. Stretch reflexes(SR) in the pups, associated with milk let-down during nursing, were timed and used as indices of OT release. In study #1, CSF(5 ul) with or without GABA (25ug) or bicuculline(BIC; 50ng), was infused intracerebroventricularly(icv) following the 3rd SR and the dam was decapitated after the 4th SR. Study #2 was similar except an inhibitor of NO synthase(N^Gnitro-l-arginine methyl ester,NAME;1000 or 500ug) or CSF was infused icv at the onset of nursing and the mother was killed at the 3rd SR. OT was quantified by RIA. Plasma levels of OT (pg/ml;$\bar{x}\pm$SEM;n=5-11) elevated by suckling (CSF, study #1: 22±4; study #2: 29±5) were unchanged after GABA (18±4), BIC (19±2) or NAME (1000ug: 30±6; 500ug: 30±4). Both BIC and NAME(1000ug) disrupted nursing behavior such that the last SR was delayed (min;$p<0.05$, Tukey's t) only by BIC (CSF vs BIC; 6±1 vs 14±3). Suckling-induced release of OT was therefore unaffected by low doses of GABA and NAME that did not disrupt nursing behavior.(Supported by RO1HD25498)

735.12

LACK OF RESPONSE TO NMDA IN RAT SUPRAOPTIC OXYTOCIN NEURONS *IN VIVO*. R. Nissen*, B. Hu, L.P. Renaud. Loeb Research Institute, Ottawa Civic Hospital, Ottawa, Ontario, Canada, K1Y 4E9.

We previously reported that NMDA receptor blockade by a non-competitive open channel blocker, ketamine, depresses spontaneous activity of supraoptic vasopressin (VP) but not oxytocin (OT) neurons (Nissen et al., 1992). The latter suggests a lack of functional NMDA receptors in supraoptic OT neurons. In the present study, the effects of specific glutamate receptor agonists were evaluated *in vivo* to determine whether OT neurons are responsive to NMDA application. Extracellular recordings were obtained from identified VP and OT neurons in pentobarbital anaesthetized Long-Evans rats. Local pressure application of NMDA (100μM) evoked activity in a dose dependent manner when applied during periods of spontaneous quiescence in 27/28 phasic VP neurons and significantly increased spontaneous firing when applied during periods of activity in 5/5 of these cells. By comparison, identical application of NMDA was associated with an increase in firing of only 1/9 OT neurons. The selective non-NMDA receptor agonists AMPA (100μM) and kainate (100μM) elevated spontaneous activity in 4/5 and 7/9 oxytocin neurons, respectively; similar excitatory responses were also observed in the majority of vasopressin neurons (21/21 and 14/15 respectively). Our results suggest that both VP and OT neurons are sensitive to non-NMDA receptor activation, while OT neurons appear to lack a response to NMDA. (Supported by MRC).

735.13

ATYPICAL PLASMA VASOPRESSIN RESPONSES TO NON-OSMOTIC STRESS IN ADRENAL DEMEDULLATED RATS. D. Jezova, A. Kiss and G. Aguilera*, DEB, NICHD, NIH, Bethesda, MD and SAS, Bratistava, Slovakia.

We have recently shown unexpected increases in hypothalamic vasopressin (VP) mRNA and plasma VP responses to acute immobilization (IMMO) in 2-month adrenal demedullated (MDX) rats with normal adrenocortical function. The role of adrenal catecholamine deficiency on the VP responses was studied in rats with splanchnic nerve section or ß-adrenergic or dopaminergic blockade. Injection of propranolol (5 mg/kg) or domperidone (2 mg/kg), 45 min prior acute IMMO in intact rats, did not affect ACTH and corticosterone responses and failed to increase plasma VP. Similarly, 10 days after splanchnic nerve section, IMMO did not increase plasma VP, despite near absent plasma epinephrine responses. In contrast to chronic MDX, after short term (12 days) enucleation of the inner adrenal zones, there was no increase in plasma VP after acute IMMO, with or without corticosterone replacement. The data show that neither the lack of acute epinephrine release during stress, nor short term absence of the medulla causes magnocellular activation. Thus, long term adaptation to MDX may suppress an inhibitory pathway which normally prevents increases in plasma VP to non-osmotic stress.

735.14

VASOPRESSIN NEURON ACTIVATION AND FOS EXPRESSION:ELECTRICAL STIMULATION OF THE CAUDAL MEDULLA OBLONGATA. S.Shioda , Y.Nakai, M.Iwase, I.Homma and M.Kawatani* Dept. of Anatomy and Physiology, Showa Univ. Sch. of Med., Tokyo 142, Japan.

The expression of Fos, the protein product of the primary response gene c-fos, was used metabolically to map the effects of unilateral electrical stimulation (150 μA at 50 Hz for 10 min) of A1 or A2 cell group in the caudal medulla oblongata. Electrical stimulation of the A1 cell group in rats anesthetized with urethane led to increase in mean arterial pressure and to bilateral increase of Fos-like immunoreactivity (FLI) in the ventral part of the supraoptic nucleus (SON) and posterior magnocellular part of the paraventricular nucleus (PVN). FLI was visible only in the vasopressin neurons but rarely in oxytocin neurons after stimulation of A1 cell group. However, electrical stimulation of A2 cell group led to increase heart rate but blood pressure was unchanged, and to bilateral increase of FLI in the SON and PVN, especially abundant in the parvocellular parts of the PVN. FLI was visible both in vasopressin and oxytocin neurons in the SON and PVN after stimulation of A2 cell group.

735.15

PHOTOPERIOD REGULATES THE MELATONIN RECEPTOR DENSITY IN THE QUAIL OPTIC TECTUM. B. Stankov[1], N. Aste[2], V. Lucini[1], S. Capsoni[1], C. Viglietti-Panzica[2], R. Nonno[2], F. Fraschini[1], G.C. Panzica[2], and B. Cozzi[1]*. [1]University of Milan, 20129 Milano and [2]University of Turin, 10128 Torino, ITALY

Male quails (*Coturnix japonica*) were kept under short (LD 8:16, n=6) or long photoperiod (LD 16:8, n=6) for 8 weeks, their brains collected around the middle of the light phase, and processed for quantitative melatonin receptor autoradiography. In both experimental groups, melatonin receptors were identified in several nuclei involved in the entrainment of circadian rhythms and processing of visual inputs (GLv, nDSV, ROT, nBOR, PPC, PT, TeO), and in the nuclei of cranial nerves responsible for the control of eye movements (nVI, EW, OM), as recently reported (Cozzi et al., 1993, Neurosci. Lett. 150:149). The apparent receptor density was approximately two times higher in the optic tectum of the short-day group. A similar difference in the optic density was evident also in the pars tuberalis. Melatonin receptors have already been described in the GLv, nDSV, and other brain nuclei in birds. Although differences in melatonin receptor density during the day have been demonstrated in the chicken brain (Brooks and Cassone, 1992, Endocrinology 131:1297), changes in the receptor density related to the photoperiod are reported here for the first time. The quail responds to increments in the dark phase of the photocycle with a dramatic alteration of its reproductive competence. These changes might be mediated through the action of melatonin on its high-affinity receptors in the brain, where their apparent density is inversely proportional to daylength.

735.16

TEMPORALLY DISTINCT RELEASE OF SEROTONIN AND MELATONIN FROM SUPERFUSED RAT PINEALOCYTES. J. Olcese*, M. Schumacher, M. Münker and C. McArdle. Institute for Hormone and Fertility Research, University of Hamburg, 2000 Hamburg 54, Federal Republic of Germany.

The present studies examined the adrenergic regulation of serotonin (5HT) and melatonin (MEL) secretion from superfused pinealocytes grown on microcarrier beads for 48h before stimulation. Indoleamine release was measured in 10 min fractions by two specific radioimmunoassays. Whereas MEL release following 1 μM norepinephrine or isoproterenol was robust but relatively sluggish (rising not until 1.5 - 2 hours after stimulation), the molar release of 5HT was considerably greater and much more rapid, occurring within 30 minutes after adrenergic stimulation. The α- agonist phenylephrine was without effect on either indoleamine. Using a 15 minute stimulation paradigm individual 5HT pulses could be elicited repetitively without similar MEL release profiles. These data support the hypothesis that different secretory mechanisms are involved for pineal 5HT and MEL. This new cell culture system should allow for a finer analysis of serotonergic function in the mammalian pineal gland.

735.17

REGULATION OF PINEALOCYTE SYNAPTIC RIBBONS (SR) BY NORADRENALINE (NE) AND SYMPATHETIC NEURONS IN A CO-CULTURE MODEL. I.A. McNulty*T. Meldgaard, L.M. Fox, S-Y. Tsai, N. Tønder. Dept. Cell Biology, Neurobiology, Anatomy, Loyola Univ. Med. Ctr., Maywood, IL 60153, and PharmaBiotec, Inst. Neurobiol., Univ. Aarhus, Denmark.

The mammalian pineal gland (PG) is characterized by SR, which reflect the PG's phylogenetic derivation from photoreceptors. More importantly, numerous studies have demonstrated numerical changes in SR related to PG melatonin production. To test the hypothesis that SR frequency is regulated by innervation from the superior cervical ganglia (SCG), a PG-SCG co-culture model was developed. PG and SCG from neonatal (5 day old) Wistar rats were mounted on glass coverslips in a clot of reconsituted chicken plasma coagulated by a drop of thrombin and cultured by the roller-tube technique (Gähwiler, BH, Neurosci. Meth. 4: 329-342, 1981). The groups included: 1) PG cultured alone, 2) PG co-cultured with SCG, and 3) PG treated with NE (10^{-5}M) added every 3 days. After 30 days, cultures were fixed, processed for routine transmission electron microscopy and SR counted by a "blind" observer. SR frequency (number/unit area of PG) was significantly higher in PG cultured alone compared to PG+SCG and PG+NE (ANOVA; $p<0.01$). SR fields (2-8 SR) were common in the PG group. Pinealocytes were highly differentiated and displayed an ultrastructure typical of that seen in situ, except that pinealocytes in the PG+NE group tended to have enlarged mitochondria and expanded Golgi saccules and endoplasmic reticulum. These findings are consistent with the hypothesis that SCG innervation regulates PG SR. Ultrastructural changes in NE-treated cultures suggest that NE effects on SR are mediated by other mechanisms.

Supported by NSF (BNS 8801726), the Danish MRC, and the Lundbeck Foundation, DK.

735.18

MULTIPLE INJECTIONS OF ISOPROTERENOL DELAY THE RAPID DECLINE OF ENDOGENOUS MELATONIN PEAK IN TURKISH HAMSTERS. S.M.Hong* and M.H.Stetson. Univ. of Delaware, Newark, DE 19716.

In a preliminary study, we found that multiple injections of isoproterenol (ISO) generate an overall stimulatory effect on induction of pineal melatonin synthesis in Turkish hamsters (Mesocricetus brandti) and that the effect is not as potent as in Syrian hamsters (Mesocricetus auratus). In order to further examine the role of beta-adrenergic components in generating the endogenous melatonin peak, Turkish hamsters were subjected to 4 injections of ISO (1 mg/kg B.W.) beginning at 0600 h with the room light off until the end of experiment (normal light onset, 0800 h). ISO or saline injections were given s.c. every two hours from 0600 h to 1200 h and pineal glands were collected under dim red light every hour from 0700 h to 1000 h and every other hour from 1000 h to 1400 h. Uninjected animals were also sacrificed every other hour from 2000 h to 0800 h. ISO injected animals showed a significantly higher content of pineal melatonin than in saline groups at 0800, 0900 and 1000 h ($p=0.0006$, 0.0225, or 0.0006, respectively, LSMeans). These results suggest that the beta-adrenergic receptors play a critical role in maintaining the endogenous melatonin peak in Turkish hamsters.

(supported by NSF Research Grant DCB87-14638)

PAIN MODULATION: PHARMACOLOGY V

736.1

THE DEVELOPMENT OF MORPHINE TOLERANCE IS ASSOCIATED WITH INCREASES IN MEMBRANE-BOUND PROTEIN KINASE C: PREVENTION BY GM1 GANGLIOSIDE D.J. Mayer*, J. Mao and D.D. Price, Dept. Anesthesiology, Medical College of Virginia, Richmond, VA 23298

In a rat model of morphine tolerance and dependence, we examined the hypotheses that the development of morphine tolerance is associated with the translocation of protein kinase C (PKC) and that agents blocking PKC translocation will prevent both the development of morphine tolerance and dependence. The translocation of PKC was measured by using an established [^{3}H]phorbol-12,13-dibutyrate binding assay ([^{3}H]PDBu), which measures primarily membrane-bound (translocated) PKC. The development of morphine tolerance was examined utilizing the tail-flick test. Once daily intrathecal (i.t.) treatment with 10 μg morphine sulfate for 8 days produced morphine tolerance and, at the same time, reliable increases in [^{3}H]PDBu binding within laminae I-II of the spinal cord dorsal horn of these same rats when examined on Day 8 as compared to saline controls ($P< 0.05$). Both the development of morphine tolerance and the increase in [^{3}H]PDBu binding were potently prevented by i.t. co-treatment of morphine with 160 nmol GM1 ganglioside (an inhibitor of PKC translocation) when tested on Day 8 as compared to saline plus morphine controls (each $P< 0.05$). GM1 ganglioside (10, 30, 60 mg/kg) given intraperitoneally at 30 min before each subcutaneous (s.c.) morphine injection (10 mg/kg) for 9 days also prevented the development of morphine dependence as indicated by reliably less naloxone (2mg/kg) precipitated jumping at 24 hr after the last morphine treatment as compared to saline controls ($P< 0.05$). The results indicate a critical role of PKC in the development of morphine tolerance and dependence and suggest that the development of morphine tolerance and dependence may be mediated through central nervous system neuroplasticity initiated by PKC translocation following neuronal excitatory amino acid receptor activation. Supported by NS 24009 and VCU Grant-in-Aid.

736.2

THERMAL HYPERALGESIA ASSOCIATED WITH THE DEVELOPMENT OF MORPHINE TOLERANCE IN RATS: ROLE OF EXCITATORY AMINO ACID RECEPTORS AND PROTEIN KINASE C J. Mao*, D.D. Price, J. Lu and D.J. Mayer, Dept. Anesthesiology, Medical College of Virginia, Richmond, VA 23298

In a rat model of morphine tolerance and dependence induced by repeated intrathecal (i.t.) morphine treatment, we examined whether thermal hyperalgesia develops in association with the development of morphine tolerance. Once daily i.t. treatment with 10 μg morphine sulfate for 8 consecutive days reliably induced tolerance to the analgesic effect of morphine (tail-flick test) as compared to saline controls ($P< 0.05$) when examined on Day 8. In association with the development of morphine tolerance, foot-withdrawal latencies to radiant heat were significantly reduced ($P< 0.05$), i.e., thermal hyperalgesia, in these same rats when tested on Day 8 before morphine treatment or Day 10 (48 hr after the last morphine treatment). Both the development of morphine tolerance and thermal hyperalgesia were reliably prevented by co-treatment of morphine with MK 801 (an NMDA receptor antagonist, 10 nmol, $P< 0.05$) or GM1 ganglioside (a protein kinase C inhibitor, 160 nmol, $P< 0.05$). In addition, a single treatment with MK 801 (10, 5, not 2.5 nmol) or CNQX (a non-NMDA receptor antagonist, 160, 80, not 40 nmol), but not GM1 (160 nmol), reliably reversed thermal hyperalgesia that had developed following repeated morphine administration on Day 10 when tested 30 min after each drug treatment (each $P< 0.05$). These results indicate that thermal hyperalgesia develops in association with the development of morphine tolerance and that NMDA/non-NMDA receptors and protein kinase C may mediate the thermal hyperalgesia. The data suggest that repeated morphine treatment may exacerbate some clinical pain states such as neuropathic pain and post-operative pain. Supported by PHS grant NS 24009 and Virginia Commonwealth University Grant-in-Aid.

736.3

BEHAVIORAL TESTING MAY PRODUCE TAIL-FLICK HYPERALGESIA IN RATS ACUTELY TREATED WITH MORPHINE. A.E. Baldwin, K.P. Myers, J.P. Willi, K.K. Newman, M.R. Dilley, & J.T. Cannon*. Department of Psychology & Neuroscience Program, University of Scranton, Scranton, PA 18510-4596.

Recently, Kaplan and Fields (1991) demonstrated hyperalgesia following naloxone injections in rats that had previously received a single morphine injection. They concluded that this was evidence for the development of acute tolerance and withdrawal.

Baldwin et al. (1992) found that simply exposing rats to a prolonged tail-flick stimulus can produce long lasting reductions of flick latencies. In the Kaplan and Fields study, all morphine-treated animals were behaviorally tested under the influence of morphine and were therefore exposed to repeated prolonged tail-flick trials. The present study examined the possibility that such testing contributes to the post-morphine hyperalgesia observed by Kaplan and Fields.

Male albino rats were tested 40 min after injection with pentobarbital (50 mg/kg i.p.). Baseline tail-flick latencies (M = 4.22 sec) were obtained at 1 min intervals. Animals (8/group) received either morphine (5 or 15 mg/kg s.c.) or saline injections. One saline and one 5 mg/kg morphine group were then tested for 40 min. The remaining 3 groups (saline, 5, and 15 mg/kg morphine) were handled at each test interval, but not exposed to the heat stimulus. All groups then received naltrexone (5 mg/kg s.c.), and were then tested for 30 min. Surface tail temperatures were obtained following each test interval for all groups.

Post-naltrexone latencies of the 5 mg/kg morphine group that was not tested while analgesic did not differ significantly from those of either saline group. Post-naltrexone latencies of the 5 mg/kg morphine group that was tested were significantly lower than all other groups. This difference was not dependent on changes in tail temperature.

These data suggest that hyperalgesia following a single morphine injection may be dependent upon behavioral testing under the influence of morphine. In light of Kaplan and Fields' demonstration that the rostral ventromedial medulla is involved in the hyperalgesia they examined, this region of the brain may also mediate the hyperalgesia observed here and in our previous work, which did not involve the administration of opiates.

736.4

DOSE-DEPENDENT DIMINISHED SENSITIVITY TO MORPHINE PLACE PREFERENCE IN RATS WITH PERSISTENT INFLAMMATORY PAIN. K. J. Sufka*. Department of Psychology, University of Mississippi, Oxford, MS 38677.

Rats display preferences to environments that have been previously paired with positively reinforcing drugs. Morphine possesses both positively (reward) and negatively (analgesic) reinforcing effects. The present research sought to determine whether animals experiencing chronic pain would display an enhanced place preference to morphine. Persistent inflammatory pain was induced by unilateral injections of complete Freund's adjuvant (0.1 ml) into the rat hindpaw. Rats received 8 counterbalanced conditioning trials (4 drug, 4 no-drug) of 60 min. each to various morphine doses (Exp. 1: 0.0, 0.1, & 1.0 mg/kg; Exp. 2 : 0.0, 3.0, & 10.0 mg/kg) in a 3 compartment (2 stimulus, 1 neutral) place-preference apparatus Significant place preference, as indexed by time spent in drug-paired chamber during a 15 min. test period, was observed in non-inflamed rats at all morphine doses tested. However, inflamed rats exhibited significant place preference only at the 3.0 and 10.0 mg/kg morphine doses. This diminished sensitivity to morphine's reinforcing effects in inflamed animals may reflect the development of tolerance resulting from activation of the endogenous opioid system during persistent inflammatory states.